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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE Nutritional and sensory quality of wheat bread supplemented with cassava and soybean flours

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Abstract: Sixteen composite flours were prepared using D-optimal constrained mixture design within a range of wheat 40–80%, cassava 10–30% and soybean 10–30% to optimize bread nutrient quality and sensory acceptability. Results obtained showed blending had a significant (p < 0.05) effect on protein, ash, energy, iron, calcium, phytate, bread loaf volume, taste and odor. Higher quantities of soybean addition increased protein, energy, mineral, phytate and condensed tannin contents, whereas cassava increased total carbohydrate contents. The optimum blending ratio for both nutritional and sensory acceptability was in the range of 49.0–71.0% wheat, 10.6–29.0% cassava and 18.2–22.0% soybean flours. Loaf volume of bread processed from less than 70% wheat flour in this study was observed to be inferior in terms of quality attributes.

Subjects: Bioscience; Food Science & Technology; Physical Sciences

Keywords: cassava; composite bread quality; D-optimal design; optimization; soybean

1. Introduction

Bread is a dietary staple in human nutrition (Dewettinck et al., 2008). Research on bread is globally conducted to improve its nutritional value (macronutrients: carbohydrates, proteins, fat and dietary fibers; micronutrients: minerals and vitamins), health supporting bioactive compounds, sensory

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PUBLIC INTEREST STATEMENT

The aim of the work was to find soybean, cassava and wheat flour blending ratio that will optimize the nutrients and sensory acceptability of composite bread. Soybean and cassava are underutilized in many countries experiencing chronic food shortage and malnutrition. Acceptable bread with improved nutrient contents and soybean bioactives can be processed maintaining wheat flours around 70% and blending the rest with soybean and cassava flours. Such practice can contribute towards minimizing wheat price for countries limited in wheat production. It supports towards soybean and cassava flours value additions and their postharvest loss reductions. It can also contribute towards alleviation of nutrition problems and increase the supply of bread that support health.

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acceptability, shelf life and to match with the affordability. In many countries, particularly in sub-Saharan Africa, bread wheat production and supply is inadequate to meet the bread eating habit of consumers, which is increasing with an increase in urbanization. One method to alleviate the shortage of wheat flour, increase the nutritional quality and bioactive contents of the bread is to use composite flours prepared from different crops like protein rich legumes, tubers rich in starches and/ or other cereal grain flours (Nwanekezi, 2013).

Cassava root is a good source of starches (about 80%) but is limited in proteins, fats and minerals (Montagnac, Davis, & Tanumihardjo, 2009). Soybean is rich in high quality proteins with balanced amino acids, lipids, minerals and bioactive compounds but is limited in starches (Gara, Lule, Malik, & Tomar, 2016; Shao et al., 2009). Wheat flour for bread has starches and functional protein glutens that favor the processing of leavened aerated bread, but is limited in fat and balanced amino acids (Goesaert et al., 2005). According to Technical Centre for Agricultural and Rural Cooperation (2007) and Yimer (2008) in Ethiopia and other eastern African countries, cassava and soybean are underutilized. Therefore, there is a need to increase the utilization of cassava and soybean as composite flours in the development of different food products such as bread. Such effort will mitigate foreign exchange bills spent on importing wheat grains, help to stabilize seasonal fluctuations in the wheat arains supply and provides employment opportunity for local processors. Thus, partial substitution of wheat flours with soybean and cassava flours has high potential to enhance the nutrient and bioactive contents of bread, and provides economic benefit for countries in short of bread wheat supply. A lot of studies on bread formulation have been commissioned in different countries, to achieve wheat flour substitution, resulting in varying degrees of success (Begum, Rakshit, & Rahman, 2011; Dhingra & Jood, 2004; Jensen, Skibsted, Kidmose, & Thybo, 2015; Mashayekh, Mahmoodi, & Entezari, 2008; Noorfarahzilah, Lee, Sharifudin, Fadzelly, & Hasmadi, 2014; Nwanekezi, 2013; Shao et al., 2009; Shoaren, Mohamed, & Carriere, 2003). Bread in which wheat flour replacement is allowed with cassava flour, about 30% (Jensen et al., 2015) and 20% (Begum et al., 2011; Eddy, Udofia, & Eyo, 2007) level of substitutions of wheat flour have been reported in previous studies to produce acceptable bread with insignificant variation, when compared to bread made with 100% wheat flour. Cassava flour is virtually starch, and is limited in amylase enzyme activity, which negatively affects the texture of the bread, by diluting wheat gluten functionality, even though it can impart white and bland taste to the bread. The starch granules morphology, composition and physicochemical properties differences between cassava and wheat starches will also contribute to the texture variations in the bread. Addition of 10% of soy flour (full-fat and defatted) (Dhingra & Jood, 2004), 11% (Shao et al., 2009), 7% (Mashayekh et al., 2008) and 30% (Shogren et al., 2003) defatted soybean flours have been reported to produce acceptable bread with quality attributes similar to the control made from 100% wheat flour. Information on appropriate blending ratios for soybean and cassava composite flours to produce bread of acceptable quality to consumers is limited. A study conducted by Udofia, Udoudo, and Eyen (2013), reported that bread with sensory acceptability next to 100% wheat flour were produced from composite of 17% cassava, 17% soybean and 67% wheat flours, however it was observed that, the nutrient content of the bread was not reported. Partial substitution of wheat flour with composite flour of soybean and cassava has high potential to enhance bread nutrients and bioactive compounds of functional food character from soybean into the baked bread (Garg et al., 2016; Shao et al., 2009). The bread loaf volume, bread mass and sensory acceptability may be negatively affected. Mixture design offers a solution to find optimum controlled processing of each raw material, to achieve bread quality, for the proportion of the blending that will lead to bread with desirable attributes. Thus, in this study from a constrained mixture design of wheat, cassava and soybean composite flours, the optimum blending ratio from response surface analysis that will maximize the nutrient contents and sensory acceptability of the bread is reported.

2. Materials and methods

2.1. Experimental materials

Bread wheat (*Denfi*) was collected from Kulumsa Agricultural Research Center, soybean (*Clark 63k*) and cassava roots (*Qulle*) were from Jimma Agricultural Research Center, Ethiopia. Selection of the

crop varieties were based on their adaptability to a wide range of agro-ecological locations in the country and importance with good agronomic performance (high yield, resistance to disease and early maturity) (Ministry of Agriculture & Rural Development, 2009). The experiments were conducted at the Postharvest Management Department and Chemistry Department laboratories of Jimma University and the Ethiopian Public Health Institute, Ethiopia.

2.2. Sample preparation

2.2.1. Cassava flour

Matured cassava roots was washed, peeled, the peeled cassava was re-washed with distilled water, sliced in to 2–3 cm size cubes, sun dried for two days, milled (heavy-duty cutting mill, SM2000/695upm, Germany) and sieved through a 0.5 mm aperture sieve.

2.2.2. Soybean flour

Soybean was cleaned, boiled (100°C, 30 min), seed coat was removed, oven dried (Leicester, LE67 5FT, England) at 60°C for about 16 h, and then milled into flours (0.5 mm sieve) using a laboratory hammer mill (Kaelkolb, D-6072 Dreich, West Germany) (Famurewa & Raji, 2011).

2.2.3. Wheat flour

Wheat grains after cleaning were milled using a laboratory hammer mill (Kaelkolb, D-6072 Dreich, West Germany) and sieved using 0.5 mm sieve. All flours were stored at refrigeration temperature (5°C) in an airtight container until used.

2.3. Experimental design and formulation of composite flours

A 16-run constrained D-optimal mixture experiment was generated using Design-Expert® (Version 8.0, Stat-Ease) software. The constraints were $40 \le$ wheat \le 80, $10 \le$ cassava \le 30 and $10 \le$ soybean \le 30; wheat + Cassava + soybean = 100. The control was 100% wheat flour.

2.4. Preparation of bread

Bread from composite flours and 100% wheat control flour were prepared following the basic straight dough method (American Association of Cereal Chemists, 2000) using ingredients: flour (400 g), sugar (12 g), salt (4 g), shortening (8 g), bread improver (8 g) and yeast (6 g). After proofing, the dough was baked (220°C for 30 min), cooled for 2 h, for loaf volume and bread mass measurement. Baked breads were packed in low-density polyethylene plastic bags and stored ($24 \pm 2^{\circ}$ C) for subsequent analysis. Results obtained from sensory analysis of bread samples after 12 h of storage at ambient temperature are reported in this article. To make the bread suitable for chemical analysis the bread samples were dried (100°C for 24 h), milled into flours and stored in an airtight container at refrigeration temperature (5°C) until analysis was done.

2.5. Nutrient contents analysis

2.5.1. Proximate composition analysis

The moisture content from the bread flour was determined by drying 5 g bread sample using an air draft oven (103° ± 1°C, 6 h) (American Association of Cereal Chemists, 2000). Crude protein content was determined after digestion of about 0.5 g sample by *micro*–Kjeldahl method to determine nitrogen content (HYP-1014 digestion and KDN-102F distillation systems, Shanghai Qian Jian Instruments CO, LTD, China) (Association of Official Analytical Chemists, 2000). Urea was used as control. Protein (%) = N (%) × 6.25. Crude fiber content was determined by taking about 1.5 g sample as portion of carbohydrate, that resisted sulfuric acid (1.25%) and sodium hydroxide (1.25%) digestion, followed by sieving (75 µm), washing, drying and ignition. Then, ash content was determined after extraction of 3.5 g sample with n-hexane by Soxhlet extraction (SZC-C, China) method (Association of Official Analytical Chemists, 2000). Ash content was determined by ashing about 5 g sample in a muffle furnace at 550°C until ashing was complete (Association of Official Analytical Chemists, 2000).

Total carbohydrate content was calculated by difference method: 100 - (%Moisture + %Crude protein + %Crude fat + %Crude fiber + %Ash) (Monro & Burlingame, 1996). Energy value (kcal per 100 g) was estimated using the Atwater conversion factor (Osborne & Voogt, 1978). Energy (kcal per 100 g) = [9 × Lipids% + 4 × Proteins% + 4 × Carbohydrates%].

2.5.2. Mineral content analysis

Iron, calcium and zinc contents were analyzed using an Atomic Absorption Spectrophotometer (Perkin–Elmer, Model 3100, USA) after dry digestion of about 5.0 g bread samples using air-acety-lene as a source of energy for atomization (American Association of Cereal Chemists, 2000). For iron, absorbance was measured at 248.3 nm wavelength and iron content was estimated from a standard calibration curve (3–8 μ g Fe/mL) prepared from analytical grade iron wire. For zinc determination, absorbance was measured at 213.8 nm wavelength and zinc content was estimated from a standard calibration curve (0.1–1.0 μ g Zn/mL) prepared from ZnO. For calcium content determination, absorbance was measured at 422.7 nm wavelength 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 (0.1–1.0 μ g Ca/mL) prepared from CaCO₂.

2.6. Anti-nutritional factors content analysis

2.6.1. Condensed tannin contents

Condensed tannin content was determined using a modified vanillin-hydrochloric acid method (Earp, Akingbala, Ring, & Rooney, 1981). Sample (about 1 g) was extracted with 10 mL of 1% HCl for 24 h. Extract (1 mL) was reacted with 5 mL vanillin-HCl reagent (8% concentrated HCl in methanol and 4% vanillin in methanol, 50:50, v/v). The absorbance of color developed was measured after 20 min at 500 nm wavelength using a UV-vis Spectrophotometer (DU-64 spectrophotometer, Beckman, USA). Catechin calibration curve was used to determine the condensed tannin contents as mg of catechin/g of sample.

2.6.2. Phytate contents

Phytate contents was determined as described in Vaintraub and Lapteva (1988) after extraction of sample with 2.4% HCl for 1 h, centrifuged and reacting sample extract (3 mL) with 1 mL of wade reagent (0.03% FeCl₃·6H₂O and 0.3% sulphosalicyclic acid in distilled water). The absorbance of sample was measured at 500 nm wavelength using a UV-vis Spectrophotometer (DU-64 spectrophotometer, Beckman, USA). The value obtained was subtracted from the blank absorbance value and the phytate content (mg/100 g sample) was estimated from the phytic acid standard calibration curve (5–36 mg/kg).

2.6.3. Cyanide content

The cyanide content in the raw cassava tuber was determined using titration method (Association of Official Analytical Chemists, 2000); in the bread, it was determined using picrate kit color method (Bradbury, Egan, & Bradbury, 1999). Bread loaf volume was measured by rapeseed displacement method and the bread mass was measured using a digital mass balance (ABJ220-4 M, WB1151070, Australia).

2.7. Sensory evaluation of bread

Sensory acceptability (taste, color, odor, texture and overall acceptability) attributes were evaluated on a five-point hedonic scale (1 = extremely dislike, 2 = dislike moderately, 3 = neither like nor dislike, 4 = like moderately and 5 = extremely like) (Linda, Debborah, Gail, & Elizabeth, 1991). Sensory evaluation was conducted by fifty untrained consumers (29 males and 21 females of age between 25 and 47), randomly recruited among staff and graduating class students of the Postharvest Management Department of Jimma University, Ethiopia. After orientation, three digit-coded samples were given in random order to panelists along with the cup of water to cleanse their mouth between sample tasting, to avoid carryover bias. The mean scores were subjected to analysis.

2.8. Statistical analysis

A D-optimal mixture design with 16 formulations of three ingredients was analyzed using Minitab Version 17 software to determine the optimum proportion of the ingredients to maximize proximate compositions, iron, zinc, calcium, bread loaf volume, and sensory acceptability response variables; and to minimize bread weight, phytate, and condensed tannins content response variables. Complete analyses of these response variables was conducted using the methods described in Montgomery (2013). The analyses included verifying that the model did not have significant lack-offit, and the normal distribution and constant variance assumptions on the error terms were valid. Independence assumption was valid through the random order of the runs. This was followed by testing the significance of each model term, and performing response variables within each of proximate composition, minerals, anti-nutrient, and sensory evaluation. This was followed by constructing an overlaid contour plot to determine the "sweet spot" that jointly optimizes all response variables for each of these groups of the response variables.

3. Results and discussion

3.1. Optimization of proximate compositions

The composite dried bread flours processed from wheat, cassava and soybean had moisture, crude fiber, protein, fat, ash, total carbohydrate and energy contents in the range of 4.87-6.15%, 2.68-4.00%, 13.05-21.2%, 4.96-11.25%, 3.78-4.23%, 53.17-70.21% and 389.6-414.73 kcal/100 g, respectively. Crude protein, fat, ash, total carbohydrate and energy contents of the bread were significantly (p < 0.05) affected by varying blending ratio as predicted by the linear and/ or quadratic models (Table 1). The highest crude protein content (21.2%) was from the formulation with 60% wheat, 10% cassava and 30% soybean (Figure 1(a)) which is associated with the highest proportion of soybean, which contains high quantity and quality proteins. High soybean blending proportion significantly increased protein, fat and energy, whereas high cassava blending ratio increased total carbohydrate content of the bread (Figure 1(d)). In the bread making performance, wheat glutens significantly (p < 0.05) affects the mixing characteristics of dough and bread loaf volume. A similar increase in the crude protein contents of the composite flour bread with soybean flour addition were reported by other authors (Mashayekh et al., 2008; Noorfarahzilah et al., 2014; Sanful & Darko, 2010; Shogren et al., 2003; Udofia et al., 2013). However, as the levels of cassava flour increased in the blend, the crude protein contents of the bread decreased. The least crude protein content was obtained in the bread processed from the blend of 65% wheat, 10% soybean and 25% cassava flour. Proteins in roots and tuber crops are very low, which is a major impediment for their extensive utilization. Such deficiency necessitates supplementing with legumes such as soybean in the food formulation.

compositions and energy content response variables								
Source	МС	Fiber	Protein	Fat	Ash	Carb	Energy	
Linear	0.261	0.997	0.001	0.068	0.001	0.037	0.001	
Quadratic	0.113	0.599	0.025	0.033	0.005	0.026	0.002	
A*B	0.980	0.507	0.068	0.776	0.001	0.771	0.819	
A*C	0.028	0.431	0.014	0.668	0.468	0.043	0.001	
B*C	0.086	0.727	0.021	0.226	0.004	0.038	0.255	
Lack-of-fit	0.259	0.273	0.268	0.097	0.099	0.128	0.078	
R_{adj}^2	87.4%	86.2%	99.9%	98.5%	94.3%	99.5%	98.2%	

Table 1. Analysis of variance (ANOVA) and lack of fit *p*-values, and adjusted *R*² for proximate compositions and energy content response variables

Notes: A = Wheat, B = Cassava, C = Soybean, MC = Moisture content, Carb = Carbohydrate.

Figure 1. Contour plots displaying proximate composition of composite bread. (a) Protein, (b) Fat, (c) Ash, (d) Carbohydrate, (e) Energy and (f) Overlaid contour plot of the five proximate compositions that shows the sweet spot (white area).



The minimum (4.96%) and maximum (11.25%) fat contents were reported from bread produced from a composite flour of 65% wheat, 25% cassava and 10% of soybean; and 60% wheat, 10% cassava and 30% of soybean, respectively. It was observed that, as the amount of soybean flour increased in bread samples, the fat content of the bread increased because of the high amount of fat in soybean (Dhingra & Jood, 2004; Garg et al., 2016; Noorfarahzilah et al., 2014). As wheat and cassava flours increased in the formulation, the fat contents decreased (Figure 1(b)) because of their low fat contents (Eddy et al., 2007; Montagnac et al., 2009). With the increase in the fat content, the bread loaf volume can increase, because it helps in the incorporation and retaining of air during the mixing of the batter (Pareyt, Finnie, Putseys, & Delcour, 2011). In addition, fat also imparts tenderness, moistness, lubricity, flavor, color and anti-bread staling qualities in the baked products.

The ash content of the composite bread samples increased from 3.84 to 4.23% with supplementation of soybean flour increase from 10 to 30%. Similar increase in ash and inorganic nutrients in soybean flour supplementation in composite bread have been reported in previous studies (Mashayekh et al., 2008; Sanful & Darko, 2010). Soybean contributed towards high ash contents in the blend because of its high mineral contents. The ash content also increased with the increase of cassava in the bread formulation; and similar ash content increase in wheat-cassava blend bread was reported (Masamba & Jinazali, 2014).

The carbohydrate contents of composite bread was influenced by the level of cassava flour supplementation and the highest (70.2%) content was found in a blending proportion of 65% wheat, 25% cassava and 10% soybean (Figure 1(d)). A similar increasing trend of carbohydrate content due to blending with cassava flours have been reported in previous studies (Eddy et al., 2007) because of high starch contents in the cassava flours. The energy content was 388.6 kcal/100 g in wheat flour, 372.9 kcal/100 g in cassava flour, and 498.5 kcal/100 g in soybean, and it varied from 389.6 to 414.7 kcal/100 g in the final composite baked bread products. The amount of energy in the final product showed a significant difference ($p \le 0.05$) in linear, guadratic, and cross-product of wheat and soybean in the model. The amount of energy obtained shown in the contour plot (Figure 1(e)) shows soybean flour supplementation imparted significant effect on the energy because of its high fat contents.

The interaction effect of wheat and soybean ingredients was significant (p < 0.05) on moisture content in the bread, probably due to the high protein content of both ingredients that contributed to high moisture absorption in the baked product. There was no significant difference (p > 0.05) in the crude fiber contents of the bread due to blending, because of removal of soybean seed coats, on soybean processing into flours. The optimum response for proximate composition (fiber-3.64%, protein—17.54%, fat—7.96%, ash—4.03%, carbohydrate—60.96%) and energy (400.23 kcal/100 g) in the composite bread flour were observed in the blend of 71% wheat, 10% cassava and 19% soybean (Figure 1(f)).

3.2. Optimization of mineral contents (calcium, iron and zinc)

The calcium and iron contents in the composite bread flour samples ranged between 12.72-17.94 mg/100 g and 5.71-6.81 mg/100 g, respectively. Results obtained revealed that with high blending proportion of soybean flours, there is an increase of Ca and Fe contents in the bread. The zinc contents (1.61–1.84 mg/100 g) did not show a significant difference both in linear and quadratic terms in the model with an increase in soybean (Table 2). Soybean flour contributed to high calcium and iron contents in the composite breads because soybean is high in mineral contents (Garg et al., 2016). The maximum response for bread mineral contents (mg/100 g) (Fe = 6.69, Zn = 1.84 and Ca = 17.92) were observed in the blend of 60% wheat, 10% cassava and 30% soybean. Mineral nutrients are important for the functioning of numerous metabolic processes in the body and their deficiency in the foods can cause different types of disorders. The study showed that it is possible to enhance the micronutrient contents of bread by blending with soybean to suppress nutritional deficiency problems.

content response variables							
Source	Iron	Zinc	Calcium	Phytate	Tannin		
Linear	0.001	0.552	0.001	0.001	0.019		
Quadratic	0.001	0.647	0.015	0.001	0.508		
A*B	0.065	0.581	0.017	0.186	0.622		
A*C	0.001	0.791	0.011	0.001	0.179		
B*C	0.001	0.947	0.521	0.001	0.543		
Lack-of-fit	0.144	0.146	0.254	0.873	0.086		
AR ² _{adj}	97.6%	93.5%	99.5%	96.3%	96.2%		

Table 2, ANOVA and lack of fit p-values, and adjusted R^2 for mineral, phytate and tapping

Notes: A = Wheat, B = Cassava, C = Soybean.

3.3. Anti-nutritional factors

The cyanide content was 65.80 ppm in fresh cassava root, 20.00 ppm in the final cassava flour and was not detectable in the final composite dried baked bread products. Even though, improved cassava variety with less cyanide content was used in this experiment, the study showed that simple home-based processing methods such as drying, chopping, fermentation and baking had reduced the cyanide content to undetectable level. Similar results were reported in other studies (Hongbété, Mestres, Akissoé, & Nago, 2009).

Phytate (40.01-78.83 mg/100 g) and condensed tannin (9.70-37.42 mg/100 g) contents of the bread were significantly (p < 0.05) affected by varying blending ratio as predicted by the linear and/ or quadratic models (Table 2). High blending proportion with soybean flours showed an increase in the phytate and condensed tannin contents of the bread. Phytate chelates minerals at the gastrointestinal conditions and makes them not bioavailable for human absorption (Liener, 2000). Condensed tannins are anti-nutritional because they form insoluble complexes with digestive enzymes and inhibit iron bioavailability. A significant reduction of phytates content in the dried baked bread as compared to found in wheat (333.9 mg/100 g), cassava (181.3 mg/100 g) and soybean flours (287.4 mg/100 g) observed shows both blending and processing has reduced the phytates. A reduction in the phytate content with fermentation was observed in composite bread of soybean and wheat flours as reported in other studies (Mashayekh et al., 2008). A slight increase in the condensed tannins contents was observed in dried baked flours compared to the amount found in the raw materials (12.2–31.4 mg/100 g) is probably contributed by Maillard reaction products produced on baking and drying from reaction of amino acid and residual sugar as reported in other studies (Dhingra & Jood, 2004; Mashayekh et al., 2008). Blending of 80% wheat, 10% cassava and 10% sovbean showed optimum reduction in anti-nutrient contents (mq/100 q) (phytate = 44.55 and condensed tannins = 11.97).

3.4. Optimization of physical characteristics of bread

Blending ratio significantly (p < 0.05) affected bread weight and loaf volume as predicted by linear and/ or quadratic models (Table 3). The highest bread loaf weight (715.9 g) was obtained from 50% wheat, 30% cassava and 20% soybean flour blend, and the lowest (675.8 g) was from 80% wheat, 10% cassava and 10% soybean flour blend. The addition of cassava (10–30%) and soybean (10–20%) flours to wheat flour increased the bread mass. The loaf volume of the bread decreased from 1300.08 to 783.31 cm³ as the proportion of both cassava and soybean flour increased. The weakening of wheat flour dough was the result of a dilution of the wheat gluten proteins structure by the added cassava and soybean flours. Cassava is deficient in sulfur-containing amino acid proteins, consequently they have a weak ability to form networks that can retain gas during dough development (Montagnac et al., 2009). Similar decrease in bread loaf volume was reported with an increase in soybean flour in breads produced from wheat and soybean flour blends (Dhingra & Jood, 2004; Mashayekh et al., 2008; Ndife, Abdulraheem, & Zakari, 2011), in other studies, when cassava flour was blended with wheat flour (Jensen et al., 2015). In this study, blending of 73% wheat, 10%

properties response variables								
Sources	Bread weight	Bread volume	Taste	Color	Odor	Texture	OA	
Linear	0.015	0.041	0.049	0.006	0.000	0.608	0.009	
Quadratic	0.066	0.001	0.001	0.071	0.000	0.121	0.066	
A*B	0.016	0.069	0.048	0.123	0.055	0.069	0.065	
A*C	0.208	0.741	0.107	0.034	0.000	0.699	0.102	
B*C	0.147	0.281	0.884	0.560	0.000	0.358	0.011	
Lack-of-fit	0.053	0.058	0.366	0.154	0.377	0.114	0.286	
R_{adj}^2	81.8%	98.4%	97.6%	90.8%	94.4%	90.4%	88.8%	

Table 3 ANOVA and lack of fit p-values and adjusted P^2 for bread physical and sensory

Notes: A = Wheat, B = Cassava, C = Soybean, OA = Overall acceptance.

cassava and 17% soybean showed optimum high bread loaf volume (1,146 cm³) and reduced bread mass (686 g).

3.5. Optimization of sensory properties of the bread

Sensory properties of the composite bread were significantly (p < 0.05) affected by blending ratio as predicted by linear and/ or quadratic models. The absence of significant lack of fit in both models (Table 3) show that the linear and/ or quadratic models fit well to the sensory attributes. Mean scores of color, taste, odor, texture and overall acceptability ranged from 4.0 to 4.3, 3.4 to 4.2, 3.7 to 3.9, 3.5 to 3.9 and 3.8 to 4.3, respectively. Sensory acceptance attributes of the bread samples increased as the proportion of wheat increased in the blends except for appearance. The acceptance of appearance as the amount of soybean flour increased from 10 to 30% (Figure 2(a)) may be attributed to Maillard reactions (Yilmaz & Toledo, 2005) between reducing sugars and amino acids in proteins, the

Wheat on (a) (b) 5.0 Wheat 4.5 Texture an.oo < 3.55 Color 10 10 3.55 -3.60 3.60 - 3.65 4.03.65 - 3.70 10.00 3.70 - 3.75 3.75 -3.80 > 3.80 Cassava 50.00 40.00 50 40 50 50,00 Soybean Cassava Sovbean Wheat 80 (d) (c) 4.25 Odour 4.00 < 3.70 10 Wheat 3.70 - 3.75 3.75 - 3.80 Taste 3.75 3.80 - 3.85 3.85 - 3.90 3,50 3.90 - 3.95 10.00 3.95 -4.00 > 4.00 Cassava 50.00 40.00 50 50 40 50.00 Soybean Soybean Cassava Wheat Color Wheat 4.05 80 -- 4.3 (e) (f) Taste 3.6 4.16 Odour 3.75 Overall ---- 3.95 10 10 acceptance Texture 10 10 < 3.8 3.6 3.8 - 3.9 -- 3.86 3.9 _ 4.0 Overall 4.0 - 4.1 acceptar 4.1 4.2 4 _ 4.3 4.2 - 4.31 4.3 40 50 50 50 50 40 Cassava Soybean Soybean Cassava

Figure 2. Contour plots and surface plots displaying sensory attributes of composite bread. (a) Bread color, (b) Bread texture, (c) Bread odor, (d) Bread taste, (e) Overall acceptance and (f) Overlaid contour plot of the five sensory analysis that shows the sweet spot (white area). lubricating and shortening effect of soybean lipids that makes bread appealing to consumer panels. The result shows that supplementation of wheat by cassava up to 30% did not significantly affect the appearance of the bread.

The observation indicates that high supplementation of non-wheat flour 30:30 (cassava: soybean) showed low scores on texture (3.5) (Figure 2(b)). High supplementation levels of other flours to wheat flour are known to reduce elastic properties of wheat flour dough leading to low gas retaining properties during fermentation (Jensen et al., 2015; Noorfarahzilah et al., 2014; Nwanekezi, 2013), which in turn reduces the texture quality of the final bread. Bread odor plays a primary role in creating consumer appeal. The odor of cassava and soybean flour supplemented bread samples scored maximum (3.9) in a ratio of 60:20:20 (wheat: cassava: soybean flours) and lowest (3.7) was for the blend of 60:10:30 (wheat: cassava: soybean flours) (Figure 2(c)). The less odor acceptance is, probably attributed to residual beany flavor of the soybean as soybean increased in the blend (Dhingra & Jood, 2004; Mashayekh et al., 2008; Ndife et al., 2011).

The inclusion of cassava (10–30%) and soybean (10–30%) had shown an effect on the overall acceptability of the final bread scoring from 3.8 to 4.3 with the highest score being recorded in 80:10:10 and the lowest in 40:30:30 (wheat: cassava: soybean) (Figure 2(e)). As in many parts of the world, consumers are familiar with the taste of the products they regularly consume. Presumably, bread with higher ratio of wheat (80%) and lower ratio of soybean flour (10%) scored the highest, which is similar to those reported in different past works (Dhingra & Jood, 2004; Eddy et al., 2007; Mashayekh et al., 2008; Udofia et al., 2013). However, changing the proportion of cassava flour from 10 to 30% have not shown a significant difference in the taste of the bread that reflects the bland taste nature of the cassava flour; and similar findings were reported (Jensen et al., 2015; Masamba & Jinazali, 2014). The sweet spot was obtained by placing a range of color 4.1-4.3, taste 3.6-4.2, odor 3.8-4.0, texture 3.6-3.9 and overall acceptance of 4.0-4.3. The optimum region in this overlaid plot was where the criteria for all five response variables (appearance, taste, odor, texture and overall acceptability) satisfied and this region was found in the range of wheat 53.5-75.7%, cassava 11.0-29.0% and soybean 11.5-25.2% (Figure 2(f)). However, the optimum proportion 74% wheat, 10% cassava and 16% soybean gave the maximum values of sensory quality responses (taste = 4.10, color = 4.08, odor = 3.87, texture = 3.8 and overall acceptability = 4.18).

3.6. Bread optimal mixture compositions

The regions of acceptability in the contour plots for protein, carbohydrate, fat, iron, calcium, bread loaf volume, taste and overall sensory attributes were superimposed to determine the optimum formulation (Figure 3). The white region in Figure 3 indicates that any point within this region represents an optimum combination of wheat, soybean and cassava flour blends. Thus, the overall





optimum values that will optimize high nutrient and reduced anti-nutrients contents of acceptable sensory attributes of the bread were in the range of wheat 49.0–71.0%, cassava 10.6–29.0%, and soybean 18.2–22.0% blending. Notwithstanding, bread attributes like loaf volume and bread mass that are processed from less than 70% wheat flour in the mixture are inferior. In the event soybean is affordable, high proportion of soybean flours (about 17%) than cassava flours and maintaining wheat flours around 70%, bread with high nutrients and bioactive contents can be processed. This is important since it favors incorporation of health supporting soybean bioactive compounds like isoflavones, which were reported to persist in the baked bread (Shao et al., 2009). Also in the event cassava flours are affordable than soybean flours, with compromised nutrient and bioactive contents in the bread as compared to high soybean flour blended bread, yet higher than 100% wheat flour bread can be processed by maintaining wheat flour around 70%, low soybean flour (10 to 13%) and the rest by blending with cassava flours.

4. Conclusions

Sixteen composite flours prepared using D-optimal constrained mixture design within a range of wheat 40–80%, cassava 10–30% and soybean 10–30% were evaluated. The optimum blending ratio for both nutritional and sensory acceptability was in the range of 49.0–71.0% wheat, 10.6–29.0% cassava and 18.2–22.0% soybean flours blending. Notwithstanding, bread attributes like loaf volume that are processed from less than 70% wheat flour in the mixture were shown to be inferior. Thus, with controlled processing of soybean and cassava flours, maintaining wheat flours around 70% and high proportion of soybean flours (about 17%) in the blend, bread with high nutrients and soybean flours in bread formulation has a significant implication in improving nutrition and reducing the rising price of bread processed from 100% wheat flours. The information could be useful for job creation for cooperatives such as women associations who can provide breads for different firms in countries that could not afford 100% wheat bread.

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Competing Interests

The authors declare no competing interest.

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