

Extruded Adult Breakfast Based on Millet and Soybean: Nutritional and Functional Qualities, Source of Low Glycemic Food

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Abstract

This study was conducted in order to develop a breakfast food with a low glycemic index from a formulation based on the pre-treated foxtail millet and soybean. The pre-treatment applied to raw materials (germination of millet, pre-cooking or roasting soybeans) have helped to improve the functional properties of blends. Flours made from germinated millet have a low estimated glycemic index, or 35.39 and 34.49 respectively for GMRS and GMPCS. During storage periods, the four blends pH decreased significantly difference ($p < 0.05$). The FA and IPV of all blends increase slightly during storage without reaching their standard values which were set at no more than 30 meq.g of iodide/1Kg for FA and 3 mg of KOH/100 g for IPV. At room temperature the blends can be stored for 90 days without preservatives.

Keywords: Germinated millet; Extrusion cooking; Low glycemic food; Fat acidity; Initial peroxide value

Introduction

According to World Health Organization (WHO) [1], in 2005, 194 million people suffering from diabetes worldwide. Two thirds of them lived in developing countries. In 2025, it will be more than 330 million people who live with diabetes. The region of the world that will have the largest number of diabetics will be the Southeast Asia with a forecast of 82 million diabetics. Deprived of food during the night, wake up our body needs a healthy dose of energy. Breakfast is the first meal of the day, is widely referred to as the most important meal of the day. Skipping breakfast does not catch up [2]. Studies on attention and working memory have shown that a breakfast rich in carbohydrates helps to maintain the same level of mental performance during morning. Nutritionists stress the importance of breakfast, especially for adults who have diabetes.

In addition, the grain are digested slowly, which result in a prolonged satiety and a gradually release of energy. It is therefore strongly advised to eat a breakfast with hot food (flakes, semolina spelled or millet with fruit and vegetables) because they provide a lasting satiety and a great appetite for lunch. Moreover the combination of vegetables cooked cereal helps eliminate toxins. Generally cereals and cereal products are the main breakfast in the most developing countries; these are maize, rice, sorghum or millet [3]. These cereals constitute important sources of carbohydrates, vitamins, minerals and fibers; indeed they contain low content of proteins and lipids [4]. The germination transforms the appearance of seeds, but also improves the nutritional value of cereals while raising their content in lysine and in tryptophan and in vitamins [5]. To improve protein and fat quantities in cereal foods, attention is focused on processing technology and use of inexpensive sources of proteins. To increase the protein content of pearl millet and improve the amino acid profile, especially lysine, the use of soybean flour as a protein supplement has been suggested. Soybean flour represents a high protein supplement relative to compositional, functional and nutritional properties; it contains approximately 40% of protein and 20% of fat. Several works have reported that animal and vegetable proteins influences lipid metabolism in human and experimental animals [6]. Soybean protein in particular, as compared with animal protein in was effective in improving hypercholesterolemia and reducing body weight [7]. Recent studies indicated that supplementation of wheat and maize

food products with soybean improve the nutritional quality of proteins, sensory and rheological properties [8,9]. Lysine is deficient in millet, but soybean is particularly high in lysine [10]. In main legumes, the level of sulphur-amino acid is low, but cereals have high level of sulphur-amino acid such as methionine. So the high lysine and low sulphur-amino acids in soybean could suitable to fortify millet products. The potential for incorporation and utilization of soybean as a source of protein in diet serves as the basis of the need for adequate processing. Soybeans have been processed in a number of ways, but a more recent processing method is extrusion cooking [1]. Low cost extrusion has been studied in a number of countries with encouraging success [11], and extrusion cooking is a versatile food processing technology that rapidly mixes and kneads feed materials at temperature of over 100°C, to cook and dry the product in a relatively short time [12].

Therefore, this study looks into the formulation of some composites blends based on whole or germinated foxtail millet and pre-cooked or roasted soybean, the functional properties, estimated glycemic index, sensory quality and shelf life of formulated blends.

Materials and Methods

Materials

Seeds of millet (Foxtail millet or *Setaria italica*) and soybean (*Glycine max* L. merr.) used for this study were bought from local market (Wuxi, China) in July 2010.

Pre-processing

Germination: The millet was germinated using the method described by Coulibaly and Chen [13]. The grain of millet were cleaned

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manually, soaked in distilled water in ration of 1:3 (p/v) for 24 hrs, and then the grains were spread on a white cotton fabric and kept wet by spraying of distilled water. After 3 days of germination, the seeds were dried at 45°C to constant weight and were grounded (Figure 1).

Pre-cooking and roasting: The seeds of soybean were cleaned manually to remove broken seeds and other extraneous materials, soaked in distilled water in ratio of 1:5 (p/v) for 24 hrs. The soaked seeds was drained: One part was put in boiled water for 30 minutes. After boiling, the seeds were cooled, dried at 50°C and grounded (Figure 1).

The second part was dried at 50°C after soaking. The dried seed were roasted at 110°C for 10min, cooling and milling (Figure 1). The milled sample was sifted in 80 mesh for soybean flour and 60 mesh for millet flour.

Proximate analysis

Samples for each flour were analyzed for moisture, protein, lipids and ash according to AOAC methods [14], and for crude fiber, the percentage fiber was obtained according to [15] method. The carbohydrate content of each blend was determined by difference:

$$\% \text{carbohydrate} = 100 - (\% \text{moisture} + \% \text{protein} + \% \text{fat} + \% \text{ash}).$$

Minerals in the samples were extracted by Mahajan and Chauhan [16]. About 1 g sample was shaken with 10 ml of 0.03M HCl for 3 hours at 37°C and then filtered. The clear extract obtained was oven-dried at 100°C and then the sample was acid-digested with diacid mixture (HNO₃:HClO₄, 5:1, v/v). The amount of extractable minerals was determined using atomic absorption spectrophotometer. Phosphorus was determined using a spectrometer at wave length 690 nm.

For the determination of the amino acids, samples of protein concentrate (100 mg) for all the samples and 5 ml 6 M HCl were added to a 50 ml stopper bottle and sealed. The air was removed by keeping

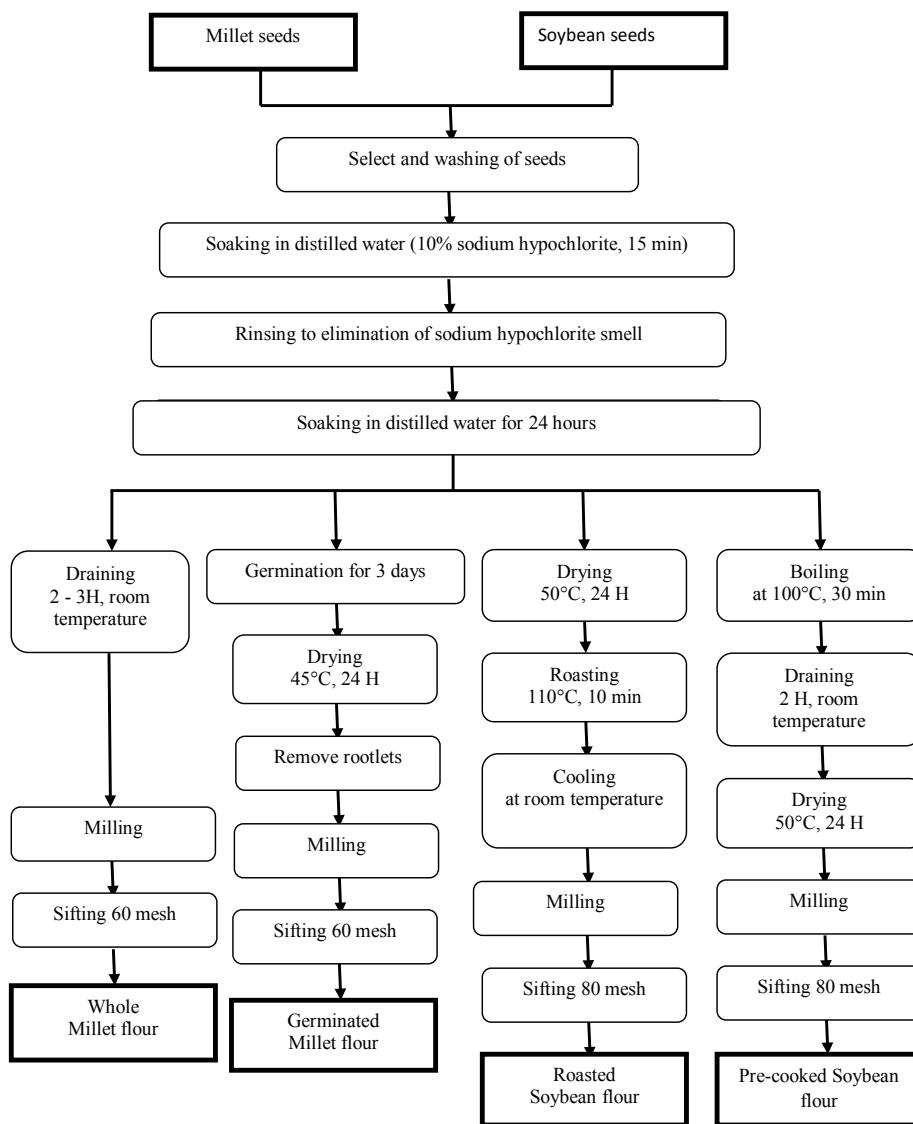


Figure1: Flow diagram for the preparation of the flour of raw materials from millet and soybean

the sample in a vacuum chamber. The sealed samples were placed in an oven at 120°C for 16 hours to hydrolyze. After hydrolysis, 5 ml of 2 mM norleucine internal standard was added and the solution was filtered in a 0.2 µl Gelman membrane filter. 1 ml of stock sample was pipetted into a 50 ml borosilicate glass serum bottle and dried in a freeze-drier. 1 ml of sodium diluent buffer (pH 2.2) was added to the freeze-dried residue and transferred to a 1.5 ml micro-centrifuge tube for HPLC analysis. The prepared samples were injected as 2.5 µl volumes and run on a Waters HPLC (Waters Corporation, Milford, MA, USA) at a flow rate of 0.4 ml/min with a Pickering sodium ion-exchange column of 4x150 mm (Pickering Laboratories, Inc., Mountain View, CA, USA) and sodium eluent (pH 3.15 and 7.40). TRIONE® ninhydrin reagent was added with post column instrument (TRIONE® ninhydrin derivatization instrument, Pickering Laboratories, Inc.). The light absorbance of amino acids was detected with an UV Visible detector (Pickering Laboratories Inc.) at 570 nm wavelength and the amino acids were quantified by comparing with standard amino acid profiles. Methionine and cysteine were determined separately by oxidation products according to the performic acid procedure of Moore [17] before hydrolysis in 6 M HCl. Tryptophan was determined after alkaline hydrolysis by isocratic ion-exchange chromatography with *o*-phthalaldehyde derivatization followed by fluorescence detection by Ravindran and Bryden [18]. Amino acid composition was reported as g/100 g of protein.

Formulation and preparation of mixture

It's based on carbohydrate, protein, and fat content of the blends. Algebraic equation is able to provide formulation that best fit the nutrient requirements for adult's food. From this design (Table 1) we obtained 4 systems of equation:

Whole millet/Pre-cooked soybean (WMPCS)

$$0.119X+0.3165Y=0.18 \quad (1)$$

$$0.0409X+0.1992Y=0.09 \quad (2)$$

$$0.7550X+0.3123Y=0.63 \quad (3)$$

$$X+Y=100 \quad (4)$$

Germinated millet/Pre-cooked soybean (GMPCS)

$$0.1342X+0.3165Y=0.18 \quad (1)$$

$$0.0384X+0.1992Y=0.09 \quad (2)$$

$$0.7385X+0.3123Y=0.63 \quad (3)$$

$$X+Y= 00 \quad (4)$$

Whole millet/Roasted soybean (WMRS)

$$0.119X+0.3165Y=0.18 \quad (1)$$

$$0.0409X+0.2008Y=0.09 \quad (2)$$

$$0.7550X+0.3280Y = 0.63 \quad (3)$$

$$X+Y=100 \quad (4)$$

Germinated millet/Roasted soybean (GMRS)

$$0.1342X+0.3165Y=0.18 \quad (1)$$

$$0.0384X+0.1992Y=0.09 \quad (2)$$

$$0.7385X+0.3280Y=0.63 \quad (3)$$

$$X+Y=100 \quad (4)$$

Of these:

The line (1) was the protein equation

The line (2) was the fat equation

The line (3) was the carbohydrate equation

The last line (4) was the representation of total mix

There were several methods by which this resolution may be done. Algebraic calculations permit to find the amounts of the flours needed. The amount of each flour to be used was got by solving these four systems of equation. The results were showed in Table 2.

Extrusion cooking

The mixed flours were extruded using a co-rotating twin screw extruder. The extruder has three independent zones and the effective cooking zone was set to 100, 120 and 140°C respectively for 1, 2 and 3 in the barrel. The length to diameter (L/D) ratio for extruder was 20:1. The diameter of the hole in the die was 6 mm with a die length of 27 mm. The moisture of different blends were adjusted by addition of a pre-determined amount of the water (12-16% moisture).The extruded products were collected and dried in oven air at 110°C for 5 min, the products were cooled, milled and tempered at 4°C before being stored into plastic bags for future analysis.

Determination of functional properties

The samples were evaluated for Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC), Hydrophilic/Lipophilic Index (HLI), Solubility (So), Swelling Power (Sp), Bulk Density (Bd) and *in vitro* protein digestibility.

Water and oil absorption capacity: WAC was determined using the method of Adebowale et al. [19] with slight modification. 1 g of each sample was dispersed in 10 ml of deionized water. After 5 minutes of stirring, the dispersion was incubate at 75°C in water bath for 30 minutes. The suspension was centrifuge at 4000 rpm for 20 minutes and the supernatant was measured into a 10 ml graduated cylinder. WAC was obtained as the difference between the initial volume of water added to the sample and the volume of the supernatant.

For OAC, 10 ml of sunflower oil were added to 1 g of each sample.

| Raw materials | Nutrients content (g / 100g raw materials) | | |
|-------------------|--|---|---|
| | Carbohydrate | Protein | Fat |
| Millet flour (X) | 75.50 ^a / 73.85 ^b | 11.90 ^a / 13.42 ^b | 4.09 ^a / 3.84 ^b |
| Soybean (Y) | 31.23 ^c / 32.80 ^d | 31.65 ^c / 31.65 ^d | 19.92 ^c / 20.08 ^d |
| Final product (%) | 63% | 18% | 9% |

^{a/b} : Carbohydrate, protein and fat content in whole millet / germinated millet

^{c/d} : Carbohydrate, protein and fat content in pre-cooked / roasted soybean

X= Amount of millet flour in the mix

Y= Amount of soybean flour in the mix

Table 1: The formulation design

| Flours | WMPCS | GMPCS | WMRS | GMRS |
|--------------------|-------|-------|-------|-------|
| Whole millet | 71.79 | - | 72.54 | - |
| Germinated millet | - | 73.80 | - | 74.98 |
| Pre-cooked soybean | 28.21 | 26.20 | - | - |
| Roasted soybean | - | - | 27.46 | 25.02 |

WMPCS: Whole millet/Pre-cooked soybean. GMPCS: Germinated millet/Pre-cooked soybean

WMRS: Whole millet / Roasted soybean. GMRS: Germinated millet / Roasted soybean

Table 2: Different flours quantities (g/100g total mix)

Samples were stirred during 5 minutes and allowed to stand for 30 minutes at room temperature. The suspension was centrifuge in the same condition as in water absorption capacity. OAC was obtained as the difference between the initial volume of sunflower oil added to the sample and the volume of supernatant.

Hydrophilic/lipophilic index: HLI was estimated as the ratio of WAC to OAC and used to define the relative affinity of flour for water and oil.

Solubility and swelling power: Solubility and swelling power of the samples were determined in triplicate according to the procedure: 1 g of each sample was suspended in 20 ml of deionized water and heated at 90°C for 1 hour in water bath with constant stirring. The suspension was cooled at 30°C and then centrifuged at 4000 rpm for 15 minutes. The supernatant was poured into aluminum dishes, decanted and weighted the swollen granules. After weighting the supernatant was dried at 110°C for 12 hours and the weight of dry solids was determined. The solubility and swelling power were determined using the formulas:

$$So = (\text{weight of supernatant dried}) / (\text{sample weight}) \times 100$$

$$Sp = (\text{weight of sediment}) / (\text{sample weight} \times (100 - So))$$

Bulk density: 10 g of the extrudates product flour was placed into 25 ml graduated cylinder and packed by gently tapping of the cylinder on a bench top, from a height of 5-8 cm. The final volume of the flour was recorded. The bulk density was recorded as grams per milliliter of sample [20].

In vitro protein digestibility: *In Vitro* Protein Digestibility (IVSPD) was determined by Hsu et al. [21], Dahlin and Lorenz [22] with slight modification. 1 g of sample was suspended in 35 ml of deionized water. After rehydration, samples were placed in a water bath at 37°C and the pH was adjusted to 8 using 0.1 N NaOH or 0.1 N HCl. Prepare trypsin solution at a concentration of 1.6 mg/ml, maintained in a ice bath and adjusted her pH to 8 with 0.1 N NaOH or 0.1 N HCl. Five milliliters of enzyme solution were added to the suspension. The pH drop was recorded 15 seconds after enzyme addition and at 2 minutes intervals for 10 minutes. Triplicate analyses were performed for each sample. The percentage protein digestibility (B) was calculated in the following equation: $B = 210.4 - 18.1A$, where A is the change in pH after 10 min.

Pasting properties

The pasting properties of the extruded products were evaluated using a Micro Visco-amilograph. Flour slurry containing 20% solids (w/w, dry basis) was heated from 50 to 95°C, held at 95°C for 15 minutes and cooled at 50°C. The pasting properties (Beginning of gelatinization (°C), pasting time (min), peak viscosity (BU), viscosity at 95°C (BU), paste stability (BU), viscosity at 50°C (BU), cooking time (min) and setback value (BU).

Hydrolysis index and estimated glycemic index

Hydrolysis Index (HI) was evaluated by Englyst et al. [23] with slight modification. 1 g of each sample and 15 ml of acetate buffer (0.1 M; pH 5) was dissolved by stirring. After equilibrating at 37°C for 5 minutes, 5 ml acetate buffer containing 2.50 IU of α -amylase from *Bacillus amyloliquefaciens* was added, followed by incubation in water bath at 37°C with shaking. Aliquots of hydrolysis solution (0.1 ml) were taken at different time intervals (30 to 180 min) and the α -amylase inactivated immediately by holding the tubes in a boiling water bath for 5 min.

1ml of acetate buffer (0.1 M; pH 5) was added and the residual starch digested to glucose by adding 30 μ l amyloglucosidase and incubating at 60°C for 45 minutes. The glucose content of the hydrolysate was determined using a dinitrosalicylic acid reagent [24]. White bread was used as a reference sample. A Hydrolysis Index (HI) was calculated as follows:

$$HI = \frac{\text{area under digestibility curve of sample (0-180min)} \times 100}{\text{area under digestibility curve of white bread reference (0-180min)}}$$

According to Ackerberg et al. [25], Bjorck et al. [26] that found a highly significant correlation ($r=0.826$) between HI and Estimated Glycemic Index (EGI). The equation of Granfeldt was used to estimate EGI. $EGI = 0.862HI + 8.198$.

Sensory evaluation

The 4 formulated samples obtained from whole or germinated millet and pre-cooked or roasted soybean were made into gruel using hot water (28g/100 ml water) and served to 20 trained students. The sample were rated based on appearance, flavor, taste, mouth feel (texture) and overall acceptability using 5 point hedonic scale scored from dislike extremely (1) to like extremely (5). The panelists were providing with clean potable water for mouth rinsing between samples.

Shelf life analysis

The shelf life of the different products was expressed by Fat Acidity (FA) and Initial Peroxide Value (IPV). Fat acidity was evaluated by AOAC [14] with slight modification. 2 g of extruded blend were weighed into a centrifuge tube. 30 ml of benzene-ethanol (2:1) mixture were added. The solutions were shaking for about 2 minutes and incubate at 25°C for 10 minutes. The resulting solution was centrifuged at 5000 rpm for 10 minutes. A 10 ml of supernatant 2 or 3 drops of phenolphthalein are added, and then the solution was titrated with 0.1 N potassium hydroxide (KOH) solution until obtaining the pink color. The FA was expressed as milligrams of KOH per 100 g of dry flour (mg of KOH/100 g).

$$FA = (5.611 \times V) / W, \text{ V: Volume of KOH, W: Weight of sample}$$

The IPV was determined by AOAC [14] with slight modification. 2 g of sample were weighed into a 100 ml conical flask. 28 ml of glacial acetic acid-chloroform (3:2) mixture were added and displace the air above the liquid with CO_2 . 1 ml of saturated potassium iodide (KI) solution was added. Shaking the flask and place it at dark place for 15 minutes. Now added distilled water until to 50 ml and mix well. The resulting solution was filtered through filter paper. Added 1 ml of 1% starch solution at filtrate and titrated with 0.01N sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) until the blue color disappeared. Carry out a blank determination simultaneously.

$$IPV = ((V_a - V_b) \times N \times 1000) / W$$

V_a: Volume of 0.01 $Na_2S_2O_3$ required for the sample

V_b: Volume of 0.01 $Na_2S_2O_3$ required for blank

The IPV was expressed as mill-equivalent grams of iodide per 1 Kg of sample (meq.g of iodide/1 Kg).

Statistical analysis

The results are presented as mean values and standard deviations of triplicate analysis. Analysis of variance and significant differences among means were tested by one way ANOVA using SAS 2003.

Results and Discussion

Proximate, minerals and amino acid composition

The proximate and mineral composition of four extruded mixed flour are presented in Table 3. In all the four extruded mixed blends, the results showed that moisture, protein, fat and carbohydrate were not significantly different ($p < 0.05$), because formulation was made according to these parameters. The fat can be a transport vehicle for fat soluble vitamins; can also provide essential fatty acids like that of n-3 and n-6 polyunsaturated fatty acids which have the ability to reduce blood level of LDL cholesterol thereby reducing the risk of coronary heart disease.

The fiber content in extruded blends which contain germinated millet flour was significantly higher than that which contain whole millet flour ($p < 0.05$). Fiber is made up of cellulose, hemicelluloses and lignin, and is usually defined as a polysaccharide plus lignin that is not digested. During germination process the fiber content increase significantly, this result was consistent with Sade [27], Ocheme and Chinma [28]. Since germination is essentially a degradative process of structural and storage molecules. The increase in fibre content could be due alterations of other components (carbohydrates, coarse cell), also may be due to starch breakdown during germination. In the extrusion process, thermal treatments can change fiber content; the fiber content tends to increase, mainly due to enzyme resistant starch fraction. We observe that, the quantities of minerals (K, P, Mg, Ca, Fe and Na) are high in the mixed blends containing the germinated millet flour. In the extruded products, the quantities of minerals tend to agree with the recommendations of FAO/WHO, 1998 [29]. These minerals are important in teeth and bone formation, also in the blood control. Phosphorus is an important mineral for energy production and is an essential component of ATP the energy store of the body. It also forms an essential part of nervous system and cell membranes. Magnesium also helps to relax blood vessels, enhances nutrient delivery by improving the blood flow and maintains the blood pressure and thus further protects the cardiovascular system.

The essential amino acid composition of whole and germinated millet, pre-cooked and roasted soybean, and all extruded blends are shown in Table 4, along with the essential amino acid composition according to the FAO/WHO/UNU, 2007 [30]. We find that different

| Nutrients | WMPCS | GMPCS | WMRS | GMRS |
|--------------|----------------|----------------|----------------|----------------|
| Moisture | 5.49 ± 0.014a | 5.71 ± 0.019a | 4.91 ± 0.034a | 5.33 ± 0.027a |
| Fat | 9.09 ± 0.03a | 8.76 ± 0.02a | 9.26 ± 0.041a | 9.05 ± 0.02a |
| Protein | 18.25 ± 0.014a | 18.37 ± 0.042a | 18.20 ± 0.005a | 18.30 ± 0.004a |
| Ash | 3.55 ± 0.025a | 3.33 ± 0.026a | 3.29 ± 0.015a | 3.34 ± 0.02a |
| Fiber | 9.72 ± 0.031b | 10.90 ± 0.01a | 9.79 ± 0.01b | 10.98 ± 0.016a |
| Carbohydrate | 63.62 ± 0.034a | 63.83 ± 0.15a | 64.34 ± 0.061a | 63.98 ± 0.35a |
| Sodium | 1.962 | 2.84 | 1.88 | 2.81 |
| Potassium | 576.48 | 639.98 | 579.98 | 640.13 |
| Iron | 5.99 | 8.95 | 6.19 | 8.99 |
| Calcium | 133.62 | 159.62 | 134.87 | 159.12 |
| Magnesium | 140.12 | 162.68 | 139.40 | 162.94 |
| Phosphorus | 550 | 575 | 548.87 | 576.11 |

Values represent means of triplicate. Values in the same line with the same letter are not significantly different ($p < 0.05$).

WMPCS: Whole millet/Pre-cooked soybean.

GMPCS: Germinated millet/Pre-cooked soybean

WMRS: Whole millet / Roasted soybean.

GMRS: Germinated millet / Roasted soybean

Table 3: Proximate (%) and mineral (mg/100g) composition of raw flours and extruded products

soybean meals contain a high amount of essential amino acids compared to those of millet. In the germinated millet flour, we noted a small change in the amino acid profile; it may be due to the transamination during germination. Germination imparted little change to the amino acid composition of the foxtail millet. In comparison with the FAO/WHO/UNU requirements of amino acids [30], the essential amino acid profile of extruded products is clearly superior. We can say that the use of soybean as a source of protein is suitable.

Water, oil absorption capacity and hydrophilic lipophilic index

Table 5 presents the results of WAC, OAC and HLI for the four extruded products. WAC of flour is an index of the maximum amount of water that it can take up and retain. The ability to absorb water is particularly important during reconstitution into the product before consumption. In our case, the WAC was significantly different ($p < 0.05$). The WAC of GMPCS (4.13 ml/g) and GMRS (4.17 ml/g) was higher than that of WMPCS (3.43 ml/g) and WMRS (3.45 ml/g). Obatolu and Cole [31] observed a similar result. The higher WAC of the extruded products which contain the germinated foxtail millet may be due to the presence of more hydrophilic carbohydrate in those flours. This also could be attributed to changes in protein quality during germination.

The OAC of WMPCS, WMRS, GMPCS and GMRS was not significantly different ($p < 0.05$). The mechanism of oil absorption may be explained as a physical entrapment of oil related off to the non polar side chain of protein. The conformational changes of starch and protein molecule could have occurred during germination and exposed less hydrophobic than hydrophilic groups.

The ratio of WAC to OAC was describing her as the Hydrophilic Lipophilic Index. The HLI of WMPCS, GMPCS, WMRS, and GMRS (Table 4) was 2.45, 2.85, 2.43 and 2.84 respectively. The HLI was significantly different ($p < 0.05$). HLI of products which contain germinated millet was slightly higher than those based on whole millet. Germination of seeds improves the WAC of the flours produced from these germinated seeds; those observations are in agreement with the results of other studies [32]. Also the heat treatment during extrusion cooking could have affected the WAC and HLI of the products [33,34].

Bulk density

The (Bd) values of different extruded flours are shown in Table 5. The (Bd) of WMPCS and WMRS was not significantly different ($p < 0.05$), it's the same finding for GMPCS and GMRS. But the extruded meals containing germinated millet flour (GMPCS, GMRS) are significantly different ($p < 0.05$) from those containing whole millet flour (WMPCS, WMRS). The Bd of blends obtained from whole millet flour (WMPCS, WMRS) was higher than that of flours obtained from germinated millet flour (GMPCS, GMRS). Njintang et al. [35] reported the same observation. This may be due to the degradation of starch during germination. Bulk density values obtained were approximately identical with those found by Edema et al. [36] for maize blends. The composite blends with germinated millet flour may serve as good breakfast food.

Solubility and swelling power

The solubility and swelling power of different extruded products are presented in Table 5. The four products are significantly different ($p < 0.05$) between them concerning the solubility and swelling power. 47.86, 56.19, 52.28 and 70.95% are respectively the solubility of WMPCS, GMPCS, WMRS and GMRS. The swelling power of WMPCS, GMPCS, WMRS and GMRS are 30.14, 35.11, 31.08 and 44.37% respectively. The

| EAA | Whole millet | Germi-nated millet | Pre-cooked soybean | Roasted soybean | WMPCS | GMPCS | WMRS | GMRS | FAO/ WHO/ UNU ^a 2007 Adult |
|---------|--------------|--------------------|--------------------|-----------------|-------|-------|------|------|---------------------------------------|
| Lys | 3.85 | 3.02 | 7.12 | 6.91 | 4.77 | 4.09 | 4.68 | 3.99 | 4.5 |
| Met | 3.06 | 3.05 | 4.30 | 3.05 | 3.41 | 3.38 | 3.06 | 3.05 | - |
| Ile | 4.59 | 3.84 | 5.07 | 5.06 | 4.72 | 4.17 | 4.72 | 4.15 | 3.0 |
| Leu | 7.6 | 6.88 | 7.25 | 7.12 | 7.50 | 6.98 | 7.47 | 6.94 | 5.9 |
| Met+Cys | 3.5 | 3.40 | 4.01 | 3.89 | 3.64 | 3.56 | 3.61 | 3.53 | 1.6 |
| Phe | 6.27 | 5.98 | 8.16 | 8.17 | 6.80 | 6.55 | 6.79 | 6.53 | - |
| Phe+Tyr | 8.71 | 7.85 | 9.33 | 9.35 | 8.88 | 8.24 | 8.88 | 8.23 | 3.8 |
| Thr | 3.68 | 2.78 | 4.39 | 4.35 | 3.88 | 3.20 | 3.86 | 3.17 | 2.3 |
| Val | 5.81 | 4.74 | 4.80 | 4.79 | 5.53 | 4.75 | 5.53 | 4.75 | 3.9 |
| His | 2.11 | 1.75 | 2.71 | 2.71 | 2.28 | 1.99 | 2.27 | 1.98 | 1.5 |
| Trp | 1.39 | 1.06 | 1.6 | 1.59 | 1.45 | 1.20 | 1.45 | 1.19 | 0.6 |

^a: FAO/WHO/UNU energy and protein requirements (2007)

EAA: Essential Amino Acid

WMPCS: Whole millet/Pre-cooked soybean.

GMPCS: Germinated millet/Pre-cooked soybean

WMRS: Whole millet / Roasted soybean.

GMRS: Germinated millet / Roasted soybean

Table 4: Essential amino acid composition (g/100g) of raw flour and the four extruded flours

| Sample | WAC (ml/g) | OAC (ml/g) | HLI | Bd (g/ml) | Sp (%) | So (%) | IVPD (%) |
|--------|--------------------------|--------------------------|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| WMPCS | 3.43 ± 0.06 ^b | 1.4 ± 0.00 ^a | 2.45 ± 0.04 ^b | 0.42 ± 0.001 ^a | 30.14 ± 0.27 ^d | 47.86 ± 0.15 ^d | 76.26 ± 0.58 ^c |
| GMPCS | 4.13 ± 0.06 ^a | 1.45 ± 0.06 ^a | 2.85 ± 0.40 ^a | 0.38 ± 0.003 ^b | 35.11 ± 0.08 ^b | 56.19 ± 0.06 ^b | 82.17 ± 0.46 ^a |
| WMRS | 3.45 ± 0.06 ^b | 1.42 ± 0.06 ^a | 2.43 ± 0.15 ^b | 0.41 ± 0.002 ^a | 31.08 ± 0.34 ^c | 52.28 ± 0.47 ^c | 73.94 ± 0.28 ^d |
| GMRS | 4.17 ± 0.1 ^a | 1.47 ± 0.06 ^a | 2.84 ± 0.13 ^a | 0.37 ± 0.002 ^b | 44.37 ± 0.45 ^a | 70.95 ± 0.81 ^a | 78.64 ± 0.28 ^b |

Values represent means of triplicate. Values in the same column with the same letter are not significantly different (p<0.05).

WAC: Water Absorption Capacity, OAC: Oil Absorption Capacity, HLI: Hydrophilic-Lipophilic Index

Bd: Bulk Density, Sp: Swelling Power, So: Solubility, IVPD: In Vitro Protein Digestibility

Table 5: Functional properties of different extruded products

| | WMPCS | GMRS | WMRS | GMPCS |
|-----|---------------|---------------|---------------|---------------|
| HI | 56.97 ± 0.15a | 31.55 ± 0.38b | 57.37 ± 0.60a | 30.50 ± 0.46b |
| EGI | 57.30 ± 0.13a | 35.39 ± 0.33b | 57.65 ± 0.52a | 34.49 ± 0.39b |

Values represent means of triplicate. Values in the same line with the same letter are not significantly different (p<0.05).

Table 6: Hydrolysis Index (HI) and estimated Glycemic index (EGI) extruded products

solubility and swelling power are higher in formulated blends which contain germinated millet flour. However, the solubility of GMRS was significantly (p<0.05) higher than in others formulated blends. The swelling power of WMPCS and WMRS was lower than GMPCS and GMRS. All this could be due to the fact that the quantity of soybean flour in WMPCS and WMRS was less than that of GMPCS and GMRS, Akingbala et al. [37], Shayo et al. [38] reported that soybean flour has no starch hence amylose. Thus addition of soybean flour reduces the percentage of starch in the blends. However these changes concerning solubility and swelling power may be due to the starch content (amylose and amylopectin chains) in different extruded flours.

In vitro protein digestibility

The IVPD results of the four extruded products are given in Table 5. Values are significantly different (p<0.05). 76.26, 82.17, 73.94 and 78.64% were respectively the *in vitro* protein digestibility values of WMPCS, GMPCS, WMRS and GMRS. Results show that the IVPD was higher in formulated blends which contain germinated millet flour and also which contain pre-cooked soybean flour. The effect of germination on protein digestibility can be related to metabolic activities during germination; it has been reported by Anigo et al. [39]. The IVPD of blends containing pre-cooked soybean flour was higher than which containing roasted soybean flour; this may be due to the effect of moist heat treatment. The moist heat treatment (pre-cooking) contributed to increase the protein digestibility of blend. The pre-

cooking may be caused an early conformational change of the protein chain in simple chain, making them more digestible. The heat treatment during extrusion cooking affected the IVPD. This observation suggests, a higher degree of cooking it's a higher protein digestibility. Heating improves digestibility due to protein denaturation which results in opening of protein structure. Dahlin and Lorenz [22] has been reported that the heating may benefit flour protein digestibility by rendering the protein more susceptible to hydrolysis due to structural changes, the destruction of enzymes inhibitors or decreases in lipid-protein, starch-protein complexes.

Estimated glycemic index and hydrolysis index

The Hydrolysis Index (HI) represents the proportion of starch that is theoretically digestible [40]. Using the HI value in the formula by Ackerberg et al. [25], Bjorck et al. [26], the EGI of WMPCS, GMPCS, GMRS and WMRS are respectively 57.30; 34.49; 35.39 and 57.65 (Table 6). According to Dietary Guidelines for Americans (DHHS/USDA, 2005), there are three class of glycemic index. High glycemic index (GI>70), intermediate glycemic index (56<GI<69) and low glycemic index (GI<55). In view of this classification, we can say that all flours are not in the class of high glycemic index foods. Millets helps to lower blood glucose levels and improves insulin response. Besides the magnesium is a co-factor in various enzymes involved in the secretion of insulin and metabolism of glucose in the body. The high calcium, high soluble fiber, low fat and low glycemic index of germinated grains

| Formulated blends | WMPCS | GMPCS | WMRS | GMRS |
|----------------------------------|---------|---------|---------|---------|
| Beginning of gelatinization (°C) | 68.4 | 69.3 | 68.4 | 68.8 |
| Pasting time (min) | 11 : 05 | 08 : 25 | 10 : 30 | 08 : 25 |
| Peak viscosity (BU) | 564 | 223 | 533 | 194 |
| Viscosity at 95°C (BU) | 239 | 28 | 267 | 20 |
| Paste stability (BU) | 52 | 168 | 29 | 159 |
| Viscosity at 50°C (BU) | 512 | 55 | 504 | 35 |
| Setback value (BU) | 334 | 26 | 334 | 16 |
| Cooking time (min) | 4 : 55 | 2 : 15 | 4: 00 | 2 : 05 |

Paste stability = difference between the peak viscosity and viscosity at 50°C

Cooking time = difference between time to reach gelatinization temperature and time to obtain maximum viscosity during heating. BU = Brabender Unit.

Table 7: Pasting characteristic of the different formulated blends

| Sample | Appearance | Flavor | Taste | Mouth feel | acceptability |
|--------|--------------|--------------|--------------|--------------|---------------|
| WMPCS | 3.71 ± 0.14d | 3.79 ± 0.12d | 3.51 ± 0.22c | 3.17 ± 0.12b | 3.05 ± 0.18d |
| WMRS | 4.79 ± 0.21c | 5.55 ± 0.23b | 5.35 ± 0.23b | 3.26 ± 0.15b | 5.17 ± 0.20b |
| GMPCS | 4.41 ± 0.13b | 4.65 ± 0.36c | 3.59 ± 0.32c | 4.89 ± 0.11a | 4.39 ± 0.12c |
| GMRS | 5.92 ± 0.18a | 6.73 ± 0.42a | 6.66 ± 0.34a | 5.23 ± 0.09a | 7.05 ± 0.14a |

Values represent means of triplicate. Values in the same column with the same letter are not significantly different (p<0.05).

Table 8: Sensory properties of different extruded products

| Room temperature | | 15 days | 30 days | 45 days | 60 days | 75 days | 90 days |
|------------------|-------|---------------|---------------|---------------|---------------|---------------|---------------|
| FA | WMPCS | 0.46 ± 0.01d | 0.69 ± 0.04b | 0.94 ± 0.01c | 1.04 ± 0.01d | 1.11 ± 0.01d | 1.12 ± 0.01d |
| | GMPCS | 0.72 ± 0.01b | 0.99 ± 0.04a | 1.16 ± 0.02b | 1.26 ± 0.06b | 1.37 ± 0.07b | 1.38 ± 0.04b |
| | WMRS | 0.56 ± 0.025c | 0.72 ± 0.05b | 0.96 ± 0.02c | 1.12 ± 0.05c | 1.20 ± 0.05c | 1.23 ± 0.01c |
| | GMRS | 0.74 ± 0.02a | 1.15 ± 0.02a | 1.39 ± 0.02a | 1.47 ± 0.01a | 1.56 ± 0.02a | 1.79 ± 0.02a |
| IPV | WMPCS | 14.33 ± 0.04d | 16.84 ± 0.02b | 18.19 ± 0.05d | 22.57 ± 0.03c | 24.67 ± 0.04c | 26.14 ± 0.01d |
| | GMPCS | 15.59 ± 0.03b | 18.18 ± 0.04c | 22.63 ± 0.04b | 25.11 ± 0.04b | 26.12 ± 0.02b | 27.42 ± 0.03b |
| | WMRS | 14.71 ± 0.02c | 16.98 ± 0.02b | 19.76 ± 0.01c | 22.95 ± 0.03c | 23.71 ± 0.03b | 25.64 ± 0.02c |
| | GMRS | 15.87 ± 0.05a | 18.84 ± 0.03a | 23.76 ± 0.04a | 25.87 ± 0.05a | 26.39 ± 0.03a | 27.91 ± 0.05a |

Values represent means of triplicate. Values in the same column with the same letter are not significantly different (p<0.05).

FA: Fat Acidity, IPV: Initial Peroxide Value

Table 9: Fat acidity and Initial Peroxide value of extruded products

is effective in controlling the blood glucose levels of diabetics [41,42]. The studies from different experiences of people taking medicine and multigrain flour showed that, the multigrain flour on consumption energized and maintained the glucose level [43]. Whereas medicines did not give such feeling because the blood sugar level went down and required energy could not be obtained from it; medicine has not provided any nutrition.

Pasting characteristics of the different formulated blends

Table 7 presents the pasting characteristics of the formulated products. The beginning of gelatinization for all formulated blends ranged from 68.4 to 69.3°C. The results gave a peak viscosity of 564, 223, 533 and 194 BU for WMPCS, GMPCS, RMRS and GMRS respectively. The setback value, pasting time, past stability, cooking time, viscosity at 95°C and at 50°C were also shown in Table 8. WMPCS and WMRS have a same gelatinization temperature, similarly to GMPCS and GMRS. This is due to the hardening effect of ingredients and the structure of starch granules after the pre-treatment of raw materials. The blends which based on the whole millet have a higher peak viscosity. The data showed low viscosity for the blends based on germinated millet flour. This reduction in viscosity is due to the starch degradation by the amylases during the germination process. Similar observation is made by Obatolu and Cole [31] who used malted or unmalted maize with soybean and cowpea to make complementary food. The paste stability of germinated based blends was higher than the whole based

blends. This explains the fact that blends was made from germinated millet had a higher swelling power. During a cooking time the changes in viscosity give indication of paste stability, also during cooling the changes could show the involve retro gradation of starch granules and the consistency of gel. The cooking time ranged between 2:05 and 4:55 min with minimum time for ease of cooking recorded in blends.

Sensory properties

Table 8 shows the results of sensory properties of extruded formulated blends. There were significant differences (p<0.05) in mouth feel, appearance, flavor, taste and overall acceptability among extruded formulated blends. The GMRS had a higher score in taste, appearance and flavor compared to the others blends. This could be due to pre-treatments performed on millet (germination) and soybeans (roasted). For overall acceptability, the GMRS had higher score. The germination eliminates the off-flavor at maximum and the roasting gives good color (brown) and flavor. Also during extrusion the color become browner, this may be due to decomposition of pigments, product expansion causing fading and chemical reactions like caramelization of carbohydrates [44]. Among extruded formulated products, GMRS was acceptable to the consumers.

Shelf life analysis

One of the principal methods of predicting the shelf life of processed food products is to monitor the level of lipid degradation

products in foods storage. The stability of the food during storage is important; some deterioration in cereal-legume blends during storage is mostly caused by fat oxidation. This reaction causes deterioration in taste, flavor, odor, color, texture, and appearance, and a decrease in the nutritional value of the foods. Fat Acidity (FA), Initial Peroxide Value (IPV) was chosen to control the products shelf life.

Table 9 presents the data of FA and IPV of extruded formulated blends.

FA increased significantly ($p < 0.05$) during storage time in all products. The blends which contain germinated millet flour had the highest rates in FA; this also may be due to lipids degradation during germination. Increase in FA during storage has been observed by Salman and Copeland [45], Saha and Dunkwal [46].

The primary products of lipids oxidation are peroxides. In all blends IPV increased significantly ($p < 0.05$) during storage periods. The blends which contain germinated millet flour had the highest rates in IPV. This agreed with the observation of Adetuyi et al. [47]. Lipids oxidation is one of the major reasons for development of off-flavors in food. The rate of lipid oxidation is influenced by storage conditions such as light exposure, moisture content, temperature, and pH and oxygen availability. The standard values of IPV and FA were set at no more than 30 meq.g of iodide/1 Kg and 3 mg of KOH/100 g respectively. In all products IPV and FA were found under the standard value. However, the four blends can be stored at room temperature for 90 days. Inhibit of fat oxidation is based on controlling some parameters (temperature, pH, moisture and oxygen concentration). Using the antioxidant is the most common method for inhibiting the fat oxidation.

Conclusion

From the results of our study we can conclude that the pre-treatments such as germination of millet, pre-cooking or roasting of soybean with a formulation as defined by the amount of protein, fat and carbohydrates have resulted meal with good nutritional and functional properties. So panelists after sensory test have appreciated more the Germinated Millet and Roasted Soybean (GMRS). In addition we found that flours made from germinated millet have a low EGI, or 35.39 and 34.49 respectively for GMRS and GMPCS. The low EGI suggest that GMRS and GMPCS could be useful in the preventing and dietary treatment of diabetes. Health is an important issue for all of us. Diabetes can be managed by slight modification in diet and low dosage of medicine. In view of the foregoing, GMPCS and GMRS can be used as a breakfast food. But minerals, lysine and vitamins in these blends are little low. It is recommended to fortify the products with minerals, vitamins and lysine according to official government standard of the country. As use correlation between in IVSD and EGI obtained by Ezeogu et al. [40], Saura-Calixto and Abia [48] allowed us to say that GMPCS and GMRS have low EGI, it would be interesting in future studies to develop a new food made from germinated millet for peoples suffering from gluten intolerance disease.

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