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Mineral, Total Carotenoids and Anti-Nutritional Contents of Complementary Food Blended from Maize, Roasted Pea and Malted Barley

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Abstract: The effect of maize, malted barley and roasted pea blending ratio on mineral, total carotenoids and anti-nutritional contents of complementary foods were evaluated. Malted barley was used as a source of amylases, roasted pea to increase protein contents and carrot (6.56g/100g) to supply pro-vitamin A carotenoids. D- Optimal mixture design was used to generate 14 formulations within constrains: maize 55-90%, pea 20-35% and malted barley 4-12 %. Iron, Zinc, Calcium and Phosphorus contents were ranged: 4.3-5.5, 2.4-3.0, 70.0-91.0, 42.0- 68.1 mg/100g, respectively, whereas total carotenoids content was ranged from 1236-2464 µg/100g. Lack of fit was significant ($p< 0.05$) for zinc, phytic acid and tannin (R^2= 98.19, 98.31 and 92.35). This shows the model generated can predict all other attributes except for zinc, phytic acid and condensed tannin contents. The optimum nutrient quality was found in the blending range of maize 55.0-68.5%, pea 27.5-35.0% and malt barley 4-10%.

Key words: Complementary Food · Maize · Malted Barley · Roasted Pea · Minerals · Anti-Nutritional Factors

INTRODUCTION

Although breast milk is adequate to meet the energy and nutrient requirements of an infant up to six months, thereafter, it is insufficient to sustain the normal growth and needs other complementary foods [1]. Complementary foods are any nutrients containing foods or liquids other than breast milk given to young children during the period of six to 24 months [1, 2]. The capacity of a weaning food to meet the protein-energy requirements depends on its nutritional quality and dietary bulkiness of the meal [3]. The bulkiness of most traditional complementary foods, their fiber and inhibitor contents are the major factors that reduce the nutritional benefits of weaning child [4, 5].

Inadequate and poor quality complementary feeding is a major cause for the high incidence of child malnutrition, morbidity and mortality in many developing countries [6]. The interaction of poverty and poor quality of complementary feeding practices are significantly affecting the developmental potential of children [7, 8]. Due to the high cost of factory processed complementary foods and poor income, most families from poor countries feed their children porridges of low energy and protein contents per unit volume processed from cereal grains and starchy roots and/or tubers [4]. This significantly contributes to growth retardation, poor cognitive development, illness and death of children [3, 7].

Micronutrients (Iron, Zinc and vitamin A) deficiencies are common in children from economically poor populations consuming diets based primarily on cereal and legume foods [9]. Public health interventions, such as supplementation or fortification of food, often target a single micronutrient deficiency, for example, Iron or vitamin A and this can jeopardize the deficiencies in other micronutrients [8]. Thus, it is important to formulate foods that contain all of these micronutrients sufficiently.

Anti-nutritional factors have negative impact on solubility or digestibility of nutrients and thereby reduce the nutrient bioavailability [10]. Of these, phytates and

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tannins can be deleterious in the complementary foods if not suppressed by processing. Many processing methods are investigated to reduce the level of anti-nutritional factors in food materials. Malting and fermentation of cereals [11] and germination, roasting, cooking and fermentation of legumes have been shown to be advantageous as it improves their nutritional qualities [12, 13]. Germination and fermentation are known to increase endogenous phytase enzyme activities, which leads to phytates degradations [12, 14]. Such practice also reduces the dietary bulkiness and the paste viscosity leading to energy dense products of the gruel because of enhanced amylase enzyme activities that will act on starches paste viscosity. Condensed tannins, which can lead to poor mineral and protein absorptions, are also suppressed with cereal decortication, legume seed coat removal, soaking and fermentation practices.

In Ethiopia, in 2014 among children under age of five, 40% are stunted, 9% are wasted and 25% are underweight [15]. These are above the global level of 24% stunted, 7.5% wasted and 14% underweight for the same year [16]. Limitation of weaning foods for complementary feeding is one of the major causes for such high prevalence of child malnutrition and growth faltering in Ethiopia [17, 18]. For example, the Ethiopian Demographic Health Survey in 2011 shows among children of 6-9 months old, only 51% received complementary feeding [17]. Complementary foods consumed by majority of Ethiopian infants and young children are based on cereal grains and/or root/tuber starchy products and are known to be deficient in energy, vitamins and minerals such as vitamin A, zinc and calcium [19]. With appropriate processing that can target anti-nutritional factors reduction, complementary foods of energy dense and improved nutrients can be formulated by complementing unexploited legumes, cereals and vegetables. Such composite can serve as a potential source of important micronutrients like Ca, Fe and Zn especially for rural communities of developing countries [3, 20]. In the previous work, the sensory acceptability of gruels, proximate nutrient and energy contents of 14 complementary foods formulated in the constrained region of maize 55-90%, pea 20-35%, malted barley 4-12 % and constant level of carrot (6.56g/100 g) blending were reported [21]. In the work, it was found the range of 55.0-68.5% Maize, 27.5-35.0% Pea and 4-10% Malt barley blending were desirable in terms of nutrient contents (5.0-5.9% moisture, 15.0-17.5% protein, 3.2-4.1% fiber and 68.9-72.4% carbohydrate) and gruel over all sensory acceptability (3.9-4.5 on five point hedonic scale). In this work, the effects of the same ingredients blending ratio that optimizes high mineral and total carotenoids and low anti-nutritional contents of the 14 complementary foods processed are studied.

MATERIALS AND METHODS

The experiment was conducted at Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM), Postharvest Laboratory in 2012, which is located at southwestern part of Ethiopia on 345 km away from Addis Ababa. Samples were collected from three different organizations. Maize (*Zea mays* var., BH660) (50 kg) was taken from Ethiopian seed enterprise Nekemte Branch and Barley (*Hordium vulgare* var., Miscal 21) (20 kg) and pea (*Pisum Sativum* var., adi) (30 kg) were taken from Holota Agricultural Research Institute, Ethiopia; carrot was obtained from Ambo University farm land.

Samples Preparation: Maize, Pea, Malt barley and carrot were processed into their respective flours prior to conducting the experiments as previously reported [21].

Experimental Design and Treatment Combinations: The experimental design was generated using D-Optimal mixture design [22]. A mixture design is appropriate when the response depends on the component proportions of the mixture and not on the component quantities. Accordingly, 14 samples as treatment combinations were generated (Table-1 & 2) within the constraints of: maize 55-90%, pea 20-35%, malted barley 4-12% and constant carrot 6.56 mg/100 g. The carrot powder was supplemented at constant level to supply pro-vitamin A carotenoids, minerals and B-vitamins [23].

Table 1: Ratio of fourteen formulations of the flour

Number of formula	Maize (%)	Pea (%)	Barely (%)
1	67.5	24.3	8.2
2	55.0	35.0	10.0
3	61.0	35.0	4.0
4	61.0	35.0	4.0
5	76.0	20.0	4.0
6	68.0	20.0	12.0
7	68.5	27.5	4.0
8	59.0	31.3	9.0
9	63.0	28.6	8.4
10	76.0	20.0	4.0
11	72.0	20.0	8.0
12	61.0	26.5	12.0
13	55.0	35.0	10.0
14	68.0	20.0	12.0

Table 2: Constraint regions of the fourteen formulations of the flour

Low	= Constraint	= High
0.55	=A: Maize	= 0.95
0.20	=B: Pea	= 0.35
0.04	=C: Barely	= 0.12

A+B+C= 1

Determination of Mineral Nutrient Contents: Iron, calcium and zinc contents were analyzed by Atomic Absorption Spectrophotometer (Perkin–Elmer, Model 3100, USA) after dry digestion of about 5.0 g bread samples using air-acetylene as a source of energy for atomization [24]. For iron, absorbance was measured at 248.3nm and iron content was estimated from a standard calibration curve (2-20µg Fe/ mL) prepared from analytical grade iron wire. For zinc determination, absorbance was measured at 213.8nm and zinc content was estimated from a standard calibration curve (0.5-5.0µg Zn/ mL) prepared from zinc metal. For calcium determination, absorbance was measured at 422.7nm after addition of 1% lanthanum (i.e., 1 mL La solution/5 mL) to sample and standard to suppress interferences. Calcium content was then estimated from standard calibration curve (2- 20 µg Ca/ mL) prepared from $CaCO_3$.

Phosphorus content was determined after digestion of about 15 mg sample and measuring of absorbance of blue colour of phosphomolybdate at 822nm with UV-Vis Spectrophotometer (DU-64 spectrophotometer, Beckman, USA) [25]. Phosphorus level was estimated from a series of standard (0.2-1.2 µg P/ mL) calibration curve prepared from K_2HPO_4.

Total Carotenoids: Stock solutions of Trans α- and â-carotenes were prepared by weighing 50 mg of each in stock solutions of 100 mL of the mobile phase used for HPLC analysis. Standard purity was checked in petroleum ether using Lambert-Beer law. Total carotenoid from homogenized carrot tissues (about 5g) and homogenously blended complementary food samples (about 5 g) were extracted with 30 mL of acetone/ethanol (50:50) solution. The extract was filtered through filter paper (42 µm) in a funnel and washed with acetone/ethanol solvent until the residue left on the filter paper became colorless. Filtrate was adjusted to 100 mL volume with acetone/ethanol. An aliquot of total carotenoid solution was placed in a 1 cm cuvette and absorbance was measured at 470 nm using UV-Vis spectrophotometer. The amount of total carotenoid was calculated as described by Gross [26] method.

$$mg \text{ carotenoid per g sample} = \frac{(\text{AxVx}10^6)}{(A^{1\%}x100xG)}$$

Where:
A = absorbance at 470 nm
V = total volume of solution
G = gram of sample
$A^{1\%}$ = specific extinction coefficient (2,500).

Determination of Anti-Nutritional Factors

Determination of Phytic Acid: Phytates content was determined as described in Vaintraub and Lapteva [27] after extraction of sample (about 0.15 g) with 2.4 % HCl for 1 h., centrifuged (3000 rpm, 30 min) and reacting sample extract (3 mL) with one mL of wade reagent (0.03 % $FeCl_3.6H_2O$ and 0.3% sulphosalicyclic acid in distilled water). The absorbance of sample was measured at 500 nm using UV-Vis Spectrophotometer (DU-64 spectrophotometer, Beckman, USA), subtracted from blank absorbance and the phytate content (mg/ 100 g sample) was estimated from phytic acid standard curve (0-40 µg/ mL).

Condensed Tannin: Condensed tannin content was determined by vanillin HCL method [28] maintaining water bath temperature at 30° C. Sample (about 200mg) was extracted with 10 mL of methanol with vortex mixing (20 min), centrifuged (3000 x g for 10 min), extract (1 mL) reacted with 5mL vanillin-HCl reagent (8% concentrated HCl in methanol and 4% vanillin in methanol, 50:50, v/v) and the absorbance of color developed was measured after 20 min at 500 nm using UV-Vis Spectrophotometer (DU-64 spectrophotometer, Beckman, USA). Catechin calibration curve was used to estimate condensed tannin contents as mg of catechin/100 g of sample.

Statistical Analysis: A D-optimal mixture design that has 14 formulations of three ingredients was analyzed using Design-Expert® 6 (Stat-Ease) to determine the optimum proportion of the ingredients that maximizes the mineral nutrient contents and minimize the anti-nutritional factors. Regression Models and contour plot were obtained using Minitab 16 statistical package. The significance test was set at P<0.05. The fitted models for all parameters were generated in three dimensional response surfaces and contour plots [22]. Graphical optimization was done to determine the optimum formulation point of Maize, Pea, Barley and carrot flours that had produced better micronutrients and reduced anti-nutritional factors. From the overlaid contour plot, sweet point of blending proportion that favored high mineral and low anti-nutrient contents were determined.

RESULTS AND DISCUSSIONS

Micronutrients: Iron, Zinc, Phosphorus and total carotenoid contents in the complimentary food flour analyzed for the 14 treatment samples are presented in Table 3. The predicted models for micronutrients were also indicated in Table 4.

Table 3: Micronutrient composition in 100 g formulations

	Ingredients in a mixture (%)			Mineral and carotenoid contents				
No.	Maize	Pea	Barely	Ca(mg)	Fe(mg)	Zn(mg)	P(mg)	Total carotenoid(µg)
1	67.5	24.3	8.2	81.5	5.2	2.6	48.8	2432.0
2	55.0	35.0	10.0	91.0	5.5	2.9	68.1	2176.0
3	61.0	35.0	4.0	87.4	4.5	2.7	52.2	1632.0
4	61.0	35.0	4.0	87.2	4.7	2.8	52.2	1632.0
5	76.0	20.0	4.0	70.0	4.3	2.3	42.1	2368.0
6	68.0	20.0	12.0	78.4	4.8	2.5	47.3	2272.0
7	68.5	27.5	4.0	82.0	4.6	2.5	44.3	2400.0
8	59.0	31.3	9.7	89.6	5.2	2.9	60.2	2016.0
9	63.0	28.6	8.4	86.5	5.1	2.8	54.0	2464.0
10	76.0	20.0	4.0	70.0	4.3	2.4	42.0	2368.0
11	72.0	20.0	8.0	74.5	4.6	2.5	46.0	2432.0
12	61.5	26.5	12.0	87.3	4.7	2.8	55.4	2080.0
13	55.0	35.0	10.0	91.0	5.3	3.0	68.0	2176.0
14	68.0	20.0	12.0	78.4	4.8	2.5	47.3	2272.0

Table 4: Predicted model for micro-nutrient content analysis

Micronutrient	*Model*	R^2 value
Calcium	$Y=0.9X_1-121.5X_2+68.4X_3+555.6X_1X_2+116.3X_1X_3+365.9X_2X_3$	0.999
Phosphorus	$Y=66.3X_1+306X_2-767.2X_3-491.1X_1X_2+779.7X_1X_3+1511.2X_2X_3$	0.999

Where: X1 = maize, X2=Pea and X3 = Barley

Calcium: The calcium content for the 14 formula of complimentary food samples was found in between range of 70.01 to 91 mg/100 g. The highest calcium content was recorded in 55% maize, 35% pea and 10% malted barley flour (formula No.2). Whereas, the least calcium content was found in 76% maize, 20% roasted pea and 4% malted barley flour (formula No.5) (Table-3). Calcium content increased with increasing of roasted pea and decreased with the increasing of maize in the formulation. Lack of fit was not significantly different for calcium content among the formulations (P>0.0.5, R^2=99.99). Our result is similar with that of Ijarotimi and Keshinro [10] who formulated complimentary food flour from germinated popcorn and Africa locust bean (70:30), germinated popcorn and bambara groundnut flour (70:30) and germinated popcorn, Africa locust bean and bambara groundnut (70:20:10). They reported 175.0, 213.0 and 170.0 mg/100 g of calcium content for germinated popcorn-African locust bean, germinated popcorn-bambara groundnut, germinated popcorn-African locust-bambara groundnut. However, our result is less than that of Raheleh and Reihaneh [29] who reported that calcium contents were 310 mg/100 g for blend of wheat and green gram; 292 mg/100 g for blend of wheat and lentil; 272 mg /100 g for blend of rice and green gram and 265 mg/100 g for the blend of rice and lentil. This may be because of the difference in proportion and type of raw material from which the product was developed. The required daily allowance (RDA) for calcium content in the complimentary foods is 400-425 mg [30]. None of the complimentary food formulated is satisfied this. Thus, increasing of the roasted pea can improve the calcium content of this result.

Iron: Iron content in the present study was found in the range of 4.3-5.5 mg /100 g. The highest iron content was recorded in blend of 55% maize, 35%roasted pea and 10 % malted barley (Formula No. 2) while the least content was recorded in the blend 76% maize, 20% roasted pea and 4% malted barley (Formula No. 5) (Table-3). Lack of fit was significantly different for Iron among the formulations (P<0.0.5, R^2=86.44). The results revealed that an increase in Fe content was observed when there was high percentage of pea in the flour. This may be attributed due to the higher concentration of iron content in the legume than the other cereals [31]. Our result resembles with that of Ghavidel and Davoodi [32] and Ijarotimi and Keshinro [10]. The recommended daily allowance (RDA) of Iron content in complementary food is to be 3.9-5.8 mg/day. This was satisfied in this study.

Zinc: Zinc content for the 14 treatment formulations had ranged from 2.3 to 3 mg/100 g. The highest zinc content was observed in the proportion of 55% maize, 35% roasted pea and 10% malted barley (Formula No. 2) (Table 3). Zinc was increased as the roasted pea increased in the formulations. Lack of fit was significantly different for Zink among the formulations (P<0.0.5, R^2=98.19). According to Dhingra and Jood [33] and Utrilla-Coello *et al.* [31], addition of legumes to cereal products increases mineral contents of complementary foods. The recommended daily allowance (RDA) of Zink content in complementary food is to be from 2.0-3.4 mg/day. Therefore, our result indicated that the formulated food samples would serve as good sources of zinc.

Phosphorus: Phosphorus content for the 14 treatment formulations had ranged from 70.01 to 91 mg/100g. The highest phosphorus content was recorded in 55% of maize, 35% of roasted pea and 10% of malted barley blend (Formula No. 2) (Table-3). Phosphorus content was increased with the increasing of pea in the formulation. Lack of fit was not significantly different for phosphorous content among the formulations (P>0.0.5, R^2=99.99). Ghavidel and Davoodi [32] and Ijarotimi and Keshinro [10] reported similar results. Dhingra and Jood [33] and Utrilla-Coello *et al.* [31] reported that it is possible to improve the phosphorus and other micronutrients contents in the complimentary foods by mixing. The required daily allowance (RDA) for phosphorus content in the complimentary foods was recommended to be 456 mg/100 g [30]. All of the complimentary foods processed in this work did not satisfy this. This can be improved again by increasing the roasted pea content in the flour.

Total Carotenoid: The total carotenoid contents in this study had ranged from 1632-2464 µg/100 g. The highest carotenoid content was observed in 63.0% maize, 28.6% pea, 8.4% malted barley blend (Formula No. 9) and the lowest was in the 61% maize, 35% pea, 4% malted barley blend (Formula No. 3&4). Lack of fit was significantly different for total carotenoids contents among the formulations (P<0.0.5, R^2=80.53). The study shows that addition of vitamin to cereal-legume or cereal-only porridges had a positive effect on other micronutrient and vitamin A status of infants and young children [34]. The RDA for carotenoid content in the complimentary food is 400 µg RE/day [35]. The total carotenoid content found from all processed complimentary foods in this work meets this requirement.

Anti-Nutritional Factors

Phytic Acid: The phytic acid content for the 14 treatment complimentary food samples had observed in between range of 2.41-2.71 mg/100 g. The highest phytic acid content was observed in 61% maize, 35% pea and 4% malted barley flours (Formula No. 3) whereas the least phytic acid content was recorded in 68% maize, 20% pea and 12% malted barley blended rations (Table-5). Phytic acid increased with the increasing of roasted pea and decreased with increasing of maize. Lack of fit was significantly different for phytic acid among the formulations (P<0.5, R^2=92.35). Fasoyiro *et al.* [36] and Ijarotimi and Keshinro [10] reported similar results. According to Gibson and Hotz [9], addition of malted cereals may increase phytase activity, which may reduce phytic acid in the food.

Condensed Tannin: The condensed tannin content for the 14 treatment complimentary food samples had ranged from 2.00-4.52 mg/100 g. The highest condensed tannin content was recorded in the blend of 61% maize, 35% pea and 4% malt barley (Formula No. 4) whereas the least condensed tannin was observed in the blend of 68% maize, 20% pea and 12% malted barley (Formula No. 6) (Table- 5). The raise of condensed tannin was observed with the increasing of pea and decreasing of malted barley blended ration in the flour. Lack of fit was significantly different for condensed tannin content among the formulations (P<0.05, R^2= 98.31). This may be because of the malting and germination of barley that increases endogenous phytase enzyme activity, which may leads to the minerals inhibitor phytate degradation [9, 13, 37]. In addition, the white varieties of pea has very low amount of anti-nutritional factors especially tannin [36].

Table 5: Phytic acid and condensed tannin contents of the complimentary foods per 100 g sample

	Ingredients in a mixture (%)				
No.	Maize	Pea	Barely	PA mg/100g	CT mg/100 g
1	67.50	24.30	8.20	2.59	3.06
2	55.00	35.00	10.00	2.70	3.70
3	61.00	35.00	4.00	2.71	4.50
4	61.00	35.00	4.00	2.69	4.52
5	76.00	20.00	4.00	2.56	3.02
6	68.00	20.00	12.00	2.41	2.00
7	68.50	27.50	4.00	2.65	3.58
8	59.00	31.30	9.70	2.67	3.50
9	63.00	28.60	8.40	2.63	3.45
10	76.00	20.00	4.00	2.56	3.05
11	72.00	20.00	8.00	2.58	2.50
12	61.50	26.50	12.00	2.59	2.79
13	55.00	35.00	10.00	2.68	3.65
14	68.00	20.00	12.00	2.41	2.04

PA = phytic acid CT = condensed tannin

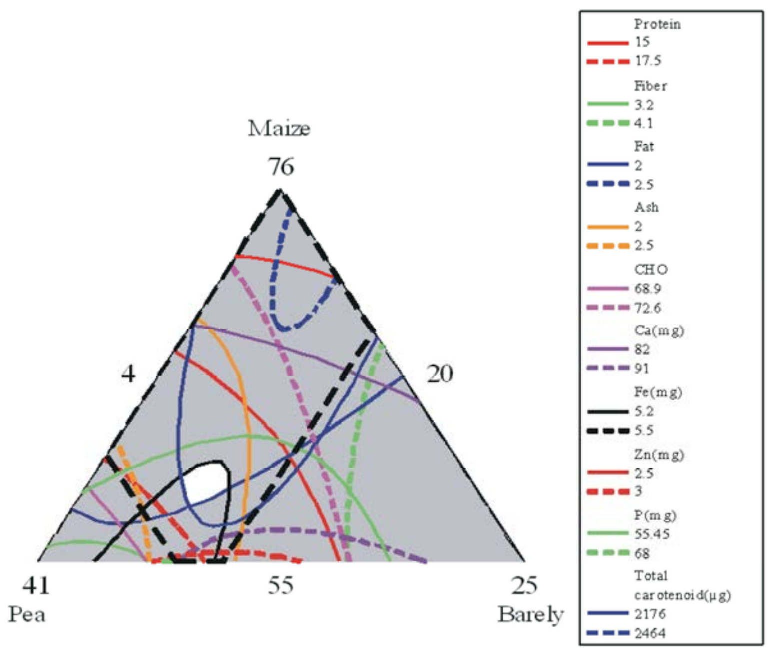

Fig. 1: Overlaid contour plot of protein, fat, fiber, ash, CHO, Ca, Fe, Zn, P and total carotenoid contents

The variety used here is *adi* (white) which might be a variety with low condensed tannin contents. Hot water treatment, soaking, boiling, dehulling and roasting reduces the tannins in legumes and cereal grains [36, 37, 38]. For instance, Fasoyiro *et al.* [36] reported that the tannin content was reduced from 4.60 g/100 g to 2.15 g/100 g in de-hulled seed, from 2.56 g/100 g in roasted seed and from 2 g/100 g in fried seed of pigeon pea.

Generally, the sweet point of mineral, ant-nutritional factors and total carotenoids content of complementary food blended in this study (82-91 mg/ 100g for calcium content, 5.2-5.5 mg/ 100g for Iron content, 2.5-3 mg/ 100g for Zinc, 55.45-68 mg/ 100g for phosphorous and 2176-24.64 µg/100 g for total carotenoid) was found in the flour samples prepared within the range of 55.0–68.5% for maize, 27.5–35.0% for roasted pea and 4–10% for malted barley (Fig. 1).

CONCLUSIONS

In the present study, the effect of blending ratio of maize, malted barley and roasted pea on micronutrient, total carotenoid and anti-nutritional factors of complimentary food was assessed. The flour was prepared by blending different ratio of Maize (55-95%), Malted barley (4-12%), Pea (20-35%) and equal amount of carrot (6.85g). Based on our findings, the micronutrient contents (Calcium, Iron, Zinc and Phosphorous) had found in between range of 70.01-91, 4.3-5.5, 2.3-3 and 70.01-91mg/100g respectively. Iron, Calcium, Zinc and Phosphorus were increased as roasted pea increased in the formulation. The total carotenoid, phytic acid and condensed tannin content were also ranged from 1632-2464 µg/ 100g, 2.41-2.71 and 2-4.52mg/100g. In general, the optimum flour nutrient quality was found to be in the range of Maize (55.0-68.5%), Pea (27.5-35.0%) and Malt barely (4-10%). The model generated in the present study can predict all other attributes except for Zinc, phytic acid and tannin contents.

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