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Nutritional Enrichment of Wheat Bread using Various Plant Proteins

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Abstract

This study examined the proximate and mineral compositions of wheat bread fortified with honey beans, soybeans and melon by using standard methods. Non-fortified wheat bread was used as the control. Results of the proximate analysis showed that wheat-melon bread had the highest moisture content (29.30 %), fat content (17.14 %), and ash content (2.08 %). Wheat-beans bread had the highest crude fibre (5.73 %) while the protein content was highest in wheat-soybeans bread (9.73 %). The protein content of the melon, bean and soya bean enriched breads increased by 9 %, 16.5 % and ~26 % respectively. Carbohydrate content increased in all samples with wheat-soybean bread having the highest and wheat-melon-bread the least. Results of the mineral analysis showed the presence of Calcium, Magnesium, Potassium, Sodium, Manganese, Iron, Copper, Zinc and Phosphorus. Meanwhile, Co, Cr, Cd, Pb and Ni were not detected in all the bread samples. The results showed increase in the mineral content of the three breads and only wheat-soybean bread showed decrease Mn. The significant increases in amounts of protein and minerals indicated that supplementation of wheat flour with soybean flour would greatly contribute to the daily dietary needs and improve the nutritional quality of wheat bread. Fortification of wheat bread with soybeans and other plant materials should therefore be encouraged.

Keywords: *Wheat bread, Soybean, honey beans, melon, proximate, minerals, enrichment*

Introduction

Wheat is a cereal that has special ability to hold gas in form of air, water vapor and carbon dioxide, hence forming foam or a spongy structure because of the presence of a protein called gluten. Other cereals such as rice, sorghum, maize, millet etc. do not possess such ability. The gluten content of wheat makes it elastic during processing such as kneading; thereby enabling it to hold the carbon dioxide that is produced during fermentation. (Mepba *et al*, 2007). Wheat is eaten all over the world and is used in producing confectionaries such as cookies, doughnuts, and noodles. The most known item of wheat product is bread. Bread is a major food consumed by many and is made by making dough of flour with water, yeast and other additional ingredients, such as butter and salt to enhance its taste and finally baked (Osuji, 2006). It has a taste that is accepted to consumers and also makes one feel full when consumed and are easily affordable (Casier *et al*., 1979; Adeleke *et al*., 2010). In a lot of homes in Nigeria, it is an important portion of a meal, especially the average and high income earners.

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The increase in demand for bread has led to high demand of white flour (Mannay and Shadaksharaswany, 2005) obtained from whole wheat grain by removing its bran and the germ in order to enhance the aesthetics and reduce early rancidity of the flour but unfortunately this process decreases its nutritive value (Dewettinck *et al*., 2008; Maneju *et al*., 2011). Recently, consumers are getting aware of the need to consume foods that have good impacts and benefits on their health; hence there is a great need to make breads from whole grain flours (Dewettinck *et al*., 2008). It is reported that consuming whole grain foods prevent many diseases like obesity, constipation, diabetes, cardiovascular diseases, irritable colon, colon cancer (Ndife and Abbo, 2009; Rodriguez *et al*., 2006; Schatzkin *et al*., 2007; Jideani and Onwubali, 2009). It is therefore required of bakers to produce food that are of good formulations and of health benefits to their consumers which will overcome the challenges relating to taste acceptability and health issues. (Ndife and Abbo, 2009; Hiller *et al*., 2011).

Honey beans, popularly called "Ewa oloyin" in Yoruba is one of the varieties of Cowpea (*Vigna unguiculata L. Walp*) belonging to the family *Leguminosae.* It is found all over the tropical region (Pasquet, 2001). Cowpea is a major source of plant protein in most developing countries like Nigeria (Adekola and Oluleye, 2007). Many people in low income nations depend on it as a source of protein in their diets (Ajayi, 2014). Cowpea seed is seen as a supplement to cereals and complement to animal protein (Stanton, 1966) and are very nutritive (Jenkins, 2000). In addition, consuming the starch of grain legumes leads to little changes in blood glucose as a result of its slow digestion when compared with starch from cereals and tubers (Jenkins, 2000).

Soybean known as *Glycine max* is of the family *leguminosae* and sub-family *papillionnideae*. (Alabi *et al*., 2001) It is also a source of protein, edible fat and oils for humans and animals (Alabi *et al*., 2001). Soybeans are very rich in nutrients with phytochemicals that can nourish and fight diseases in the body. It is a good source of trace elements like zinc, manganese and copper and has all the essential amino acids (Basman *et al*., 2003; Dhingra and Jood, 2002; Olaoye *et al*., 2006).

Citrullus lanatus; melon, is of the family *cucurbitaceae*. It is called egusi in Yoruba. It is grown mainly with early maize and yam as crop interplant in many locations of the country (Mabalaha *et al.,* 2007). *Citrullus lanatus* is found in tropical and temperate areas as economically essential vegetable crops all over the world (Paris, 2001). In Nigeria, melon seeds are used to make delicious soups and have special and tasteful aroma, therefore they are used as a flavoring agent in soups, sauces and stews (Onyeike and Achera, 2002). Based on earlier research, melon seeds have little protein and some oil content which make them essential raw materials for cosmetic and food industries. Fat, protein, minerals, vitamin C and vitamin B_2, carbohydrate and Riboflavin are found in melon (Lazos, 1986). They can enhance and improve good balanced diet (Fokou *et al.,* 2004) and can be used as composite ingredient.

Huge amount of money is spent on managing flour mills and importation of wheat flour in the African continent (Hart *et al*., 1970). In a bid to decrease the high foreign exchange spent on wheat importation and building flour mills, attempts have been made to produce composite flour (Odedeji and Odetayo, 2010). White wheat flour is poor in protein content as a result of the elimination of the bran and germ parts (Young, 2001) in an attempt to enhance the shelf life of the flour and its products (Maneju *et al*., 2011).

It is therefore necessary to fortify wheat bread with protein sourced from plants in order to increase the nutritive content of bread. This will greatly reduce the high foreign exchange spent on the importation of wheat, bring down the demand and at the same time producing protein–enriched bread (Giami *et al*., 2004; Olaoye *et al*., 2006). Money gained from reduced importation of wheat can be used to build more infrastructures for our country for the benefit of the people.

Materials and methods

Collection of samples

All samples (Wheat flour, Honey beans, Soy beans and Melon) were purchased from Ilishan market, Ogun state, Nigeria.

Preparation of samples

The unwanted particles were removed from the grains by hand picking. The beans were washed, peeled and dried. The soybeans were crushed and winnowed while the melon were washed and dried. The dried seeds were grounded into fine and homogeneous powder and kept in air tight plastic containers until usage. The wheat flour, the three sources of protein, baking soda, yeast were mixed in a fixed proportion. Salt, Sugar, Butter, water were also added to taste. The mixing ratio of flours used for bread production is shown in Table 1. The wheat bread was baked at the bakery of Babcock University High School, Ilishan-Remo, Ogun state, Nigeria.

Table 1: Mixing ratio of flours used for bread production

Bread Samples	Wheat flour (g)	Honey Beans (%)	Soy-Beans (%)	Melon (%)
Wheat bread	500	X	X	X
Wheat + honey beans	500	15	X	X
Wheat + soya beans	500	X	15	X
Wheat + melon	500	X	X	15

X= not present ; % = % of other flours relative to Wheat flour

Proximate analysis

The proximate analysis of the samples was carried out according to AOAC, 2010 method.

Moisture content

A washed crucible was dried at 100 °C for 30 minutes in an oven and allowed to cool in a desiccator. This was repeated until a constant weight was obtained (W_0). Two grams (W_1) of the sample was added into the pre-weighed crucible and weighed (W_2). It was placed in an oven at 105 °C for about 4 – 5 hours after which the crucible was removed, cooled in a desiccator and weighed (W_3). The process was repeated until a constant weight was obtained. Moisture content was calculated as a percentage of initial weight of the sample

$$\text{Moisture \%} = \frac{W_2 - W_3}{W_1} \times 100$$

Fat content

Two grams (W_0) of the sample was weighed into a pre-weighed muslin cloth and weighed (W_1). The muslin cloth containing the sample was subsequently dropped in the condenser of an assembled soxhlet apparatus. The extraction was carried out for six hours with 250 mL of petroleum ether. The muslin cloth together with the extracted sample was dried in the oven at about 60 °C for 30 minutes until a constant weight (W_2) was obtained. The difference in weight before and after extraction was the measure of the fat content in the sample.

$$\% \text{ Fat content} = \frac{W_1 - W_2}{W_0} \times 100$$

Crude fibre

Two grams of defatted sample was weighed (W_0) and poured in a 250 mL beaker. 100 mL of 0.12 M Sulphuric acid was added to the beaker and boiled for 30 minutes, filtered and washed with distilled water three times. The residue was transferred into the 250 mL beaker and 100 mL 0.12 M Sodium Hydroxide was added, boiled for 30 minutes, filtered and washed with hot water. The residue was transferred to a pre-weighed crucible and allowed to dry in an oven at about 100 ^{0}C until a constant weight was obtained. This was cooled in the desiccator and the weight was recorded (W_1) .The crucible was put in a muffle furnace at about 550 ^{0}C for 4 hours, cooled in a desiccator and weighed again (W_2)

$$\text{Crude fibre content } \% = \frac{W_1 - W_2}{W_0} \times 100$$

Ash content

Two grams of the sample (W_1) was added to a pre-weighed crucible and weighed (W_2). It was then incinerated in the muffle furnace at 550 ^{o}C for 4 hours. The crucible with its content was cooled in a desiccator and weighed (W_3). Ash content was calculated as;

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Crude protein

0.5 grams of the sample was weighed into a Kjeldahl flask. 15 mL of concentrated Sulphuric acid and 1 gram of the selenium catalyst were added into the flask. The mixtures were heated on a digestion block in a fume cupboard for 3 hours at 350 ^{0}C. After the mixture became clear and allowed to cool, 10 mL of distilled water was carefully added to avoid caking. The content was later transferred to Kjeldahl distillation apparatus. 60 mL of 40 % NaOH solution was added and the mixture distilled into receiving flask containing 25 ml of 2 % Boric acid solution and few drops of mixed indicator. Hydrochloric acid (0.1 N) was standardized with sodium carbonate solution and used to titrate the mixture until a pink color appeared. The average titre values were used to calculate the crude protein content. Titre values were also obtained for the blank solution. The protein content was calculated as follows;

$$\% \text{ N} = \frac{14 \times (V_1 - V_2) \times \text{Normality of Acid}}{1000 \times \text{Weight of sample (g)}} \times 100$$

% crude protein = % N x 5.70

where

V_1 = Titre value of sample, Blank V_2 = Blank titre value

5.70 = Protein factor for Wheat flour (AACC and AOAC International, 1995)

14 = Atomic mass of Nitrogen

Carbohydrate content

This was determined mathematically by subtracting the sum of percentages of all the nutrients already determined from 100. That is;

% Carbohydrate = 100 - (% moisture + % Crude Fibre + % Crude Protein + % Crude Fat + %Ash).

Energy value

The energy content that could be obtained in food when consumed in kcal/100 g, was calculated using the factors: 4 for protein and carbohydrate and 9 for fat (Marero *et al*., 1988; Bradbury *et al*., 1991).

Mineral analysis

0.5 g of sample was weighed into a crucible and incinerated in a muffle furnace at a temperature of 550 ^{o}C for 6 hours by which time the samples completely turned ash. The resulting ash was dissolved in 20 mL of 10 % HNO_3. The solution was boiled for 10 min, filtered and made up to mark in a 100 mL volumetric flask with distilled water. 20 mL of the filtrates was then transferred to sample bottles, accurately labeled and used for analysis. Reagent blank was concomitantly prepared by boiling 20 mL HNO_3 (10 %) solution for 10 min, filtered and made up to mark with distilled water in a 100 ml volumetric flask. Mineral content (Ca, Mg, K, Na, Mn, Fe, Cu, Zn, P, Co, Cr, Cd, Pb and Ni) was determined using Atomic Absorption Spectrophotometer (Buck Scientific Model 2010 VGP) except phosphorus which was determined with a UV-Visible Spectrophotometer (LaboMed SPECTRO SC).

Result and Discussion

The results of the proximate analysis and mineral composition of the fortified wheat bread samples are shown in Tables 2 and 4 respectively. The results showed that the moisture contents of the bread samples ranged from 13.89 to 29.30 % with the melon bread having the highest while the honey beans bread had the least. The relatively low moisture content of the honey beans and soybeans fortified breads was similar to the report of Eddy *et al.,* (2007) while the relatively high moisture in the melon fortified bread was also similar to the report of Njintang *et al*., (2008). The low moisture content observed in honey bean and soybeans fortified bread samples suggests that it would have a relatively long shelf life. This is because high moisture content supports microbial growth thus leading to chemical degradation and spoilage of the food (Ezeama, 2007). All the bread samples had increase in their fat content relative to the control (15.42 %) with the highest fat content found in melon bread (17.14 %), followed by honey beans bread (16.89 %) and soybeans bread (15.78 %). This will increase the solubility of the fat soluble vitamins and improve their nutritional and the textural qualities of the bread sample. Crude fiber content was highest in honey beans bread (5.73 %) followed by control (5.28 %,). Meanwhile, soybeans and melon bread had 1.12 % and 2.78 % of crude fibre respectively. Schneeman (2002) stated that the metabolic and gastrointestinal system function well with a good intake of crude fibre. Crude fibre (cellulose and lignin) estimation affords an index for evaluation of dietary fibre whose deficiency has been implicated in a variety of gastrointestinal disorder. Crude fibre is also reported to reduce the occurrence of carcinoma of the colon (Eddy *et al*., 2007). The ash content is generally used as a quality measure for the assessment of the functional properties of foods (Hofman, *et al*., 2002) and related to the mineral content of the food (Abulude, 2005; Cubadda *et al*., 2009). The ash content of fortified bread samples and control sample were relatively equal except in melon bread that had the lowest ash content (1.53 %). Protein content was found to increase in all the blends with soybeans bread having the highest protein content (9.73 %), followed by honey beans bread (8.91 %), melon bread (8.41 %) and the control sample with the least (7.74 %)**.** The percentage protein increase in fortified wheat breads compared to the control was 16.53 %, 25.84 %, and 8.79 % for honey beans bread, soybeans and melon breads respectively (Table 3). The increase in the protein content due to fortification was different from the report of Mongi *et al.,* (2012), who fortified wheat bread with cocoyam. This showed that all the blends used for this research are good sources of protein. Proteins are primary source of amino acid which is the building block of body cells. It also acts as antibodies to maintain and repair damage cells or tissues in the body. The carbohydrate content of the fortified bread sample ranged from 49.25-53.61 %. The soybeans bread had the highest value (53.61 %) while melon bread had the least (49.25 %). However, carbohydrate contents of the fortified bread samples were higher than that of the control (Table 2).

Table 2: Proximate Composition of Wheat and Fortified Wheat Bread

Proximate Parameters	*Food Samples*				
	Wheat Only (control)	*Wheat + Honey Beans*	*Wheat + Soy Beans*	*Wheat + Melon*	*Daily Value (g)*
Moisture (%)	23.05 ± 0.63	13.89 ± 0.69	17.88±0.04	29.30 ±0.51	NA
Crude Fat (%)	15.42 ± 0.48	16.89 ± 0.37	15.78 ± 0.41	17.14± 0.52	70
Crude Fibre (%)	5.28 ± 0.23	5.73 ± 0.34	1.12 ± 0.47	2.78 ± 0.25	30
Ash (%)	2.08 ± 0.34	1.97 ± 0.30	2.03 ± 0.41	1.53 ± 0.28	NA
Crude Protein (%)	7.74 ± 0.14	8.91 ± 0.24	9.73 ± 0.36	8.41 ± 0.36	50
Carbohydrate (%)	46.47 ± 0.52	52.61± 0.49	53.36 ± 0.47	49.25±0.54	310
Energy value (Kcal)	1.78 ± 0.64	1.99 ± 0.45	1.97 ± 0.53	1.55 ±0.57	8700

Average weight of a loaf of bread = 199.8 g. The data are mean of two determinations. NA= not available

Table 3: Percentage Protein increase in Composite Wheat Bread

Food Component	*Wheat Bread only (control)*	*Wheat + Honey Beans*	*Wheat + SoyBeans*	*Wheat +Melon*
Protein (g)	0.0387	0.0451	0.0487	0.0421
%Protein increase		16.53	25.84	8.79

Calculation is based on 0.5 g of bread baked with the flours

Table 4: Mineral Composition of Wheat and Fortified Wheat Bread

Mineral (mg/g)	*WH/Loaf*	*WB/Loaf*	*WSYB/Loaf*	*WM/Loaf*	*Daily Value[a]*
Ca	0.05 ± 0.02	0.04 ± 0.01	0.08 ± 0.02	0.09 ± 0.03	1000-1200 mg[a]
Mg	0.44 ± 0.02	0.58 ± 0.02	0.64 ± 0.03	0.62 ± 0.01	310-420 mg[a]
K	2.71 ± 0.03	3.01 ± 0.02	3.04 ± 0.01	2.21 ± 0.03	4.7 g[c]
Na	6.41 (1281.88) ± 0.02	7.64 ± 0.02	8.02 ± 0.03	5.94 ± 0.01	1.3-1.5 g[c]
Mn	0.01 ± 0.01	0.01 ± 0.03	0.01 ± 0.02	0.01 ± 0.01	1.8-2.3 mg[b]
Fe	0.05 ± 0.01	0.06 ± 0.03	0.06 ± 0.02	0.05 ± 0.01	8-18 mg[b]
Cu	0.004 ± 0.02	0.004 ± 0.02	0.006 ± 0.02	0.003 ± 0.01	900 µg[b]
Zn	0.01 ± 0.01	0.04 ± 0.02	0.04 ± 0.03	0.04 ± 0.01	8-11 mg[b]
P	2.28 ± 0.02	2.59 ± 0.03	2.99 ± 0.03	2.49 ± 0.02	700 mg[a]
Co	ND	ND	ND	ND	-
Cr	ND	ND	ND	ND	25-35 µg
Cd	ND	ND	ND	ND	-
Pb	ND	ND	ND	ND	-
Ni	ND	ND	ND	ND	-

WH = Wheat bread, WB= Wheat + honey beans, WSYB = Wheat+ soybeans, WM = Wheat +melon, Data are averages of two determinations, a = Institute of Medicine, 1997, b = Institute of Medicine, 2001, c = Institute of Medicine, 2004.

Table 4 shows that nine minerals were found in all the samples. In honey beans bread, there was increase in magnesium, potassium, sodium, manganese, iron, zinc and phosphorus and a decrease in calcium and copper when compared with the control. In soybeans bread, there was increase in all the minerals but a slight decrease in calcium relative to the control bread. Melon bread showed increase in calcium, magnesium, iron, zinc and phosphorus but a decrease in potassium, sodium, manganese and copper relative to the control bread. In terms of daily requirements, most of the minerals contained more than required except potassium and phosphorus. Cobalt, Chromium, Cadmium, Lead and Nickel which have been noted as threat to live were not found in all the bread samples. This means that the wheat and fortified bread samples are safe and free from contaminants that could pose threat to human life by their consumption and are hereby healthy.

Conclusion

Based on the results of this study, fortification of wheat flour with soybeans, honey beans and melon significantly enhanced the nutritional value of wheat bread especially in terms of protein and essential minerals. This will provide a single meal which is cheaper and with more readily available source of protein. Also, use of plant protein will stimulate more production and reduce wheat importation thereby, creating wealth, ensuring food security and reducing overdependence on wheat importation. Production of composite bread especially with soybeans should be greatly considered on a commercial level.

Authors Contribution

This research work was carried out by all authors. The author OA designed the study, performed statistical analysis and wrote the first draft of the manuscript. All authors contributed to the analysis of the samples, collection of data and development of the final manuscript. All authors also read and approved the final manuscript.

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