JIMMA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEVELOPMENT OF NUTRIENT DENSE, OPTIMIZED NOVEL COMPLEMENTARY FOOD FROM LOCAL CEREAL-LEGUME BLENDS ENRICHED WITH *DABI TEFF* (*Eragrostis Teff)* GROWN IN WESTERN ETHIOPIA

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Jimma, Ethiopia

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A PhD Dissertation Submitted to the School of Graduate Studies of Jimma University, Department of Nutrition and Dietetics in fulfillment of the requirements for the award of the degree of Doctor of Philosophy (PhD) in Human Nutrition

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Dedication

I dedicate this dissertation work to my late parent, Raga’e Ararso, mother and, Ethiyane Chewaka, sister.

Acronyms and Abbreviations

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CI	Confidence interval
CSB	Corn Soya Blend
CSB+	Corn Soya Blend Plus
CSB++	Corn Soya Blend Plus Plus
dL	Deciliter
EDHS	Ethiopia Demographic and Health Survey
EPHI	Ethiopian Public Health Institute
FAO	Food and Agriculture Organization
FGDs	Focus Group Discussions
FNPS	Food and Nutrition Policy Strategy
g	Gram
ICF	International Classification of Functioning, Disability and Health
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
IDA	Iron deficiency Anemia
KIIs	Key Informants Interview
ISO	International Organization for Standardization
IYCF	Infant and young child feeding
kg	Kilogram
L	Liter
mg	Milligram
mL	Milliliter
mon	Month
h	Hours
HCL	Hydrochloric acid
s	Seconds
^{0}C	Degree Celsius
NGO	Non-Governmental Organization

NNP	National Nutrition Program
PAHO	Pan American Health Organization
PEM	Protein-energy malnutrition
PhD	Doctor of Philosophy
Ph:Ca	Phytate to calcium molar ratio
Ph*Ca:Zn	Phytate by calcium to zinc molar ratio
Ph:Fe	Phytate to iron molar ratio
Ph:Zn	Phytate to zinc molar ratio
RDA	Recommended Dietary Allowance
RNI	Recommended Nutrient Intake
SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
SSA	Sub-Saharan Africa
UNICEF	United Nations International Children's Emergency Fund
Viz	Videlicet (meaning, namely)
USA	United State of America
WFP	World Food Program
WHO	World Health Organization

"The Youngest are Hardest Hit by Nutritional Deficits - Hunt for Provision of Nutritionally Adequate Food and Health Care Services for Sustainable and Timely Action - Implication for National Economic Development." **(Scaling-Up Nutrition,** A framework for action, 2011)

Table of Contents

Acknowledgments i

Dedication iii

Acronyms and Abbreviations iv

Summary xi

Chapter 1: General Introduction 1

1.1 Malnutrition-an overview 2

1.1.1 Protein-energy malnutrition in children 3

1.1.2 Micronutrient malnutrition in children 5

1.2 Impact of childhood malnutrition 9

1.3 Causes of child malnutrition 10

1.4 Strategies to alleviate child malnutrition 12

1.5 Food based strategy- a sustainable approach to alleviate child malnutrition 13

1.6 Energy and nutrient requirements from complementary foods 16

1.7 Blending cereal and legume ingridients for complementary food development 18

1.8 Anti-nutrient contents 20

1.9 Sensory evaluation 21

1.10 Traditional usage, social beliefs and cultivation trends of *dabi teff (Eragrostis teff)* 22

1.11 Selection of raw meterials for complementary food formulation 22

1.11.1 *Dabi teff* (*Eragrostis teff*) 24

1.11.2 Barley (*Hordeum vulgare*) 25

1.11.3 Oats (*Avena sativa*) 26

1.11.4 Field pea (*Pisum sativum L.*) 26

1.11.5 Maize (*Zea mays L.*) 27

1.11.6 Linseed (*Linum usitatissimum*) 27

1.12 Knowledge gap-drivers of this study 29

1.13 Research Objectives and Hypothesis 33

1.13.1 Objectives 33

1.13.2 Hypotheses of the study 34

1.14 Dissertation Layout 35

Chapter 2: Materials and Methods 37

2.1 Description of the study area 38

2.2 Study Design 38
2.3 Methods for the Scoping Review 39
2.4 Methods for the Ethnographic Qualitative Study 41
2.4.1 Study design 41
2.4.2 Sampling technique and study participants selection procedure 41
2.4.3 Data collection tools 42
2.4.4 Tool validation and pilot-testing 43
2.4.5 Data collection procedures 43
2.4.6 Trustworthiness of the data 44
2.4.7 Data analysis 44
2.5 Methods for the Experimentation 46
2.5.1 Sample collection 46
2.5.2 Samples processing 47
2.5.3 Flour preparation and handling 49
2.5.4 Experimental design 50
2.6 Statistical analysis and model evaluation 61
2.7 Variable optimization and validation 62
2.8 Data quality assurance 64
2.9 Ethical considerations 64
2.10 Dissemination of findings 64
Chapter 3: Nutritional Enrichment of Traditional Complementary Foods Using Underutilized Nutritious Plant Foods and their Nutritional Potential and Health Benefits in sub-Saharan Africa: -A Scoping Review 66
Abstract 67
Introduction 68
Methods 71
Results 71
Discussion 76
Conclusion 86
Chapter 4: Ethnographic Qualitative Study to Uncover the Traditional Usage, Social Beliefs and Cultivation trends of *Dabi Teff* (*Eragrostis teff*), A Typical Farmer Variety Teff, Grown in Western Ethiopia 87

Abstract 88
Introduction 89
Methods 92
Results 92
Discussion 102
Conclusion 105
Chapter 5: Optimization of a Formula to Develop Energy and Nutrients Dense Novel Complementary Food from Cereal-Legume Blends Enriched with *Dabi Teff* Grown in Western Ethiopia 106
Chapter 5.1: Optimization of *Dabi Teff*-Field Pea Based Energy and Protein-Dense Novel Complementary Food with Improved Sensory Acceptability Using D-optimal Mixture Design 107
Abstract 108
Introduction 109
Materials and Methods 113
Results 113
Discussion 126
Conclusion 133
Chapter 5.2: Optimization of a Formula to Develop Iron-Dense Novel Composite Complementary Flour with a Reduced Phytate/Minerals Molar Ratio from *Dabi teff*-Feld Pea Based Blends Using a D-Optimal Mixture Design 134
Abstract 135
Introduction 136
Materials and Methods 140
Results 141
Discussion 156
Conclusion 162
Chapter 6: Effects of Blending Ratios Variation on Macro- and Micronutrients, Phytate/Minerals Molar Ratio and Sensory Acceptability of *Dabi teff*-Field Pea Based Novel Composite Complementary Flours 164
Chapter 6.1: Effects of Blending Ratio Variation on Macronutrient Compositions and Sensory Acceptability of *Dabi teff*-Field Pea Based Novel Composite Complementary Flours 165

Abstract 166
Introduction 167
Methods and Materials 169
Results 169
Discussion 181
Conclusion 182
Chapter 6.2: Effects of Blending Ratios Variation on Micronutrient Compositions and Phytate/Minerals Molar Ratios of *Dabi teff*-Field Pea Based Novel Composite Complementary Flours 184
Abstract 185
Introduction 186
Methods and Materials 189
Results and Discussion 190
Conclusion 203
Chapter 7: Energy, Protein and Iron Densities of *Dabi Teff*-Field Pea-Based Optimized Novel Complementary Flour and Its Contribution to Daily Energy and Nutrients Demand by 6–23-months-old Children 204
Abstract 205
Introduction 206
Materials and Methods 211
Results 211
Discussion 221
Conclusion 227
Chapter 8: General Discussion, Conclusion and Recommendations for Policy Actions and Further Research 229
8.1 General introduction 230
8.2 General discussion on the main findings of the project work 232
8.3 Implications of the dissertation works 236
8.3.1 Implication of the need to develop nutrient-dense complementary food 236
8.3.2 Implication of cereal-legume blends in complementary food development 237
8.3.3 Implications of incorporating *dabi teff* to the cereal-legume blends for complementary foods development 239
8.3.4 Implication of the proper selection and processing techniques applied 241

8.3.5 Implication for formulation and optimization 242
8.4 Application areas of the project work findings 243
8.5 Limitations of the study 244
8.6 Conclusions 245
8.7 Recommendations for policy and further research 248
8.7.1 Recommendations for policy actions 248
8.7.2 Recommendation for further research 249
References 252
Annex I - Curriculum Vitae of the Author 268
Annex II-Ethnographic Qualitative Data Collection sheet 273
Annex III- Sensory Evaluation Format 275
Annex IV: Ethical Approval 276
Annex V- Preliminary Key Micronutrient Analysis Results of *Dabi teff* 277
Annex VI- Declaration 278

Summary

Complementary feeding determines children's growth and survival with implications on health, education, work capacity and productivity in later life and on national economic development. Consequently, the provision of nutritionally adequate, safe and acceptable complementary food to ensure the nutritional well-being of children in early life is more emphasized. There is urgency and opportunity to explore the possibility of developing nutritionally adequate complementary foods based on locally available cereals and legume blends by inclusion of nutritious underutilized plant foods.

Poor quality complementary foods due to high dietary bulk, lower energy, protein and iron densities, lower intake of bioavailable iron and inadequate intake of daily required nutrients by children coupled with inappropriate feeding practices are leading to a great risk of nutritional deficiencies. These are among the prime causes of persistent and unacceptably high protein-energy and micronutrient malnutrition in infants and young children. Proper food selection, combination and preparation practices that affect the nutrient density, bioavailability and acceptability compounded by age-specific feeding, caregiver's nutrition knowledge and socio-economic statutes of the family are among the known integral components of caregiving to infants and young children feeding (IYCF).

The food-based approach is a sustainable way of providing a nutritionally adequate complementary diet through diversification of locally available foods either through own production or purchase from the local market, proper selection, preparation and adequate feeding. The approach also employs the enrichment of traditional foods using nutritious local foods, where if the enriched food is consumed on a regular and frequent basis, it will maintain body stores of nutrients more efficiently and effectively than intermittent supplements. This is an important advantage to growing children who need a sustained supply of macro- and micronutrients for growth and development particularly in rural and semi-urban poor who don't have purchasing power of commercial fortified foods. The use of indigenous locally available food ingredients to develop complementary products could provide an opportunity to utilize the use of unreached/untouched golden crops to prepare good quality home-made complementary foods for IYCF.

The main aim of this PhD dissertation work was to develop a nutrient dense optimized novel complementary food from locally available cereal-legume blends through the incorporation of *dabi teff*, the underutilized crop to be used for IYCF in Ethiopia. Before diving into the detail dissertation works, a scoping review, ethnographic qualitative study and preliminary test on the key micronutrient contents of the principal ingredient (*dabi teff)* were conducted **(Annex V)**. Proper controlled processing techniques were applied to the selected cereals, legume and oilseed crops. The energy, protein and iron densities of the developed complementary product were compared with the traditional complementary food and the Cerifam® faffa flour (the popular commercial complementary flour in Ethiopia) and checked against the Food and Agriculture Organization and World Health Organization (FAO/WHO) recommended standards. The effects of blending ratio variation on the macro- and selected micro-nutrient compositions of the formulations were investigated.

Finally, the likely contribution of the optimized novel complementary product to daily energy and nutrient damans by 6-23 months children was checked/modeled against the recommended dietary allowance (RDA) set by the Pan American Health Organization and World Health Organization (PAHO/WHO) child feeding guidelines. This was to verify whether the new product could demonstrate the energy and nutrients adequacy to expect positive nutritional outcomes following feeding of infants and young children. To realize these, the dissertation work was organized into seven interrelated studies in Nedjo district, Ghimbi Zone, Western Ethiopia.

The summary of the major findings from each chapter of the study was presented as follows: **Chapter 3** presents a scoping review of studies conducted in sub-Saharan Africa (SSA) on the possibility of enriching traditional staple foods using nutritious underutilized plant foods including the effects of common processing techniques on the nutritional value of the resultant complementary products. The review showed that underutilized plant foods such as amaranth seeds, moringa olifera leaf, baobab fruit pulp, Bambara ground nut, chickpea, red teff, soya bean, spinach, peanut and orange-fleshed sweet potato were used to enrich traditional complementary diets. All the studies have proven that enrichment of the staple diets with underutilized nutritious plant foods has resulted in the development of nutritionally dense complementary foods, meeting standards, specifically, with improved micronutrient contents such as iron, calcium, zinc magnesium, phosphorous, and potassium. The most common processing techniques applied were

found to be fermentation, germination, malting, extrusion cooking, dehulling and roasting resulting in the improvement of the nutritional values and bioavailability of micronutrients in the complementary foods developed.

Chapter 4 presents an exploratory ethnographic qualitative study on traditional usage, social beliefs and cultivation trends of *dabi teff* (*Eragrostis teff*), a typical farmer variety teff grown in Nedjo district, Western Ethiopia: including its usage as complementary food. The result showed that there is a profound tradition in the usage of *dabi teff*, where the participants had shown to have life-long practices of preparing and enjoying a number of food forms from *dabi teff* which are highly praised in their community. Yet, the practice of using *dabi teff* as a complementary food is not common and the crop is usually reserved for the nutritional treatment of sick, delivered women and bone-fractured persons. The findings showed that there is a deep-rooted social belief linked to *dabi teff* among the communities in the area and it was claimed that the crop has strong nutritional potential and is prized as a medicinal food. All the participants argued that eating the different food forms, particularly the "mooqa manyee" (gruel type made from bull hooves and *dabi teff* flour) and "cafaqoo" (a blouse-like food made from *dabi teff*), it increases blood volume "dhiiga dabalaa", boosts energy/strength "humna dabalaa", and repairs/strengthens the backbone and fractured bones "dugda jabeessa". It was further claimed that if "mooqa manyee" is served to a recently delivered mother, she would recover from her backbone pain shortly and fast weight gain (postnatal nutrition), and if served to a bone-fractured person he/she would be healed fast and the participants praised the crop using a quote, 'if we have *dabi teff* crop in our home, we feel like we have a medicine at home'. Yet, it was told that the cultivation of the crop is declining than the past.

Chapter 5 presents the optimization of a formula to develop energy and nutrients dense novel complementary food from cereal-legume blends through incorporation of *dabi teff*. **Chapter 5.1** presents the potential of including *dabi* teff, the underutilized crop grown in western Ethiopia into pre-processed local food crops *viz*., germinated maize, roasted barley, roasted field pea, dehulled oats and linseed to develop energy and protein-dense optimized novel complementary food with improved sensory acceptability. The findings have shown that field pea and linseed contained a significantly higher ($P < 0.05$) protein content at 20.95% and 20.57%, respectively. The newly formulated complementary foods contained a significantly higher protein (1.4–1.6 times) and protein density (1.31–1.56 times) compared with the control and fulfilled the

FAO/WHO recommendation. The optimal formula was identified at 34.66% *dabi teff*, 25% barley, 15% oats, 15.34% field pea, 5% linseed and 5% maize flour ratios with response values at overall optimization to be 5.57% moisture, 15.74% protein, 5.09% fat, 2.26% ash, 2.88% fiber, 73.05% carbohydrate, 380.43 kcal/100 g energy and 4.12 sensory acceptability score. The optimal was found to contain an energy density of 1.27 kcal/g and a protein density of 4.14 g/100kacl. These findings showed that protein and energy-dense optimized novel complementary food with improved sensory acceptability can be developed from locally available cereal-legume blends by the inclusion of *dabi teff* and can be used as a sustainable food-based strategy to alleviate protein-energy malnutrition among children in developing countries like Ethiopia.

Chapter 5.2 presents the potential of incorporating *dabi teff* into other pre-processed local food crops, *viz.*, germinated maize, roasted barley, roasted field pea, dehulled oats, and linseed, to develop optimized iron-dense novel composite complementary food with a reduced phytate/minerals molar ratio (a proxy indictor of *in-vivo* minerals bioavailability). The findings have shown that *dabi teff* had a significantly higher ($P < 0.05$) iron content at 86.5 mg/100 g, iron density at 24.53 mg/100 kcal, and calcium content at 123.59 mg/100 g. The new formulations had a significantly higher iron content (3.31–4.36 times), iron density (3.25–4.27 times), and calcium content (1.49–1.58 times) as compared to the control flour, and fulfilled FAO/WHO recommendations. The optimal formula was identified at 34.66% *dabi teff*, 25% barley, 15% oats, 15.34% field pea, 5% linseed, and 5% maize flour ratios, with response values at the overall optimization as 32.21 mg/100 g iron, 77.51 mg/100 g calcium, 2.59 mg/100 g zinc, 0.233 phytate/iron molar ratio (Ph:Fe), 0.067 phytate/calcium molar ratio (Ph:Ca), 3.43 phytate/zinc molar ratio (Ph:Zn), and 6.63 phytate by calcium to zinc molar ratio (Ph*Ca:Zn). The optimal was found to contain 2.01 times higher iron content than the standard and 4.44 times higher than the control, as well as an iron density of 8.47 mg/100 kcal, which was 4.39 times higher than that of the control. These findings showed that iron-dense optimized novel composite complementary food with enhanced bioavailability can be developed and used as a sustainable food-based strategy to alleviate iron deficiency anemia among children in less developed countries such as Ethiopia.

Chapter 6 presents the effects of blending ratio variation on macro- and micronutrients, phytate/minerals molar ratio and sensory acceptability of *dabi teff*-field pea-based novel composite complementary flours. **Chapter 6.1** presents the effects of blending ratio variation on

the macro-composition and sensory acceptability of *dabi teff*-field pea-based novel composite complementary flours. Evidence has shown that 'the response of a mixed system is determined by the proportions of the various ingredients that add up to one, not by the combination's total amount'. Our findings showed that all the responses were significantly different ($P < 0.05$) among the different blends except for fat content, ranging from 14.58% to 17.21% for protein, 4.22% to 5.59% fat, 2.01% to 2.60% ash, 2.68% to 3.96% fiber, 68.08% to 70.76% utilizable carbohydrate, and 378.82 to 386.9 kcal/100 g gross energy. The sensory acceptability score was ranged from 3.4 to 4.4. The linear model was shown to be significant ($P < 0.05$) and adequate to describe variation in moisture, protein, and ash contents. An increase in the ratio of field pea in the blends significantly increased ($P < 0.05$) protein, ash, fiber, and energy whereas the interactive effects between *dabi teff and* field pea significantly increased the sensory acceptability of the blends. These findings showed that varied proportions of the ingredients had a significant effect on the response variables, and were used as bases to develop a wholesome product to fight protein-energy malnutrition among children.

Chapter 6.2 presents the effect of blending ratios variation on micro-compositions and phytate/minerals molar ratios of pre-processed local *dabi teff*-field pea-based novel composite complementary foods. Evidence shows that 'Mixtures of multiple grains at varied ratios can provide multiple and higher micronutrients than a single (mono) grain'. The results have shown that the mineral contents were significantly different ($P < 0.05$) among the blends (as affected by components ratios variation) and ranged from 24.01–31.58 mg/100 g for iron, 73.46 -78.81 mg/100 g for calcium, and 2.33–2.61 mg/100 g for zinc, 113.9–124.22 mg/100 g for magnesium, 364.77–497.48 mg/100 g for potassium, 281.03–308.54 mg/100 g for phosphorous and 3.46–6.25 mg/100 g for sodium contents, respectively. The phytate/minerals molar ratios were shown to be significantly different among the blends except phytate/calcium molar ratio (Ph:Ca), ranged from 0.232–0.344 for phytate/iron molar ratio (Ph:Fe), 0.067–0.085 for (Ph:Ca), 3.356–4.18 for phytate/zinc molar ratio (Ph:Zn) and 6.457– 7.943 for phytate by calcium to zinc molar ratio (Ph*Ca:Zn). A linear model was shown to be significant ($P < 0.05$) and adequate to describe variations in iron, zinc, Ph:Fe, Ph:Zn and Ph*Ca:Zn. The result has shown a remarkably linear increase in iron and calcium contents with an increased *dabi teff* ratio in the blends accompanied by a significant decrease ($P < 0.05$) in phytate/minerals molar ratios. The findings showed that increasing *dabi teff* ratio in the blends notably increased iron content with reduced Ph:Fe molar

ratio, providing the bases for developing iron-enriched novel composite complementary food with improved iron bioavailability to combat iron deficiency anemia among children.

Chapter 7 presents the likely contribution of the optimized novel complementary food to daily energy and nutrient demand by 6–8, 9–11 and 12–23-month-old children. Based on various previous studies, it was estimated that the serving amount of complementary food for 6-8 months, 9-11 months and 12-23 months old children to be 75 g, 100g and 150 g per meal and the children should be served three times a day. The energy density of the newly optimized complementary product is 1.27 kcal/g. When adjusted for these estimated daily serving amounts and taking the energy density into account, the developed nutritionally enriched optimized novel complementary food from the locally available cereal-legume blends; blends of the cereals (maize, barley and oats) and the legumes (field pea and linseed) combined with the incorporation of *dabi teff* has shown the likely contribution of more than 100% of daily demands for energy and protein. Notably, it has been shown to contribute more than 200% of daily iron demand (at moderate bioavailability) by all the age groups (6–8 months, 9–11 months and 12–23-months-old children) as modeled against the recommended dietary allowance (RDA) set by PAHO/WHO Guiding Principles for Complementary Feeding of the Breastfed Child.

In conclusion, this PhD project work provides evidence of the possibility of developing a nutritionally enriched/dense optimized novel complementary food from local cereal-legume blends through the incorporation of *dabi teff*, the underutilized food crop grown in western Ethiopia and the application of proper processing techniques on the ingredients. Child nutrition emphasizes the importance of protein density and quality, adequate iron and omega-3 polyunsaturated fatty acid during complementary feeding, where our new optimized product would certainly contain a substantial amount of these nutrients as a result of the presence of particularly field pea, maize, *dabi teff* and linseed flours in the formulations. The findings have shown that the developed complementary food has demonstrated the energy and key nutrient adequacy to expect positive nutritional outcomes (anthropometric indices and iron status) following the feeding of infants and young children in Ethiopia. **Chapter 8** presents a summary of the main findings, implications of the dissertation work, limitations of the study, general conclusion and recommendations for policy actions and further research prospectives.

1

Chapter 1:

General Introduction

1.1 Malnutrition-an overview

Despite significant economic advances in the past decades and substantial food supplies, as well as the support made to households diversified food production and consumption, malnutrition remained a major public health problem and a big global challenge [1, 2]. Lack and/or overtaking of nutrients by the people is largely prevalent in several parts of the world [2]. FAO *et al.* [3] reported that more than half of the global populations are not adequately nourished and there is an increasing focus on the global malnutrition challenge. Hunger, micronutrient deficiencies, overweight and obesity co-exist in most countries of the world and there is a worry of providing adequate nutritious food to the projected 10 billion people by 2050 [3].

The FAO *et al.* [2], report estimated that 768 million people in the world are still hungry, underscoring the immense challenge of achieving the Zero-Hunger target by 2030. It was also estimated that out of the global undernourished people, more than half (418 million) live in Asia and Africa respectively followed by Latin America and Caribbean regions. The report further stated that more than one-third (282 million) of those people live in African countries. On the other hand, healthy diets were unaffordable for around 3 billion people in the world.

In developing countries including Africa, malnutrition is recognized as a broader problem than just insufficient intake of dietary energy and protein, the so-called “undernourishment” or hunger, it also encompasses two other dimensions; micronutrient deficiencies and over nutrition (overweight and obesity) that are collectively referred as the ‘triple burden’ of malnutrition [4].

Micronutrient deficiency is among the greatest public health significance in the world today [5]. It is a serious life-threatening condition that affects the body in the same way as that of vitamin or essential amino acid deficit does [6]. Research on the causes of growth failure over the past two decades focused on protein and energy intake.However, currently there has been a shift in the focus on ensuring whether micronutrient needs are met [7].

1.1.1 Protein-energy malnutrition in children

The World Health Organization (WHO) [8] defines malnutrition as "the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions". Protein-energy malnutrition (PEM) is one of the most important public health problems and poor physical functioning in many developing countries, of which Africa countries are mostly affected [9]. Protein-energy malnutrition alone is the cause of more than half of the deaths occurring in children per year in developing countries [10, 11].

Indeed, under-nutrition affects several millions of people worldwide where mainly children under-five are among the most visible victims. They are the most susceptible segment of a population to PEM-related characteristic growth impairment due to their high demand for protein and energy during this acute period and vulnerability to infections [12].

Protien-energy malnutrition which is also referred to us “the silent emergency of the world” is an immediate cause of poor nutritional status that include; stunting, wasting and underweight. Under this circumstance, the important indicators of PEM are height, weight, skin fold thickness and arm circumference with reference to age [13]. In 2020, 149.2 million (22%) of under-five children had stunted growth, 45.4 million (6.7%) suffered from wasting and 38.9 million (5%) were overweight globally, where children from Asia and Africa were the most affected [2, 14]. Under-nutrition contributes to about 45% of child deaths in under-five years resulting in 3.1 million deaths every year [15]. **Figure 1.1** shows the percentage of children under 5 affected by stunting, by country, 2020.

In Africa, 61.4 million under-five children were stunted, 12.1 million were wasted and 10.6 million were overweight, where this figure is 22.1 million, 3.5 million and 2.7 million in Eastern Africa, respectively [14]. In Ethiopia, approximately more than 350,000 children are estimated to die each year mainly from preventable and treatable infectious diseases complicated by malnutrition [16] and children in rural areas are more likely affected by under-nutrition than their urban counterparts [17, 18]. According to the Ethiopian Public Health Institute (EPHI) and the International Classification of Functioning, Disability and Health (ICF) survey (2019), 37% of

children under-five years were stunted, 21% were underweight, 7% were wasted, 2.9% overweight [19].

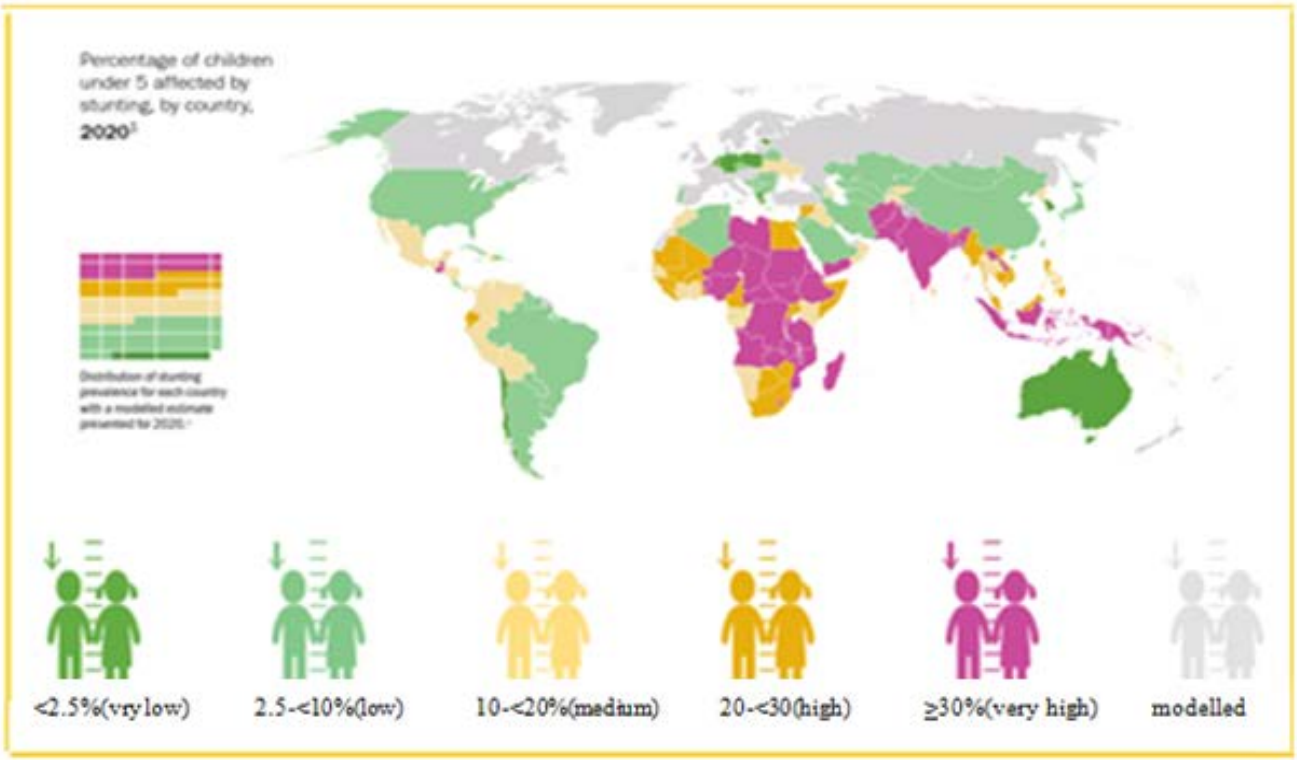

Figure 1. 1: Percentage of children under 5 affected by stunting, by country, 2020.

Source: UNICEF, WHO, World Bank Group joint malnutrition estimates, 2021 edition [14]
Available at: http://www.who.int/teams/nutrition-and food safety/databases/nutgrowthdb.

Protien-energy malnutrition usually ends in the form of kwashiorkor, marasmus, marasmic-kwashiorkor and edematous as a clinical manifestation and has been associated with co-morbidities such as anemia, malaria and diarrhea [20, 21] and equally considered as a risk factor for death in children [11]. Nassar *et al*. [22] stated that PEM causes notable morphological changes in the brains of children in many developing countries. These changes affect the intellectual potential of those who survived and limit their capacity to become part of the competitive world. It also affects both structure and functioning of the brain with a wide range of cognitive deficits reported in malnourished children due to which special attention should be given to omega 3 and omega 6 polyunsaturated fatty acids [23].

1.1.2 Micronutrient malnutrition in children

Micronutrient malnutrition in infants and young children became a major concern to public health nutrition just since the World Summit for Children in the late 1980s [24]. The focus expanded from just protein and energy malnutrition to include vitamin A, iodine and iron deficiencies. There is also an emerging interest in zinc and copper nutrition. Control of Vitamin A deficiency, iron deficiency anemia and iodine deficiency disorders remain a big challenge for nutritionists and health workers [25].

About 50% of all children under five suffer from one or more of these key micronutrient deficiencies that inflict anemia, neural tube defects, cretinism, blindness (xerophthalmia), mental retardation and loss of productivity on tens of millions of people in the world [15]. The World Health Organization (WHO) estimated that more than two billion people, which is one in every three people worldwide are deficient in key vitamins and minerals, particularly vitamin A, iron, iodine, zinc and folate, of which developing countries are the most affected [26]. These deficiencies are mostly superimposed by PEM and reveal to us a 'hidden hunger' [27], where the signs of undernutrition and/or hunger are less visible as a person may have access to a sufficient amount of calories but lack an adequate amount these micronutrients.

Adequate intake of micronutrients is crucial, especially during early childhood, pregnancy and lactating periods [28]. Food and Agriculture Orgnaization and World Health Orgnanization (FAO/WHO) [29] recommends micronutrient supplementation through the use of mainly tablets, capsules, oil solutions or powders or syrups to help alleviate the problems of micronutrients at the community level. These approaches are practical and widely applicable in providing micronutrients to pregnant women, under-five children and postnatally depressed women; nonetheless, the program has limited coverage and is often unreachable to rural areas in practice. Thus still, iron, zinc, calcium, and thiamine, riboflavin and pyridoxine have been identified as problem nutrients for infants and young children beyond 6 months of age in developing countries [6].

The contribution of micronutrients from breast milk declines with a child's age and would be almost zero after 12 months for all micronutrients [30]. The percentage of total daily requirement

for micronutrients needed from complementary foods may range from 30 to 100%. Meeting such high requirements, particularly for resource-poor people calls for searching for other promising alternatives such as exploring local foods and developing nutritious food products and promoting them in the society [30].

Animal-source foods, such as meat, liver, and fish, can provide for the demand as they contain higher bioavailable minerals. However, these products are often expensive and unaffordable for low-income households, particularly the rural poor with additional religious concerns [31]. Consequently, in developing countries, it was suggested that extending child breastfeeding beyond 2 years is the first line of defense against key micronutrient deficiencies, such as iron deficiency anemia, although the iron content of human milk is relatively low [6].

The major causes of these micronutrient deficiencies include inadequate dietary intake, poor monotonous diets of low in micronutrients, low bioavailability (impaired absorption), losses during cooking and infections. These may be compounded by a lack of knowledge of the nutritional values of varied diets and the importance of foods rich in micronutrients as well as the role of dietary inhibitors and enhancers that interfere with the absorption and utilization of the micronutrients [32].

1.1.2.1 Iron deficiency

Iron is required to make hemoglobin concentration and for energy metabolism. Hemoglobin is an iron-rich protein that gives a red color to the blood cells and used for the circulation of oxygen from the lungs to the rest of the body. The causes of anemia are multifaceted, where iron deficiency is known to be the major cause. Anemia, also called “iron-poor blood”, is a condition in which a person’s blood has a low number of red blood cells, or the red blood cells don’t have enough hemoglobin. Iron deficiency anemia is the most common nutritional disorder in the world, even in developed countries that call for a sustainable proposed strategy to alleviate the problem [33]. It affects all age groups of the under privileged population in developing countries, and iron deficiency is also common among vulnerable groups such as adolescent girls and the elderly in developed countries too [34]. Iron deficiency anemia has adverse effects on mental, motor and emotional development in children and later cognitive performance [35]. It has been

shown that the prevalence of iron deficiency anemia (hemoglobin concentration <11mg/dL and ferritin <12 µg/L) is high in infants and this is exacerbated by malaria infection [33]. Global estimates show that the highest prevalence of anemia was 47.4% among under-five children with the prevalence rising to 64.6% in Africa [36] and in Ethiopia is 57% anemic [17]. The nutritional status of children in Ethiopia by administrative region is presented in **Figure 1.2.**

The causes of iron deficiency anemia in children include inadequate dietary iron intake and intestinal parasitic infections such as hookworm [33]. Iron reserves at birth and the small amount of highly bioavailable iron in breast milk (0.35 mg iron/L) are adequate to meet the iron requirements of normal birth weight breast-fed infants for the first six months of life [35]. An increased requirement for iron occurs between 6 and 12 months of life, a need that cannot be met by breast milk alone [37]. However, in developing countries iron deficiency is exacerbated by the fact that complementary foods are based on mixtures of cereals and legumes that are rich in iron-chelating ligands such as phytic acid [37] and tannins, those hampering its absorption. Thus, in most developing countries, complementary foods do not provide sufficient iron, zinc and vitamin B6. Even in the USA, iron and zinc were identified as problem nutrients in the first year of life, despite the availability of iron-fortified products. Dairy products are a good source of some nutrients, such as calcium, but do not provide sufficient iron unless they are fortified. In sub-Saharan Africa, iron deficiency and anemia are still a major public health problem impacting the health and economic potential of many countries [36].

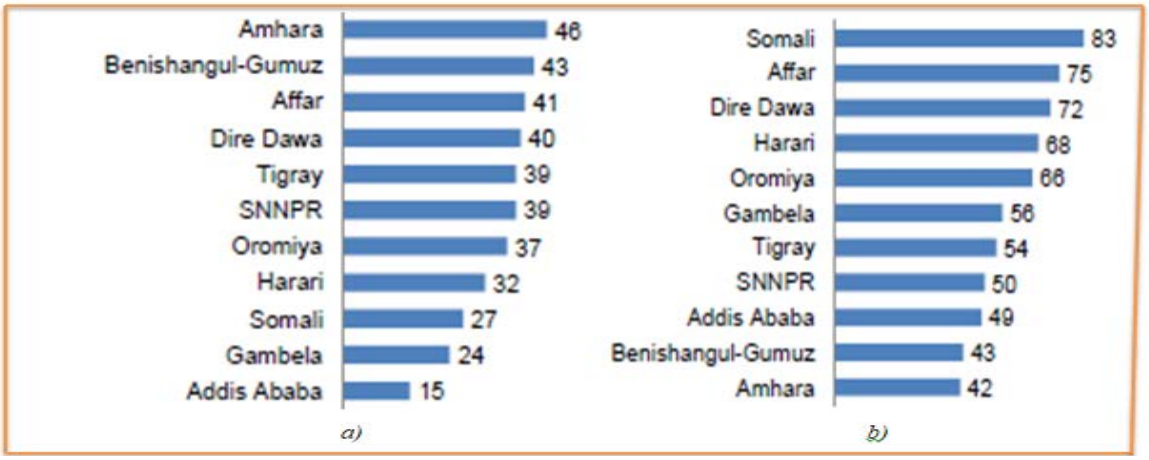

Figure 1. 2. Nutritional status of children in Ethiopia by region. a) *Percentage of children under age 5 who are stunted.* b) *Percentage of children under age 5 who are anemic.*
Source: EDHS, 2016 [17]

1.1.2.2 Zinc deficiency

Zinc is a vital micronutrient, and its deficiency leads to stunted growth and infectious diseases. Globally, it was estimated that zinc deficiency is responsible for approximately 16% of lower respiratory tract infections, 18% of malaria, and 10% of diarrhea cases [38]. Zinc promotes normal growth and development and is an element in enzymes that work with red blood cells which move carbon dioxide from tissues to lungs [33]. Clinical symptoms of marginal zinc deficiency are depressed immunity, impaired taste and smell, impaired memory and decreased spermatogenesis. Single zinc supplementation during infancy has been found to result in significantly improved growth. Zinc, copper and selenium are linked together in cytosolic defense against reactive oxygen and nitrogen species [39]. Milk zinc concentrations are quite high in the early weeks postpartum, averaging >3 mg/L at 2 weeks, but then decline sharply over the early weeks of lactation, resulting in a longitudinal decline in zinc intake from breast milk [39].

1.1.2.3 Calcium deficiency

Calcium plays a role in neuro-transmission, muscle contraction, components of bones and teeth blood coagulation (a regulator of blood clotting) and skeletal support. Calcium deficiency usually leads to stunted growth in children and bone loss or osteoporosis in adults [40]. Severe bone mineral deficiency in infants and young children is associated with nutritional rickets [41]. Vitamin D deficiency is the main cause of rickets in young infants; however, very low calcium intakes may be partially or primarily responsible for the disease in some children [41]. Daily calcium intake of less than 300 mg may pose a risk for the development of rickets notwithstanding normal vitamin D status [41]. Low calcium intakes in older infants and young children can be attributed, in most cases, to the consumption of cereal-based diets with little variety, high in phytate and less access to dairy products. In general populations, adequate calcium intake has been associated with reduced risk for chronic diseases such as osteoporosis and hypertension. Calcium can be obtained from foods naturally rich in calcium such as milk and milk products, calcium-fortified foods, supplements or a combination of these sources [42].

1.2 Impact of childhood malnutrition

In addition to the public health problems, there are also huge economic impacts of malnutrition on national economic development. The Global Nutrition Report [43], presented that more than 11% of annual gross domestic product losses in Asia and Africa are due to undernutrition and the major micronutrient deficiencies. Ethiopia is the most affected country where its productivity (GDP) loss due to undernutrition accounts for 16% [18, 44].

Malnutrition constrains the ability of children to fulfill their potential to the fullest and limits the role they can play in national development [45]. Poor nutrition in early life appears in the working-age population through lower physical and intellectual development. Growth faltering that occurred during the first two years of life would end up with irreversible stunting, impaired cognitive development, lifetime loss of income earning potential, diminished learning and school achievements, lower productivity, greater susceptibility to infectious disease and increased morbidity and mortality as shown by many longitudinal studies [45, 46].

Adults who were well-nourished in childhood increased their workload capacity by nearly 50% over their malnourished peers [47]. Bagnall-Oakeley *et al*. [48] reported malnourished children are likely to earn 20% less than their well-nourished counterparts even when they come from the same economic background. It has been documented that malnutrition in young children causes disturbances in the morphological and functional development of the central nervous system, thus affecting the cognitive and emotional development of the child [23]. Eliminating malnutrition in childhood has a paramount effect on adulthood productivity. For example, it was reported that eliminating anemia can lead to a 5 to 17% increase in adult productivity, which adds up to 2% of GDP increases in the worst-affected countries [49].

In Africa, many countries aspire to achieve middle-income status with vibrant, dynamic and knowledge-led economies. But this aspiration is being hampered by the damage done to today's generation by a lack of proper nutrition during their child hood [48]. Generally, malnutrition, both protein-energy and micronutrient malnutrition has a profound negative consequence on the growth, well-being, school achievements, productivity, health and survival of children [50].

1.3 Causes of child malnutrition

Inadequate dietary intake (in terms of quantity and quality), low bioavailability (the body's inability to absorb and utilize nutrients), losses during processing/cooking, inappropriate age-specific feeding practices and infectious diseases are the major immediate causes of child malnutrition. These may be compounded by a host of underlying factors related to poverty, including food insecurity (availability and affordability of nutritious food), healthy environment, access to water, hygiene and sanitation, health services and opportunities for safe physical activity [2, 14], which find their roots in factors that can vary from conflict, climate variability, economic slowdowns and downturns from scarce natural resources to high and volatile food prices; poor governance to demographic growth, poor infrastructure and limited resources are factors that contribute to the staggering levels of child malnutrition [2, 51].

The vital causes of child malnutrition are the lack of nutrition-specific interventions that directly impact the children's nutritional status; that requires the promotion of proper child nutrition practices including: adequate maternal nutrition before and during pregnancy and while lactating time; optimal breastfeeding (giving colostrum, exclusive breastfeeding from 0-6 months, continue breastfeeding up to 24 months); timely introduction and optimal complementary feeding in early childhood (initiating complementary feeding at 6 months and fulfilling the principle of frequency, adequacy, density, utilization/hygiene and active feeding criteria (FADUA); the reduction of micronutrients deficiencies (e.g. vitamin A and iron supplements) and the prevention and community management of severe acute malnutrition [14, 52, 53]. Failure to achieve these components predisposes infants and young children to malnutrition, growth retardation, infection disease and increased risk of mortality, reduced motor and cognitive development, reduced learning and working capacity [54]. Conceptual frame work on reducing causes of child malnutrition with emphasis on child feeding and health care practices is illustrated in **Figure 1.3.**

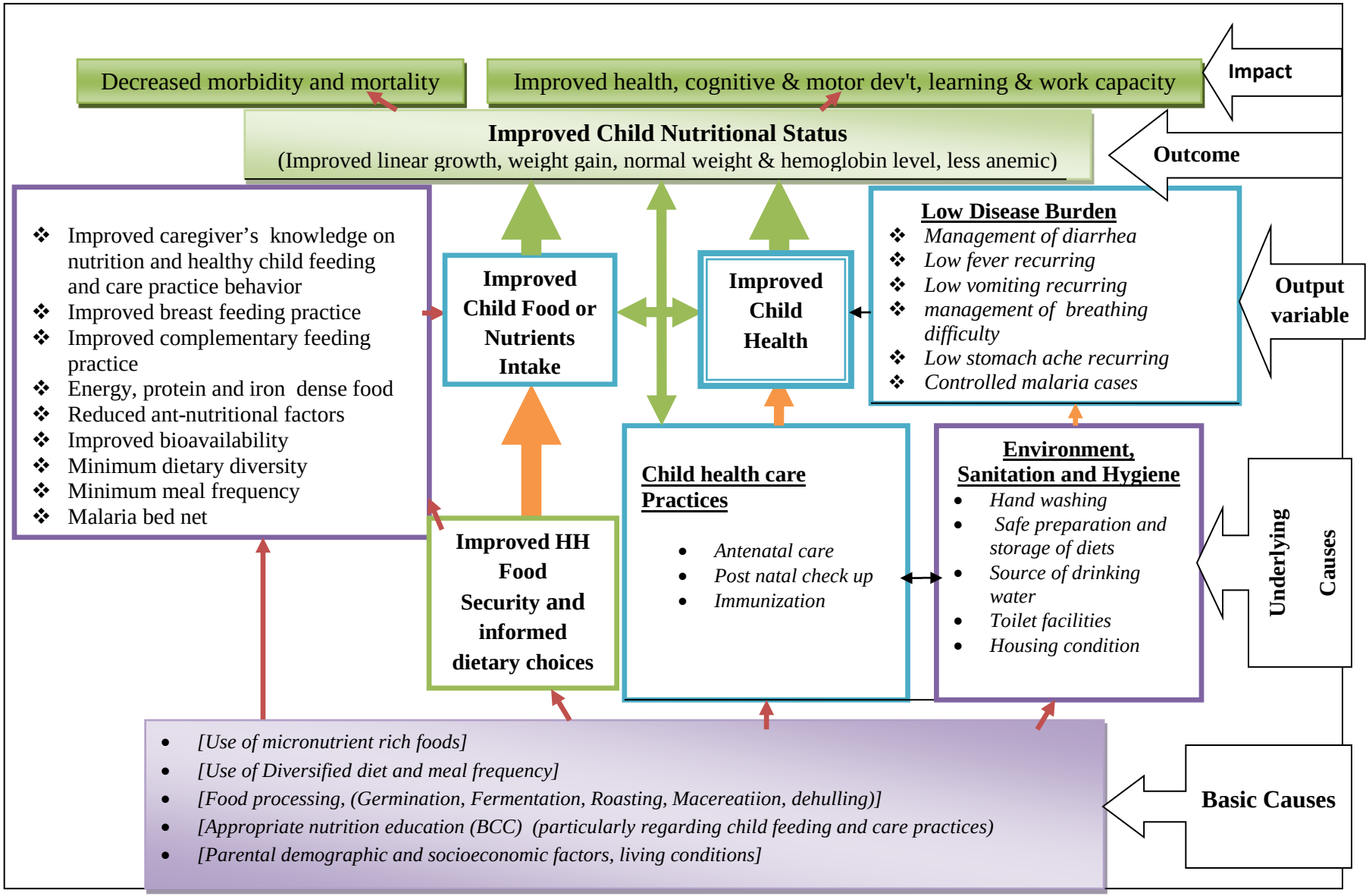

Figure 1. 3: Conceptual frame work on reducing causes of child malnutrition with emphasis on child feeding and health care practices (Source: Adapted from [18, 55].

1.4 Strategies to alleviate child malnutrition

Overcoming both protein-energy and micronutrient malnutrition of a population is a critical pre-condition for ensuring appropriate national economic development imapct. Countries need to adopt and support a comprehensive approach that addresses the causes of malnutrition and the often associated “hidden hunger” which rest intrinsic to poverty and unsustainable livelihoods [15].

The significant effect that nutritional deficiencies and excesses have on the health status of populations has been well recognized. Alleviation of these imbalances forms part of most national food and nutrition as well as health policies as both adequate food and good nutrition have been declared as basic human rights [56]. Under ideal conditions of food access and availability, food diversity can satisfy the micronutrient and energy needs of the general population. Unfortunately, for many people in developing countries access to a variety of micronutrient-rich foods is not possible as many communities are living under conditions of poverty [32].

Many international and national efforts or programs were conducted towards addressing these problems (particularly during the critical periods) including nutritional supplementation, fortification of staple foods and modification of traditional diets to meet specific requirements by different segments of a population [57].

Until recently, the focus on preventing micronutrient deficiencies was primarily on micronutrient supplementation for children which is essential for high-risk populations and emergencies but cannot provide long-term nutrition, social and economic benefit [51] and it fails to recognize the root causes of malnutrition and to assist communities and households to feed and nourish themselves adequately [32]. Since 2008, commercial fortified food products such as powdered milk and peanut butter and supplementation such as ready-to-use therapeutic foods (Plumpy’Nut, F-75, F-100) have been proposed as effective strategies for combating PEM in children. Additionally, supplementation of vitamin A and zinc, fortification of iron and salt iodization and biofortification were ranked among the top five investments (deworming and other nutrition

programs at school) and community-based nutrition promotion were also among the top 10 development “buys” to reduce the micronutrient deficiencies in children [57].

In Ethiopia, efforts were being made to implement a multi-sectorial plan of high-impact nutrition-specific and nutrition-sensitive interventions such as the Multi-Sectoral Nutrition Coordination and Integration Guideline (2017) [58], and the School Feeding Program (2019), the Sekota-Declaration (2017-2020) [59] and National Nutrition Program-II (2016-2020) [60] to end child under-nutrition including stunting and eradicating micronutrient deficiencies by 2030. However, the country is still experiencing one of the worst situations where PEM and micronutrient deficiencies (iron, zinc and vitamin A) are unacceptably high among infants and young children. Commercial fortification and micronutrient supplementation have been in place for a long time, however these strategies posed limitations, they have been found to be costly, not sustainable and not accessible (often beyond the reach of the poor).

1.5 Food based strategy- a sustainable approach to alleviate child malnutrition

Due to the limitations of many strategies stated above such as supplementation and fortification, the correction of malnutrition underlines the importance of another new sustainable strategy, the “food-based strategy” [52, 61]. Food-based strategy has been recognized as the principal way of combating child malnutrition and some other forms of interventions could serve as a complement [25]. The strategy also employs the inclusion of indigenous, acceptable, nutrient-rich including neglected foods together with simple home-based processing techniques to combat macro and micronutrient deficiencies and improve the nutritional status of infants that recently deserved more attention [62]. Dietary diversifications to include micronutrient-rich foods in the diet appear more feasible than other methods in most developing countries [25].

Food-based strategies are often described as a sustainable approach because the process empowers individuals and households to take ultimate responsibility over the quality of their diet through own production of nutrient-rich foods and informed consumption choices. These strategies are said to be “the ideal long-term goal toward which society strives-provision for

assurance of access to a nutritionally adequate diet achieved through the diversity of food availability, wise consumer selection, proper preparation and adequate feeding". Additionally, the approach is demonstrated to be cost-effective and can be adapted to different cultural, and dietary traditions and locally feasible strategies [52, 61].

The food-based approach offers a number of advantages over other interventions (supplementation and fortification) aimed at preventing and controlling malnutrition. These include if consumed on a regular and frequent basis, enriched foods will maintain body stores of nutrients more efficiently and more effectively than will intermittent supplements. This is an important advantage to growing children who need a sustained supply of micronutrients for growth and development and to women of reporoductive age who need to enter periods of pregnancy and lactation with adequate nutrient stores [15]. Fortification or enrichment (either processed or home complementary foods) is the most feasible option in most circumstances given the cost of iron-rich foods (such as liver or meat) [63].

The Codex-Alimentarius guideline for complementary foods advocates promotion on food-based approach and dietary diversification as a crucial strategy for combating life-threatening nutritional deficiencies [28]. Further, WHO [62] recommends the use of nutritious, indigenous and underutilized/marginalized foods with simple home processing techniques such as germination and fermentation among others to yield nutrient and energy-dense complementary foods as well as improved bioavailability of key micronutrients including iron, zinc and calcium. Gibson *et al*. [64] and Gibson *et al*. [31] suggested that all the approaches would be efficacious if the micronutrients notably iron, zinc and calcium content of the product have enhanced bioavailability and acceptability and the food is consumed in appropriate quantities by infants and young children.

Food and agricultural Organization (FAO) advocates for nutrition-sensitive agriculture that increases access, availability and consumption of a variety and diversity of safe, good-quality foods, including food fortification, as a sustainable strategy for improving the nutritional status of populations [32]. The development of food-based dietary guidelines by FAO and WHO [65], recognizes this and notes that there are economic constraints that limit food supply at the

household level, focusing on the combination of foods that can meet nutrient requirements rather than on how each specific nutrient is provided in adequate amounts. The food-based guidelines are based on the fact that people eat food, not nutrients or tablets. Food-based dietary guidelines can then be prepared that indicate what aspects of the diet could be modified to improve nutrition [66].

Most studies also recommend that priority be given to food-based strategies to combat child malnutrition. A study by Jalal *et al*. [67], suggests that it is feasible to promote production and consumption of micronutrient-rich foods among populations with deficiencies. In addition to improving micronutrient status, such approaches are hypothesized to have other beneficial nutrition effects and to have positive impacts on household income. Research by Takyi [68], has also recommended the use of the underutilized locally available food materials that are inexpensive and high in micronutrients for child food preparation. The poverty reduction strategy in many African countries explicitly calls for the promotion of food-based approaches to reduce malnutrition [69].

The food-based strategy employs the use of both animal and plant-source foods ranging from traditional produce to commercially and centrally processed ones like the corn-soya blends. In developing countries, economic and/or religious reasons are the major constraints to use animal source foods as staple and child diets. Thus, the formulation and development of complementary foods from locally and readily available nutrient-rich food commodities/produce has received a lot of attention in many developing countries.

A “nutritous food first” approach encourages eating frequent, small, and high-energy and protein meals to address child malnutrition. In sub-Saharan Afrric (SSA), complementation of cereals with locally available legumes such as soy beans, groundnuts, chickpeas, cowpeas and pigeon peas among others have been tried as a protein source to manage PEM with mixed results [70]. United States Agency for International Development (USAID) and the World Food Programme (WFP) have promoted corn-soy-blend plus (CSB+) and corn-soy-blend plus-plus (CSB++), food supplements utilized in the management of moderate acute malnutrition with mixed results [71].

1.6 Energy and nutrient requirements from complementary foods

Infants and young children need adequate energy and a nutrient-dense diet both through exclusive breast-feeding followed by quality complementary feeding **(Table 1.1)** to support the rapid rate of physical and mental growth that occurs in the first two years of life [63, 72]. In the first 6 months after birth, breast milk intake continues to make a substantial contribution to the energy and nutrient intake requirements by infants [72], particularly for protein and many of the vitamins after which the milk is no longer sufficient and children transfer from ideal and uncontaminated breast milk to poor-quality complementary foods (often regular family food) with the highest chance of vulnerability to malnutrition and infectious diseases, particularly in developing countries [28].

Inadequate intake (both in quantity and quality) of age-specific essential nutrients together with infectious diseases is the prime immediate cause of child malnutrition leading to PEM and micronutrient-related growth impairments; the anthropometric deficits (stunting, wasting, underweight), anemia, impaired cognitive development, reduced productivity and earning potential and finally increased morbidity and mortality [19].

A review of studies on complementary foods in developing countries by Dewey and Brown [6] showed that the protein density (2.0 to 3.3 g/100 kcal) was lower than the required level (3.0 to 5.5 g/100 kcal) whereas it met the standard level of energy density (0.6 to 1.0 g/100 kcal). Of course, the amount of complementary food needed to satisfy the energy and nutrient requirements of children is a function of energy and nutrient density that can be provided by complementary foods [73].

Complementary foods are needed to fill the gap in energy, protein, iron and other essential nutrients, among those provided by breastfeeding and the total nutritional requirements of the infants. The total energy requirements of healthy, breastfed infants are approximately 615 kcal/d at 6-8 months, 686 kcal/d at 9-11 months and 894 kcal/d at 12-23 months of age [6]. The average breast-milk energy intake provides 413, 379 and 346 kcal/d at 6-8, 9-11 and 12-23 months, respectively [53]. Thus to fill the energy and nutrient gap, between what is provided by breast

milk and the total requirements, FAO/WHO [28] and WHO/UNICEF [53] recommend the energy intakes that should be derived from complementary foods for infants and young children in developing countries are approximately 200 kcal, 300 kcal and 550 kcal per day for 6-8 months, 9-11 months and 12-23 months old age groups, respectively, based on an assumed average breast milk intake of 630 mL per day [6]. This is because breast-milk intake declines with age, while the energy and nutrients needed from complementary foods increase as the child grows [63].

However, in developing countries infectious disease such as diarrhea has a growth-limiting effect, affecting appetite and energy expenditure and an increase in metabolic rate in normal reaction to fever (the aggravating factors), accounting for a higher energy requirement. As a result, infants and younger children fail to attain their full potential growth and development, suffer from long-term deprivation of energy, and nutrients and consequently chronic PEM, often with concurrent micronutrient deficiencies and high incidence of morbidity [14]. Thus, infants and younger children need special foods of adequate nutrient density, consistency, of good texture and are fed more often than adults.

In addition to protein and energy adequacy, infants and young children require an adequate amount of micronutrients such as iron, zinc, calcium and vitamins to meet their physiological needs, particularly in the first 2 years of life and they are prone to micronutrient deficiencies and associated diseases [57]. Ahmed *et al*. [74] stated that globally, 7.3 % of the disease burden in children is accounted for by micronutrient deficiencies. The contribution of dietary minerals from breast milk declines with a child's age and would be almost zero after 12 months, which makes these nutrients limiting nutrients [30]. The percentage of total daily micronutrient requirement from complementary foods may range from 30 to 100 %. For instance, for 9–11-month-old children, about 97 % of iron, 86 % of zinc, 72 % of calcium, 76 % of magnesium, 81 % of phosphorus and 73 % of sodium requirements of children 6–24 months should come from complementary food, assuming average breast milk intake [6].

The PAHO and WHO [55] recommend iron intake by children at 6–8, 9–11 and 12–23 months should be 11mg/d, 11mg/d and 7 mg/d, respectively, where these figures are 4.1, 4.3 and 5.8 mg/d for zinc and 400, 400 and 500 mg/d for calcium for the respective age groups. Meeting

such high demand is unlikely to be attained through traditional complementary food, which calls for searching for other promising alternatives such as exploring locally available underutilized/forgotten nutritious plant foods and developing nutritionally enriched optimized complementary foods, especially enriched with micronutrients.

1.7 Blending cereal and legume ingridients for complementary food development

Globally, there is an increasing tendency to shift from consuming animal-source foods, especially highly processed meat products, to plant-source foods because of the increased health risks associated with excess processed meat consumption [75]. In the past, starting from the 1930s, there was a general belief that people needed to eat animal foods to obtain high-quality protein, the ‘Great Protein Fiasco’, to prevent PEM in children, notably ‘Kwashiorkor in Africa’ [88], but later, nutritionists recognized the relative importance of plant proteins and the need to formulate multi-mixes of local food stuffs rather than commercial mixtures, where wheat/chickpea mixtures were successful by the WHO in 1966 [88].

Foods from cereal and legume grains at varied ratios can provide multiple and higher macro-and micronutrients than a single (mono) variety grain. This shows that the response of a mixture system is determined by the ratio variation of the various ingredients that add up to one, not by the total combination's amount of the mixture. This concept aroused the interest of nutritionists in examining the effects of mixing different grains at varied mixing proportions on the macro- and micronutrients. Thus, exploring different locally available, underutilized, affordable and nutrient dense plant foods (the dietary diversity approach) and blending them together after the application of simple pre-processing is the best option [28] to get multiple and higher micro-nutrients from their mixture and to provide nutrient-dense foods that are easily absorbable and bioavailable with improved sensory acceptability as a main drive to prevent child malnutrition [76].

Table 1.1: Recommended energy and nutrient needs for healthy and average breast fed children aged 6-23 months old

Age	From Breast milk (kcal/day)	From CF (kcal/day)	Total Energy Needed (kcal/day)	Protein g/day	Iron mg/day	Zinc mg/day	Calcium mg/day	Meal Frequency per day
6-8 months	413	200	615	9.1	11	4.1	400	2-3 times
9-11 months	379	300	686	9.6	11	4.3	400	3-4 times
12-23 months	346	550	894	10.9	7	5.8	500	3-4 times
References	[53, 55, 63]							

1.8 Anti-nutrient contents

Cereals and legumes are highly nutritious staples, but they have high anti-nutritional contents that limit their nutritional quality. Anti-nutrients such as phytate, tannin, trypsin inhibitor, cyanogenic glycosides and oligosaccharides are naturally occurring constituents mainly in cereals and legumes. These anti-nutritional contents antagonize the absorption of one or more nutrients through non-digestible insoluble complex formation, particularly with minerals and reduce the bioavailability of the minerals to the human body [77, 78].

Enzymatic processing, such as germination and fermentation, or non-enzymatic processing, such as roasting, soaking, maceration, and dehulling, are among the most common and globally accepted processing techniques for reducing the deleterious effects of anti-nutrients and increasing the nutrient density and bioavailability of key micronutrients as well to boost sensory qualities of food products [79]. The application of these processing techniques on selected cereal and legume food ingredients used in the formulation of complementary food could contribute in reducing the deleterious effects of the anti-nutrients that might present and increase the nutrient density and bioavailability of micronutrients. The detail descriptions and effects of the different processing techniques on improving the nutritional values (benefits) of the resultant food products are presented in **Chapter 3** below.

However, the anti-nutrients in cereals and legumes contain bioactive compounds that have been reported to exert health-promoting properties in contrary to their deleterious effects in nutrient absorption. These compounds vary considerably in their biochemistry and can be in the form of proteins, glycosides, phytates, tannins, saponins, alkaloids and polyphenols. These compounds possess antimicrobial, antiviral, antiparasitic, antioxidant, neuroactive, cytotoxic and neurotoxic actions, which provide beneficial contributions towards health [80]. They can also play lowering of chronic inflammation and the chance of diabetes mellitus and lower obesity [81] and cardiovascular disease [82]. Hence, the anti-nutrients in cereals and legumes need to be evaluated against these health-benefit properties. They have a free radical scavenging activity against oxidation and thus alleviate the onset and progression of cancer and cardiovascular diseases.

1.9 Sensory evaluation

Sensory evaluation is a scientific method that suggests, measures, analyses and interprets responses to food products as perceived through the senses of sight, smell, touch, taste and sound [83]. In determining how food product affects consumers, senses are one of the most important goals for food product development, industries and also for nutritionists and dieticians who develop healthier recipes. The human senses act as gatekeepers of human bodies, and healthy foods would be picked only if consumer's senses accept them. Therefore, consumer reactions on acceptability of the newly formulated food products as perceived by their five senses are considered as vital measures of food development. Sensory evaluation is of paramount concern especially when new food product development is targeting consumers. In the first place, there is a problem of 'picky eating and food jags' in infants and young children group and if the food is not pleasant or tastes good, they may refuse to consume it.

Since no apparatus can substitute for senses in testing foods, humans are used as instrumental test subjects [83]. These studies are becoming more prevalent despite the potential biases of humans and the costs involved [83]. In sensory evaluation, panelists are asked to rate the product for color, appearance, aroma, flavor, taste, mouth feel, texture and overall acceptability using a score of hedonic rating scale. This evaluation relies on people's ability to communicate their feelings of liking or disliking. The hedonic scale is popular because it may be used with untrained people as well as experienced panel members (Bruce, 2010). Many studies have shown that a 5-point hedonic scale was well appropriate to conduct a sensory evaluation of a complementary porridge formulated from local food ingridients using untrained or semi-trained panelists [70]. A 5-point hedonic scale was applied in the present sensory evaluation of porridges is shown in **Annex III**. The environment in which the sensory test is conducted should be carefully controlled. Samples must be prepared in a uniform fashion with codes so as not to influence panelists` perception on the foods` quality [83]. The sensory evaluation sessions should be monitored by the principal researchers.

1.10 Traditional usage, social beliefs and cultivation trends of *dabi teff (Eragrostis teff)*

Plant foods that are either cultivated or edible wild species have a substantial contribution to food and nutrition security, medicinal value and sources of income generation [84]. However, many plant-foods in SSA are underutilized and/or forgotten and are marginalized.

The rural populations in Ethiopia have a rich knowledge concerning the use of cultivated and wild plants as food and medicine [85, 86]. Using traditional plant origin-foods is still very common especially in rural areas of developing countries including Ethiopia where elders and other knowledgeable community members are the key sources of plant lore [86]. However, this indigenous knowledge among the rural communities is affected by fragile traditional skills (older people replaced by younger) that are likely to be lost when people immigrate to towns or to other regions; can be lost by lifestyle changes, the westernization or industrialization, when the young people ignore to practice and pass down the traditional practices in their community [85].

Dabi teff, a typical farmer variety teff (landrace), is one of the teff varieties marginally cultivated or underutilized/forgotten in Western Ethiopia. *Dabi teff* is the 'afaan oromoo' language name for an early maturing variety of dark red teff and the people in western Ethiopia have a strong connection with the cultural and medicinal values, social claims and preparation schemes linked to *dabi teff*. Thus, an exploratory ethnographic qualitative study needs to be conducted to elicit and collect ethnocultural information in the community to document the traditional usage, medicinal value, social beliefs and cultivation trends of *dabi teff* in the area.

1.11 Selection of raw meterials for complementary food formulation

Underutilized plant foods are plant species whose potential contribution to the national economy has not been adequately explored due to little or no attention to their production, consumption, and utilization [87]. The sub-Saharan African region is endowed with a rich diversity of “orphan” crops that are underutilized/forgotten and marginalized, yet rich in nutrients, especially micronutrients that are unavailable in staple foods. They have also medicinal values and are sources of income generation [84]. The global facilitation unit for underutilized species defines it as “those plant species whose potential are not fully exploited to contribute to food security and

poverty alleviation". They hold huge potential for reducing the impact of malnourishment and hidden hunger, particularly in children. The potential for their use in enriching major staple foods in Africa is currently a novel approach and holds a brighter prospect for alleviating poverty and macro- and micronutrient malnutrition [87].

Padulosi *et al*. [88] and Conti *et al*. [89] reported that there are numerous neglected and underutilized species known in SSA and categorized them under cereals, legumes, tubers, and leafy vegetables and concluded among other things that underutilized species could represent an opportunity to enhance food security and empower women [87]. Despite the availability and nutritional benefits of these promising underutilized species, their use and incorporation into our everyday diet need investigation and promotion [87]. Because they are entirely ignored by agricultural researchers, plant breeders and policymakers. Therefore, increased attention has to be given to such potential food crops in terms of research and development and policy frameworks that could promote their extensive agricultural production, industrial utilization and home consumption [90].

Several strategies have been used to formulate baby food [84, 85] through a combination of locally available food ingridients that complement each other in such a way that they provide the recommended daily allowance for infants. Therefore, the development of complementary foods based on locally available food ingridients has been suggested by the Integrated Child Development Scheme and Food and Agriculture Organization (FAO) to combat malnutrition among mothers and children of low socio-economic groups [28].

Commercial-formulated complementary foods currently available are not affordable to many low-income families [70]. Most complementary food research and developments done, including those in Ethiopia, were cereal-based such as maize, wheat, sorghum, oats and other root crops. Such food produces bulky complementary foods hence reducing the nutrient and energy content of the complementary foods. It is therefore, profoundly important to search for such nutritious underutilized plant foods and incorporate them into the traditional complementary foods to enhance the nutritional qualities of the final product and be able to develop energy, protein and iron-dense optimized novel complementary food as a main driver of this study.

The underutilized/forgotten crop, *dabi teff* grown in Western Ethiopia was explored for its nutritional potential by incorporating it into other locally grown food crops and which are easily accessible, affordable, cultivable and nutritious selected food crops including field pea, barley, oats, maize and linseed **(Figure 2.1)** to produce an acceptable and nutrient-dense optimized novel complementary food. This can be used as sustainable food-based strategy to combat child malnutrition in Ethiopia and Africa in general. *Dabi teff* is well known and its nutritional and medicinal values are highly praised among the community in Western Ethiopia, hence the ease of incorporating it into other foods. Further, an ethnographic qualitative study on traditional usage and social claims linked to *dabi teff* needs to be investigated. In sub-Saharan Africa, in Ethiopia in particular, there are indigenous knowledgeable people who are well equipped with incorporating nutritious food materials into stable diets that would be more sustainable and an option to alleviate nutritional deficiencies among the wide ranges of population segments.

1.11.1 *Dabi teff* (*Eragrostis teff*)

In Ethiopia, the food culture of teff is both historical and part of the country's antiquity. *Dabi-teff* is a farmer variety teff (**Figure 1.4)**. It is one of the underutilized and marginalized food crops grown in western Ethiopia, Oromia region. It could be used to produce complementary food for infants and younger children and for the preparation of important traditional foods. *Dabi teff* is the 'afaan oromoo' language name for an early maturing variety of dark red teff **(Figure 1.4)**. There are many social beliefs linked to *dabi teff* regarding its nutritional and health benefits among people in Western Ethiopia.

Based on these social beliefs and personal long-time observations, a preliminary study on some selected micronutrient contents of *dabi teff* was conducted and found that it contains a considerable amount of iron at 122.66 mg/100 g, calcium at 190 mg/100 g, and zinc at 1.8 mg/100 g **(Annex V)**, which created a pivotal springboard to carry out intensive research work. The lower incidence of anemia in Ethiopia is presumed to be due to such a high iron content of red teff [91]. But the problem is, the practice of preparing complementary food for infants and young children using red teff grains including *dabi teff* is low which could be the reason for the high prevalence of anemia (57%) in under-five children in Ethiopia [17]. Therefore, the

incorporation of *dabi teff* into complementary food formulation, particularly for its high iron content is nutritionally feasible.

Figure 1.4 *Dabi teff* grain sample (Photo by the researcher)

1.11.2 Barley (*Hordeum vulgare*)

Barley is one of the most important staple food crops in Ethiopia. Research shows that barley fiber contains beta-glucans and toco-trineols, chemicals known to lower serum cholesterol levels [92]. In Ethiopia, barley is the fifth most important cereal crop next to maize, tef, sorghum and wheat. It is often used as dehulled and roasted flour to prepare a large variety of recipes, including soups, gruels, porridge, breads *Injera* as well as local beverages *Tella* and *Borde.*

Traditional recipes, such as "*Besso"* (roasted barley flour moistened with water) and "*Chuko"* (roasted barley mixed with butter and some spices), which have a longer shelf life, can be prepared from barley. Other food forms such as "*Kolo" (*de-hulled and roasted barley) are usually served as a snack food and "*Kinche"* (grits cooked into thick meal snack) is the most popular recipe from barley. It can also be used in the preparation of gruel as complementary food. Barley is mainly prized as a source of carbohydrates, where its protein content is also

considerable, but with lower values in lysine and methionine. This could be the reason why most traditional barley recipes are prepared or consumed along with legumes or animal products to supplement these deficient amino acids. All these descriptions of barley justify the necessity of its consideration for complementary food formulations.

1.11.3 Oats (*Avena sativa*)

Oat is mostly produced in rural and semi-urban areas in Ethiopia where its husbandry is similar to that of other cereals such as barley and wheat. It is commonly rolled or crushed into oatmeal, or milled into fine oat flour. It is widely used for the production of ready-to-eat breakfasts such as bakery goods, snacked food, infant food, and other products. Oat is a gluten-free crop and is usually mixed with wheat to produce high-quality leavened bread [93]. It is generally considered healthy and nutritious food that is known to lower cholesterol levels, particularly reducing the lower density lipoprotein (LDL or bad cholesterol) in humans and possibly can be used to manage the risk of heart disease [93].

Oats have appreciable oil content and thus a higher energy density. Oat contains a good amount of protein with a relatively higher amino acid balance. Oat protein is nearly equivalent in quality to soy protein, meat, milk, and egg protein as shown by the WHO.
It is also known to contain significant amounts of soluble dietary fiber, dietary minerals (Fe, Zn, Mg, P and Cu) and vitamins (thiamin, vitamin E, folate, and niacin). Oat is usually dehulled before preparing food from it. In view of its good nutritional profile, low allergy bearing, flavor compatibility and cost, oats are a useful component of infant foods. This justifies the importance of blending oats in complementary foods formulation as sources of essential nutrients and healthy food [93].

1.11.4 Field pea (*Pisum sativum L.*)

Field pea is an excellent source of dietary protein, starch, micronutrients, fiber and many health-promoting benefits. It contains 20-29% protein and it is a potential protein-energy source. Field pea is known to contain higher amounts of lysine which is the limiting amino acid in cereals [94]. All the essential amino acid requirements by human beings can be met by the consumption of field peas except the sulfur-containing amino acids. The higher protein and fiber of field peas

help to regulate the digestion of starches by slowing down the breakdown of carbohydrates and regulating blood sugar which can be used to manage type 2-diabetes. Based on the nutritional and health benefits of field peas, its incorporation into complementary food formulation is justifiable [94].

1.11.5 Maize (*Zea mays L.*)

From time immemorial, maize has been an important annual cereal crop of the world belonging to the family Poaceae. It is the third leading crop in the world after rice and wheat [69]. Maize is popular for its high nutritional significance, rich in macronutrients like starch, fiber, protein and oil along with micronutrients like vitamin B complex, ß-carotene and essential minerals such as magnesium, zinc, phosphorus and copper [95].

Maize oil contains 56% polyunsaturated fatty acids. The presence of essential fatty acids, especially linoleic acid in maize oil plays an important role in the diet by maintaining blood pressure, regulating blood cholesterol levels, and preventing cardiovascular maladies [107]. Maize protein is known for its sulfur-containing amino acids, cystine and methionine, but low in lysine and very low in tryptophan. It is well established that children at high risk of stunting may have limitations of essential amino acids in their diet such as tryptophan and lysine [95]. Evidence has shown that lysine and tryptophan deficiencies in cereals can be complemented with legumes that are rich in lysine and tryptophan to compensate for the deficiency of these essential amino acids in cereal [96]. The un-enriched maize-based complementary foods have been implicated as the main causes of PEM among children in developing countries [97]. Therefore, based on the nutritional and health benefits of the maize crop, it can be used for the formulation of nutritious and healthy complementary food for infants.

1.11.6 Linseed (*Linum usitatissimum*)

Linseed is an underutilized oilseed, but a rich source of protein and dietary fiber. Its oil is a leading source of α-Linolenic acid or omega-3 polyunsaturated fatty acid among plant foods [98]. The exceptional feature of nutritional and health significance of linseed is its abundance of poly-unsaturated fatty acids particularly omega-3 and omega-6 fatty acids which are the structural material for nervous tissue, including the brain. Its oil is also rich in alpha- and

gamma-tocopherol and vitamin K. Consumption of linseed oil is also associated with a lower risk for coronary heart disease, morbidity and mortality and it helps in preventing or treating a variety of diabetic complications and modulates coagulation in diabetic patients [98]. Therefore, there is a need to promote the utilization of linseed in the development of complementary food for its significance of omega-3 and omega-6 fatty acids for the cognitive development of infants and young children. Incorporation and utilization of linseed oil in our day-to-day life is also recommended owing to its health-promoting features [98].

Incorporation of these selected raw materials with remarkable and varied nutritional and medicinal values and investing on their synergetic nutritional and sensory acceptability was not yet examined. Each of thelocally produced ingredients was carefully and thoughtfully selected to add one or more nutrients to their mixture system and to develop nutritionally complemented and balanced child diets. This is supported by FAO [28] codex alimentarius on complementary feeding guidelines that describe mixtures of cereals, legumes, pulses/oilseed constituting an appropriate source of nutrients and energy, essential fatty acids and limiting amino acids with many functional and health benefits as well as improved organoleptic characteristics. Diverse foods also give infants and young children a chance to appreciate various food flavors and textures, all of which are vital for later developing healthy eating habits [28]. The study employed proper globally accepted processing techniques that could reduce the deleterious effects of the anti-nutrients that might be present in the raw materials and increase the nutrient density, bioavailability of micronutrients and palatability of the complementary food.

In particular, despite the preliminary findings, the social beliefs and the traditional usage, to date the potential of incorporating *dabi teff* into complementary flour formulations in combating protein-energy and micronutrient malnutrition in infants and younger children has not yet been examined. Thus, this research was aimed at the inclusion of *dabi teff* into other pre-processed food ingridients *viz*. germinated maize, roasted barley, roasted field pea, dehulled oats, and minimally cooked linseed by formulating and optimizing a complementary food and determining their nutritional quality, sensory acceptability and anti-nutrients to minerals molar ratio using D-optimal mixture design and to identify the optimal condition (sweet spot) based on the overall desirability function of the multi-response numerical optimization. The formulation and optimization of complementary foods with these indigenous and locally available food

ingredients could provide an opportunity to utilize the use of unreached/untouched golden crops for IYCF. In addition, the use of such locally available nutrient-dense ingredients in the development of infant complementary foods development would help to enhance nutritional quality of home-made foods.

Stat-Ease Design Expert ® software version 11 (D-optimal mixture design) was used to find the best proportion of ranged mixture components through predictive equations that allow the application of mathematical algorithms, and making it possible to determine the optimal conditions for a formulation of novel products or an industrial process [99, 100]. Thus, it was for this typical reason that the design was adopted in the current study to formulate and optimize composite flours to develop nutritionally enriched novel complementary food for infants and young children from the nutrient-rich locally available farm produce in Western Ethiopia.

Thus, this PhD dissertation work focused on the blending of local foods ingridients including; 1) cereals that are recognized as good sources of carbohydrates and sulfur-containing amino acids, methionine and cysteine; 2) legumes that are good sources of high quality protein, containing lysine and tryptophan and provide energy [72] and thirdly to provide essential micronutrients from underutilized nutritious plant food, *dabi teff* that complement each other to develop wholesome complementary foods suitable for infants and young children meeting the PAHO/WHO [55] energy and estimated nutrient needs from complementary foods.

1.12 Knowledge gap-drivers of this study

The period of complementary feeding determines a child's growth and survival and has short and long-term implications for long-term health status, work capacity in later life, improved cognitive development and learning capacity, and increased productivity of children to contribute to national economic development. The promotion of breast feeding has received much attention in the past decade; however, it can not provide adequate nutrition as a child's age increases. This triggers the provision of safe, acceptable and nutritionally adequate complementary foods to ensure optimal growth and development in children [57].

The global strategy for infant and young child feeding states that infants should be exclusively breastfed for the first six months of life to achieve optimal growth, development, health and survival, and thereafter, receive nutritionally adequate and safe complementary foods while breastfeeding continues for up to two years or beyond [101]. According to the 2013 *Lancet* series on child and maternal nutrition, appropriate complementary feeding is one among ten core interventions that have the potential to reduce child mortality by 15% [102].

However, traditional complementary foods utilized in developing countries including Ethiopia are predominantly starchy cereal-based, often monotonous and are characterized by low nutritive value; low protein and energy density, low micronutrient density and high anti-nutritional factors that jeopardize the nutrient bioavailability of complementary foods [31, 63, 71]. Due to this, the very high demand for macro-and micronutrients is unlikely to be met by infants and young children using traditional complementary foods to support their rapid growth and development. On the other hand, infants and young children have a small stomach (lower gastric capacity) and can only consume small amounts of food at a time which triggers the importance of frequently feeding them energy and nutrient-dense diets to fill their growth and developmet requirements.
WHO recommends daily consumption of animal-source foods for their higher bioavailable (quality) nutrient density which plant-based complementary food alone cannot provide [55]. However, animal source foods are expensive and the access of resource-constrained people to such nutrient-rich foods of animal products, fruits and vegetables is limited [103].

Traditional complementary foods could be nutritionally enhanced through proper selection and combination of local cereal-legume blends enriching with underutilized nutritious food products. These nutritious underutilized plant foods are substantial sources of proteins, carbohydrates, essential fatty acids, minerals, vitamins, phytochemicals, and dietary fiber and contain important functional values [104]. Notably, they provide essential micronutrients that complement staple crops, strengthen local foods, and could play a major role in improving the micronutrient content of diets [105]. Amagloh *et al*. [106] stated a careful selection of nutritionally promising ingredients in complementary food development is more likely to have a positive impact in meeting energy and nutrient demand as recommended by codex Alimentarius commission guide lines for formulated foods for infants and young children. The Integrated Child Development

Scheme and FAO suggest the development of complementary foods based on locally available foods to combat malnutrition among mothers and children of low socio-economic groups [28]. Additionally, the World Health Assembly [107] emphasizes the provision of safe and appropriate processed complementary foods using indigenous nutrient-rich foodstuffs.

In Ethiopia, there are many indigenous, local underutilized crops whose nutritional and medicinal potential are not yet uncovered. It is, therefore, profoundly important to search for such nutritious underutilized/indigenous food crops with promisingly higher energy and nutrient contents and incorporate them into traditional foods that can additively provide super-quality complementary food. Several studies have been carried out to formulate baby food [108, 109, 110] through a combination of locally available crops. But the inclusion of *dabi teff*, the underutilized/marginalized food crop grown in western Ethiopia into other cereals and legumes to develop nutrient-dense, optimized complementary food for infant and young children is not yet examined. The present preliminary study showed that *dabi teff* contained a considerable amount of iron at 122.66 mg/100g **(Annex V)** and the study has projected that incorporating this crop into traditional complementary food could be a remedy to reduce the unacceptably high rate of anemia in children at 57% [17]. This approach can result in enhanced nutritional qualities (improved energy and nutrient densities) of the resultant complementary product as a main driver of this study. This study could also help in the the operationalization of the recently formulated 'Ethiopian food and nutrition policy strategy that supports the development and promotion of complementary foods production from local produce that meet acceptable standards [56] as a second major driver.

The dissertation work is therefore a knowledge contribution to address the PEM and micronutrient deficiencies in children in Nedjo district and its area through proper selection and combination of locally available and affordable cereal-legume blends enriched with *dabi teff* to develop nutritious composite novel complementary foods that can be used to alleviate child malnutrition. The cereals that are carefully selected include maize, barley and oats, which contributed higher carbohydrates and energy. The legumes include field pea and linseed which were identified as good sources of protein, fat and important minerlas, typically linseed is a leading source of α-Linolenic acid, omega-3 and omega 6 polyunsaturated fatty acids among plant foods [111, 112]. Prticularly, the third selected was *dabi teff*, which is an

underutilized/forgotten cereal crop identified as a rich source of iron and calcium. Accordingly, all the selected cereals and legumes contributed a substantial amount of the required nutrients (protien, fat, minerals). These justify the possibility of developing a multi-mix nutritionally complemented wholesome and nutrient-dense complementary food product. These locally produced crops can easily be accessible either through own production or from an open local market with affordable prices to the communities. The approach can be used to develop nutritious home-based complementary foods meeting standards in an affordable and sustainable way, particularly by rural and poor urban mothers to feed their infants and young children during the complementary feeding period with potential for micro processing business.

1.13 Research Objectives and Hypothesis

1.13.1 Objectives

General objective: The main objective of this PhD dissertation work is: Development of Nutrient Dense, Optimized Novel Complementary Food from Local Cereal-legume Blends Enriched with *Dabi teff (Ergarostis teff)* Grown in Western Ethiopia

Specific Objectives:

1. To summarize the existing evidence on the possibilities of using nutritious underutilized plant foods to enrich traditional complementary foods in sub-Sahara Africa: their nutritional and health benefits: a scoping review.

2. To explore and document traditional usage, social beliefs and production trends of *dabi teff* (*Eragrostis teff*), the underutilized/forgotten crop grown in western Ethiopia: including its usage as a complementary food: Ethnographic qualitative study.

3. To identify the potential of including *dabi teff,* into pre-processed local food crops *viz*., germinated maize, roasted barley, roasted field pea, dehulled oats and linseed to develop energy and protein-dense optimized novel complementary food with improved sensory acceptability.

4. To identify the potential of including *dabi teff,* into pre-processed local food crops *viz*., germinated maize, roasted barley, roasted field pea, dehulled oats and linseed to develop iron-dense optimized novel composite complementary food with a reduced phytate/minerals molar ratio.

5. To determine the contribution of the newly developed *dabi teff*-field pea-based optimized novel complementary food to the daily energy, protein and iron demands by 6-23 months old children.

6. To identify the effects of blending ratios variation on the macronutrient compositions and sensory acceptability of *dabi teff*-field based novel composite complementary formulations

7. To identify the effects of blending ratios variation on micronutrient compositions and phytate/minerals molar ratios of *dabi teff*-field pea-based novel composite complementary formulations

1.13.2 Hypotheses of the study

1. The newly developed *dabi teff*-field pea-based optimized complementary product would have improved macro-and micronutrient compositions, particularly an improved energy, protein and iron densities than the control and the Cerifam® faffa flours and can meet FAO/WHO standards.

2. The newly developed optimized complementary product would have increased *in-vitro* minerals bioavailability and boosted sensory acceptability as compared to the traditional complementary food (control)

3. There would be a direct positive correlation between *dabi teff* ratio variation and iron content in the formulation.

4. The likely daily contribution of the newly developed optimized novel complementary product to children's daily energy, protein and iron demand would be higher when modeled/checked against the recommended dietary allowance (RDA) set by PAHO/WHO feeding guideline.

1.14 Dissertation Layout

For the purpose of presenting this PhD project work, the dissertation was organized into **8 Chapters** with their respective descriptions.

Chapter 1 presented a short and comprehensive background information of each study (articles) including an overview of global malnutrition challenge, protein-energy and micronutrient malnutrition in under-five children, causes and impacts of child malnutrition, international and national strategies attempted so far to alleviate child malnutrition, the food-based strategy, objectives and hypothesis; **Chapter 2** presented materials and methods of the study, including study area, study design and graphical abstract showing the whole study process; in **Chapter 3,** the possibility of nutritional enrichments of traditional complementary foods using underutilized nutritious plant foods in SSA: their nutritional potential and health benefits was reviewed; in **Chapter 4,** the traditional usage, social beliefs and cultivation trends of *dabi teff*, including its usage as complementary food was explored and documented; in **Chapter 5**, Optimization of a formula to develop energy and nutrients dense novel complementary food from cereal-legume blends through incorporation of *dabi teff* was presented, in **Chapter 5.1**, Optimization of *dabi teff*-field pea based energy and protein dense novel complementary food with improved sensory acceptability using D-optimal mixture design was presented; **Chapter 5.2** presented 'Optimization of a formula to develop iron-dense novel composite complementary flour with a reduced phytate/minerals molar ratio from *dabi teff*-field pea-based blends using a D-optimal mixture design'; in **Chapter 6**, effects of blending ratio variation were presented; in **Chapter 6.1**, 'Effects of blending ratio variation on macronutrient compositions and sensory acceptability of *dabi teff*-field pea-based novel composite complementary foods was elaborated; **Chapter 6.2** presented 'Effects of blending ratio variation on micronutrient compositions and phytate/minerals molar ratios of *dabi teff*-field pea based novel composite complementary flours' in **Chapter 7,** 'Energy, protein and iron densities of *dabi teff*-field pea-based optimized novel complementary flour and its likely contribution to daily energy and nutrients demand by 6–23-months-old children was demonstrated as modeled against the PAHO/WHO standard child feeding guidelines'; and **in Chapter 8,** the general discussion and conclusions from our findings, and recommendations for policy actions and further research were drawn. The schematic pictorial layout of the dissertation is shown in **Figure 1.5.**

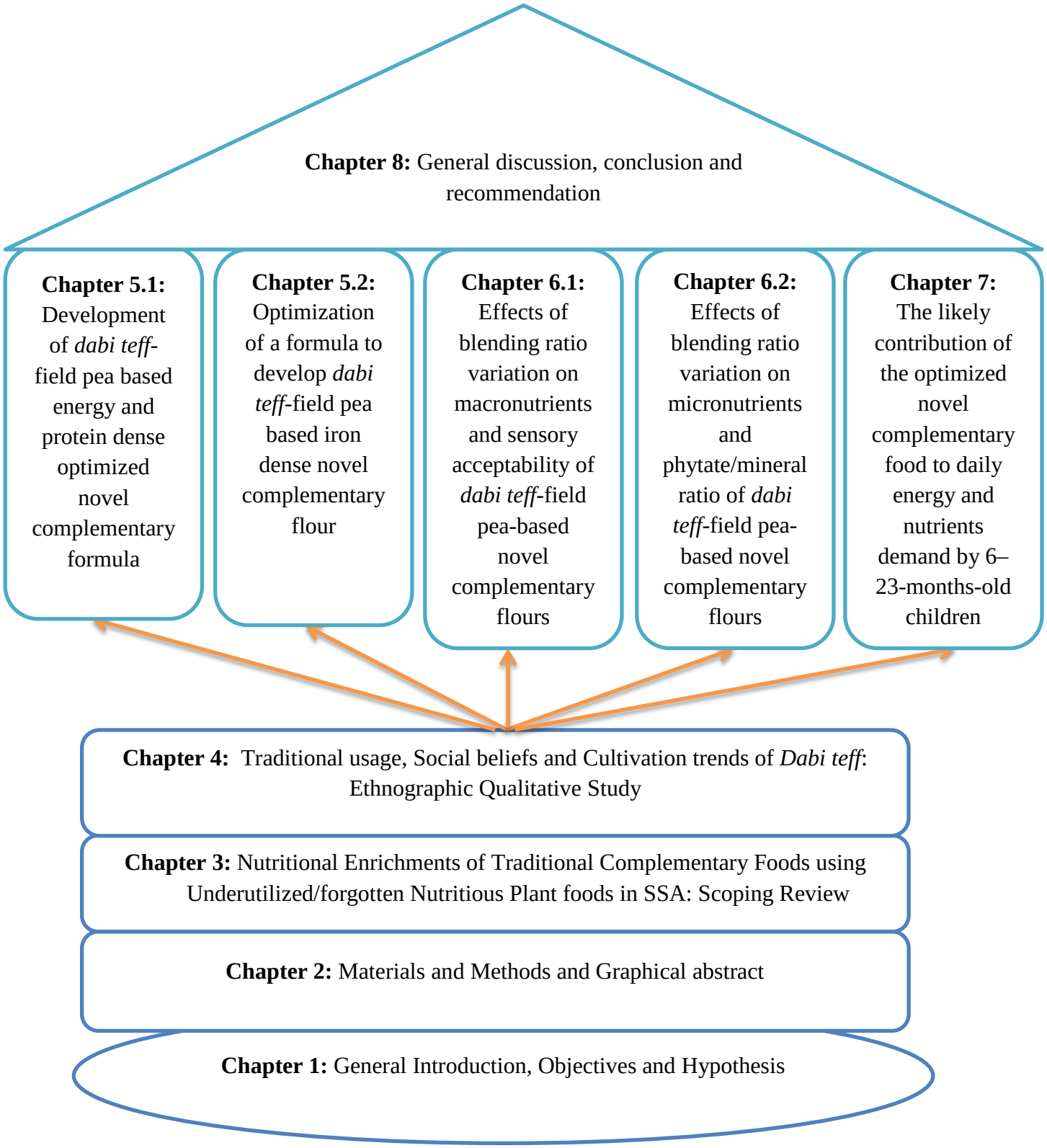

Figure 1.5: Schematic pictorial layout presentation of the dissertation

2

Chapter 2:

Materials and Methods

2.1 Description of the study area

The study was conducted (centered) in Nedjo district, Ghimbi Zone, Oromia region, Western Ethiopia **(Figure 2.1)**. There are 23 districts in Ghimbi zone. Nedjo district was purposively selected as it is known for growing *dabi teff*. Nedjo district has 34 sub-districts or kebele's (the smallest administrative unit in Ethiopia). Nedjo town is located at 575 km away to the west of Addis Ababa, the capital city. Geographically, Ghimbi zone is bordered by Qellem Wollega zone in the west, East Wollega zone in the east, Benishangul Gumuz region in the north and Ilu Abba Bor Zone in the south. The zone has a latitude and longitude of 9°10′N 35°50′E/9.167°N 35.833°E with an elevation between 1845 and 1930 meters above sea level.

According to Ethiopia Central Statistical Agency [113], the Zone has a total population of 1,350,415, of which 671,538 are men and 678,877 women and 92.42% of the population are rural inhabitants and it covers an area of 10,833.19 square kilometers. Majority of the inhabitants in Ghimbi zone is the Oromo ethnic group whose main economic activities and dominant means of livelihood/mainstay is subsistence agriculture including cultivating starchy cereals such as maize, barley, sorghum, teff, finger millet, oats; grain legumes such as beans, peas and lentils; oil seeds such as niger seed, linseed, soybean, sesame seed and rape seed; root and tuber crops such as potatoes, sweet potatoes and 'anchote'; and vegetable production [114] which are components of the major staple meals in the area. The zone is also known for its coffee-producing area and moderate livestock production such as cattle, sheep, goats and poultry.

2.2 Study Design

This PhD project work was conducted in three major parts and it employed three designs: **Part I:** Evidense synthesis on "Nutritional enrichment of traditional complementary foods using underutilized plant foods in sub-Saharan Africa: generation of evidence-based information on the possibility of using nutritious underutilized plant foods to enrich starchy traditional staple foods: their nutritional and health benefits. **Part II:** Uncovering the traditional usage, social beliefs and production trends of *dabi teff* (*Eragrostis teff*) using exploratory ethnographic qualitative study design through focus group discussion (FGD) and KIIs interviews. **Part III:** Experimental study

design to generate laboratory results of the formulations. Optimization of a formula was employed using Stat-Ease-Design-Expert®, D-Optimal Mixture Design.

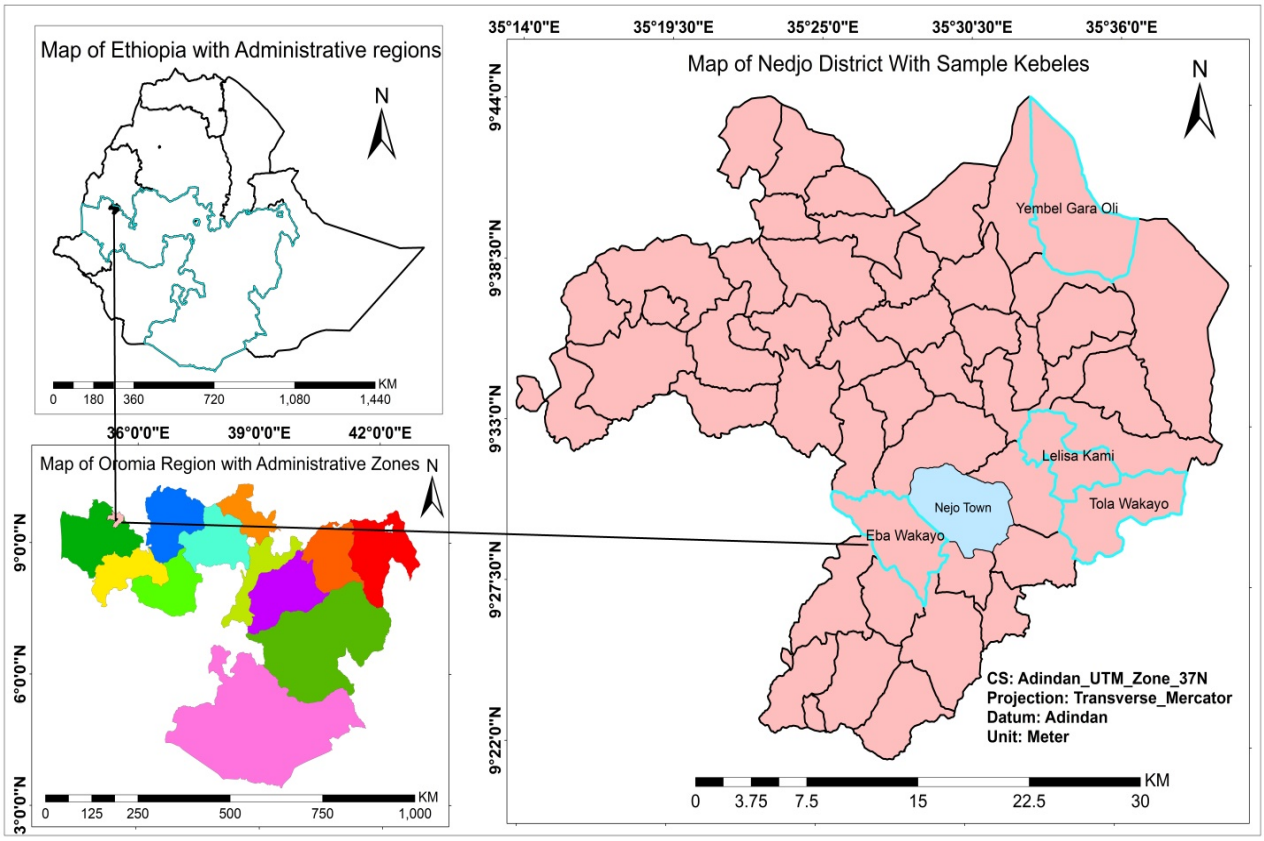

Figure 2.1: Map of the study area, showing the study kebeles in Nedjo district, Western Ethiopia (By the researcher)

2.3 Methods for the Scoping Review

Three stages of paper searching were employed to obtain the final eligibale papers for reviewing. These include initial searching of papers in webpages, searching for key words and screening for retrived articles for eligibility. A search was conducted on Google Scholar, Web of Science, Scopus and PubMed online databases using the key search terms “traditional complementary foods” “underutilized,” “plant foods”,” enrichments”, “health benefits”, and “Africa South of Saharan” to guide screening of articles. Search techniques such as the use of Boolean operators

(AND, OR, and NOT) to create a comprehensive search were applied. The review included studies from 2002 to 2022.

The main criteria for inclusion (eligibility) of the retrieved papers were (a) studies that have focused on the enrichment of traditional complementary foods using underutilized plant foods, (b) studies where maize, sorghum, wheat, millet, rice, teff, oats and cassava were considered as the main staple ingredients of the traditional complementary foods formulation (c) studies done geographically in SSA and (d) studies where food processing techniques were applied to improve the resultant nutritional quality of complementary food products.

On the other hand, articles were excluded when: a) the major staple diets are out of the scope of this dissertation; b) studies where commercial fortificants were added to the traditional complementary foods as an ingredient c) studies that were conducted out of the SSA region, d) studies where processing methods were not applied; and e) studies where there is no comparator.

All the research articles retrieved were exported to the EndNote reference manager where duplicates were removed. The screening process was performed in three steps; first, the titles of all articles were critically screened in order to ensure that they were aligned with our review topic. Secondly, the abstracts of the selected articles were screened to remove studies not fulfill our inclusion criteria. Finally, the full texts of the articles were critically assessed for eligibility and reviewed to compile the pertinent information required. This information consists of the following: traditional staple ingredients, underutilized plant foods used for enrichments, processing methods applied, the resultant nutritional enhancement, country and references.

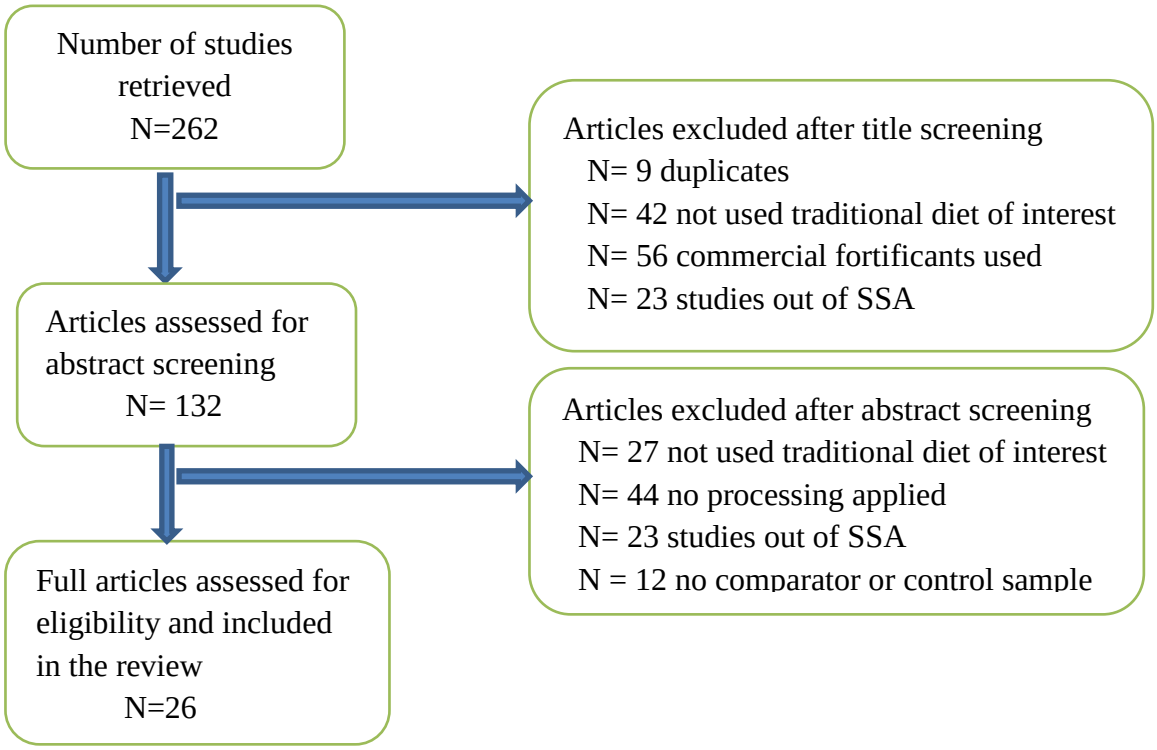

Figure 2.2: Search of publications related to nutritional enrichment of traditional complementary foods using underutilized nutritious plant foods in SSA-A scoping review

2.4 Methods for the Ethnographic Qualitative Study

2.4.1 Study design

A community-based exploratory ethnographic qualitative study design was employed to elicit and collect deep ethnocultural information in the community using a focus group discussion (FGD) and key informants interviews (KIIs). This design was used to best explore the traditional usage, social beliefs and cultivation trends linked to *dabi teff* and it worked well to uncover the way the traditional values are practiced and praised in the community. The study assumed the interpretivist point of view that allows multiple interpretations of the same phenomenon (traditional practices linked *dabi teff*) among the community Nedjo district in western Ethiopia.

2.4.2 Sampling technique and study participants selection procedure

Selection of the study participants accounted first visiting the zonal agricultural bureau to identify the potential *dabi teff* growing area in the zone where Nedjo district with its sub-

districts, the kebele's (the smallest administrative unit) was identified. Then, in consultation with the district administrators, four kebele's with a good history of growing *dabi teff* and also near the main road were purposively selected to arrive at the study participants. The names of the four selected Kebele's were Eba waqayyo kebele, Tola Waqayyo kebele, Yambal kebele and Lalisa kami kebele.

Male and female elders aged around 50 (the grandmothers/fathers) who were perceived to have good traditional knowledge and beliefs regarding *dabi teff* were purposely selected for the FGD nominated by the agricultural development army (DA) in the village. The KIIs were comprised of the DA and knowledgeable community members who were also purposively selected.

The criteria for FGD eligibility were older male and female born and lived (believed to have lived experience and perception) in the study area, able to understand and speak the local language and give their consent **(Annex II).** The eligibility criteria for the KIIs were education level (college level) and being knowledgeable and able to understand and speak the local language.

2.4.3 Data collection tools

An open-ended semi-structured FGD guide interview question was used for both the FGD and KIIs **(Annex II)** to elicit and collect ethnocultural information including the traditional usage, the social beliefs and the cultivation trends, its usage as a complementary food, the storage quality and some other qualities of *dabi teff*. Any new experience arising from the participants was able to build on each other's ideas and used for the enrichment of the information. One FGD sessions were conducted from each of the four purposively selected kebeles' comprising of 8-10 participants, totally four FGD were conducted which was assumed to be enough to capture more than 90% of themes to be generated from our homogenous study participants. Totally 38 FGD discussants were participated. Concerning the KIIs, four key informants from each kebele's were interviewed including three knowledgeable/educated community members and one agricultural development army (DA) worker in the village. Totally 16 key informants were participated. The number of FGDs and KIIs were determined based on the level of information saturation which

was determined by transcribing the discussions of each day's session. The homogeneity of the respective FGD participants was maintained by sex. Male and female discussants were interviewed separately to freely express their ideas.

The guide questions **(Annex II)** were first prepared in English language and translated into "afaan Oromoo" language by the researcher for easy communication and back translated to English language to confirm the consistency of the tool to generate qualitative original data.

2.4.4 Tool validation and pilot-testing

For the purpose of tool validation, three experts, two cultures and tourism experts and one agricultural development army (DA) were employed who were working in the district office. For the purpose of pilot testing, four potential participants (two male and two female) were employed. They were purposively selected knowledgeable people (similar to subjects of the final study) and they were interviewd two times from an area similar to the study area. The purpose of this pilot testing was to obtain feedback concerning the appropriateness, the content validity, the easy understanding of all the questions and the estimated time required to complete the session. Finally, the data collection tool was reviewed and adjusted based on the feedback obtained. The data were analyzed manually using subsequent standard steps where all the information collected was transcribed verbatim, reviewed, through read and internalized, color-coded, categorized and synthesized into themes, translated and reported.

2.4.5 Data collection procedures

Data were collected in December 2022 and the discussion was facilitated by the principal researcher (PhD fellow) and two assistants, who were trained before the actual data collection period. The two assistants were composed of one cultural and tourism expert and one development army (DA) having tertiary level education and working in the study area. These research assistants did not have any relationship with the participants before the commencement of the study. The participants were gathered at village posts on non-market and weekend days to

avoid barriers to participate and the discussion was held face-to-face where none of them declined to participate in the study.

The facilitators introduced themselves to the participants and warmly welcomed and invited them to introduce themselves because they were a group of strangers and finally relationship was established between them. Then after, the objective of the discussion was briefed to them followed by initiating the discussion session one by one based on the guide using the local language. The information generated by each participant was audio-taped using digital recorders and also by handwritten notes using a notebook with the help of the research assistants. This was done to ensure triangulation of the data with the record and/or refer to the notebook if the recorder fails. The FGD discussion took from 60 to 70 min while the key informants' interview took from 40 to 50 min. Redundancies of ideas were considered to be information saturation and were removed every evening during the transcription of the day's work and preliminary analysis. The study partcipants were further probed to refelect more deeply on the meaning of their comments and new questions were added, whenever an information gap was identified.

2.4.6 Trustworthiness of the data

The quality of qualitative research is assured by meeting standards of trustworthiness through addressing credibility and transferability. To satisfy credibility, participants from different kebele's were included. Additionally, focus group discussions, in-depth interviews, and field notes were used in the analysis of the data. The study provided descriptions of the setting, sampling procedure, eligibility criteria, tool validation and pilot testing, interview processes, and findings to strengthen the transferability to different areas. Validity and reliability were ensured by triangulation of the data gathered from the interviews with the information obtained at the FGD. Further we have returned the transcribed text (results) to some participants (member-checks) for reviewing for any correction and ensure reliability.

2.4.7 Data analysis

An inductive approach was employed and the collected data were analyzed manually using the guideline of systematic text condensation lens [202]. The principal researcher together with the assistants (the research team) carefully listened to the audio-record and reviewed the note book.

The information obtained was checked against the objectives and finally it was transcribed verbatim. The transcript was read thoroughly and repeatedly to grasp and internalize a thorough sense of the overall content in the texts to identify central meaningful units in the transcribed material and reviewed. The contents were color-coded by the research team. Major categories of information were captured and synthesized to develop themes. Any diverse cases linked to the codes, categories and themes were corrected through team discussions. Sections of the discussions were quoted verbatim, and a few were modified to enhance readability and finally translated from the local language (afaan Oromoo) to the English language.

For presentation in the results section, summaries of the findings were condensed (texts with similar colors were pooled) and narrated under each of the identified themes or constructs and substantiated with powerful quotes as illustrations to persuade the claims. Steps in the conduct, analysis and reporting of this study were arranged according to the guidelines outlined in the consolidated criteria for reporting qualitative research (COREQ) [203]. The subsequent steps of the data analysis are presented in **Table 2.1** below.

Table 2.1. Steps of the ethnographic qualitative data analysis, Nedjo district, Western Ethiopia, 2022.

Steps	Description
Transcription of data	The audiotape recorded was carefully listened and the handwritten notes were reviewed and transcribed verbatim (word perfect).
Internalizing the data	Grasping and internalizing of the whole information was achieved by thoroughly and repeatedly reading the transcript to get the real meaning of words from the participants and reflective notes that were taken.
Generating color codes	After reading thoroughly, similar texts were color-coded line by line attempting to categorize major information.
Categorizing captured information	Major categories of information were captured (texts with similar coded colors were pooled together) to form potential themes
Synthesizing themes	The pooled texts were grouped together and themes were developed guided by the objectives followed by interpretation.
Reviewing themes	The themes were checked with the coded data extracts and assessed in terms of how well they represented the entire data set.
Defining and naming of themes	The names and definitions of each theme were refined and a short description (short paragraph) of each theme was developed by the principal investigator.
Narration and producing a report	A completed report of the analysis was written by the principal researcher by narrating each of the emerged themes, substantiated with powerful quotes and relating to the original research question. Finally, the report was read and reviewed by all the authors.

2.5 Methods for the Experimentation

2.5.1 Sample collection

The food crops *viz*., *dabi teff (Eragrostis teff (Zucc.) farmer variety*), maize (*Zea mays* L.), barley (*Hordeum vulgare*), white field pea (Pisum sativum), oats *(Avena. sativa)* and linseed (*Linum*

usitatissimum) were purchased from an open market of Nedjo town **(Figure 2.3)**. The availability of these crops in the area was verified by an inspection survey of Nedjo market as well as by asking mothers about the traditional usage of the crops as complementary foods and their affordability. Literature-based nutrient content of each crop was taken into account during the selection process which was later verified by laboratory experimentation (nutrients analysis of each crop). The crops were wisely selected to add one or more essential nutrients to their mixture to be nutritionally complemented. Apparently, uninfected samples (visual inspections like the absence of black spots and discoloring) were purchased from the center and corners of the market on two different market days to assure representativeness. Two indigenous older people were volunterly participated during the purchasing to critically identify the *dabi teff* grain in the market. Each piece of the six crops purchased from the different corners was packed separately in polyethylene bags and transported to the Ethiopian Institute of Agricultural Research (Food Science and Nutrition Laboratory) and mixed using a laboratory mixer (Hot Sale DZ-10 L laboratory mixer, China) to get a homogeneous, 3 kg of composite crop samples each.

2.5.2 Samples processing

It is not only the proper selection of nutritionally important crops, but it is also the processing techniques applied that determine the quality of the final product. Accordingly, the collected crop samples underwent various controlled processing techniques. In brief, *dabi teff* was manually cleaned by winnowing to remove chaffs, straw, dust and other extraneous and washed with tap water and sundried. Other cereals (maize, barley and oats) and the legumes (field pea and linseed) were sorted out from sands, sticks, stones and defective seeds, later washed and sundried for two days and were ready for further individual processing **(Figure 2.3)**. Due to the small size of *dabi* teff seeds, it was made into whole-seed milled flour which could be the reason for the higher nutrient contents of the crop. Barley and oats samples were soaked in clean tap water for 2 h. Then the water used for soaking was drained off and the crops were immediately decorticated (while the seeds were still wet) using a wooden decorticator and the hulls were removed by winnowing.

The technique of maize seed germination was adapted from the method previously described by Rasane *et al.* [115] with little modification. Briefly, the maize grains were soaked in water (1:3

w/v) for 3 h to achieve hydration, drained, spread on a clean jute sack placed on a wooden platform and covered with another jute sack for germination at room temperature (25 ^{0}C ± 2 ^{0}C) for 72 h. Water was sprayed every 12 h to stay humid (60% relative humidity). A 72 h germination time was used to obtain maximum amylase activity according to Rasane *et al*. [115] After 72 h, the germinated seeds were rinsed, drained for 10 min, transferred to aluminum trays and dried in an air oven at 40 ^{0}C for 5 h to terminate the germination process. Then the dried germinated sample was roasted at 120 ± 5 ^{0}C for 10 min and allowed to cool.

Figure 2.3: Collected crop samples (Photo by the researcher)

The crop samples *viz*., barley, oats and field pea were minimally roasted in an oven at 120 ^{0}C for 20 min until light brown colored and then cooled to room temperature (25 ± 2 ^{0}C) as described by Rasane *et al*. [115]. The roasting process was carefully controlled to prevent over-roasting and the formation of undesirable Maillard reactions that may lead to protein quality damage. The linseed sample was minimally cooked for 5 min at 90 ^{0}C as described by Castro-Alba [116] with a small amount of water to condition the seeds to release oil from the oil cells and later sundried without draining the water used for cooking.

2.5.3 Flour preparation and handling

All the six pre-treated samples were milled into flours using a standard miller (Cyclotec 1093 sample mill, Foss Analytical, Sweden) to obtain smooth and consistent particle sizes and sieved through a 0.5 mm mesh sieve size. The flours were then packed in air-tight high-density polyethylene bags as described by the American Association of Cereal Chemists [117]. Then the flour samples were separately coded as X1-*Dabi Teff* flour, X2-Barley flour, X3-Oats flour, X4-Field pea flour, X5-Lin seed flour and X6-Germinated maize flour and stored safely at room temperature till formulation.

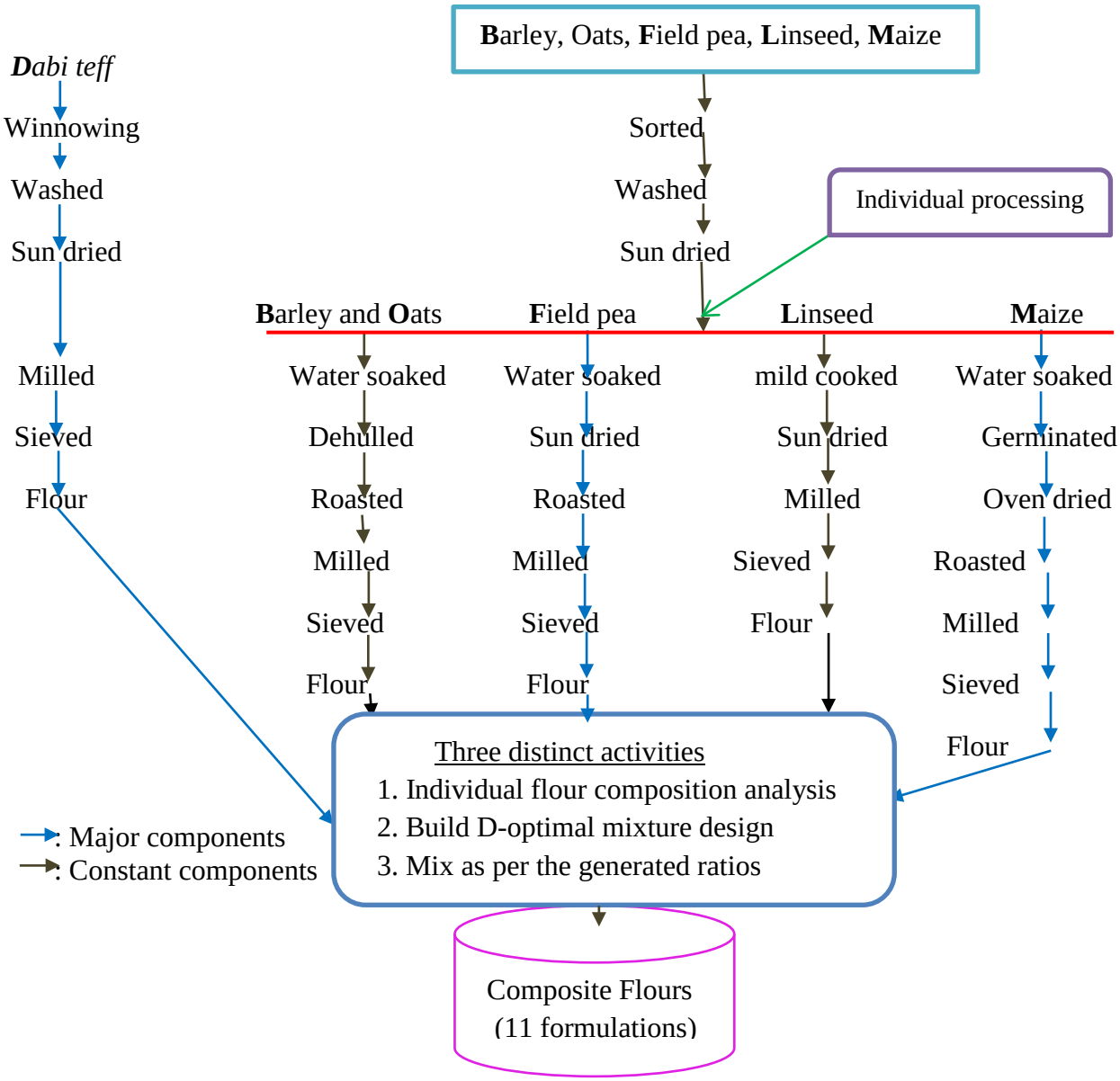

Figure 2.4: Process flowchart diagram of the crop samples and the formulations

2.5.4 Experimental design

2.5.4.1 Flour formulations

Approximately 100 g of flour from each of the six components was taken separately before generating the formulations to determine the macro-and micronutrient compositions of each ingredient **(Table 5.1 and 5.9)**.

Stat-Ease Design-Expert® software version 11 (Randomized Mixture Design, D-Optimal, Minneapolis, USA, 2018) was used to generate the formulation design matrix **(Table 2.1)**.

Defining the range of each component in the formulations was based on three considerations, including, first, targeting to attain the FAO/WHO [28] macro- and micronutrient recommendation where moisture, protein, fat, ash, fiber, carbohydrate and energy contents to be $\leq$5, $\geq$15, 10-25, $\leq$3, $\leq$5, 64$\pm$4% and 400-425kcal/100g, respectively. Moreover, the guideline says that the energy from protein (protein-calorie) should be between 6–15% of the total energy from a complementary food, fat-energy to be 20–30% of the total energy with the remaining energy 55–65% should be generated from a carbohydrate source and iron requirements should be 16 mg/100g, calcium 500 mg/100g and zinc 3.2 mg/100g from complementary food to be nutritionally adequate, secondly aligning the Ethiopian complementary feeding guideline [118], which specifies an enriched complementary food should be prepared using one third of legumes/pulses and three-fourth of cereals, and thirdly should account for the macro- and micronutrient contents of each ingredient **(Table 5.1 and 5.9)**. To obtain the high carbohydrate-energy requirement for children and to attain the targeted considerations, the other components including barley, oats and linseed were set constant in the blends which were thoughtfully designed to maintain the higher carbohydrate–energy requirements.

The macro- and micronutrient composition analysis results of each ingredient (Table 5.1 and 5.9) were recorded (customized) 'into' Nutrisurvey linear programming software (Version 2007) for analysis to estimate and define ranges of major components **(Table 5.2 and 5.10)**. By estimating the amount of a meal to be served to 1-3 years old children to be 75 g (solid portion) per meal and adjusting for the required number of meals per day (3 times), several trials (iterations) were

made to define percentage ranges **(Table 2.2)** of the major components (*dabi teff*, field pea and maize). This was done by entering range-related amounts into the software, which were combined with the constant components, and the output was examined. The final generated output, the percentage fulfillment by the meal of the mixture as compared to the Reference Nutrient Intake (RNI) as set by the Pan American Health Organization PAHO/WHO (219) for 1-3-year-old children was found to be 84-152%, 90-188%, 107-195%, 41-61%, 612-1045%, 133-225% and 229-400% for energy, protein, carbohydrate, fat, iron, zinc and for magnesium, respectively **(Table 5.2)** which corresponded to 20-35% of *dabi teff*, 0-30% of field pea, 5-20% of germinated maize, while the rest were set constant at 25% barley, 15% oats and 5% linseed which were used as constraints for generating the formulation design matrix **(Table 2.2).**

The conventional flour (a commonly used cereal-based complementary food in Nedjo area) was used as control, constructed from 80% barley flour and 20% ungerminated maize flour, approximately $3/4^{th}$ of barley and $1/4^{th}$ of maize (personal communication or information collected from some skilled caretakers/mothers assisted by primary health extension works in the village). The reason why barley and oats were kept constant was that, these cereals are naturally good energy and carbohydrate sources and they can contribute higher carbohydrate-energy to the formulations to provide the required value set by the standard and also to fulfill the Ethiopian complementary feeding guideline and it was thoughtfully designed to maintain the higher carbohydrate-energy requirements from complementary foods. Cerifam® faffa flour (popular commercial complementary/weaning flour in Ethiopia) was used as a comparator.

The nutrisurvey analysis output showed that the percentage fulfillment of the reference nutrient intake (RNI) was a good indicator of adequate range to seek out an optimal point (sweet spot) and constraints for the component were well-defined to generate the formulation design matrix. Ranges for the major components were, therefore, defined **(Table 2.2, top right)** majorly based on the percentage fulfillment range (lower-upper %) of protein and energy requirements by the formulated meal from the mixture of those ingredients as suggested by the software **(Table 5.2, last raw).** The aim of the optimization was to identify the best ingredient proportion that could provide the best response values of macro- and micronutrient compositions to develop nutrient and energy-dense novel complementary food with improved sensory acceptability and

bioavailability from local cereal-legume blends incorporated with *dabi teff* that can fulfill the energy and nutrient requirements of infants and young children to alleviate protein-energy and micronutrient malnutrition to ensure their optimal growth and development.

D-optimal mixture design was run to generate the formulation design matrix using the range defined **(Table 2.2).** The six components were constrained to generate a total of 16 experimental runs with 6 central points and 5 replicates to provide 11 experimental runs. The amount in grams of individual components generated in each formulation (experimental run) was carefully weighed on a digital balance gravimetrically and blended together. The blended flours were thoroughly mixed using an electrical blender for 3 min at 200 rpm to homogenize the flours. They were then packed and sealed in high-density polyethylene bags and stored in a refrigerator at 4°C till analysis.

2.5.4.2 Porridge preparation

A thick and consistent porridge was prepared by mothers acquainted with good cooking skills from all the formulations **(Table 2.2)** and the control flour following the method outlined by Onabanjo *et al*. [119] with slight modifications. Briefly, 300 g of the composite flour was mixed with 500 mL of clean tap water in a saucepan to make a slurry and put aside. Eight hundred milliliter (800 mL) of the water was boiled in a stainless steel pan and once the water reached boiling point, the previously prepared slurry was added to the boiled water and allowed to cook for 10–15 min on an electric stove with continuous stirring using special stainless stirrer to avoid coagulation until the desired consistency was reached. The porridge was allowed to cool to a mild or at a serving temperature around 40 ^{0}C. The usual (commonly consumed cereal-based complementary food in the sample collection area) was used as a control constructed from 80% barley flour and 20% un-geminated maize flour in consultation with skilled mothers/caretakers. All the prepared porridges were subjected to sensory evaluation as fresh as possible by mother panelists where these mother panelists were generally consumer panelists.

2.5.4.3 Macronutrient-compositions analysis of individual components and the formulation

All the laboratory analyses were conducted at the Ethiopian Institute of Agricultural Research (Food Science and Nutrition Laboratory) which is certified by the International Organization for Standardization (ISO), ISO-17025:2017 by the International Laboratory Accreditation Cooperation (ILAC). Associations of Official Analytical Chemists [120] modified methods were used to determine the macro-compositions of the individual components and formulated flours. The moisture content was determined by air convection oven drying method (Model No. DHG-9123 A, Sweden) using the method described by 925.10 [120], for 1 h at 130 ± 3 ^{0}C. Crude protein content was determined by Kjeldahl (Kjeltec 8400, Auto Sample Systems, Foss Analytical, Sweden) using a nitrogen conversion factor of 6.25 following the official method 954.10 [120]. Soxhlet method (Soxtec 8000, Tecator Line, Foss Analytical, Sweden) was used to determine crude fat content by N-Hexane extract according to the method number 2003.06, [120]. Ash content was determined using the combustion method by box type muffle furnace (Model No. SX2-4-10 GJ, China) at 550 ◦C for 4 h following method 923.03 [120]. Crude fiber content was determined by Fibertec 8000, Foss analytical, Sweden, following official method 978.10, [120]. Utilizable carbohydrate was estimated by difference: 100 - (% Moisture +% Crude protein +% Crude fat +% Crude fiber + % Ash) as indicated by Ayele *et al*. [121]. Gross energy (kcal/100 g) of individuals and formulated flours was computed based on FAO/WHO [122] recently amended in 2021 codex guideline by multiplying the values obtained for energy-yielding nutrients (crude protein, crude fat, and utilizable carbohydrate) with Atwater conversion factors where Energy (kcal/100 g) = [(% crude fat x 9) + (% crude proteins x 4) + (% utilizable carbohydrates × 4)].

Determination of energy and protein density of the optimized formula

The energy density of complementary foods, expressed as kcal/g, can be calculated by dividing the total energy from complementary foods by the amount of complementary foods served to children, taking into account the theoretical gastric capacity of infants and young children to be 30g/kg body weight per meal and the required number of meals to be served to the children per day for the respective age group. However, the theoretical gastric capacity may not usually be

functional due to a lack of responsive feeding, inappropriate consistency of foods, or due to underlying health conditions where the children take less [126].

The amount of food needed per day by children in their respective age groups to satisfy their energy and nutrient demands is a function of the amount of energy and nutrient densities that can be provided by complementary foods. Abeshu *et al.* [127] estimated that for complementary food having an energy density range of 0.6–1.0 kcal/g, the amount of food served to provide the daily energy requirement is 263.3g for 6-8-month-old, 317.09g for 9-11month old, and 334.92g for 12-23month old children for the study done in Wolayita Zone of Ethiopia and the children could consume an average amount of 212.82g of the complementary food per day due to their low functional gastric capacity. Another report by Wasswa *et al.* [128] stated that the amount of porridge eaten by children in rural settings of Uganda was estimated 200 g per day for age 6-12 months old children. Based on these two reports, we have estimated the amounts of our optimized novel complementary foods to be served to an age group of 6-8months, 9-11 and 12-23 months old children to meet their daily requirements to be 225 g, 300 g and 450 g, respectively (i.e 75g, 100g and 150g per meal) for the respective age group and 3 meals/day. Protein-density or protein energy ratio was calculated by dividing the protein content of the optimal flour by its corresponding optimal energy value expressed as g/100kcal.

2.5.4.4 Sensory evaluation of the porridges

The sensory quality of the formulated flour porridges was examined for its color, aroma, taste, mouth-feel and overall sensory acceptability by the mother panelist. Each porridge sample was evaluated by 48 untrained healthy panelists comprising mothers/caretakers having babies between 6 and 24 months. The selection of the mothers was purposive and assisted by the community health extension workers where mothers with at least primary education and who are familiar with quality characteristics of porridge for children were targeted. The evaluation was conducted at around 9:30 a.m. (morning) to ensure that they were not hungry and that it was not immediately after a meal. The porridges were served on clean, odor-free identical plates coded with 3-digit random numbers **(Annex III)** in randomly arranged order at the serving temperature.

The panelists were requested to evaluate each sample after they were briefed about scoring of a sensory attribute using a 5-point hedonic scale representing; 5-like very much, 4-like moderately, 3-neither like nor a dislike, 2-dislike moderately and 1-dislike very much. The panelists were provided with mild-heated water before and after each tasting of the porridge to rinse (expectorate) their mouths to prevent bias of evaluation and avoid the carry-over effect and waited for 1 minute before testing the next sample. The traditional way to report on the hedonic scale is to sum the values given to each selected food attribute and create a score for each respondent and use it to represent a particular trait. The evaluation process was attentively instructed and controlled by the researcher.

2.5.4.5 Dietary mineral analysis of the individual components and the formulations

Analysis of aqueous solutions by inductively coupled plasma-optical emission spectrometry (ICP-OES) was employed for the simultaneous determination of the dietary mineral contents (Fe, Zn, Ca, Mg, K, P, and Na) of the individual components. This method was also used for the analysis of the formulated composite complementary flours with a radial plasma observation method [123]. All the measurements were performed using a sector arcos optical emission spectrometer optimized with a small volume and 32 linear charge-coupled device (CCD) detectors in the wavelength range between 130 and 770 nm, and they were simultaneously analyzed. A nebulizer was used to introduce the sample solution into the argon gas field plasma, and the energy excitement was preceded in the single spectra. The energy was expressed in terms of intensity, which was directly proportional to concentration; the concentration was calculated on the linear graph of the standard concentration and the corresponding intensities. All the measuring conditions, such as plasma power, gas flow, torch positions, and measuring time, were configured for concentration measurements. According to the standard, the calibration and standardization of the spectra method were performed, and standardization was performed daily. It is a quick procedure for correcting measuring intensities so that the correct concentration is obtained using the original calibration curve.

2.5.4.6 Determination of anti-nutritional factors (non-nutrient contents)

Phytate content determination

Latta's and Eskin's [124] method was used to determine the phytate content after sample extraction with 10 mL of 0.2 N HCl for 1 h, centrifuged (Sigma 2-16KC, UK), and the clear supernatant sample extract (3 mL) was reacted with 2 mL of Wade reagent (0.03% FeCl3.6H2O and 0.3% sulfosalicylic acid in distilled water) and vortexed mixed for 5 s. The absorbance of the sample was measured at 500 nm using a UV–visible spectrophotometer (V-630, Jasco, USA). The phytate concentration was calculated from the difference between the absorbance of the blank (3 mL of 0.2 N HCl + 2 mL of Wade reagent) and that of the assayed sample. It was calculated using a phytic acid standard curve prepared under the same condition, and the result was expressed as mg/100 g.

Condensed tannin content determination

Traditionally, tannins have been classified into condensed and hydrolyzable tannins, based on their hydrolytic properties in hot water or their response to the tannase enzyme. In the present study, condensed tannin (proanthocyanidins) was analyzed by the Vanillin–HCl method of Price *et al*. [125]. The sample was milled until it could pass through a ≤ 750 μm sieve. Approximately 200 mg of the sample was weighed into a screw-capped test tube and extracted with 10 mL methanol by vortex-mixing for 20 min. Then, it was centrifuged at 3,000 rpm for 10 min. The Vanillin (5 mL) reagent was mixed with 1 mL of the sample extract at 1 min intervals into a one-test sample set, and for the blank (1 mL), only 0.5 mL of concentrated HCl was added in methanol 4% at 1 min intervals. The supernatant was used for the analysis after warming it along with the Vanillin-HCl reagent in a water bath at 30°C. One milliliter of the sample extract was taken in duplicate for each sample (one for mixing with the Vanillin reagent and the other as a blank) to be deducted from sample absorbance. Then, it was immediately incubated in a water bath at 30°C for 20 min. After 20 min, the absorbance was immediately measured/read on a spectrophotometer at 500 nm in 1 min intervals, as per the sequence used for mixing. The sample absorbance was deducted from the blank, and the value was estimated from the catechin equivalent (CE) standard curve. A standard curve absorbance (y) against the catechin concentration (x) was prepared from the catechin. Standard solution readings and the slope were

computed, and the tannin concentration was determined from the equation of the curve and expressed as mg/100 g.

$$CE = CC * \frac{VM}{VE * Wt}$$

Where CE = catechin equivalent; CC = catechin concentration (mg/mL); VM = volume made up (mL); VE = volume of extracts and Wt = weight of the sample.

Tannin in mg/g = $\frac{(As-Ab)-Intercept}{Slope \times D \times W}$

Where: As = Sample Absorbance; Ab = Blank Absorbance; D = Density of solution (0.791 g/mL); W = Weight of sample in grams.

2.5.4.7 Phytate/minerals molar ratios (in-vitro minerals bioavailability)

The phytate/minerals molar ratio for each formulation for iron, calcium, and zinc was computed using the method of Norhaizan and Nor Faizadatul [126]. The amount of phytate and minerals in each formulation was divided by their respective atomic weight (phytate: 660 g/mol; Fe: 56 g/mol; Zn: 65 g/mol; and Ca: 40 g/mol), and the phytate/mineral molar ratio was obtained by dividing the phytate mole with the mole of the respective minerals. The molar ratios found were compared with the acceptable critical limits [28], including the phytate/calcium molar ratio (Ph:Ca) (<0.24), the phytate/iron molar ratio (Ph:Fe) (< 1), the phytate/zinc molar ratio (Ph:Zn) (<15), and the Ph*Ca:Zn molar ratio (<200), which are used as proxy indicators of good mineral bioavailability or uncompromised absorption.

2.5.4.8 Determination of energy and protein density of the optimized formula

The protein-density or protein energy ratio was calculated by dividing the protein content of the optimal flour by its corresponding optimal energy value expressed as g/100 kcal.

The energy density of complementary foods, expressed as kcal/g, can be calculated by dividing the total energy from complementary foods by the amount of complementary foods served to children, taking into account the theoretical gastric capacity of infants and young children to be 30 g/kg body weight per meal and the required number of meals to be served to the children per

day for the respective age group. However, the theoretical gastric capacity may not usually be functional due to a lack of responsive feeding, inappropriate consistency of foods, or due to underlying health conditions where the children take less [6]. The amount of food needed per day by children in their respective age groups to satisfy their energy and nutrient demands is a function of the amount of energy and nutrient densities that can be provided by complementary foods. Abeshu *et al*. [127] estimated that for complementary food having an energy density range of 0.6–1.0 kcal/g, the amount of food served to provide the daily energy requirement is 263.3 g for 6-8-month-old, 317.09 g for 9–11month old, and 334.92 g for 12–23month old children for the study done in Wolayita Zone of Ethiopia and the children could consume an average amount of 212.82 g of the complementary food per day due to their low functional gastric capacity. Another report by Wasswa *et al*. [128] stated that the amount of porridge eaten by children in rural settings of Uganda was estimated at 200 g per day for aged 6–12 months old children. Consequently, based on these two reports, we have estimated the amounts of our optimized novel complementary foods to be served to an age group of 6–8 months, 9–11 and 12–23 months old children to meet their daily requirements to be 225 g, 300 g and 450 g, respectively (i.e 75 g, 100 g and 150 g per meal) for the respective age group and 3 meals/day.

PAHO/WHO [219] and WHO [265] complementary feeding guidelines recommend that 5 meals/day is needed for a lower energy density of 0.6 kcal/g, 4 meals/day for 0.8 kcal/g and 3 meals/day when the energy density is at least 1.0 kcal/g. The standard also specifies that meal frequency that is greater than necessary may lead to excessive displacement of breast milk and preparing and feeding five meals per day requires a considerable amount of time and effort by caregivers. This may prompt them to hold prepared food over from one meal to the next, thereby potentially increasing the risk of microbial contamination which needs to be considered when developing messages regarding meal frequency due to which 1 to 2 nutritious snacks per day, such as a piece of fruit or a piece of bread would be recommended that takes less time for preparation and may also be less likely to displace breast milk [219].

2.5.4.9 Determination of minerals density of the optimized formula

The mineral density of the optimized flour was calculated by dividing the mineral content at the optimal condition by its corresponding optimal energy value expressed as mg/100 kcal.

2.5.4.10 Contribution of the optimized complementary food to energy and nutrient demand

Based on various previous studies, it was estimated that the serving amount of complementary food for 6-8 months, 9-11 months and 12-23 months old children to be 75 g, 100g and 150 g per meal and the children to be served three times a day. By adjusting for these estimated daily serving amounts, the likely contribution of the developed optimized novel complementary food to daily energy and nutrient demand by 6–8, 9–11 and 12–23 month-old children was computed by checking/modeling against the recommended dietary allowance (RDA) set by PHAO/WHO [55] child feeding guideline. Finally, the likely contribution was presented in percentages. The overall process of the dissertation works was presented by a graphical abstract in **Figure 2.5** below.

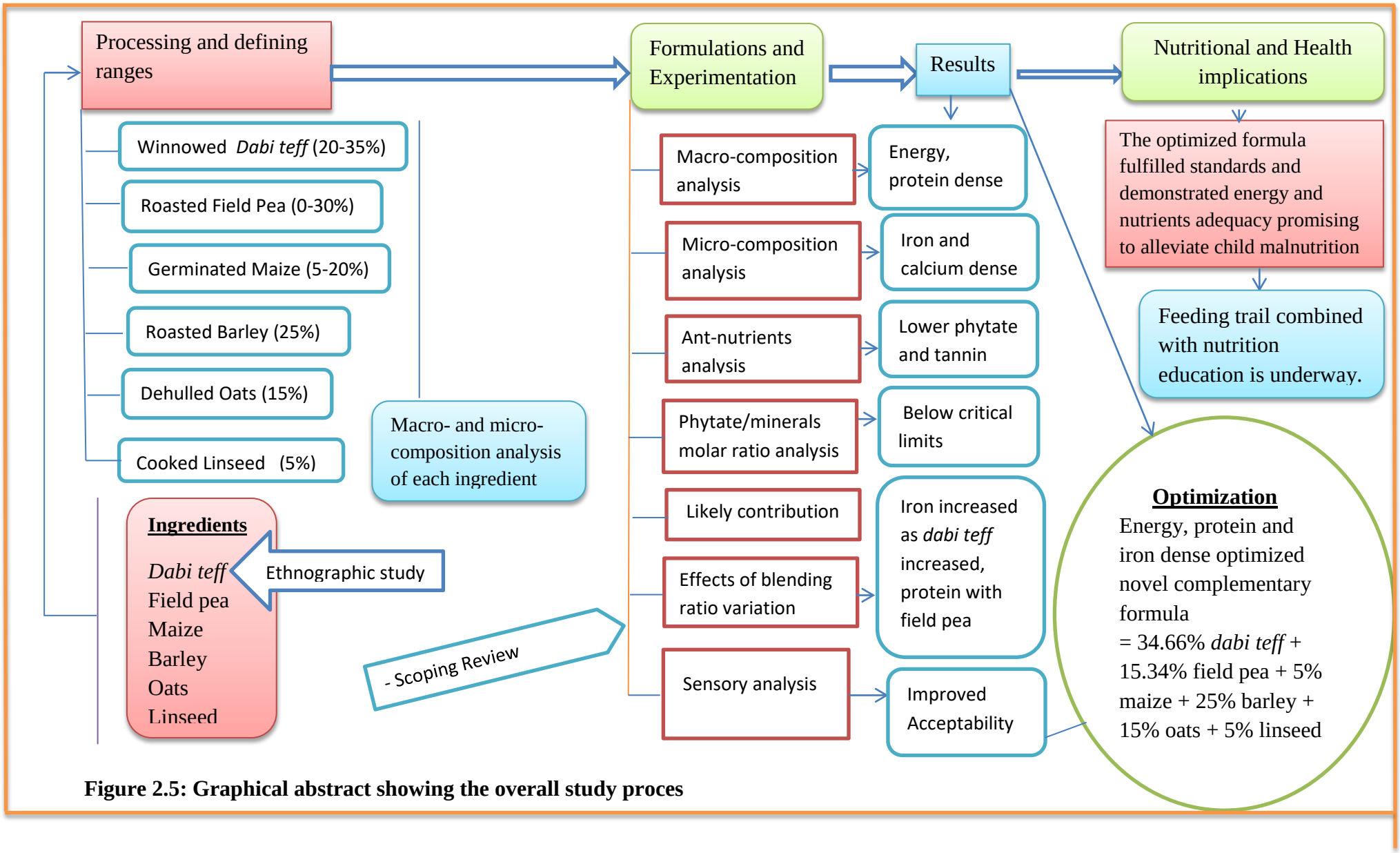

Figure 2.5: Graphical abstract showing the overall study proces

2.6 Statistical analysis and model evaluation

All the laboratory analysis results of the 11 formulations **(Table 2.2)** were subjected to Scheffe's polynomial multiple regression analysis using Stat-Ease Design Expert® software version 11 (D-optimal mixtures design). The mixture components were considered model terms, and the mixture regression was designated as the model fitting. Linear, quadratic, cubic, and special cubic models and interactive effects of the independent variables were fitted for the evaluation of macro-compositions, overall sensory acceptability (**Table 5.3**), dietary minerals, phytate, tannin, and phytate/mineral molar ratio (**Table 5.11**). Analysis of variance (ANOVA) of the design expert was performed to develop models (mathematical equations) and determine the goodness of fit (significance) of the models developed and to show the relationship between Xs' (individual linear, quadratic, cubic and interactive effects of the components) and Ys' (the response variables). These effects (relationships) were further verified by running the 'Model Graphs' test through '2D' (contour plot) and 'trace plots' graphs.

Linear and polynomial regression models were judged (verified) to be adequate and significant using F-statistic at a probability (P) of 0.05, 0.01, and 0.001 and the coefficient of determination R^2. R^2% is the percentage variation of the response (dependent) variable that can be explained by its linear relationship with the independent variable, and it measures the degree of fitness of a regression model. The closer the R^2 value is to unity (1), the closer R^2adj is to R^2pred (with a difference of less than 0.2) and the adequate precision value greater than 4 indicates the better (the satisfactory) the model fits the actual data and adequate to specify the correct relationship between response (Ys') and independent variables (Xs') [129]. The R^2% value should be at least 80% to have a good regression model fit [129].

The developed models from the D-Optimal mixture design employed a formula:

$$Y = \beta 1X_1 + \beta 2X_4 + \beta 3X_6 + \beta 4X_1X_4 + \beta 5X_1X_6 + \beta 6X_4X_6 + \beta 7X_1X_4X_6 + €$$

Where Y represents the response variables to be modeled, and β1, β4 and β6 are the coefficient of the independent variables X_1, X_4, and X_6 and € the random error of the model. X_2, X_3 and X_5 were the constant independent variables and did not enter into model terms.

Normality and constant variance assumptions of the error terms were checked to determine whether a model meets the assumptions of the analysis which was later verified that the assumptions were fulfilled. Furthermore, datasets of the macro-composition, sensory attributes of the porridges, dietary minerals and anti-nutrient contents of the individual and formulated flours were statistically analyzed using a one-way ANOVA of SPSS (IBM version 24, Chicago, USA) to declare statistically significant differences between the formulated flours, control, and the Cerifam® faffa flour. All the data collected were in triplicate. The mean nutrient contents of the optimized were checked against the critical limits set by FAO/WHO [28]. In Addition, the nutrient contents of the optimized, the Cerifam® faffa (the popular commercial complementary flour in Ethiopia) and the control flours were checked for any significant differences. The energy density (kcal/g), protein, carbohydrate and fat densities (g/100kcal), and iron, calcium and zinc densities (mg/100kcal) of the optimized flour were compared to PAHO/WHO [219] recommendations and finally, the likely daily energy and nutrients contribution by the optimized complementary flour, the Cerifam® faffa and the control flour were computed for 6-8 months old, 9-11 months old and 12-23 months old children after adjusting for an estimated daily amount to be served to the children and checked/modeled against the recommended daily allowance (RDA) as per the PAHO/WHO [219].

Levene's test was conducted to check the equal variance assumptions ($P > 0.05$, should be non-significant). Tukey's honestly significant difference (HSD) post-hoc test was conducted for pairwise multiple comparisons (mean difference separation test), and significant differences were declared at a value of p of <0.05.

2.7 Variable optimization and validation

After regression analysis of the experimental data, numerical and graphical optimization techniques were employed to identify the optimum formula (sweet spot) using the design expert (D-optimal mixture design). Simultaneous numerical values of independent variables and multi-response optimization were performed by setting the desired goals for each independent and dependent (response) variable. In brief, the major components used, *dabi teff* flour, field pea flour, and germinated maize flour were set in the range, and the responses were set to maximize energy, protein, ash, fat, and carbohydrate, iron, calcium and zinc whereas to minimize moisture,

crude fiber, phytate and phytate/minerals molar ratios within the experimental ranges **(Table 5.4, 5.12 and Table 7.2).** In addition to goal setting, the relative importance of each dependent variable in the overall optimization solution was set to be '++++' for protein and carbohydrate while it was set as '+++++' for iron, calcium, zinc, overall acceptability and for phytate/minerals molar ratios while the remaining were kept at default which is '+++'. The number of the '+' signs represents the relative importance or weight to be given to the parameter under optimization. This is greatly important because the overall desirability function value of any optimization process is majorly 'Goal or criteria setting dependent'.

Graphical optimization was carried out by the superimposition of contour plots for all the responses with respect to the component ratios. To confirm the validity (accuracy) of the models, a laboratory experiment was conducted on the component ratios identified at the optimal formula, and the obtained laboratory results were then compared with the optimal response values at the optimal condition and with the values of the control flour and finally reported as the formula with the optimal nutritional and sensory profile of all the possible combinations or solutions. One-way ANOVA using SPSS (IBM version 24, Chicago, USA) was conducted to compare the means and declare the statistically significant differences between the predicted response values, the laboratory analysis (validation value), and the control value. Furthermore, two sample *t*-tests were conducted to compare the predicted optimal response values with the validation (laboratory analysis) values to confirm the validity and adequacy of the model equations to predict the optimal response values at a statistical significance level of $p < 0.05$.

They were finally reported as the formula with the optimal nutritional (both macro- and micro-composition contents), optimal energy and nutrient density, daily energy and nutrient-adequate complementary food with improved sensory acceptability and enhanced bioavailability of all the possible combinations or solutions. In conclusion, the protocol and the data generation methods were explained in detail in this method section to allow reproducibility of the work elsewhere.

2.8 Data quality assurance

Data collection procedures were checked and controlled by the principal investigator and the research team and every problem faced during the data collection was reported, discussed and appropriate solutions were made during the ethnographic qualitative study. The data were analyzed manually following standard steps and the trustworthiness of the information was checked. The laboratory experimentations were done in triplicate and the collected datasets were expressed as mean and standard deviation. The sensory evaluation was described as mean score from the panelist likert scale rating.

2.9 Ethical considerations

The study protocol was approved by Jimma University Post Graduate Doctoral School and ethical clearance was given by the Institutional Review Board (IRB) of Jimma University, Institute of Health Science, Ethiopia with reference number of IHRPGS/395/2019. Oral informed consent was also obtained from the sensory panelists.

2.10 Dissemination of findings

The findings were shared with the scientific community through publications in peer-reviewed journals, presentations at scientific meetings, and national/international conferences. Additionally, a policy brief is being prepared to exploit underutilized plant foods grown in Ethiopia for their contributions to food and nutrition security, sources of income generation and national economic development.

Table 2.2: D-optimal mixture design matrix, formula code, mixture ratio, control and individual flours and constraints with their limits.

Std. order	ID	Run order	Mixture ratio (%)						Limits of mixture components			
Standard	ID	Formulation code	X1	X2	X3	X4	X5	X6	Total	Constraints	Lower	Upper
2	0	F1	27.5	25.0	15.0	15.0	5.0	12.5	all	X1	20	35
7	10	F2	26.123	25.0	15.0	23.877	5.0	5.0	100	X2	25	25
10	4	F3	30.457	25.0	15.0	19.543	5.0	5.0		X3	15	15
11	5	F4	20.000	25.0	15.0	29.988	5.0	5.012		X4	0	30
1	2	F5	28.793	25.0	15.0	6.207	5.0	20.0		X5	5	5
9	3	F6	35.000	25.0	15.0	11.884	5.0	8.116		X6	5	20
8	1	F7	21.705	25.0	15.0	13.295	5.0	20.0				
4	7	F8	34.385	25.0	15.0	6.476	5.0	14.139				
5	8	F9	20.000	25.0	15.0	19.54	5.0	15.46				
3	6	F10	22.735	25.0	15.0	22.515	5.0	9.75				
6	9	F11	29.604	25.0	15.0	10.076	5.0	15.32				
		Control	0	80	0	0	0	20				
		Dabi teff	100	0	0	0	0	0				
		Barley	0	100	0	0	0	0				
		Oats	0	0	100	0	0	0				
		Field pea	0	0	0	100	0	0				
		Linseed	0	0	0	0	100	0				
		Germinated maize	0	0	0	0	0	100				

Key: Std-Standard, ID-design identity, x_1-*Dabi Teff*, x_2-Barley, x_3-Oats, x_4-Field Pea, x_5-Lin Seed, x_6-Germinated Maize, F1-F11-Formula Code for each Formulation.

3

Chapter 3:

Nutritional Enrichment of Traditional Complementary Foods Using Underutilized Nutritious Plant Foods and their Nutritional Potential and Health Benefits in sub-Saharan Africa: -A Scoping Review

(Submitted)

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Redrafted from: Diriba Chewaka Tura, Tefera Belachew, Dessalegn Tamiru, Kalkidan Hassen Abate. ***Nutritional enrichment of traditional complementary foods using underutilized nutritious plant foods in sub-Saharan Africa: their nutritional potential and health benefits -A scoping review***

Abstract

In sub-Saharan Africa, starchy foods such as maize, wheat, sorghum, millet and cassava still represent the bulk ingredients of traditional complementary foods. There is increasing attention to indigenous underutilized plant foods due to their nutritional potential and health benefits. Thus, the objective of this review was to summarize the existing evidence on the possibility of enriching traditional complementary foods using nutritious underutilized plant foods, including the effects of common processing techniques on the nutritional value of the resultant complementary products in sub-Saharan Africa. Google Scholar, Web of Science, Scopus and PubMed online databases were searched and the inclusion and exclusion criteria were applied on the retrieved articles for screening. The review revealed that the enrichment of staple traditional complementary foods using underutilized plant foods has been studied well. Maize, a predominant stable crop used for the formulation of complementary food was enriched with amaranth seeds, moringa olifera leaf and others that resulted in the enhancement of protein, iron, calcium, zinc, phosphorus, magnesium, copper, iodine, manganese and potassium. Similarly, the enrichment of sorghum with amaranth, spinach, soybean, and pigeon pea reported to enhance protein, energy, fats, calcium, iron, potassium and zinc contents of the resultant complement products. The others, cassava enriched with pigeon pea, Bambara groundnut, moringa and cowpea; wheat with quinoa, lupine, amaranth, orange-fleshed sweet potato, groundnut, mango and spinach; oats with soybean, linseed and premix (moringa leaf powder, fenugreek); teff-maize, teff-wheat, teff-oats and teff-barley based enriched with pea, sesame, soybean, chickpea and spinach. All these studies reported enrichment of the staple diets using underutilized plant foods resulted in nutritionally dense meeting standards, specifically, micronutrients such as iron, calcium, zinc and magnesium. Fermentation, germination and roasting were the most processing used. In conclusion, the enrichment of traditional complementary foods with underutilized plant foods combined with the processing has been proven to increase the nutritional potential, especially protein and the key micronutrients. Such could help to develop nutritionally adequate complementary foods and it was suggested to include the approach into nutrition policy and program planning as a sustainable food-based strategy in alleviating child malnutrition in sub-Saharan African countries.

Key words: Enrichment, traditional foods, underutilized plant foods, Sub-Saharan Africa

Introduction

Sub-Saharan Africa (SSA) is one of the economically deprived regions of the world, where most of the countries in the region are categorized under the least developed countries and half of the population lives below the poverty line [130]. The majority of the households in the region are unable to afford to purchase commercial or fortified complementary foods. As a result, they rely on traditional or homemade complementary foods that are usually cereal-based often added with root and tuber crops [130].

The SSA region is endowed with a lot of indigenous underutilized plant foods that are rich in nutrients, especially micronutrients that are unavailable in staple or traditional diets. However, in most of the home-made traditional complementary foods in the region, there is over reliance on starchy staples such as wheat (*Triticum aestivum*), Sorghum (*Sorghum bicolor*), finger millet (*Eleucine coracana*), teff (*Eragrostis tef*), maize (*Zea mays*), pearl millet (*Pennisetum glaucum*), guinea millet (*Urochloa deflexa*) and African rice (*Oryza glaberrima*); root and tuber crops such as sweet potato (*Ipomea batatas*), cassava (*Manihot esculenta* Crantz), yam (*Dioscorea cayenensis)* and taro (*Colocassia esculenta*) [131, 132] and [133] which represent the bulk ingredient of traditional complementary foods and are among the major causes that led to persistently high malnutrition levels in the SSA region.

Underutilized or marginalized or neglected or forgotten food crops have been defined as crops that have not been previously classified as major staple crops, in particular, crops that have previously been under-researched, and currently occupy low levels of utilization and are predominantly limited to smallholder and communal farming areas [134]. These are crops whose potential contributions to the national economy have not been adequately explored due to little or no attention to their production, consumption, and commercialization [135]. The global facilitation unit for underutilized species defines it as “those plant species whose potential are not fully exploited to contribute to food security and poverty alleviation”. They have superior nutritional content and immense potential to contribute to food and nutritional security and reduce the impact of malnutrition and hidden hunger particularly in children [83, 136].

Currently, it is suggested that besides more sustainable production of conventionally used foods, introduction of the marginalized or underutilized crops should be focused on, to ensure both increased sustainability of food systems and dietary diversity [137]. There is an increasing interest in introducing forgotten plants and creating foods and products that could contribute to human health and nutrition in innovative ways [137, 138]. The potential use of underutilized plant foods in enriching major staple foods in Africa is currently a novel approach and holds a brighter prospect for alleviating poverty and macro- and micronutrient malnutrition [83].

Padulosi *et al*. [100] and Conti *et al*. [101] reported that there are numerous neglected and underutilized plant foods known in SSA and categorized them under cereals, legumes, tubers, roots and leafy vegetables and concluded among other things that underutilized plant foods could represent an opportunity to enhance food and nutrition security, income generation, empower the poor marginalized people. Underutilized plant foods have important nutritional and functional values, presenting sustainable sources of proteins, carbohydrates, essential fatty acids, vitamins, minerals, phytochemicals, and dietary fiber [80, 81]. In particular, they provide essential micronutrients, complement staple crops, strengthen local traditional foods, and could play a major role in improving the micronutrient content of the diets of millions of people worldwide [137].

Underutilized plant foods have also numerous positive health benefits mainly due to their constituents. Antioxidant, anti-inflammatory, and anticarcinogenic properties additionally add to the importance of these crops [137] and they are naturally gluten-free [101]. Their nutritional composition classifies them as beneficial food sources with the potential to reduce the risk of developing several non-communicable diseases [139]. Moreover, the use of underutilized indigenous plant foods provides benefits not only in terms of nutritional and health benefits; they also ensure the continuity of traditional cultures, prevent loss of food heritage, and preserve a connection to local land race and knowledge of local plants, in terms of cultivation, storage and food preparation methods [137].

Despite the availability, nutritional and health benefits of these promising underutilized pant foods, their use and incorporation into our everyday diet need investigation and promotion [83], because they are entirely ignored by agricultural researchers, plant breeders and policymakers.

Therefore, increased attention has to be given to such foods in terms of research and development and policy frameworks that could promote their extensive agricultural production to improve yields [102], industrial utilization as well as home consumption.

Staple traditional complementary foods in SSA have low energy and nutrient density and contain inhibitory ligands such as phytic acid and tannic acid that limit the digestion and absorption of protein and essential micronutrients such as iron, calcium, zinc and other divalent and trivalent micronutrients [64]. As a result, infants in rural areas in SSA countries are vulnerable to trace mineral deficiencies exacerbated by low body stores at birth because of prematurity and/or low birth weight, induced by poor maternal nutritional status during pregnancy [140]. Some key recommendations to improve the nutritional value of traditional complementary foods are to design proper formulation and enrichments of the main bulk ingredients with nutritious underutilized plant foods by putting a special focus on micronutrient-rich foods [141]. Several studies have shown that this is the best strategy that is safe and effective in preventing micronutrient deficiencies [83].

The World Health Organization [62] recommends the use of nutritious indigenous underutilized plant foods in combination with staple foods and the application of some appropriate processing technologies such as germination and fermentation among others to yield nutrient and energy-dense complementary foods as well as improved bioavailability of key micronutrients. Kamotho [69] has also recommended the use of underutilized locally available food materials that are inexpensive and high in micronutrients for child food formulations. Therefore, the incorporation of these nutrient-rich underutilized plant foods could be the solution to the dual challenges, undernutrition and hidden hunger, which are manifested most in developing countries with a noticeable symptom of growth stunting and impaired capacity to resist diseases mostly among children, [83, 130]. This scoping review is therefore; to summarize the existing evidence on nutritional and health benefits of enriching traditional complementary foods using nutritious underutilized plant foods in SSA. It also explores the effects of various processing techniques on the nutritional value of food products and suggests the way forward for effective utilization of the underutilized/marginalized food crops for their nutritional and health outcome among children in SSA.

The ultimate goal is to identify possible evidences from the existed findings that can be pursued (pave the way) for further research in using indigenous underutilized nutrient-rich plant foods for enriching traditional staples to address child malnutrition in SSA. The main questions under the review include: To what extent have underutilized pant foods been used in enriching traditional staple diets? What nutritional potential/enhancements do the underutilized plant foods have in complementing essential macro and micronutrients in complementary food products? What processing techniques were applied to improve the nutritional values of the final products? To address these questions, this scoping review targeted to research done on enrichments of traditional complementary foods using underutilized plant foods where the traditional stable diets commonly used in SSA include: maize, sorghum, wheat, millet, rice, teff, oats, cassava and sweet potato.

Methods

Details of the searching process of articles from the known databases, criteria for inclusion and exclusion of the retrieved papers were well described in **Chapter 2.**

Results

From the online database search, 262 articles **(Figure 2.2)** were retrieved in November 2022. After removing duplicates 253 records were screened based on their titles and abstracts. Most of the articles retrieved did not meet the inclusion criteria and 121 records were excluded based on title (42 did not use the traditional staple diet of our interest, 56 records where commercial fortificants were added to the traditional complementary foods as an ingredient and 23 studies were out of SSA) while 106 studies were excluded based on the abstract review (27 not used traditional staple diet of our interest, 44 records where no food processing techniques were applied and 23 records were out of SSA) and in 12 studies there were no comparators or control samples. Totally 236 studies were excluded and 26 articles met our inclusion criteria with full-text publications **(Figure 2.2)** that aligned with the title of our scoping review; nutritional enrichment of traditional complementary foods using underutilized plant foods in SSA.

A summary of the nutritional enrichment of traditional complementary foods using underutilized plant foods and the main staple traditional ingredients used is presented in **Table 3.1**. The table shows that the main staple traditional ingredients used in the production of enriched complementary food products were maize, sorghum, wheat, millet, rice, teff, oats and cassava with the corresponding underutilized plant foods used for enrichments, the resultant nutritional enhancements and the common processing methods applied. The final included studies were from Nigeria (n=12), Uganda (n=2), Ethiopia (n=5), Ghana (n=5), Kenya (n=1) and Benin (n=1) which could best represent the SSA region.

Table 3.1: Nutritional enrichment of traditional complementary foods using underutilized plant foods in SSA

Traditional staple foods	Underutilized plant foods for enrichment	Common processing methods applied	Nutritional enhancements/benefits	Country	References
Maize	Amaranth	Roasting	Increased protein, iron, calcium and zinc	Kenya	[142]
	Amaranth, Chickpea	Soaking, germinated	Improved iron, zinc, calcium, protein and fat	Ethiopia	[143]
	Baobab fruit pulp, Moringa Oleifera	Soaking, fermentation	Increase calcium and iron contents of the meal	Nigeria	[144]
	Moringa oleifera	Germination combined with fermentation	Improved macro and micro compositions, phosphorus, magnesium, copper, iron, iodine, manganese and zinc potassium and calcium and beta-carotene levels	Ghana	[145]
	Soybean, peanut, Moringa oleifera leaf	Soaking and roasting	Increased in protein and micronutrient	Nigeria	[146]
	Banana, soybean	Drying	Improved protein, carbohydrate potassium and sodium appreciable levels of iron, zinc, and manganese.	Ghana	[147]
	Soybean, OFSP	Roasting	Improve the β-carotene or vitamin A content	Ghana	[148]
Sorghum	Amaranth	Roasting	Improved protein, micronutrients	Nigeria	[149]
	Soybean,	Malting, blanching	Improved protein, fat, energy, calcium, potassium, sodium, iron and zinc contents.	Nigeria	[150]
		Roasting, fermentation	Improved the protein content and consumer acceptability		[151]

	African yam bean			Nigeria	
Cassava	Pigeon pea flour	Boiling, Drying	Protein and ash contents were improved	Nigeria	[152]
	Soybean, melon seed, and Moringa oleifera	Roasting	Protein, fat, and ash increased	Nigeria	[153]
	Bambara ground nut	Soaking, boiling	Enhanced protein and fat contents, and energy content increased in the composite flour	Nigeria	[154]
Sweet Potato	Bambara groundnuts	Extrusion cooking	Increased magnesium, phosphorous, potassium, and iron	Uganda	[155]
	Moringa oleifera	Germination	Improved the lycopene, beta-carotene, protein, and fat contents	Nigeria	[156]
	Cowpea, peanut	Drying, milling	Improved protein, fat, and functional properties	Uganda	[157]
Millet	Soybean, sorghum, sesame	Germination	Improved in protein, manganese, phosphorous, iron vit-K, calcium and B-vitamins	Nigeria	[158]
Rice	Baobab fruit pulp, Moringa leaf	Cooking	Improved calcium, iron, and zinc	Benin	[159]
	Moringa oleifera leaf	Drying. Milling	Better mineral, contents, Zn, Mn, and Fe and high β-carotene levels	Ghana	[160]
	Soy bean, groundnut	Soaking, dehulling	Improved the mineral, protein content, and dietary fibre content	Nigeria	[[161]
	Quinoa, lupine, amaranth	Roasting	Improved nutritional content in terms of protein and mineral composition	Ghana	[162]

Wheat	Orange-fleshed sweet potato	Washing, milling	Increased ash, fibre β-carotene, and quality of the bread	Ethiopia	[163]
	Groundnut, mango, and spinach	Defatting	Improved protein, fats and micronutrients	Nigeria	[164]
Oats	Soybean, linseed and premix	Extrusion cooking	Improved protein, fat, ash, iron, calcium and β-carotene of the mix	Ethiopia	[165]
Red teff	Soybean, papaya fruit	Malting	Improved protein, fat and energy, mineral and β-carotene	Ethiopia	[166]
Teff-maize	Pea, sesame, spinach	Germination, dehilling, roasting			
Teff-wheat	Bean, sesame, spinach	Germination, dehilling, roasting	Improved protein, energy, fat, iron, calcium and magnesium contents	Ethiopia	[167]
Teff-barley	Chikpea, sesame, spinach	Germination, dehilling, roasting			
Teff-Oats	Soyabean, sesame, spinach	Germination, dehilling, roasting			

Discussion

Staple food ingredients are normally foods, which are eaten regularly to supply the body with energy and nutrients. The majority of staple ingredients frequently used for the production of traditional complementary foods in SSA are starchy foods which are used by several formulations as the main source of carbohydrates. These starchy foods are on average comprised of 46% cereals, 20% root and tuber, and a small amount around 7% of animal products [51]. The major cereals include maize, sorghum, wheat, millet, rice, teff, oats, barley and finger millet whereas the root and tuber crops comprise cassava, yam, sweet potato and taro. Amongst cereals used in the region, maize is the predominant one, while cassava is the favored tuber crop [130].
According to FAO/WHO [130], it is recommended that complementary foods should mainly contain good sources of carbohydrates, proteins, minerals and vitamins to meet the nutritional requirements by children. However, the consumption of starchy foods by children and another segment of the population in SSA does not provide the total nutritional needs and the majority of children suffer from malnutrition, especially micronutrient deficiency, “the hidden hunger” and related diseases. Therefore, to alleviate these challenges, there is a need to search for and incorporate inexpensive, locally available nutrient-rich underutilized plant foods that can complement and enrich complementary products prepared using the staple food ingredients.

Legumes have been mainly used as the source of proteins by various formulators of complementary products, notably soybean (*Glycine max*), [168], cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogaea*) [169], and several varieties of beans (kidney, red, Fava) (*Phaseolus vulgaris*) [91, 170]. Some leguminous plants, which are also known to be locally available and require simple processing (like voandzou [*Vigna subterranean*] and Bambara groundnut [*Vigna subterranea*]), are also been used in complementary food formulation, as they contain high proteins and low in anti-nutritional factors such as phytates and tannins [169]. Fishes and other seafood as the source of protein have been used scarcely [170, 171] and this can be justified by the fact that this class of proteins is generally not affordable for families with low income.
Since fruits like papaw (*Carica papaya*) and mango (*Mangifera olifera*), vegetables like carrot (*Daucus carota*) and pumpkin (*Cucurbita pepo*), and leafy vegetables like moringa (*Moringa*

oleifera), baobab (*Adansonia digitata*), spinach (*Spinacia oleracea*]) and orange-flesh sweet potatoes [*Ipomea batatas*] are rich sources in minerals (iron, calcium, magnesium, etc.) and provitamin A (α and β - carotene, β - cryptoxanthin), they have been used to fight iron and vitamin A deficiencies [146, 172].

Recently, cereals and pseudo-cereals such as teff (*Eragrostis teff)*, finger millet (*Eleucine coracana*), buckwheat *(Fagopyrum esculentum)*, quinoa *(Chenopodium quinoa)* and amaranth (*Amaranthus caudatus)* are getting attention to be included in complementary food formulations for their excellent nutritional value. They contain considerable amounts of iron, calcium, zinc, magnesium, etc. contents, are gluten-free, and are good sources to prevent diseases like hyperlipidemia, osteoporosis and celiac diseases [173]. Considering teff in particular, Ebba [174] has reported that there are 34 different varieties/cultivars of teff identified in Ethiopia based on their morphological characteristics. Daba [27] has mentioned that 32 varieties of teff have been and are being researched by the teff research program of the Ethiopian Institute of Agricultural Research so far. These researchers are mainly focused on morphology, yield and genetic improvements, yet their nutritional potential and health benefits are largely untapped. *Dabi teff*, a typical farmer variety teff (landrace), is one of the teff varieties marginally cultivated in western Ethiopia, where there are many social beliefs and health benefits linked to it among the community and its nutritional potential to be incorporated into staple traditional complementary foods to enhance the nutritional values of the resultant product is not yet researched.

Though staple food ingredients and nutritious underutilized plant foods can complement each other to provide nutritionally wholesome complementary products, the bioavailability of some key micronutrients and protein digestibility might be limited due to the anti-nutrients present in these foods. Some of the anti-nutrients of concern in complementary foods include; trypsin inhibitors, phytates, tannins, oxalates, cyanogenic glycosides, oligosaccharides and fibers which are naturally occurring constituents majorly in cereals and legumes that antagonize the absorption of one or more key nutrients.

Trypsin inhibitors reduce the digestibility of proteins through the inhibition of digestive enzymes (e.g. trypsin to digest protein), phytates and tannins inhibit iron, calcium and zinc absorption

through non-digestible insoluble complex formation [175] while fibers inhibit the absorption of vitamin A and reduce the bioavailability of these nutrients to the human body. [89]. As a result of these deleterious effects, common processing techniques need to be applied to each ingredient before the formulation of complementary foods to eliminate or reduce the ant-nutritional factors below the acceptable critical limits to guarantee the in-vivo (efficacy) absorption of the nutrients. The most common and globally accepted processing techniques that have been applied and effective by researchers in SSA to reduce the effects of anti-nutrients in the formulation of complementary foods include; soaking, germination, fermentation, roasting, dehulling, blanching and steaming [168, 169, 172] **(Table 3.1)**. These processing techniques were justified to be effective in reducing the deleterious effects of anti-nutrients and increasing the nutrient density and bioavailability of key micronutrients as well as boosting sensory qualities of food products [91]. For example, very high trypsin inhibitory activity in dry kidney beans reduced progressively by 51% after soaking for 12 hours, while tannins and phytic acids reduced to 46% and 36%, respectively [176].

Many other researchers have reported that the processing techniques have considerably reduced the levels of anti-nutrients below the critical [168, 169, 172]. From these findings then researchers recommend the application of proper pre-processing on each ingredient; both the staple food ingredients and the underutilized plant foods for efficient utilization of their nutritional contents. The summary of common pre-processing techniques applied to staple food ingredients in SSA is presented in **Table 3.1** together with the underutilized plant foods. Thus, the revealed nutritional enhancement reported by the researchers in this table was the combined effect of incorporating the underutilized plant foods and the processing techniques applied, usually meant to reduce the anti-nutritional factors. Most of the processing techniques are very common and are traditionally applied among most households in the SSA region.

Soaking has been an indigenous technique mainly applied on cereals and legumes for softening and faster cooking. It is widely applied at both household and industrial scales at varying soaking times usually from 8 h to 72 h at ambient temperature [168, 169]. It is the most important pre-operation in the germination or fermentation process of cereals. During cereals soaking for food making, a natural fermentative acidification takes place and this is regarded as important for food

quality. Soaking cereal or legumes improves the bioavailability of amino acids (especially lysine, niacin, and thiamine) in edible grains [177]. It also results in the passive diffusion of water-soluble phytates and oligosaccharides which can then be removed by decanting the water [178]. Soaking also removes other anti-nutritive factors, such as saponins, trypsin inhibitors, and polyphenols. Oversoaking may lead to the decrease of some nutrients. Studies have shown that soaking of soybean for 72 h resulted in a decrease of protein and soaking of Bambara groundnut for 4 h leads to a decrease of minerals [168, 169]. This shows that soaking conditions need to be controlled/optimized for the maximal reduction of anti-nutrients at the same time minimizing the loss of nutrients.

Roasting is a thermal processing that reduces microorganisms, destroys insects, and inactivates trypsin enzyme inhibitors, thus improving protein digestibility, and amino acids bioavailability, improving the keeping (storage) and nutritional quality of food products [177]. Additionally, roasting improves the sensory qualities where it enhances the flavor and taste of food products through starch dextrinization [28]. Many reports also show that during roasting total phenols and tannins were decreased [179]. But at higher temperatures, protein would be denatured [180] and produces undesirable flavors and darkened colors due to heat-enhanced chemical reactions (both in cereals and legumes) due to which the roasting process has to be well controlled/optimized (time and temperature control) [181].

Germination is an indigenous technique practiced in many African countries. Germination or malting is a bioprocess technology that enhances the nutritive value of cereals and legumes through the formation of enzymes that eliminate or reduce the anti-nutritional and indigestible factors in legumes [182]. The process might, in particular, increase protein digestibility and enhance the absorption and bioavailability of micronutrients, including vitamins such as riboflavin (B2), pyridoxine (B6), vitamin C, vitamin E, and antioxidants (21). Additionally, germination helps in the degradation of the phytate-mineral complex and polyphenol-protein complex and also decreases condensed tannin, thus facilitating mineral and protein absorption. Furthermore, the enzyme α-amylase synthesized during germination hydrolyzes starch to dextrin (dextrinization) and maltose (referred to us the amylase-rich food technology) and reduces its water-holding capacity, thereby reducing the viscosity of thick cereal-legume porridges without

dilution with water while simultaneously enhancing or maintaining their energy and nutrient densities for infants and young children who have limited gastric capacity [28, 52].

Fermentation has been mainly performed on cereals and tubers which are used as sources of carbohydrates in the formulation of complementary foods. Fermentation constitutes an important process that helps to lower the content of anti-nutrients (phytates, tannins, and polyphenols) of cereal grains and increase the nutritional composition [183, 184]. The process activates several endogenous starches hydrolyzing enzymes such as alpha-amylase and maltase which degrade starch into maltodextrins and simple sugars [183], thus improving the digestibility of starch as well as the bioavailability of some minerals like magnesium, phosphorus, calcium and iron [172]. Moreover, during fermentation with microorganisms like *Lactobacillus, Leuconostoc* and *Lactococcus*, there is the production of flavoring components such as lactate, propionate, butyrate acetate, 1- propanol, isoamyl acetate, ethyl acetate, 3- methyl-1-butanol and acetone, which improve the sensory acceptability of the resulting food products. This shows fermentation improves organoleptic properties as well as extends the shelf life of food products [185].

Dehulling is removing the fibrous outer layers of grains. The dehulling reduces the levels of anti-nutrients in cereals and legumes. These reductions in anti-nutrients will improve the bioavailability of minerals. Thus, dehulling is a viable means of improving the nutritional quality of cereals and legumes that have anti-nutritive factors in the seed coat [186].

As described, the common processing techniques could effectively reduce the anti-nutrients (phytates, tannins, oxalates and protein inhibitors) content of cereals and legume foods used in the formulation of complementary foods, thus leading to the improvement in the digestibility of proteins, improving the bioavailability of key micronutrients and improvement of the palatability of food matrix (roasting and fermentation). However, some processing such as soaking and germination might lead to losses of nutrients by leaching. Thus, it is worth noting that optimum processing conditions which can lead to maximum reduction of anti-nutrients and minimum losses/damage of key nutrients such as proteins and minerals need to be determined. Yet, not only the processing methods but also the mixing ratio need to be optimized because it is associated with sensory acceptability. Thus, there needs to be a controlled processing condition

to obtain maximum nutrients and acceptability and this can be achieved through counseling/teaching the community, particularly mothers/caretakers for its utilization at the household level. Moreover, the study of the effect of combined processing techniques on the nutritional quality of foods might be another interesting area of future research.

Food fortification or enrichment is defined as the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups. WHO defines food fortification as the practice of deliberately increasing the content of an essential micronutrient (vitamins and minerals) in food, to improve the nutritional quality of the food and provide a public health benefit with minimal risk to health [83].

Nutritional enrichments would be effective if traditional staple foods were used as bases to add the required micronutrients that are generally lacking or not available in sufficient quantities in consumers' diets. This approach has been practiced since the early days of food enrichments to target specific health conditions such as iodine deficiency through the iodization of salt, anemia through the fortification of cereals with iron and vitamins, vitamin A-fortified margarine, and neural tube defects through the fortification of wheat flour with folic acid [26].

However, in SSA, the best alternative way of food enrichment could be the incorporation of nutrient-rich underutilized plant foods into staple foods. Food-to-food fortification or enrichment of traditional foods using underutilized nutrient-rich plant foods would be more sustainable and an option to alleviate nutrient deficiencies in SSA, because there are traditional practices of incorporating nutritious foods into staple foods by indigenous people in the region and some authors, have reported the health benefits of these underutilized plant foods [187, 188].

In this review, the discussion of enriching traditional staple foods using indigenous underutilized plant foods is more focused on the most commonly used plant foods in SSA. In this regard, underutilized nutritious plant foods including amaranth, moringa oleifera, Bambara groundnut, baobab fruit pulp, orange-fleshed sweet potato, quinoa, lupine, teff and spinach **(Table 3.1)** were the target of interest in the studies included in this review. The reason why more attention was

given to these underutilized plant foods was that they are proven to be potentially nutrient-rich, particularly higher micronutrients and there are social beliefs linked to the health benefits of these foods in the community in SSA. The enrichment of traditional complementary foods by incorporating such plant foods is a novel approach in formulating new nutritious food products. Therefore, the subsequent discussions have made its center of summarizing this review based on these plant foods and presenting them one by one in each paragraph.

Nutritional enrichment of traditional complementary foods using the underutilized nutrient-dense plant foods mentioned and others could provide a huge benefit in combating malnutrition challenges. Numerous researchers have attempted to prove the nutritional potential of these crops which is shown in **Table 3.1.** Firstly, maize, a predominant staple food in SSA would be a major crop to be enriched for nutritional enhancement of the resulting final product. Kamotho *et al*. [142] developed enriched maize flour with grain amaranth flour and revealed that it enhanced the protein, iron, calcium, and zinc contents of the final flour significantly and concluded that 40% grain amaranth gave the best result. This shows that using grain amaranth could provide a cheap and effective means of reducing hidden hunger such as iron and zinc deficiencies, particularly among children. Another study by Zebdewos *et al.* [143] developed complementary food from maize, check pea and amaranth and the result has shown an increase in iron, zinc, and calcium; protein and fat; and a low phytate level compared with the control.

Moringa oleifera leaf powder was used to enrich various dishes like complementary porridges, sauce and groundnut soup and it increased the levels of key micronutrients like iron, magnisum, zinc, cipper and beta-carotene content of the diets. Moreover, the enriched diets were preferred by children and could be used as a means for the utilization of micronutrients present in Moringa oleifera by children [145]. Shiriki *et al*. [146] developed a complementary food from the blend of maize, soybean and Moringa oleifera leaves. Proximate composition, energy, mineral and β-carotene flour of the blends were assessed. The report shows that the protein and carbohydrate of the blends differed, but the ash, fat, crude fiber and energy of both blends were comparable. The mineral content of the blend was higher than that of the control.

Adejuyitan *et al*. [144] also studied the proximate composition of moringa fortified with yellow maize to obtain a fermented gruel from “ogi”. The moringa leaf was processed into powder and

was substituted with yellow maize flour to produce “ogi”. The report shows that the enrichment of “ogi” with moringa leaves at 10% substitution enhanced the nutritional quality of “ogi”. There are many more studies using moringa enriched products some of which are also presented in **Table 3.1** above.

Enrichment of sorghum grain with amaranth has also shown improvements in the general macro- and micronutrient contents of the final product [149]. Furthermore, composite flour made from local maize, banana, and soybean had high levels of potassium and sodium and appreciable levels of iron, zinc, and manganese. Physicochemical analyses showed total carbohydrate was relatively high in all the composite flours with a substantial amount of crude protein. The proximate results and mineral content made the banana composite flour an ideal product for weaning babies and infants [189].

Soybean, a crop shown to contain higher protein has widely been used as a functional ingredient in complementary food enrichment [190]. The effect of soybean flour enrichment on the chemical and physicochemical properties of malted sorghum flour was investigated [150], and the results have shown that the protein content of the flour mixes increased from 7.3% to 19.2%. The blend containing 40% soybean flour substitution had the highest protein, fat, ash, and fiber content. The mineral content increased while the anti-nutritional factors decreased as the soy flour substitution increased in the mixes. The functional properties of the flour decreased while the pasting viscosity and pH increased [150], this further provides great evidence for using underutilized plant foods for enriching traditional complementary foods with great nutritional potential.

Bambara groundnut is one of the underutilized plant foods typically used for the enrichment of traditional staple crops in SSA with positive results. Sweet potato, another major staple food in SSA was enriched with Bambara Groundnut in substitution with orange-fleshed sweet potato (OFSP) in the formulation of a snack increasing the magnesium, phosphorous, potassium, and iron contents in the composite snacks. The study showed that the development of OFSP substituted with Bambara groundnut up to 40% enhances the nutritional quality of the products and retains sensory properties with an acceptable consumer score, and with color and sweetness being the main drivers [150]. The sweet potato was also enriched with cowpea and peanut at a

percentage of 25% and 15%, respectively, and it provided a nutritionally dense complementary product for infant weaning, and subsequently, the functional properties of the flour were improved. Ijarotimi and Keshinro [108] formulated a complementary food from the mixture of fermented popcorn, African locust bean and Bambara groundnut seed flour and the report shows that the formulated samples were sources of high-quality protein of almost adequate or more than adequate essential amino acids and energy values. Hence, the formulated complementary foods are better than the traditional ones, namely "ogi", and comparable to commercial complementary food such as Cerelac in terms of nutrient composition.

The enrichment of cereal-legume-based traditional complementary food with orange-fleshed sweet potato (OFSP) was studied [148]. It was concluded that OFSP flour has the potential to be used at 25% replacement level in the soy-enriched roasted maize meal formulation, and OFSP is a useful ingredient with the potential to improve the β-carotene or vitamin A content. This would help alleviate vitamin A deficiency of children in Ghana and other countries with similar problems.

Cassava is one of the most consumed staple diets in SSA. It contributes substantial amounts of energy, carbohydrates, minerals (iron and calcium) and vitamins (A and C) through consumption of both its roots and leaves whereas protein is the most limiting nutrient in cassava root. Studies were conducted to correct the protein and the other deficit nutrients in cassava using underutilized plant foods. Aniedu and Aniedu [151] have conducted enrichment of cassava root flour with African yam bean (toasted, fermented or untreated) at a level of 5%, 10% and 20% and it was reported that the protein content of the composite flour was appreciably increased. It was also shown that the protein content of the composite kept increasing as the mixing ratio of the African yam bean kept increased. The study concluded that there was a significant increase in protein content after the enrichment of cassava root flour with the African yam and the study recommended the use of such product to increase protein intake to combat protein-energy malnutrition prevalence in most cassava-consuming areas of Nigeria. Another study conducted by Ezinwanyi and Ndaeyo [153] on the enrichment of cassava with soybean, melon seed and moringa seed flours at 5% substitution level to produce a cassava-based product called "gari". The study concluded that it led to significant improvements in nutritive values of the final "gari"

products with increased in protein, fat, and ash content and the study recommended the final developed product can thus present a viable and sustainable means of tackling nutrient deficiencies, particularly protein energy malnutrition Nigerian population.

Enrichment of teff-maize-based, teff-wheat-based, teff-barley-based, and teff-oats based complementary foods with pea, bean, chickpea, soybean, sesame and spinach was studied in Ethiopia [167] and it was concluded that blending of locally available cereal-legumes enriched with sesame and spinach in the formulation of complementary food with the application traditional technologies such as soaking, dehulling, germination, and roasting resulted in a significant improvement of the nutritional quality of the developed formulas. The study revealed that mixing 40 teff, 30% maize, 20% pea, 5% sesame, and 5% spinach; 40 teff, 30% wheat, 20% bean, 5% sesame, and 5% spinach; 40 teff, 30% barley, 20% chickpea, 5% sesame, and 5% spinach; 40 teff, 30% oats, 20% soybean, 5% sesame, and 5% spinach resulted in nutritional enhancement of protein, energy and problem micronutrients such as iron, zinc, vitamin A and water-soluble B-vitamins. The studies further revealed that the formulated diets were better than the comparator diet (the Cerifam faffa) and met the recommended levels for the entire nutrients and recommended that the formulated diets have a huge positive impact to be used as complementary foods in a poor rural and urban setting to alleviate child malnutrition.

In summary, **Table 3.1** shows the results of enriching major traditional staple foods with typical underutilized plant foods with common processing techniques applied in SSA and their nutritional potential and health benefits. Though not narrated in detail in this review, most of the researchers who looked into the effect of blending ratio have reported that, as the ratio of the underutilized plant foods used increased, the nutrients under investigation have also often increased. It is therefore recommended that such important review findings need to be included in nutritional policies and programs, supported by nutrition education that could lead to a sustainable food-based strategy to enrich traditional complementary foods with key nutrients in reducing child malnutrition in SSA. Increased awareness of the importance of food composition in human health with an intensified consumer's interest in nutritious plant-based foods could bring behavioral change in the community to utilize such plant foods.

Conclusion

There are numerous underutilized plant foods in SSA with proven nutritional potential and promising health benefits to combat food and nutrition insecurity in rural households, enrich and diversify diets and reduce malnutrition at the grass-root level. More importantly, the nutritional enrichment of traditional complementary foods using underutilized plant foods has proven useful for enhancing the nutritional value of most staple starchy foods in the region. This review also showed evidence that the application of common processing technologies on complementary food ingredients has reduced the anti-nutrients and enhanced the nutritional value and bioavailability of key micronutrients in the final products. Thus, it is worth recommending identifying, processing, and incorporating the underutilized nutritious food crops into the major staple crops such as maize, wheat, millet, sorghum, teff, rice, oats cassava, sweet potato and others to provide more nutritious home-based complementary products to address child malnutrition challenges in rural communities in SSA. Furthermore, this review provides evidence-based information of enriching staple foods to address one of the components of the Ethiopian national nutrition strategy that states the need for ‘micronutrient fortification or enrichments’ particularly for children, pregnant and lactating mothers. The exceptional proven nutritional potential and promising health benefits of underutilized plant foods indicated that increased promotion, domestication, commercialization and consumption of these plants should be strongly supported. Interventions based on underutilized plant foods could be cost-effective investments in combating child malnutrition and ensuring their optimal growth and development. Thus, the efficacy (feeding trial) of enriching complementary foods with underutilized plant foods needs to be studied to prove its direct potential in reducing child undernutrition and hidden hunger to verify the real impact of the enriched products on nutritional outcomes. Further, the safety and acceptability of the enriched product need to be investigated.

4

Chapter 4:

Ethnographic Qualitative Study to Uncover the Traditional Usage, Social Beliefs and Cultivation trends of *Dabi Teff* (*Eragrostis teff*), A Typical Farmer Variety Teff, Grown in Western Ethiopia

(Submitted)

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Abstract

The rural Ethiopian populations have a rich traditional knowledge and beliefs concerning the use of cultivated and wild plants as a food and medicine. However, most traditional knowledge and practices are fragile. This study aimed to explore and document the traditional usage, social beliefs and cultivation trends of *dabi teff* (*Eragrostis teff*) grown in Western Ethiopia: including its usage as a complementary food. An exploratory ethnographic qualitative study design was employed through focus group discussions and key informants in-depth interviews using purposively selected elder discussants and knowledgeable community members. A semi-structured questionnaire was used to collect primary data. Data were analyzed manually where the information collected was transcribed verbatim, reviewed, through read and internalized, color-coded, categorized and synthesized into themes, translated and reported. Three major themes have emerged. The first theme was traditional usage. The participants had life-long traditional usages of preparing and enjoining a number food forms from *dabi teff*. Most of the participants said that the use of *dabi teff* as a complementary food is not common. The second theme was social beliefs. It was claimed that the crop has strong nutritional potential and it is prized as a medicinal food (nutraceutical). All the participants argued that, after eating the different food forms, particularly the "mooqa manyee" (gruel), a recipe prepared from *dabi teff* flour mixed with bulls hooves; it increases blood volume, boosts energy/strength, and repairs/strengthens the backbone and fractured bones. Serving "mooqa manyee" to a recently delivered mother results in fast recovery from her backbone pain and weight gain while serving to a bone fractured person, he/she would be healed fast and the participants praised the crop using a quote, 'if we have *dabi teff* at home, we feel like we have a medicine at home'. Yet it was told that its cultivation is declining than the past. The findings showed that there are profound traditional usages and social beliefs linked to *dabi teff* among the communities in Western Ethiopia. It was suggested that the traditional practices linked to the crop need to be incubated/preserved for its nutritional and health benefits.

Keywords: *Dabi teff*, traditional usage, social belief, cultivation trend, medicinal food

Introduction

Plants are the most important sources of food for human beings mainly due to their availability and low cost compared to animal sources. The importance of plants to man, directly or indirectly is demonstrated by the useful products from both cultivated and wild species related to their numerous uses as sources of energy, nutritional values, medicinal values, structural and many other co-products [191]. Among plant foods, cereal crops occupy a remarkable and central position in ensuring food and nutrition security due to their high worldwide production, containing considerable amounts of energy, proteins, carbohydrates, minerals, vitamins, essential fatty acids, dietary fiber and certain hormone precursors [192].

Despite such a substantial contribution to food and nutrition security, having medicinal value and sources of income generation, many plant foods across the world are underutilized and/or forgotten [96]. The sub-Saharan African region is endowed with a rich diversity of such plant foods, where little or no attention was given in terms of research and development and policy framework that could promote their extensive agricultural production, marketing, industrial utilization as well as home consumption [96].

The rural populations in Ethiopia have a rich knowledge concerning the use of cultivated and wild plants as food and medicinal plants [97, 98] and this type of indigenous knowledge is applied to preserve plant foods as a means of survival during times of food shortage in fighting against food insecurity challenges. Using traditional plant foods is still very common especially in rural areas of developing countries including Ethiopia because there are elders and other knowledgeable community members who are the key sources of plant lore [98]. However, this indigenous knowledge among the rural communities is affected by fragile traditional skills (older people replaced by younger) that are likely to be lost when people immigrate to towns or to other regions; can be lost by lifestyle changes, the Westernization or industrialization, when the young people ignore to practice and pass down the traditional practices in their community [97]. Therefore, local and indigenous knowledge should be valued as a vital instrument in future policies for agriculture and food security and should be carefully considered and documented, when possible with the cooperation of indigenous and local populations.

Teff (*Eragrostis teff*) is an ancient tropical super cereal food where both its origin and a major center of diversity is in Ethiopia [193], and it was first domesticated between 4000-1000 BC [194]. Teff grain was reported to be discovered in a pyramid which is thought to date back to 3359 BC [195]. According to the report by Ebba [174], there are 34 different varieties/cultivars of teff identified in Ethiopia based on their morphological characteristics.

Teff is an important part of Ethiopia's cultural heritage and national identity. Nowadays teff's international popularity is rapidly growing and it was labeled as one of the latest *superfoods* of the 21st century [27]. The use of teff grain as foodstuff is already well known in some parts of the world like the Netherlands, North America, Australia, Canada, South Africa and Kenya where its cultivation trial has shown a wide range of adaptations [103, 174, 195]. Seeking to ensure food security for the Ethiopian population and to protect local markets from inflation or to keep domestic prices low, the export of non-processed teff has been banned since 2006 [196]. Due to this, teff is mostly traded domestically where more than 70% of the Ethiopian population use teff as a traditional staple meal [103].

Teff is primarily cultivated and used as a staple food in the western, northern and central parts of Ethiopia and it is the dominant cereal crop in terms of production. Until recently, 99% of the global teff production took place in Ethiopia, making the teff value chain localizable [27]. The common vernacular name of the crop is derived from the Amharic word "teffa" which means "lost" due to the small size of the grain and how easily it is lost if dropped, *Taafi* (Oromifa) and *Taf* (Tigrigna) which are the main language of the Ethiopian people [195]. Teff grows in both drought-stressed and water-logged soil conditions and it has a short growing season with low water/rainfall of (450–550 mm) requirements and a temperature range of 10–27°C [174, 193].

Over the past two decades, teff has become an issue of agronomic, nutritional, food technological, microbiological, chemical and physical research programs [195]. It is highly nutritious with its grain protein content (10-12%) similar to other cereals [27]. Teff protein is compared with egg protein and with an ideal protein for children between two to five years old. A lot of scholars have also demonstrated that teff starch has a low glycemic index (decreases the

incidence of diabetes), the reason why this microscopic grain is initiating a big war between different grain producers and processors [195].

Besides providing protein and calories, teff is a high source of minerals, such as calcium, zinc, magnesium, phosphorous, copper, aluminum, barium, thiamine and particularly iron [197], which are the most limiting minerals in developing countries. The content of iron and calcium in teff is higher as compared to other cereals such as wheat, barley and oats [197]. It has an excellent amino acid composition, with lysine levels higher than wheat or barley [197]. Teff is also a gluten-free crop and is gaining popularity as an alternative grain for people with gluten sensitivity called celiac disease [198] and this qualifies the crop as a nutrceutical food. Teff has many health benefits where most Ethiopians hardly suffer from diseases like anemia, osteoporosis, diabetes and celiac disease [199]. Presently, the only effective treatment for celiac disease is a lifelong gluten-free diet [195]. Teff may help in maintaining glycemic control and can be used for the management of diabetic diseases because teff consumption does no result in the rise of blood glucose levels because of its low glycemic index [200].

Dabi teff, a typical farmer variety (landrace), is one of the teff varieties marginally cultivated or underutilized/forgotten in Western Ethiopia. *Dabi teff* is the 'afaan oromoo' language name for an early maturing variety of dark red teff. Farmers in Wollega and Illuababor, western Ethiopia, cultivate *dabi teff* either for its grain seeds or its straw. The contribution of *dabi teff* grains to household food and nutrition security is considerable when viewed through a food security lens. *Dabi teff* can grow under low rainfall requirements and this property together with an early maturing, makes the crop a unique variety teff amenable to climate-smart agriculture.

The key underlying cause of child malnutrition among households with low socio-economic status is a lack of purchasing power of quality foods for their children and they consume foods of low nutritive value. Price-wise, the red teff varieties grown in Ethiopia including *dabi teff* are the cheapest. For example [201], has reported that the market price of mixed teff and red treff were 24% and 55% less than the white teff. This statement specifies that the price of *dabi teff* is quite affordable for all socio-economic classes. In addition to lower prices, red teff varieties have been implicated in the low incidence of anemia in Ethiopia which is presumed to be due to the grain’s high iron content [103]. But paradoxically, according to the EPHI and the ICF [19] report, 57%

of under-five children in Ethiopia are anemic which leads to the suspicion that there are low or nil practices of using red teff as an ingredient in complementary food preparation. It is also reported that preschool children in Africa, including Ethiopia, have some of the highest rates of anemia in the world, nearly 56% [195].

There are many social beliefs regarding the nutritional and health benefits of *dabi teff* among rural elderly people in particular and the consumers in general where they prize it as a medicinal food (Personal communication). Thus, this qualitative study was aimed at exploring the traditional usage including the preparation schemes, the social beliefs, the cultivation trends, usage as complementary food and some other qualities of *dabi teff* grown in western Ethiopia. It mainly focuses on the preparation and consumption pattern of *dabi teff* as well as the rooted social belief linked to *dabi teff* in the community.

Methods

A detailed description of the study area and the selected Kebele's, study design, **s**ampling technique and study participants' selection procedure, data collection tools, tool validation and pilot testing and data collection procedure were all presented in **Chapter 2.**

Results

Background characteristics of the study participants

A total of fifty- four (54) participants were involved in this study. The FGD was comprised of 20 elder males and 18 elder females while the KIIs comprised of 10 males and 6 females. They represent a wide age range (32-63 years) and their educational status ranges from no formal education to secondary level and above **(Table 4.1)**.

Reviewing, through reading and internalization of the transcripts resulted in the emerging of three major themes that were synthesized into heading titles followed by short paragraphs and triangulated or substantiated with powerful quotes from the participants.

Table 4.1 Socio-demographic characteristics of the study participants (n=54), Nedjo district, Western Ethiopia, 2022.

Characteristics of participants	Categories	Frequency	Percent (%)
Age range	32-63	-	-
Sex	Male	30	55.56
	Female	24	44.44
Educational status	No formal education	20	37.04
	Primary	16	29.63
	Secondary and above	18	33.33
Occupational status	Employee	10	18.52
	Housewife/farmer	38	70.37
	Daily laborer	6	11.11

The major emerged themes were:

- Traditional usage including food preparation protocols/schemes
- Social beliefs linked to *dabi teff* in the community
- *Dabi teff* cultivation trends from the past to present

Traditional usage of *Dabi teff* including its food preparation schemes

According to the information and experiences generated from the study participants, it was understood that there is a very similar traditional usage of *dabi teff*. People in western Ethiopia are highly connected with the traditional usage of *dabi teff* where the participants had life-long practices of preparing and enjoining different forms favorite foods from *dabi teff* which are highly praised and 'liked very much' in the community.

All participants involved in the FGD stated,

"... many traditional food formss such as muke/mooqa (gruel), kita (sweet dry unleavened bread), cumboo, cafaqoo, cacabssa, cuukoo, unkuroo (the favorite cultural dishes), bread and injera/budena (pancake-like local bread) can be prepared from dabi teff…"

Further, the FGD participants described about the special food form called “mooqa manyee” (a gruel prepared from *dabi teff* and bulls hooves) that was also supported by the key informant's interviewers. A 62-year-old FGD female participant describe the preparation scheme of ‘mooqa manyee’ as,

“:.. “mooqa manyee” is a recipe prepared from bulls hooves and dabi teff. Firstly, the nails of the hooves would be removed using fire, then other unwanted parts like the skin would be cleaned and it would be chopped at its joint points. Then it would be cooked overnight where its meat and bones would be separated apart. A gruel from either dabi teff alone or mixed with oats and/or barley flour would be prepared separately and the cooked hooves will be added to it, and then mixed together while it is on fire adding other required ingredients (homemade spices like garlic and salt). The oromo people like to add what is called “amole chew” salt, not the table salt, no butter is added to it, it will be mixed in a wider big pan/dish, called “sataate”, after which it would be served to a family member as a ‘muke’ while it is hot…”.

The 'kita' *(sweet dry unleavened bread)* is usually consumed during breakfast and snack time. Fresh 'kita' of *dabi teff* has a distinctive flavor smell that people sense from a distance when passing by a house where 'kita' is being baked from *dabi teff* flour. One of the key informant interviewers, the DA said,

“… when I go in the village to visit farmers, I know where dabi teff kita is being prepared. It always causes me a mouth-watering stimulation to eat it because of the good flavor I sense…”.

Further, the participants described the preparation and consumption scheme of the food form, “Cafaqoo” prepared from *dabi teff*. One of the FGD discussants elaborated,

“… “Cafaqoo” is a small rounded bolus-like food, prepared by breaking fresh 'kita' into smaller pieces and mixing it with some amount of refined butter. During 'Cafaqoo' preparation, more salt is added than usual and it will be eaten more often as a dinner meal and it evokes one more water to drink. 'Cafaqo' is prepared with the addition of spices such as onion, garlic, black cumin and cardamom and was used as "sinqi" in a long time back when traders go a long distance for marketing (more than a week time walk on foot)...”

In addition to the different traditional food forms, home-brewed traditional alcoholic drinks could also be prepared from *dabi teff* and used as income generation. One of the 52 years of FGD female participants described,

"… I have been preparing alcoholic beverages such as tella (local opaque beer) and katikala (local spirit) by using dabi teff as an ingredient for a long period in my life and I have been earning money by selling it. The tella and katikala from dabi teff is even very strong…"

The development of nutritious complementary foods from locally available and forgotten/underutilized food crops has received a lot of attention in low-income countries. The participants were asked whether they use *dabi teff* for the preparation of child food and it was responded that, it is not common.

Most of the participants responded that,

"…the use of dabi teff as a complementary food is not common. We usually use other crops such as barley, maize, oats, and chickpeas for the preparation of complementary food for our children. We mostly use dabi teff for nutritional treatment of sick, delivered women and bone-fractured persons and we often reserve it for such events. But rarely some people might add as an ingredient for weaning food preparation…"

One Key informant interviewer suggested,

"… even though it is not common practice to prepare complementary food from dabi teff, there is no foreseen problem if it can be used as a complementary food, even since it is rich in iron content, it would be good food for the children's development, it would increase their blood volume…".

Social beliefs linked to *dabi teff* in the community

Almost all the participants shared common beliefs linked to *dabi teff* in the community. Most of the social beliefs were associated with the consumption of the different food forms that were described earlier. These beliefs were composed under energy value, medicinal value and workforce efficiency.

The beliefs or *the lived* experience associated to the consumption of "mooqa manyee" was described by one of the FGD participants where it was said,

"…a person who ate "mooqa manyee" became stronger and braver the next day. A male who has eaten such food form shouldn't approach a female for a sexual act because it is believed that he might harm her. So he shouldn't approach her by any means on the day he has eaten it. He can approach the next day. It is also used as a medicinal food...".

The medicinal values of "mooqa manyee"; were potentiated by one key informan by verifying its health values that result in positive health and nutritional outcomes when served to the member of the family who needs special nutritional treatments, the food therapy. One elder female in the FGD that was also supported by a key informant interviewer stated,

"… when "mooqa manyee" is served to a recently delivered mother, she would recover from her backbone pain shortly and fast weight gain (postnatal nutrition) and if served to a bone fractured person he/she would heal fast, it is also used for the nutritional treatment of sick people, the actual observed outcome …".

The beliefs were further supported by the female FGD participants, who said,

"… after eating the different food forms, particularly the "mooqa manyee" and "cafaqoo", it increases blood volume "dhiiga dabalaa," boosts energy/strength "humna dabalaa," and repairs/strengthens the backbone and fractured bones "dugda jabeessa…"

A 54-year female FGD participant raised the improvement of workforce efficiency following the consumption of "Mooqa manyee and Cafaqo" and she stated,

"… "Mooqa manyee and Cafaqoo" are usually prepared for males who are going to plough or till farm land because they will not get tired and/or feel hungry the whole day. It gives males more energy to work hard without tiring …"

Trends of *dabi teff* cultivation (from past to present)

There is an old popular tradition saying, "Daabiin angafa midhaaniiti", meaning *dabi teff* is a leading crop of all cereals, known among the Oromo people in the study area. All of the discussants (the FGD and key informants) have noted that they are very familiar with *dabi teff* and it has been cultivated in their area since ancient times. *Dabi teff* produce can either be used for home consumption or for earning money by selling it in the market. It is still commonly

cultivated by many farmers in the area and much praised among the community, but most of them are arguing that its cultivation is declining nowadays and they fear that its cultivation will be forgotten in the long run.

One of the male FGD participants stated that,

"… dabi teff is well known in our area and it is a historical crop that people's minds are associated with the golden quality of this crop. Mostly we use dabi teff grain for home consumption or sell it to earn money. He further stated that its cultivation pattern from past to present is a bit declining. He added, when we think of dabi teff cultivation, mostly its traditional and medicinal value is coming into our mind which is less considered among the younger people…"

Another FGD participant supported this idea and mentioned,

"... yes, dabi teff has been cultivated since ancient times and our community has a special opinion/perception for dabi teff, they just see it as a medicinal food and give special respect for the food forms prepared from it…"

Further, the participants from the key informants stated,

"… if we have dabi teff crop in our home, we feel like we have a medicine at home. Most of the time, it is reserved for "the time of problem" to be used as treating of a family member who needs special nutritional treatment such as by preparing its gruel and serving to delivered women and sick person…"

Contribution in addressing food security

Between June and early August, households in most part of Ethiopia are in a food crisis every year. All the participants (FGD and Key informants) have described that *dabi teff* reaches in a short time for harvest since its cultivation. Thus they appreciated the contribution of *dabi* teff in ensuring their food security, particularly during the summer season when there is food shortage in most households. One male FGD participant said,

"… due to the very short harvesting period of dabi teff, dabi teff is harvested twice within one rainy season (1st, during the onset of Kiremt rainfall called 'daabi gannoo' & 2nd late rain fall after mid-September called 'daabi birraa'. We usually call dabi teff 'Ji'a-Lame' to mean a crop reaches to harvest in two months as it usually reaches to harvest in 70-80 days…"

This early maturing and less rainfall requirement make *dabi* a unique variety teff amenable to climate-smart agriculture. One of the participants said,

"… in addition to cultivating dabi teff on the usual farm land by application of fertilizers, it can also be cultivable on irrigation land during the winter time…".

Thrashing system of *dabi teff*

Dabi teff threshing is carried by squashing over the cut crop collected on a flat surface and driving cattle (oxen) over it. It can also be thrashed by using a special wooden stick. A 63-year-old FGD participant stated,

"… before trashing dabi teff, we habitually prepare a flat surface in the appropriate place and seal it using cow dang. Women perform the sealing activity and they mix the cow dung with water (thick mixed) and it would be slowly dried so that the cow dang would get epoxied to the flat surface. He mentioned this is done to ensure that it would not get cracked and to prevent seed lost during the trashing. To avoid the seed from soil contamination or to prevent any soil from entering into the sealed floor, the women create a kind of embankment (dike) surrounding all the sealed floor, called 'timjjesssu in afaan oromoo'. After it gets dried well the women check it and clean the dried sealed floor surface using a cleaner called "dry harata" to make it a very clean surface with no cow dung debrits that can contaminate the seeds. The women do the cleaning in the late afternoon of the previous day to trash it in the late morning (from 10-11 o'clock) of the next day using wooden sticks…".

The trashing system was substantiated by another FGD participant who said,

"… to trash dabi teff, we use a special wooden stick which is prepared technically by bending one end of the wooden stick looking like a fork and when we beat, we would not beat directly down to the floor but it has its own beating fashion (inclined beating) that only expertise can perform it. This is particularly to avoid cracking of the sealed flour thereby avoiding soil contamination and seed loss.."..

The same idea was again supported by another key informant's interviewer mentioning,

"… in case any tresh grund floor cracking is observed, the women would re-seal it and it has to be waited for one day to till drying. Now time people have also started to use what we call 'Shara' that they place it on the flattened surface…" **(Figure 4.1).**

a) *Dabi teff* harvest

b) Trashing (photo by the researcher)

Figure 4.1. *Dabi teff* harvest and trashing system

Separation of the grain from its thrash (chaffs or *'galaba/chidd'*)

First, the straw would be removed using the beating stick and the separation of teff grain from its chaffs is carried out by throwing high in wind the trashed grain into the air following the difference in aerodynamic or air-bellowing properties. During the separation of the threshed materials, the fundamental forces involved are the weight/gravity of the seeds and the aerodynamic wind drag. One key informant interviewer stated,

"… to separate the thrashed material from the seed, we use a martial called sefed/gundoo **(Figure 4.2)***, a kind of fan woven prepared from grass stem. This operation is carried out by lifting the fan woven above our height or standing on a wooden footstool and letting it drop smoothly so that the blowing wind will separate the seeds from the chaff by following the wind direction* **(Figure 4.3)**. *The winnowing has its own technique in that we see the direction of the wind and we move around to adjust ourselves to the direction the wind below. If the wind speed is stronger we stop it for a while until the wind calms down and become moderate. Unless the*

seeds will be blown away because of its smaller weight. The remaining inert materials are removed by fanning with a material called Afarssa/Maragabiya (small sefed or stif dried cattle skin)…".

Another participant supported the same idea by stating,

"…If there is no wind bellowing at all, there is a common traditional practice that males whistle-out believing that wind would come and it usually happens (It is a kind of ordering nature (wind) to below obeying the whistle sound…".

Figure 4.2. Sefed or gundoo (photo by the researcher

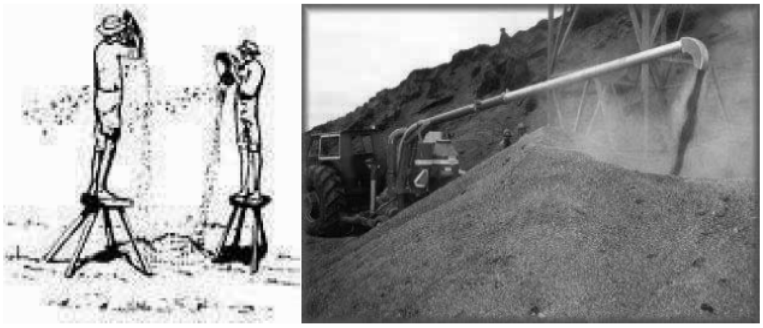

Figure 4.3. Small and medium level teff grain cleaning.

Source: [195] Available at: www.cd3wd.com/.../X0027S/ES/X0027S04.HTM

Storage quality of *dabi teff*

Dabi teff seed could stay for a long time after harvest if properly stored. If it is stored in a dry environment it can stay for a longer period without being damaged by pests and spoilage.

This was explained by a male FGD participant,

"… dabi teff grain has good storage quality that it will not be damaged by any pests and it can stay for many years, up to 4 to 5 years even more without damage if stored under normal conditions. They call such dabi teff "Daabi dilbii" to mean the dabi teff which was stored and stayed for long times after harvest. He stated that such type is more preferred for 'Muk' (gruel) preparation…".

Contribution in house construction

The discussants were further probed for any other use of *dabi teff* and all the participants (FGD and key informants) expressed that on top of its seed harvest for consumption and medicinal importance, the trashed straw is composed of fine stems used for plastering (finishing) mud hut walls, mud silo making and contributes for house construction.

Most FGD participants said,

"… when we have planned to construct a house in the coming year, we cultivate dabi teff a head, carefully collect and reserve the straw of dabi teff for using it for plastering mud hut walls. This is because the straw is very fine and it cements the mud together preventing it from cracking…".

Contribution for animal forage

The participants, when they are expressing the additional values of *dabi teff*, they have stated the forage potential of *dabi teff* straw. After trashing, they accumulate the straw and store it in a safe dry place. Most of the discussant describes,

"… the value of dabi teff is not only the seed, but the straw is also very useful for animal feeding. We store the straw in a safe place till summer time comes to feed our cattle. It is specially used for feeding bulls or oxen after plowing or tilling farmland because it is believed that the oxen

would become strong. We also use it for feeding other animals like milking cows to provide more milk and horses…"

One male FGD participant stated the following substantiating this opinion,
"…. during past times, we also mix dabi teff and finger millet flours to prepare leavened bread by adding some salt to it and used to feed oxen. It is believed that when the ox fed such kind of food, it would get more strength and we don't observe a feeling of tiredness from the ox when tilling land…".

Discussion

Dabi teff is a marginally cultivated and/or underutilized food crop grown in Western Ethiopia. The result of this study showed that there are many traditional usages, social beliefs and cultivation trends associated to *dabi teff* among the communities in the study area. In-home consumption, *dabi teff* can be used for the preparation of important traditional healthy foods such as *injera/budena* (pancake-like local bread), *kitta/maxino/cacabsa* (unleavened bread), *atmit or muk/mooqa* (gruel), 'mooqa manyee', *fetfet/fafato, cumboo, cafaqoo and cacabssa (the favorite cultural dishes)* and bread for all age groups including children in Ethiopia [204, 205]. Another traditional usage of *dabi teff* is the preparation of homemade local brews or local alcoholic drinks such as such as *tela/farsoo* and *katikala/arakee that women sell for income* generation.

This study has generated the real information/knowledge deep from the community is crucially important to design appropriatefood-based nutrition intervention strategies that would be mostly successful because it partcipatorly provided the actual production and consumption practices or the real dietary patterns and beliefs in the area. Scholars call such an approach, 'a simulation analysis.' A food belief study allows for the identification of positive and negative social cognitive factors that may influence both production and consumption of a crop. The FAO and WHO [29] recommend for collection of detailed baseline information on traditional practices and food beliefs prior to designing and initiating food-based intervention programs.
In addition to the enjoyment of many traditional food forms of *dabi teff*, the result shows that there are also many social beliefs associated to *dabi teff*. The participants reflected many health and nutritional benefits followed eating the different food forms, particularly the 'mooqa manyee

and cafaqoo, which increases blood volume “dhiiga dabalaa,” boosts energy/strength “humna dabalaa,” and repairs/strengthens the backbone and fractured bones “dugda jabeessa.

The findings from this study showed that *dabi teff* grain matures in less than 90 days due to which it is harvested twice within one rainy season, during the onset of *Kiremt* rainfall, and late rainfall after mid-September, thus it allows a good double cropping option following the harvest of crops such as maize in the late September. Between June and August, households in most parts of Ethiopia are in a food shortage every year [206]. However, households in West Ethiopia address their food security problem during such food shortage times by cultivating *dabi teff* at the early onset of *Kiremt* rainfall showing its potential in responding to the food insecurity challenge.

Despite the aforementioned traditional usage (the preparation and consumption schemes), social beliefs, nutritional and health benefits of *dabi teff*, there are various shortcomings in its production. Some of the major ones are low productivity, laborious production from its cultivation till trashing, its need of commercial fertilizers and overemphasizing other staple crops such as maize, sorghum and barley. Further, the young populations are showing ignorance to take the traditional practices and pass down to the next generation. Therefore, the chances of *dabi teff* in continuing as highly liked and praised food among the community in western Ethiopia is doubtful because traditional knowledge linked with *dabi teff* is in danger of eroding and disappearing.

This study revealed that the threshing of *dabi teff* and its separation from the chaffs is carried in a very controlled condition that the contamination of its seed with the soil is almost none. Some scholars think that the high iron content of teff is due to contamination from iron-rich soil ground on the external surface of the grains. However, a higher value was obtained using fresh teff from the plant, threshed in the laboratory [207]. Actually, the iron content of teff might vary depending on the crop variety and soil conditions, but still, it contains high iron content wherever sampled.

Some others claim that the iron is actually embedded in the grain walls and considered as a dietary source of iron along with the actual true iron content of the grain itself [27]. Despite the arguments, it has been proved that teff grain has high iron content. One point is that not all soils of teff growing areas are ferruginous and the other point is only a small portion of bulk teff is

contaminated with soil even in the traditional threshing style. The threshing grounds are made with care to avoid soil contamination by sealing with animal dung and teff straw mixture. In research stations, teff is harvested and threshed with care not on the ground and hence, low chance of soil contamination but still teff is reported with high iron content [27]. *Dabi teff* straw is composed of fine stems used mainly for reinforcing mud for plastering wooden walls of buildings. The straw is stored and used as an important livestock feed, especially during the dry season [193]. Cattle prefer teff straw than straw from any other cereal and its price is higher than that of other cereals. Hence teff crops including *dabi teff* can be used as a dual or multipurpose crop, which is both as a cereal and as forage feed *[208]*.

One of the findings from this study was the storage quality of *dabi teff*. Thus it has a high economic value as its grain can be kept for many years in practical when kept in any kind of storage facility without being seriously damaged by common storage insect pests. Some farmers store maize and other pulse crops along with dabi tef. This is due to the minute size of the *dabi teff* seeds, which could hamper the movement of weevils inside the seeds and due to the low level of oxygen by closing air holes, insects can not grow inside and damage the crops. In summary, we found out that *dabi teff* is primarily worth as a traditional food, medicinal food and good energy source and one of the highly valued food commodities in western Ethiopia.

The important finding from this study is that, even though the community has strong traditional practices and connection with *dabi teff*, there was less practice of using it as a complementary food, not only in the study area but also, there is a less practice of using red teff as a child food in the general population in Ethiopia which could be the reason for the prevalence of higher rate of anemia (57%) in under-five children [17]. WHO [62] recommends the use of nutritious indigenous or staple foods together with traditional processing technologies such as fermentation and germination among others to produce nutrient and energy-dense complementary foods and need to be promoted. We, the researchers suggested that enriching child food through incorporating *dabi teff* flour might be nutritionally promising and can address both the protein-energy and micronutrient malnutrition for optimal growth and development of children.

Conclusions

Dabi teff is an ancient indigenous cereal crop cultivated in western Ethiopia. There are a number of traditional usages and social beliefs in the community that are linked with *dabi teff*. It is believed in the community that *dabi teff* has many health benefits in bringing positive health and nutritional outcomes when its different food forms typically “mooqa manee” is served to a family member requiring special nutritional treatment. It has shown that *dabi teff* has good storage quality, and can play a substantial role in ensuring food security, house construction and animal forage. *Dabi teff* can be used as an iron-rich source for complementary food preparation. The big worry associated to *dabi teff* is that its cultivation is declining nowadays, and there is fear that all those traditional usages and social beliefs would be forgotten in the long run. Enriching staple diets with *dabi teff* could be very essential in alleviating nutritional challenges as well as preventing the total extinction of this plant food. Future policies should encourage investing in the preservation and protection of traditional knowledge linked to *dabi teff*. Thus, it was suggested that *dabi teff* cultivation should be integrated into Ethiopian agricultural program packages and the general community should aware the new generation and pass down about the traditional usage and beliefs of this healthy food to incubate/preserve its values. Moreover, the usage of *dabi teff* as a complementary food ingredient needs to be promoted through nutrition education to mothers/caretakers in the study area, Nedjo district, western Ethiopia.

5

Chapter 5:

Optimization of a Formula to Develop Energy and Nutrients Dense Novel Complementary Food from Cereal-Legume Blends Enriched with *Dabi Teff* Grown in Western Ethiopia

Chapter 5.1:

Optimization of *Dabi Teff*-Field Pea Based Energy and Protein-Dense Novel Complementary Food with Improved Sensory Acceptability Using D-optimal Mixture Design

Redrafted from: Diriba Chewaka Tura, Tefera Belachew, Dessalegn Tamiru, Kalkidan Hassen Abate. ***Optimization of dabi teff-field pea based energy and protein-dense novel complementary food with improved sensory acceptability using D-optimal Mixture Design.*** *Heliyon. 2023; 9: e19029. doi.org/10.1016/j.heliyon.2023.e19029. Elsevier. August 12.*

Abstract

Protein-energy malnutrition is unacceptably high among children in developing countries due to inadequate required nutrients and poor quality of complementary foods characterized by low protein and energy density and often monotonous. Thus, this research was aimed at examining the potential of incorporating *dabi teff*, the underutilized/marginalized crop into pre-processed local food crops *viz*., germinated maize, roasted barley, roasted field pea, dehulled oats and linseed to develop energy and protein-dense optimized novel complementary food with improved sensory acceptability. Nutrisurvey software was employed to define ranges and they were constrained at 20-35% *dabi teff*, 0-30% field pea and 5-20% maize, while the rest were set constant at 25% barley, 15% oats and 5% linseed. Eleven experimental runs were generated from the six mixture components using D-optimal mixture design, Stat-Ease Design Expert® software version 11. A 5-point Hedonic scale was used to evaluate the sensory attributes. 'Scheffe' regression was used to fit and test model adequacy and numerical multi-response optimization was performed to identify optimal points using the Design expert. Field pea and linseed contained significantly higher ($P < 0.05$) protein at 20.95% and 20.57%. The newly formulated products contained significantly higher protein (1.4-1.6 times) and protein density (1.31-1.56 times) as compared to the control and fulfilled the recommended standard. The optimal was identified at 34.66% *dabi teff*, 25% barley, 15% oats, 15.34% field pea, 5% linseed and 5% germinated maize flour ratios with response values at overall optimization to be 5.57% moisture, 15.74% protein, 5.09% fat, 2.26% ash, 2.88% fiber, 73.05% carbohydrate, 380.43 kcal/100g energy and 4.12 sensory acceptability score. It contained an energy density of 1.27 kcal/g and protein density of 4.14 g/100kacl. These findings showed that optimized *dabi teff*-field pea-based novel complementary food can be developed and used as a sustainable food-based strategy to combat protein-energy malnutrition among children in western Ethiopia.

Key Words: *Dabi-teff*, mixture optimization, protein and energy-dense, sensory acceptability, novel complementary food

Introduction

Malnutrition, in all its forms, remained a major public health problem globally and the world is off-truck or at slow momentum to achieve the Zero-Hunger target by 2030 [2]. The problem is further worsened by the COVID-19 pandemic where the number of undernourished people continued to rise since then. In most African countries, the vicious cycle of malnutrition is endemic due to poverty, food insecurity and infectious diseases. Protein-energy malnutrition (PEM), 'the silent emergency of the world' is an immediate cause of child mortality, morbidity and anthropometric deficits; stunting, wasting and underweight. It has hunted and continued to hunt mankind since the dawn of history and is by far the most lethal form of malnutrition [91, 209].

Indeed, undernutrition affects several millions of people worldwide where mainly children under five years are among the most visible victims to nutritional deficiencies and most susceptible to PEM-related growth impairments due to their high demand for protein and energy during this period [210]. In 2020, 22% of under-five children were stunted, 6.7% were wasted and 5.7% were overweight worldwide [2, 14]. Overall undernutrition contributes to about 45% of child deaths in under-five years resulting in 3.1 million deaths every year [211]. In Africa, 61.4 million of under-five children were stunted, 12.1 million were wasted and 10.6 million were overweight [14]. According to the Ethiopian Public Health Institute and the International Classification of Functioning, Disability and Health (ICF) survey [19], 37% of children under five years were stunted, 21% were underweight, 7% were wasted, and 2.9% were overweight.

The importance of proper nutrition in the first 1,000 days of life (conception up to 24 months) and beyond is well-recognized in children. Growth faltering occurred during this critical period would end up with irreversible stunting, impaired cognitive development, diminished learning and earning capacity, greater susceptibility to infectious disease and increased morbidity and mortality as shown by many longitudinal studies [45, 46]. Catch-up growth can be attempted but it is mostly at the later stage of growth faltering which is commonly observed during recovery periods after illness, after addressing causes of growth failure and post-natally after severe intrauterine growth restriction [212] and ending impractical and even poses a later-life risk of chronic disease in most cases [213].

The significantly higher prevalence of malnutrition in under-five children occurs during or after the introduction of complementary foods because of the poor quality and inadequate required nutrients for optimal growth and development [214]. Traditional complementary and weaning foods in developing countries including Ethiopia are characterized by low nutritive value (often monotonous), low protein and energy density, high bulk density, high viscosity, and high anti-nutrients and there is a suboptimal complementary feeding practice [74, 215].

Commercial fortified food products such as powdered milk and peanut butter and supplementation such as ready-to-use therapeutic foods (Plumpy'Nut, F-75, F-100) were proposed as effective strategies for combating child malnutrition where these strategies posed limitations in developing countries. In Ethiopia, efforts were being made to implement a multi-sectorial plan of high-impact nutrition-specific and nutrition-sensitive interventions such as the [60] and [59] to end child under-nutrition including stunting by 2030 [56]. However, the country is still experiencing one of the worst situations where PEM is unacceptably high among under-five children.

The food-based approach has currently received much attention as a better strategy where it uses a combination of production and consumption of nutrient-rich foods among children, and pregnant and lactating women [52, 61]. It is considered as the best approach since it is cost-effective, sustainable and can be adapted to different cultural, dietary traditions and locally feasible strategies. This approach focuses on the combination of diverse staple/indigenous foods that can meet nutrient requirements and is based on the fact that people eat foods, not nutrients or supplements [216]. Diverse foods also give infants and young children a chance to appreciate various food flavors and textures, all of which are vital for later developing healthy eating habits [82]. The FAO/WHO [28] codex alimentarius on complementary feeding guidelines describes that mixtures of cereals, legumes, pulses/oilseed can constitute an appropriate source of nutrients and energy, essential fatty acids and limiting amino acids with many functional and health benefits as well as improved organoleptic characteristics. Therefore, there is a need to ensure both nutrient densities and sensory acceptability when developing new complementary foods.

Local food ingridients can be modified through the use of various processing techniques like germination, soaking, fermentation and extrusion cooking which can result in the reduction of anti-nutrients, improve energy and nutrient densities of complementary foods, increase the bioavailability of key micronutrients as well as enhance sensory and palatability of foods [91].

Across the world, many plant foods are underutilized/marginalized while they can play a substantial role in ensuring food and nutrition security, having nutceuticals value and sources of income generation [96]. The sub-Saharan African region is endowed with a rich diversity of such plant foods, where little or no attention was given in terms of research and development and policy framework that could promote their extensive agricultural production, marketing, industrial utilization as well as home consumption.

In Ethiopia, the food culture of teff is both a historical, integral part of the country's antiquity. It is mostly traded domestically and more than 70% of the Ethiopian population use teff as a traditional staple meal [103]. The gluten-free property of teff grains as reported by Bultosa [198] qualifies the crop as a medicinal food (nutraceuticals) to be used as a good alternative diet for celiac disease patients. *Dabi* teff *(Eragrostis teff*), a farmer variety teff, is one of the underutilized/marginalized or forgotten food crops grown in Ethiopia (Oromia region) that could be utilized to combat protein-energy and micronutrient malnutrition and can be used for the preparation of important traditional healthy foods such as *injera/budena* (pancake-like local bread), *kitta/maxino/cacabsa* (unleavened bread), *atmit or muk/mooqa* (gruel), *fetfet/fafato, cumboo and cafaqoo (*the favorite cultural dishes*)* and bread for all age groups including children in Ethiopia [204, 205].

Dabi teff is the 'afaan oromoo' language name for an early maturing variety of dark red teff. The early maturing property makes the crop to be harvested twice within one rainy season (at early rainfall called "*daabii gannoo*" and late rainfall called "*daabii birraa*") (personal experience). An early maturing and low rainfall requirement makes *dabi* teff a unique variety teff amenable to climate-smart agriculture. Farmers in Wollega and Illuababor, western Ethiopia, cultivate *dabi* teff either for its grain seeds or its straw. The contribution of *dabi* teff grains to household food and nutrition security is considerable when viewed through a food security lens while the straw

is composed of fine stems used for plastering (finishing) mud hut walls during house construction and rated higher among others for animal fodder, especially oxen feeding (personal experience). There are many social beliefs regarding the nutritional and health benefits of *dabi teff* among rural elderly people in particular and the consumers in general where they prize it as a medicinal food (Personal communication).

The key underlying cause of child malnutrition among households with low socio-economic status is a lack of purchasing power of quality foods for their children and they consume foods of low nutritive value. Price-wise, the red teff varieties grown in Ethiopia including *dabi teff* are the cheapest. For example, [201] reported that the market price of mixed teff and red treff were 24% and 55% less than the white teff. This statement specifies that the price of *dabi teff* is quite affordable for all socio-economic classes. In addition to lower prices, red teff varieties have been implicated in the low incidence of anemia in Ethiopia which is presumed to be due to the grain's high iron content [103].

Some previous studies have been conducted on the nutrient contents of different teff varieties grown in Ethiopia with comparable results with the present finding [27, 166, 198] and the formulation of complementary food by inclusion of the different teff varieties has also been conducted [217, 218] with lower results with the current finding. These scholars obtained the teff varieties from Ethiopian agricultural research centers some of which are released and others on the accession stage. *Dabi teff* is however traditionally known and acceptably consumed among the producers and the samples were directly collected from the popular market. It is therefore, we suggest the crop needs to be integrated into the Ethiopian agricultural research program for its intensive research, extensive production and industrial utilization.

Despite the social beliefs then, to date, the potential of including *dabi teff* into complementary flour formulations in combating protein-energy malnutrition in infants and younger children is not yet examined. Thus, this research was aimed at examining the potential of including *dabi teff*, the underutilized food crop into pre-processed local food crops *viz*., germinated maize, roasted barley, roasted field pea, dehulled oats and minimally cooked linseed to develop energy and protein dense optimized novel complementary food with improved sensory acceptability and

finally to suggest the potential of *dabi teff*-field pea based novel complementary food to be used as a sustainable food-based strategy to combat protein-energy malnutrition among children in less developed and resource-limited countries including Ethiopia.

Materials and Methods

Details of the methods related to the crop sampling, processing, flour preparation and handling were described in **Chapter 2**. Literature-based nutrient content of each crop was taken into account during the selection process which was later verified through compositional analysis **(Table 5.1).**

Results

Macronutrient composition of the individual processed flours

The mean and standard deviation of the macro-composition of the individual components that underwent the various processing steps were presented in **Table 5.1.** A significant difference ($P < 0.05$) was observed in mean values of the macro-composition among the individual components and ranged between 4.32- 9.03% for moisture, 8.58-20.95% protein, 2.58-36.08% fat, 1.42-3.96% ash, 2.12-7.46% fiber, 28.24-77.63% utilizable carbohydrate and 350.26-519.96 kcal/100g for gross energy, respectively. The fat content was similar ($P > 0.05$) in all the components except in linseed flour where it was significantly higher ($P < 0.05$) than the others **(Table 5.1)**. This table indicates that field pea and linseed had significantly higher protein content at 20.95% and 20.57%, respectively with their highest protein density at 5.98 and 3.96-g/100kcal, respectively followed by oats flour at 12.5% protein content while the protein content of *dabi teff* was determined at 10.74%

Table 5.1. Macro-nutrients and energy values of the flours from processed individual crops

Individual flours	Macro-composition (%)						Gross Energy (Kcal/100g)	PER (g/100kcal)	PEL
	Moisture	Protein	Crude Fat	Total Ash	Crude Fiber	Carbohydrate			
Dabi teff	9.03±0.10^{a}	10.74±0.04^{a}	3.94±0.58^{a}	3.96±0.03^{a}	3.76±0.16^{a}	68.57±0.51^{a}	352.70^{a}	3.045	12.180
Roasted Barley	4.32±0.33^{b}	11.62±0.03^{b}	2.58±0.43^{a}	1.73±0.09^{b}	2.12±0.07^{b}	77.63±0.06^{b}	380.22^{b}	3.056	12.224
Dehulled Oats	7.16±0.06^{c}	12.45±0.06^{bc}	5.69±0.03^{a}	1.92±0.01^{bc}	2.78±0.07^{c}	70.00±0.09^{a}	381.01^{bc}	3.268	13.071
Roasted Field pea	5.80±0.01^{bc}	20.95±0.02^{d}	2.74±0.01^{a}	2.60±0.03^{d}	7.46±0.07^{d}	60.45±0.08^{c}	350.26^{a}	5.981	23.925
Cooked Linseed	6.32±0.25^{ce}	20.57±0.30^{de}	36.08±1.45^{b}	3.45±0.01^{e}	5.34±0.11^{e}	28.24±1.61^{d}	519.96^{d}	3.956	15.824
Germinated Maize	7.68±1.13^{ac}	8.58±0.13^{f}	4.61±0.95^{a}	1.42±0.01^{bf}	2.47±0.03^{bcf}	75.24±0.03^{be}	376.77^{be}	2.277	9.109

Key: Values are means ± standard deviation of the triplicate determinations. Values in the same column followed by different superscripts are significantly different at $P < 0.05$. PER-protein energy ratio, PEL-protein energy level.

Table 5.2: Nutrisurvey analysis of the food records (customized) to define the ranges of each component in the formulations

		Calculated energy and nutrient values of the formulated meal by Nutrisurvey							
Components	Proportion (%) (lower-upper)	Amount eaten range/day (g)	Energy range (kcal)	CHO range (g)	Protein range (g)	Fat range (g)	Iron range (mg)	Calcium range (mg)	Zinc range (mg)	Magnesium Range (mg)
Dabi teff	20-35	45-79	158.7-278.7	30.9-54.2						
Roasted Barley	25	56	212.9	43.5						
Dehulled Oats	15	34	129.5	23.8						
Roasted Field pea	0-30	0-68	0-238.3	0-41.1						
Cooked Linseed	5	12	62.4	3.4						
Germinated Maize	5-20	11-45	41.4-169.5	8.3-33.9						
Analyzed value (range) for the formulated meal using our components			604.9-1091.5*	109.9-199.9 (73-74%)	19-39.8 (13-15%)*	10-14.8 (15-12%)	49-83.6*	87.8-176.1	4-6.7*	183-320.3
Recommended value for 1-3 years old child as generated by the 'Nutrisurvey' software			718.3	102.5 (>55%)	21.2 (12%)	24.4 (<30%)	8	600	3	80
Percentage fulfillment from our formulated meal (range) (lower-upper)%			84-152*	107-195*	90-188*	41-61	612-1045*	15-29	133-225*	229-400*

*Showing the blended complementary flours would be promising to attain the PAHO/WHO recommendations.

In addition to the significantly higher protein content, linseed flour was determined to contain significantly higher fat content at 36.08% as expected from an oil seed. The fat content was similar (P >0.05) and significantly lower-far below 6% in all the other components. The ash content of *dabi teff* flour was significantly higher (P <0.05) at 3.96% among the others followed by linseed flour at 3.45% while germinated maize showed the lowest ash at 1.42%. The fiber content of linseed was significantly higher at 5.34% preceded by field pea with the highest fiber content at 7.46%. *Dabi teff* flour had shown fiber content at 3.76% while the remaining components contained fiber blow 3%. The carbohydrate content of roasted barley was significantly higher at 77.63% followed by germinated maize flour at 75.24% with the lowest value for linseed at 28.24% and the carbohydrate content of *dabi teff* was determined at 68.57% which was comparably high as that of oat flour at 70%.

A relatively lower energy value was computed for *dabi teff* flour at 352.7 kcal/100g as compared to the other components which could be attributed to its higher moisture content (9.03%). The lowest energy value was computed for field pea flour at 350.26 kcal/100g while roasted barley, oats and germinated maize flours had shown comparably higher energy values at 380 kcal/100g whereas the highest energy value at 519.96kcal/100g was determined for linseed flour **(Table 5.1).**

Macronutrient contents of the formulated complementary flour

Model fitting and testing model adequacy

The fitted models were found to be adequate and significant for most response variables based on the F-statistic (the ANOVA regressions outputs); the P-value, the coefficient of determinations, R^2 and the agreement between R^2_{adj} and R^2_{pred}. **Table 5.3** showed that the linear models were adequately fitted for moisture, protein and ash contents and significant at P-values of <0.01, <0.001 and <0.05. This shows that x_1, x_4 and x_6 (*dabi teff*, field pea and germinated maize) were the significant model terms for these compositions. The quadratic model was adequately fitted for fat and significant at a P-value of <0.05. This shows that the linear model terms (x_1, x_4, and x_6) and the interactive model terms (x_1x_4, x_1x_6 and x_4x_6) were significant model terms for fat content.

On the other hand, the cubic model was significantly fitted for carbohydrates and significant at P-values of <0.05 where the linear model terms (x_1, x_4, x_6) and the interactive model terms x_1x_6, x_4x_6 (x_1- x_6), and x_4x_6(x_4-x_6) were the significant model terms for carbohydrate at P-value less than 0.05. Special cubic and linear models were suggested by the software for fiber and energy contents, respectively, but the models were non-significant (P >0.05).

Macro-composition of the formulated complementary flours

The mean moisture, protein, fat, ash, fiber, carbohydrate and energy contents of the new formulations were determined to be 4.89, 15.49, 4.91, 2.23, 3.48, 68.99% and 382.14kcal/100g, respectively **(Table 5.5).** When compared to the [28] guideline for the nutrient content of formulated complementary foods, the mean value of the new formulations fulfilled the critical limits for all the macro-compositions except for fat where the guideline recommends moisture, protein, fat, ash, fiber, carbohydrate and energy contents to be $\leq$5, $\geq$15, 10-25, $\leq$3, $\leq$5, 64$\pm$4% and 400-425kcal/100g, respectively.

The moisture content of the formulations ranged from 4.41-5.74% and the mean moisture was significantly lower (P <0.05) than that of the control and 'Cerifam® faffa' flour at 6.15 and 6.5%, respectively. The protein content of the new formulations ranged from 14.58-17.21% where it showed significantly higher (P <0.05) protein content (1.4-1.6 fold) and protein density (1.31-1.56 fold) than the control flour and slightly higher than the Cerifam® faffa flour. In this study, fat contents of the formulations ranged from 4.22- 5.59% and there was no significant difference (P >0.05) between the mean fat content of the formulations, the control at 3.78% and the Cerifam® faffa flour at 4%.

The ash content of the formulated flours ranged from 2.01 to 2.60% where some of the formulated flours had significantly higher (P <0.05) ash content as compared to the control. The fiber content of the new formulations ranged from 2.68 to 3.96% which was slightly higher than that of the control and Cerifam® faffa flour at 2.65% and 3%, respectively. The carbohydrate content of the new formulations ranged from 68.08-70.76% which was slightly lower than that of the control and Cerifam® faffa flour at 74.41% and 76%, respectively and this could be due to the barley and maize which made the control and the wheat and soybean in the Cerifam®. The energy content of the formulations ranged from 378.82 to 386.9 kcal/100g and it was significantly higher (P <0.05) than the control flour at 374.74 kcal/100g.

Table 5.3: Models fitted for macro-nutrients, sensory evaluation and statistical outputs showing significance and model adequacy

	Macro-Compositions							SE
Model's P-value (model terms)	MC	Protein	Fat	Ash	Fiber	CHO	Energy	OA
Linear (x_1,x_4,x_6)	0.0049 #**	0.0005 #***	0.334	0.0211#*	0.3442	0.1790	0.3108#^	0.2196
Quadratic (x_1^2, x_4^2, x_6^2)	0.3313	0.4721	0.0030#**	0.7957	0.9237	0.0545	0.8144	0.0977
Special Cubic	0.5132	0.5436	0.9767	0.8995	0.2875#^	0.4498	0.3075	0.9245
Cubic	0.3819	0.4444	0.8637	0.9694	0.6022	0.0483#*	0.7794	0.0452
Sp. Quartic vs Quadratic	0.1780	0.4675	0.5789	0.8310	0.3177	0.3527	0.5419	0.0311#*
Interaction terms								
x_1x_4	-	-	0.0102	-	0.5564	0.0560	-	0.3471
x_1x_6	-	-	0.0064	-	0.2962	0.1521	-	0.0319
x_4x_6	-	-	0.0009	-	0.5077	0.0406	-	0.5901
$x_1x_4x_6$	-	-	-	-	0.2875	0.9808	-	0.0308
F-statistics (ANOVA)								
Model F-value	11.15	22.23	16.61	6.49	0.64	257.55	1.36	31.58
Lack-of-Fit	PE0	PE0	PE0	PE0	PE0	PE0	PE0	PE0
R^2	0.75	0.8487	0.9432	0.6188	0.4899	0.9996	0.2534	0.9921
R^2%	75	84.87	94.32	61.88	48.99	99.96	25.34	99.21
ADP	8.53	12.961	12.72	6.979	2.856	55.029	2.76	19.95
Model	0.0049*	0.0005*	0.0039*	0.0211*	0.7025^	0.0483*	0.3108^	0.0311*

Key: Sp.-special, vs-versus, PE0- pure error zero, R^2%-coefficient of determination, ADP-adequate precision, MC-moisture content, CHO-carbohydrate, SE-sensory evaluation, OA-overall acceptability, #-suggested, #*, #** and #***-model is suggested and significant at P<0.05, at P<0.01 and at P<0.001, respectively, #^ -suggested and not significant, * and ^ model is significant and not significant respectively, x_1-*dabi teff*, x_4- field pea, x_6- germinated maize.

Table 5.4: Goals set, the relative importance of each variable and the optimal values at optimal conditions identified.

Name	Goal	Lower Limit	Upper Limit	Importance	Optimum value
Independent variables					
A:*Dabi teff* Flour	In range	20	35	3	34.66
D:Field pea Flour	In range	0	30	3	15.34
F:Maize Flour	In range	5	20	3	5
Barley flour	Constant	25	25	3	25
Oats flour	Constant	15	15	3	15
Linseed flour	Constant	5	5	3	5
Dependent variables					
Moisture (%)	minimize	4.41	5.74	3	5.57
Crude Protein (%)	maximize	14.58	17.21	4	15.74
Crude Fat (%)	maximize	4.22	5.59	3	5.09
Ash (%)	maximize	2.01	2.6	3	2.26
Crude Fiber (%)	minimize	2.68	3.96	3	2.88
Carbohydrate (%)	maximize	66.91	70.76	4	73.05
Energy (kcal/100g)	maximize	378.82	386.9	3	380.43
Overall Acceptability	maximize	3.4	4.4	5	4.12

Sensory evaluation results

The mean values of the sensory evaluation score were significantly different ($P < 0.05$) among the formulated porridges (as affected by components ratio variations) and ranged between 3.4 -4.8 for color, 2.8 -4.8 for aroma, 3.0 -4.4 for taste, 3.2 -4.5 for mouth feel and 3.4 -4.4 for overall acceptability with mean overall sensory acceptability score of 3.99 among all the porridges **(Table 5.6)**. The control porridge had 3.5, 4.2, 3.8, 3.9 and 3.4 for color, aroma, taste, mouth feel and overall acceptability, respectively. With these determined sensory mean scores, it can be judged that the porridges prepared from the newly formulated complementary foods were liked much and acceptable as high as 96% by mothers/caregivers who participated as sensory panelists.

Table 5.5: Macro-nutrients and energy values of the formulated fours with their corresponding protein-energy density (PER) and FAO/WHO (1991) recommendation

Formulations	Macro-compositions (%)						Gross Energy	PER	PEL
	Moisture	Protein	Crude Fat	Total Ash	Crude Fiber	Carbohydrate	(Kcal/100g)		
F1	4.58±0.01^{a}	15.08±0.25^{a}	4.22±0.04^{a}	2.31±0.08^{a}	3.68±0.06^{a}	70.13±0.26^{a}	378.82^{a}	3.981	15.293
F2	4.61±0.25^{a}	16.51±0.16^{b}	4.75±0.01^{a}	2.45±0.01^{a}	3.56±0.03^{a}	68.12±0.13^{a}	381.27^{a}	4.330	17.321
F3	5.74±0.10^{a}	15.93±0.05^{ab}	4.78±0.06^{a}	2.25±0.06^{a}	3.22±0.01^{b}	68.08±0.04^{a}	379.06^{a}	4.203	16.810
F4	4.68±0.54^{a}	17.21±0.17^{bc}	5.14±0.40^{a}	2.60±0.01^{ab}	3.46±0.04^{a}	66.91±0.29^{b}	382.74^{a}	4.496	17.986
F5	5.05± 0.16^{a}	14.63±0.02^{a}	5.59±0.57^{a}	2.14±0.01^{ac}	3.82±0.06^{a}	68.77±0.33^{a}	383.91^{b}	3.811	15.243
F6	5.52±0.42^{a}	15.27±0.32^{ab}	4.92±0.69^{a}	2.20±0.11^{a}	2.68±0.01^{c}	69.41±048^{a}	383^{c}	3.987	15.948
F7	4.50±0.01^{ab}	15.17±0.04^{ab}	5.32±0.06^{a}	2.05±0.02^{ad}	3.74±0.03^{a}	69.22±0.08^{a}	385.44^{d}	3.936	15.743
F8	5.45± 0.03^{a}	14.65±0.35^{a}	5.30± 0.11^{a}	2.09±0.18^{ae}	3.96±0.06^{ag}	68.55±0.14^{a}	380.5^{a}	3.850	15.401
F9	4.41±0.03^{ab}	14.80±0.09^{a}	4.62±0.33^{a}	2.35±0.02^{a}	3.66±0.06^{a}	70.16±0.19^{a}	381.42^{a}	3.880	15.521
F10	4.43±0.04^{ab}	16.53±0.25^{bd}	4.34±0.12^{a}	2.11±0.03^{af}	3.78±0.06^{a}	68.81±0.08^{a}	380.42^{a}	4.345	17.381
F11	4.88± 0.04^{a}	14.58±0.04^{a}	5.06±0.19^{a}	2.01±0.19^{ag}	2.71±0.32^{cd}	70.76±0.03^{a}	386.9^{e}	3.768	15.074
Mean	4.8955	15.4873	4.913	2.233	3.479	68.993	382.135	4.053	16.156
Control	6.15±0.82^{c}	10.77±0.52^{e}	3.78± 0.79^{a}	2.24±0.01^{a}	2.65±0.03^{ce}	74.41±2.15c	374.74f	2.874	11.496
Cerifam®Faffa	6.5^{c}	14^{a}	4^{a}	2.5^{a}	3^{b}	76^{c}	396^{g}	3.54	14.14
Optimized	5.57	15.74	5.09	2.26	2.88	73.05	380.43	4.14	16.55
FAO/WHO	≤5	>15	10-25	≤3	≤5	64±4	400-425	<5.5	6-15

Key: Values are means ± standard deviation of the triplicate determinations. Values in the same column followed by different superscripts are significantly different at P <0.05. The corresponding component ratio for each formula code (F1- F11) is well described in **Table 2.1** earlier. PER-protein energy ratio; PEL-protein energy level.

Table 5.6: The mean score of the sensory evaluation of porridges prepared from the formulated complementary flours

Porridge from Formulations	Mixture components ratio (%)						Sensory Attributes				
	DTF	BF	OF	FPF	LSF	GMF	Colour	Aroma	Taste	MF	OA
FP1	27.50	25	15	15.00	5	12.50	3.5±0.97[a]	3.8±0.92[a]	3.4±0.52[a]	3.8±1.32[a]	3.9±0.74[a]
FP2	26.123	25	15	23.877	5	5.00	3.7±1.06[a]	3.7±0.82[a]	3.4±1.17[a]	4.2±0.79[a]	4.2±1.05[a]
FP3	30.457	25	15	19.543	5	5.00	3.9±0.57[a]	3.3±0.82[a]	3.2±0.92[a]	3.6±0.84[a]	4.2±1.08[a]
FP4	20.00	25	15	29.988	5	5.012	3.4±0.82[a]	3.6±0.82[a]	3.4±0.70[a]	3.3±1.20[a]	4.0±0.94[a]
FP5	28.793	25	15	6.207	5	20.00	4.0±1.2[a]	4.8±1.02[b]	4.1±1.10[a]	4.1±0.99[a]	3.4±0.82[a]
FP6	35.00	25	15	11.884	5	8.116	4.8±0.74[b]	4.0±0.99[a]	4.2±0.92[a]	4.0±0.92[a]	4.4±0.92[b]
FP7	21.705	25	15	13.295	5	20.00	4.0±1.35[a]	4.2±0.92[a]	3.8±1.14[a]	4.2±0.57[a]	3.9±0.99[a]
FP8	34.385	25	15	6.476	5	14.139	4.7±0.99[c]	4.4±0.52[a]	4.1±0.95[a]	4.2±1.14[a]	3.8±0.52[a]
FP9	20.00	25	15	19.540	5	15.460	4.1±0.57[a]	3.8±1.03[a]	3.9±0.88[a]	4.5±0.95[a]	4.3±1.16[a]
FP10	22.735	25	15	22.515	5	9.750	4.0±0.82[a]	2.8±0.88[a]	3.0±0.94[b]	3.2±1.32[b]	3.8±1.05[a]
FP11	29.604	25	15	10.076	5	15.320	4.3±0.62[a]	4.4±0.70[a]	4.4±0.97[a]	4.2±12[a]	4.0±1.03[a]
						Average	4.04	3.89	3.72	3.94	3.99
Control porridge	0	80	0	0	0	20	3.5±1.25[a]	4.2±0.63[a]	3.8±0.63[a]	3.9±0.88[a]	3.4±0.63[a]

Key: FP1-FP11- porridges of the coded formulations, DTF- *dabi teff* flour, BF-barley flour, FPD- field pea flour, LSF-linseed flour, GMF-germinated maize flour, MF-mouth feel, OA- overall acceptability.

Values are means ± Standard deviation of the sensory scores. Values in the same column followed by different superscripts are significantly different at $P < 0.05$.

Variables optimization and validation

An optimal complementary food that can meet energy and protein density requirements can be obtained from nutritious cereal-legume blends to develop a super-quality complex child food. In the present study, from the multi-response numerical optimization, the optimal formula (sweet spot) at overall optimization was identified to be at 34.66% *dabi teff* flour, 25% barley, 15% oats, 15.34% field pea, 5% linseed and 5% germinated maize flours with the predicted optimal value for the macronutrients to be 5.57% for moisture, 15.74% for protein, 5.09% fat, 2.26% ash, 2.88% fiber, 73.05% carbohydrate, 380.43kcal/100g for energy and 4.12 for overall sensory acceptability score, respectively **(Table 5.4)** where the 3-D surface graph showing the maximum selected desirability function at overall optimal condition was found to be 0.651 **(Figure 5.1)**. Results obtained at optimum conditions showed significantly higher protein (1.46 fold), and fat (1.35 fold) as compared to the control **(Table 5.4)**.

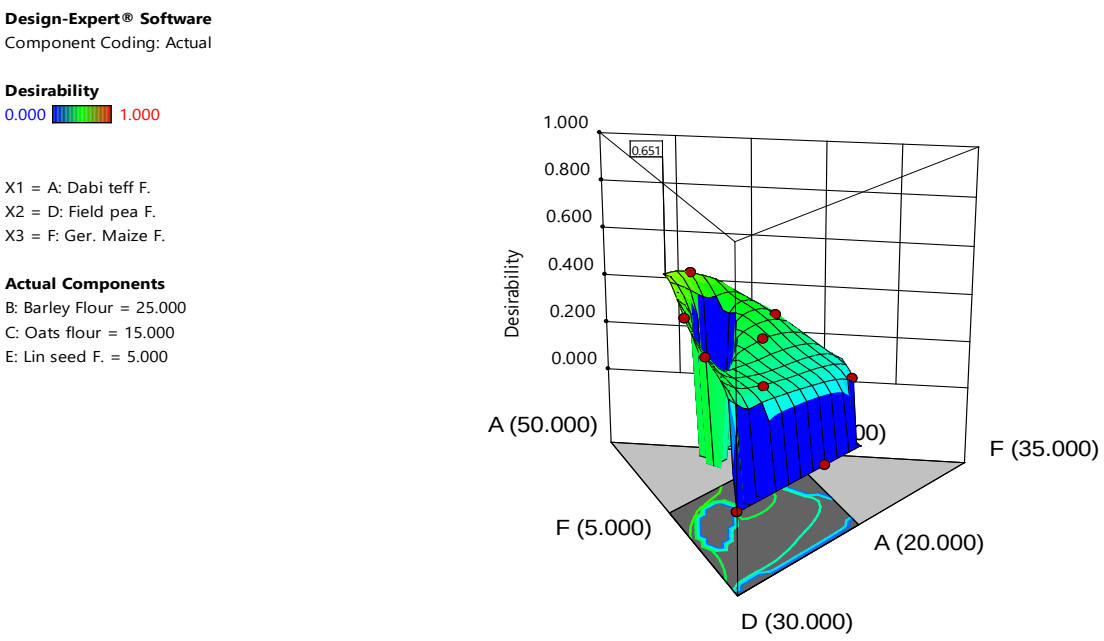

Figure 5.1: 3-D surface graph showing the desirability function of each response variable and the combined desirability function.

The following figure shows the material balance design of the defined ranges of the ingridents, their nutrient contents and the optimal condition identified **(Figure 5.2)**.

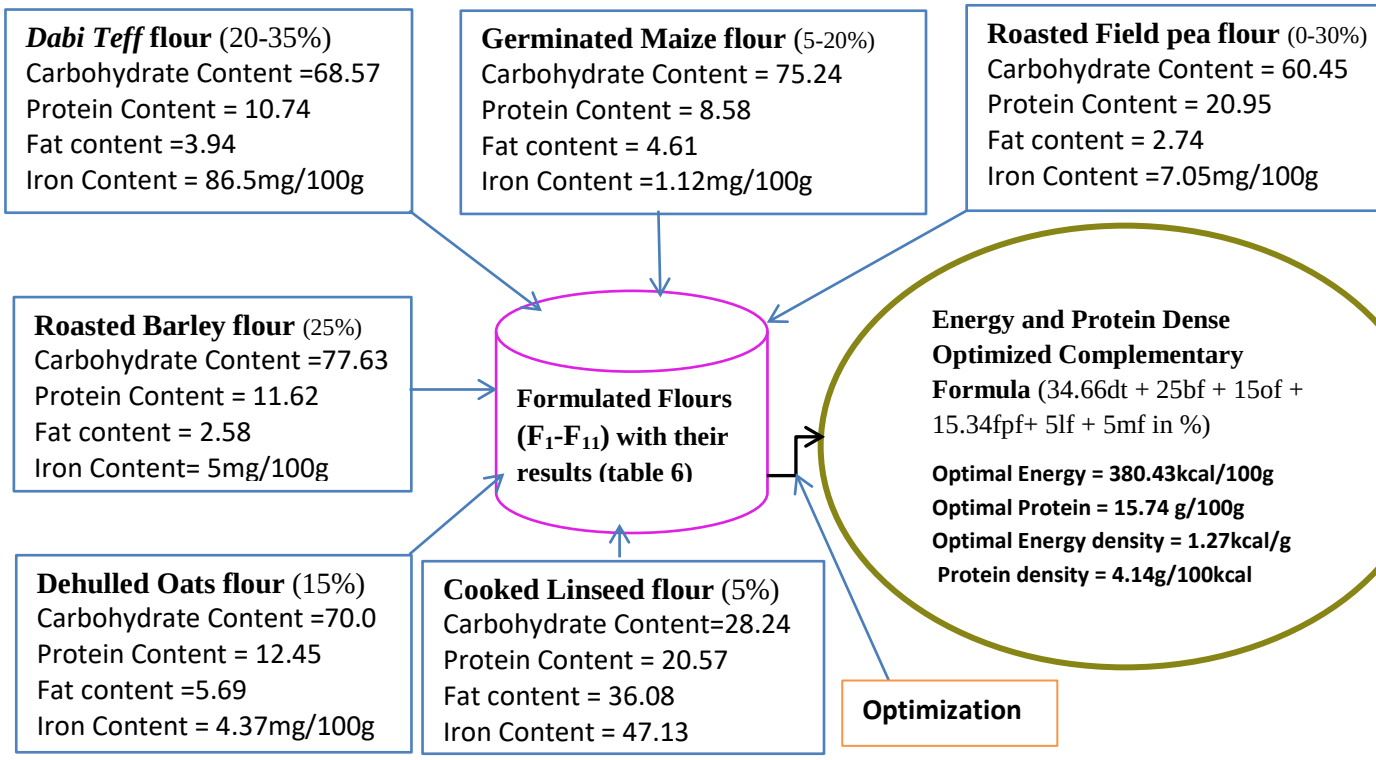

Figure 5.2. Material balance design showing individual flour ranges, their nutrient contents and the optimal condition. Key: dt = *dabi teff*, bf = barley, of = oats, fp = field pea, lf = linseed, mf = maize flours

Table 5.7. Experimental validation of optimal condition and comparison with the control flour.

Response variables	Optimized value	Validation value	Contro value
Moisture	5.57[a]	5.34a	6.15[b]
Protien	15.74[a]	15.86[a]	10.77[b]
Fat	5.09[a]	5.12[a]	3.78[b]
Ash	2.26[a]	2.21[a]	2.24[a]
Fiber	2.88[a]	2.85[a]	2.65[a]
Carbohydrate	73.05[a]	68.82[a]	74.41[a]
Energy	380.43[a]	384.80[a]	374.74[b]
Overall acceptability	4.12[a]	4.34[a]	3.45[b]

Key: Values are means ± Standard deviation of the triplicate determinations. Values in the same raw followed by the same superscripts are not significantly different at P >0.05.

To validate the optimal condition, the ratio of each component at the optimized formula was blended and laboratory analysis of nutrient compositions of the blend was conducted where the validation analysis result was 5.34%, 15.86%, 5.12% 2.21%, 2.85%, 68.82%, 384.80kcal/100g for moisture, protein, fat, ash, fiber, energy, respectively. This showed there was a good agreement (accuracy of the model solution) between the optimal values at optimal conditions and the validation results **(Table 5.7).**

The optimization process was verified using the graphical optimization technique **(Figure 5.3)** where the overlay counter plot was generated for the most important variables to identify the optimum regions. Any design points that fall within the yellow region (with the very conservative silver-gray region) in the overlay plot represent an optimal combination of *dabi teff* flour, field pea and germinated maize flours in combination with the other constant components that can bear optimal macronutrient contents and overall sensory acceptability within 95% confidence interval (CI).

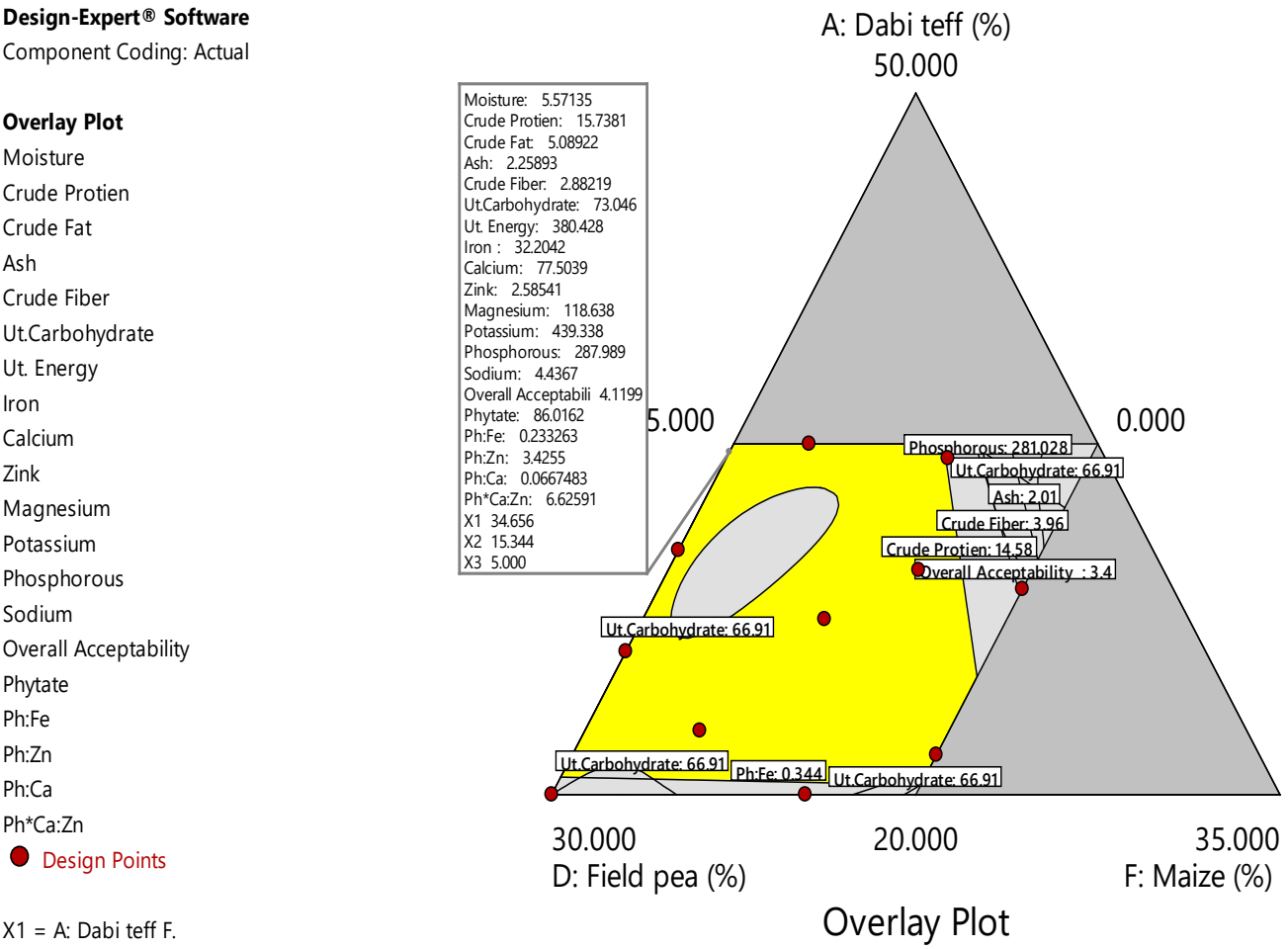

Figure 5.3. Graphical overlay contour plot for the overall graphical optimization

When compared to the [28] guideline for nutrient content requirements of formulated complementary foods, the optimized complementary flour adequately met the recommended critical limits for all the macro-compositions except for fat content at $\leq$5, $\geq$15, 10-25, $\leq$3, $\leq$5, 64$\pm$4% and 400-425kcal/100g for moisture, protein, fat, ash, fiber, carbohydrate and energy contents, respectively.

The 'novelty' of the optimized complementary flour developed would be accredited to the incorporation of *dabi teff* flour containing high iron at 86.5 mg/100g (determined by the authors) with good bioavailability (Ph:Fe molar ratio far below the critical limit) that greatly evidenced the social beliefs of this typical farmer variety teff. In addition, linseed flour which is a leading source of α-Linolenic acid, and omega-3 polyunsaturated fatty acid would make the product super and the approach novel.

Energy and macro-nutrient densities of the optimized complementary flour

The calculated energy and nutrient densities of the optimized flour are presented in **Table 5.8.** As described earlier, we have estimated the amount of complementary food to be served to children per day at 6-8 months, 9-11 and 12-23months old to be 225g, 300g and 450g, respectively. After adjusting for these daily amounts, the energy density of our optimized complementary flour for all the age groups was computed to be 1.27 kca/g. The protein and carbohydrate densities were determined at 4.14 g/100kcal and 19.20 g/100kcal, respectively.

Table 5.8. Energy and nutrient densities of complementary flours and Codex standards

Parameters	Optimal flour	Control flour	PAHO/WHO	Cerifam®
Energy density (kcal/g)	1.27	1.25	≥0.8	1.32
Protein density (g/100kcal)	4.14	2.87	>5.5	3.54
Fat density (g/100kcal)	1.34	1.01	2.5-4.5	1.01
Carbohydrate density (g/100kcal)	19.20	19.86	-	19.19

According to PAHO/WHO standard, the energy density of processed cereal-based complementary foods for older infants and young children should be ≥0.8kcal/g [219] and from **Table 5.8,** it can be shown that the newly optimized novel complementary flour can considerably meet the PAHO/WHO standards for energy, protein and carbohydrate densities of complementary foods required for infants and young children.

Codex Alimentarius committee recommends the protein density of cereal-based formulated complementary foods for infants and young children should not exceed 5.5 g/100 kcal [28] and the protein density of our optimized complementary flour was 4.14 g/100kcal which was near to the upper threshold (5.5 g/100kcal) and it was 1.44 times higher than the protein density of the control (traditional) and 1.2 times higher than the Cerifam® faffa flours.

Discussion

Macro-compositions are among the key nutritional quality indicators of food components and are usually the basis for establishing the nutritional profile and overall sensory acceptability of the final product [220]. In this study, the macro-compositions of each component were examined to

justify the bases for the inclusion of the crop as a potential candidate in the formulations and it was aimed to add one or more essential nutrients to the mixture. The compositions of each component varied significantly and this could be attributed to group differences (some are cereals and others are pulses/legumes) and due to the individual samples pre-processing techniques applied.

In agreement with the present findings, Munshi *et al.* [221] reported the protein content of linseed at 18.2%. Our result indicated that field peas and linseed were super sources of protein which justified their inclusion in the formulation to develop protein-dense complementary food. This higher protein content of field pea and linseed was attributed to the legume and pulse crops naturally containing higher amounts of protein than cereals. Further, the incorporation of field peas in the lysine-limited cereal is an excellent complement which further justifies the use of field peas as a feasible protein supplement in the development of cereal-based complementary foods. The mean protein content at 10.74% of *dabi teff* flour was in agreement with Bultosa [198] who reported a range of 8.7-11.1% with a mean of 10.4% for 13 teff varieties grown in Ethiopia and also with Daba [27] who reported protein content of 10.7 for DZ-01-2423 teff variety. On the contrary, the observed protein content of *dabi teff* was higher than the report by Agza *et al*. [222] which was 8.43% for '*kora*-DZ-Cr-438' teff variety.

The present observed findings of fat and energy content of linseed were slightly lower than that reported by Munshi *et al.* [221] at 42% and 534 kcal/100g, respectively. Linseed is a popularly cultivated oil crop by the community in the sample collection area (Nedjo district) and its oil is among the leading sources of α-Linolenic acid, omega-3 polyunsaturated fatty acid from plant foods [73, 116] which is crucially important for brain neural and cognitive development in utero and early childhood [223]. Such super quality and accessibility of linseed signifies its selection as a potential crop in the current formulations. The fat content of *dabi teff* flour was 3.94% which is relatively higher than the fat contents of teff grains reported by Daba [27] at 3-3.2%.

The higher ash content is an indicator of the higher total mineral content of the crop where the ash content of *dabi teff* in the present finding was high which was comparably agreed with the report by Woldemariam *et al.* [224] that red teff had an ash content of 3.2% whereas the report by Bultosa [198] showed lower mean ash content at 2.45% of 13 teff varieties. The observed fiber in all the individual components reasonably agreed with the codex standard where the value

for *dabi teff* was consistent with the previous study by Bultosa [198] that showed mean fiber content of 13 teff varieties at 3.3 % and Woldemariam *et al*. [224] also reported a similar result at 3.51% of fiber content in red teff.

The higher carbohydrate content was observed in the cereal components where they are naturally known to contain higher starch than legumes and oil seeds. The lower carbohydrate value observed for linseed was in agreement with the report by Munish *et al*. [221] at 29% which could probably be compromised by its higher fat and protein content.

The energy density of a food is critical for energy intake by infants and young children and this can be maintained by selecting energy-rich food components to increase the energy density of formulated products. The comparably higher observed energy values of the individual components justified the selection of the food crops in these formulations to develop energy-dense complementary food. The highest amount of energy content computed for linseed may perhaps be due to its higher fat content than the other components because fat provides double as much energy (9kcal/gram) as compared to protein and carbohydrate.

Even though, it may not make sense to compare the nutrient contents of cereals and legumes because of their different natural physiognomies, in the present study, we have compared them simply because they were selected components and to justify that cereals and legumes need to be mixed to nutritionally complement each other to develop wholesome and nutritionally complete end product. Aligned with this, the nutrient content of *dabi teff* such as protein, fat, carbohydrate, and energy, though lower than some of the other current components, results were incomparably higher (in some cases similar) than other cereals such as sorghum [103].

In model fitting, almost all the models generated were declared to be adequate to describe the effects of independent variables (all the possible model terms) on the response variables. Meaning changes in the response variables could adequately be described (adequate predictive power) by the model terms in the developed mathematical model as a function of component ratio variations.

A linear model was adequately fitted and significant to describe changes (adequate predictive power) in moisture, protein and ash contents as a function of blending ratio variations whereas the quadratic model was adequately fitted and significant for fat content and the cubic model adequately fitted for carbohydrate content. Meaning changes in fat can adequately be described by the linear and quadratic model terms as a function of the blending ratio variations and the cubic medal terms for carbohydrates. Though a linear model was suggested by the software for energy contents, it was non-significant and has no predictive power in describing changes in energy content as a function of component ratio variations in the blends. This finding was similar to the report by Tadesse *et al.* [225] that the combination variation of bulla, pumpkin and germinated amaranth had no predictive effect in describing the energy contents of complementary mixes.

This study has shown that incorporating *dabi teff* which is a nutritious forgotten/underutilized food crop into other pre-processed local food crops (filed pea, germinated maize, barley, oats and linseed) to formulate and develop energy and protein-dense novel complementary food could be a potentially promising alternative than traditional (monotonous) child food. The lower moisture content observed in the newly formulated complementary food could be attributed to the processing techniques applied. A lower moisture content of less than 5% is recommended for formulated complementary flour [28] which is an indicator that the flour has longer storage stability that is required particularly among rural mothers in developing countries because there is a usual practice of preparing a relatively large amount of complementary flour and use it for a longer time to avoid frequent preparation.

The current observed protein content of the newly formulated complementary food was higher than the report by Desalegn *et al*. [217] for the formulation of complementary food from Chickpea (*kabulli variety*), red teff (*DZ-0199 variety*) and quality protein maize (*BHQPY-545 variety and Ipomea batata, Tulla variety*) that protein ranged from 10.42 to 12.70%. The higher protein content of the new formulations could be attributed to the higher protein content of field pea and linseed flours in the formulations as well as due to the germinated maize which could be attributed to the mobilization of storage nitrogen and the synthesis of enzymatic proteins by the sprouting maize seeds during germination. Mariam [54] reported that complementary food

products from cereal-legume combinations of two or more components have better protein (overall nutritive value) than products from a single plant food.

Regarding fat content of the currently new formulations, comparatively higher fat content was reported by Walle and Moges [218] from the formulation of complementary food from maize, wheat, barley, chickpea, soya bean and lentil ranging from 5.8 to 8.22%. The lower fat content may call for finding other sources and there is a common practice among Ethiopian mothers/caretakers that they add a spoonful of spiced refined cow butter during child food preparation that may match the fat content. In consistent to the present finding, Birhanu *et al*. [217] reported a similar result for the formulation of complementary food from Chickpea (*kabulli variety*), red teff (*DZ-0199 variety*) and quality protein maize (*BHQPY-545 variety and Ipomea batata, Tulla variety*) that ash ranged from 1.61 to 3.07%.

In agreement with the present findings, Mezgabo *et al*. [166] reported that the fiber content ranged between 2.33 to 3.6% for complementary porridge formulation from red teff, malted soybean flour and papaya fruit powder. The fiber content was in the recommended range and although consumption of dietary fiber-rich products provides various health benefits, the importance of food fiber is not over-emphasized in child nutrition and is required to be very low or nil in complementary food [226].

The contribution of carbohydrates as an energy source in complementary foods is considerably high and it must be digestible enough for infants and young children to be able to obtain the amount of energy required. The current finding fairly agrees with the report by Mezgabo *et al*. [166] that carbohydrate content ranged from 55.43 to 69.68% for complementary porridge formulation from red teff, malted soybean flour and papaya fruit powder. The present observed value of energy content was amenable with the report by Mezgabo *et al.* [166] that the energy content of complementary porridge formulation from red teff, malted soybean flour and papaya fruit powder ranged from 376.30 to 385.56 kcal/100g. On the other hand, there was a slightly lower energy content of the current formulations than the FAO/WHO [28] recommendation (400 kcal//100g) and the Cerifam® faffa flour (396 kcal/100g) which may be attributed to the lower fat content in the new formulations.

Sensory attributes are of paramount concern especially when new food product development is targeting infants and young children. In the first place, there is a problem of 'picky eating and food jags' in this age group and if the food is not pleasant or good taste, they may refuse to consume it and this is why mothers most often tend to add more sugar to make child porridge sweeter. In this study, color, aroma and overall sensory acceptability attributes of the formulated porridges were more preferred than that of the control porridge by mothers panelists and there would be a high likelihood that the new products will also be liked much by their infants and young children. The observed optimal overall sensory acceptability score at 4.12 out of the five hedonic scales of the formulated porridge was judged to be high and much liked. This enhanced sensor acceptability could be attributed to the multiple grains and the processing technique applied which imparted good flavor, aroma and mouth feel to the newly formulated porridges.

Dewey and Brown [6] reported that cereals contain a limited amount of essential amino acid (lysine) while pulses and peas are lacking in cysteine, methionine and tryptophan therefore giving the bases for complementing each other by blending and optimizing to correct for the essential amino acid deficits to be able to develop fairly complete protein (containing all essential amino acids) product. Equally, child nutrition emphasizes the importance of quality protein, omega-3 polyunsaturated fatty acid and adequate iron during complementary feeding [223] where our new optimized product would certainly contain a good amount of these nutrients as a result of the presence of field pea, linseed and *dabi teff* flours in the formulations.

Regarding the overall desirability function of the present study, it was in agreement with Talabi *et al.* [227] who reported a similar desirability function of 0.65 during the optimization of complementary meal from a mix of yellow maize, sorghum, millet, soybean, groundnut, crayfish and fish, additionally, Keyata *et al*. [228] reported a similar desirability function of 0.625 for the overall optimization of complementary food from sorghum, soybean, karkade and premix.

Feeding energy and nutrient-dense foods provides children with the required energy/nutrients for proper growth and development as well as their daily demands would be fulfilled by fewer meal frequencies. The energy and nutrient intake/kg body weight by children would be maintained by

increasing the energy and nutrient density of their diet and not by an increase in the intake amount or volume.

In the current study, it was unable to compare the energy density of our optimized complementary food with some past reports because of the wrong energy density calculation which said, “Simply dividing the energy contents of the complementary foods by 100”. For example, Tenagashaw *et al.* [229] reported energy density of complementary food formulated from blends of teff, soya bean and orange flashed sweet potato ranged from 3.70 to 3.76 kcal/g and Ayele *et al.* [121] reported the energy density of complementary foods from kocho, pumpkin fruit, red kidney bean and maize at 3.3 kcal/g following the same calculation which was over-exaggerated. Even higher energy-dense foods have energy density of 1.07–1.46 kcal/g where in this case the approximate quantity of complementary food that would meet the energy needs of the children would be 137–187 g/day at 6-8 months, 206-281 g/d at 9-11 months, and 378-515 g/d at 12-23 months [30, 219]. Conversely, the current energy density of our optimized novel complementary food was higher than the energy density reported by Abebe *et al.* [230] where the traditional corn-based porridge consumed as complementary foods in rural villages of Sidama zone of Southern Ethiopia contains an energy density of 0.53 kcal/g, while kocho-based contained 0.49kcal/g, both of which contained very low energy levels.

In agreement with the present finding, Tenagashaw *et al*. [229]) reported that the protein density of complementary foods from blends of teff, soya bean and orange flashed sweet potato was in the range of 3.50 to 4.79 g/100 kcal. It also agreed with the report by Ayele *et al*. [121] where the protein density of an optimized formula from kocho, pumpkin fruit, red kidney bean and maize was 3.0 g/100 kcal. Alternatively, the protein density of homemade complementary foods in Wolayita zone of Ethiopia contained lower protein density ranging from 2.13–2.48 g/100kcal as reported by Abeshu *et al*. [30]. This showed that the new optimized novel complementary food is superior to the traditional ones, met the recommended standard and can provide the daily protein and energy demands by 6-23 months old children if served the amount estimated for the respective age group and fed three times per day.

Conclusion

This study demonstrated that optimized energy and protein-dense novel complementary food with improved sensory acceptability can be developed by the inclusion of *dabi teff*, the underutilized/forgotten food crop into locally available pre-processed food crops *viz*., germinated maize, roasted barley, roasted field pea, dehulled oats and linseed flours that can fulfill the FAO/WHO standard and exhibit superior quality over the traditional complementary food and the Cerifam® faffa flour. Each of the components used contributed an important nutrient of interest to develop a nutritionally complemented optimized product. The results indicated that the optimized condition was identified at 34.66% *dabi teff*, 25% barley, 15% oats, 15.34% field pea, 5% linseed and 5% germinated maize flour ratios which were found to contain 15.74 g/100g protein, 5.09 g/100g fat, 2.26 g/100g ash, 2.88 g/100g fiber, 73.05 g/100g carbohydrate, 380.43 kcal/100g energy, 4.14 g/100kacl protein density and 1.27 kcal/g energy density. The lower fat content can be matched by looking for additional fat-energy-rich legumes. The findings suggested that the developed *dabi teff*-field pea-based optimized novel complementary food can be used as a sustainable food-based strategy to combat protein-energy malnutrition among children in less developed countries like Ethiopia.

Chapter 5.2:

Optimization of a Formula to Develop Iron-Dense Novel Composite Complementary Flour with a Reduced Phytate/Minerals Molar Ratio from *Dabi teff*-Feld Pea Based Blends Using a D-Optimal Mixture Design

Redrafted from: Diriba Chewaka Tura, Tefera Belachew, Dessalegn Tamiru, Kalkidan Hassen Abate. **Optimization of a formula to develop iron-dense novel composite complementary flour with a reduced phytate/minerals molar ratio from *dabi teff*-field pea-based blends using a D-optimal Mixture design.** *Frontiers in Nutrition. 2023; 10:1244571*. doi:10.3389/fnut.2023.1244571. frontiersin.org. *Oct 11*.

Abstract

Iron deficiency anemia is one of the major public health problems in children associated with inadequate intake of bioavailable iron. Thus, this research was aimed at incorporating *dabi teff*, an underutilized crop into other pre-processed local food crops *viz*., germinated maize, roasted barley, roasted field pea, dehulled oats and linseed to develop optimized iron-dense novel composite complementary flour with a reduced phytate/minerals molar ratio. Nutrisurvey software was employed to define ranges and they were constrained at 20-35% *dabi teff,* 0-30% field pea and 5-20% maize, while the rest were set constant at 25% barley, 15% oats and 5% linseed. Eleven experimental runs were generated from the six mixture components using Stat-Ease Design-Expert® software version 11, D-optimal. Inductively Coupled Plasma-Optical Emission Spectrometry was used for the determination of micronutrients. 'Scheffe' regression was used to fit and test model adequacy and numerical multi-response optimization was performed using the Design-Expert® to identify optimal points. *Dabi teff* had a significantly higher ($P < 0.05$) iron content at 86.5 mg/100g, iron density at 24.53 mg/100kcal and calcium content at 123.59 mg/100g. The new formulations had a significantly higher iron (3.31-4.36 times), iron density (3.25-4.27 times) and calcium (1.49 -1.58 times) as compared to the control flour and fulfilled FAO/WHO recommendations. The optimal formula was identified at 34.66% *dabi teff,* 25% barley, 15% oats, 15.34% field pea, 5% linseed and 5% maize flour ratios with response values at overall optimization to be 32.21 mg/100g iron, 77.51 mg/100g calcium, 2.59 mg/100g zinc, 0.233 phytate to iron molar ratio (Ph:Fe), 0.067 phytate to calcium molar ratio (Ph:Ca), 3.43 phytate to zinc molar ratio (Ph:Zn), and 6.63 phytate by calcium to zinc molar ratio (Ph*Ca:Zn) and it contained iron at level that is 2.01 times higher than the standard, 4.44 times higher than the control and iron density at 8.47 mg/100kcal that was 4.39 times higher than the control. These findings showed that optimized *dabi teff*-field pea based iron dense novel composite complementary flour with enhanced bioavailability can be developed and used as a sustainable food-based strategy to combat iron deficiency anemia among children in less developed countries like Ethiopia.

Key Words: *Dabi-teff,* Optimization, Iron dense, Phytate/minerals molar ratio, Novel complementary food

Introduction

Micronutrient malnutrition is one of the serious, life-threatening public health problems both in developed and developing countries [7]. Its deleterious effect is more significant in children under five years where more than 50% of the children worldwide suffer from one or more key micronutrient deficiencies and it often co-exists with infectious diseases [211]. The adverse health consequences of micronutrient deficiency in children include impaired physical and cognitive development, diminished learning and earning capacity, and reduced immune competency which may be difficult to reverse by nutrition interventions [46, 231].

The most often deficient micronutrients in developing countries include iron, zinc, calcium, vitamin A and iodine among others and their deficiency is mostly superimposed by protein-energy malnutrition, revealed to us a 'hidden hunger' [232]. Iron deficiency is the most prevalent micronutrient deficiency affecting around two billion people in the world. Anemia is a disease with multiple causes, both nutritional anemia (iron and vitamin deficiencies) and non-nutritional anemia (infection and blood loss). Among nutritional anemia, iron deficiency anemia is most common in children worldwide [233]. Global estimates show that the highest prevalence of anemia was 47.4% among under-five children with the prevalence rising to 64.6% in Africa [234]. According to the Ethiopian Public Health Institute (EPHI) and ICF [19] survey, 57% of children under-five years were anemic.

Micronutrient deficiency-related growth impairments usually occur during the complementary feeding period due to the high demand for varied micronutrients at this age [235]. Hallberg and Rossander [236] reported that micronutrient malnutrition especially anemia is most prevalent in children between 6 and 24 months of age than at any other time in the life cycle and the major causes are inadequate dietary intake of bioavailable iron, malaria and parasitic infections. For example, for 9-11 months old children, about 97% of iron, 86% of zinc, 72% of calcium, 76% of magnesium, 81% of phosphorus and 73% of sodium should come from complementary food, assuming average breast milk intake [6, 12, 219].

Zinc is one the vital micronutrients and its deficiency lead to stunted growth and infectious diseases. Globally it was estimated that zinc deficiency is responsible for about 16% of lower respiratory tract infections, 18% of malaria and 10% of diarrhea cases [38]. Calcium serves as a component of bones and teeth, a regulator of nerve and muscle function as well as blood clotting and many more functions and its deficiency usually leads to stunted growth in children and bone loss or osteoporosis in adults [40].

Traditional complementary foods in developing countries, including Ethiopia are predominantly cereal-based, often monotonous and are characterized by low nutritive value, low micronutrient density and high ant-nutritional factors such as phytates, tannin and other phenolic compounds and fiber which jeopardize the nutrient bioavailability of the products [31, 74]. Thus, meeting the very high demand for micronutrients by infants and young children using traditional complementary food is unlikely to be achieved. Animal-source foods like meat, liver and fish can provide the demand and they contain higher bioavailable minerals, however, these products are often expensive and unaffordable by low-income households particularly the rural poor with additional religious concerns [31]. Consequently, in developing countries, it was suggested that extending child breastfeeding beyond two years is the first line of defense against key micronutrient deficiencies such as iron deficiency anemia although the iron content of human milk is relatively low [6].

Micronutrient supplementation and fortification were proposed as effective strategies to improve mineral bioavailability and combat micronutrient deficiencies [237] though they posed limitations in developing countries due to less accessibility to such programs. In Ethiopia, efforts were being made to implement a multi-sectorial plan of high-impact nutrition-specific and nutrition-sensitive interventions such as the Multi-Sectoral Nutrition Coordination and Integration Guideline (2017), the Nutrition-Sensitive Agriculture Strategy (2016-2020), the School Feeding Program (2019) to end child under-nutrition including micronutrient deficiencies by 2030 [56, 238]. However, the country is still experiencing one of the worst situations where micronutrient deficiencies including iron, zinc and vitamin A deficiencies are unacceptably high among infants and young children. This calls for searching for other promising alternatives such as exploring locally available underutilized/forgotten nutritious food crops to develop an

optimized micronutrient-dense composite complementary flour, which has been suggested to be the best food-based approach [52, 61].

The food-based or the dietary change approach is a combination of nutrient-rich food production and consumption-oriented among children, pregnant and lactating women. The approaches provide several micronutrients at once, affordable and sustainable and can be adapted to different cultural, dietary traditions and locally feasible strategies [52, 61]. It focuses on the combination of diversified foods that can meet the nutrient requirements of the target group and is based on the fact that people eat foods, not nutrients or supplements [32, 91]. The FAO/WHO [28] codex alimentarius on complementary feeding guidelines describes that mixtures of cereals, legumes, pulses/oilseed can constitute an appropriate source of nutrients and energy, essential fatty acids and limiting amino acids with many functional and health benefits as well as improved organoleptic characteristics. Enzymatic processing like germination and fermentation or non-enzymatic processing such as roasting, soaking, maceration and dehulling are among the most known techniques to reduce the deleterious effects of anti-nutrients and increase the nutrient densities and bioavailability of key micronutrients [91].

The crucial effects of roasting crops includes that it reduces microorganisms and destroys insects; inactivates trypsin enzyme inhibitors thus improving protein digestibility, amino acids bioavailability and keeping qualities as well as enhances the flavor and taste of food products through dextrinization of starch [28].

Germination is a bioprocess technology that might in particular increase protein digestibility; enhances the absorption and bioavailability of micronutrients including vitamins such as riboflavin (B_2), pyridoxine (B_6) and vitamin C, vitamin E and antioxidants [52]. Additionally, germination helps in the degradation of phytate-mineral complex and polyphenol-protein complex and also decreases condensed tannin thus facilitating mineral and protein absorption. Furthermore, the enzyme α-amylase synthesized during germination hydrolyzes starch to dextrin (dextrinization) and maltose (referred to us the amylase-rich food technology) and reduces its water-holding capacity thereby reduces the viscosity of thick cereal-legume porridges and enhances or maintain their energy and nutrient densities for infants and young children who have limited gastric capacity [28, 52].

Maceration is a hydrothermal processing that involves hydration and heating, enabling the release of micronutrients from the plant matrix and improving the nutrient availability of foods by destroying (leaching) of anti-nutritional compounds. This wet-heat treatment of foods had been attributed to the increased solubility of some minerals like iron, calcium and magnesium causing the leaching of the micronutrient into the processing water, thereby leading to the bulk micronutrient losses over a long period of maceration. Thus, short-time maceration is recommended to avoid such bulk losses of key micronutrients [239]. The sub-Saharan African region is endowed with a rich diversity of 'orphan crops' having improved nutritional and medicinal values as well as sources of income generation [96]. Little or no attention was given to such crops in terms of research and development and policy framework that could promote their extensive agricultural production, industrial utilization as well as home consumption. *Dabi*-teff *(Eragrostis teff*), a farmer variety teff, is one of the underutilized/marginalized or forgotten food crops grown in Ethiopia (Oromia region) that could be utilized to combat micronutrient malnutrition.

Dabi teff is the 'afaan oromoo' language name for an early maturing variety of dark red teff due to which the crop is harvested twice within one rainy season (at early rain fall called "*daabii* gannoo" and late rain fall called "*daabii* birraa"). An early maturing and low rainfall requirement makes *dabi teff* a unique variety teff amenable to climate-smart agriculture. Farmers in Wollega and Illuababor, western Ethiopia, cultivate *dabi teff* either for its grain seeds or its straw. The contribution of *dabi teff* grains to household food and nutrition security is considerable when viewed through a food security lens while the straw is composed of fine stems used for plastering (finishing) mud hut walls during house construction and rated higher among others for animal fodder, especially oxen feeding (personal experience). Red teff varieties including *dabi teff* grown in Ethiopia are lower in price as compared to the white varieties and have been implicated in the low incidence of anemia which is presumed to be due to the red teff grain's high iron content [27].

There are many social beliefs regarding the nutritional and health benefits of *dabi teff* among the consumers that after eating the different food forms of *dabi teff* products such as *'kita/maxinoo* (unleavened bread), *muk/mooqa* (gruel), *mooqa manyee and cafaqoo (the favorite cultural*

dishes)', it "*dhiiga dabalaa*" to mean it increases blood volume, "*humna dabalaa*" meaning it boosts energy/strength, "*dugda jabeessa*" meaning it repairs/strengthening back bone and fractured bone which could be implicated for its high calcium content where *dabi teff* is prized as medicinal food among the rural elderly people in particular and the consumers in general (Personal communication).

Based on these social beliefs and personal long-time observations, a preliminary study on selected micronutrient contents of *dabi teff* was conducted where it was found to contain a considerable amount of iron at 122.66 mg/100g, calcium at 190 mg/100g and zinc at 1.8 mg/100g (preliminary findings by the researcher) **(Annex V)** which created a pivotal springboard to carry out intensive research work.

Despite the social beliefs and the preliminary findings, to date, the potential of incorporating *dabi teff* into complementary flour formulations in combating micronutrient malnutrition in infants and younger children is not yet examined. Thus, this research was aimed at incorporating *dabi teff* into pre-processed local food crops *viz*., germinated maize, roasted barley, roasted field pea, dehulled oats, and minimally cooked linseed for optimization of a formula to develop iron-dense novel composite complementary flour from *dabi teff*-field pea based blends and to identify the optimal formula (sweet spot) with enhanced bioavailability using D-optimal mixture design and to suggest the promising potential of *dabi teff*-filed pea based novel composite complementary food to combat iron deficiency anemia among children in Ethiopia and in Africa in general.

Materials and Methods

Details of the methods related to the crop sampling, processing, flour preparation and handling were described in **Chapter 2**. Literature-based nutrient content of each crop was considered during the selection process which was later verified through micro-composition analysis **(Table 5.9).**

Results

Dietary mineral contents of the individual processed flours

In the current study, seven different dietary minerals were studied for their contents in the individual components as well as in the formulated composite flours. Iron, calcium and zinc were more emphasized than other minerals in the formulated composite flours because these micronutrients are the limiting micronutrients in diets of infants and young children which are strongly associated with child physical growth, mental and cognitive development and deficiency disease related to them are most common [6].

In this study, the mean and standard deviations of mineral contents of the individual components used were presented in **Table 5.9**. A significant difference ($P < 0.05$) was observed in mean values of mineral contents among the components, ranging between 1.12 and 86.5 mg/100g for iron, 15.78 and 123.59 mg/100g for calcium, 1.56 and 6.63 mg/100g for zinc, 78.87 and 279.86 mg/100g for magnesium, 260.2 and 720.14 mg/100g for potassium, 227.58 and 488.97 mg/100g for phosphorous and 1.57 and 7.91 mg/100g for sodium **(Table 5.9)**. *Dabi teff* flour has shown the highest ($P < 0.05$) amount of iron and calcium content at 86.5 mg/100g and 123.59 mg/100g, respectively, compared to the other components **(Table 5.9)**. The second higher iron content was recorded for linseed flour at 47.13 mg/100g whereas the remaining components contained significantly lower iron, with germinated maize being the least at 1.12 mg/100g.

The calcium content of *dabi teff* was significantly higher ($P < 0.05$) at 123.59 mg/100g than the others, followed by roasted field pea flour at 59.99 mg/100g. Linseed flour contained a significantly higher ($P < 0.05$) amount of zinc content at 6.63 mg/100g, followed by roasted barley flour at 2.46 mg/100g while *dabi teff* flour showed lower zinc content at 1.94 mg/100g. Similarly, linseed contained significantly higher ($P < 0.05$) magnesium content at 279.86 mg/100g followed by *dabi teff* flour at 142.48 mg/100g.

Anti-nutritional factors (phytate and tannin) of the individual processed flours

The mean phytate contents of the component were significantly different at P value of < 0.05 and ranged between 80.6 mg/100g and 158.9 mg/100g. The tannin content was similar among the components, except for oats flour, which had a significantly higher ($P < 0.05$) tannin content at 25.55 mg/100g as compared to the others **(Table 5.13)**. *Dabi teff* contained 92.8 mg/100g of phytate and 14.07 mg/100g of tannin whereas field pea contained a relatively higher phytate at 158.89 mg/100g.

Table 5.9: Dietary minerals content of the individual components processed flour

Individual flour components	**Micronutrient Contents (mg/100g)**						
	Iron	Calcium	Zinc	Magnesium	Potassium	Phosphorous	Sodium
Dabi teff	86.50±0.42[a]	123.59±1.02[a]	1.94±0.04[a]	142.48±0.39[a]	297.97±3.97[a]	237.14±1.43[a]	4.90±0.31[a]
Roasted Barley	5.00±0.06[b]	32.49±0.76[b]	2.46±0.03[b]	87.48±1.06[b]	355.45±6.11[b]	279.09±2.13[b]	3.94±0.40[a]
Dehulled Oats	4.37±0.07[bc]	26.33±0.59[c]	2.28±0.03[c]	78.87±0.44[c]	319.04±6.74[c]	261.23±2.32[c]	1.57±0.51[b]
Roasted Field pea	7.05±0.19[d]	59.99±0.61[d]	2.28±0.02[cd]	89.54±1.44[bd]	720.14±4.41[d]	227.58±7.82[a]	1.69±0.77[bc]
Cooked Linseed	47.13±0.33[e]	28.83±0.23[e]	6.63±0.10[e]	279.86±2.23[e]	563.28±6.02[e]	488.97±0.78[b]	7.91±0.51[d]
Germinated Maize	1.12±0.02[f]	15.78±0.45[f]	1.56±0.06[f]	80.91±0.47c[f]	260.20±1.67[f]	236.48±4.07[a]	3.73±0.47[a]

Values are means ± Standard deviation of the triplicate determinations. Values in the same column followed by different superscripts are significantly different at $P < 0.05$.

Table 5.10: Nutrisurvey analysis of the food records (customized) to define the ranges of each component in the formulations

Components	Proportion (%) (lower-upper)	Calculated energy and nutrient values of the formulated meal by Nutrisurvey								
		Amount eaten range/day (g)	Energy range (kcal)	CHO range (g)	Protein range (g)	Fat range (g)	Iron range (mg)	Calcium range(mg)	Zinc range (mg)	Magnesium Range (mg)
Dabi teff	20-35	45-79	158.7-278.7	30.9-54.2						
Roasted Barley	25	56	212.9	43.5						
Dehulled Oats	15	34	129.5	23.8						
Roasted Field pea	0-30	0-68	0-238.3	0-41.1						
Cooked Linseed	5	12	62.4	3.4						
Germinated Maize	5-20	11-45	41.4-169.5	8.3-33.9						
Analyzed value (range) for the formulated meal using our components			604.9-1091.5*	109.9-199.9 (73-74%)	19-39.8 (13-15%)*	10-14.8 (15-12%)	49-83.6*	87.8-176.1	4-6.7*	183-320.3
Recommended value for 1-3 years old child as generated by the 'Nutrisurvey' software			718.3	102.5 (>55%)	21.2 (12%)	24.4 (<30%)	8	600	3	80
Percentage fulfillment from our formulated meal (range) (lower-upper)%			84-152*	107-195*	90-188*	41-61	612-1045*	15-29	133-225*	229-400*

*Showing the blended complementary flours would be promising to attain the PAHO/WHO recommendations.

Micronutrient contents of the formulated composite complementary flour

Model fitting and testing model adequacy

The fitted models were found to be adequate and significant for most response variables based on the F-statistic (the ANOVA regressions outputs); the P-value, the coefficient of determinations R^2, and the agreement between R^2_{adj} and R^2_{pred}. In other words, the developed models were declared to be adequate for describing the effects of determinant variables (all the possible model terms) on the response variables. **Table 5.11** shows that the linear model was adequately fitted for iron, zinc, Ph:Fe, Ph:Zn and Ph*Ca:Zn contents at P-values of <0.05, whereas for calcium, the same linear model was suggested by the software, but it was not significant (P >0.05).

This shows that x_1, x_4 and x_6 were the significant model terms for these variables with their corresponding p-values at 0.0001, 0.0164 and 0.1959 for iron, zinc and calcium, respectively. This means that changes in iron, zinc, Ph:Fe, Ph:Zn and Ph*Ca:Zn can adequately be described by the linear models (adequate predictive power) as a function of the component ratio variations in the formulations.

Table 5.11: Models fitted for responses and statistical outputs showing the significance of regression models and model adequacy

Model's P-value (model terms)	Mineral Contents			Phytate/Minerals Molar Ratio			
	Iron	Calcium	Zinc	Ph:Fe	Ph:Zn	Ph:Ca	Ph*Ca:Zn
Linear (x_1,x_4,x_6)	0.0001#***	0.1959 #^	0.0164#*	0.0001#***	0.0269#*	0.101	0.0240 #*
Quadratic (x_1^2, x_4^2, x_6^2)	0.2371	0.4114	0.4476	0.8767	0.4914	0.156#^	0.6042
Special Cubic	0.4765	0.4444	0.1574	0.6340	0.6445	0.837	0.7531
Cubic	0.2183	0.9156	0.8113	0.3527	0.5676	0.648	0.3427
Sp. Quartic vs Quadratic	0.5088	0.8675	0.2753	0.0623	0.3208	0.455	0.3105
Interaction terms							
x_1x_4	-	-	-	-	-	0.036	-
x_1x_6	-	-	-	-	-	0.122	-
x_4x_6	-	-	-	-	-	0.502	-
$x_1x_4x_6$	-	-	-	-	-	-	-
F-statistics (Anova)							
Model F-value	59.3	2.01	7.18	63.43	5.88	3.66	6.16
Lack-of-Fit	PE0	PE0	PE0	PE0	PE0	PE0	PE0
R^2	0.9368	0.3347	0.6423	0.9407	0.5949	0.786	0.6064
R^2%	93.68	33.47	64.23	94.07	59.49	78.56	60.64
ADP	18.63	3.73	6.94	19.25	6.35	5.4	6.46
Model	0.0001*	0.1959^	0.0164*	0.0001*	0.0269*	0.09^	0.0240*

Key: Sp.-special, vs-versus, PEO- pure error zero, R^2%-coefficient of determination, ADP-adequate precision Ph:Fe-phytate to iron, Ph:Zn-phytate to Zinc, Ph:Ca-phytate to calcium, #-suggested, #*, #** and #***-model is suggested and significant at $P < 0.05$, at $P < 0.01$ and at $P < 0.001$, respectively, #^ -suggested and not significant, * and ^ model is significant and not significant respectively, x_1-*dabi teff*, x_4- field pea, x_6- germinated maize.

Table 5.12: Goals set, the relative importance of each variable and the optimal values at optimal conditions identified

Name	Goal	Lower Limit	Upper Limit	Importance	Optimum value
Independent variables					
A:*Dabi teff* Flour	In range	20	35	3	34.66
D:Field pea Flour	In range	0	30	3	15.34
F:Maize Flour	In range	5	20	3	5
Barley flour	Constant	25	25	3	25
Oats flour	Constant	15	15	3	15
Linseed flour	Constant	5	5	3	5
Dependent variables					
Iron (mg/100g)	maximize	24.007	31.5801	5	32.21
Calcium (mg/100g)	maximize	73.4631	78.1767	5	77.51
Zink (mg/100g)	maximize	2.326	2.612	5	2.59
Magnesium (mg/100g)	maximize	113.899	124.223	3	118.64
Potassium (mg/100g)	maximize	364.768	497.482	3	439.33
Phosphorous (mg/100g)	maximize	281.028	308.538	3	287.99
Sodium (mg/100g)	maximize	3.455	6.246	3	4.44
Phytate (mg/100g)	minimize	85.2	104.12	5	86.01
Ph:Fe	minimize	0.232	0.344	5	0.233
Ph:Zn	minimize	3.356	4.18	5	3.43
Ph:Ca	minimize	0.067	0.085	5	0.067
Ph*Ca:Zn	minimize	6.457	7.943	5	6.63

Dietary mineral contents of the formulated composite complementary flours

The mean iron, calcium, zinc, magnesium, potassium, phosphorous and sodium contents of the newly formulated composite complementary flours were determined to be 28.12 mg/100g , 76.25 mg/100g and 2.47 mg/100g, 120.57 mg/100g, 443.99 mg/100g, 293.25 mg/100g and 4.52 mg/100g, respectively **(Table 5.14)**. The new formulations incorporated with *dabi teff* flour had a significantly higher ($P < 0.05$) iron content (3.31-4.36 fold), iron density (3.25-4.27 fold), calcium content (1.49 -1.58 fold), magnesium content (1.18-1.29 fold), and sodium (1.54 fold) as compared to the control flour. Zinc content was almost similar among the formulations.

In this study, the mean iron content of the formulations ranged from 24.01 to 31.58 mg/100g whereas the mean calcium content ranged from 73.46 to 78.18 mg/100g. The mean zinc content ranged from 2.33-2.61 mg/100g **(Table 5.14)**. Zinc content of the formulated flours was slightly lower than the control flour at 2.64 mg/100g and that of the Cerifam® faffa flour at 5 mg/100g. The magnesium content of the new formulations ranged from 113.9-124.22 mg/100g while sodium content ranged from 3.46-6.25 mg/100g. There was no significant difference ($P > 0.05$) between the sodium content of the formulations and the control. The phosphorous and potassium contents of the newly formulated complementary flours ranged from 281.03-308.54 mg/100g and 364.77-497.48 mg/100g, respectively (Table 6.6).

Anti-nutritional factors (Phytate and Tannin) of the formulated complementary flours

The mean values of phytate and tannin were determined to be 95.91 mg/100g and 14.40 mg/100g, and there observed a significant difference ($P < 0.05$) among the new composite formulations where their contents ranged from 85.2-104.12 mg/100g for phytate and from 8.35-23.1 mg/100g for tannin level **(Table 5.15)**. The phytate content of the new formulations was significantly lower ($P < 0.05$) as compared to that of the control at 126.54 mg/100g, and this could be attributed to the processing methods applied on the individual mixture components. However, tannin content was similar among most of the formulations and also to that of the control, which was determined at 12.42 mg/100g.

Table 5.13: Anti-nutrient contents and phytate/minerals molar ratio of the individual components processed flours

Individual flours	Ant-nutrients (mg/100g)		Phytate/mineral molar ratio			
	Phytate	**Tannin**	**Ph:Fe**	**Ph:Ca**	**Ph:Zn**	**Ph*Ca:Zn**
Dabi teff	92.8±001[a]	14.07±2.55[a]	0.091±0.01[a]	0.046±0.01[a]	4.711±0.01[a]	14.556±0.01[a]
Roasted Barley	148.28±0.37[b]	12.84±0.59[a]	2.516±0.01[b]	0.277±0.01[b]	5.936±0.01[b]	4.822±0.01[b]
Dehulled Oats	152.2±0.1[c]	25.55±1.27[b]	2.955±0.01[c]	0.35±0.03[c]	6.574±0.01[c]	4.328±0.01[c]
Roasted Field pea	158.9±0.1[d]	11.82±0.01[a]	1.912±0.01[d]	0.161±0.01[d]	6.864±0.01[d]	10.294±0.01[d]
Cooked Linseed	118.4±0.28[e]	12.86±0.54[a]	0.213±0.01[e]	0.249±0.01[be]	1.759±0.01[e]	1.268±0.01[e]
Germinated Maize	80.6±0.01[f]	13.01±1.51[a]	6.106±0.03[f]	0.31±0.01[f]	5.088±0.01[f]	2.007±0.01[f]
		Critical value	<1	<0.24	<15	<200

Key: Values are means ± Standard deviation of the triplicate determinations. Values in the same column followed by different superscripts are significantly different at P <0.05.

Phytate/minerals molar ratio of the formulated composite complementary flours (In-vitro mineral bioavailability)

The mean phytate/minerals (iron, zinc, and calcium) molar ratio of the newly formulated composite complementary flours is presented in **table 5.15**. Phytate/iron molar ratio (Ph:Fe) ranged from 0.232 to 0.344 among the formulations with the mean value of 0.29, which was far below the critical limit of 1 **(Table 5.15)**. Phytate/calcium molar ratio (Ph:Ca) of the newly formulated complementary flours ranged from 0.067 to 0.085, with a mean value of 0.076, which was below the critical limit of 0.24:1; where above this limit, calcium absorption can be impaired [31]. Phytate/zinc molar ratio (Ph:Zn) ranged from 3.356 to 4.18 among the formulations, with a mean value of 3.83, while the phytate by calcium to zinc molar ratio (Ph*Ca:Zn) ranged from 6.457 to 7.943 among the formulations with a mean value of 7.29, which was far below the critical limits of 200.

Variables optimization and validation

From the multi-response numerical optimization, the optimal formula (sweet spot) for the overall optimization was identified to be at 34.66% *dabi teff* flour, 25% barley, 15% oats, 15.34% field pea, 5% linseed and 5% germinated maize flours, with the predicted optimal value for the dietary minerals at 32.21 mg/100g for iron, 77.51 mg/100g for calcium, 2.59 mg/100g for zinc, 118.64 mg/100g for magnesium, 439.33 mg/100g for potassium, 287.99 mg/100g for phosphorous, 4.44 mg/100g for sodium, 86.01 mg/100g for phytate, 0.233 for the Ph:Fe molar ratio, 3.43 for the Ph:Zn molar ratio, 0.067 for the Ph:Ca molar ratio and finally, 6.63 for the Ph*Ca:Zn molar ratio, respectively **(Table 5.12)** where the maximum selected combined desirability function was 0.651 **(Figure 5.4).** Results obtained at optimum conditions showed significantly higher iron (4.44 fold), calcium (1.57 fold), and magnesium (1.23 fold) than that of the control; thus, the optimized novel composite complementary flour demonstrated superiority ($P < 0.05$) in terms of minerals, specifically in terms of iron content, over the traditional complementary flour **(Table 5.14).**

Table 5.14: Dietary mineral contents of the formulated composite complementary fours

Formulations	Micronutrient Contents (mg/100g)							
	Iron	**Calcium**	**Zinc**	**Magnesium**	**Potassium**	**Phosphorous**	**Sodium**	Iron density
F1	28.51±0.17^{a}	73.46±0.85^{a}	2.42±0.02^{a}	113.90±2.01^{a}	424.25±10.25^{a}	288.35±4.31^{a}	3.46±0.66^{a}	7.53
F2	27.86±0.12^{a}	76.48±0.12^{b}	2.58±0.04^{a}	121.52±0.25^{b}	482.48±5.71^{b}	297.87±5.04^{a}	4.64±0.05^{a}	7.31
F3	30.71±0.23^{bc}	76.63±0.10^{bc}	2.61±0.02^{b}	120.64±0.72^{bc}	488.25±6.10^{bc}	296.06±5.26^{a}	4.29±0.04^{a}	8.10
F4	24.01±0.09^{bd}	76.19±0.11^{bd}	2.50±0.03^{a}	120.54±1.07^{bd}	467.67±2.91^{bd}	308.54±2.14^{ab}	6.25±0.05^{b}	6.27
F5	30.33±0.41^{bc}	76.07±0.18^{bc}	2.42±0.06^{a}	122.72±0.02^{be}	364.77±10.59^{be}	291.03±3.58^{a}	3.97±0.41^{a}	7.90
F6	31.58±0.37^{bc}	78.18±0.33^{e}	2.45±0.06^{a}	118.22±2.30^{f}	427.37±0.79^{ac}	283.96±6.37^{ac}	4.60±0.68^{a}	8.25
F7	24.38±0.09^{bd}	75.25±0.05^{bf}	2.33±0.08^{ad}	122.18±0.28^{bg}	437.62±3.74^{ab}	292.29±3.20^{a}	4.04±0.07^{a}	6.33
F8	31.20±0.12^{bc}	77.57±0.05^{bg}	2.52±0.06^{a}	118.07±0.08^{h}	386.47±7.42^{cf}	281.03±2.04^{ac}	4.57±0.03^{a}	8.20
F9	24.51±0.17^{bd}	75.89±0.05^{bh}	2.39±0.09^{af}	121.07±0.24^{b}	497.48±4.20^{bg}	298.94±4.40^{a}	4.27±0.04^{a}	6.43
F10	25.68±0.09^{b}	76.22±0.06^{bd}	2.46±0.08^{a}	123.19±0.11^{be}	479.65±9.48^{bh}	300.15±2.00^{a}	5.69±0.09^{ad}	6.75
F11	30.58±0.09^{bc}	76.81±0.11b^{j}	2.52±0.04^{a}	124.22±0.18^{be}	427.87±10.08^{ab}	287.55±4.61^{a}	3.92±0.02^{c}	7.90
Mean	28.12	76.25	2.47	120.57	443.99	293.25	4.52	7.36
Control	7.25±0.11^{b}	49.45±0.06^{i}	2.64±0.07^{c}	96.45±0.22^{i}	398.67±6.95^{i}	282.65±3.14^{ad}	4.07±0.04^{a}	1.93
Optimal	32.21	77.52	2.59	118.64	439.33	287.99	4.44	8.47
FAO/WHO	16	500	3.2	-	-	-	-	0.8-4
Cerifam® Faffa	6	100^{m}	5	-	-	-	-	-

Key: Values are means ± Standard deviation of the triplicate determinations. Values in the same column followed by different superscripts are significantly different at P <0.05. The corresponding component ratio for each formula code (F1- F11) is well described in **Table 2.1**

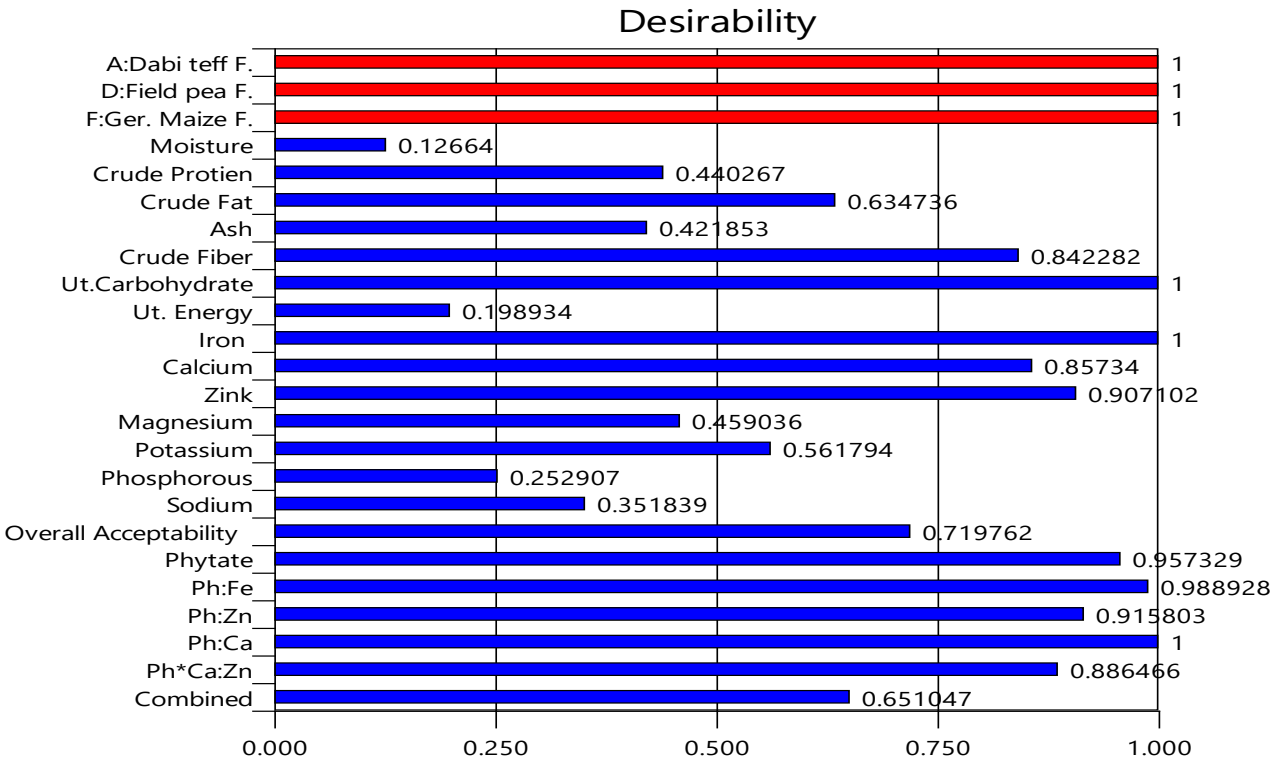

Figure 5.4: Bar graph showing the desirability functions of each variable and the combined desirability function.

Figure 5.5 shows the material balance design of the defined ranges of the components, their nutrient contents and the optimal condition identified.

To validate the optimal condition, the ratio of each component at the optimized formula was blended, and laboratory analysis of the micronutrient compositions of the blend was conducted. The validation analysis result were 31.76 mg/100g for iron, 76.88 mg/100g for calcium, 2.53 mg/100g for zinc, 119.61 mg/100g for magnesium, 440.66 mg/100g for potassium, 291.48 mg/100g for phosphorous, 4.48 mg/100g for sodium, 90.93 mg/100g for phytate, 0.236 for Ph:Fe, 3.52 for Ph:Zn, 0.074 for Ph:Ca and 7.22 for Ph*Ca:Zn. These values and the predicted response values at optimal conditions were significantly higher at P value <0.05 than the control flour, except for zinc and phosphorous contents **(Table 5.16)** which showed the superiority of the optimized composite novel complementary flour over the control flour. Further, the predicted response values derived from the model equations at optimal and the experimental (validation) values obtained under laboratory analysis were presented in **Table 5.17**, which showed there was

no significant difference (P >0.05) between the predicted optimal response values and the validation (laboratory analysis) values except for potassium (slight difference). This showed that there was a good agreement between the predicted optimal response values at optimal conditions and the validation values, thereby confirming the validity and adequacy (accuracy) of the model equations to predict optimal response values. The optimization process was verified using the graphical optimization technique **(Figure 5.3)** where the overlay counter plot was generated for the most important variables to identify the optimum regions. Any design point that falls within the yellow region (with the very conservative silver-gray region) in the overlay plot represents an optimal combination of *dabi teff* flour, field pea and germinated maize flours in combination with the other constant components that can bear optimal micronutrient contents with reduced phytate/minerals molar ratio to the minimum within 95% confidence interval (CI).

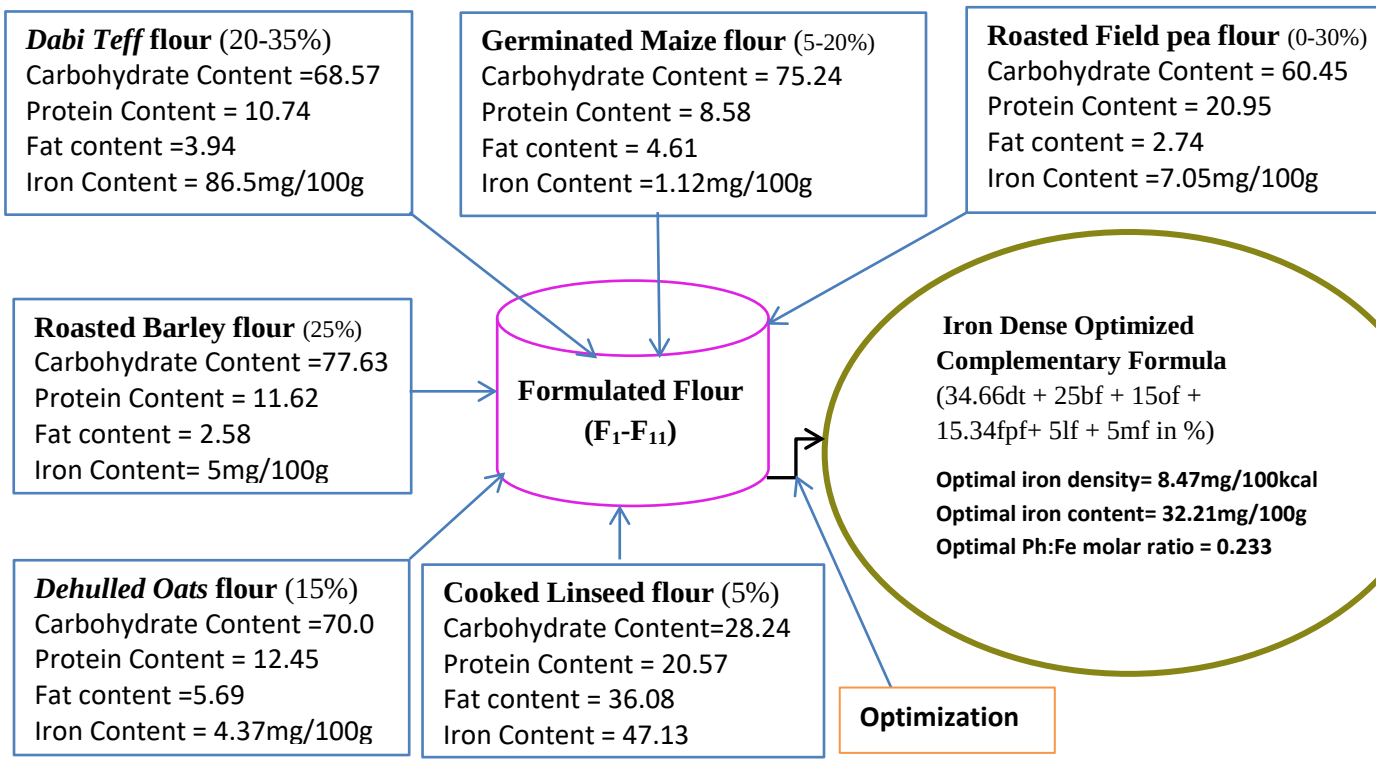

Figure 5.5. Material balance design showing individual flour ranges and their nutrient contents and the optimal condition identified.

Key: dt = *dabi teff*, bf = barley, of = oats, fp = field pea, lf = linseed, mf = maize flours

Micro-nutrient densities of the optimized complementary flour

The micro-nutrient densities of the optimized, the control, the Cerifam® faffa flour and the PAHO/WHO [219] recommendations for complementary food for average breastfed children were presented in **Table 5.18**. The iron density of the optimized complementary flour at 8.47 mg/100kcal was compared to the recommendation accounted for high, moderate and low bioavailability. The recommended value for 6-8, 9-11 and 12-23 months-old children are 2.5 mg/100kcal, 1.5 mg/100kcal and 0.5 mg/100kcal when accounted for high bioavailability, 4.0 mg/100kcal, 2.4 mg/100kcal and 0.8 mg/100kcal when accounted for moderate bioavailability, and 7.7 mg/100 kcal, 4.6 mg/100 kcal and 1.6 mg/100kcal when accounted for low bioavailability, respectively [219, 240, 241]. The determined zinc density of the new formulations was at 0.68 mg/100kcal while the calcium density was 20.37 mg/100kcal.

Table 5.15: Anti-nutrient contents and phytate/minerals molar ratio of the formulated composite complementary flours

Formulations	Mixture components ratio (%)						Anti-nutrient contents (mg/100g)		Phytate/minerals molar ratio (In-vitro Bioavailability)			
	DTF	BAF	OF	FPF	LSF	GMF	Phytate	Tannin	Ph:Fe	Ph:Zn	Ph:Ca	Ph*Ca:Zn
F1	27.50	25	15	15.00	5	12.50	102.72 ± 1.19^{a}	15.48 ± 2.29^{a}	0.306 ± 0.01^{a}	4.180 ± 0.03^{a}	0.085 ± 0.01^{a}	7.677 ± 0.01^{a}
F2	26.123	25	15	23.877	5	5.00	96.8 ± 01^{b}	13.01 ± 1.51^{a}	0.295 ± 0.01^{a}	3.695 ± 0.01^{b}	0.077 ± 0.01^{a}	7.065 ± 0.01^{b}
F3	30.457	25	15	19.543	5	5.00	97.1 ± 01^{b}	17.23 ± 3.1^{a}	0.268 ± 0.01^{a}	3.664 ± 0.01^{bc}	0.077 ± 0.01^{a}	7.019 ± 0.01^{bc}
F4	20.00	25	15	29.988	5	5.012	96.94 ± 0.99^{b}	18.06 ± 2.97^{ab}	0.343 ± 0.01^{ab}	3.819 ± 0.01^{d}	0.077 ± 0.01^{a}	7.274 ± 0.01^{d}
F5	28.793	25	15	6.207	5	20.00	99.38 ± 2.08^{b}	14.49 ± 2.31^{a}	0.278 ± 0.01^{a}	4.044 ± 0.01^{e}	0.079 ± 0.01^{a}	7.691 ± 0.01^{a}
F6	35.00	25	15	11.884	5	8.116	86.27 ± 1.12^{c}	10.64 ± 1.19^{ac}	0.232 ± 0.01^{be}	3.468 ± 0.01^{f}	0.067 ± 0.01^{a}	6.778 ± 0.15^{e}
F7	21.705	25	15	13.295	5	20.00	97.61 ± 0.4^{b}	11.44 ± 1.27^{a}	0.340 ± 0.03^{ae}	4.126 ± 0.01^{g}	0.079 ± 0.01^{a}	7.762 ± 0.01^{a}
F8	34.385	25	15	6.476	5	14.139	85.2 ± 0.86^{c}	23.1 ± 2.26^{b}	0.232 ± 0.01^{bc}	3.356 ± 0.01^{h}	0.067 ± 0.01^{a}	6.457 ± 0.01^{f}
F9	20.00	25	15	19.540	5	15.460	97.3 ± 0.16^{b}	8.35 ± 1.26^{ac}	0.337 ± 0.01^{ag}	4.009 ± 0.01^{i}	0.078 ± 0.01^{a}	7.607 ± 0.01^{a}
F10	22.735	25	15	22.515	5	9.750	104.12 ± 0.13^{a}	8.56 ± 0.85^{ac}	0.344 ± 0.01^{af}	4.168 ± 0.01^{a}	0.083 ± 0.01^{a}	7.943 ± 0.01^{g}
F11	29.604	25	15	10.076	5	15.320	91.52 ± 0.21^{d}	18.06 ± 1.44^{ab}	0.254 ± 0.01^{ad}	3.577 ± 0.01^{j}	0.072 ± 0.01^{a}	6.868 ± 0.01^{e}
Average							95.91	14.04	0.29	3.83	0.076	7.29
Control	0	80				20	126.54 ± 0.82^{e}	12.42 ± 1.09^{a}	1.481 ± 0.03^{e}	4.721 ± 0.01^{k}	0.155 ± 0.01^{b}	5.836 ± 0.01^{i}
					Recommended values (Critical limits)				<1:1	<15:1	<0.24:1	<200:1

Key: Values are means ± Standard deviation of the triplicate determinations. Values in the same column followed by different superscripts are significantly different at $P < 0.05$.

Table 5.16: Experimental validation of optimal condition and comparison with the control flour.

Response variables	Optimized value	Validation value	Contro value
Iron (mg/100g)	32.21[a]	31.76[a]	7.25[b]
Calcium (mg/100g)	77.51[a]	76.88[a]	49.45[b]
Zinc (mg/100g)	2.59[a]	2.53[a]	2.64[a]
Magnesium (mg/100g)	118.64[a]	119.61[a]	96.45[b]
Potassium (mg/100g)	439.33[a]	440.66[a]	398.67[b]
Phosphorous (mg/100g)	287.99[a]	291.48[a]	282.65[a]
Sodium (mg/100g)	4.44[a]	4.48[a]	4.07[b]
Phytate (mg/100g)	86.01[a]	90.93[a]	126.54[b]
Ph:Fe	0.233[a]	0.236[a]	1.48[b]
Ph:Zn	3.43[a]	3.52[a]	4.72[b]
Ph:Ca	0.067[a]	0.074[a]	0.16[b]
Ph*Ca:Zn	6.63[a]	7.22[a]	5.84[b]

Key: Values are means ± Standard deviation of the triplicate determinations. Values in the same raw followed by the same superscripts are not significantly different at P >0.05.

Table 5.17: Predicted optimal response values and Experimental (validation) values for verfication

Response variables	Optimized value	Validation value
Iron (mg/100g)	32.21[a]	31.76[a]
Calcium (mg/100g)	77.51[a]	76.88[a]
Zinc (mg/100g)	2.59[a]	2.53[a]
Magnesium (mg/100g)	118.64[a]	119.61[a]
Potassium (mg/100g)	439.33[a]	440.66[b]
Phosphorous (mg/100g)	287.99[a]	291.48[a]
Sodium (mg/100g)	4.44[a]	4.48[a]
Phytate (mg/100g)	86.01[a]	90.93[a]
Ph:Fe	0.233[a]	0.236[a]
Ph:Zn	3.43[a]	3.52[a]
Ph:Ca	0.067[a]	0.074[a]
Ph*Ca:Zn	6.63[a]	7.22[a]

Key: Values are means ± Standard deviation of the triplicate determinations. Values in the same raw followed by the same superscripts are not significantly different at P >0.05.

Table 5.18: Micronutrient densities of the composite complementary flours and Codex standards

Computed responses (mg/100kcal)	Optimal flour	Control flour	PAHO/WHO	Cerifam®
Iron density	8.47	1.93	0.8-4	1.52
Calcium density	20.37	13.20	>80	25.25
Zinc density	0.68	0.70	0.6-1.6	1.26
Magnesium density	31.19	25.74	-	-
Phosphorous density	75.70	75.43	-	-

Discussion

Minerals are vital nutrients for life. They are essential for healthy brain, bone and body development as well as growth regulators in children [7]. In the present study, the iron content of *dabi teff* determined to be 86.5 mg/100g was much higher than that of the commonly recommended animal source foods for iron such as liver (17.9 mg/100g edible portion) and red beef meat (3.79 mg/100g) [242]. However, the problem with plant-source iron is its non-heme form limiting its absorption. This problem can be resolved by suggesting the consumption of vitamin-C-rich foods such as citrus fruits. When compared to the FAO/WHO [28] recommendation, *dabi teff* was shown to contain 5.4 times higher iron content than the requirement from complementary food and it contained an iron density of 24.53 mg/100kcal given its energy value to be 352.70 kcal/100g [243] making the crop a super iron bank. Contrary to the present findings, a lower level of iron content (38 mg/100g) was reported by Mezgebo *et al.* [166] for the popular red teff grown in Ethiopia. Similarly, Daba [27] reported lower iron content of the red-teff (Dz-01-1681) variety at 20.6 mg/100g. The high iron level of *dabi teff* determined in this study magnified the importance of incorporating the crop into other pre-processed local food crops to develop iron-dense optimized novel composite complementary products.

In agreement with the present findings, Woldemariam *et al.* [224] reported the calcium content of the popular red teff at 102.96 mg/100g. Even though it may not be as such reasonable to compare the nutrient contents of cereals and legumes because of their different natural physiognomies, in the present study, we have compared simply because they were the selected

components. We have compared them to justify that cereals and legumes need to be mixed to nutritionally complement each other to develop a wholesome and nutritionally complete end product. Aligned with this, the nutrient content of *dabi teff*, specifically zinc and magnesium were incomparably higher (in some cases similar) than other cereals such as sorghum [103] although they were lower than some of our current components.

Compared to the critical limits, the phytate and tannin content of all the individual components were shown to be below the maximum tolerable limit at 200 mg/100g for phytate and 560 mg/100g for tannin, respectively [31, 37]. These two anti-nutrients are of concern in limiting the absorption and bioavailability of nutrients from cereals and legumes foods, but currently, there would not be a remarkable problem foreseen because of their determined low level in all our components. Specifically, *dabi teff* flour which contained the highest iron and calcium content **(Table 5.9)** had the lowest phytate and tannin content as well as the phytate/iron molar ratio which was calculated to be 0.091 **(Table 5.13)** which was far below the critical limit (<1) [31]. This demonstrated the good proxy indicator of iron abortion and bioavailability from *dabi teff*-field pea-based complementary food products. These lower anti-nutrient values of *dabi teff* and others, add to the justification of our components to be the best candidates in the formulations.

Iron is a vital nutrient necessary for building and maintaining healthy red blood cells and for making hemoglobin [7]. When compared to FAO/WHO [28] guideline for the nutrient content of formulated complementary foods, the mean value of the new formulations had significantly higher ($P < 0.05$) mean iron content (1.76 fold) where the recommended value is at 16 mg/100g whereas none of the formulations met the recommendations for calcium and zinc at 500 mg/day and 3.2 mg/100g, respectively which may call for searching other calcium and zinc rich food sources for matching.

Compared to the present findings, Mezgabo *et al*. [166] reported a wider range of iron content (9.38-34.86 mg/100g) for complementary porridge formulation from red teff, malted soybean flour and papaya fruit powder, but it fairly agrees with our results. Contrarily, the present finding was higher than the report by [244] who reported that by supplementing wheat bread with 30 percent teff flour, the iron content of the bread was more than doubled whereas, in our

formulations, it was more than tripled (3.31-4.36 fold) than the control at *dabi teff* range of 20-35%. Similarly, when compared to Cerifam® faffa flour, the new formulations had significantly higher ($P < 0.05$) iron content (4.0-5.26 fold).

Contrary to the present finding, Mezgebo *et al*. [166] reported higher calcium content at 293.57 mg/100g for complementary porridge formulation from red teff, malted soybean flour and papaya fruit powder and the report associated the high calcium content of the formulations with the higher calcium content in soybean flour. Cerifam® faffa flour had significantly higher ($P < 0.05$) calcium content than our new formulations as well, which could be attributed to the milk powder contained in the product.

Furthermore, contrary to the present findings, Mezgebo *et al*. [166] have reported higher zinc content which ranged from 4.05-5.58 mg/100g for complementary porridge formulation from red teff, malted soybean flour and papaya fruit powder. Magnesium is a cofactor that aids in protein synthesis in the human body and its deficiency in children leads to stunted growth [7]. In the current study, there observed high mean magnesium content of the newly developed complementary flour at 120.57 mg/100g. Literature supports that cereals, legumes and oilseeds contain a good amount of magnesium and it was because of the presence of these components in the current formulation which was responsible for the higher magnesium content.

Sodium is an essential mineral mostly responsible for the maintenance of extra-cellular fluid and normal blood pressure and for other several physiological functions. The moderate use of salt in some foods is appropriate and it is neither safe nor reasonable to avoid salt from infant's and younger children's diets [53]. In this study, the sodium content of the new formulations was far below the recommended dietary allowance (RDA) for infants (370 mg/day) and young children (1000 mg/day) [53]. This very low sodium content might call for the addition of a small amount of iodized salt during complementary porridge preparation. The new formulations had a vital amount of phosphorous at 293.25 mg/100g where its principal role in the human body is that it combines with calcium to make bone and teeth, used to release energy from carbohydrates and fat during metabolism (ATP production).

The present findings showed that phytate and tannin contents in the formulated flours were below the maximum tolerable level at 200 mg/100g [31, 37] for phytate and at (560 mg/100g) for tannin, respectively which indicates both the anti-nutrients might not possibly inhibit the digestion, absorption and bioavailability of minerals and protein from the formulated complementary food.

The anti-nutrient/minerals molar ratio have got more nutritional implications than the anti-nutrient contents of food. That is the degree of interaction between the minerals and the anti-nutrients to form insoluble complexes determines the absorption inhibitory effect, thus influencing the bioavailability of minerals under study. In the human body, this effect can be predicted by calculating molar ratios of the anti-nutrients to minerals [116] which shows the proxy indicator of minerals bioavailability. The mean calculated value of Ph:Fe molar ratio for the formulation at 0.29 was far below the critical value at 1:1 ratio where a value greater than this has a negative effect on iron absorption; and it was even far less than 0.4:1 which is the preferable Ph:Fe molar ratio [37, 245]. The critical limit for (Ph:Zn) is 15:1 **(Table 5.15)** where a value greater than this limit is associated with low estimated bioavailability, where 5-15 and below 5 Ph:Zn molar ratio are associated with moderate and high estimated zinc bioavailability, respectively, with zinc absorption corresponding to 15, 30, and 50%, respectively [245]. The calculated value in the current study falls in the third category (Ph:Zn below 5) showing higher estimated bioavailability with an expected 50% zinc absorption rate.

In infants and young children, zinc absorption is not directly related to dietary phytate intake but indirectly related to the dietary calcium content. Higher calcium content in foods induces the dietary phytate to reduce zinc absorption by potentiating the negative effect of phytate through the formation of insoluble phytate-calcium-zinc complexes and calcium itself does not impair zinc absorption regardless of whether the phytate is high or low [246]. Thus, the presence of higher calcium content in diets has been suggested as a better predictor of zinc absorption and Ph*Ca:Zn molar ratio is the preferred indicator of zinc absorption from foods rather than the Ph:Zn molar ratio, where the Ph*Ca:Zn molar ratio greater than the critical limit (200:1) may lead to lowered/poor zinc bioavailability [116]. In the present findings, the Ph*Ca:Zn molar ratio, was incomparably lower than this critical limit, which showed the reduced likelihood of

zinc mal-absorption expected from complementary diet. In reality, not calcium from a conventional diet, but only calcium supplementation is expected to impede zinc absorption [37, 245]. This was confirmed by the past report by Hemalatha *et al*. [247] which stated that zinc absorption and bioavailability is unlikely to be impaired by the calcium contents of foods prepared from cereals and pulses. The probable reason for these low phytate/mineral molar ratios in the present study may be the effect of soaking, germination, roasting, an dehulling processes applied on the selected components used. Gibson *et al*. [248] reported that molar ratios of phytate/mineral were improved (lowered) by practicing processing techniques such as roasting, germination and fermentation or through enriching complementary foods with animal-source foods.

Optimized composite complementary flour that can meet iron density requirements with reduced phytate/minerals molar ratios can be obtained from nutritious cereal-legume blends to develop a super quality complex child food. Child nutrition emphasizes the importance of quality protein, omega-3 polyunsaturated fatty acid and adequate iron during complementary feeding [28, 219] where our new optimized product would certainly contain a substantial amount of these nutrients as a result of the presence of particularly, field pea, maize, *dabi teff* and linseed flours in the formulations.

The present computed phytate/minerals molar ratios of the optimized complementary flour were far below the critical limits at <1, <0.24, <15 and <200 for Ph:Fe, Ph:Ca, Ph:Zn and Ph*Ca:Zn, respectively as set by FAO/WHO [28] indicating the unlikely absorption inhibition effect of the phytate on the minerals.

Regarding the maximum combined desirability function, Talabi *et al*. [227] has reported a similar desirability function with the present study at 0.65 during optimization of complementary meal from a mix of yellow maize, sorghum, millet, soybean, groundnut, crayfish and fish. Additionally, Keyata *et al*. [228] reported a similar desirability function of 0.625 for the overall optimization of complementary food from sorghum, soybean, karkade and premix.

When compared to the FAO/WHO [28] guideline for nutrient content requirements of formulated complementary flour, the newly optimized novel complementary flour adequately

met the recommended standard for iron at 16 mg/100gm. This showed that the optimized complementary flour could demonstrate iron content of two times higher than the recommendation which showed the superiority of our optimized complementary flour over the recommended standard and the control flour. It may not be surprising that the optimized complementary flour had two times more iron content (32.21mg/100g) than the recommended at 16mg/100g because *dabi teff* flour had shown extraordinarily high iron content at 86.5 mg/100g (Table 5.9).

Zinc met the recommended value (3.2 mg/100g) at a marginal of 80.94% which might be taken as a considerable amount whereas calcium did not meet the recommendation at 500 mg/100g.
The 'novelty' of the newly optimized novel composite complementary flour developed would be attributed to the incorporation of *dabi teff* flour containing high iron (86.5 mg/100g) with good bioavailability (Ph:Fe molar ratio far below the critical) that greatly evidenced the social beliefs of this typical farmer variety teff. In addition, linseed flour which is a leading source of α-Linolenic acid, and omega-3 polyunsaturated fatty acid would make the product super.

According to PAHO/WHO [219], complementary feeding guideline, the iron density of cereal-legume-based complementary foods for infants and young children (6-23 months) should range from 0.8-4.0 mg/100kcal when accounted for moderate iron bioavailability and the iron density of our current optimized complementary flour was computed to be 8.47 mg/100kcal given the energy value of our optimized complementary flour to be 380.43 kcal/100g [243] which was 2.12-10.59 times higher than the recommended value when accounted for moderate bioavailability and it was 4.39 and 5.57 times higher than the control and the Cerifam® faffa flour, which could be accredited to the higher iron content of *dabi teff* flour.

This finding showed that the newly optimized complementary flour could even meet the recommendation when accounted for the low bioavailability of iron density at 7.7 mg/100 kcal. Hence, the newly optimized complementary flour could demonstrate to meet the iron density requirements for infants and young children aged 6–23 months and the children can meet their daily iron demand if properly fed. The present finding was higher than the iron density of complementary blends of teff, soya bean and orange flashed sweet potato that range from 2.42 to

5.19 mg/100kcal as reported by Tenagashaw *et al*. [229]. Iron in plant foods exists in non-heme form and it may also bind with anti-nutrients found in cereal-legume foods where its absorption can be impaired [37]; but in the current case, the pre-processing technique applied might reduce the effect of the anti-nutrients as a result of which the Ph:Fe molar ratio was far below the critical.

PAHO/WHO [219] recommends zinc density at 1.6 mg/100kca, 1.1mg/100g and 0.6 mg/100g for 6-8 months, 9-11 and 12-23 months of age children from complementary food when accounted for lower bioavailability [241] and the current determined zinc density was at 0.68 mg/100kcal showing the fulfillment for 12-23 months old children while at marginal level (61.82%) when accounted for the requirements for 9-11 months old children and below the recommendation for 6-8 months old children. The present finding was higher than the zinc density of complementary food at 0.4 mg/100kcal as reported by Ayele *et al*. [121] while it was lower than the report by Tenagashaw *et al*. [229] with a zinc density range of 1.41 to 1.49 mg/100kcal.

In the current study, the calcium density could only be demonstrated to meet 25.46% of the required calcium density in formulated complementary for infants and young children as set by the codex standard (>80 mg/100kcal) [28]. The present finding was slightly lower than the report by Ayele *et al*. [121] where the calcium density was at 24.1 mg/100kcal but by far lower than the report by Tenagashaw *et al*. [229] with a calcium density range of 60.68 to 67.84 mg/100kcal from complementary blends of teff, soya bean and orange-flashed sweet potato which could be attributed the variety of soya bean and the orange-fleshed sweet potato used. Of the examined micronutrients, calcium density was found to be lower than the required where it was frequently reported to be low in cereal-based complementary foods [204, 218].

Conclusion

Micronutrients malnutrition especially iron deficiency anemia is most prevalent in children under two years than at any other time in the life cycle. In the present study, the incorporation of *dabi teff* into other pre-processed local food crops provided iron-dense optimized novel complementary flour that can meet the FAO/WHO guideline recommendations and exhibit

improved in-vitro iron bioavailability over the traditional and the Cerifam® faffa flour. Each of the components used contributed important limiting micronutrients such as iron, calcium and zinc to develop a micronutrient-enriched optimized product, where *dabi teff* grain, a typical farmer variety teff was found to contain iron at 86.5 mg/100g, iron density at 24.53 mg/100kcal making the crop super iron bank. The optimized flour had an iron density at 8.47 mg/100kcal. The results showed that the iron content of the optimized flour was two times higher and iron density was 2.12-10.59 times higher than the recommended value from complementary food when accounted for moderate bioavailability. It was even shown to meet the recommendation at low bioavailability. The findings further showed that the optimum novel complementary flour contained 4.44 times higher iron content, 1.57 times higher calcium content and 1.23 times higher magnesium content, as compared to the control. The optimized flour was also shown to contain 5.57 times higher iron density as compared to the Cerifam® faffa flour and the phytate to iron molar ratio was very low showing higher bioavailable iron. Specifically, the findings show that *dabi teff* is a good dietary source of iron from cereals. Thus in Ethiopia, where the consumption of animal-source foods is low and where nearly all iron requirement (>97%) should come from complementary food after 6 months, the promotion of *dabi teff*, the underutilized/forgotten food crop in the formulation of complementary foods is required. Utilization of *dabi teff* could serve as an inexpensive and sustainable way of obtaining iron demand by children for their optimal growth and development that can aid as a sustainable food-based intervention strategy to combat iron deficiency anemia.

6

Chapter 6:

Effects of Blending Ratios Variation on Macro- and Micronutrients, Phytate/Minerals Molar Ratio and Sensory Acceptability of *Dabi teff*-Field Pea Based Novel Composite Complementary Flours

Chapter 6.1:

Effects of Blending Ratio Variation on Macronutrient Compositions and Sensory Acceptability of *Dabi teff*-Field Pea Based Novel Composite Complementary Flours

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Abstract

The response of a mixed system is determined by the proportions of the various ingredients that add up to one, not by the combination's total amount. This research aimed at examining the effects of blending ratios variation on macro-composition and sensory acceptability of *dabi teff*-field pea-based novel composite complementary flours. Standard methods (AOAC, 2016, *Official Methods of Analysis of the Association of Official Analytical Chemists*) were used to determine macronutrients. The sensory attributes were evaluated using a 5-point hedonic scale. The ingredients were constrained at 0-30% field pea, 20-35% for *dabi teff*, and 5-20% for maize, while the remaining were set constant at 5% linseed, 15% for oats and 25% for barley. D-optimal was used to examine the effects of blending ratio variation on the responses. All the responses were significantly different ($P < 0.05$) among the blends except for fat content, ranging from 14.58 to 17.21% for protein, 4.22 to 5.59% fat, 2.01 to 2.60% ash, 2.68 to 3.96% fiber, 68.08 to 70.76% utilizable carbohydrate and 378.82 to 386.9 kcal/100g gross energy. The sensory acceptability score ranged from 3.4 to 4.4. The linear model was significant ($P < 0.05$) and adequate to describe variation in moisture, protein and ash contents. An increase in the ratio of field pea had significantly increased ($P < 0.05$) protein, ash, fiber and energy. The interactive effect between *dabi teff* and field pea significantly increased the sensory acceptability in the blends. These findings showed that varied proportions of the ingredients had a significant effect on the responses, used to develop a wholesome product to combat protein-energy malnutrition among children.

Key Words: *Dabi teff*, Blending ratios variation, Macronutrients, Sensory acceptability, Novel complementary flour

Introduction

Globally, there is an increasing tendency to shift from consuming animal-source foods, especially from consuming highly processed meat products, to plant-source foods because of the increased health risks associated with excess processed meat consumption [87]. In the past, starting from the 1930s, there was a general belief that people need to eat animal foods to obtain high-quality protein, the 'Great Protein Fiasco', to prevent protein-energy malnutrition in children notably 'Kwashiorkor in Africa' [88] but later, nutritionists have recognized the relative importance of plant proteins and the need to formulate multi-mixes of local food stuffs than commercial mixtures where wheat/chickpea mixtures were successful by WHO in 1966 [88]. In developing countries like Ethiopia, plant foods are the major staple diets and major food choices due to economic and religious reasons.

The nutritional and sensory qualities of plant-based foods need to be improved especially when to be used for child feeding. When a new food product development is targeting infants and young children, special concern should be given to the sensory attributes of the product. This is because; food acceptance by the target consumers is largely determined by sensory qualities rather than the nutritional value of the food [248, 249]. Foods derived from a variety of grains are more likely to contain several important nutrients and provide infants and young children an opportunity to experience a range of flavors, aromas, tastes, and textures, all of which are crucially important in building healthy eating habits in their later life [82].

Searching for different locally available, underutilized, affordable and nutritious plant foods and blending them together (the food-based approach) is the best option [52, 61] to get an increased and multiple nutrients with enhanced sensory acceptability from their mixture and to provide a wholesome food product (containing all the essential amino acids and poly-unsaturated fatty acids) as a main drive to prevent child protein-energy malnutrition [28, 88].

The complementary feeding time (6 to 24 months of age) is one of the critical times in a child's life when the children transfer from breast milk to complementary feeding because the breast milk cannot satisfy the child's developing nutritional needs after six months [115] and infant's transfer from an ideal, nutritious, adequate and uncontaminated breast milk to complementary

foods (often regular family food) [70]. FAO/WHO underscored that complementary foods should not replace (but complement) breastfeeding [28, 230].

Traditional complementary foods are characterized by low nutritional value (often monotonous) and known to contain high bulk density, high viscosity, low protein, and low energy density used in most developing countries including Ethiopia. There are also suboptimal complementary feeding practices [74, 215] which would result in elevated protein-energy malnutrition in children. According to the Ethiopian Public Health Institute (EPHI) and the International Classification of Functioning, Disability and Health (ICF) survey, 37% of children under-five years were stunted, 21% were underweight, 7% were wasted, and 2.9% were overweight [19].
Recently in Africa, cereal foods were complemented with locally available legumes such as soybeans, groundnuts, cowpeas and pigeon peas among others as a protein source to improve the protein quality of the mix and varied results were reported [70, 250].

Foods from multiple cereal-legume blends are more likely to contain multiple or essential nutrients than foods from a single (mono grain) which aroused the interest of nutritionists in examining the effects of mixing different grains at varied mixing proportions. Thus, it was hypothesized that blending different cereals and legumes which are expected to be rich in nutrients at varied ratios accompanied by proper processing techniques would be promising to provide increased macronutrients with improved sensory acceptability.

Dabi teff is the 'afaan oromoo' language name for an early maturing variety of dark red teff. Due to its early maturing property, *dabi teff* is harvested twice within one rainy season (at early rainfall called "*daabi* gannoo" and late rainfall called "*daabi* birraa") [243]. The rural elderly people in particular and the consumers in general, value *dabi teff* as a medicinal food and there are many social beliefs regarding the nutritional and health benefits of this crop [243]. Hence in this study, the effects of blending ratios variation on macronutrient compositions and sensory acceptability of pre-processed *dabi teff*-field pea-based novel composite complementary flours were examined to develop wholesome composite complementary flour to combat protein-energy malnutrition among children.

Methods and Materials

Details of the methods related to the crop sampling, processing, flour preparation and handling were described in **Chapter 2**.

Results

Model fitting and testing model adequacy

The fitted models were found to be adequate and significant for most response variables based on the F-statistic (the ANOVA regressions outputs); the P-value and the coefficient of determinations. Normality and constant variance assumptions were fulfilled.

Table 5.3 showed that the linear models were adequately fitted for moisture, protein and ash contents and significant at P-values of <0.01, <0.001 and <0.05. This shows that x_1, x_4 and x_6 (*dabi teff*, field pea and germinated maize) were the significant model terms for these compositions. This means changes in moisture, protein and ash can adequately be described by the linear models (adequate predictive power) as a function of the component ratio variations in the blends where the regression equation was shown in **Table 6.1.** Quadratic model was adequately fitted for fat and significant at P-value of <0.05. This shows that the linear model terms (x_1, x_4, and x_6) and the interactive model terms (x_1x_4, x_1x_6 and x_4x_6) were significant model terms for fat content. The linear model terms (*dabi teff*, filed pea and germinated maize) had shown a synergetic effect whereas the interactive model terms (interactive effects) (*dabi teff* by field pea, *dabi teff* by germinated maize and field pea by germinated maize) had shown a significant antagonistic effect on fat content in the blends at P-value <0.05 which was also shown in **Table 5.3** with repression equation for fat.

On the other hand, a cubic model was significantly fitted for carbohydrates and significant at P-values of <0.05 where the linear model terms (x_1, x_4, x_6) and the interactive model terms x_1x_6, x_4x_6 (x_1- x_6), and x_4x_6(x_4-x_6) were the significant model terms for carbohydrate at P-value less than 0.05. Meaning changes in carbohydrates can be described by the linear, quadratic and cubic model terms as a function of the component ratio variations in the blends. x_1 (*dabi* teff), x_1x_6 (*dabi teff* by germinated maize) and x_4x_6 (field pea by germinated maize) had a synergetic effect

on the carbohydrate whereas x_4, x_6, x_1x_4 had shown an antagonistic effect on carbohydrate content shown in table 5 with the regression equation for carbohydrate. Likewise, the special quartic model was significantly fitted for overall sensory acceptability and was significant at P value of 0.05.

Special cubic and linear models were suggested by the software for fiber and energy contents, respectively, but the models were non-significant (P-values >0.05), with no predictive power in describing changes in fiber and energy content as a function of components ratio variations in the blends, regression equation found in **Table 5.3.**

The predictive regression models developed for the relationship between the independent variables (Xs') and the dependent variables (Ys') in terms of macronutrient compositions and sensory acceptability of the blends were presented in **(Table 6.1)** for moisture, crude protein, crude fat, ash, utilizable carbohydrate, gross energy and overall sensory acceptability, respectively. The coefficients with single factor (linear model terms) (X_1, X_4, and X_6) represent the independent effect of a particular determinant variable while coefficients with two of the factors (X_1X_4, X_1X_6, and X_4X_6) represent the interaction between the factors (interactive model terms). A positive sign in front of the regression model terms (mathematical algorism) is an indication of a synergetic relationship, while a negative sign indicates an antagonistic relationship.

Table 6.1: ANOVA regression models showing effects of the independent variables (Xs') on dependent variables (Ys') in the blends

Dependent variables (Ys')	Regression Models with Xs' (Actual Equations)	Model
Moisture (Y_1)	$0.129969\ x_1 + 0.056327\ x_4 + 0.0406071\ x_6$	Linear
Crude Protein (Y_2)	$0.267207\ x_1 + 0.350487\ x_4 + 0.219951\ x_6$	Linear
Crude Fat (Y_3)	$0.195395\ x_1 + 0.228208\ x_4 + 0.508282\ x_6 - 0.007827\ x_1x_4 - 0.0146967\ x_1x_6 - 0.013247\ x_4x_6$	Quadratic
Ash (Y_4)	$0.037258\ x_1 + 0.052819\ x_4 + 0.031447\ x_6$	Linear
Crude Fiber (Y_5)	$-0.077218\ x_1 - 0.12638\ x_4 - 0.386782\ x_6 + 0.012573\ x_1x_4 + 0.023008x_1x_6 + 0.036897\ x_4x_6 - 0.001532\ x_1x_4x_6$	Special Cubic
Utilizable Carbohydrate (Y_6)	$7.08547\ x_1 - 1.53572\ x_4 - 29.974\ x_6 - 0.0920234\ x_1x_4 + 0.872668\ x_1x_6 + 0.741828\ x_4x_6 + 1.03834e\text{-}05\ x_1x_4x_6 - 0.00550154\ x_1x_4\ (x_1 - x_4) - 0.0186895\ x_1x_6\ (x_1 - x_6) - 0.00724686\ x_4x_6\ (x_4 - x_6)$	Cubic
Gross Energy (Y_7)	$6.88819\ x_1 + 6.91064\ x_4 + 7.13471\ x_6$	Linear
Overall Acceptability (Y_8)	$2.90276\ x_1 + 4.00009\ x_4 + 4.02192\ x_6 + 2.62446\ x_1x_4 - 14.8958\ x_1x_6 + 1.18348\ x_4x_6 + 140.849\ x_1^2x_4x_6 - 51.8023\ x_1x_4^2x_6 + 42.694\ x_1x_4x_6^2$	Special quartic

Key: x_1- *dabi teff*, x_4- field pea, x_6- germinated maize

Effects of blending ratios variation on macronutrient compositions of the composite complementary flours

The response of a mixture system is determined by the ratio variation of the various ingredients that add up to one, not by the combination's total amount. In this study, the mean and standard deviation of energy and macronutrient composition of the blended complementary flours were summarized in **Table 5.5** Mean difference separation results showed that all the macro-composition were significantly different ($P < 0.05$) among the blends (as affected by the component ratios variation) except for fat, where their content ranged from 4.41 to 5.74% for moisture, 14.58 to 17.21% protein, 4.22 to 5.59% fat, 2.01 to 2.60% ash, 2.68 to 3.96 fiber, 68.08 to 70.76% utilizable carbohydrate and 378.82 to 386.9 kcal/100g gross energy, respectively. These results were within the acceptable critical limits set by FAO/WHO [28] guideline except for fat content at $\leq$5%, $\geq$15%, 10-25%, $\leq$3%, $\leq$5%, 64$\pm$4% and 400 to 425 kcal/100g for the respective composition parameters The control flour had contained 6.15%, 10.77%, 3.78%, 2.24%, 2.65% and 74.41% and 374.74 kcal/100g for the respective parameters mentioned **(Table 5.5).**

The observed mean moisture content of the current flour blends was significantly lower ($P < 0.05$) than the moisture content of the control flour at 6.15% and the lower moisture content could be attributed to the processing techniques applied. With regard to the effect of component ratio variation in the blends, there observed an increase in the moisture content with an increase in the ratio of *dabi teff* flour in the blends as shown by 2D contour and trace plots **(Figure 6.1)**.

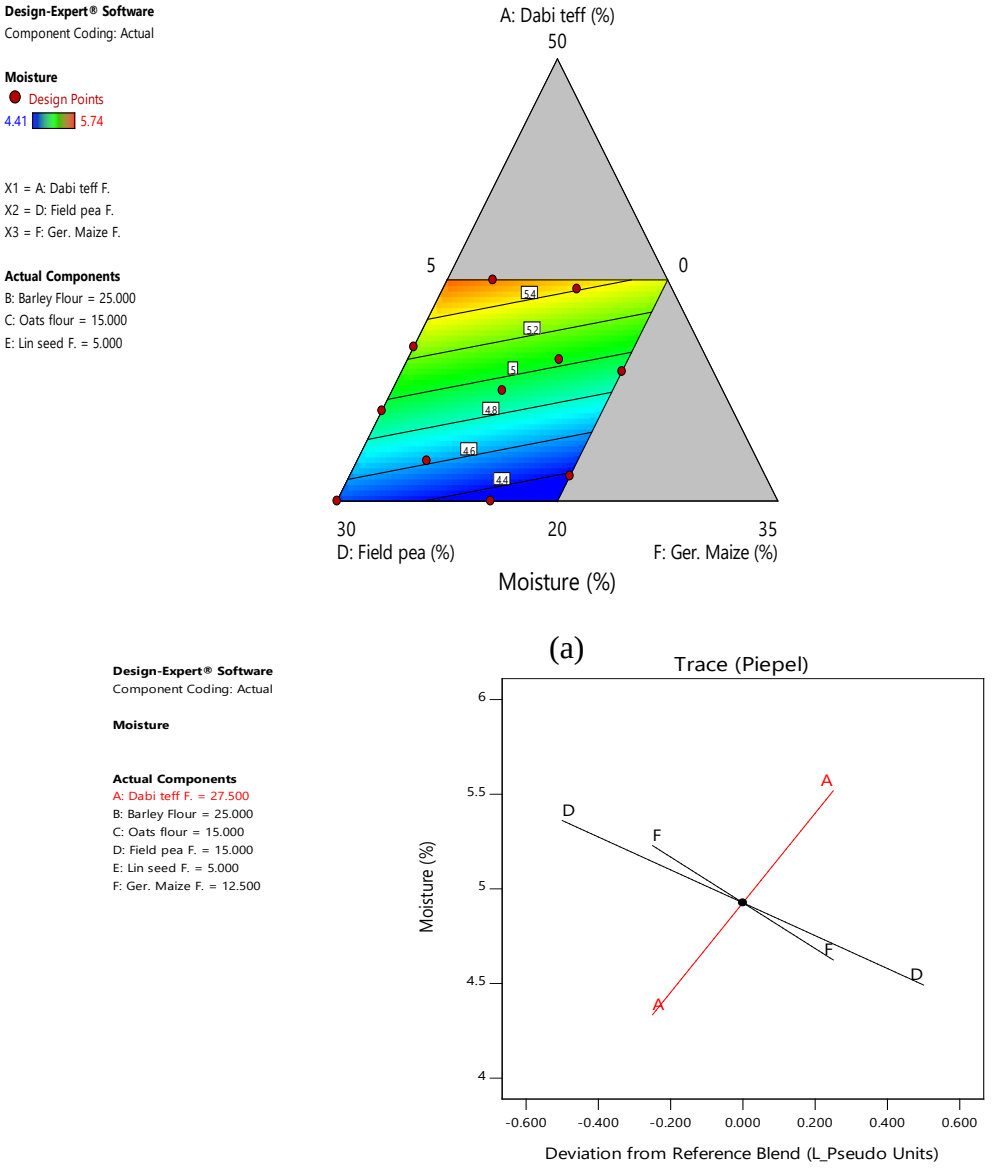


Figure 6.1: Model graphs showing the effects of the major components ratios on moisture content. (a) 2D contour plot. (b) Trace plots.

The observed protein content in the blends was 1.4 to 1.6 times higher than that of the control flour which was found to be significant at P <0.05. With regard to the effect of component ratio variation among the blends, there observed a linear increasing effect of protein content with an increased ratio of field pea which could be attributed to the naturally higher protein contents in pulses/legumes foods. It could be observed from **Table 5.5** that at the highest proportion of field

peas at 29.98%, the protein content was at the highest value at 17.21% (F4) whereas it was the lowest at 14.58% (F11) where the ratio of field pea was lower at 10.08% in the blends. On the contrary, there observed a decreasing effect of protein content as *dabi teff* and germinated maize flour proportion increased as shown by 2D contour and trace plots (**Figure 6.2)**.

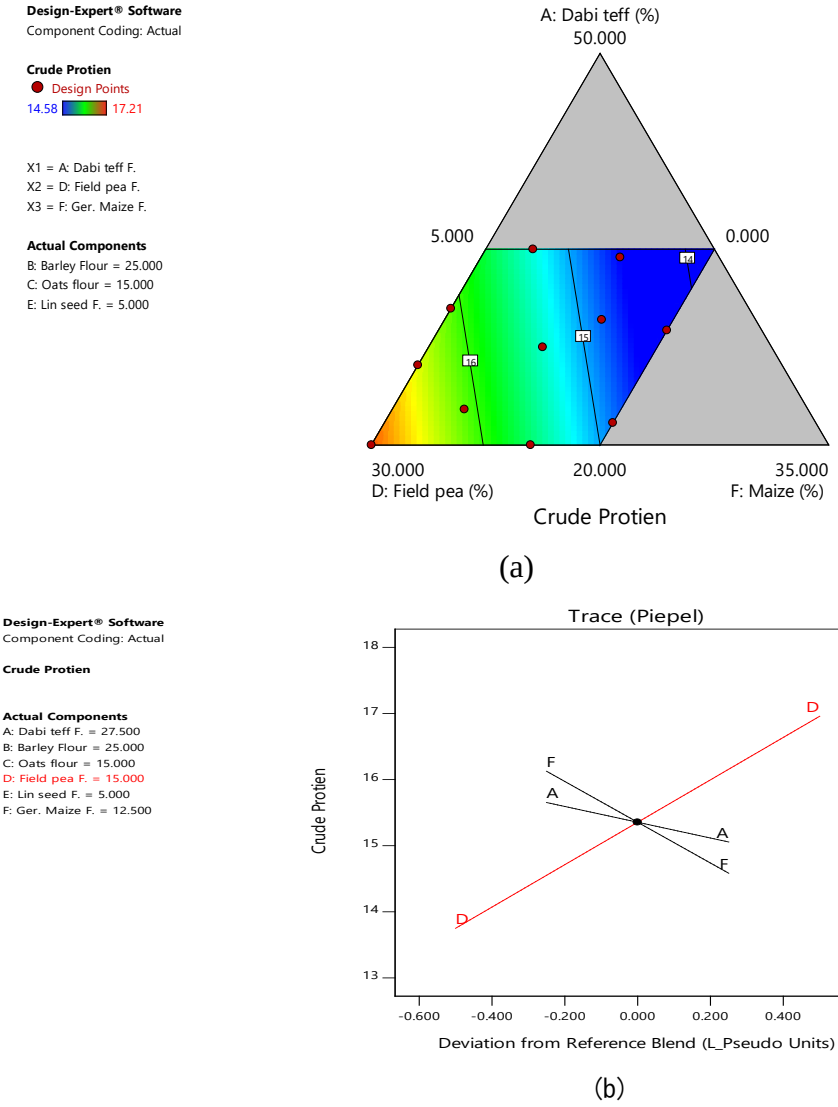
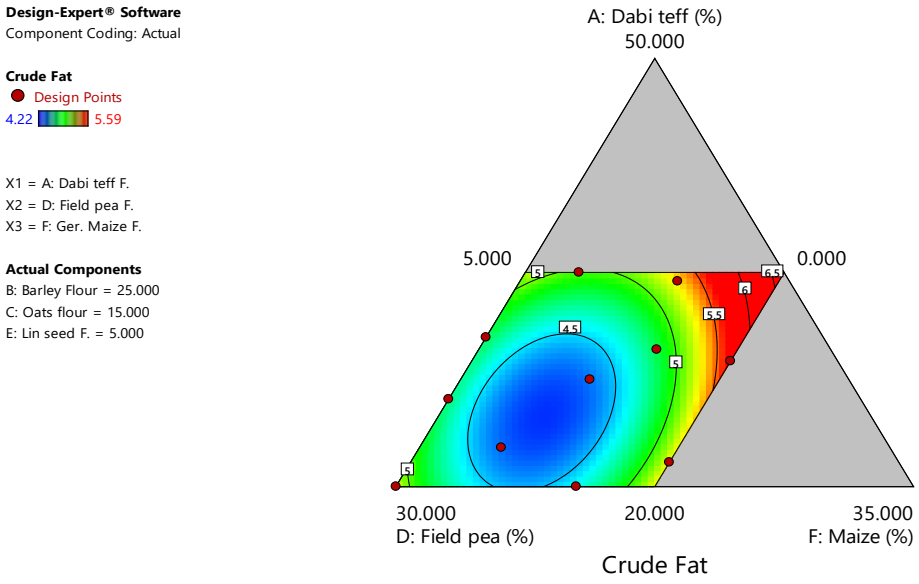


Figure 6. 2. Model graphs showing the effects of the ratios of the major components on protein content. (a) 2D contour plot. (b) Trace plots.

The mean fat content of the blends at 4.91% was slightly higher than that of the control flour at 3.78%. It had shown the highest fat content at 5.59% (F5) and the lowest at 4.22% (F1) **(Table 5.5).** As far as the effect of component ratio variation is concerned, fat content was majorly

determined by the field pea and germinated maize ratios in the blends as shown by 2D contour and trace plots **(Figure 6.3)**.



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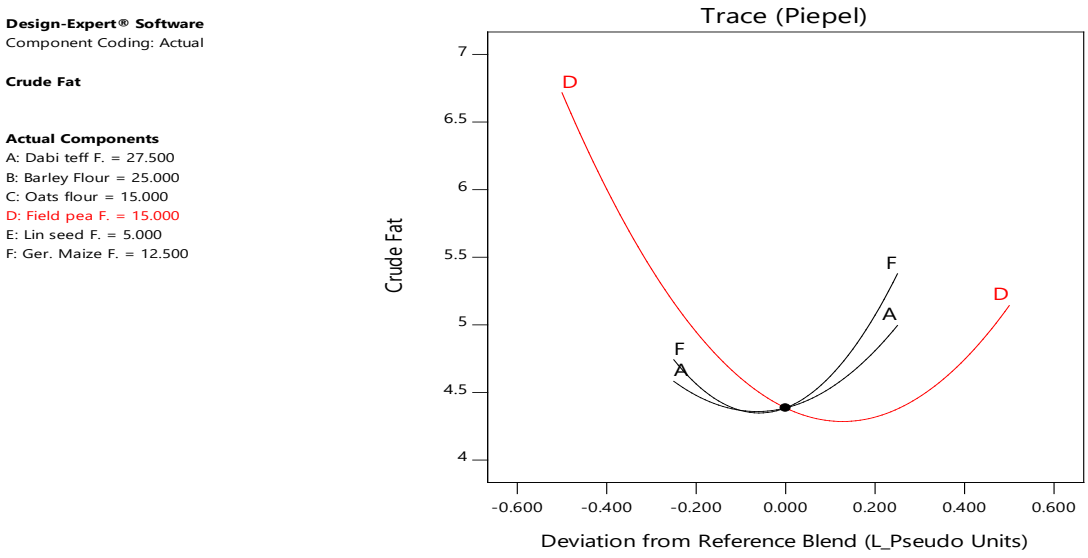
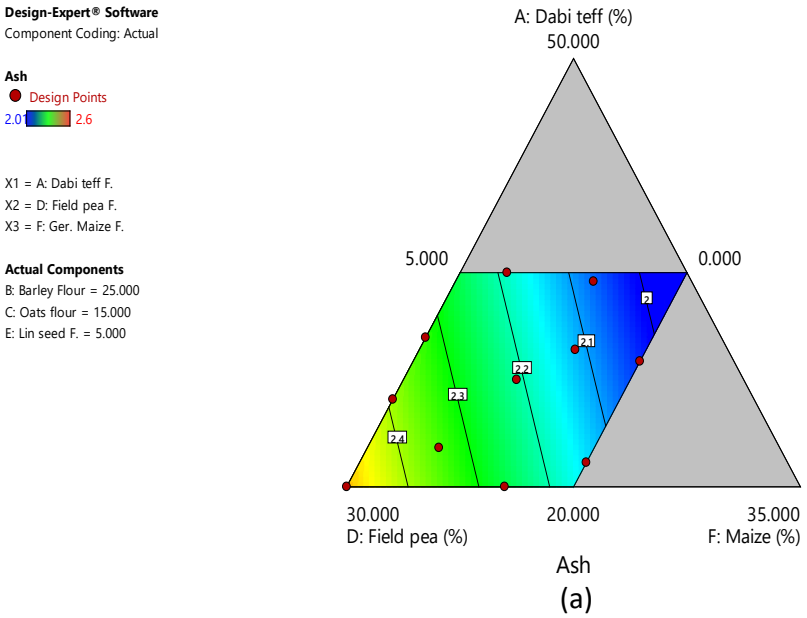


(b)

Figure 6.3. Model graphs showing the effects of the major components ratios on fat content. (a) 2D contour plot. (b) Trace plots.

The mean ash content of the blends at 2.23% was similar to that of the control flour at 2.24%, the highest at 2.60% (F4) and the lowest at 2.01% (F11). Regarding the effect of component ratios variation, there observed a linear increasing effect in ash content with an increased ratio of field

pea. On the contrary, there observed a decreasing effect of ash content as *dabi teff* and germinated maize flour proportion increased in the blends as shown by 2D contour and trace plots **(Figure 6.4).**



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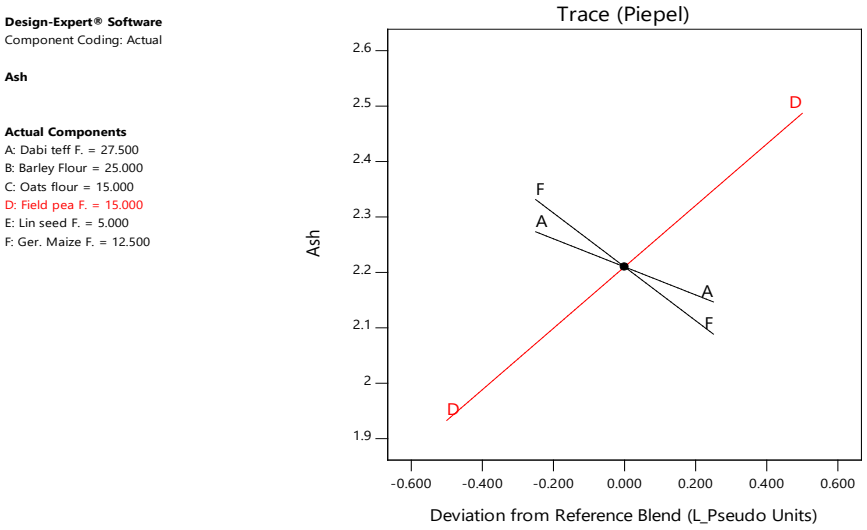


(b)

Figure 6.4. Model graphs showing the effects of the major components ratios on ash content. (a) 2D contour plot. (b) Trace plots

The mean fiber content of the blends at 3.48% was slightly higher than that of the control at 2.65% with the highest and lowest value at 3.96% (F8) and 2.68% (F6), respectively **(Table 5.5)**.

Field pea and germinated maize had a major effect in determining the fiber content among the blends. As field pea and germinated maize ratio increased, fiber content also increased in the blends as shown by 2D contour and trace plots **(Figure 6.5)**.

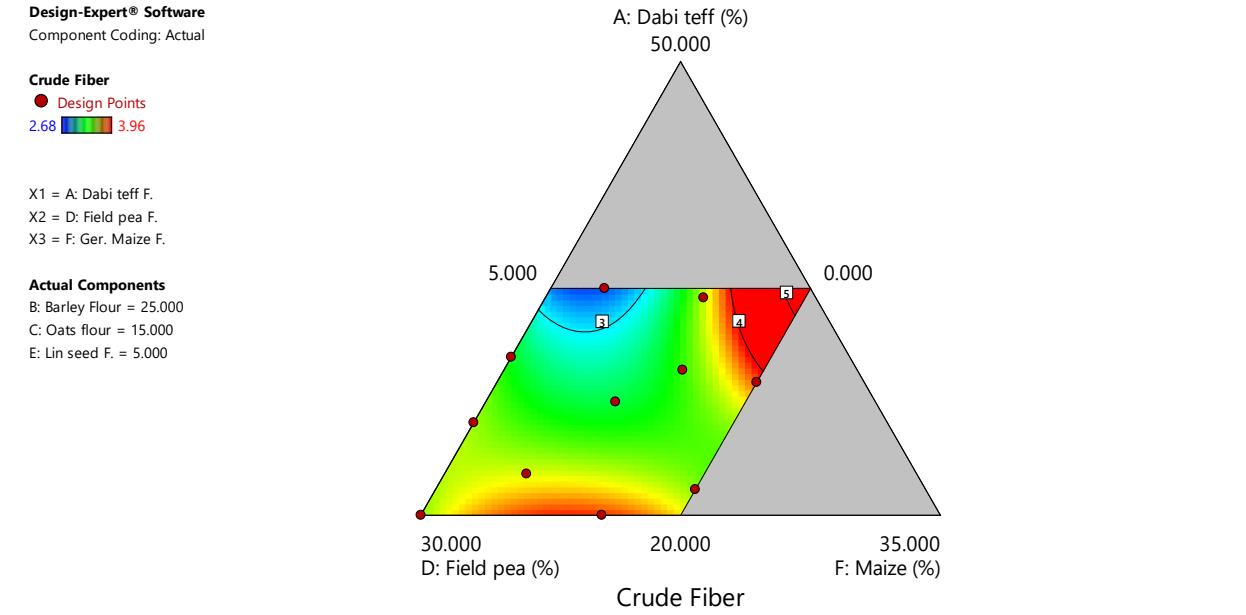
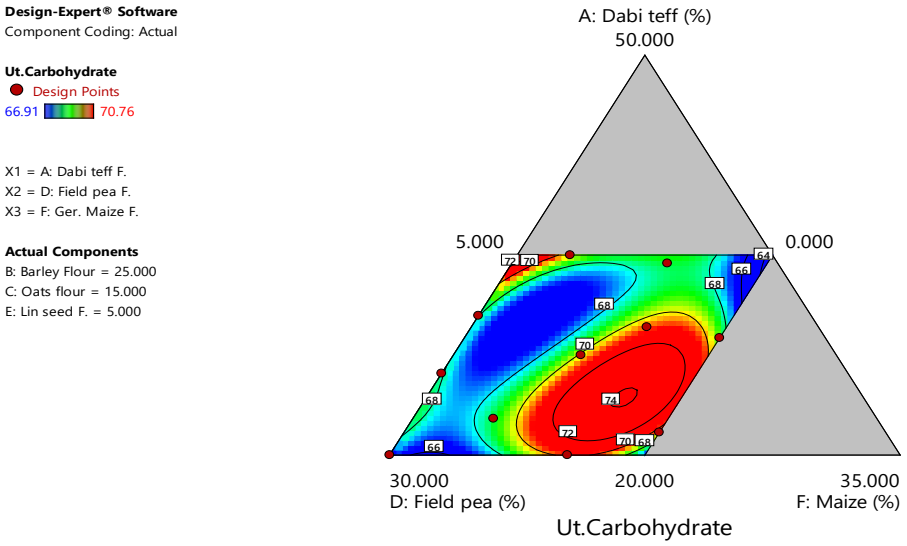


Figure 7.5: Model graphs showing the effects of the major components ratios on fiber content. (a) 2D contour plot. (b) Trace plots.

The mean carbohydrate content of the blends at 68.99% was slightly lower than that of the control at 74.41% which could be attributed to barley and maize from which the control was

constructed with the lowest and highest values at 68.08% (F3) and 70.76% (F11), respectively **(Table 5.5).** There observed an increasing effect of carbohydrates as the ratio of *dabi teff* and germinated maize flours were increased in the blends as shown by 2D contour and trace plots **(Figure 6.6)** which could be attributed to cereals containing higher carbohydrate content than legumes.



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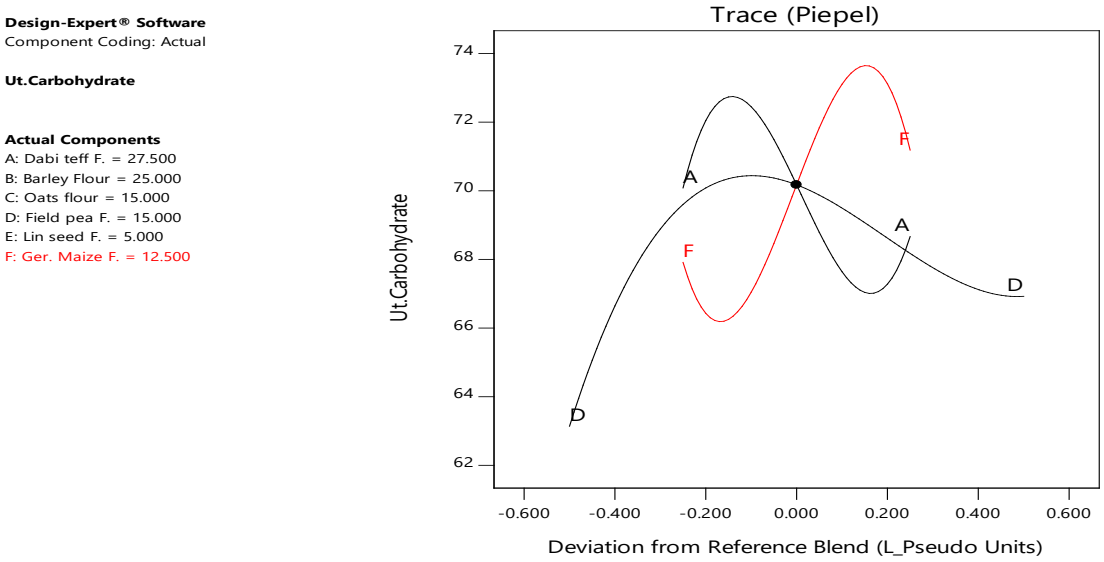
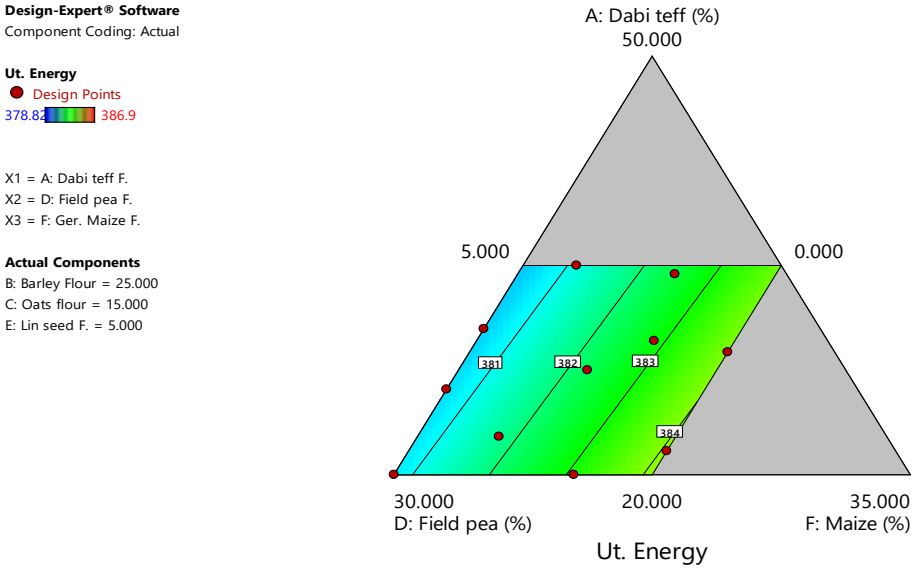


(b)

Figure 6.6. Model graphs showing the effects of the major components ratios on carbohydrate content. (a) 2D contour plot. (b) Trace plots.

The mean energy contents of the blends at 382.14 kcal/100g were significantly higher ($P < 0.05$) than the control flour at 374.74 kcal/100g with its highest and lowest value at 386.9 kcal/100g

(F11) and at 378.82 kcal/100g (F1) **(Table 5.5).** Regarding the effect of component ratio variation in the blends, there observed a linear increasing effect in energy content with an increased ratio of germinated maize and this could be due to the higher energy in cereals. On the other hand, there observed a decreasing effect in energy content as *dabi teff* and field pea flour ratios increased in the blends as shown by 2D contour and trace plots **(Figure 6. 7)**.



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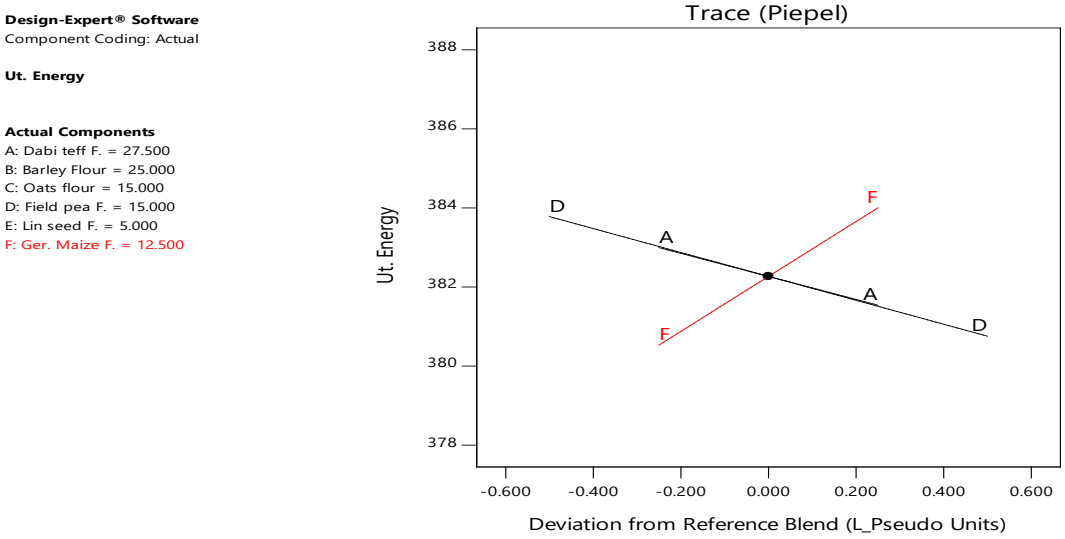
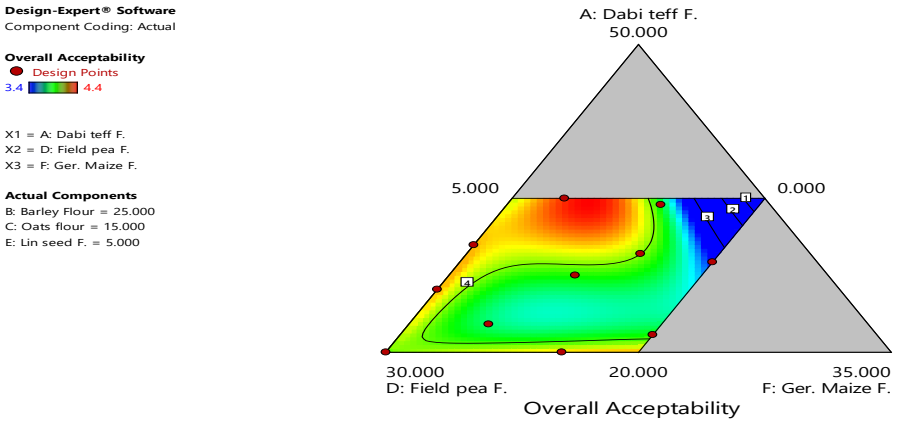


(b)

Figure 6.7. Model graphs showing the effects of the major components ratios on energy content. (a) 2D contour plot. (b) Trace plots**.**

Effects of blending ratios on sensory attributes of the complementary porridges

In the present study, the mean values of sensory evaluation score were significantly different (P <0.05) among the blends (as affected by components ratio variations) and ranged between 3.4 -4.8 for color, 2.8 -4.8 for aroma, 3.0 -4.4 for taste, 3.2 -4.5 for mouth feel and 3.4 -4.4 for overall sensory acceptability, respectively. The control porridge had 3.5, 4.2, 3.8, 3.9 and 3.4 for color, aroma, taste, mouth feel and overall acceptability, respectively **(Table 5.6).** The overall sensory acceptability score of the blended porridges was higher (liked much) than that of the control with the highest and lowest scores at FP6 (4.4) and FP5 (3.4) which might be due to the multiple grains and the processing technique applied which imparted good flavor and aroma to the newly formulated porridges. Regarding the effect of component ratios variation, there observed a linear increasing effect in the overall sensory acceptability score with an increased proportion of field pea and *dabi teff* flours while it showed a negative relationship with germinated maize flour in the blends as shown by 2D contour and trace plots **(Figure 6.8).**



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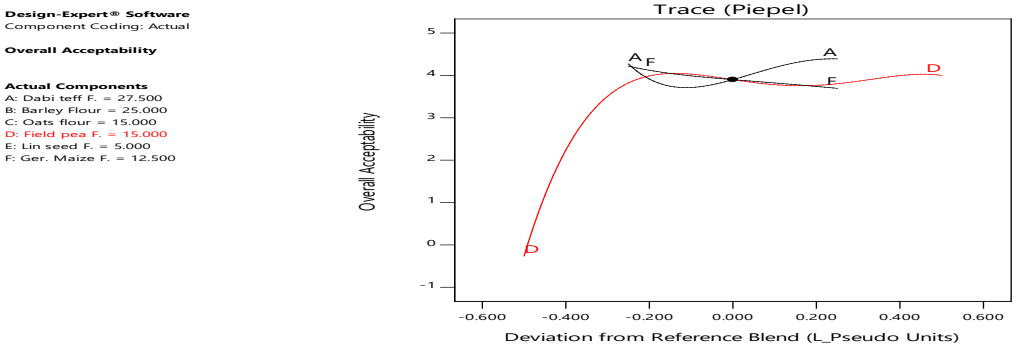

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Figure 6.8. Model graphs showing effects of the major components ratios on overall sensory acceptability score (a) 2D contour plot. (b) Trace plots.

Discussion

The ‘novelty’ of this study would be accounted for the incorporation of *dabi teff* flour into the blends containing high iron (86.5 mg/100g) and linseed which is a leading source of α-Linolenic acid, omeg-3 polyunsaturated fatty acid that would make the blends super.

In agreement with the present finding, Fikiru *et al*. [251] reported the protein content ranged from 13.0-18.5% from the blends of maize, pea and malted barley flours where protein content was increased with increasing ratio of pea and decreased with increasing maize proportion in the blend. Additionally, Mezgebo *et al*. [166] reported that increasing proportions of legumes/pulses in food formulations increases the protein content. Mariam [54] reported that complementary food products from cereal-legume combinations of two or more components have better protein (overall nutritive value) than products from a single plant food. Such phenomenon can be well explained by the FAO/WHO [28] codex alimentarius commission on complementary feeding guidelines. The guidelines describes that mixtures of cereals, legumes, pulses/oilseed can constitute an appropriate source of nutrients and energy, essential fatty acids and limiting amino acids with many functional and health benefits as well as improved organoleptic characteristics.

Fikiru *et al*. [251] reported lower fat content that ranged from 1.8-2.51% from the blends of maize, pea and malted barley flours while the report agreed with the present finding that fat content increased with an increasing maize flour ratio in the blends. It is a fact that when non/lower-fat-containing components are added to a relatively higher fat-containing component, it most likely reduces the concentration of fat in the total sum of the mixture. This is because it is certain that fat content (any response) is a function of a ratio in a mixture.

The current finding was lower than the report by Fikiru *et al*. [251] that ash content ranged from 1.5-2.5% from the blends of maize, pea and malted barley flours and there was an increase in ash content as an increased proportion of pea in the blends which agreed with the current observation.

The present findings agreed with many reports that stated adding legumes into food products increases ash content. [248] supported addition of legumes into cereals could lead to higher ash and mineral content. In agreement with the present finding, Fikiru *et al*. [251] reported that the

fiber content from blends of maize, pea and malted barley flours ranged between 3.1- 4.1% and increased with increasing pea and maize flour.

Inconsistent to the present finding, Fikiru *et al*. [251] reported that the carbohydrate contents of complementary flour from maize, pea and malted barley ranged from 68.9–74.1% and an increase in carbohydrate content with increasing maize flour ratio. However, our finding was slightly higher than the report by Mezgabo *et al*. [166] that carbohydrate content ranged from 55.43 to 69.68% for complementary porridge formulation from red teff, malted soybean flour and papaya fruit powder and an increase of carbohydrates with increased red teff ratio.

Furthermore, our study agreed with the report of Tadesse *et al*. [225] that the component variation of bulla, pumpkin and germinated amaranth had no predictive effect in describing the energy contents of complementary blends. The determined mean value of energy content in the current blends was amenable with the report by Mezgabo *et al*. [166] that the energy content of complementary porridge formulation from red teff, malted soybean flour and papaya fruit powder ranged from 376.30 to 385.56 kcal/100g and increased with an increasing of malted soya flour. However, Fikiru *et al*. [251] reported a lower energy content of complementary flour from maize, pea and malted barley ranging from 364.4-371.0 kcal/100g and energy content increased with an increase of malted barley.

The finding of the overall sensory acceptability score in the present study was lower than the report by Mezgebo *et al*. [166], which found that the overall sensory acceptability score ranged from 4.84-4.97 for complementary porridge formulation from red teff, malted soybean flour and papaya fruit powder and it was accounted for malted soya flour ratio in its formulations.

Conclusion

In most developing countries, complementary foods are monotonous and are characterized by low protein and energy density which is an immediate cause of protein-energy malnutrition in children. Foods from multiple grains at some defined mixing ratios are more likely to contain an increased and multiple or essential nutrients than foods from a single (mono) grain. The present

study had shown that the blending ratios variation of the various ingredients (*dabi teff*, fileld pea and germinated maize with the other constant ingredients) had a significant effect on the macronutrients and the sensory attributes of the mixture except the fat content. An increase in the ratio of field pea significantly increased the protein, ash, fiber and energy content of the blends while an interactive effect between field pea and *dabi teff* flours had a synergetic effect in increasing the overall sensory acceptability. Such effects can provide the bases to optimize the mixture ratios to develop wholesome product that can be used to combat protein-energy malnutrition among children.

Chapter 6.2:

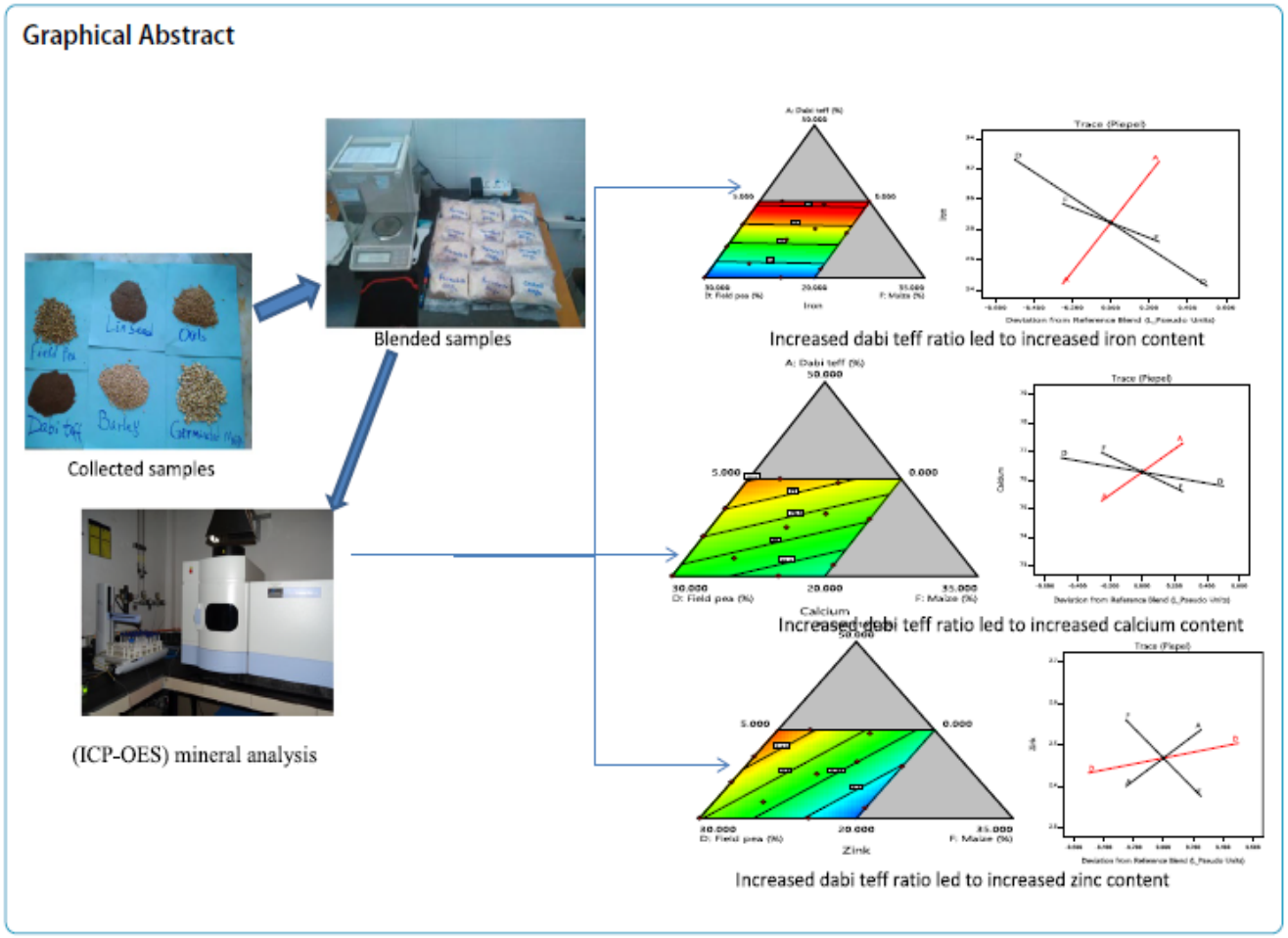
Effects of Blending Ratios Variation on Micronutrient Compositions and Phytate/Minerals Molar Ratios of *Dabi teff*-Field Pea Based Novel Composite Complementary Flours

Redrafted from: Diriba Chewaka Tura, Tefera Belachew, Dessalegn Tamiru, Kalkidan Hassen Abate. ***Effects of blending ratios variation on micronutrient compositions and phytate/minerals molar ratios of dabi teff-field pea based novel composite complementary flours.*** *Food Production, Processing and Nutrition*. 2024; https://doi.org/10.1186/s43014-023-00203-7. Springer Nature. *2024*.

Abstract

Mixtures of multiple grains at varied ratios can provide multiple and higher micronutrients than a single grain. Thus, this research was aimed at examining the effect of blending ratios variation on micro-compositions and phytate/minerals molar ratios of pre-processed local *dabi teff*-field pea-based novel composite complementary flours. Inductively Coupled Plasma-Optical Emission Spectrometry was used to determine dietary minerals. Nutrisurvey software was employed to define ranges of the mixture components and they were constrained at 20-35% for *dabi teff*, 0-30% field pea and 5-20% maize, while the remaining were set constant at 25% barley, 15% oats and 5% linseed. Design-Expert ® software version 11, D-optimal was used to generate eleven experimental blends and to examine the effects of blending ratio variation on the responses. Mean mineral contents were significantly different ($P < 0.05$) among the blends (as affected by component ratios variation) and ranged from 24.01-31.58 mg/100g for iron, 73.46 -78.81 mg/100g for calcium, and 2.33-2.61 mg/100g for zinc contents. The phytate/minerals molar ratios were significantly different among the blends except phytate/calcium molar ratio (Ph:Ca), ranged from 0.232-0.344 for phytate/iron molar ratio (Ph:Fe), 0.067-0.085 for (Ph:Ca), 3.356-4.18 for phytate/zinc molar ratio (Ph:Zn) and 6.457-7.943 for phytate by calcium to zinc molar ratio (Ph*Ca:Zn). A linear model was significant ($P < 0.05$) and adequate to describe variations in iron, zinc, Ph:Fe, Ph:Zn and Ph*Ca:Zn. There was a remarkably linear increase in iron and calcium contents with an increased *dabi teff* ratio in the blends accompanied by a significant decrease ($P < 0.05$) in phytate/minerals molar ratios. The findings showed that increasing *dabi teff* ratio in the blends notably increased iron content with reduced Ph:Fe molar ratio, providing the bases for developing iron-dense novel composite complementary flour with improved iron bioavailability to combat iron deficiency anemia among children.

Key Words: *Dabi teff*, Effect of blending ratios, Micronutrients, Phytate/minerals molar ratios, Novel complementary food



Introduction

In Africa, the consumption of animal foods which are good sources of high bioavailable dietary minerals is low because of affordability and religious reasons. This low consumption of animal-source foods combined with suboptimal complementary feeding practices [252, 253], resulted in higher micronutrient malnutrition among children. For example, the prevalence of anemia among under-five children is about 64.6% in Africa [234] and according to the Ethiopian Public Health Institute (EPHI) and International Classification of Functioning, Disability and Health (ICF) survey, 57% of children under-five years were anemic [19]. In Ethiopia, the lower consumption of animal-source food is majorly associated with economic and religious reasons due to which plant foods are the major staple diets and major food choices among the people. Thus, micronutrients and their bioavailability from plant foods need to be improved especially when to

be used for IYCF. This is generally because micronutrients from plant sources are not in easily absorbable forms like the non-heme forms of iron and inorganic forms of zinc as well as the presence of high mineral absorption inhibitors referred to us the anti-nutrients [248].

The contribution of micronutrients from breast milk declines with a child's age and would be almost zero after 12 months for all micronutrients and nearly all iron requirements (>97%) should come from complementary foods after 6 months [30]. Exploring different locally available, underutilized, affordable and nutritious plant foods (the dietary diversity approach) and blending them together after the application of simple pre-processing is the best option [28] to get multiple and higher micro-nutrients from their mixture and to provide dietary minerals rich foods or food with easily absorbable and bioavailable minerals as a main drive to prevent child micronutrient malnutrition [88].

Complementary feeding (from 6-24 months of age), is one of the most critical periods in children's life during when 'milk from the mother's breast' which is an ideal child food, can no longer meet the increasing nutrient needs of the growing child [115] and infant's transfer from nutritious, adequate and uncontaminated breast milk to complementary foods (often regular family food). It should be underscored that complementary foods do not replace (but complement) breastfeeding [28, 230]. The significantly higher prevalence of micronutrient malnutrition in under-five children occurs during or after the introduction of complementary foods because of the poor quality and inadequate required micronutrient nutrients for the optimal growth and development of the children [70].

Traditional complementary foods in developing countries including Ethiopia are characterized by low nutritive value, low micronutrient density and high anti-nutrients [74, 215] and children are more susceptible to micronutrient deficiencies due to poor minerals bioavailability, inadequate dietary intake and low intake of animal food sources [254].

Currently, the bioavailability of minerals and protein is the top buy than their higher amounts in the food system because of the nutritional implication that nutrients need to be absorbed and efficiently utilized for normal body function. The bioavailability of nutrients/minerals is affected by factors like chemical forms of the nutrients in foods (e.g. heme and non-heme forms of iron, organic and inorganic forms of zinc), interactions between nutrients and other organic

components including anti-nutrients, dietary fiber, ascorbic acids and by the processing or preparation practices applied [248].

Despite cereals, and legumes in particular being highly nutritious, the problem associated with the higher anti-nutritional contents present in these crops limits their nutritional quality. Anti-nutrients such as phytate, tannin, trypsin inhibitor, cyanogenic glycosides and oligosaccharides are naturally occurring constituents majorly in cereals and legumes which antagonize the absorption of one or more minerals through non-digestible insoluble complex formation, particularly with positively charged divalent minerals and reduce the bioavailability of the minerals as well as prevent their efficient utilization in human body [89, 90], thus, required to be eliminated or reduced below their critical limits by proper treatments. However, phytates and tannins have been identified to have some health benefits for instance; they can play a high antioxidant and anti-carcinogenic activity [92], lowering chronic inflammation and the chance of diabetes mellitus and lower obesity [93] and cardiovascular disease [94]. Hence, the anti-nutritional effects of phytate and tannin need to be evaluated against these health-benefit properties. That is, they can play a free radical scavenging activity against oxidation and thus alleviate the onset and progression of cancer and cardiovascular diseases.

Enzymatic (use of phytase and amylase enzymes) processing such as germination and/or fermentation 'the amylase-rich food technology' or non-enzymatic processing like roasting, soaking and dehulling are the globally accepted low-cost methods used to reduce the anti-nutrients and their adverse interference with minerals absorption such as iron, calcium and zinc [31, 91, 254]. Dietary factors such as ascorbic acid, meat, poultry and fish are also important enhancers of non-hem iron (plant-source) and zinc absorption [255, 256]. But in many developing countries, meat is too expensive and consumed on special occasions only, if not at all, where it is even unthinkable for people with poor settings or limited resources in developing countries to purchase meat regularly.

Processing and blending of cereals, legumes and oilseeds constitute a good source of micronutrients with many health benefits as well as a reduced phytate/minerals molar ratio or enhanced bioavailability [28]. Thus, it was hypothesized that mixing different locally available

cereals and legumes such as *dabi teff*, field pea and others which are expected to be rich in micronutrients accompanied by proper processing techniques would be promising to provide multiple and higher micronutrients with improved bioavailability.

Dabi teff is the 'afaan oromoo language ' name for an early maturing variety of dark red teff. The early maturing property makes the crop to be harvested twice within one rainy season (at early rainfall called "*daabi gannoo*" and late rainfall called "*daabi* birraa"). Farmers in Wollega and Illuababor, western Ethiopia, cultivate *dabi teff* either for its grain seeds or its straw. The contribution of the grains to household food and nutrition security is considerable when viewed through a food security lens while the straw is composed of fine stems used for plastering (finishing) mud hut walls during house construction and rated higher among others for animal fodder, especially oxen feeding [243]. There are many social beliefs regarding the nutritional and health benefits of *dabi teff* among the consumers that after eating its different food form such as *'kita/maxinoo* (unleavened bread), *muk/mooqa* (gruel), *mooqa manyee and cafaqoo (the favorite cultural dishes)*', it "*dhiiga dabalaa*" to mean it increases blood volume, "*humna dabalaa*" meaning it boosts energy/strength, "*dugda jabeessa*" meaning it repairs/strengthening backbone and fractured bone which could be implicated for its high calcium content where *dabi teff* is prized as medicinal food among the rural elderly people in particular and the consumers in general (unpublished observations by the researchers) . Hence, in this study, the effects of blending ratios variation on micronutrient compositions, anti-nutritional factors and phytate/minerals molar ratio of pre-processed *dabi teff*-field pea-based novel composite complementary flours were examined. The findings from this study can be used to provide the bases for developing *dabi teff*-field pea-based novel composite complementary food to be used as a sustainable food-based strategy in combating iron deficiency anemia among children.

Methods and Materials

Details of the methods related to the crop sampling, processing, flour preparation and handling were described in **Chapter 2**.

Results and Discussion

Model fitting and testing model adequacy

The fitted models were found to be adequate and significant for almost all response variables based on the F-statistic (the ANOVA regressions outputs); the P-value, the coefficient of determinations R^2 and the agreement between R^2_{adj} and R^2_{pred} **(Table 5.11)**. Normality and constant variance assumptions were fulfilled; that normal plots of residuals were well aligned around the actual line for most response variables showing a well-fitted model design.

Table 5.11 shown that the linear model was adequately fitted for iron, zinc, Ph:Fe, Ph:Zn and Ph*Ca:Zn contents at P-values of <0.05 whereas for calcium the same linear model was suggested by the software but not significant or no predictive power (P >0.05). This shows that x_1, x_4 and x_6 were the significant model terms for these variables with their corresponding P-values at 0.0001, 0.0164 and 0.1959 for iron, zinc and calcium, respectively. Meaning changes in iron, zinc, Ph:Fe, Ph:Zn and Ph*Ca:Zn can adequately be described by the linear models (adequate predictive power) as a function of the component ratios variation in the blends.

The predictive regression models developed for the relationship between the independent (Xs') and dependent (Ys') in terms of mineral contents, anti-nutrients and phytate/minerals molar ratio of the blends was presented in **Table 6.2** for iron, zinc, calcium, magnesium, phytate, Ph:Fe, Ph:Zn, Ph:Ca and Ph*Ca:Zn, respectively. The coefficients with single factor (linear model terms) (X_1, X_4, and X_6) represent the independent effect of a particular determinant variable while coefficients with two of the factors (X_1X_4, X_1X_6, and X_4X_6) represent an interaction between the factors (interactive model terms). A negative sign in front of the regression model terms is an indication of an antagonistic relationship while a positive sign indicates a synergetic relationship or synergetic effect.

Preliminary study and Nutrisurvey analysis result

Prior to blending the components together, a preliminary study on selected micronutrient contents of the typical component, the *dabi teff* was conducted and the result showed that it contained a considerable amount of iron at 122.66 mg/100g, calcium at 190 mg/100g and zinc

at1.8 mg/100g **(Annex V)**. In this study, the mean and standard deviations of the micronutrient contents of the individual components used were presented in **Table 5.9** which were customized 'into' the Nutrisurvey software. The generated output from the Nutrisurvey analysis showed the percentage fulfillment by the meal of the mix (blend) of our components as compared to the recommended nutrient intake (RNI) to be 612-1045%, 133-225% and 229-400% for iron, zinc and magnesium, respectively **(Table 5.10)** which corresponds to 20-35% of *dabi teff*, 0-30% of field pea, 5-20% of germinated maize, 25% of barley, 15% of oats and 5% of linseed meal mixture, respectively which were used as constraints for generating the blending matrix.

Table 6.2: Regression models showing the effects of the independent variables (Xs') on the dependent variables (Ys') in the blends

Dependent variables (Ys')	Regression Models with Xs' (Actual Equations)	Model
Iron (Y_1)	$0.784574\ x_1 + 0.243021\ x_4 + 0.257295\ x_6$	Linear
Calcium (Y_2)	$1.45713\ x_1 + 1.3401\ x_4 + 1.2887\ x_6$	Linear
Zinc (Y_3)	$0.050019\ x_1 + 0.0444216\ x_4 + 0.0340729\ x_6$	Linear
Phytate(Y_4)	$-0.678836\ x_1 + 0.031137\ x_4 - 0.419523\ x_6 + 0.152411\ x1x_4 + 0.162141x1x_6 + 0.0262255\ x_4x_6$	Quadratic
Ph:Fe (Y_5)	$0.00131006\ x_1 + 0.00927607\ x_4 + 0.00910206\ x_6$	Linear
Ph:Zn (Y_6)	$0.0467844\ x_1 + 0.0847505\ x_4 + 0.100725\ x_6$	Linear
Ph:Ca (Y_7)	$-0.000937918\ x_1 - 0.000335364\ x_4 - 0.000883061\ x_6 + 0.000147022\ x_1x_4 + 0.00016075\ x_1x_6 + 3.61222e\text{-}05\ x_4x_6$	Quadratic
Ph*Ca:Zn (Y_8)	$0.0947537\ x_1 + 0.158155\ x_4 + 0.183046\ x_6$	Linear

Key: x_1- *dabi teff*, x_4- field pea, x_6- germinated maize.

Effects of blending ratios on dietary mineral contents of complementary flour blends

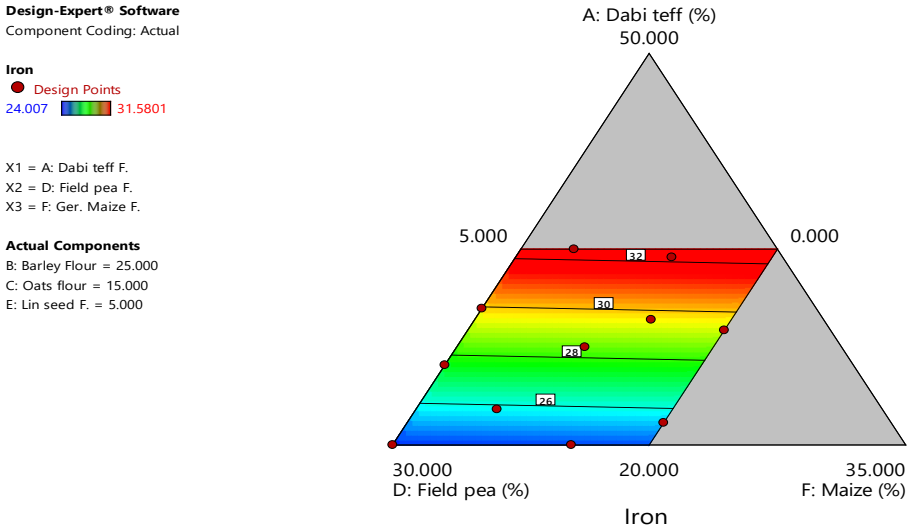
The mean and standard deviation of mineral contents of the eleven blends of complementary flours were summarized in **Table 5.14.** Results of mean difference separation showed that all the mineral contents were significantly different ($P < 0.05$) among the blends (as affected by components ratios variation) and ranged from 24.01-31.58 mg/100g for iron, 73.46 -78.81 mg/100g for calcium, 2.33-2.61 for zinc, 113.9-124.22 mg/100g for magnesium, 364.77-497.48 mg/100g for potassium, 281.03-308.54 mg/100g for phosphorous and 3.46-6.25 mg/100g for

sodium, respectively. The control flour contained 7.25 mg/100g, 49.45 mg/100g, 2.64 mg/100g, 96.45 mg/100g, 398.67 mg/100g, 282.65 mg/100g and 4.07 mg/100g for iron, calcium, zinc, magnesium, potassium, phosphorous and sodium, respectively (Table 6.6). The iron and calcium contents of the blended flours were significantly higher ($P < 0.05$) than that of the control flour whereas zinc content was slightly lower at 2.64 mg/100g. This finding showed that the iron content of the blends exceeds (1.5-1.97 times higher than) the recommended value by FAO/WHO [28] at 16 mg/100g in formulated complemented food for infants and older children. On the other hand, calcium and zinc contents of the blends were below the recommendations at 500 mg/100g for calcium and 3.2 mg/100g for zinc which was in agreement with the report by Gibson *et al*. [31] that an iron, calcium and zinc deficits were observed in indigenous complementary foods usually consumed by infants and young children in the southern part of Ethiopia.

Contrary to the current findings, Mezgabo *et al*. [166] reported a wider range of iron content at 9.38-34.86 mg/100g but it fairly agrees and it was further reported a higher calcium content at 293.57 mg/100g for complementary porridge formulation from red teff, malted soybean flour and papaya fruit powder where the report associated the high calcium content of the formulations with the higher calcium content of soybean. The zinc content of the current blends was lower than the report by Mezgebo *et al*. [166] that it ranged from 4.05-5.58 mg/100g for complementary porridge formulation from red teff, malted soybean flour and papaya fruit powder and the higher zinc content was associated with malted soya flour ratio in the formulation.

In this study, out of the seven minerals experimented **(Table 5.14)**, the effects of component ratio variation in the blends on the major three minerals (iron, calcium and zinc) were more emphasized and narrated because of their relatively higher importance to be present in complementary food that determines child growth and development. There observed a remarkably linear increasing effect in iron content with an increased proportion of *dabi teff* (the single influencing mixture component) as shown by 2D contour and trace plots **(Figure 6.9)** which could be due to the significantly higher iron content of *dabi teff* flour at 86.5 mg/100g **(Table 5.9).** It can be seen from **Table 5.14** that at the maximum ratio (35%) of *dabi teff* in the

blends, the iron content was at the maximum value of 31.58 mg/100g (F6) whereas at the lowest *dabi teff* ratio (20%), the iron content was at the lowest value of 24.01 mg/100g (F4). These findings agreed with the report by Mezgebo *et al*. [166] that the iron content had a direct positive association with the amount of red teff flour ratio in the porridge flour formulation from red teff flour, malted soya flour and papaya flour powder. But in contrary, the effect of *dabi teff* ratio variations in our blends was higher than the report by Alaunyte *et al*. [244] who reported that by supplementing wheat bread with 30 percent teff flour, the iron content of the bread was more than doubled where in our blends, it was more than tripled (3.31-4.36 fold) than the control at *dabi teff* range of 20-35% in the blends. On the other hand, there observed a negative relationship between iron content and germinated maize as well as field pea flour ratios in the blends. The probable reason for this could be the very low iron contents of these two components **(Table 5.9).**



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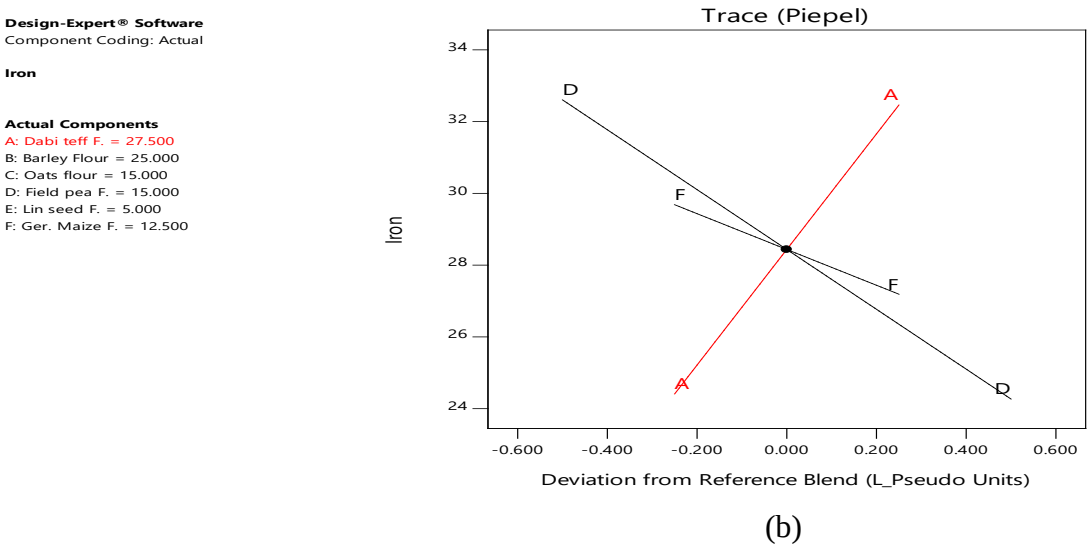
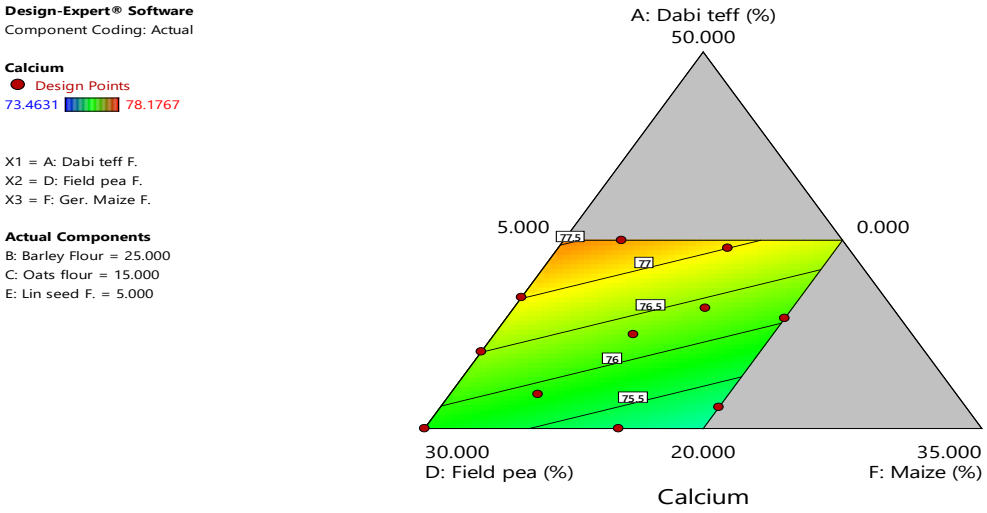


(b)

Figure 6.9. Model graphs showing the effects of the major components ratios on iron content. (a) 2D contour plot. (b) Trace plots.

Similarly, there was a linear increasing effect in calcium content with an increased proportion of *dabi teff* flour ratio in our blends as shown by 2D contour and trace plots **(Figure 6.10)** which could be due to the significantly higher calcium content of *dabi teff* flour among the other components. On the other hand, there observed a negative relationship between calcium content and germinated maize as well as field pea flour ratios in the blends though the decrease in calcium content in relation to field pea was not as sharp as that of germinated maize. The probable reason for this could be the very lower calcium contents of germinated maize flour than the field pea flour **(Table 5.9).** On the contrary to the present finding, Mezgebo *et al*. [166] reported that calcium content increased with an increase in soybean ratio (a pulse crop) in complementary food formulation from red teff flour, malted soya flour and papaya flour powder.



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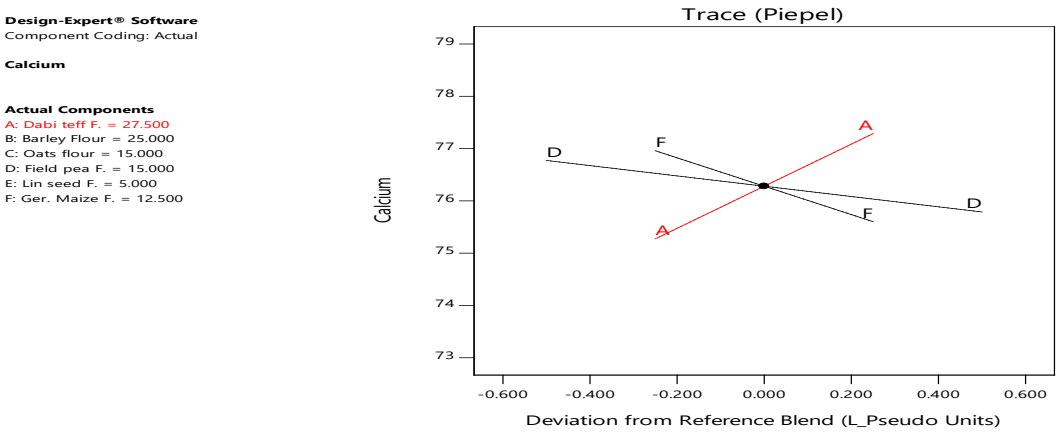
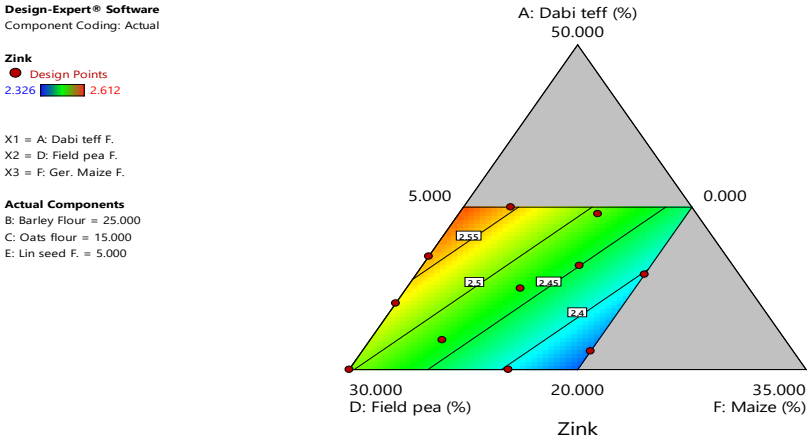


(b)

Figure 6.10. Model graphs showing the effects of the major component ratios on calcium content. (a) 2D contour plot. (b) Trace plots.

Concerning zinc content, there observed a moderately increasing effect with an increased proportion of *dabi teff* and field pea flours while it showed a negative relationship with germinated maize flour in the blends as shown by 2D contour and trace plots **(Figure 6.11)**. This finding agrees with the report by Mezgebo *et al*. [166] that zinc content increased as the ratio of soybean flour increased in complementary flour formulation which is also a pulse crop as that of our field pea.



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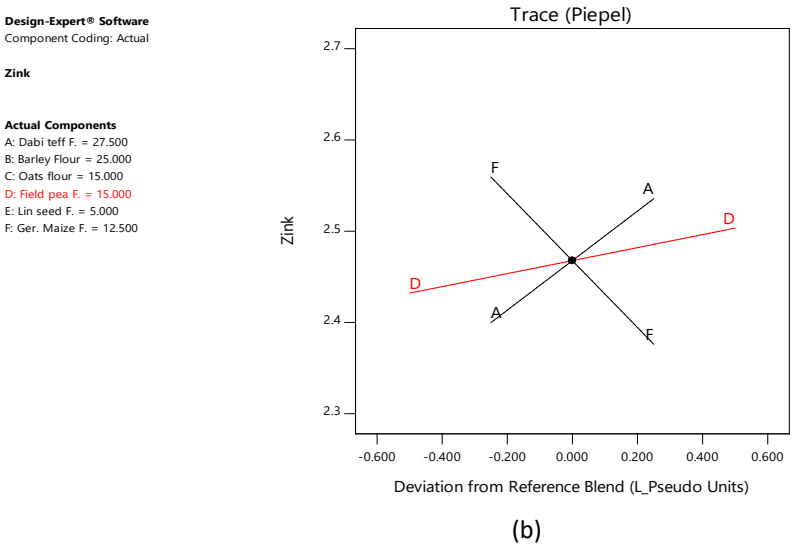


(b)

Figure 6.11. Model graphs showing the effects of the major components ratios on zinc content. (a) 2D contour plot. (b) Trace plots.

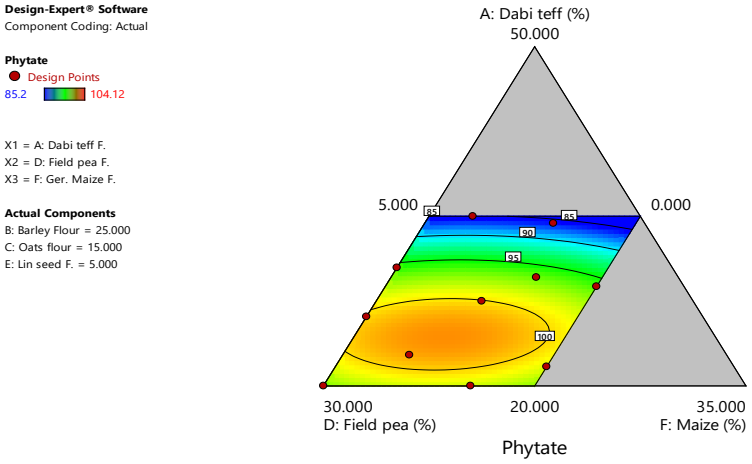
The 'novelty' of this study would be accounted for the incorporation of *dabi teff* flour into the blends containing high iron (86.5 mg/100g) and linseed which is a leading source of α-Linolenic acid, omega-3 polyunsaturated fatty acid that would make the blends super. The nutritional composition, particularly the iron content of *dabi teff* is quite different (much higher) from the other popular red teff varieties grown in Ethiopia. For example, [166] reported a lower level of iron content at 38 mg/100g for the popular red teff grown in Ethiopia. Similarly, Daba [27] reported a lower iron content of the red-teff (Dz-01-1681) variety at 20.6 mg/100g, which makes *dabi teff* a unique and super iron bank.

Effects of blending ratio variation Anti-nutritional factors (Phytate and Tannin) of the complementary flour blends

The mean and standard deviation of anti-nutrient contents of the blended complementary flours were summarized in **(Table 5.15).** The anti-nutrients among the blends ranged from 85.2-104.12 mg/100g for phytate and from 8.35-23.1 mg/100g for tannin content, respectively and they were significantly different ($P < 0.05$) among the blends. The first blending (F1) and the tenth blending (F10) had shown a significantly higher ($P < 0.05$) phayate content at 102.72 and 104.12 mg/100g as compared to the rest of the blends while the control had shown incomparably higher phaytate content than all the blends at 126.54 mg/100g.

There observed an increasing linear effect in phytate content as a function of field pea flour, where, as a field pea flour ratio increased in the blends, the phytate content linearly increased

where this was inconsistent with the report by Mezgebo *et al*. [166] that phytate content increased with an increase in the soybean ratio in the formulation of complementary food. But there observed a decrease in phytate content with an increasing ratio of *dabi teff* flour ratio in the blends as revealed by 2D contour and trace plots **(Figure 6.12).** The lowest phytate content was observed in the sixth blend (F6) at 86.27 mg/100g and eighth blend (F8) at 85.2 mg/100g where the ratio of *dabi teff* flour at these two blends was at the highest ratio. This suggested that *dabi teff* enriched novel food products would be less likely to exhibit absorption inhibition due to the lower phytate content observed in *dabi teff* enriched products.



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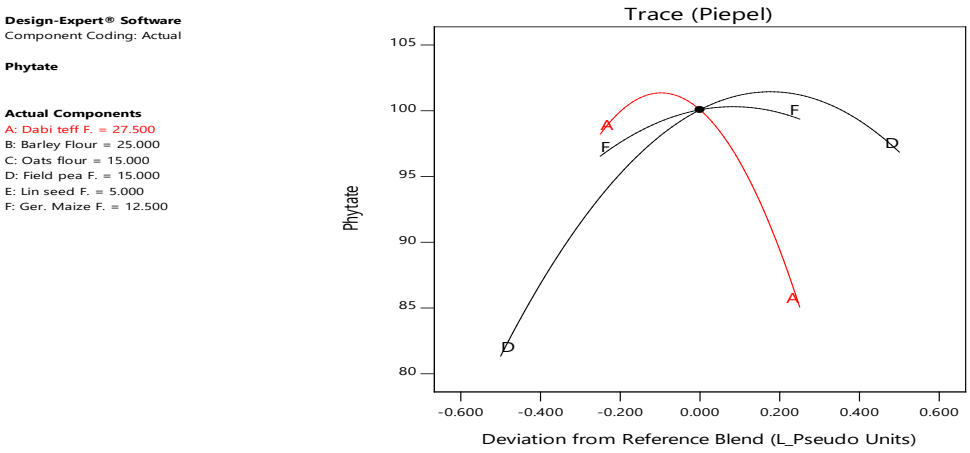


(b)

Figure 6.12 Model graphs showing the effects of the major components ratios on phytate content. (a) 2D contour plot. (b) Trace plots.

Regarding tannin content of the flour blends, the eighth blend (B8) shown significantly higher ($P < 0.05$) tannin content followed by the fourth blend (F4) and eleventh blend (F11) at 23.1, 18.06

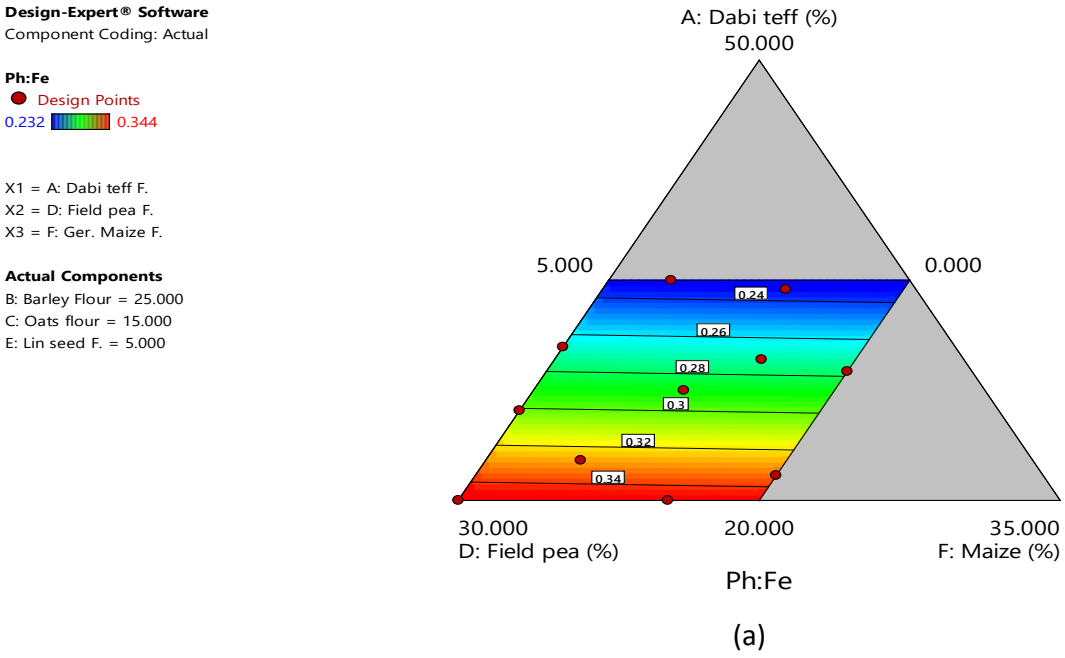
and 18.06 mg/100g, respectively while the lowest tannin content was observed in the ninth blend (F9) at 8.35 mg/100g and the control had a similar tannin content (12.42 mg/100g) as the rest of the blends **(Table 5.15)**. Tannin content was inversely correlated to *dabi teff* flour typically revealed by the sixth blend (F6), lower tannin content (10.64 mg/100 g) at highest *dabi teff* ratio (35%) and ninth blend (F9), lowest tannin content (8.35 mg/100 g) at lowest *dabi teff* ratio (20%) also it was inversely correlated to field pea flour ratio as revealed by the eighth blend (F8), highest tannin (23.1 mg/100 g) at lower field pea ratio (6.48%) and tenth blend (F10), lowest tannin (8.56 mg/100 g) at higher field pea flour (22.74%). Thus it can be suggested that *dabi teff*-field pea-based novel complementary food would pose a low association with the tannic acid absorption inhibition effect.

Effects of blending ratios on phytate/minerals molar ratio (In-vitro mineral bioavailability)

In nutrition science, the anti-nutrients/minerals molar ratio is more interesting than the level of the anti-nutrient content in foods because of its implication in the absorption and bioavailability of minerals. In this study, the mean values of Ph:Fe, Ph:Ca, Ph:Zn and Ph*Ca:Zn among the blends ranged from 0.232-0.344, 0.067-0.085, 3.356-4.18 and 6.457-7.943, respectively **(Table 5.15).** There was a significant difference ($P < 0.05$) in mean values of phytate/minerals molar ratios except Ph:Ca among the blended composite complementary flours. The mean Ph:Fe molar ratio among the blends at 0.29:1 was below the critical limit at 1:1, above which iron absorption can be impaired [31]. A value greater than 1:1 ratio has a negative effect on iron absorption; where the calculated values in the present study were all less than 0.4:1 which is a preferable Ph:Fe molar ratio [37, 116, 245].

The Ph:Fe molar ratio showed a strong inverse relationship with the proportion of *dabi teff* flour in the blends where the lowest molar ratio was observed in the sixth blend (F6) and eighth blend (F8) at 0.232 (at the highest proportion of *dabi teff* flour at 35% and 34.385%) and the highest Ph:Fe molar ratio was observed in seventh (F7) and tenth blends (F10) at 0.340 and 0.344, respectively where the proportion of *dabi teff* flour was at the minimum ratios. This inverse relationship was revealed in 2D contour and trace plots **(Figure 6.13).** The Ph:Fe molar ratio in this study showed a direct (linear) relationship with the proportion of field pea flour in the blends (increasing field pea flour ratio resulted in increased Ph:Fe content and the vice-versa). There

was also a linear relationship between Ph:Fe molar ratio and the proportion of germinated maize flour in the blends but moderate **(Figure 6.13).**



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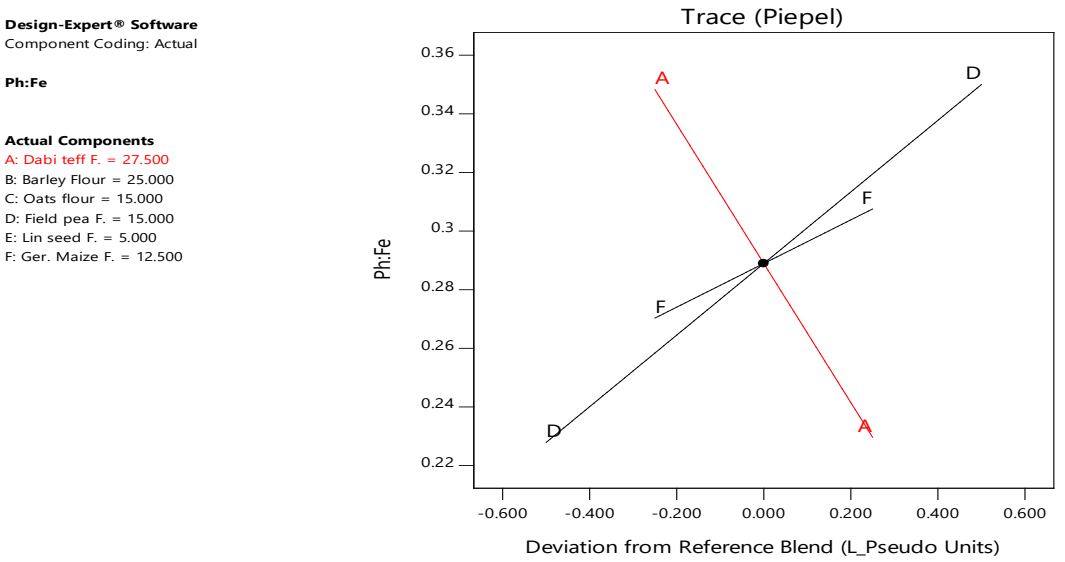
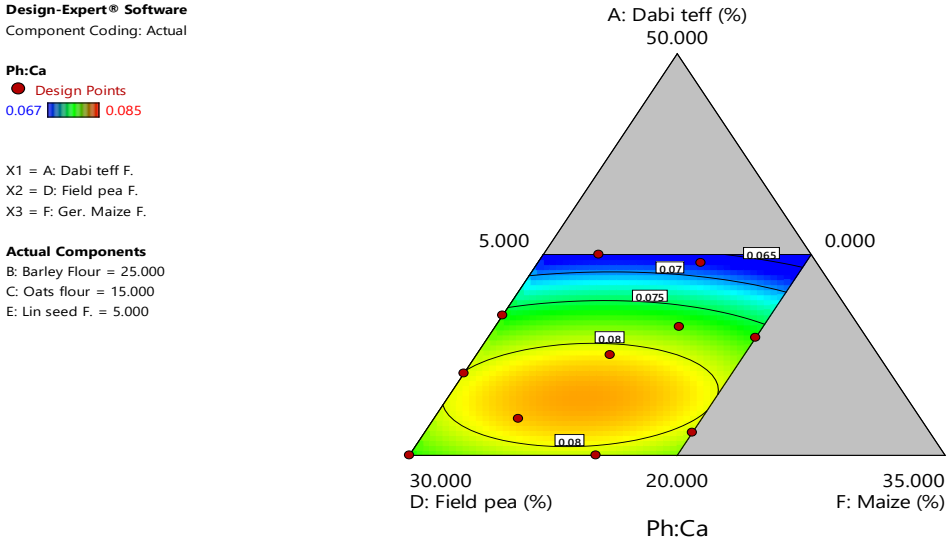


(b)

Figure 6.13. Model graphs showing the effects of the ratios of the major components on Ph:Fe content. (a) 2D contour plot. (b) Trace plots.

The mean Ph:Ca molar ratio among the blends at 0.076:1 was below the critical limit at 0.24:1, above which calcium absorption can be impaired [31]. The relationship between the proportions

of *dabi teff* flour and Ph:Ca molar ratio was inverse in the blends with the lowest determined values of 0.067 at the maximum ratio of *dabi teff* flour at 35% and 34.385% (F6 and F8), respectively while the highest determined values of Ph:Ca at 0.083 and 0.085 were at minimum proportion ratio of *dabi teff* flour ratio at 22.73% and 27.5%, respectively and the relationship was revealed by 2D contour and trace plots **(Figure 6.14).** The germinated maize flour ratio was also negatively correlated to Ph:Ca molar ratio while field pea flour ratio in the blends was positively correlated to Ph:Ca molar ratio.



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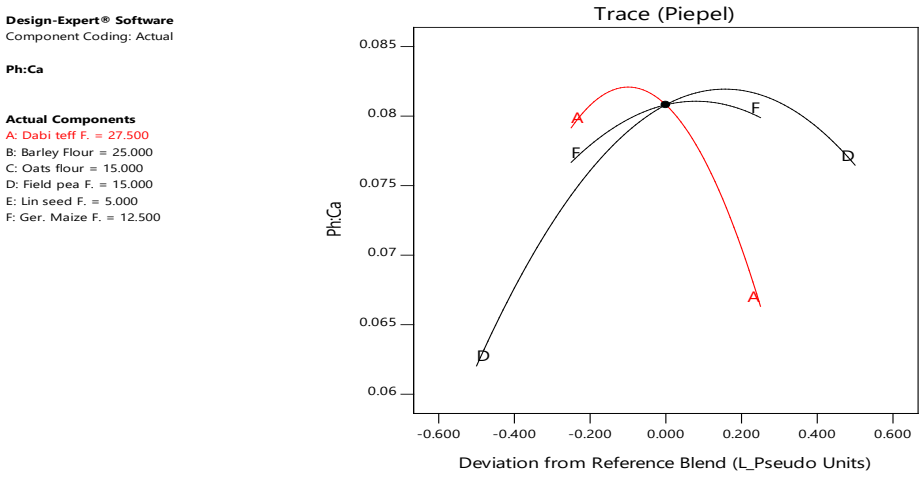

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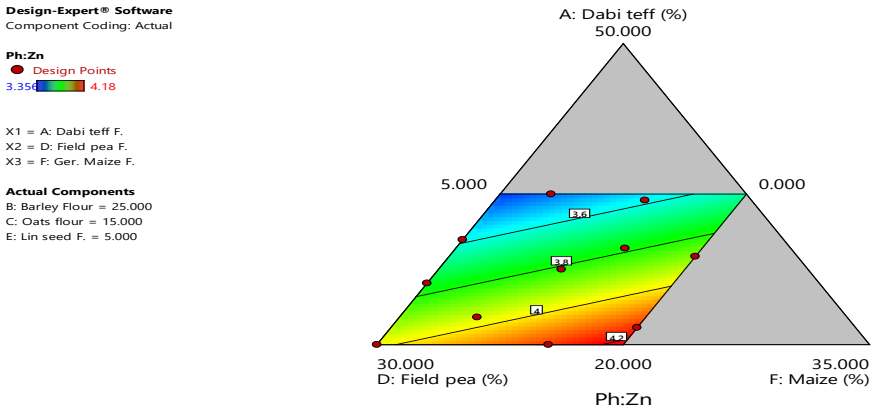
Figure 6.14. Model graphs showing the effects of the ratios of the major components on Ph:Ca content. (a) 2D contour plot. (b) Trace plots.

The mean Ph:Zn molar ratio among the blends at 3.83:1 was lower than the critical limit of 15:1 **(Table 5.15).** A value greater than the critical limit is associated with low estimated bioavailability, where 5-15 and below 5 Ph:Zn molar ratio are associated with moderate and high estimated bioavailability, respectively, with zinc absorption corresponding to 15%, 30%, and 50%, respectively [116, 245]. The calculated value in this study falls in the third category (Ph:Zn below 5) showing higher estimated bioavailability with an expected 50% absorption rate. The relationship between the proportion of *dabi teff* flour and Ph:Zn molar ratio was strong inverse, where the lowest Ph:Zn values (3.468 and 3.356) were at the sixth blend (F6) and eighth blends (F8) (35% and 34.385% ratio of *dabi teff*) which was the maximum ratios and the relationship was revealed by 2D contour and trace plots **(Figure 6.15).** A direct relationship was observed between Ph:Zn molar ratio and the proportion of field pea as well as germinated maize flour among the blends (i.e an increase in the ratio of the two components in the blends resulted in an increased Ph:Zn molar ratio).



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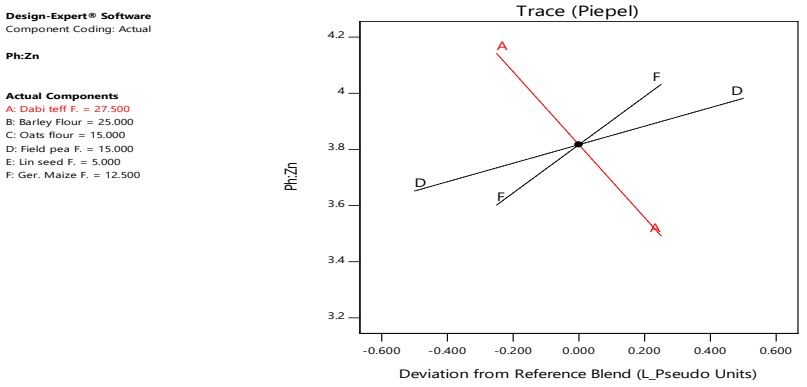

(b)

Figure 6.15. Model graphs showing the effects of the ratios of the major components on Ph:Zn content. (a) 2D contour plot. (b) Trace plots.

In line with the present finding, Castro-Alba *et al*. [116] have reported a significant negative correlation (P <0.05) between Ph:Fe and cereals and a positive correlation between Ph:Zn and dry legumes. In contrary to the present finding, Castro-Alba *et al*. [116] has reported a negative correlation between Ph:Ca and dry legume which could be attributed to different factors.

The mean range of Ph*Ca:Zn molar ratio among the blends at 7.29:1was found to be far below the critical limits which is 200:1 above which zinc absorption would be impaired. In the same fashion, the relationship between *dabi teff* flour proportion and Ph*Ca:Zn molar ratio was strongly inverse where the value was at a minimum at 6.457 and 6.778 with its corresponding maximum *dabi teff* flour ratio at 34.385% and 35% (F8 and F6), respectively and the relationship was revealed by 2D contour and trace plots **(Figure 6.16).** The relationship between Ph*Ca:Zn molar ratio and field pea flour as well as germinated maize flour proportion was direct, where the Ph*Ca:Zn molar ratio kept increasing as the proportion of these two flours increased in the blends.

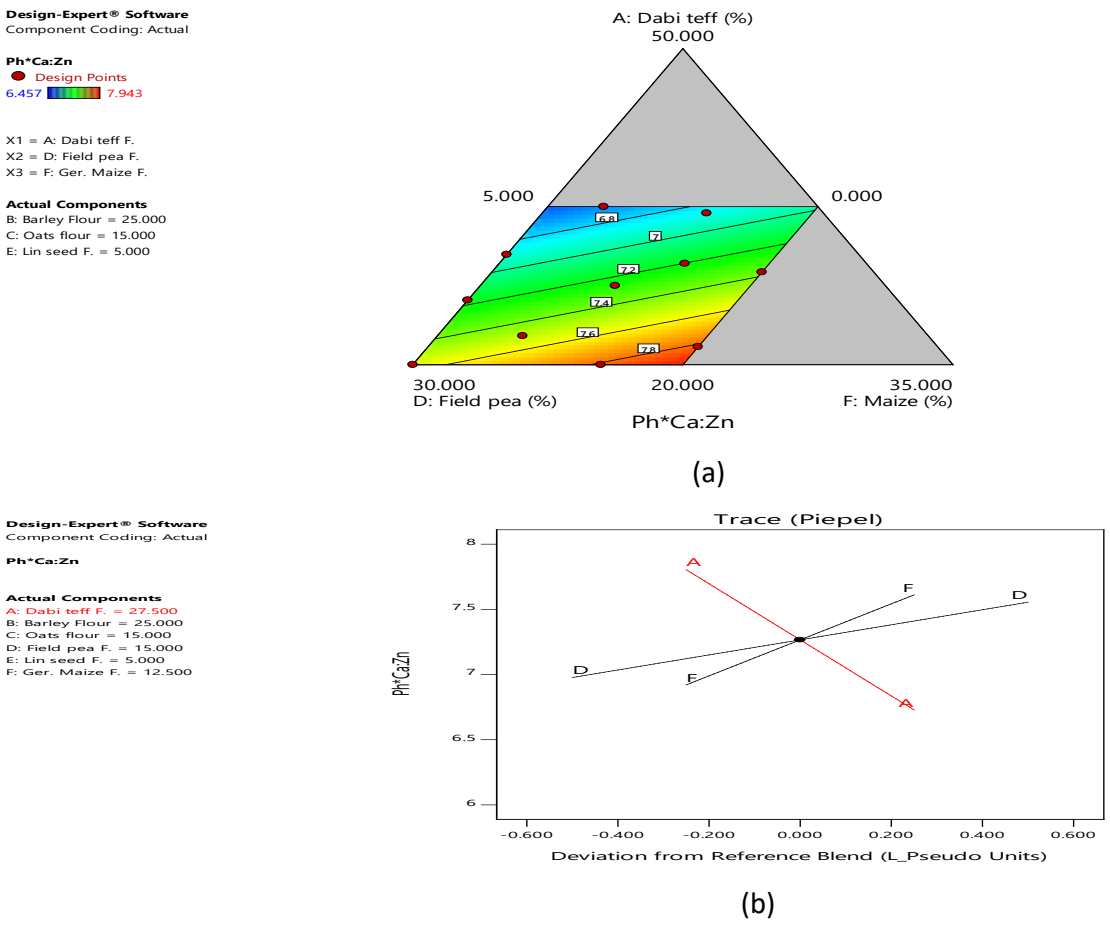

Figure 6.16 Model graphs showing the effects of the ratios of the major components on Ph*Ca:Zn content. (a) 2D contour plot. (b) Trace plots.

From the present findings, it can be generalized that the inhibitory effect of phytate on iron, calcium and zinc absorption and bioavailability would be insignificant (unlikely to be markedly compromised) because the molar ratios computed for the minerals were far below the critical limit above which their absorption and utilization would be impaired. The probable reason for these low phytate/mineral molar ratios might be the effect of the pre-processing techniques applied; soaking, germination, roasting, and dehulling processes on the components used which is supported by the report by Gibson *et al*. [248] that molar ratios of phytate/mineral were improved (reduced) by processing techniques such as roasting, germination, and fermentation.

Conclusion

Multiple grains at varied mixing ratios are more likely to contain multiple and higher micronutrients than grains from a single (mono) grain. The present study demonstrated that complementary fours that can compose the limiting minerals (iron, zinc and calcium) in an increased level could be prepared from *dabi teff*-field pea blends. The ratio variation of the major components has shown a significant effect on the dietary mineral contents as well as on the phytate/minerals molar ratios of the blends. There was a remarkably linear increase in iron and calcium contents of the blends with an increased *dabi teff* ratio accompanied by a significant decrease in phytate/minerals molar ratio which is a good proxy indicator of higher iron bioavailability. The findings revealed that increased iron content accompanied by its enhanced bioavailability (the reduced phytate/iron molar ratio) can be prepared from *dabi teff*-field pea-based local food crops, providing the bases for developing iron-dense novel composite complementary flours that can serve as a sustainable food-based strategy to alleviate iron deficiency anemia among children.

7

Chapter 7:

Energy, Protein and Iron Densities of *Dabi Teff*-Field Pea-Based Optimized Novel Complementary Flour and Its Contribution to Daily Energy and Nutrients Demand by 6–23-months-old Children

Redrafted from: Diriba Chewaka Tura, Tefera Belachew, Dessalegn Tamiru, Kalkidan Hassen Abate. **Energy, protein and iron densities of *dabi teff*-field pea-based optimised novel complementary flour and its contribution to daily energy and nutrients demand by 6–23-months-old children.** *British Journal of Nutrition. 2024;131. doi:10.1017/S0007114523002581. Cambridge University Press. 2024. Nov 08.*

Abstract

Inadequate intake of age-specific energy and nutrients is among the prime immediate causes of child malnutrition. Thus, this study aimed to analyze the energy, protein and iron densities of pre-processed *dabi teff*-field pea-based optimized novel complementary flour and its contribution to daily energy and nutrient demand by 6-8, 9-11 and 12-23 months old children. The optimal formula at overall optimization was identified to be 34.66% *dabi teff*, 25% barley, 15% oats, 15.34% field pea, 5% linseed and 5% maize with response values of 15.74% protein, 5.09% fat, 2.26% ash, 2.88% fiber, 73.05% carbohydrate, 380.43 kcal/100g energy, 32.21 mg/100g iron, 77.51 mg/100g calcium and 2.59 mg/100g zinc. The energy density of the optimized novel complementary flour was 1.27 kcal/g which fulfilled the PAHO/WHO recommendation (≥0.8 kcal/g), protein density was 4.14 g/100kcal and the iron density was 8.47 mg/100kcal which was 2.12 to 10.59 times higher than the recommended value where the optimal had demonstrated to contribute more than 100% of the daily energy and protein demand and notably more than 200% of daily iron demand at moderate bioavailability (0.8-4 mg/100kcal) and it even met at low bioavailability (7.7 mg/100kcal). These findings showed that the daily recommended dietary allowance for energy, protein and iron could be attained by the developed *dabi teff*-field pea-based optimized novel complementary flour and its contribution to the children's daily energy and nutrients demand met the standard, where the product can be used as food-based nutrition intervention to sustainably manage protein-energy malnutrition and iron deficiency anemia in children.

Key Words: *Dabi-teff*, novel complementary food, nutrient density, iron adequacy, 6-23 months old chuldren

Introduction

Adequate nutrition during childhood is a key factor to support the rapid rate of physical growth and mental development that occurs in the first 2 years of life [74, 84] as well as for healthy life and future productivity of children [257, 258]. Stunting or declined linear growth of children usually occurs during the period of complementary feeding, particularly around the end of the second year of life and ends irreversible [235]. Additionally, iron deficiency anemia is more prevalent in early childhood (between 6 and 24 months) than at any other time in the life cycle where the major causes are inadequate dietary intake of bioavailable iron, malaria and parasitic infections [236, 241]. During this critical time, there is a high dietary requirement of iron per body weight, where some health firms recommend that infants should be primarily introduced at first to iron-rich complementary foods such as iron-fortified or meat foods to meet their iron requirements and alleviate long-life developmental impairments [259, 260].

Infants and young children need adequate energy and a nutrient-dense diet both through exclusive breastfeeding followed by quality complementary feeding [74, 84, 261]. In the first 6 months after birth, breast milk is adequate for energy and nutrient requirements by infants [84] after which the milk is no longer sufficient and children transfer from ideal and uncontaminated breast milk to poor-quality complementary foods (often regular family food) with the highest chance of vulnerability to malnutrition and infectious diseases [28, 230].

The energy density of a food refers to the amount of calories per unit weight of the food and is expressed as kcal/g whereas nutrient density refers to the amount in grams of macro-and micronutrient nutrients in a food per 100kcal [85, 146]. The energy needs from complementary foods for infants with “average” breast milk intake in developing countries are approximately 200 kcal per day at 6-8 months of age, 300 kcal per day at 9-11 months of age, and 550 kcal per day at 12-23 months of age [219, 240]. According to FAO/WHO [28] guidelines, the critical values of nutrient contents in formulated complementary food should be 400-425 kcal/100g for energy, $\geq$15% for protein, 10-25% fat, 64$\pm$4% carbohydrate, 16 mg/100g iron, 500 mg/100g for calcium and 3.2 mg/100g for zinc to be nutritional adequate [28].

Complementary foods need to be far more nutritious compared to family foods to fill the energy and nutrient gap. It should contain protein of high-biological value composing the essential

amino acids. Yet, the opposite is the case in low-income countries. Apart from a major source of essential amino acids; protein is a good energy source at times of energy deprivation [262, 263]. Low energy intake accompanied by inadequate intake of protein leads to protein-energy malnutrition, the commonest lethal form of malnutrition revealed to us the 'the silent emergency of the world' [264]. Thus, infants and young children need adequate protein where the recommended daily amount of protein in grams for a 6-8-month-old child is 9.1 g, for 9-11 months is 9.6 g and for 12-23 months old child is 10.9 g [219]. The ratio of energy from protein to the total energy content of a given food which is referred to us the protein energy ratio, is one of the indicators used to assess the adequacy and quality of protein in complementary foods. The codex allementrous commission [28] specifies that the daily energy needs from protein (protein-calorie) should be between 6-15% of the total daily energy requirements and typically should not exceed 15%. This is because while protein is still crucially required for growth and development, a higher protein-calorie ratio beyond 15% in complementary foods may increase the renal solute load (the non-metabolized nitrogenous end products) and interfere with appetite [265].

In addition to protein, infants and young children require an adequate amount of micronutrients such as iron, zinc, calcium and vitamins to meet their physiological needs, particularly in the first two years of life and they are prone to micronutrient deficiencies and associated diseases [263, 266]. Ahmed *et al.* [86] stated that globally, 7.3% of the disease burden in children is accounted for by micronutrient deficiencies. The contribution of dietary minerals from breast milk declines with a child's age and would be almost zero after 12 months which makes these nutrients limiting nutrients [30]. The percentage of total daily micronutrient requirement from complementary foods may range from 30 to 100%. For instance, for 9-11 months old children, about 97% of iron, 86% of zinc, 72% of calcium, 76% of magnesium, 81% of phosphorus and 73% of sodium requirements of children 6 to 24 months should come from complementary food, assuming average breast milk intake [6, 219].

PAHO and WHO [219] recommend iron intake by children at 6-8 months, 9-11 months and 12-23 months should be 11 mg/day, 11 mg/day and 7 mg/day, respectively where these figures are 4.1 mg/day, 4.3 mg/day and 5.8 mg/day for zinc and 400 mg/day, 400 mg/day and 500 mg/day for calcium for the respective age groups. Meeting such high demand is unlikely to be attained

through traditional complementary food which calls for searching for other promising alternatives such as exploring locally available underutilized/forgotten nutritious cereals and legume crops and developing optimized complementary flour.

The theoretical gastric capacity of infants and young children is 30 g/kg body weight per meal but their functional gastric capacity is much lower than this figure in usual settings. For instance, Abeshu *et al*. [127] have reported that the functional gastric capacity of 6-23-month-old children on average was around 20 g/kg body weight per meal for the study done at Wolayita Zone of Ethiopia, whereas Baye *et al*. [204] had reported a very low functional gastric capacity at 9 g/kg body weight per meal for 12-23 months children for the study done at North Wollo of Ethiopia. Because of this lower functional gastric capacity, children need to be fed energy and nutrient-dense food to satisfy their daily demands and served more frequently than adults by WHO [265]. PAHO/WHO [219] recommends the required minimum energy density from processed cereal-legume-based complementary food should be 0.8 kcal/g, protein density should be up to 5.5 g/100kcal and iron density needs to be 0.8-4 mg/100kcal where the required number of meals to be served to children per day was estimated to be 2-3 times for 6-8 months old children and 3-4 times for 9-23 months old children to provide adequate daily energy and nutrients requirements by children with additional one or two nutritional snacks based on the appetite of the child.

Application of some simple processing techniques like fermentation, germination, soaking, roasting and dehulling can improve the nutrient densities and absorption of cereal-legume foods as well as boost organoleptic qualities [28]. Dietary factors such as meat, poultry, fish and citrus fruits are also good sources and important enhancers of non-heme iron and zinc absorption from plant foods [255, 256]. But in many developing countries, these products are too expensive and consumed on special occasions only, if not at all, where it is even unthinkable to purchase meat by people with poor socio-economic classes. Currently, the development of energy and nutrient-dense complementary foods from local and readily available nutrient-rich cereals and legumes combinations has received a lot of attention in many developing countries since it is cost-effective, sustainable and can be adapted to different cultural, and dietary traditions and locally feasible strategies [52, 61]. The food-based dietary guidelines by the International Life Science Institute [267] focus on the combination of foods that can meet nutrient requirements rather than

on how each specific nutrient is provided in adequate amounts. This guideline was based on the fact that people eat foods, not nutrients or food tablets [32]. Recently, nutrition has gotten increasing attention with stronger political involvement both at the national and international levels. For instance, the Ethiopian food and nutrition policy and strategy which was recently formulated supports the development and promotion of local production of complementary foods meeting acceptable standards as on one of its operationalizing the strategy [56].

Generally, inadequate intake (both in quantity and quality) of age-specific essential nutrients together with infectious diseases are the prime immediate causes of child malnutrition leading to anthropometric deficits (stunting, wasting, underweight), anemia, impaired cognitive development and finally increased morbidity and mortality. According to the Ethiopian Public Health Institute survey [19], 37% of children under five years old were stunted, 21% were underweight, 7% were wasted, 2.9% were overweight and 57% anemic. Thus, it was hypothesized that the development of energy, protein and iron-dense complementary foods from locally available food crops including the underutilized/marginalized crops that could meet acceptable standards for energy and nutrient requirements by children and its promotion could be used to sustainably manage child malnutrition and its aligned consequences.

The sub-Saharan African region including Ethiopia is endowed with a rich diversity of ‘orphan crops’ having improved nutritional and medicinal values as well as sources of income generation [35]. Little or no attention was given to such crops in terms of research and development and policy framework that could promote their extensive agricultural production, industrial utilization as well and home consumption. In Ethiopia, the food culture of teff grains, ‘a widely utilized orphan crop’ is both historical, an integral part of the country’s antiquity and mostly traded domestically where more than 70% of the Ethiopian population use teff grains as a traditional staple meal.

Dabi-teff (Eragrostis teff), a farmer variety teff grain, is one of the underutilized/marginalized or forgotten food crops grown in Ethiopia (Oromia region) that could be utilized to combat macro- and micronutrient malnutrition. *Dabi teff* is the 'afaan oromoo language' name for an early maturing variety of dark red teff grain. The early maturing property of the grain makes the crop to be harvested twice within one rainy season (at early rainfall called "*daabi* gannoo" and late

rainfall called "*daabi* birraa"). Farmers in Wollega and Illuababor zones, western Ethiopia, cultivate *dabi teff* either for its grain seeds or its straw. The contribution of the grains to household food and nutrition security is considerable when viewed through a food security lens while the straw is composed of fine stems used for plastering (finishing) mud hut walls during house construction and rated higher among others for animal fodder, especially oxen feeding [243]. There are many social beliefs regarding the nutritional and health benefits of *dabi teff* among rural elderly people in particular and consumers in general (Personal communication).

Price-wise, the red teff varieties grown in Ethiopia including *dabi teff* are the cheapest. For example, Rashid and Negasa [201] reported that the market price of mixed teff and red treff was 24% and 55% less than the white teff. In addition to lower prices, red teff varieties have been implicated in the low incidence of anemia in Ethiopia which is presumed to be due to the grain's high iron content [103]. The other cereals and legume components included in our current formulation for optimization were thoughtfully selected to develop a wholesome and nutritionally complete novel complementary product. For example, the legumes/pulses field pea and linseed were selected because of their naturally containing high amounts of protein and to complement the lysine-limited cereals. Additionally, linseed is a popularly cultivated oil crop by the community in the sample collection area and its oil is among the leading sources of α-Linolenic acid, omega-3 polyunsaturated fatty acid from plant foods [73] which is crucially important for brain neural and cognitive development in utero and early childhood [223].

On the other hand, the cereals maize, barley and oats were selected because of their high carbohydrate contents and also for complementing the cysteine, methionine and tryptophan-limited legumes. All these nutritional qualities of the components would make the complementary product developed novel and superior quality. Hence in this study, the energy, protein and iron densities of *dabi teff*-field pea-based optimized novel complementary flour and its contribution to daily energy and nutrient demand by 6-23 months old children was investigated in comparison to FAO/WHO [28] recommended nutrient contents of complementary foods and PAHO/WHO [219] recommended daily energy and nutrients requirements for 6-23 months old children to ensure adequate energy and nutrients daily intake from the optimized complementary flour for children's optimal growth and development.

Materials and Methods

Details of the methods related to the crop sampling, processing, flour preparation and handling were described in **Chapter 2**.

Results

Macro- and mineral compositions of each crop and the formulated complementary flours

The mean macro-compositions of each crop samples collected were presented in **Table 7.1** where field pea and linseed flours were determined to contain a significantly higher ($P < 0.05$) protein content at 20.95% and 20.57%, respectively while barley, oats, maize and linseed were determined to contain a significantly higher ($P < 0.095$) energy value at 380.22 kcal/100g, 381.01 kcal/100g, 376.77 kcal/100g and 519.96 kcal/100g, respectively which were the bases to develop protein and energy dense complementary flour. On the other hand, *dabi teff* has shown to contain significantly higher ($P < 0.05$) iron and calcium content at 86.50 mg/100g and 123.59 mg/100g, respectively which was the bases to develop iron-dense novel complementary flour. The mean macro-compositions of the formulated complementary flours that were subjected to the optimization process were 4.89%, 15.49, 4.91, 2.23, 3.48, 68.99% and 382.14 kcal/100g for moisture contents, protein, fat, ash, fiber, carbohydrate and energy contents, respectively whereas the mean dietary mineral contents of the formulated complementary flours that were subjected to the optimization process were 28.12 mg/100g, 76.25 mg/100g, 2.47 mg/100g, 120.57 mg/100g, 287.99 mg/100g, and 439.33 mg/100g for iron, calcium, zinc, magnesium, phosphorus and potassium, respectively **(Table 7.3).**

Table 7.1: Major macro-and micronutrient compositions of each crop sample collected

Crop Samples	Macro-Compositions (g/100g)					Dietary minerals (mg/100g)					
	Protein	CHO	Fat	Ash	Energy(kcal/100g)	Iron	Zinc	Calcium	Magnesium	Phosphorous	Potassium
Dabi Teff	10.74[a]	68.57[a]	3.94[a]	3.96[a]	352.70[a]	86.50[a]	1.94[a]	123.59[a]	142.48[a]	237.14[a]	297.97[a]
Roasted Field pea	20.95[b]	60.45[b]	2.74[a]	2.60[b]	350.26[a]	7.05[b]	2.28[a]	59.99[b]	89.54[b]	227.58[a]	720.14[b]
Roasted Barley	11.62[a]	77.63[c]	2.58[a]	1.73[c]	380.22[b]	5.00[b]	2.46[a]	32.49[c]	87.48[b]	279.09[b]	355.45[c]
Dehulled Oats	12.45[a]	70.00[a]	5.69[a]	1.92[c]	381.01[b]	4.37[b]	2.28[a]	26.33[d]	78.87[b]	261.23[b]	319.04[c]
Cooked linseed	20.57[b]	28.24[d]	36.08[b]	3.45[a]	519.96[c]	47.13[c]	6.63[b]	28.83[d]	279.86[c]	488.97[c]	563.28[d]
Germinated Maize	8.58[c]	75.24[c]	4.61[a]	1.42[c]	376.77[b]	1.12[d]	1.56[c]	15.78[e]	80.91[b]	236.48[a]	260.20[a]

Key: Values are means of the triplicate determinations. Values in the same column followed by different superscripts are significantly different at $P < 0.05$.

Table 7.2: Goals set, the relative importance of the key variable and the optimal values at optimal conditions.

Variables Name	Goal	Lower Limit	Upper Limit	Importance	Optimum value
Independent variables					
A:*Dabi teff* Flour	In range	20	35	3	34.66
D:Field pea Flour	In range	0	30	3	15.34
F:Maize Flour	In range	5	20	3	5
Barley flour	Constant	25	25	3	25
Oats flour	Constant	15	15	3	15
Linseed flour	Constant	5	5	3	5
Dependent variables					
Protein (%)	maximize	14.58	17.21	4	15.74
Fat (%)	maximize	4.22	5.59	3	5.09
Ash (%)	maximize	2.01	2.6	3	2.26
Crude Fiber (%)	minimize	2.68	3.96	3	2.88
Carbohydrate (%)	maximize	66.91	70.76	4	73.05
Energy (kcal/100g)	maximize	378.82	386.9	3	380.43
Iron (mg/100g)	maximize	24.007	31.5801	5	32.21
Calcium (mg/100g)	maximize	73.4631	78.1767	5	77.51
Zink (mg/100g)	maximize	2.326	2.612	5	2.59

Table 7.3: Macro-and micronutrient compositions of the formulated mean, the optimized, the Control and FAO/WHO specifications

Complementary flours	Macro-Compositions (g/100g)					Dietary minerals (mg/100g)					
	Protein	CHO	Fat	Ash	Energy kcal/100g	Iron	Zinc	Calcium	Magnesium	Phosphorous	Potassium
Mean of the formulated	15.49[a]	68.99[a]	4.91[a]	2.23[a]	382.14[a]	28.12[a]	2.47[a]	76.25[a]	120.57[a]	293.25[a]	443.99[a]
Optimized flour	15.74[a]	73.05[b]	5.09[a]	2.26[a]	380.43[a]	32.21[a]	2.59[a]	77.52[a]	118.64[a]	287.99[a]	439.33[a]
Control flour	10.77[b]	74.41[b]	3.78[b]	2.24[a]	374.74[b]	7.25[b]	2.64[a]	49.45[b]	96.45[b]	282.65[a]	398.67[b]
Cerifam®Faffa flour	14[c]	76c	4[a]	2.5[a]	396[c]	6[c]	5[b]	100[c]	-	-	-
FAO/WHO value	≥15	64±4	10-25	<3	400-425	16	3.2	500	-	-	-

Key: Values are means of the triplicate determinations. Values in the same column followed by different superscripts are significantly different at P <0.05.

Macro- and miconutrient compositions of the optimized complementary flours

The macro- and mineral compositions of the optimized novel complementary flour, the formulated mean, the control and the FAO/WHO [28] critical limits were presented in **Table 7.3** where the overall optimized formula was identified to be 34.66% *dabi teff*, 25% barley, 15% oats, 15.34% field pea, 5% linseed and 5% germinated maize flours with response values of 15.74% protein, 73.05% carbohydrate, 5.09% fat and 380.43 kcal/100g energy, 32.21 mg/100g iron, 77.52 mg/100g calcium and 2.59 mg/100g zinc, 118.64 mg/100g magnesium, 287.99 phosphorous and 439.33 mg/100g for potassium at the optimal point with the overall desirability function of 0.651 **(Figure 5.4)**. The control flour contained 10.77%, 74.41%, 3.78%, and 374.74kcal/100g for protein, carbohydrate, fat and energy contents, respectively whereas it had 7.25 mg/100g for iron, 49.45 mg/100g calcium, 2.64 mg/100g zinc, 96.45 mg/100g magnesium, 282.65 mg/100g phosphorus and 398.67 mg/100g for potassium, respectively. Cerifam® faffa flour contains 14% protein, 76% carbohydrate, 4% fat and 396 kcal/100g and it contains 6 mg/100g iron, 5 mg/100g zinc and 100 mg/100g calcium, respectively **(Table 7.3)**. Both the formulated mean and the optimized value fulfilled the FAO/WHO [28] critical standard limit. The energy, protein and iron contents of the optimized and the formulated mean were significantly higher ($P < 0.05$) than the values for the control and the Cerifam® faffa flours while there was no significant difference ($P > 0.05$) between the ash and zinc contents of the optimized, the formulated mean, the control and the Cerifam® faffa flours.

The validation analysis result was 5.34%, 15.86%, 5.12% 2.21%, 2.85%, 68.82%, 384.80 kcal/100g, 31.76 mg/100g, 76.88 mg/100g and 2.53 mg/100g for moisture, protein, fat, ash, fiber, energy, iron, calcium and zinc contents, respectively **(Table 7.4).** This showed that there was a good agreement between the predicted response values at optimal conditions and the validation laboratory values. The optimization process was verified using the graphical optimization technique **(Figure 5.3)** where the overlay counter plot was generated for the most important variables to identify the optimum regions. Any design points that fall within the yellow region (with the very conservative silver-gray region) in the overlay plot represent an optimal combination of *dabi teff* flour, field pea and germinated maize flours in combination with the other constant components that can bear optimal macro-and micronutrient contents within 95% confidence interval (CI).

Energy and macro-nutrient densities of the optimized, control flours and PAHO/WHO recommendation

The energy and macro-nutrient densities of the formulated mean, the optimized, the control flours and the PAHO/WHO [219] recommendations for complementary food for average breastfed children were presented in **(Table 7.5).** After adjusting for the amount of food to be served to the children, the energy density of our current optimized complementary flour for all the age groups (6-8 months, 9-11 months and 12-23 months old children) was computed to be 1.27 kca/g. The protein density of our optimized complementary flour was computed to be 4.14 g/100kcal and which was 1.44 times higher than the protein density of the control (traditional) flour while the fat density was computed to be 1.34 g/100kcal and the carbohydrate density of the optimized flour was computed as 19.20 g/100kcal.

Table 7.4: Experimental validation of optimal condition and comparison with the control flour.

Response variables	Optimal value	Validation value	Contro value
Protien (g/100g)	15.74^{a}	15.86^{a}	10.77^{b}
Fat (g/100g)	5.09^{a}	5.12^{a}	3.78^{b}
Ash (g/100g)	2.26^{a}	2.21^{a}	2.24^{a}
Fiber (g/100g)	2.88^{a}	2.85^{a}	2.65^{a}
Carbohydrtae (g/100g)	73.05^{a}	68.82^{a}	74.41^{a}
Energy (kcal/100g)	380.43^{a}	384.80^{a}	374.74^{b}
Iron (mg/100g)	32.21^{a}	31.76^{a}	7.25^{b}
Calcium (mg/100g)	77.51^{a}	76.88^{a}	49.45^{b}
Zinc (mg/100g)	2.59^{a}	2.53^{a}	2.64^{a}

Key: Values are means of the triplicate determinations. Values in the same raw followed by the same superscripts are not significantly different at P >0.05.

Minerals densities of the optimized, the control flours and PAHO/WHO recommendation

The dietary minerals densities of the formulated mean, the optimized and the control flours and the PAHO/WHO [219] recommendations for complementary food for average breastfed children were presented in (**Table 7.5**). The iron density of our optimized complementary flour was

computed to be 8.47 mg/100kcal and it was 4.39 times higher than the control which could be accredited to the higher iron content of *dabi teff* flour at 86.50 mg/100g **(Table 7.1)** which was one of the major ingredients in our formulations. The zinc density of our optimized flour was determined at 0.68 mg/100kcal and the calcium density was determined to be 20.37 mg/100kcal.

Table 7.5: Energy and nutrient densities of the formulated mean, the optimal and the control flours and PAHO/WHO standards

Parameters	Formulated mean	Optimal	Control	PAHO/WHO
Energy density (kcal/g)	1.27	1.27	1.25	≥ 0.8
Protein density (g/100kcal)	4.05	4.14	2.87	≤ 5.5
Fat density (g/100kcal)	1.55	1.34	1.01	2.5-4.5
Carbohydrate density (g/100kcal)	18.05	19.20	19.86	-
Iron density (mg/100kcal)	7.36	8.47	1.93	0.8-4
Calcium density (mg/100kcal)	19.95	20.37	13.20	>80
Zinc density (mg/100kcal)	0.65	0.68	0.70	0.6-1.6

Source of PAHO/WHO: Codex Stan 074-1981, Rev. 1-2006; PHO/WHO, 2001

Contribution of the optimized complementary flour

Contribution of the optimized Complementary Flour to daily energy and Protein demand by 6-23 Months of children

Table 7.6 shows the required daily meal frequency and the estimated amount of solid portions of complementary food per meal for age groups of 6-8 months, 9-11 months and 12-23 months old children and the recommended daily requirements of energy and protein from a complementary food based on PAHO/WHO Guiding *Principles for Complementary Feeding of the Breastfed Child.* After adjusting for the amount of complementary food a child in its respective age group needs to be served, the likely daily contribution (in percentage) of the currently developed optimal complementary food to the recommendation and the daily requirements were computed for energy and protein. It was further compared with the relative contribution of the control and Cerfifam® faff flour (the popular commercial complementary flour in Ethiopia). In the above section, it was estimated that the daily amounts of complementary foods to be served to an age

group of 6-8 months, 9-11 months and 12-23 months old children to be 225 g, 300 g and 450 g, respectively. Based on adjusting for this estimated daily serving amount and the daily requirements of the nutrients, the currently developed optimized complementary product could likely contribute up to 143%, 127% and 104% of daily energy requirements for the age group of 6-8 months, 9-11 and 12-23 months old child, respectively (Table 9.6). Similarly, after adjusting, the optimized complementary flour could be able to contribute up to 130%, 164% and 217% of the daily protein requirements for the respective age groups. This contribution of the optimized complementary flour to the PHO/WHO recommended requirements (demand) was significant (more than 100%) and the product can considerably fulfill the energy and protein demand by the children under each respective age group which showed the protein-energy deficit would be less likely to occur if the children would be fed the estimated serving amount properly.

Table 7.6: Contribution of our optimized flour to the daily energy and protein demands by 6-23 months old children modeled against recommendation by PAHO/WHO (2001).

	Age group					
Nutrient intake	6-8 months		9-11 months		12-23 months	
Number of servings (meal)	3 times		3 times		3 times	
Amount of solid portion per meal (g)	75		100		150	
Energy RDA from CF (kcal/day)	**200**		**300**		**550**	
Flour type	Adjusted	% CTB	Determined	%CTB	Adjusted	% CTB
Optimized formula	285.32	142.66	380.43	126.81	570.65	103.75
Control	281.06	140.53	374.74	124.91	562.11	102.20
Cerifam® Faffa	297	148.50	396	132	594	108
Protein RDA from CF (g/day)	**9.1**		**9.6**		**10.9**	
Flour type	Adjusted	% CTB	Determined	%CTB	Adjusted	% CTB
Optimized formula	11.81	129.78	15.74	163.96	23.61	216.61
Control	8.08	88.76	10.77	112.19	16.16	148.53
Cerifam® Faffa	10.5	115.38	14.0	145.83	21.0	192.66

Key: RDA-recommended dietary allowance (PAHO/WHO, 2001), g-gram, CF-complementary food, %CTB- percentage contribution.

The relative contributions of the optimal, the control and the Cerfifam® faffa flour to the daily energy and protein demand by the children were presented in **Table 6** which was calculated to be 143%, 141% and 149% by the optimal, the control and Cerifam® faffa flours for the 6-8 months children, 127%, 125% and 132% for the 9-11 months children and 104%, 102% and 108% for the age group of 12-23 months old children, respectively. When compared to the control and the Cerfifam® faffa flour, the contribution of energy by the optimized complementary flour was slightly higher than that of the control flour and slightly lower than that of the Cerfifam® faffa flour for the entire age category. This relatively comparable energy content of the control and Cerifam® faffa flour to the optimal flour could be attributed to the cereals, barley and wheat flours in their formulation which are claimed as higher energy-containing cereals. The relative contribution of the optimal complementary flour to the daily protein demand was calculated to be higher than both the control and Cerfifam® faffa flour and was determined to be 130%, 89% and 115% by the optimal, control and Cerifam® faffa flours for the 6-8 months children, 164%, 146% and 112% for the 9-11 months children and 217%, 148% and 193% for the age group of 12-23 months old children, respectively **(Table 7.6).**

Contribution of the optimized complementary flour to daily iron, calcium and zinc demand by 6-23 months old children

Table 7.7 shows the required daily meal frequency and the estimated amount of solid portions of a complementary food per meal for age groups of 6-8 months, 9-11 months and 12-23 months old children and the recommended daily requirements (RDA) of iron, calcium and zinc from a complementary food based on PAHO/WHO Guiding *Principles for Complementary Feeding of the Breastfed Child.* After adjusting for the estimated daily serving amount and the daily requirements of the minerals as well as based on the assumption of moderate bioavailability, the currently optimized complementary flour was computed to contribute up to 220%, 293% and 690% of the daily iron requirement for 6-8 months, 9-11 months and 12-23 months old children, respectively with daily iron demand of 11 mg, 11 mg and 7 mg by 6-8 months, 9-11 months and 12-23 months old children, respectively **(Table 7.7).** This contribution of the optimized complementary flour to the PHO/WHO recommended requirements (demand) for iron was significant (more than 200%) and the product can considerably fulfill the iron demand by the children under each respective age group which showed the iron deficiency anemia would be less likely to occur if the children would be fed the estimated serving amount properly.

Regarding zinc, the optimized flour was demonstrated to contribute up to 47.32%, 60.23% and 67.07% of daily zinc demand of 4.1 mg, 4.3 mg and 5.8 mg by the respective age groups. On the other hand, the new optimized *dabi teff*-field-based complementary flour could only contribute up to 14.53%, 19.37% and 23.3% of daily calcium requirement (demand) of 400 mg, 400 mg and 500 mg by the respective age group **(Table 7.7).**

Table 7.7. Contribution of our optimized flour to the daily iron, calcium and zinc demands by 6-23 months old children as modeled against the recommendation by PAHO/WHO (2001).

	Age group					
Nutrient intake	6-8 months		9-11 months		12-23 months	
Number of servings (meal)	3 times		3 times		3 times	
Amount of solid portion per meal (g)	75		100		150	
Iron RDA from CF (mg/day)	**11**		**11**		**7**	
Flour type	Adjusted	% CTB	Determined	%CTB	Adjusted	%CTB
Optimized formula	24.16	219.61	32.21	292.82	48.32	690.29
Control	5.44	49.45	7.25	65.91	10.86	155.14
From Cerifam® Fafa	4.5	40.91	6	54.55	9	128.57
Zinc RDA from CF (mg/day)	**4.1**		**4.3**		**5.8**	
Flour type	Adjusted	% CTB	Determined	%CTB	Adjusted	%CTB
Optimized formula	1.94	47.32	2.59	60.23	3.89	67.07
Control	1.98	48.29	2.64	61.39	3.96	68.28
Cerifam® Faffa	3.75	91.46	5	116.28	7.5	129.31
Calcium RDA from CF (mg/day)	**400**		**400**		**500**	
Flour type	Adjusted	% CTB	Determined	%CTB	Adjusted	%CTB
Optimized formula	58.13	14.53	77.5	19.37	116.25	23.25
Control flour	37.09	9.27	49.45	12.36	74.18	14.84
Cerifam® Faffa	75	18.75	100	25	150	30

Key: RDA- recommended dietary allowance (PAHO/WHO, 2001), g-gram, mg-milligram, CF-complementary food, %CTB- percentage contribution. All the calculations were based on the moderate bioavailability of the minerals.

Regarding the relative iron contribution by the optimized complementary flour to the daily iron demand by the children, the optimal flour had a significantly higher (non-comparably higher) percentage contribution than both the control and Cerfifam® faffa flour where it was calculated to be 220%, 50% and 41% by the optimal, the control and the Cerifam® faffa flours for the 6-8 months children, 293%, 66% and 55% for the 9-11 months old and 690%, 155% and 129% for the age group of 12-23 months old children, respectively **(Table 7.7).** This higher iron contribution by the optimized complementary flour was ascribed to the higher iron content of *dabi teff* flour at 86.5mg/100g **(Table 7.1).**

On the other hand, the relative contribution by the optimized complementary flour to daily zinc demand was comparable with that of the control flour and while it was lower than that of the Cerifam® faffa flour for all the age groups where it was calculated to be 47%, 48% and 91% by the optimal, the control and the Cerifam® faffa flours for the 6-8 months children, 60%, 61% and 166% for the 9-11 months old and 67%, 68% and 129% for the age group of 12-23 months old children, respectively **(Table 7.7)** and this higher zinc content of the Cerifam® faffa flour could be attributed to the ingredients used during its formulation. Regarding the relative contribution of the three flours to daily calcium demand; the optimized, the control and the Cerifam® faffa flour were found to be very low for all the age groups ranging from 9% to 30% **(Table 7.7)** with relatively higher contribution by Cerfifam® faffa flour which could account for the milk powder in its formulation.

Discussion

The complementary crop samples collected have shown to contain varied and higher energy and nutrient contents and each of the crop samples was designed to add one or more nutrients to their mixture which were used to develop nutritionally complemented optimized novel complementary flour.

When compared to the FAO/WHO [28] recommended guideline for nutrient content requirements of formulated complementary foods, the mean macronutrients values of the formulations and the response values at the optimal condition of the currently developed complementary flour were adequately met the recommended critical limits for all the macro-

compositions except for fat content at ≤5, ≥15, 10-25, ≤3, ≤5, 64±4% and 400-425kcal/100g for moisture, protein, fat, ash, fiber, carbohydrate and energy contents, respectively (**Table 7.3**). Similarly, the mineral contents adequately met the recommended critical limits for iron at 16mg/100gm for both the formulated mean and the optimized complementary flours (Table 9. 3) where the mean formulation was 1.76 times higher and the optimal value was 2.01 times higher than the recommendation for iron content. The calcium and zinc contents, however, did not meet the recommendation at 500 and 3.2mg/100g, respectively, though zinc could meet up to 77.19% of the required value which was marginal.

According to PAHO/WHO [219] complementary feeding guidelines, the energy density of cereal-based complementary foods for infants and young children should be ≥0.8 kcal/g. Hence, our optimized complementary flour had demonstrated to meet beyond the minimum energy density requirements from complementary foods at 1.27 kcal/g for infants and young children aged 6–23 months old and the children can meet their daily energy demand if served the estimated amounts for the respective age group described in the above section and if fed three times per day as the guideline specifies that 3meals/day would satisfy the energy demand of the children if the complementary food contains an energy density of at least 1 kcal/g.

It was unable to compare the energy density of our current optimized product with some past reports because of the wrong energy density calculation which said, “Simply dividing the energy contents of the complementary foods by 100”. For example, Tenagashaw *et al*. [229] reported energy density of complementary food formulated from blends of teff, soya bean and orange flashed sweet potato ranged from 3.70 to 3.76 kcal/g and Ayele *et al*. [121] reported the energy density of complementary foods from kocho, pumpkin fruit, red kidney bean and maize at 3.3 kcal/g following the same calculation which was over exaggerated. Even higher energy-dense foods have energy density of 1.07–1.46 kcal/g where in this case, the approximate quantity of complementary food that would meet the energy needs of the children is 137–187 g/day at 6-8 months, 206-281 g/d at 9-11 months, and 378-515 g/d at 12-23 months old as specified by PAHO/WHO [30, 219] recommendation. However, the current energy density determined was higher than the energy density reported by Abebe *et al*. [230] where the traditional corn-based porridge consumed as complementary foods in rural villages of Sidama zone of Southern

Ethiopia contained an energy density of 0.53 kcal/g, while kocho-based contained 0.49 kcal/g, both of which were very low energy density levels.

The FAO/WHO Codex Alimentarius committee recommends the protein density of cereal-based formulated complementary foods for infants and young children should not exceed 5.5 g/100 kcal (upper threshold) where the determined protein density of our optimized complementary flour at 4.14 g/100kcal was near to the upper threshold. This showed that our optimized complementary food is super and can meet the recommended standard and could provide the daily protein demand by 6-23-month-old children if served the estimated amount in the above section for the respective age group and fed three times per day. This finding was in agreement with the report by Tenagashaw *et al*. [229] where the protein density of complementary foods from blends of teff, soya bean and orange flashed sweet potato was in the range of 3.50 to 4.79 g/100 kcal. It also agreed with the report by Ayele *et al*. [121] where the protein density of an optimized formula from kocho, pumpkin fruit, red kidney bean and maize was 3.0 g/100 kcal. Alternatively, the protein density of homemade complementary foods in Wolayita zone of Ethiopia contained lower protein density ranging from 2.13–2.48 g/100kcal as reported by Abeshu *et al*. [230].

Fat is among the major factors determining the energy density of foods; it provides essential fatty acids needed for the effective absorption of fat-soluble vitamins and it boosts the sensory qualities of processed foods, PAHO/WHO [219]. The fat density determined at 1.34 g/100kcal in our optimized flour was below the recommended standard set by codex (2.5- 4.5 g/100 kcal) for cereal-based complementary foods for infants and young children, FAO/WHO [28]. This finding was in agreement with the report by Tenagashaw *et al*. [229] that the fat density of complementary foods from blends of teff, soya bean and orange flashed sweet potato was in the range of 1.26-1.66 g/100kcal where it did not even meet the minimum standard value which calls for additional fat-energy source. The current carbohydrate density of the optimized flour determined at 19.20 g/100kcal agreed with the report by Tenagashaw *et al*. [229] that the carbohydrate density ranged from 18.22-20.05 g/100kcal and this higher carbohydrate density could be attributed to the cereal-legume blends used to optimize the complementary flour which were claimed to contain higher carbohydrate contents and maybe also due to the pre-processing techniques applied.

Dietary minerals such as iron, calcium and zinc are the limiting nutrients in the diets of infants and young children [6]. The iron density of our optimized flour as compared to the recommendation by PAHO/WHO [219] complementary feeding guideline of cereal-legume based complementary foods for infants and young children (6-24 months) accounted for high, moderate and low bioavailability of iron where the recommended value is 2.5 mg/100kcal, 1.5 mg/100kcal and 0.5 mg/100kcal for 6-8 months, 9-11 and 12-23 months old children when accounted for high bioavailability, while it is 4.0 mg/100kcal, 2.4 mg/100kcal and 0.8 mg/100kcal when accounted for moderate bioavailability and 7.7 mg/100 kcal, 4.6 mg/100 kcal and 1.6 mg/100kcal when accounted for low bioavailability for 6–8 month, 9–11, and 12–23-month-old children, respectively [219, 240, 241].

The iron density of our current optimized complementary flour which was computed to be 8.47 mg/100kcal was 2.12-10.59 times higher than the recommended value when accounted for moderate bioavailability. This finding showed that the optimized complementary flour could even meet the recommendation when accounted for the low bioavailability of iron density at 7.7 mg/100 kcal. Hence, it had demonstrated to meet the iron density requirements from the complementary foods for infants and young children aged 6–23 months and the children can meet their daily iron demand if properly fed. Such products with higher iron content can be considered as meat substitutes given the iron bioavailability would be comparable with that of red meat and the 'novelty' of this study would be accredited to the incorporation of *dabi teff* flour into the formulations containing high iron at 86.50 mg/100g **(Table 7.1)** and the presence of linseed which is a leading source of α-Linolenic acid, omega-3 polyunsaturated fatty acid that would make the formulated and optimized product super.

The present finding was higher than the iron density of complementary blends of teff, soya bean and orange flashed sweet potato that range from 2.42 to 5.19 mg/100 kcal as reported by Tenagashaw *et al*. [229]. Iron in plant foods exists in non-hem form and it may also bind with ant-nutrients found in cereal-legume foods where its absorption can be impaired [37]; but in our case, the pre-processing technique applied could reduce the effect of the anti-nutrients in inhibiting absorption.

Zinc is an important mineral used for the management of diseases such as diarrhea, pneumonia and sometimes malaria in children. It is also used for tissue growth and maintenance and wound healing where its deficiency leads to stunted growth though its effect is less recognized and referred to us the 'hidden hunger' [85]. PAHO/WHO [219] recommends zinc density at 1.6 mg/100kcal, 1.1 mg/100g and 0.6 mg/100g for 6-8 months, 9-11 and 12-23 months of age children from complementary food when accounted for lower bioavailability [241]. In the present study, the determined zinc density at 0.68 mg/100kcal has shown fulfillment for 12-23 months old children while at a marginal level (61.82%) when accounted for the requirements for 9-11 months old children and below the recommended for 6-8 months old children. The present finding was higher than the zinc density of complementary food at 0.4 mg/100kcal as reported by Ayele *et al*. [121] while it was lower than the report by Tenagashaw *et al*. [229] with a zinc density range of 1.41 to 1.49 mg/100kcal.

The calcium density of our optimized flour determined at 20.37 mg/100kcal could only meet 25.46% of the required calcium density in formulated complementary food for infant and young children as set by codex standard (>80 mg/100kcal) FAO/WHO [28]. The present finding was slightly lower than the report by Ayele *et al*. [121] which was 24.1 mg/100kcal but by far lower than the report by Tenagashaw *et al*. [229] with a calcium density range of 60.68 to 67.84 mg/100kcal from complementary blends of teff, soya bean and orange flashed sweet potato which could be attributed the variety of soya ben and the orange-fleshed sweet potato used.
Collectively speaking, our optimized complementary food was super iron, protein and energy sources meeting the FAO/WHO [28] and PAH/WHO [219] recommended standard and could provide the daily energy, protein, carbohydrate, iron and zinc (to a considerable extent) demands by 6-23 months old children if served 225g of the optimized complementary food for 6-8 months, 300g for 9-11 months and 450g for 12-23 months old children and feed three times per day accompanied by additional nutritional snacks during any signs of hunger and satiety and average breastfeeding.

Of the examined nutrients, the determined fat density at 1.34 g/100kcal and calcium density at 20.37 mg/100kcal **(Table 7. 5)** were found to be unfavorably lower than the required standard at 2.5-4.5 g/100kcal of fat density and at >80 mg/100kcal of calcium density where these two

nutrients were frequently reported to be lower in cereal-based complementary foods [204, 218, 229]. The lower fat and calcium densities might be matched by advising mothers/caretakers to add calcium and vitamin-rich foods such as eggs, dried meat powder (quanta powder), refined butter and vegetable oil (the usual practice), mashed vegetables and replacing water with milk as well as adding mashed vegetables during porridge preparation for infants and young children which might aid to enhance energy density and to meet the recommendations. The energy density of a meal can be increased from 0.6 kcal/g to 1.0 kcal/g by adding one teaspoon of oil/butter or one piled teaspoon of mashed avocado or some amount of meat to 100 g of porridge [268].

PAHO/WHO [6, 219] recommends that the energy needs from complementary foods for infants and young children with average breast milk intake in developing countries is approximately 200 kcal, 300kcal and 550 kcal per day for 6–8 months, 9–11 months and, at 12–23months of age children, respectively. Complementary foods are expected to be energy-dense and fill these deficits of daily energy and nutrient demand by the children apart from the amount obtained from breastfeeding [127]. Among the energy-yielding macronutrients, protein should provide 6-15% of total daily energy requirements which corresponds to 9.1g, 9.6g and 10.9g of protein per day for 6-8months, 9-11 months and 12-23 months old children, respectively PAHO/WHO [219].

From the other energy-yielding macronutrients, the standard states 55-65% of total daily energy requirements should come from carbohydrates and the suggested amount ranging from 30-45% need to come from fat energy [6]. From the optimal points of the optimized complementary flour, the contribution of energy from protein and carbohydrate to the total energy of the complementary flour was 16.55% and 76.81%, respectively which was adequately considerable and agreed with the recommended values whereas the energy from fat was determined to be 12.04% which was below the desired range.

From these findings, it could be deduced that the contribution of iron that can be obtained from the optimized *dabi-teff* field pea-based complementary flour to the daily iron demand by the children was extraordinarily high that it could even meet the daily requirement under low bioavailability. Thus, the product could demonstrate to fill the huge gap of iron demand by

children that should come from complementary food (97%) and can serve as a sustainable food-based nutrition intervention against iron deficiency anemia.

It might not be surprising that the optimized complementary flour could demonstrate to contribute a higher amount of iron because *dabi teff* flour had iron content at 86.5 mg/100g which was one of the major ingredients in our formulations. It is unlikely that the high levels of iron in the optimal complementary flour can cause any danger to infants and young children since iron overload in body fluid is mostly hereditary where the intestine continues to absorb iron at a high rate despite the excess iron builds up in the body tissue. Iron deficiency is the most common problem rather than its toxicity and some adverse health effects of iron overload like fatigue, mental depression, abnormal heartbeat, intestinal side effects and diabetes are severe with alcohol abusers because alcohol damages the intestine and impairs its defense against absorbing too much iron [269]. However, the use of such high iron-containing food in malaria-endemic areas needs to be stressed to avoid complications and may require following the WHO/UNICEF recommendation [270].

The contribution of zinc by the currently optimized complementary flour was lower as compared to the report by [217] for the formulation of complementary food from Chickpea (*kabulli variety*), red teff (*DZ-0199 variety*) and quality protein maize (*BHQPY-545 variety and Ipomea batata, Tulla variety*) that the zinc content fulfilled 81-88.34% of the recommended daily allowance which could be attributed to variety and component variation. In agreement with the present findings, [271] reported that composite flour from orange-fleshed sweet potato, soya bean and carrot met 22-23% of the daily recommended allowance for calcium for the 1-3 years age group.

Conclusion

Nutritional well-being in early childhood is a major driver for optimal growth and development, healthy life, improved school performance as well as improved future productivity and earning potential of children. Children should be nourished with age-specific daily adequate energy and nutrient demands. In the present study, the optimized *dabi teff*-field pea-based novel complementary flour was demonstrated to provide adequate energy, protein and iron densities

meeting the standard and show the likely contribution of more than 100% of daily demands for energy and protein, and notably more than 200% of daily iron demand by all the age groups (6-8 months, 9-11 months, and 12-23 months old children) as per the by PAHO/WHO recommendation at moderate bioavailability where it even met at lower iron bioavailability. Thus, children aged 6-23 months old can meet their daily energy and nutrient demand if served the estimated amount of the novel complementary food and fed there times a day. The density and daily contribution of zinc from the developed product were marginal whereas the fat and calcium densities and their daily contribution by the product were inadequate which might require matching. This study gives the basis for the optimization of cereal-legume blends from locally available, affordable, and underutilized food crops that can serve as sustainable food-based nutrition intervention strategies to fight against protein-energy malnutrition and iron deficiency anemia in children.

8

Chapter 8:

General Discussion, Conclusion and Recommendations for Policy Actions and Further Research

8.1 General introduction

Under-nutrition and micronutrient deficiencies remain major public health problem globally. Beside, there is a worry of providing adequate nutritious food to the projected 10 billion people by 2050 [3]. Furthermore, FAO *et al*. [4] reported that healthy diets were unaffordable for around 3 billion people in the world due to high and volatile food prices. This projected growth in global population with the attendant increase in food demands, especially in the African region, calls for focusing and devoting more attention to extensive agricultural production, sustainable production of conventional foods, intensive research in finding for new nutritious food varieties with increased yield, supportive nutrition policy framework and program planning and give special focus to underutilized or marginalized plant foods which have a great potential to influence and improve food security and dietary diversity [78] for African nations and promote sustainable growth and development.

In sub-Saharan Africa, macro- and micronutrient deficits in complementary diets are most common and it is usually associated with bulk and/or monotonous ingredients of traditional cereal-based complementary foods, high anti-nutritional contents, losses and quality damage during processing and lack of purchasing power of quality/healthy foods among others. This led to re-curring protein-energy and micronutrient malnutrition with its health consequences in children. Despite abundant global food supplies by WHO and UNICEF, malnutrition, particularly protein-energy and micronutrient malnutrition is widely persistent among infants and young children in the region [2, 209]. It was agreed that by 2030 all countries should end all forms of malnutrition; including achieving the internationally agreed target of the World Health Assembly to reduce the total number of stunted children under-five by 40% of its 2010 level by the year 2025 [272].

The present research was aimed to alleviate the double burden of malnutrition, the protein-energy and micronutrient malnutrition in children where children's nutritional status must be improved by the prevention of both the macro and micronutrient deficits by the development of energy and nutrient-dense complementary foods. Some key recommendations to improve the nutritional value of traditional complementary foods are to design proper formulation and enrichments of the main ingredients with nutritious underutilized plant source foods by putting a special focus on micronutrient-rich ones [141]. Research by Kamotho [69] recommended the use

of underutilized locally available food crops that are inexpensive and high in micronutrients for child food formulations. In the current work, incorporation of nutrient-rich underutilized plant foods and incorporated it into staple diets and attempts to develop energy and nutrient-dense super-quality complementary food which could be the solution to the dual challenges, undernutrition and hidden hunger. These challenges are manifested most in developing countries with a noticeable symptom of growth stunting and impaired capacity to resist diseases [83, 130] which results in reduced work and earning capacity in children and reflect in reduced national economic development. In Ethiopia, the consequences of under-nutrition and micronutrient deficiencies are more serious and higher numbers of children die each year from malnutrition complicated with preventable and treatable infectious diseases [16] where children in rural poor are more affected than their urban counterparts [18].

Both protein-energy and micronutrient malnutrition have strong negative consequences on growth and development, healthy life, school performance as well as future productivity and earning potential, and survival of children [50]. Thus, overcoming these consequences by ensuring nutritional well-being in the early life of children is a critical pre-condition for national development. In Ethiopia, efforts were being made to implement a multi-sectorial plan of high-impact nutrition-specific and nutrition-sensitive interventions such as the School Feeding Program, the Sekota-Declaration (2017-2020) [59] and National Nutrition Program-II (2016-2020) [60] to end child under-nutrition including stunting and eradicating micronutrient deficiencies by 2030. However, the country is still experiencing one of the worst situations where protein-energy malnutrition and micronutrient deficiencies (iron, zinc and vitamin A) are unacceptably high among infants and young children.

Food-based interventions have been identified as the best approach for ending malnutrition in under-five children [273]. As a food-based approach, formulation and development of enriched composite complementary foods from locally and readily available foods has received a lot of attention in many developing countries to address protein-energy and micronutrient malnutrition in children sustainably [70]. Amagloh *et al*. [79] stated a careful selection of nutritionally promising local ingredients in complementary food development have more likely to have a

positive impact in meeting energy and nutrient demand as recommended by codex Alimentarius commission guidelines for formulated foods for infants and young children.

Consequently, this PhD dissertation work was aimed at development of nutritionally enriched optimized novel composite complementary food through proper selection and combination of locally available and affordable cereal-legume blends (blends of *dabi teff*, barley, maize, oats, field pea and linseed) to address protein-energy and micronutrient malnutrition among children in Western Ethiopia. The dissertation work was specifically aimed to introduce a new crop *dabi teff,* the underutilized nutritious crop into complementary food formulation to develop energy, protein and iron-dense optimized novel complementary food with improved sensory acceptability and nutrient bioavailability from cereal legume blends that can be used for IYCF to sustainably fight against protein-energy malnutrition and iron deficiency anemia. To make this dissertation work effective, *dabi teff* and the other cereals and legumes used were purchased from Nedjo open market in Nejdo district, western Ethiopia for development of nutritious complementary food and the following major findings were drawn.

8.2 General discussion on the main findings of the project work

Linked to our published articles from this PhD work, the major findings were summarized from the project are briefly described below.

The scoping review revealed that enrichment of traditional complementary foods using underutilized nutritious plant foods has proven to increase the nutritional potential of the resultant complementary foods, meeting standards, especially protein and the key micronutrients such as iron, calcium, zinc magnesium, phosphorous, and potassium contents were shown to increase significantly in the enriched products than the starchy traditional complementary foods and could help to develop nutritionally adequate complementary products. It was also learned from the review that the most common processing techniques applied such as fermentation, germination and roasting resulted in the improvement of the nutrition values of the complementary foods developed. Moreover, the review also identified that cereals and pseudo-cereals such as teff (*Eragrostis teff)*, finger millet (*Eleucine coracana*), buckwheat *(Fagopyrum esculentum),* quinoa *(Chenopodium quinoa)* and amaranth (*Amaranthus caudatus)* are getting attention for inclusion in complementary food formulations for their excellent nutritional value. They contain considerable amounts of iron, calcium, zinc, magnesium, etc. contents. They are

also gluten-free and are good sources to prevent diseases like hyperlipidemia, osteoporosis and celiac diseases [173]. Yet, *dabi teff*, which is one of the teff varieties marginally cultivated in western Ethiopia, where there are many social beliefs and health benefits linked to it among the community. Further, its nutritional potential to be incorporated into traditional complementary foods to enhance the micronutrient values of the resultant product is no yet researched **(Chapter 3).**

The ethnographic qualitative study has shown that there are a number of traditional usage and social beliefs in the community that are linked to *dabi teff*. The community believes that *dabi teff* has many health benefits in bringing positive health and nutritional outcomes when its different food forms typically "mooqa manyee" is served to a family member requiring special nutritional treatment, the food therapy. There is also an understanding in the community that *dabi teff* is a rich source of iron. The study further revealed that *dabi teff* cultivation plays a substantial role in ensuring food security in the area but its cultivation is reported to be declining nowadays and there is a fear of total extinction of the crop and loss of the traditional usages and social beliefs among the community in the long run **(Chapter 4)**.

The energy and protein contents as well as the sensory acceptability of the formulated and the optimized complementary food have exhibited a significant superiority ($P < 0.05$) over the traditional complementary food and the Cerifam® faffa flour and fulfilled the FAO/WHO [28] standard. However, the fat content was found to be below the standard. It was proven from this study that energy and protein-dense/enhanced optimized novel complementary food could be developed from the inclusion of *dabi teff*, the underutilized/forgotten food crop into locally available pre-processed food crops *viz*., germinated maize, roasted barley, roasted field pea, dehulled oats and linseed flours. It was further shown that each of the components used contributed an important nutrient of interest to develop a nutritionally complemented optimized novel complementary product where field pea and linseed crops were found to contribute a significantly higher protein (**Chapter 5.1**).

The iron content of the formulated and the optimized complementary food has shown to be significantly higher ($P < 0.01$) than the traditional complementary food and the Cerifam® faffa flour and fulfilled (more than twofold) the FAO/WHO [28] standard. However, the calcium content did not meet the standard and zinc content was found to be at a marginal level. Further,

this study has shown that the phytate/minerals molar ratios (the proxy indicators of the *in-vivo* mineral bioavailability) of the formulations and the optimized complementary food were significantly lower where the foods have exhibited and improved *in-vitro* iron bioavailability over the traditional and the Cerifam® faffa flour. This shows that the anti-nutritional factors assayed in the blends were within the tolerable limits, and may not therefore pose serious problems to nutrient availability and utilization. The findings have further shown that the iron met the standard when accounting for moderate bioavailability and was even shown to meet the standard at low bioavailability. From this study, it was revealed that the incorporation of *dabi teff* into other pre-processed local food crops provided iron-dense optimized novel complementary food. It was also shown that each of the components used contributed the important limiting micronutrients including; iron, calcium, and zinc, for the development of a micronutrient-enriched optimized product, where *dabi teff*, the underutilized/forgotten crop was found to contain extraordinary iron content, signifying the crop as a super iron bank **(Chapter 5.2)**.

The blending ratio variation of the major ingredients (*dabi teff*, field pea, and germinated maize) together with those kept constant ingridients (maize, barley, and oats) had shown a significant effect on the macronutrient contents and the sensory attributes of their blends. The finding has shown that an increase in the ratio of field pea within the range had significantly increased ($P < 0.05$) the protein, ash, fiber, and energy content of the blends, while an interactive effect between field pea and *dabi teff* flours had a synergetic effect on increasing the overall sensory acceptability **(Chapter 6.1).**

The blending ratio variation of the major ingredients (*dabi teff*, field pea, and germinated maize) together with those kept constant (maize, barley, and oats) had shown a significant effect on the micronutrient contents and the phytate/minerals molar ratios of their blends. The study has shown that as *dabi teff* ratio increased within the range, there was a remarkably linear increasing effect in iron and calcium content in the blends accompanied by a significant decrease ($P <0.01$) in phytate/minerals molar ratios. The finding has shown that there was a notable increase in iron contents as a function of *dabi teff* increase in the blends **(Chapter 6.2)**.

Based on various previous studies, it was estimated that the serving amount of complementary food for 6-8 months, 9-11 months and 12-23 months old children to be 75 g, 100 g and 150 g per meal and the children should be served three times a day taking the energy density of our new

optimized complementary product into account, which is 1.27 kcal/g. Based on adjusting for these estimated daily serving amounts, our developed nutritionally enriched optimized novel complementary food from the locally available cereal-legume blends; blends of the cereals (maize, barley and oats) and the legumes (field pea and linseed) combined with *dabi teff* has shown the likely contribution of more than 100% of daily demands for energy and protein, and notably more than 200% of daily iron demand (at moderate bioavailability) by all the age groups (6–8 months, 9–11 months and 12–23-months-old children) as modeled against the recommended dietary allowance (RDA) set by PAHO/WHO [55] Guiding Principles for Complementary Feeding of the Breastfed Child. The finding has shown that the daily iron demand could even be met at lower iron bioavailability **(Chapter 7)**.

In summary, the above-described findings demonstrated that energy, protein and iron-dense optimized novel complementary food with improved sensory acceptability and iron bioavailability can be developed from a proper selection and combination of locally available cereal and legumes which can exhibit a superior quality over the traditional complementary foods and the Cerifam® faffa flour and fulfilled the FAO/WHO [28] recommended standard. From the findings, it was observed that there was a remarkably linear increasing effect in iron and calcium content as a function of *dabi teff* ratio increase in the blends accompanied by a significant decrease in phytate/minerals molar ratios, a proxy indicator of the improved *in-vivo* iron bioavailability following feeding infant and young children. This finding has proven that the inclusion of *dabi teff*, the underutilized crop into other locally available processed crops has significantly increased the key micronutrients, notably iron contents of the resultant blend which signified the crop as a super iron bank. The findings has also shown that the protein content of the blends increased as a function of field pea increase, which is an indicator of legume crops naturally contain more protein than cereals. Moreover, from the findings, it was shown that the expected intake or the likely contribution of the optimized novel complementary food to protein and energy daily demand by 6-23 months old children was more than 100% and notably more 200% for iron when modeled against the recommended dietary allowance (RDA) set by PAHO/WHO. To this end, our generated hypothesis presented in the last part of **chapter 1** were tested and verified to be accepted as revealed by this summary of the findings. However, the developed optimized novel complementary food did not meet the recommended standard for fat

and calcium content and its zinc content was at a marginal level which may require matching for these nutrients by fat energy source and/or dairy products.

8.3 Implications of the dissertation works

8.3.1 Implication of the need to develop nutrient-dense complementary food

The period of complementary feeding determines a child's growth and survival and has short and long-term implications for psycho-motor development, earning potential, and workload capacity in later life [50]. Therefore, special focus needs to be given to the nutritional improvement of complementary food and feeding practices to well-nourish infants and young children to mitigate the negative consequences of early-life poor nutrition and ensure lifelong productivity loss [57]. The global strategy for infant and young child feeding states that, infants should be exclusively breastfed for the first six months of life to achieve optimal growth, development, health and survival, and thereafter, receive nutritionally adequate, acceptable and safe complementary foods while breastfeeding continues for up to two years or beyond [57, 63].

Children's demand for energy, protein and micronutrients especially iron, zinc, calcium and vitamin-A is high in their first two years of life [210]. Thus, they need foods of adequate energy and nutrient density to meet their demand for optimal growth and development. However, most of the households in a rural and semi-urban poor setting of Ethiopia including the study area, Nedjo district, often lack purchasing power of commercial or fortified complementary foods, as a result of which they rely on home-made traditional complementary foods. Yet, the home-made traditional complementary foods in Ethiopia are predominantly starchy cereals, often monotonous and are characterized by low nutritive value; low protein and energy density, low micronutrient density and high anti-nutritional factors that jeopardize the nutrient bioavailability of complementary foods and there is a sub-optimal feeding practices [205]. Consequently, intake of adequate simultaneous multiple nutrients are unlikely to be met from the cereal-based traditional complementary foods when compared to WHO recommendations from complementary foods. On the other hand, infants and young children have a small gastric capacity and can only consume small amounts of food at a time which triggers the importance to feed them nutritionally enriched, energy and nutrient-dense diets to fill their demands.

A recent strategy called, the food-based approaches are often described as a sustainable approach, because the approach empowers households to take ultimate responsibility over the quality of their diet mainly through the own-production of nutrient-rich foods or purchasing locally available and affordable diets and informed dietary choices. These strategies are said to be “the ideal long-term solution towards prevention of macro- and micronutrient deficits through obtaining of nutritionally adequate diverse foods, wise consumer selection, proper preparation and adequate feeding”. The use of locally available food crops to formulate a complementary diet agrees with the recently formulated ‘Ethiopian food and nutrition policy strategy that supports the development and promotion of local production of complementary foods meeting acceptable standards [56], calling for the use of available food crops in various communities in the country to prepare food for infants and young children. Such strategies are particularly important in developing countries where gross malnutrition has largely been attributed to inadequate intake of energy and nutrient-dense complementary foods due to the inability of households to afford nutritious and healthy diets (especially animal source foods). In most cases, there is an improper selection of nutritious food crops that could provide a balanced diet might result in a poor quality complementary diet.

8.3.2 Implication of cereal-legume blends in complementary food development

As a food-based approach, formulating and developing of nutritionally enriched complementary foods from locally available cereal-legume multi-mixes through the application of proper processing techniques to correct the nutrient deficits of the resultant complementary food has got increasing attention in many developing countries rather than cereal-based solely. Such an approach is supported by the Food and Agriculture Organization and World Health Organization [28] codex alimentarius on complementary feeding guidelines that describe mixtures of cereals, legumes, pulses/oilseed can constitute an appropriate source of nutrients and energy, essential fatty acids and limiting amino acids with many functional and health benefits. It further expresses that multi-mixed foods can provide improved sensory characteristics and give infants and young children a chance to appreciate various food flavors and textures, all of which are vital for later developing healthy eating habits [52]. Carbohydrates, fat, protein, minerals and vitamins are particularly important both in quantity and quality when formulating or preparing

diets for infants and young children, where requirements must be met for rapid growth, development and maintenance of good health.

In Ethiopia, particularly in the study area, Nedjo district, western oromia, there is a substantial production of starchy cereals such as maize, barley, sorghum, teff, finger millet, oats; legumes such as beans, peas and lentils and oil seeds such as linseed and soybean (identified through market survey and during ethnographic study) which can bear the bases for the development of a nutritionally enriched complementary food from proper combination of these cereals and legume.

The concept of cereal–legume combination is for the complementation of nutrients from each other and it has been particularly applied to the development of infant complementary foods with amplified protein quality. Nutritionally, the high protein content of legumes increases the protein content of cereal-based complementary foods and supplements the deficient amino acids [274]. Legumes are principally replacing milk and other sources of animal proteins, which are expensive and not readily available in poor rural settings as suitable substitutes for high-quality protein. Thus, supplementing cereals with legumes improves the nutritional quality of cereal-based foods by complementing their limited amino acids [274]. Specifically, cereals are generally fair in sulphur-containing amino acids; methionine and cysteine while legumes are good sources of lysine and tryptophan [72].

Therefore, blending locally available cereal and legume crops for complementary food production can effectively augment the intake of an affordable and more nutritionally balanced protein source, which would most likely alleviate the challenge of PEM in in our study area as well as in Ethiopia. However, to be considered wholesome (balanced) for infants and young children, complementary foods must contain other essential nutrients meeting standards such as the key micronutrients including iron, zinc and calcium which can be obtained from underutilized/marginalized nutritious crops and fat requirements where special emphases should be given to omega 3 and omega 6 polyunsaturated fatty acids in addition to protein density and quality. Based on this, the present project work employed proper selection, processing and combination of locally available and affordable cereal-legume blends accompanied by the incorporation of *dabi teff*, the underutilized crop widely grown in Nedjo area.

Dabi teff which was identified as very rich source of iron and calcium; field pea, a legume identified to be rich source of protein; maize, barley and oats, cereals which are identified to be higher sources of carbohydrates and linseed which is identified as rich source of protein and fat, and typically a leading source of α-Linolenic acid, omega-3 and omega 6 polyunsaturated fatty acids among plant foods were blended to develop a nutritionally enriched optimized novel complementary food to be used for infant and young child feeding to sustainably address PEM and micronutrients deficiencies, particularly the iron deficiency in children in the study area. Feeding this newly developed product could result in optimal growth and development as well as improved lifelong productivity of children who are growing in the area in particular and in Ethiopia in general.

8.3.3 Implications of incorporating dabi teff to the cereal-legume blends for complementary foods development

Many studies in SSA has proven that indigenous underutilized plant foods have nutritional potential and health benefits; where they contain more protein, energy and especially micronutrients that are unavailable in traditional staple foods. There are also reports that underutilized plant foods are naturally gluten-free and play anti-oxidant properties that signify their medicinal values.

There is a need and an opportunity in SSA countries to explore such nutritious underutilized/forgotten plant foods and incorporate them into the main traditional foods to produce nutrient-adequate complementary food that might meet the World Health Organization's estimated energy and nutrient requirements from complementary foods [83]. The use of underutilized plant foods in enriching major staple foods in SSA is currently novel approach in formulating new nutritious food products and holds a brighter prospect for alleviating hunger and macro- and micronutrient malnutrition [83]. *Dabi teff* is an indigenous underutilized/forgotten and marginalized farmer variety teff widely grown in western Ethiopia.

From the ethnographic qualitative study, it was found that there are general beliefs in the community that *dabi teff* has many health benefits in bringing positive health and nutritional outcomes when its different food forms are served to a family member requiring special nutritional treatment, the food therapy. It was also raised that even though the use of *dabi teff* as

a complementary food is not very common, people sometimes add it as an ingredient for complementary food preparation in the form of gruel, porridge, kita/mexinoo and “cafaqoo” and serve it to children **(Chapter 4).**

In this dissertation work, it was found out that *dabi teff* had a significantly higher iron content at 86.5 mg/100 g individually and the iron content of our optimized complementary food was two-fold than the FAO/WHO recommendations **(Chapter 6.1)** and it was shown that the likely contribution of the optimized novel complementary food to daily iron demand by 6-23 months old children was more than 200% (at moderate bioavailability) as modeled against the recommended dietary allowance (RDA) by PAHO/WHO child feeding guidelines **(Chapter 7)**. This shown that *dabi teff* is a good dietary source of iron from cereals that can serve as an inexpensive and sustainable way of meeting children’s iron demand and its incorporation into locally available cereals and legumes to develop an iron-enriched complementary food can be used as a food-based intervention strategy to alleviate iron deficiency anemia.

Thus, in Ethiopia, where it was reported that prevalence of anemia in children is unacceptably high at 57% [17], where the consumption of animal-source foods is low and where nearly all iron requirements (>97%) should come from complementary foods after 6 months [6] the incorporation and promotion of *dabi teff* in the formulation of complementary foods is very supported as an implication of this study.

In the report of EDHS [17], the prevalence of anemia in women 15-49 years of age was as low as 24%, where the iron requirement of this productive age group is very high and it was also reported that for male of 15-49 years of age the prevalence of anemia was only 15%. This lower incidence of anemia in this age group can be implicated to the presumed high iron content of teff, particularly the red teff [103] which is the staple diet in the Ethiopia population, frequently and regularly consumed where they can secure their iron requirements and body stores. Furthermore, from this report, it can also be inferred that the iron from red teff might got higher bioavailability and its utilization might have resulted in higher hemoglobin level, a lower incidence of anemia. This might be attributed to the common processing techniques particularly the fermentation technique usually applied during the preparation of foods from teff that can be responsible for the degradation of phytic acid, making iron uncompromised for absorption. On the other side, the same report has shown that the prevalence of anemia in under-five children in Ethiopia was 57%

which would raise a suspect that there is a very low or nil practice of using red teff as an ingredient in complementary food preparation which was practically justified from our ethnographic study in Nedjo area. Therefore, the incorporation of *dabi teff* into other locally available cereal-legume food crops has high implications and should be promoted to develop nutritionally enriched optimized novel complementary foods for child feeding particularly to address the iron deficiency and anemia in general that is persistently high.

8.3.4 Implication of the proper selection and processing techniques applied

Prior to the collection of the crop samples, the nutritional contents of each complementary crop identified in the study area were searched in the literature and the particular crops used were wisely selected to add one or more essential nutrients to their mixture for complementation. The implication of this account for, even though there are numerous crops in the study area, the community has to get an informed choice of nutrient-rich foods from all the available food crops.

From the laboratory analysis results, it was found that the cereals and legumes employed in the development of nutritionally enriched complementary contained a considerable amount of macro- and micronutrients. However, anti-nutrients such as phytate, tannin, trypsin inhibitor, cyanogenic glycosides and oligosaccharides are naturally occurring constituents majorly in cereals and legumes which antagonize the absorption of one or more nutrients through non-digestible insoluble complex formation, particularly with minerals and reduce the bioavailability of the minerals to the human body [89, 90]. Local foods can be modified through the use of various processing techniques like germination, soaking, roasting, fermentation, dehulling and extrusion cooking which can result in the reduction of anti-nutrients, improve energy and nutrient densities of complementary foods, increase bioavailability of key micronutrients as well as enhance sensory and palatability of foods [91].

Thus, the germinating, roasting, soaking, dehulling and cooking processing applied on our respective ingredients were meant to reduce the effect of these anti-nutrients and the phytate/minerals molar ratio where the resultant complementary food has got an increased level of nutrient density and improved the in-vitro bioavailability of iron, calcium and zinc as well boosted the sensory qualities of our formulated complementary foods. The mechanism by which

these processing techniques bring an effect is well elaborated in **Chapter 3.** In addition to reducing the anti-nutrient contents, the lower moisture content observed in the formulated complementary foods was attributed to the processing techniques applied where lower moisture content is an indicator of the foods to have longer storage stability. A lower moisture content of foods is particularly required among rural mothers in developing countries including Ethiopia because there is a usual practice of preparing a relatively large amount of complementary flour and use it for a longer time to avoid frequent preparation.

Sensory qualities are of paramount concern especially when new food product development is targeting infants and young children. If the food is not pleasant or good taste, they may refuse to consume it. The mother panelists judged the porridge from the formulated complementary foods and rated “liked much” that can be transferred to their infants and young children. This enhanced sensor acceptability could be attributed to the multiple grains, the cereals and legumes multi-mix and the processing technique applied which imparted good flavor, aroma and mouth feel to the newly formulated porridges **(Chapter 5.1).**

8.3.5 Implication for formulation and optimization

Optimization in the current context is defined as finding out the most desirable combinations of mixture ingredients that can provide the best product attributes (to identify the best proportion ratios of ingredients that can bear the best nutritional and sensory profile of the developed product). The D-optimal mixture design employed in this study was used to find the best proportion of ranged mixture components through predictive equations that allow the application of mathematical algorithms, making it possible to determine the optimal conditions for the formulation of novel products. Thus, the overall implication of the optimization work was to identify the vital formulation conditions (the optimal mixture components) from the trivial many solutions that can provide the optimal nutritious and acceptable magical optimal complementary food product that can fulfill the daily energy and nutrients demand by infants and young children if served the estimated amount per meal and fed three times a day where a positive nutritional outcomes are more likely to be expected from the efficacy study on children in the study area.

Therefore, the optimization work would provide scientifically proven information to the community to choose a healthy and nutritious diet from different combinations of food groups as supported by the Ethiopian food and nutrition policy implementation [275]. Specifically, the community in Nedjo area would get information about what type of food crops they should choose (our properly selected locally available crops including *dabi teff*) and how much (what amount) of each crop they should mix or blend together to prepare energy and nutrient dense composite novel complementary food by utilizing food crops in their locality to feed their children.

It also provides qualitative information concerning the nutritional profiles of the optimized novel complementary foods formulated from the combinations of locally available food crops. The production and utilization of this optimized complementary food as infant and young child feeding would more likely improve the health and nutritional wellbeing, and thereby reduce morbidity and mortality rates among infants and young children in Ethiopia. Moreover, the findings of this study would be useful in nutrition and health programs and to update the Ethiopian food composition tables and complementary feeding dietary guidelines by considering these local crops for achieving the development goals in child feeding.

8.4 Application areas of the project work findings

The findings of this PhD dissertation work would have practical application in the production of iron-enriched homemade complementary food preparation using local food crops and also for baby food production at industrial level targeting infants and young children, school-going children, pregnant and lactating mothers as well as women of reproductive age group starting from the menarche stage aiming to meet optimal levels of energy, protein and iron requirements.

It could be utilized to meet the optimal levels of bioavailable iron. The higher iron density with improved *in vitro* bioavailability of the newly developed cereal-legume-based product could increase the hemoglobin concentration of the blood, which allows for more oxygen to be transmitted in the body and aids in higher resistance and increased endurance during athletics performance. For example, recent studies have shown that athletic runners require 30–70% more iron due to losses from foot strike hemolysis and gastrointestinal blood loss [276]. Thus, the consumption of the newly developed product (which can be translated to athletics food

production) could provide this higher iron requirement among athletic runners, thereby being a magnificent implication in sports nutrition.

8.5 Limitations of the study

In laboratory experimentations, there are lower possibilities of errors that might affect the findings, because experimentations allow a high degree of control over laboratory situations. In this study, the instrumentations used were sophisticated and working correctly; the protocols used were standard and updated **(Chapter 2)**. However, there were some limitations that need worth mentioning. As noted from the ethnographic study, *dabi teff* is harvested twice within one rainy season, but during the sample collection from the open market of Nedjo town, the season of the harvest was not considered which might be suspected to affect the results.

It was contextualized by referring to studies done in Ethiopia when estimating the daily serving amount of our optimized complementary food, which might have minor effects on the finding of the likely contribution of our product to daily energy and nutrient demand by the children. The determined likely contribution of this product to fat, calcium and zinc demand by the children were below the standard which shows our formulation lack animal source food or other fat energy source as a limitation. Another limitation of this study could be attributed to the soaking process applied on the ingredients where unless controlled, soaking may lead to the leaching of nutrients into the soaking water which was later drained off and this could result in the loss of some nutrients such as protein and the micronutrients. Such limitation provides the basis for optimization of processing conditions for maximal reduction of the anti-nutrients present in cereals and legumes and at the same time for minimizing the loss of the key nutrients. In addition, the *in-vitro* approaches of estimating the mineral's bioavailability have obvious limitations even though they can provide good proxy indicators (the *in-vivo* bioavailability) before the expensive nutrition intervention or efficacy studies are performed. WHO recommends seven dietary diversity for infant and young children feeding where the minimum was defined as having received 4 out of 7 food groups. But our currently formulated optimal complementary food did not accommodate the other required food groups such as from animal source, dairy source, fruit and vegetables which is another limitation of the study.

8.6 Conclusions

The ever-increasing child population in sub-Saharan Africa with an attendant increase in nutritious and wholesome foods calls for giving more attention to sustainable extensive agricultural production, households own diversified food production and consumption (the food-based approach), intensive research for new nutritious food varieties with increased yield, strong child nutrition policy strategy and also strong considerations to underutilized/forgotten nutritious foods which could play a great role in ensuring food and nutrition security. Children require adequate food, both in quantity and quality where nutritional well-being in early childhood has a supreme positive effect on optimal growth and development, lifelong healthy life, improved school performance as well as improved future productivity and earning potential of children. There is a saying, “Childhood is a father of a man”. This shows that children should be well-nourished with age-specific daily adequate energy and nutrient demands to become vibrant adulthood. However, in developing countries including Ethiopia, energy and nutrient deficits in complementary diets are most common, habitually associated with bulk and/or monotonous ingredients of traditional complementary foods, high anti-nutritional contents, losses and quality damage during processing, lack of purchasing power of quality foods and lack of informed choice to healthy and nutritious local foods. This led to re-curring protein-energy and micronutrient malnutrition with their major negative public health consequences where children in rural poor are more affected than their urban counterparts.

The goal of this project is to develop a nutritionally enriched optimized novel complementary food from locally available cereal-legume blends for IYCF in addressing this endemic public health problem. In Ethiopia, Nedjo district in particular, ranges of cereals such as maize, barley, sorghum, teff, finger millet, oats; legumes such as beans, peas and lentils and oil seeds such as linseed and soybean are grown and/or accessible in the market. The area is also known for the production of *dabi teff*, the underutilized/forgotten nutritious food crop. The main initial intention of this project work was to introduce *dabi teff* into complementary food formulations to develop a nutrient-dense complementary product. Incorporation of underutilized/forgotten nutritious food crops into complementary food formulation is currently a novel approach to alleviate the double burden of malnutrition, protein-energy and especially micronutrient

malnutrition in children by prevention of both the macro and micronutrient deficits in complementary diets.

Generally, it was possible to formulate and develop a nutritionally enriched optimized novel complementary food through proper selection, processing and combination of locally available cereal-legume blends accompanied by incorporation of *dabi teff*, the underutilized/forgotten crop widely grown in Nedjo area that could be used as home-based complementary foods where mothers can feed their children during the complementary feeding period. Each of the ingredients used contributed an important nutrient of interest to develop a nutritionally complemented optimized product. *Dabi teff* had an extraordinarily high contribution to the iron density of the formulations; field pea had contributed to higher protein density; maize, barley and oats contributed to energy and carbohydrate density and linseed contributed to higher protein and fat content, and perhaps contributed to α-Linolenic acid, omega-3 and omega 6 polyunsaturated fatty acids to the formulations. The optimal magic formula at overall optimization was identified at 34.66% *dabi teff*, 15.34% field pea, 5% maize, 25% barley, 15% oats, 5% linseed with response values of 15.74% protein, 5.09% fat, 2.26% ash, 73.05% carbohydrates, 380.43 kcal/100 g energy, 32.21 mg/100 g iron, 77.51 mg/100 g calcium, 2.59 mg/100 g zinc, 4.12 sensory acceptability that provided 1.27 kcal/g energy density, 4.14 g/100 kcal protein density and 8.47 mg/100 kcal iron density, respectively.

In summary, the following conclusions were drawn based on the main findings:

- There are profound traditional usages, preparation schemes and social beliefs linked to *dabi teff* among communities in Nejdo district, Western Ethiopia and there is a claim that the crop has strong nutritional potential, and health benefits and is prized as a medicinal food (nutraceutical).
- Energy and protein-dense optimized novel complementary food meeting FAO/WHO standards were developed with improved sensory acceptability by the inclusion of *dabi teff* into the locally available cereals and legume blends. The developed optimized complementary food exhibited superior quality over the traditional complementary food and the Cerifam® faffa flour.
- Iron-dense optimized novel complementary food meeting FAO/WHO standards was developed with a reduced phytate/iron molar ratio which is a good proxy indicator of

improved *in-vivo* iron bioavailability by the inclusion of *dabi teff* into the locally available cereals and legume blends. The findings show that *dabi teff* is a good dietary source of iron that can serve as an inexpensive and sustainable way of meeting children's iron demand for optimal growth and development.

- The ratio variation of the major components has shown a remarkable effect on the macro-compositions and sensory acceptability of the blends. An increase in the ratio of field pea in the formulations has resulted in increased protein, ash, fiber and energy content while an interactive effect between field pea and *dabi teff* had a synergetic effect on increasing sensory acceptability of the blends.

- The ratio variation of the major components has shown a significant effect on the dietary mineral contents as well as on the phytate/minerals molar ratios of the blends. There was a remarkably linear increase in iron and calcium contents with a function of *dabi teff* ratio increase in the blends accompanied by a significant decrease in phytate/minerals molar ratios. Particularly, the increase in *dabi teff* ratio in the blend has resulted in a notable increase in the iron content of the blend.

- The optimized novel complementary food has shown the likely high contribution of more than 100% of daily demands for energy and protein, and notably more than 200% of daily iron demand by 6-23 months old children when modeled against the recommended dietary allowance set by PAHO/WHO child feeding guideline. The density and daily contribution of zinc from the developed product were marginal, whereas the fat and calcium densities and their daily contribution by the product were inadequate, which might require matching.

The ultimate long-term goal of the PhD project was to demonstrate whether the newly developed energy, protein and iron-dense novel composite complementary product with enhanced bioavailability and sensory acceptability could be used as an amenable and sustainable food-based strategy to alleviate the underlying and immediate causes of child malnutrition and support the optimal growth and development, and allied nutritional and health outcomes among children in Ethiopia and Africa in general and to ensure that the children could bring the required life-long positive impact on national development.

8.7 Recommendations for policy and further research

Given complementary feeding determines a child's growth and survival with implications on health, education, work capacity and productivity in later life and on national economic development, the provision of nutritionally adequate and acceptable complementary food developed using indigenous nutrient-rich food crops to ensure the nutritional well-being of children in early life need to be more emphasized. There is urgency and opportunities to explore the possibility of producing nutrient-adequate complementary foods based on locally available cereals and legumes with inclusion of nutritious underutilized nutrient-dense crops like *dabi teff* in this study.

Poor quality complementary foods due to high dietary bulk, low energy and nutrient density coupled with inappropriate feeding practices are leading to a great risk of nutritional deficiencies and are among the prime causes of recurring and unacceptably high protein-energy and micronutrient malnutrition in infants and young children. Proper food selection, combination and preparation practices that affect food safety, nutrient density and acceptability compounded by feeding practices, caregiver's nutrition knowledge and socio-economic statutes are among the known integral components of caregiving in relation to IYCF. Based on this background, the following points are recommended for policymakers and further research to address protein-energy and micronutrient malnutrition and improve the nutritional status of infants and young children in Ethiopia.

8.7.1 Recommendations for policy actions

- Zonal and regional agricultural offices need to give due attention to the production of locally available nutritious and healthy food crops including *dabi teff*.

- Government bodies including the Ministry of Health, Ministry of Agriculture and the Ministry of Child and Women's Affairs need to establish an integrated initiative in the production, consumption and promotion of *dabi teff* in Ethiopia to address iron deficiency and anemia in children sustainably. Thus, needs to be integrated into the Ethiopian agricultural research program for its extensive production, industrial utilization, and home consumption.

- In addition to the sustainable production of nutritionally important conventional foods, introduction of the underutilized/marginalized crops should be focused on ensuring the increased sustainability of food systems, and dietary diversity and improving market access, including the production of home yards, the nutrition-sensitive agriculture.
- The operationalization of the recently formulated Ethiopian food and nutrition policy strategy need to be promoted to provide quantitative and qualitative information to the community so that they can get an informed choice from what food crop is available in their localities including indigenous underutilized food crops to enrich home-made complementary foods with micronutrient-rich food such as an iron-rich *dabi teff* crop in our case.
- International non-government organizations like the UNICEF, FAO, WHO, Save the Children, USAID, World Bank and others who are interested in child welfare should give more attention to the food-based approach rather than intermittent supplementation. The food-based nutrition interventions need to be promoted to help the community enrich the complementary diet with nutrient-rich local foods.
- The Ethiopian food composition tables and complementary feeding dietary guidelines need to be updated to include local underutilized food crops like *dabi teff* and their recipes and include the bioavailability of limiting micro-nutrients such as iron, calcium and zinc to make the nutrients density and adequacy to be known. Such effort requires the involvement of policymakers, research institutions, the agriculture sector and non-governmental organization working on food composition database establishing and updating.

- Promote incorporation of *dabi teff* into selected locally available cereals and legumes to formulate and develop a nutritionally enriched optimal novel complementary food needs to be promoted in Nedjo and nearby area in particular and in Ethiopia in general for infant young child feeding.

8.7.2 Recommendation for further research

Generally, this PhD dissertation work is novel in its approach and focused on exploring nutritious underutilized plant food (*dabi teff*) and introducing it into complementary food formulation to develop a nutritionally enriched optimized novel complementary food for child feeding. Such an approach needs to be encouraged, especially in Ethiopia because there are various untouched micronutrient-rich underutilized plant foods in the country with huge potential

to contribute to food and nutrition security, and national economic development and can alleviate child malnutrition in particular. Intensive impact-full research in finding for new nutritious food varieties needs to be conducted and given more attention so that proven pieces of evidence can be generated for policy discourse.

Thus, the major findings obtained from this dissertatin work need to be substantiated with further research including:

- Exploration of underutilized/marginalized plant foods found in Ethiopia need to be explored to fully exploit them and tackle malnutrition immense nutritional potential to enrich the bulky ingredients of traditional complementary foods to alleviate undernutrition and the hidden hunger in children.
- In addition to *dabi teff* which was determined to be a rich source of dietary iron, linseed which was one of our ingredients is a popularly cultivated oilseed by the community in Nedjo area. Literatures support that linseed oil is a leading source of omega-3 polyunsaturated fatty acid from plant foods which is crucially important for brain neural and cognitive development in utero and early childhood. It would therefore be recommended to be further explored as a local source of this important fatty acid for the cultivation of linseed and inclusion in complementary food formulation.
- Strengthen food-based approaches and behavior change communications to ensure mothers/caretakers and families are educated to produce or prioritize and purchase locally available nutrient-rich foods products. They also need to be educated regarding the benefits of preparing complementary food from multiple foods that multiple macro- and micronutrients can be obtained from local mixed foods. It would therefore be recommended to deliver nutrition education to mothers/caretakers on how to prepare nutritionally enriched/dense complementary foods (the optimized formula) and feed their children including the serving amount and frequency and also how to safely and hygienically handle the food.
- Conducting a randomized controlled feeding trial to determine the efficacy of the newly developed *dabi teff*-field-based optimized novel complementary food in improving nutritional status (anthropometric indices and iron status) among infants and young children is worthful recommendation.

- In this dissertation work, we have employed conventional (standard) processing techniques, and we recommend process optimization for further study, particularly the time-temperature optimization for the maximal reduction of the anti-nutrients and to keep the quality of the developed complementary food against aflatoxin and rancidity.
- The concept behind cereal–legume-oilseed multi-mix combination is for the complementation of nutrients from each other where it has been particularly applied to the development of infant complementary foods with amplified nutrients. Linked to this, amino-acid profiling and fatty acid profiling (the mono, poly and omega-3) of the mix is recommended to be examined.
- Food safety, particularly the microbial safety of complementary foods is recommended to be studied to determine the shelf life of the product.
- The addition of other food groups such as animal-source foods, dairy source (milk), fruits and vegetables to child food formulation is worth recommended for future to address the recommended dietry diversity for children. The addition of animal and dairy sources can also be used to match the lower levels of calcium, zinc and fat content observed in our optimized complementary food.

There should be a continuous policy discourse between researchers and policymakers using evidence from experimental data and change into policy language (Policy Briefs) to introduce underutilized/marginalized plant foods into child nutrition programming.

References

1. FAO and WHO, *The nutrition challenge: food system solutions*. 2018, World Health Organization.
2. FAO, *et al*., *The State of Food Security and Nutrition in the World 2021: Transforming food systems for food security, improved nutrition and affordable healthy diets for all*. Vol. 2021. 2021: Food & Agriculture Org.
3. FAO, *et al*., *The state of food security and nutrition in the world 2017: Building resilience for peace and food security. Rome, FAO*. 2017.
4. FAO, *et al*., *The state of food security and nutrition in the world 2019: safeguarding against economic slowdowns and downturns. Rome, FAO*. Vol. 2019. 2019: Food & Agriculture Org.
5. Walter, T., *et al*., *Effectiveness of iron-fortified infant cereal in prevention of iron deficiency anemia.* Pediatrics, 1993. **91**(5): p. 976-982.
6. Nkesiga, J., J.O. Anyango, and P. Ngoda, *Optimization of Protein, Total Minerals and Vitamin A Content of Orange-fleshed Sweet Potato, Amaranth Seed and Soybean Flour Blends.* Gl J Food Sci Nutri: GJFSN-121 2021.
7. Dewey, K.G. and K.H. Brown, *Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs.* Food Nutr Bull, 2003. **24**(1): p. 5-28.
8. WHO. *Interactions of Nutrition and Infection. WHO. Monograph series No.57*. in *Ser*. 1968.
9. Temba, M.C., *et al*., *The role of compositing cereals with legumes to alleviate protein energy malnutrition in Africa.* International journal of food science & technology, 2016. **51**(3): p. 543-554.
10. Kinabo, J., *Nutrition in Africa in a global economy: perspectives challenges and opportunities.* African Study Monographs, 2001. **22**(3): p. 103-122.
11. Muller, O. and M. Krawinkel, *Malnutrition and health in developing countries.* Cmaj, 2005. **173**(3): p. 279-286.
12. Kabir, I., *et al*., *Determinants of inappropriate complementary feeding practices in infant and young children in Bangladesh: secondary data analysis of Demographic Health Survey 2007.* Matern and Child Nutrition 2012. **8**(1): p. 11-27.
13. Jones, J.M., *The methodology of nutritional screening and assessment tools.* Journal of Human Nutrition and Dietetics, 2002. **15**(1): p. 59-71.
14. UNICEF, WHO, and W. Bank, *Levels and trends in child malnutrition: key findings of the 2021 edition of the joint child malnutrition estimates*. 2021, World Health Organization: Geneva. p. 1-32.
15. Hodge, J., *Food fortification: a techno-fix or a sustainable solution to fight hidden hunger. The Future of Global Relations.* The future of global relations, 2014: p. 1-68.
16. Asres, G. and A.I. Eidelman, *Nutritional assessment of Ethiopian Beta-Israel children: a cross-sectional survey.* Breastfeeding Medicine, 2011. **6**(4): p. 171-176.
17. EDHS, *Ethiopia Demographic and Health Survey; Central Statistical Agency Addis Ababa, Ethiopia.* The DHS Program ICF, Rockville, Maryland, USA, 2016.
18. UNICEF, *The cost of hunger in Ethiopia. The social and economic impact of child undernourishment in Ethiopia summary report. UNICEFAddis Ababa, Ethiopia. NEW York, NY, USA. Ethiopia. NEW York, NY, USA,*. . 2014.

19. EPHI and ICF, *Ethiopia Mini Demographic and Health Survey 2019:Key indictors*. 2019, EPHI and ICF: Rockville, Maryland, USA.
20. Ernest, N., P. Akpan, and E. Uko, *Reduced levels of some iron parameters of protein energy malnourished children in Calabar, Nigeria.* Journal of Biology, Agricultural and Healthcare, 2013. **3**: p. 114-120.
21. Une, L. and S. Gupta, *Micronutrients and antioxidant status in children with protein energy malnutrition.* 2013.
22. Nassar, M.F., *et al*., *Language skills and intelligence quotient of protein energy malnutrition survivors.* Journal of tropical pediatrics, 2012. **58**(3): p. 226-230.
23. Kar, B.R., S.L. Rao, and B. Chandramouli, *Cognitive development in children with chronic protein energy malnutrition.* Behavioral and Brain Functions, 2008. **4**(1): p. 1-12.
24. Bailey, R.L., K.P. West Jr, and R.E. Black, *The epidemiology of global micronutrient deficiencies.* Annals of Nutrition and Metabolism, 2015. **66**(Suppl. 2): p. 22-33.
25. Barber, L.I., P.C. Obinna-Echem, and E.M. Ogburia, *Proximate composition micronutrient and sensory properties of complementary food formulated from fermented maize, soybeans and carrot flours.* Sky Journal of Food Science, 2017. **6**(3): p. 033-039.
26. Das, J.K., *et al*., *Micronutrient fortification of food and its impact on woman and child health: a systematic review.* Systematic reviews, 2013. **2**(1): p. 1-24.
27. Daba, T., *Nutritional and soio-cultural values of teff (Eragrostis tef) in Ethiopia.* International Journal of Food Science and Nutrition, 2017. **2**(3): p. 50-57.
28. FAO/WHO, *Guidelines on formulated complementary foods for older infants and young children, CAC/GL 8â€“1991, REV 2013*. 1991.
29. FAO and WHO, *Guidelines on food fortification with micronutrients.* World Health Organization, Food and Agricultural Organization of the United Nations: Geneva, Switzerland, 2006. **2006**: p. 1-376.
30. Abeshu, M.A., A. Lelisa, and B. Geleta, *Complementary Feeding: Review of Recommendations, Feeding Practices, and Adequacy of Homemade Complementary Food Preparations in Developing Countries - Lessons from Ethiopia.* Front Nutr, 2016. **3**(41): p. 1-9.
31. Gibson, R.S., *et al*., *A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability.* Food Nutr Bull, 2010. **31**(2): p. 134S–146S.
32. Thompson, B. and L. Amoroso, *Combating micronutrient deficiencies: food-based approaches*. 2011: Cabi.
33. Godswill, A.G., *et al*., *Health benefits of micronutrients (vitamins and minerals) and their associated deficiency diseases: A systematic review.* International Journal of Food Sciences, 2020. **3**(1): p. 1-32.
34. Bhaskaram, P., *Immunobiology of mild micronutrient deficiencies.* British Journal of Nutrition, 2001. **85**(S2): p. S75-S80.
35. Lynch, S.R. and R.J. Stoltzfus, *Iron and ascorbic acid: proposed fortification levels and recommended iron compounds.* The Journal of nutrition, 2003. **133**(9): p. 2978S-2984S.
36. McLean, E., *et al*., *Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993â€“2005.* Public health nutrition, 2009. **12**(4): p. 444-454.
37. Hurrell, R. and I. Egli, *Iron bioavailability and dietary reference values.* Am J Clin Nutr, 2010. **91**(5): p. 1461S–1467S.

38. WHO, J., *Protein and amino acid requirements in human nutrition.* World Health Organization technical report. Geneva: Technical Report Series, No. 935, 2007(935).
39. Lind, T.r., *et al*., *A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development.* The American journal of clinical nutrition, 2004. **80**(3): p. 729-736.
40. Raihan, M. and C. Saini, *Evaluation of various properties of composite flour from oats, sorghum, amaranth and wheat flour and production of cookies thereof.* International Food Research Journal, 2017. **24**(6): p. 2278-2284.
41. Abrams, S.A. and S.A. Atkinson, *Calcium, magnesium, phosphorus and vitamin D fortification of complementary foods.* The Journal of nutrition, 2003. **133**(9): p. 2994S-2999S.
42. Miller, G.D., J.K. Jarvis, and L.D. McBean, *The importance of meeting calcium needs with foods.* Journal of the American College of Nutrition, 2001. **20**(2): p. 168S-185S.
43. WHO, *From Promise to Impact: Ending Malnutrition by 2030*. 2016, Geneva: World Health Organization.
44. Global Panel, *The Cost of Malnutrition: Why policy Actions is Urgent.London, UK: Global Panel on Agriculture and Food Systems for Nutrition.* 2016.
45. Kathryn, D., *Guiding Principles for Complementary Feeding of the Breastfed Child.* Pan American Health Organization (PAHO)/World Health Organization (WHO). Available online: https://www. who. int/nutrition/publications/guiding_principles_compfeeding_breastfed. pdf (accessed on16 August 2018), 2001.
46. Arage, G., *et al*., *Consequences of early life exposure to the 1983-1985 Ethiopian Great Famine on cognitive function in adults: a historical cohort study.* BMJ Open, 2020. **10**(9): p. e038977.
47. Chetail, S., C. Bergman, and A. Mottram, *Nutrition-sensitive agriculture programming: A nutrition-sensitive approach for market-based agricultural projects.* Oregon: Mercy Corps. Portland, 2015.
48. Bagnall-Oakeley, H., *et al*., *Nutrition sensitivity: How agriculture can improve child nutrition.* United Kingdom: Save the Children. St John's Lane, London, 2014.
49. Horton, S., *et al*., *Scaling up nutrition: What will it cost?* 2009: World Bank Publications. Washington DC.
50. Parker, S. and C. Tubbs, *Aggregate economic shocks during middle childhood.* Children and Youth in Crisis, 2012: p. 149.
51. FAO, I., and WFP,, *State of Food Insecurity in the World: 2014: Strengthening the Enabling Environment for Food Security and Nutrition*. 2014: Food & Agriculture Organization of the UN (FAO).
52. Ruel, M.T. and C.E. Levin, *Assessing the potential for food-based strategies to reduce Vitamin A and iron deficiencies.* FCND briefs, 2000(92).
53. WHO/UNICEF, *Preparation and use of food-based dietary guidelines. Report of a joint FAO/WHO consultation. WHO technical report series n 880.* 1998.
54. Mariam, S., *Nutritive value of three potential complementary foods based on cereals and legumes.* African Journal of Food, Agriculture, Nutrition and Development, 2005. **5**(2): p. 1-14.

55. PAHO/WHO, *PAHO/WHO: Guiding principles for complementary feeding of the breastfed child. Division of Helath Promotin and Protection. Food and Nutrition program*. 2001.
56. FNPS, *Federal Democratic Republic of Ethiopia National Food and Nutrition Strategy, Addis Ababa, Ethiopia*, 2021.
57. WHO and UNICEF., *Global strategy for infant and young child feeding.WH/A55/2002/REC/1 Annex 2. Geneva*. 2003.
58. Ayele, S., E.A. Zegeye, and N. Nisbett, *Multi-sectoral nutrition policy and programme design, coordination and implementation in Ethiopia*. 2020.
59. Seqota-Declaration, *The Seqota Declaration: Innovation Phase Investment Plan (2017-2020)*. 2018, Federal Democratic Republic of Ethiopia Addis Ababa, Ethiopia.
60. NNP, I., *The National Nutrition Program*. 2016.
61. Usha, R., M. Lakshmi, and M. Ranjani, *Nutritional, sensory and physical analysis of pumpkin flour incorporated into weaning mix*. Malaysian Journal of nutrition, 2010. **16**(3): p. 379-387.
62. WHO, G., *Global strategy for infant and young child feeding. Geneva, WHO. Available from: : <http://www.who.int/nutrition/publications/infantfee>*. 2003.
63. Dewey, K.G. and S. Adu-Afarwuah, *Systematic review of the efficacy and effectiveness of complementary feeding interventions in developing countries*. Matern Child Nutr, 2008. **1**(Suppl 1): p. 24-85.
64. Gibson, R.S. and E. Ferguson, *Assessment of dietary zinc in a population*. The American journal of clinical nutrition, 1998. **68**(2): p. 430S-434S.
65. FAO and WHO, *Preparation and use of food-based dietary guidelines: report of a joint FAO/WHO consultation*, in *Preparation and use of food-based dietary guidelines: report of a joint FAO/WHO Consultation*. 1998. p. vi, 108-vi, 108.
66. Michaux, K.D., J.K. Foley, and E. Boy, *Food-Based Approaches for Combating Iron Deficiency*. Nutritional anemia, 2020: p. 339-350.
67. Jalal, F., *et al.*, *Serum retinol concentrations in children are affected by food sources of Â²-carotene, fat intake, and anthelmintic drug treatment*. The American journal of clinical nutrition, 1998. **68**(3): p. 623-629.
68. Takyi, E.E., *Children's consumption of dark green, leafy vegetables with added fat enhances serum retinol*. The Journal of nutrition, 1999. **129**(8): p. 1549-1554.
69. Kamotho, S.N., *FORTIFICATION OF MAIZE FLOUR WITH GRAIN AMARANTH FOR IMPROVED NUTRITION*. 2020, JKUAT-AGRICULTURE.
70. Muhimbula, H.S. and A. Issa-Zacharia, *Persistent child malnutrition in Tanzania: Risks associated with traditional complementary foods (A review)*. 2010.
71. WFP, *Technical specifications for the manufacture of: corn soya blend for young children and adultsâ€"CSB plus, version 2.1*. 2010, United Nations World Food Programme Rome (Italy).
72. Vallinoto, P., E.G. Moreira, and V.A. Maihara, *Estimation of daily dietary intake of essential minerals and trace elements in commercial complementary foods marketed in Brazil*. Food Chemistry Advances, 2022. **1**: p. 100039.
73. van der Merwe, J., *et al.*, *Optimizing the introduction of complementary foods in the infant's diet: a unique challenge in developing countries*. Matern Child Nutr, 2007. **3**(4): p. 259-70.

74. Ahmed, T., M. Hossain, and K.I. Sanin, *Global burden of maternal and child undernutrition and micronutrient deficiencies.* Annals of Nutrition and Metabolism, 2013. **61**(suppl 1): p. 8-17.
75. Mayer Labba, I.-C., *et al.*, *Nutritional Composition and Estimated Iron and Zinc Bioavailability of Meat Substitutes Available on the Swedish Market.* MDPI, Nutrients, 2022. **14**(19): p. 390-3.
76. McLaren, D., *The great protein fiasco.* The Lancet, 1974. **304**(7872): p. 93-96.
77. Bouchenak, M. and M. Lamri-Senhadji, *Nutritional quality of legumes, and their role in cardiometabolic risk prevention: a review.* Journal of medicinal food, 2013. **16**(3): p. 185-198.
78. Graham, R.D., J.M. Humphries, and J.L. Kitchen, *Nutritionally enhanced cereals: A sustainable foundation for a balanced diet.* Asia Pacific Journal of Clinical Nutrition, 2000. **9**(S1): p. S91-S96.
79. Ndagire, C.T., *et al.*, *Optimized formulation and processing protocol for a supplementary beanâ€‐based composite flour.* Food Science & Nutrition, 2015. **3**(6): p. 527-538.
80. Roy, M.K., *et al.*, *Antioxidant potential, anti-proliferative activities, and phenolic content in water-soluble fractions of some commonly consumed vegetables: Effects of thermal treatment.* Food chemistry, 2007. **103**(1): p. 106-114.
81. Bouchenak, M. and M. Lamri-Senhadji, *Nutritional quality of legumes, and their role in cardiometabolic risk prevention: a review.* J Med Food, 2013. **16**(3): p. 185-98.
82. Thorisdottir, B., *et al.*, *Legume consumption in adults and risk of cardiovascular disease and type 2 diabetes: a systematic review and meta-analysis.* Food Nutr Res, 2023. **67**(2023): p. 2023.
83. Learning, B. and S.E. Choi, *Chapter 3 Sensory evaluation. Food Science: An Ecological Approach, .* 2014: p. 84-111.
84. Magbagbeola, J., J. Adetoso, and O. Owolabi, *Neglected and underutilized species (NUS): a panacea for community focused development to poverty alleviation/poverty reduction in Nigeria.* Journal of Economics and International Finance, 2010. **2**(10): p. 208.
85. Abebe, D., and, and A. Ayehu, *Medicinal Plants and Enigmatic Health Practices of Northern Ethiopia. B.S.P.E, Addis Ababa, 419-431.* 1993.
86. Dechassa Lemessa, *Ethiopia: Rapid Assessment Mission Report: Konso Special Woreda, SNNPR. UN-EUE Field Mission Report, 29 to 31 August 1999, Addis Ababa.* 2010.
87. Aboagye, L.M., *et al.*, *Underutilized Species Policies and Strategies; Analysis of Existing National Policies and Legislation That Enable or Inhibit the Wider Use of Underutilized Plant Species for Food and Agriculture in Ghana. Council for Scientific and Industrial Research, Kumasi, 472/a.*. 2007.
88. Padulosi, S., J. Thompson, and P. Rudebjer, *Fighting poverty, hunger and malnutrition with neglected and underutilized species (NUS): needs, challenges and the way forward.* 2013.
89. Conti, M.V., *et al.*, *Potential role of neglected and underutilized plant species in improving womenâ€™s empowerment and nutrition in areas of sub-Saharan Africa.* Nutrition Reviews, 2019. **77**(11): p. 817-828.
90. Qaim, M., *Role of new plant breeding technologies for food security and sustainable agricultural development.* Applied Economic Perspectives and Policy, 2020. **42**(2): p. 129-150.

91. Gebremariam, M.M., M. Zarnkow, and T. Becker, *Teff (Eragrostis tef) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review.* Journal of food science and technology, 2014. **51**: p. 2881-2895.
92. Grando S and G. H, *Food uses of barely. International Center for Agricultural Research in the Dry Areas (ICARDA).* 2005.
93. Welch, R.W., *Oat in Human Nutrition and Health.* 1995.
94. Wu, D., *et al*., *A Comprehensive Review of Pea (Pisum sativum L.): Chemical Composition, Processing, Health Benefits, and Food Applications.* MDPI, foods, 2023. **12**.
95. Adeyeye, S.A.O., A.O. Adebayo-Oyestoro, and S.A. Omoniyi, *Quality and sensory properties of maize flour cookies enriched with say protein isolate.* Cogent food and agriculture. 2017.
96. Ijarotimi, S.O. and O.O. Keshinro, *Determination of nutrient composition and protein quality of potential complementary foods formulated from the combination of fermented popcorn, African locust and bambara groundnut seed flour.* Polish Journal of Food and Nutrition Sciences, 2013. **63**(3).
97. Tufa, M., *et al*., *Development and nutritional assessment of complementary foods from fermented cereals and soybean. . Journal of Food Science and Nutrition,*, 2016. **2**: p. 1-8.
97. Preethi, M.B. and B.V. Chimmad, ***Utilization of linseed for value addition to supplementary food****. *Karnataka J. Agric. Sci.*,, 2010. **23**(5): p. 765-768.
99. **Galvan, D.**, *et al*., *Recent Applications of Mixture Designs in Beverages, Foods, and Pharmaceutical Health: A Systematic Review and Meta-Analysis.* MDPI, foods, 2021. **10**.
100. Orives, J.R., *et al*., *Multiresponse optimisation on biodieselobtained through a ternary mixture of vegetable oil and animal fat: Simplex-centroid mixture design application. .* Energy Convers. Manag, 2014. **79**: p. 398-404.
101. Dewey, K.G. and S. Adua Afarwuah, *Systematic review of the efficacy and effectiveness of complementary feeding interventions in developing countries.* Maternal & child nutrition, 2008. **4**: p. 24-85.
102. Bhutta, Z.A., *et al*., *Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost?* The Lancet, 2013. **382**(9890): p. 452-477.
103. Nga, T.T., *Acceptability and impact on anthropometry of a locally developed ready-to-use therapeutic food in pre-school children in Vietnam* Nutrition Journal, 2013. **12**(1).
104. **Dhliwayo, T.C.**, *et al*., *A review of traditional grain-based complementary foods for children aged 6-23 months in selected African countries* Nor. Afr. J. Food Nutr 2022. **6**(14): p. 115-125.
105. Knez, M., M. Rani, and M. Mirjana Gurinovi, *Underutilized plants increase biodiversity, improve food and nutrition security, reduce malnutrition, and enhance human health and well-being. Let's put them back on the plate!* Nutrition Reviews., 2023. **00**: p. 1014.
106. Amagloh, F.K., *et al*., *Complementary food blends and malnutrition among infants in Ghana: A review and a proposed solution.* 2012.
107. WHO, *Complementary feeding: report of the global consultation, and summary of guiding principles for complementary feeding of the breastfed child*. 2003: World Health Organization, Geneva, Switzerland.

108. Hassan, Z., N. Sebola, and M. Mabelebele, *The nutritional use of millet grain for food and feed: a review.* Agriculture & food security, 2021. **10**: p. 1-14.
109. Saleh, A.S., *et al*., *Millet grains: nutritional quality, processing, and potential health benefits.* Comprehensive reviews in food science and food safety, 2013. **12**(3): p. 281-295
110. Teye, E., *et al*., *Delivering the nutritional needs by food to food fortification of staples using underutilized plant species in Africa.* International Journal of Food Science, 2020. **2020**.
111. Horwitz, A., E.T. Kennedy, and C.P. Howson, *Prevention of micronutrient deficiencies: tools for policymakers and public health workers.* 1998.
112. Lee, S.H., *et al*., *Dietary soluble flaxseed oils as a source of omega-3 polyunsaturated fatty acids for laying hens.* Poultry Science, 2021. **100**(8): p. 101276.
113. CSA, *Summary and statistical report of the 2007 population and housing census. population size by age and sex: Addis Ababa, Ethiopia.* 2007.
114. FAO, *Ethiopia Nutrition Profile – Nutrition and Consumer Protection Division, Food and Agriculture Organization.* 2008.
115. Rasane, P., *et al*., *Reduction in phytic acid content and enhancement of antioxidant properties of nutricereals by processing for developing a fermented baby food.* Journal of food science and technology, 2015. **52**: p. 3219-3234.
116. Castro-Alba, V., *et al*., *Phytate, iron, zinc, and calcium content of common Bolivian foods and their estimated mineral bioavailability.* Food Sci Nutr, 2019. **7**(9): p. 2854-2865.
117. AACC, *American Association of Cereal Chemists, 11th edition, Cereals and Grains Associations Technical Communities, Washington DC.* . 2000.
118. Federal Democratic Republic of Ethiopia, M.o.H., *Complementary feeding of children 6 up to 24 months old for IYCF trainers and promoters*. 2012: Addis Ababa, Ethiopia.
119. Onabanjo, O., *et al*., *Nutritional evaluation of four optimized cassava-based complementary foods.* African Journal of Food Science, 2008. **2**(12): p. 136-142.
120. AOAC, *Official Methods of Analysis of the Association of Official Analytical Chemists, twentieth ed. AOAC International, Washington, DC.* 2016.
121. Ayele, D.A., *et al*., *Optimization of nutritional and functional qualities of local complementary foods of southern Ethiopia using a customized mixture design.* Food Sci Nutr, 2021. **10**(1): p. 239-252.
122. FAO/WHO, *Guideline on nutritional lebelling. Codex alimentarius-International Food Standards.* 1985.
123. de Souza, R.M., L.G. LeocÃ¡dio, and C.L.c.P. da Silveira, *ICP OES simultaneous determination of Ca, Cu, Fe, Mg, Mn, Na, and P in biodiesel by axial and radial inductively coupled plasma-optical emission spectrometry.* Analytical letters, 2008. **41**(9): p. 1615-1622.
124. Latta, M. and M. Eskin, *A Simple and Rapid Colorimetric Method for Phytate Determination. Journal of Agricultural and Food Chemistry.* 1980. **28**: p. 1313-1315.
125. Price, M.L., S.S. Van, and L. Butler, G, *A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain.* J. Agric. Food Chem 1978. **26**(5): p. 1214-1218.
126. Norhaizan, M.E., Jr. and A.W. Nor Faizadatul Ain, *Determination of phytate, iron, zinc, calcium contents and their molar ratios in commonly consumed raw and prepared food in malaysia.* Malays J Nutr, 2009. **15**(2): p. 213-22.

127. Abeshu, M.A., *et al*., *Assessment of Caregiver's Knowledge, Complementary Feeding Practices, and Adequacy of Nutrient Intake from Homemade Foods for Children of 6-23 Months in Food Insecure Woredas of Wolayita Zone, Ethiopia.* Front Nutr, 2016. **3**(32): p. 2016.
128. Wasswa, M., *et al*., *Proximate composition and sensory characteristics of refractance window dried cowpea composite porridges.* African Journal of Food, Agriculture, Nutrition and Development, 2021. **21**(10): p. 18965-18979.
129. Nahemiah, D.N., Iro Badau, and M. Halidu, *Application of Response Surface Methodology (RSM) for the Production and Optimization of Extruded Instant Porridge from Broken Rice Fractions Blended with Cowpea.* International Journal of Nutrition and Food Sciences, 2016. **5**(2).
130. Ngaha, W.D., *et al*., *Nutritional quality of formulated complementary foods and their biological effects for tackling malnutrition in Sub-Saharan Africa (SSA) countries.* European Journal of Nutrition & Food Safety, 2020. **12**(12): p. 91-103.
131. Ali, A., *et al*., *Effect of substitution of wheat flour with taro flour on some properties of weaning food formula.* Journal of Applied Sciences Research, 2013. **9**(6): p. 3985-3991.
132. Ikpeme-Emmanuel, C., J. Okoi, and N. Osuchukwu, *Functional, anti-nutritional and sensory acceptability of taro and soybean based weaning food.* African Journal of Food Science, 2009. **3**(11): p. 372-377.
133. Taylor, J. *Traditional African Grains*. in *Traditional grains for low environmental impact and good health. Seminar/workshop organised by MISTRA and IFS, September*. 2008.
134. Haddad, L., *Ending undernutrition: Our legacy to the post 2015 generation. Background framing paper for the Nutrition for Growth Conference. June. London*. 2013.
135. Aboagye, L.M., *et al*., *Underutilized species, policies and strategies.* Analysis of existing national policies and legislation that enable or inhibit the wider use of underutilized plant species for food and agriculture in Ghana, Council for Scientific and Industrial Research, Kumasi, 2007. **472**.
136. Hunter, D., *et al*., *The potential of neglected and underutilized species for improving diets and nutrition.* Planta, 2019. **250**: p. 709-729.
137. Knez, M., M. RaniÄ‡, and M. GurinoviÄ‡, *Underutilized plants increase biodiversity, improve food and nutrition security, reduce malnutrition, and enhance human health and well-being. Let us put them back on the plate!* Nutrition Reviews, 2023: p. nuad103.
138. Gregory, P.J., *et al*., *Crops For the Future (CFF): an overview of research efforts in the adoption of underutilised species.* Planta, 2019. **250**: p. 979-988.
139. Drub, T.F., et al., *Sorghum, millet and pseudocereals as ingredients for gluten-free whole-grain yeast rolls.* International Journal of Gastronomy and Food Science, 2020. **23**: p. 100293.
140. Lim, K.H., *et al*., *Iron and zinc nutrition in the economically-developed world: a review.* Nutrients, 2013. **5**(8): p. 3184-3211.
141. Schlossman, N., *Delivering improved nutrition: dairy ingredients in food aid products.* Food and Nutrition Bulletin, 2016. **37**(1_suppl): p. S6-S13.
142. Kamotho, S., F. Kyallo, and D. Sila, *Biofortification of maize flour with grain amaranth for improved nutrition.* African Journal of Food, Agriculture, Nutrition and Development, 2017. **17**(4): p. 12574-12588.

143. Zebdewos, A., *et al*., *Formulation of complementary food using amaranth, chickpea and maize improves iron, calcium and zinc content.* African Journal of Food, Agriculture, Nutrition and Development, 2015. **15**(4): p. 10290-10304.
144. Adejuyitan, J., *et al*., *An evaluation of some properties of baobab fruit powder and ogi mixes.* Transnational Journal of Science and Technology, 2012. **2**(7): p. 99-102.
145. Glover-Amengor, M., *et al*., *Micronutrient composition and acceptability of Moringa oleifera leafâ€fortified dishes by children in Adaâ€East district, Ghana.* Food Science & Nutrition, 2017. **5**(2): p. 317-323.
146. Shiriki, D., M.A. Igyor, and D.I. Gernah, *Nutritional evaluation of complementary food formulations from maize, soybean and peanut fortified with Moringa oleifera leaf powder.* Food and nutrition sciences, 2015. **6**(05): p. 494.
147. Twum, L.A., *et al*., *Physicochemical and elemental analyses of banana composite flour for infants.* 2015.
148. Bonsi, E., W. Plahar, and R. Zabawa, *Nutritional enhancement of Ghanaian weaning foods using the orange flesh sweetpotato.* African Journal of Food, Agriculture, Nutrition and Development, 2014. **14**(5).
149. Peiretti, P., *Amaranth in animal nutrition: A review.* Livestock Research for Rural Development, 2018. **30**(5): p. 2018.
150. Bolarinwa, I.F., *et al*., *Malted sorghum-soy composite flour: preparation, chemical and physico-chemical properties.* Journal of Food Processing & Technology, 2015. **6**(8): p. 1.
151. Aniedu, C. and O.C. Aniedu, *Fortification of cassava fufu flour with African yam bean flour: implications for improved nutrition in Nigeria.* 2011.
152. Ashaye, O., O. Olanipekun, and S. Ojo, *Chemical and nutritional evaluation of biscuit processed from cassava and pigeon pea flour.* J Food Process Technol, 2015. **6**(12): p. 521.
153. Ezinwanyi, A.Y. and E.N. Ndaeyo, *Proximate compositions, physicochemical and sensory properties of gari fortified with soybean, melon seed and moringa seed flours.* International Journal of Nutrition and Food Sciences, 2017. **6**(2): p. 105-110.
154. Olapade, A., Y. Babalola, and O. Aworh, *Quality attributes of fufu (fermented cassava) flour supplemented with bambara flour.* International Food Research Journal, 2014. **21**(5).
155. Honi, B., *Development of Orange Fleshed Sweet Potato and Bambara groundnut-based snacks for School children in Tanzania.* Makerere University, Makerere, Uganda, 2016.
156. Fausat Lola, K., B. Mutiat Adebanke, and S.-O. Halimat Olamide, *Physical and chemical characteristics of moringa-fortified orange sweet potato flour for complementary food.* Hrvatski Ä□asopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam, 2017. **12**(1-2): p. 37-43.
157. Honi, B., I.M. Mukisa, and R.J. Mongi, *Proximate composition, provitamin A retention, and shelf life of extruded orangeâ€fleshed sweet potato and bambara groundnutâ€based snacks.* Journal of Food Processing and Preservation, 2018. **42**(1): p. e13415.
158. Namiki, M., *Nutraceutical functions of sesame: a review.* Critical reviews in food science and nutrition, 2007. **47**(7): p. 651-673.
159. Agossadou, J., *et al., Effect of consumption of foods fortified with baobab fruit pulp and moringa leaf powder on the nutritional status of children.* RUFORUM Working Document Series, 2016. **14**(2): p. 979-988.

160. Gloverâ€□Amengor, M., *et al*., *Micronutrient composition and acceptability of Moringa oleifera leafâ€□fortified dishes by children in Adaâ€□East district, Ghana.* Food Science & Nutrition, 2017. **5**(2): p. 317-323.
161. Madukwe, E., I. Obizoba, and O. Chukwuka, *Nutrient assessment of processed rice (Oryza sativa), soybean (Glycine max Merr) flours/groundnut (Arachis hypogea) paste and sensory attributes of their composites.* 2013.
162. HofmanovÃ¡, T.a., M. HruÅ¡kovÃ¡, and I. Å vec, *Evaluation of wheat/non-traditional flour composite.* Czech Journal of Food Sciences, 2014. **32**(3): p. 288.
163. Kidane, G., *et al*., *Nutritional analysis of vitamin A enriched bread from orange flesh sweet potato and locally available wheat flours at Samre Woreda, Northern Ethiopia.* Current Research in Nutrition and Food Science Journal, 2013. **1**(1): p. 49-57.
164. Ocheme, O.B., et al., *Proximate composition, functional, and pasting properties of wheat and groundnut protein concentrate flour blends.* Food Science & Nutrition, 2018. **6**(5): p. 1173-1178.
165. Forsido, S.F., *et al*., *Nutritional and sensory quality of composite extruded complementary food.* Food Science & Nutrition, 2019. **7**(2): p. 882-889.
166. Mezgebo, K., T. Belachew, and N. Satheesh, *Optimization of red teff flour, malted soybean flour, and papaya fruit powder blending ratios for better nutritional quality and sensory acceptability of porridge.* Food Sci Nutr, 2018. **6**(4): p. 891-903.
167. Yohannes, T.G., *et al*., *Developing and nutritional quality evaluation of complementary diets produced from selected cereals and legumes cultivated in Gondar province, Ethiopia.* Current Research in Nutrition and Food Science Journal, 2020. **8**(1): p. 291-302.
168. Agume, A.S.N., N.Y. Njintang, and C.M.F. Mbofung, *Effect of soaking and roasting on the physicochemical and pasting properties of soybean flour.* Foods, 2017. **6**(2): p. 12.
169. Adebowale, A., *et al*. *Effect of soaking pre-treatments on nutritional composition and functional properties of bambara groundnut (vigna subterranea) flour*. in *II International Symposium on Underutilized Plant Species: Crops for the Future-Beyond Food Security 979*. 2011.
170. Tiencheu, B., *et al*., *Formulation and nutritional evaluation of instant weaning foods processed from maize (Zea mays), pawpaw (Carica papaya), red beans (Phaseolus vulgaris) and mackerel fish meal (Scomber scombrus).* American Journal of Food Science and Technology, 2016. **4**(5): p. 149-159.
171. Ajibola, C., T. Fagbemi, and O. Osundahunsi, *Nutritional Quality of Weaning Foods Formulated from Maize Gruel'Ogi'and Crayfish Using Combined Traditional Processing Technology.* Advances in Research, 2016. **6**(4): p. 1-11.
172. Tedom, W., *et al*., *Optimal conditions of steam blanching of spinach (Spinacia oleracea), a leafy vegetable consumed in Cameroon.* International Journal of Nutritional Sciences and Food Technology, 2020. **6**(4): p. 1-8.
173. Rupa, A.Z., and, and A.T.M.A. Rahim, *Ethnographic survey on cereal and pseudocereal in Bangladesh perspective* Food Research 2023. **7**(2): p. 222-229.
174. Ebba, T., *T'ef (Eragrostis tef) cultivars: morphology and classification.* . Experimental Bulletin 66, Addis Ababa University, College of Agriculture, Ethiopia., 1975.
175. Mohammed, Z. and U. Ahmad, *Formulation and nutritional evaluation of a complementary food blend made from fermented yellow maize (Improved variety),*

soybean and African cat fish meal. Nigerian Journal of Biotechnology, 2021. **38**(1): p. 98-108.
176. Ramakrishna, V., P.J. Rani, and P.R. Rao, *Anti-nutritional factors during germination in Indian bean (Dolichos lablab L.) seeds.* World Journal of Dairy and Food Sciences, 2006. **1**(1): p. 6-11.
177. Mubarak, A., *Nutritional composition and antinutritional factors of mung bean seeds (Phaseolus aureus) as affected by some home traditional processes.* Food chemistry, 2005. **89**(4): p. 489-495.
178. Hotz, C. and R.S. Gibson, *Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets.* The Journal of nutrition, 2007. **137**(4): p. 1097-1100.
179. Ekpa, O., *et al*., *Sub-Saharan African maize-based foods-processing practices, challenges and opportunities.* Food Reviews International, 2019. **35**(7): p. 609-639.
180. Sade, F.O., *Proximate, antinutritional factors and functional properties of processed pearl millet (Pennisetum glaucum).* Journal of Food Technology, 2009. **7**(3): p. 92-97.
181. Son, S.-J. and S.-P. Lee, *Physicochemical and functional properties of roasted soybean flour, barley, and carrot juice mixture fermented by solid-state fermentation using Bacillus subtilis HA.* Food Science and Biotechnology, 2011. **20**: p. 1509-1515.
182. Abdelrahaman, S.M., *et al*., *Antinutritional factor content and hydrochloric acid extractability of minerals in pearl millet cultivars as affected by germination.* International journal of food sciences and nutrition, 2007. **58**(1): p. 6-17.
183. Adebiyi, J.A., *et al*., *Food fermentation and mycotoxin detoxification: An African perspective.* Food Control, 2019. **106**: p. 106731.
184. Olagunju, O.F., *et al*., *Effects of fermentation on proximate composition, mineral profile and antinutrients of tamarind (Tamarindus indica L.) seed in the production of daddawa-type condiment.* LWT, 2018. **90**: p. 455-459.
185. Chaves□Lopez, C., *et al*., *Traditional fermented foods and beverages from a microbiological and nutritional perspective: the Colombian heritage.* Comprehensive reviews in food science and food safety, 2014. **13**(5): p. 1031-1048.
186. Adeoti, O. and O. Osundahunsi, *Nutritional characteristics of maize-based complementary food enriched with fermented and germinated Moringa oleifera seed flour.* International Journal of Food Science, Nutrition and Dietetics, 2017. **6**(2): p. 350-357.
187. Affonfere, M., *et al*., *RUFORUM Working Document Series (ISSN 1607-9345) No. 14 (2): 969-977. Available from http://repository. ruforum. org.* 2016.
188. Sankhon, A., I. Amadou, and W.-R. Yao, *Application of resistant starch in bread: processing, proximate composition and sensory quality of functional bread products from wheat flour and African locust bean (Parkia biglobosa) flour.* Agricultural Sciences, 2013. **4**(05): p. 122.
189. Twum, L.A., *et al*., *Physicochemical and elemental analyses of banana composite flour for infants.* 2013.
190. Singh, P., *et al*., *Functional and edible uses of soy protein products.* Comprehensive reviews in food science and food safety, 2008. **7**(1): p. 14-28.
191. Gibson, R.S., L. Perlas, and C. Hotz, *Improving the bioavailability of nutrients in plant foods at the household level.* Proceedings of the Nutrition Society, 2006. **65**(2): p. 160-168.

192. Grivetti, L.E. and B.M. Ogle, *Value of traditional foods in meeting macro-and micronutrient needs: the wild plant connection.* Nutrition research reviews, 2000. **13**(1): p. 31-46.
193. Ketema, S., *Tef (Eragrostis Tef (Zucc.) Trotter). Promoting the Conservation and Use of Underutilized and Neglected Crops Series No. 12. Institute of Plant Genetics and Crop Plant Research, Gatersleben and Int. Plant Genetic Resources Institute, Rome, 52.* 1997.
194. Roseberg, R.J., *et al*., *Yield and quality of teff forage as a function of varying rates of applied irrigation and nitrogen.* Klamath Experiment Station, 2005. **1069**: p. 119-136.
195. Dijkstra, A., *et al*., *Survey on the nutritional and health aspects of teff (Eragrostis Tef).* Memorias, red-alfa lagrotech, comunidad europea, cartagena, 2008. **321**.
196. FAO, *Analysis of incentives and disincentives for teff in Ethiopia." Retrieved from FAO website http://www.fao.org/3/a-at474e.pdf.* 2013.
197. Mengesha, M.H., *Chemical composition of teff (Eragrostis tef) compared with that of wheat, barley and grain sorghum.* Economic Botany, 1966. **20**(3): p. 268-273.
198. Bultosa, G., *Physicochemical characteristics of grain and flour in 13 tef [Eragrostis tef (Zucc.) Trotter] grain varieties.* Journal of Applied Sciences Research, 2007. **3**(12): p. 2042-2051.
199. Gamboa, P.a. and L.V. Ekris, *Survey on the nutritional and health aspects of teff (Eragrostis tef). Memorias Red-Alfa Lagrotech, Comunidad Europea Cartagena, .* 2008: p. 319-382
200. Viljamaa, M., *et al*., *Coeliac disease, autoimmune diseases and gluten exposure.* Scandinavian journal of gastroenterology, 2005. **40**(4): p. 437-443.
201. Rashid, S. and A. Negassa, *Policies and performance of Ethiopian cereal markets.* Ethiopia strategy support program.ESSP II Working Paper No. 21, 2011. **2.**
202. **Eliot, a.** and **Associates.**, *Guideline for conducting focus group dicussion* 2005.
203. Tong, A., P. Sainsbury, and J. Craig, *Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups.* International journal for quality in health care, 2007. **19**(6): p. 349-357.
204. Baye, K., *et al*., *Nutrient intakes from complementary foods consumed by young children (aged 12-23 months) from North Wollo, northern Ethiopia: the need for agro-ecologically adapted interventions.* Public Health Nutr, 2012. **16**(10): p. 1741-50.
205. Temesgen, M., *Nutritional status of Ethiopian weaning and complementary foods: a review.* Open Access Sci Rep, 2013. **2**(2): p. 1-9.
206. NET/WFP, F., *Famine Early Warning Systems Network. ETHIOPIA Food Security Outlook.* 2018.
207. Sufian S and L.R. Pittwell, *Iron content of teff (Eragrostis abyssinica). .* 1968.
208. Ketema, S., *Phenotypic variations in Tef (Eragrostis tef) germplasm. morphological and agronomic traits. a catalog.* 1993.
209. Addi, H., Y.T. Reddy, and N. Gandla, *Study of prevalence of protein energy malnourishment in children of 1 to 5 years age* European Journal of Molecular & Clinical Medicine, 2022. **09**(08): p. 178-183.
210. Yue, T., *et al*., *Global Burden of Nutritional Deficiencies among Children under 5 Years of Age from 2010 to 2019.* Nutrients, 2022. **14**(13).
211. Hodge, J., *Food fortification: A "techno-fix" or a sustainable solution to fight hidden hunger. The Future of Global Relations,* in *The Future of Global Relations*, B. Dederichs-Bain, W. Senior Policy Advisor, and W.-C. Ramm, Editors. 2014. p. 1-68.

212. Shim, J.O., *et al*., *Effect of nutritional supplement formula on catch-up growth in young children with nonorganic faltering growth: a prospective multicenter study.* Nutr Res Pract, 2020. **14**(3): p. 230-241.
213. Alderman, H., *et al*., *Evidence of Impact of Interventions on Growth and Development during Early and Middle Childhood*. 2017, Washington (DC): Child and Adolescent Health and Development.
214. Yirga, A.A., *et al*., *Factors affecting child malnutrition in Ethiopia.* Afr Health Sci, 2019. **19**(2): p. 1897-1909.
215. Gibson, R., *et al*., *Dietary strategies to combat deficiencies of iron, zinc, and vitamin A in developing countries: development, implementation, monitoring, and evaluation.* Food and Nutrition Bulletin, 2000. **21**(2): p. 219-231.
216. Webb, P., *et al*., *Modelling the potential cost-effectiveness of food-based programs to reduce malnutrition.* Glob Food Sec, 2021.
217. Desalegn, B.B., K. Abegaz, and E. Kinfe, *Effect of blending ratio and processing technique on physicochemical composition, functional properties and sensory acceptability of quality protein maize (QPM) based complementary food.* International Journal of Food Science and Nutrition Engineering, 2015. **5**(3): p. 121-129.
218. Walle, H. and D. Moges, *Optimization of cereal-legume blend ratio to enhance the nutritional quality and functional property of complementary food.* Ethiopian Journal of Science and Technology, 2017. **10**(2): p. 109-122.
219. Dewey, K., *PAHO/WHO: Guiding principles for complementary feeding of the breastfed child. Division of Helath Promotin and Protection. Food and Nutrition program.* 2001.
220. Adesanmi, A.R., S.A. Malomo, and T.N. Fagbemi, *Nutritional quality of formulated complementary diet from defatted almond seed, yellow maize and quality protein maize flours.* Food Production, Processing and Nutrition, 2020. **2**: p. 1-12.
221. Munshi, R., A. Kochhar, and A. Kaur, *Nutrient selection and optimization to formulate a nutrient bar stable on storage and specific to women at risk of osteoporosis.* J Food Sci Technol, 2020. **57**(8): p. 3099-3107.
222. Agza, B., R. Bekele, and L. Shiferaw, *Quinoa (Chenopodium quinoa, Wild.): As a potential ingredient of injera in Ethiopia.* Journal of Cereal Science, 2018. **82**: p. 170-174.
223. Campoy, C., *et al*., *Omega 3 fatty acids on child growth, visual acuity and neurodevelopment.* Br J Nutr, 2012. **2012**(107).
224. Woldemariam, F., *et al*., *Optimization of amaranthsâ€“teffâ€“barley flour blending ratios for better nutritional and sensory acceptability of injera.* Cogent Food & Agriculture, 2019. **5**(1): p. 1565079.
225. Tadesse, A.Y., *et al*., *Nutritional and sensory quality of complementary foods developed from bulla, pumpkin and germinated amaranth flours.* Nutrition & Food Science, 2018. **49**(3): p. 418-431.
226. Amadou, I., M.E. Gounga, and G.-W. Le, *Millets: Nutritional composition, some health benefits and processing-A review*. Emirates Journal of Food and Agriculture, 2013. **25**(7): p. 501-508.
227. Talabi, J., S. Makanjuola, and E. Egbagbara, *A mixture design approach to developing a cereal-based complementary meal for better nutritional quality.* African Journal of Food, Agriculture, Nutrition and Development, 2021. **21**(9): p. 18748-18766.

228. Keyata, E.O., *et al*., *Optimization of nutritional and sensory qualities of complementary foods prepared from sorghum, soybean, karkade and premix in Benishangul-Gumuz region, Ethiopia.* Heliyon, 2021. **7**(9): p. e07955.
229. Tenagashaw, M.W., *et al*., *Nutrient density of complementary foods formulated from a blend of teff, soybean and orange-fleshed sweet potato.* International Journal of Food Science and Nutrition Engineering, 2017. **7**(4): p. 61-69.
230. Abebe, Y., *et al*., *Nutritive value and sensory acceptability of corn-and kocho-based foods supplemented with legumes for infant feeding in Southern Ethiopia.* African Journal of Food, Agriculture, Nutrition and Development, 2006. **6**(1): p. 1-19.
231. Bernard, T., *et al*., *Nutritive value, functional and sensory attributes of weaning foods formulated from egg white, fermented maize, pawpaw and beans.* American Journal of Food Science Nutrition Research, 2021. **3**(3): p. 22-30.
232. Fahmida, U., *et al*., *Complementary feeding recommendations based on locally available foods in Indonesia.* Food Nutr Bull, 2014. **35**(4 Suppl): p. S174-9.
233. Badham, J., M.B. Zimmermann, and K. Kraemer, *The guidebook nutritional anemia*. 2007: Task Force Sight and Life, Basel, Switetzerland.
234. McLean, E., *et al*., *Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005.* Public Health Nutr, 2009. **12**(4): p. 444-54.
235. Onyango, A.W., *et al*., *Complementary feeding and attained linear growth among 6-23-month-old children.* Public Health Nutr, 2014. **17**(9): p. 1975-83.
236. Hallberg, L. and L. Rossander, *Improvement of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid, and ferrous sulphate on iron absorption from a simple Latin American-type of meal.* Am J Clin Nutr, 1984. **39**(4): p. 577-83.
237. Hotz, C. and B. McClafferty, *From harvest to health: challenges for developing biofortified staple foods and determining their impact on micronutrient status.* Food Nutr Bull, 2007. **28**(28): p. S271-S279.
238. Geda, N.R., *et al*., *Infant and young child feeding practices in Ethiopia: analysis of socioeconomic disparities based on nationally representative data.* Arch Public Health, 2021. **79**(1): p. 021-00555.
239. Monanu, M.O., E.A. Anyalogbu, and E.N. Onyeike, *Effect of Maceration Time on Micronutrient Concentrations of Canarium schweinfurthii Pulp Flour.* Advances in Research, 2014. **2**(11): p. 605-617.
240. Michaelsen, K.F., *Complementary Feeding of Young Children in Developing Countries: a Review of Current Scientific Knowledge: edited by Kenneth Brown, Kathryn Dewey, and Lindsay Allen, 1998, 178 pages, softcover. World Health Organization, Geneva*. 2000, Oxford University Press.
241. Ogbonnaya, J.A., *et al*., *Energy, iron and zinc densities of commonly consumed traditional complementary foods in Nigeria.* British Journal of Applied Science & Technology, 2012. **2**(1): p. 48-57
242. Valenzuela, C., *et al*., *Total iron and heme iron content and their distribution in beef meat and viscera.* Biol Trace Elem Res, 2009. **132**: p. 103-111.
243. Tura, D.C., *et al*., *Optimization of dabi teff-field pea based energy and protein dense novel complementary food with improved sensory acceptability using D-optimal mixture design.* Heliyon, 2023. **9**(8): p. e19029.

244. Alaunyte, I., *et al*., *Improving the quality of nutrient-rich Teff (Eragrostis tef) breads by combination of enzymes in straight dough and sourdough breadmaking.* Journal of Cereal Science, 2012. **55**(1): p. 22-30.
245. Magallanes-LApez, A.M., *et al*., *Variability in iron, zinc and phytic acid content in a worldwide collection of commercial durum wheat cultivars and the effect of reduced irrigation on these traits.* Food Chem, 2017. **237**: p. 499-505.
246. Miller, L.V., K.M. Hambidge, and N.F. Krebs, *Zinc Absorption Is Not Related to Dietary Phytate Intake in Infants and Young Children Based on Modeling Combined Data from Multiple Studies.* J Nutr, 2015. **145**(8): p. 1763-9.
247. Hemalatha, S., K. Platel, and K. Srinivasan, *Zinc and iron contents and their bioaccessibility in cereals and pulses consumed in India.* Food chemistry, 2007. **102**(4): p. 1328-1336.
248. Gibson, R.S., L. Perlas, and C. Hotz, *Improving the bioavailability of nutrients in plant foods at the household level.* Proc Nutr Soc, 2006. **65**(2): p. 160-8.
249. Samuel, T.M., *et al*., *A narrative review of childhood picky eating and its relationship to food intakes, nutritional status, and growth.* Nutrients, MDPI, 2018. **10**(12): p. 1992.
250. Karakochuk, C., *et al*., *Treatment of moderate acute malnutrition with ready-to-use supplementary food results in higher overall recovery rates compared with a corn-soya blend in children in southern Ethiopia: an operations research trial.* The American journal of clinical nutrition, 2012. **96**(4): p. 911-916.
251. Fikiru, O., *et al*., *Nutritional quality and sensory acceptability of complementary food blended from maize (Zea mays), roasted pea (Pisum sativum), and malted barley (Hordium vulgare).* Food Sci Nutr, 2016. **5**(2): p. 173-181.
252. Bolton, L., *Animal sourced foods (ASF): evidence on stunting and programmes to increase consumption.Institute of Development Studies.* 2019.
253. FAO, *Livestock Technical Guidance to Harness the Potential of Livestock for Improved Nutrition of Vulnerable Populations in Programme Planning. Nutrition FAO: Rome, Italy.* 2020.
254. Soetan, K. and O. Oyewole, *The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review.* African Journal of Food Science, 2009. **3**(9): p. 223-232.
255. Etcheverry, P., *et al*., *Effect of beef and soy proteins on the absorption of non-heme iron and inorganic zinc in children.* Journal of the American College of Nutrition, 2006. **25**(1): p. 34-40.
256. Hallberg, L. and L. Hulthan, *Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron.* The American journal of clinical nutrition, 2000. **71**(5): p. 1147-1160.
257. Michaelsen, K.F. and F.R. Greer, *Protein needs early in life and long-term health.* Am J Clin Nutr, 2014. **99**(3): p. 718S-22S.
258. Skau, J.K., *et al*., *The use of linear programming to determine whether a formulated complementary food product can ensure adequate nutrients for 6- to 11-month-old Cambodian infants.* Am J Clin Nutr, 2014. **99**(1): p. 130-138.
259. Helath-Canada, *Nutrition for healthy term infants: recommendations from birth to six months.* Can J Diet Pract Res, 2012. **73**(4): p. 204.
260. Qasem, W. and J. Friel, *The Recommended First Complementary Foods: A Review of the Literature.* J Pediatr Neonatal Care, 2015. **2**(2): p. 00069.

261. Arsenault, J.E. and K.H. Brown, *Zinc intake of US preschool children exceeds new dietary reference intakes.* Am J Clin Nutr, 2003. **78**(5): p. 1011-7.
262. Forsido, S.F., *et al*., *Complementary feeding practices, dietary diversity, and nutrient composition of complementary foods of children 6-24â€‰months old in Jimma Zone, Southwest Ethiopia.* J Health Popul Nutr, 2019. **38**(1): p. 019-0172.
263. WHO, G., *Global strategy for infant and young child feeding.* 2003.
264. Rolfes, S.R., K. Pinna, and E. Whitney, *Understanding normal and clinical nutrition*. 2008: Cengage learning.
265. WHO, *Proceedings of the WHO UNICEF WFP UNHCR informal consultation on the management of moderate malnutrition in under-5 children. Geneva, Oct. 2008.* Food Nutr Bull, 2009. **30**: p. S464-74.
266. Huffman, S.L. and D. Schofield, *Consequences of malnutrition in early life and strategies to improve maternal and child diets through targeted fortified products.* Matern Child Nutr, 2011. **3**(Suppl 3): p. 1-4.
267. ILSI, *Preventing Micronutrient Malnutrition: A Guide to Food-based Approaches: why Policy Makers Should Give Priority to Food-based Strategies*. 1997: ILSI Press.
268. Wolmarans, P., E. Kunneke, and R. Laubscher, *Use of the South African Food Composition Database System (SAFOODS) and its products in assessing dietary intake data: part II: invited review.* South African Journal of Clinical Nutrition, 2009. **22**(2): p. 59-67.
269. Diress, G. and M.L. Endalifer, *Effect of alcohol consumption on haemoglobin level among non-pregnant reproductive age women in Ethiopia: a cross-sectional secondary data analysis of the 2016 Ethiopian Demographic Health Survey*. BMJ Open, 2022. **12**(2): p. e046458.
270. Zlotkin, S., *et al*., *Effect of iron fortification on malaria incidence in infants and young children in Ghana: a randomized trial.* Jama, 2013. **310**(9): p. 938-947.
271. Adetola, O.Y., O.O. Onabanjo, and A.H. Stark, *The search for sustainable solutions: Producing a sweet potato based complementary food rich in vitamin A, zinc and iron for infants in developing countries.* Scientific African, 2020. **8**: p. e00363.
272. WHO, *SEA/RC65/17-Key issues and challenges arising out of the Sixty-fifth World HealthAssembly and the 130th and 131st sessions of the WHO Executive Board*. 2012, WHO Regional Office for South-East Asia.
273. Compaore, W.R., *et al*., *Nutritional properties of enriched local complementary flours.* Advance Journal of Food Science and Technology, 2011. **3**(1): p. 31-39.
274. Ejigui, J., *et al*., *Improvement of the nutritional quality of a traditional complementary porridge made of fermented yellow maize (Zea mays): Effect of maizeâ€“legume combinations and traditional processing methods.* Food and Nutrition Bulletin, 2007. **28**(1): p. 23-34.
275. FNP, *Federal Democratic Republic of Ethiopia. National Food and Nutrition Policy, Addis Ababa, Ethiopia.* 2018.
276. Micronutrients and N. Board, *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. 2002: Dietary Reference Intakes.

Annex I - CURRICULUM VITAE OF THE AUTHOR

1. PERSONAL INFORMATION

Name: **Diriba Chewaka Tura**

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2. QUALIFICATIONS

B.Sc, M.Sc. and PhD fellow:-Lecturer of Food Science and Nutrition, Food Biochemistry, All Nutrition courses

3. EDUCATIONAL BACKGROUND

Instruction	Duration	Place	Qualification
University: PhD fellow	Since 2017	Jimma University	On research work
University: Post Graduate studies	2007-2009	Addis Ababa Un.	M.Sc. in Food Science & Nutrition
University: Higher Education	1997-2000	Addis Ababa Un.	B.Sc. in Chemistry
High School: High School	1993-1996	Lalo Aira Sec.School	ESLCE Certificate

4. PUBLICATIONS IN PEER-REVIEWED JOURNALS

Tura, D.C., Belachew, T., Tamiru, D. and Abate, K. H. (2024). Energy, protein and iron densities of *dabi teff*-field pea-based optimised novel complementary flour and its contribution to daily energy and nutrients demand by 6–23-month-old children. *Bri. J. of Nutri. Cambridge University Press*. doi:10.1017/S0007114523002581

Tura, D.C., Belachew, T., Tamiru, D. and Abate, K. H. (2023). Optimization of a formula to develop iron-dense novel composite complementary flour with a reduced phytate/minerals molar ratio from *dabi teff*-field pea-based blends using a D-optimal mixture design. *Frontiers in Nutrition Journal.* Doi:10.3389/fnut.2023.1244571

Tura, D.C., Belachew, T., Tamiru, D. and Abate, K. H. (2023). Effects of blending ratios variation on micronutrient compositions and phytate/ minerals molar ratios of *dabi teff*-field pea based novel composite complementary flours. *Journal of Food Production, Processing and Nutrition.* Springer Nature. https://doi.org/10.1186/s43014-023-00203-7

Tura, D.C., Belachew, T., Tamiru, D. and Abate, K. H. (2023). Effects of blending ratio variation on macronutrient compositions and sensory acceptability of *dabi teff*-field pea-based novel composite complementary flours. *Food Sci and Nutri J.* Wiley. DOI: 10.1002/fsn3.3925

Tura, D.C., Belachew, T., Tamiru, D. and Abate, K. H. (2023). Optimization of *dabi teff*-field pea-based energy and protein-dense novel complementary food with improved sensory acceptability using D-optimal mixture design. Heliyon. Elsevier https://doi.org/10.1016/j.heliyon.2023.e19029

Chewaka, D., Tadesse,. M. (2017). Effect of Processing on Anti-nutritional Factors and Sensory Qualities of 'Hepho', a Black Climbing Bean (*Lablab purpureus* L.) Flour. *Journal of Food Science and Quality Management:* Vol.60: 22-27

Tadesse,. M., **Chewaka, D**. (2017). Effect of Processing on Proximate Composition and Mineral content of "hepho" a Black Climbing bean (*Lablab purpureus L.*) flour. *Journal of Food and Nutrition Sciences:* **5**(1):16-22

Chewaka, D., Abate, D., Urga, K. (2014). Growth Inhibition of Grain Spoilage Fungi by Selected Herbs and Spices Essential Oils. *Science, Technology and Arts Research Journal Sci. Technol. Arts Res. J.* PP: 135-140

Chewaka, D. (2013). Antifungal Efficacy of Herb and Spice Essential Oils. Lambert academic Publishing

Manuscripts - under review

Nutritional enrichment of traditional complementary foods using underutilized nutritious plant foods in sub-Saharan Africa: their nutritional potential and health benefit: A scoping review.

Uncovering the Traditional Usage, Social Beliefs and Cultivation Trends of *Dabi Teff* (*Eragrostis teff*), A Typical Farmer Variety Teff Grown in Western Ethiopia: Ethnographic Qualitative Study.

Manuscripts – Under preparation

Adherence to Iron/Folate Supplementation and Associated Factors among Pregnant Women Attending Antenatal Care in Gidami District Health Centers, Wollega, Ethiopia.

Complementary Feeding Practices and Associated Factors among mothers of children aged 6 months to 2 Years in Nole Kaba Woreda, Western Wollega Zone, Ethiopia

Assessment of nutritional outcome of ready-to-use therapeutic food and associated factors among HIV/AIDS adult clients in Nekemte Referral Hospital, Nekemte, west Oromia, Ethiopia

Nutritional status and major determinants of malnutrition among children under five years of age in Horo district, Horo Guduru Wollega Zone of Oromia regional state, Ethiopia.

Assessment of nutritional status and its associated factors among pregnant women attending Antenatal Care in Nekemte town, East Wollega, Ethiopia

Workshop attended

Research paper presenter on 4th International Research Conference, Bule Hora University, May 25, 2024.

5. SHORT TERM TRAINING AND CERTIFICATES

- ✓ ***Certificate of presentation*** *on the 4th international research conference on Indegnous Knowlwgw for Innovation and Societal Transformation hosted by Bule Hora University.*
- ✓ ***Certificate of excellence*** *in reviewing an article from the European Journal of Nutrition and Food Safety, in recognition of an outstanding contribution for the quality of the journal.*
- ✓ ***Certificate:*** *'Research Project Grant Writing' by NORAD through NORHED project from Stavanger University, Norway.*
- ✓ ***Certificate:*** *Manuscript preparation and Publication for postgraduate students at Wollega University.*
- ✓ ***Certificate:*** *Local Economic Development: towards local agribusiness cluster development by Center for Development and Innovation (CDI), Ede, The Netherlands.*
- ✓ ***Certificate:*** *Governance and Food Safety in International Food Chains by Center for Development and Innovation (CDI), Ede, The Netherlands*
- ✓ ***Certificate:*** *"Quality Management System (ISO 9001:2000), Food Safety Management System (ISO 22000:2005, HACCP,GHP and GMP) and system maintenance at quality Inspection, Testing and Certification Services P.L.C (QITC)"*
- ✓ ***Certificate:*** *Statistical Process Control at Quality and Authority of Standard of Ethiopia.*
- ✓ ***Certificate:*** *National symposium on Science, Technology and Innovation for national development (Chairperson)*
- ✓ ***Certificate:*** *National Symposium on Trends and Challenges in Adoption of Science, Technology and Innovation in Local Development Endeavors (Rapporteur).*
- ✓ ***Certificate:*** *The Higher Diploma Program (HDP)*

6. NARRATION OF PERSONAL GENERAL PROFILE

Mr. Diriba Chewaka Tura was born in Ethiopia, Wollega. He has achieved a BSc degree in chemistry from Addis Ababa University leading onto MSc degree in the same University and hold masters of science in Food Science and Nutrition. Mr. Diriba has joined Wollega University, Ethiopia as a Lecturer and Researcher. He is currently a PhD fellow at Jimma University under department of Nutrition and Dietetics, Institute of Health, Jimma, Ethiopia. Now he is doing his PhD project entitled "Development of Nutrients Dense Optimized Novel Complementary Food from Locally Available Cereal-legume Blends in Combination with *Dabi teff (Eragrostis teff)* for Infants and Young Child Feeding in Ethiopia." Diriba has more than twelve high-impact articles published in peer-reviewed journals and one book entitled "Antifungal Efficacy of Herb and Spice Essential Oils", he has attended more than ten international and national scientific conferences and training and coached (advised) more than fifteen master's thesis papers in the area of Nutrition. He is a member of the Food and Nutrition Society of Ethiopia (FoNSE).

Annex II-Ethnographic Qualitative Data Collection Sheet

Focus group discussion

Informed consent for of the FGD discussant for the study entitled "Uncovering the Traditional Usage, Social Beliefs and Cultivation Trends of *Dabi Teff* (*Eragrostis teff*), A Typical Farmer Variety Teff Grown in Western Ethiopia, Nedjo district: Ethnographic Qualitative Study."

FGD discussants: Male and female elders (the grandmothers/fathers) born and lived in the Nedjo area.

Investigators: Mr. Diriba Chewaka, Prof. Kalkidan Hassen and Prof. Tefera Belachew

Part I – Information sheet

Introduction: Good morning/afternoon! My name is _____. We represent the research team from Jimma University. We are conducting FGD discussions with *male and female elders* who were selected purposively or nominated and are believed to have a lived experience related to *dabi teff* and speak the local language.

Purpose of the research

The purpose of the study is to explore the traditional usage, social beliefs and cultivation trends of *dabi teff* (*Eragrostis teff*), a typical farmer variety teff grown in Western Ethiopia. The findings of the study will be used to generate the preparation and consumption pattern of *dabi teff* as well as the rooted social belief linked to *dabi teff* in the community including the practice of using the crop as a complementary food.

Risks and discomfort

The discussion may take some of your time. However, we will try to make it short and to the point and guide you through the FGD guide question. There are no right or wrong answers to the focus guide questions. We want to hear many different viewpoints and would like to hear from everyone. We hope you can be honest even when your responses may not be in agreement with the rest of the group.

Benefits There will be no direct benefit to you, but based on the findings, we will design a wider research (full project) and this is to get prehand information regarding *dabi teff* usage.

Confidentiality: In respect for each other, we ask that only one individual speak at a time in the group. Confidentiality will be maintained and information from the discussion will be used for the purpose of the study only.

Right to refuse or withdraw

You can choose whether or not to participate in the focus group and stop at any time. Although the focus group will be tape-recorded, your responses will remain anonymous and no names will be mentioned in the report. However, your active participation and genuine responses have paramount importance in gathering pertinent information regarding *dabi teff*.

I understand this information and agree to participate fully under the conditions stated above:

Signature/fingerprint of the participant

Signature/fingerprint _____ date _____

Respondents background

Age _____, Educational status _____

Key Question guide for Ethnography focus group discussion (FGD) regarding *dabi teff*.

Semi-structured questionnaire	Expected outcome
1. What are the preparation and consumption schemes of *dabi teff* (traditional usages)?	The different food forms that can be prepared from *dabi teff* and the eating pattern.
2. What are the nutritional and health benefits associated with *dabi teff*?	Knowledge of the role of healthy foods in nutritional treatment.
3. What are some social beliefs or claims linked to *dabi teff*	To generate social beliefs or claims rooted in the community regarding *dabi teff*.
4. How is the cultivation trend of *dabi teff* in the area?	The pattern of cultivation trend from the past to current.
5. In what duration does *dabi teff* reach for harvest?	The number of days *dabi teff* reaches for harvest. Its role to ensure food security in harsh times.
6. What type of crops do you use for the preparation of complementary food in this area?	Identifying the locally available crops for complementary food preparation.
7. Do you use *dabi teff* for the preparation of complementary food?	The practice of using *dabi teff* as complementary food
8. Other than its seeds, is there any purpose of cultivating *dabi teff*.	Multi-purpose of *dabi teff*.
9. How is the trashing and cleaning system of *dabi teff* seeds?	Understand the possible contamination with soil.
10. For how long can you store *dabi teff* without damage?	Storage quality without deterioration
11. Any other benefits of *dabi teff* cultivation?	Other contributions of *dabi teff* cultivation beyond food purpose

We have completed our discussion. Thank you very much!

Annex III- Sensory Evaluation Format

5-point Hedonic Test

You are provided with twelve (12) coded samples of complementary porridge on the plate.

Instructions:

Please grade the samples according to the scale provided below by filling in the table against each sample. Rinse your mouth with clean water before and after tasting each food sample.

5. Like very much
4. Like moderately
3. Neither like nor dislike
2. Dislike moderately
1. Dislike very much

Sample Code	Color	Aroma	Taste	Mouth-feel	Overall Acceptability
759					
308					
194					
707					
258					
206					
342					
608					
304					
437					
821					
976					

Any other comments you have? _____

Signature _____

Thank you for your participation!!!

Annex IV: Ethical Approval

JIMMA UNIVERSITY
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Ref.No JHRPGD/395/2019
ቀን
Date 16/09/2019

Institutional Review Board (IRB)
Institute of Health
Jimma University
Tel : +251471120945
E-mail : zeleke.mekonnen@ju.edu.et

To: Mr. Dirriba Chewaka

Subject: Ethical approval of research protocol

The IRB of institute of health has reviewed your research project entitled:

"Title: Effect of Dabi Teff blended optimal child food formulation and nutrition education on nutritional and iron status of infant and young children (6-59 months) in Western Wollega Zone, Oromia Region, Ethiopia"

This is to notify that this research protocol as presented to the IRB meets the ethical and scientific standards outlined in national and international guidelines. Hence, we are pleased to inform you that your protocol is ethically cleared.

We strongly recommended that any significant deviation from the methodological details indicated in the approved protocol must be communicated to the IRB before they are implemented.

With regards!

Zeleke Mekonnen (PhD)
Associate Professor, Health
Research and Postgraduate
Director

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Annex V- Preliminary key micronutrient analysis results of *Dabi teff*

JIJE LABOGLASS Pvt. Limited Company	JIJE Analytical *Testing* Service Laboratory	Doc. No: JATSL/F5.10-3	Page 1 of 1	
			Ver. No: 04	Effective Date: July 08, 2017
	Analytical Test Report			
Customer Name	Diriba Cheweka	Test Report No	037-1	
Contact Person:	Diriba Cheweka	Reported date:	01/02/2019	
Sample Type:	Food (dabi-teff)	Test Request No:	Not Specified	
Sample Source:	Wollega	Tel:	0911714320	
Sample collected by:	Diriba Cheweka	Fax:	Not Specified	
Sample Collected Date:	15/12/2018	E-mail:	senyidd@gmail.com	
Sample Received Date:	01/01/2019	Tested by:	LA-09	
Sample Condition	Normal	Date tested:	24/01/2019 -30/01/2019	

S/N	Lab No	Customer ID	Unit (mg/100gm)		
			Ca	Fe	Zn
1	J-0225/0101/19		190.00	122.66	1.80

S/N	Parameters	Test Methods
1	Calcium (Ca)	Modified AOAC Official Method 985.35-EDTA Titrimetric Method
2	Iron (Fe)	Modified AOAC Official Method 985.35-Flame AAS
3	Zinc (Zn)	Modified AOAC Official Method 985.35-Flame AAS

Remark:

1. This test report is only for specific sample(s) which has been tested by JIJE Analytical *Testing* Service Laboratory.
2. This test report replaces a test report number 037 which is issued on 31/01/2019.

Verified by

Name	Signature	Date
Gemechu Sorsa	.....	01/02/19 .....

Technical Signatory

Authorized by

Name	Signature	Date
Mulugeta Terefe	.....	..... 01/02/19

Laboratory Manager

Tel : +251-11-835 30 65/66, 37207 01/ 02
Fa x : +251-011-3720700
P.O. Box: 70077

E-mail: jije@ethionet.et
info@jijelaboglassplc.com
Web site: www.jijelaboglassplc.com

Physical Address: Nifas Silk Lafto Sub City, Kebele 01, House Number New, Lebu, 200 m off the main road by the side of Abtam Center, Addis Ababa, Ethiopia.

Annex VI- Declaration

I, the undersigned, do hereby declare that:

(a) This **PhD Dissertation work** is the bona fide record of my original research work written by me.

(b) No part of the dissertation work has ever been presented in this or any other university at any time for the award of any higher degree.

(c) All quotations, references and materials used have been duly acknowledged.

Name: **Diriba Chewaka Tura**,
Signature: _____
Date: _____
Place: Jimma University, School of Graduate Studies
Date of submission: _____

This dissertation has been submitted for examination with my approval as supervisor.

Candidate's First Supervisor
Name: Professor Kalkidan Hassen Abatew
Signature: _____
Date: _____
Candidate's Second Supervisors
Name: Professor Tefera Belachew
Signature: _____
Date: _____

Name: Doctor Dessalegn Tamiru
Signature: _____
Date: _____