

EFFECT OF FERMENTATION MEDIA AND TIME ON PHYSICOCHEMICAL AND SENSORY PROPERTIES OF SOYBEAN POWDERS

¹Ugwuona F.U., ²Obeta N.A., ²Asogwa I.S. and ³Sabo T.R.

¹Department of Food Science & Technology,
Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria

²Department of Food Science & Technology, Faculty of Agriculture,
University of Nigeria, Nsukka, Enugu State, Nigeria

³Department of Home Science & Management, Faculty of Agriculture,
Nasarawa State University, Keffi, Nigeria

*Corresponding author's email: nwamaka.obet@unn.edu.ng

ABSTRACT

Fermentation improves quality of food, and is exploited in processing soymilk powder. Unfortunately, there is no unified fermentation procedure for producing powdered soymilk in Nigeria. A fermentation condition to produce high nutritional and most acceptable powdered soymilk is ideal for Nigerians. This study evaluated the effect fermentation media and time on physicochemical and sensory properties of powdered soymilk. Seven batches (600 g each) of soybean seeds were fermented, the first three in neutral water, sample A for 4 h, B for 16 h in the same water, and C for 16 h but changing the water every 4 h. The next two were fermented in alkaline solution for 16 h, D in the same water, and E with changing the water every 4 h. The last two were fermented for 16 h in acidic solution, Fin the same solution and G with changing the water every 4 h. The beans were processed into cooked soy flour and analyzed for physicochemical and sensory properties. Fermentation enhanced better quality than soaking; fermentation time and medium pH significantly ($p < 0.05$) induced variations in quality of the powder. Soaking produced soy powder with highest carbohydrate (44.47 %) and fibre (1.355%) but significantly ($p < 0.05$) low in protein, minerals and crude fat. Continuous 16-h fermentation in the same medium produced soy powders with lowest phytochemical contents. Soaked (4 h) soybean in neutral water (A) produced powder with 39.50% protein, 44.47% carbohydrates, 1.35% fibre, 5.58% fat and 2.75% ash while continuous fermentation for 16 h in the same water produced powder (B) with 42.47% protein, 41.71% carbohydrate, 1.22% fibre, 5.81% fat and 2.42% ash. Continuous 16-h fermentation was better than changing the medium; and neutral medium was better than acidic and alkaline medium. All the soy powder high sensory scores (≥ 5) and were acceptable to the panelists. Thus, 16-h continuous fermentation in neutral medium was more cost effective and produced soybean powder of better quality than fermenting in alkaline and acidic media.

Key words: fermentation time, quality, soaking media, soybean powders

INTRODUCTION

The current global economic recession and poor income make it impossible for many families to afford animal protein. An alternative rich plant protein is usually sourced to ameliorate protein energy problems among the low-income earners. Soybean (*Glycine max*) is a leguminous crop. The seed flour is a good source of protein, particularly for families with low income who cannot afford animal protein. Soybeans are highly recognized globally for their high nutritional value and excellent functional properties. The seed flour is rich in most essential amino acids, particularly lysine, arginine, cysteine, leucine and methionine (Giampietrol *et al.*, 2004), thereby making it a relatively inexpensive source of protein. Soybean flour is also rich in minerals, especially calcium,

potassium, magnesium, iron, zinc and copper (FAO, 2009). It is also an excellence source of vitamins including thiamine, riboflavin and niacin (Singh *et al.*, 2000). Soy flour is a good source of protein, essential fatty acids, dietary fiber and many important bioactive compounds such as is flavones. Important bioactive compounds found naturally in soy flour have been proven to relieve menopausal symptoms such as hot flashes, maintain healthy bones and prevent prostate, breast and colorectal cancers (Ugwuona, 2009). The content and profile of these food nutrients and bioactive compounds vary depending on soybean varieties and processing methods employed (Prestamo *et al.*, 2000). Soy foods are healthy because of the high quantity of protein and these bioactive ingredients in them (Sneller, 2003).

Globally, there is increasing awareness of the potential value of soybean flour as a relatively cheap source of protein in the diet. Incorporation of soybean flour into locally produced weaning and adult foods is used to combat protein-energy malnutrition among the low-income families.

Unfortunately, soybean has many anti-nutritional factors, including phytates, tannins, polyphenols, trypsin inhibitors and many oligosaccharides, notably raffinose and stachyose (Prodanov *et al.*, 2004), which have to be removed before its nutrient potentials could be fully harnessed. Most vital metallic minerals are bound with phytates, making them not releasable to the human enzymes for digestion and utilization. Thus, raw soybeans need to be properly processed to reduce many of these anti-nutritional factors, improve taste and texture of soy flour, making it more acceptable to consumers before consumption. Processing also improves functional properties of soybean powder for more technological application.

Soaking, boiling and fermentation are common processing methods used in producing soybean flours, but these methods have their limitations. Soaking breaks down many anti-nutritional factors such as protease inhibitor, phytic acid and α -galactoside linkages due to partial or total solubilization and removal (Prodanov *et al.*, 2004). Extended soaking in tropical climates can lead to undesirable microbial deterioration while cooking for a long time is time- and energy-consuming and cost-ineffective. Also, soaking for too long can cause loss of many essential water-soluble vitamins and minerals (Akande and Fabiyi, 2010). Fortunately, soaking in alkaline solution (e.g., sodium bicarbonate solution) has been shown to reduce cooking time for a number of legumes (Singh *et al.*, 2000).

There is currently no standardized soaking time, pH and fermentation period to achieve high quality soy flour. It is therefore the thrust of this study to investigate the effect of soaking time, pH, changing of water during soaking and fermentation on quality characteristics of soy flour.

MATERIALS AND METHODS

Materials

Soybean (*Glycine max*) seeds and food grade chemicals (citric acid and Sodium bicarbonate) were purchased from retail stocker at Lafia modern market in Lafia, Nasarawa State, Nigeria. All reagents used for analysis were of analytical quality and were purchased from a registered Chemical agent, Lavans Nig. Ltd, Nsukka, Enugu State.

Processing of Soybean Flours

Alkaline, neutral and acidic media were used for soaking and fermentation of soybean seeds. Potable water served as the neutral media. The acidic solution was prepared by dissolving 40 g of citric

acid in 4 L of distilled water (1% citric acid) while the alkaline solution was by dissolving 25 g of NaHCO_3 in 5 L of distilled water (0.5% NaHCO_3).

Soybean seeds were cleaned and divided into seven batches of 600 g each. First batch (A) was soaked in 2 L of potable water for 4 h to aid dehulling. The second (B) and third (C) batches were each fermented in 2 L of potable water for 16 h but with water content of C being changed every 4-h intervals. The fourth (D) and fifth (E) batches were each fermented in 2 L of alkaline (0.5% NaHCO_3) solution for 16 h and with the water content of E being changed at 4-h intervals. The sixth (F) and seventh (G) batches were fermented in 2 L of 1% citric acid solution for 16 h but with the water content of G being changed at 4-h intervals.

After soaking and fermentation, seeds were dehulled manually, washed severally with clean potable water and the dehulled seeds (nibs) cooked in boiling water for 30 min. The cooked nibs were drained, allowed to cool, sun-dried (48 h) and then oven-dried (48 h, 55°C). The dried nibs were milled in to powder with attrition mill (model GX 160). The powder was sieved through 0.20 mm pore sieve to remove fibrous materials, re-milled, packed in polyethene bags and placed in airtight plastic container. The containers were stored in the laboratory cupboard and analyzed for physico-chemical and sensory properties within three days.

Determination of Nutrient Composition of the Soybean Powders

Moisture, crude protein (% N \times 6.25), fat, fibre and ash contents of the soy flours were determined in triplicates using standard procedures of the Association of Official Analytical Chemist (AOAC, 2000). The moisture content was calculated as loss in weight of soy flour after drying at 105°C for 4 h in hot air oven; and crude protein (N \times 6.25) content was estimated after digestion with concentrated H_2SO_4 in Micro Kjeldhal unit.

Total lipid (fat) was estimated by exhaustive extraction of a sub sample (5 g) of each soy flour with petroleum ether (boiling point: 40-60°C) using a Soxhlet apparatus. Digestible carbohydrate (excluding fibre) was determined by difference.

The residual weights after incinerating 5 g of each of the dry soy flours at 600°C for 2 h in a muffle furnace were the ash contents and were expressed as percentage of the original weights of samples. Each of the ash samples was dissolved in 2 ml of concentrated hydrochloric acid (HCl), filtered and then washed with deionized water through Whatman no. 540 filter papers into dilution tubes. These were made up to 25 ml marks with deionized water prior to mineral analysis (AOAC, 2000). The filtrates were used to determine calcium (Ca), sodium (Na), Iron (Fe), Magnesium (Mg) and potassium (K) contents of the samples, using atomic absorption spectrophotometer (ASS) (A.

Analyst 300, Perkin Elmer, Morwalk, Conn, U.S.A). Phosphorus was determined using the Vanadomolybdate method (AOAC, 2000).

Determination of Phytochemical Constituents

Determination of B-carotene contents

Soy flour (1 g) was dissolved in 10 ml of acetone in 30 ml conical flask and swirled for about 2 min. This was allowed to stand for 30 min with repeated shaking at 5 min. intervals to extract the β -carotene in the sample. This was rested for 5 min, and the upper layer decanted into a test tube. Five ml of benzene was added and the mixture shaken gently and allowed to rest for 2 min. Two distinct layers were observed, and the upper layer was obtained using a separating funnel. This was used to determine total β -carotene content in the samples (Singleton *et al.*, 1999). Absorbance was read using spectrophotometer (Spectronic 21 days, Multon Roy, Rochester Ny, U.S.A.) at 453 nm, and β -carotene content calculated as follow:

$$(\text{Abs} \times 10 \times 1/5 \times 10^4) / 3370.$$

Determination of total phenol

Total phenol content was determined using Folin-ciocalteu method (Roesler *et al.*, 2006). To do this, the various soy flours, 0.5 ml aliquot of freshly prepared extracts (from 1 g of flour in 25 ml of distilled water) was mixed with equal volume of water, 0.5 ml Folin-Ciocalteu's reagent and 2.5 ml of saturated solution of sodium carbonate (Na_2CO_3). The mixture was centrifuged (3000rpm, 20 min) (Beckman coulter Ltd Palo, Alto, Calitonia, U.S.A) and absorbance of supernatant measured after 40 min at 725 nm. Garlic acid was prepared at concentrations of 0.0, 3.0, 6.0, 12.0, 18.0, 24.0 and 30.0 $\mu\text{g ml}^{-1}$ to plot total phenol standard curve. Total phenol content was extrapolated from the standard curve using the absorbance values and expressed as garlic acid equivalents (GAE 100 g^{-1}).

Determination of tannin content

The condensed tannin content of the soy flours was determined by the method of Price and Butler (1977). The soy flour samples (1 g) were added to 10 ml of distilled water, and mixed with 0.5 ml of 0.1 M FeCl_3 in 0.1 N HCl and 0.5 ml of 0.008 $\text{MK}_3\text{Fe}(\text{CN})_6$. The mixture was allowed to stand for 1 min., and absorbance was read at 720 nm. Tannin content was extrapolated from a standard curve (prepared with tannic acid at concentrations of 0.0, 0.01, 0.04, 0.08, 0.15, 0.20, 0.50 and 1.0 mg ml^{-1}) and expressed as tannic acid equivalents (TAE 100 g^{-1}).

Determination of phytate content

Phytate content was determined using the method of AOAC (2000). The soy flour samples (4.0 g) were soaked in 100 ml of 0.2 N hydrochloric acid (HCl) solution for 3 h and then filtered through Whatman NO. 2 filter paper. The filtrates (25 ml)

were pipetted into 50 ml conical flasks, and 5 ml of 0.3% ammonium thiocyanate solution added, after which 53.5 ml of distilled water was added and the mixtures were titrated against standard Iron (III) chloride solution containing 0.00195 $\text{g Fe}^{3+} \text{ml}^{-1}$ until a brownish yellow colour persisted for 5 min. The phytate content of the flours was calculated from the titre value and expressed as percentage phytate.

Functional Properties

Water absorption capacity

Water absorption capacity of the soy flour samples was determined using the method of Abbey and Ibe (1988) with slight modification. One gram of each of the flour samples was mixed with 10 ml of distilled water in a centrifuge tube. The suspension was agitated for one hour on a griffin flask shaker after which it was centrifuged for 15 min at 2200 rpm. The volume of water or oil on the sediment water was measured. Water and oil absorption capacities were calculated as ml of water or oil absorbed per gram of flour respectively.

Least gelation concentration

Least gelation concentrations for the various flour samples were determined using the method of Abbey and Ibeh (1988). Each flour sample was mixed at eight different concentrations of 2, 4, 6, 8, 10, 12, 16 and 20 g per 100 ml of distilled water in separate test tubes. This gave a total of 56 test tubes of flour suspensions. The test tubes were heated for 1 h in a boiling water bath (Beckman coulter Ltd Palo, Alto, Calitonia, U.S.A), cooled rapidly under running tap water and further cooled for 2 h in a refrigerator at 4°C. The least gelation concentration was regarded as that concentration at which the sample from the inverted test tube did not fall or slip.

Viscosity analysis

Apparent viscosities were determined at 80°C using a Brookfield DVII+Viscometer (Brookfield Engineering Laboratory Incorporation., Stoughton, Mass., U.S.A.). A 10% soy flour suspension in distilled water was prepared at room temperature (26±2°C) and stirred mechanically for 2 h using a mechanical stirrer. This was cooled to 8°C, transferred into the viscometer tube and the apparent viscometer determined. Three readings were taken per replication, and two replications were conducted.

Sensory Analysis

Trained panellists (15) of ages 17-27 years, comprising 9 females and 6 males were drawn from 21 volunteers from the staff and students of Faculty of Agriculture, Lafia, Nasarawa State University, Lafia, Nigeria. Prior to the selection, volunteers were interviewed to assess their familiarity with soy products, and how often they consume these products. The best 15 who proved familiarity with soy products were selected and trained according to the spectrum methodology of

Mellgaard *et al.* (1991). Predetermined sensory attributes of soy flour were used for them to become familiar with definitions and references to these when tested. Each sample was rated on perceived intensities of standard sensory attributes (acceptability, mouth-feel, flavour, texture and colour) using a 9-point Hedonic scale with 1 = disliked extremely, 2 = disliked very much, 3 = disliked moderately, 4 = disliked slightly, 5 = neither liked nor disliked, 6 = liked slightly, 7 = liked moderately, 8 = liked very much and 9 = liked extremely. Scores were collated and analysed.

Statistical Analysis

Data generated from the study were analyzed using analysis of variance while means with significant ($p \leq 0.05$) difference were separated with Fisher's least significant difference (LSD) using Statistical Package for Social Sciences (SPSS) version 13.0.

RESULTS AND DISCUSSION

Effect of Fermentation Time and Soaking Media on Proximate Composition of Soybean Powders

Table 1 shows the proximate composition of soy powders produced from soybeans fermented in different media at different time intervals. The moisture content (%) of the flours ranged from 5.59 in D to 7.72 in F. Both fermentation and pH of the media significantly ($p < 0.05$) influenced moisture, protein, carbohydrate, ash and fat contents of the soy powders. Also, changing the water at 4 h intervals during 16 h fermentation influenced the moisture contents of the soy powders. Soybean powders sample fermented in alkaline pH had significantly ($p < 0.05$) higher moisture content (Sample F with moisture content of 7.72%) than every other sample. At 16 h fermentation, sample B from neutral medium had 6.45% moisture, C from alkaline medium had 6.85% and D from acidic medium had 5.59% moisture. Moisture contents of these soy flours were below the maximum 14.5% for safe storage of food at ambient condition in the tropics. This shows that moisture content is an indicator of storability of food at ambient condition because moisture content greater than 14.5% supports microbial growth and enhances spoilage (AACC, 2000). Fermentation increased protein content from 39.50 % in the unfermented sample (A) to highest value of 47.55% in the sample (F) fermented in alkaline medium for 16 h with changing of water at 4 h intervals. However, protein content of the sample fermented in the same alkaline medium without changing the water for 16 h decreased from 39.50% to 39.06%. On the other hand, when the beans was fermented for 16 h without changing the water, protein content increased from 39.50% to 42.47% in neutral medium but when fermented for 16 h with changing of water at 4 h intervals (sample E), the protein content was 40.63%. Thus, changing water at time intervals during fermentation decreased protein content of

soybean in both neutral and alkaline media. The same trend was observed when fermenting in acidic medium. Protein content increased from 39.49% in the unfermented sample to 47.29% when fermenting without changing the water but this value decreased down to 44.00% when fermenting and changing the water at 4 h intervals. This implies that changing the water medium at time intervals during fermentation aids leaching out of water-soluble part of the protein from the soybean.

Effect of Fermentation Time and Soaking Media on Mineral Composition of Soybean Powder

Table 2 shows the mineral composition of soy powder produced from soybean seeds subjected to fermentation conditions. Both fermentation time and the fermenting medium jointly affected mineral contents of the soy powders. Fermentation generally improved iron (1.49 mg 100 g⁻¹ in F to 5.09 mg 100 g⁻¹ in G) and phosphorus (10.21 mg 100 g⁻¹ in C to 16.32 mg 100 g⁻¹ in G) contents as against 1.00 mg 100 g⁻¹ iron and 10.07 mg 100 g⁻¹ phosphorus in the unfermented sample (sample A). Iron is an important constituent of haemoglobin found in the blood. De Villota *et al.* (1981) emphasized the importance of iron in oxygen carriage in blood. According to National Academy of Science (2004) the recommended daily allowance of iron is between 8 to 18 mg day⁻¹.

Soy powder produced from 16 h fermentation in neutral medium (sample B) had the highest calcium (440.44 mg 100-g⁻¹) and magnesium (3.76 mg 100-g⁻¹) contents among all the samples. The different fermenting media and processes improved some mineral and decreased others in the flour samples as compared with the unfermented control (sample A) sample. Soy flour from 16 h fermentation in neutral medium (sample B) had the highest contents of calcium (440.44 mg 100-g⁻¹) and magnesium (3.76 mg 100-g⁻¹) while soy powder from 16 h fermentation in acidic medium (G) with changing of solution at 4 h intervals had the highest contents of iron (5.09 mg 100-g⁻¹), phosphorus (16.32 mg 100-g⁻¹) and potassium (41.95 mg 100-g⁻¹). However, soy powder from 16 h fermentation in alkaline medium (sample C) without changing of water had the highest content of sodium (1.53 mg 100-g⁻¹). The relative significantly ($p < 0.05$) highest contents of iron and phosphorus in sample G suggests acidic solution as the best fermenting medium for hydrolysing iron from phytates and phytic acid which are highly present in soybeans.

The mineral contents in the fermented samples were at variance when compared with those in the unfermented control sample (A). The mineral contents of the powders produced from different fermenting mediums varied significantly ($p < 0.05$). The powders were rich in calcium (220.43 in C to 440.44 mg 100-g⁻¹ in B), potassium (33.33 in C to 41.95 mg 100-g⁻¹ in G) and phosphorus (6.86 in F to 16.32 mg 100-g⁻¹ in G) contents but relatively low in sodium (1.04 in E to 1.53 mg 100-g⁻¹ in C) contents.

Table 1: Proximate composition of the soybean powders

Samples	Moisture (%)	Protein (%)	Carbohydrates (%)	Crude fibre (%)	Fat (%)	Total ash (%)
A	6.36 ^c ±0.51	39.50 ^f ±0.22	44.47 ^a ±0.71	1.35 ^a ±0.01	5.58 ^d ±0.01	2.75 ^a ±0.06
B	6.45 ^d ±0.44	42.47 ^d ±0.14	41.71 ^d ±0.42	1.22 ^d ±0.01	5.81 ^a ±0.01	2.42 ^f ±0.05
C	6.85 ^b ±0.52	39.06 ^e ±0.18	44.08 ^b ±0.44	1.14 ^c ±0.14	5.25 ^f ±0.02	2.63 ^b ±0.03
D	5.59 ^e ±0.34	47.29 ^b ±0.21	37.16 ^f ±0.38	1.35 ^a ±0.03	5.62 ^c ±0.00	2.54 ^d ±0.04
E	6.47 ^c ±0.44	40.63 ^c ±0.36	43.34 ^c ±0.22	1.32 ^b ±0.00	5.65 ^b ±0.03	2.60 ^c ±0.05
F	7.72 ^a ±0.17	47.55 ^a ±0.16	35.42 ^e ±0.11	1.27 ^e ±0.01	5.45 ^c ±0.04	2.60 ^c ±0.02
G	6.16 ^f ±0.13	44.00 ^c ±0.11	41.29 ^c ±0.25	1.27 ^e ±0.01	4.89 ^e ±0.01	2.44 ^e ±0.01
SEM	0.038	0.001	0.005	0.002	0.002	0.034

Values are means of 3 determinations. Means on the same column with different superscripts differed significantly ($p < 0.05$). A - soy powder from soybean soaked for 4 h in neutral water; B, C and D - soy powders from soybean fermented 16 h, respectively in the same neutral, alkaline and acidic water; E, F and G - soy powders from soybean fermented 16 h, respectively in neutral, alkaline and acidic water with changing water at 4 h intervals

Table 2: Mineral composition of the soybean powders

Samples	Calcium (mg 100-g ⁻¹)	Magnesium (mg 100-g ⁻¹)	Iron (mg 100-g ⁻¹)	Phosphorous (mg 100-g ⁻¹)	Potassium (mg 100-g ⁻¹)	Sodium (mg 100-g ⁻¹)
A	420.43 ^b ±1.77	3.24 ^d ±0.00	1.00 ^e ±0.01	10.07 ^f ±0.01	41.56 ^c ±1.32	1.40 ^c ±0.00
B	440.44 ^a ±1.01	3.76 ^a ±0.03	2.23 ^c ±0.01	12.20 ^b ±0.01	35.67 ^d ±1.51	1.42 ^d ±0.02
C	220.43 ^e ±1.09	2.64 ^f ±0.00	2.34 ^d ±0.02	10.21 ^c ±0.00	33.33 ^e ±1.22	1.53 ^a ±0.01
D	306.38 ^d ±2.01	2.76 ^e ±0.00.2	3.62 ^b ±0.02	11.74 ^c ±0.03	35.34 ^c ±2.00	1.45 ^b ±0.01
E	281.37 ^f ±1.23	3.61 ^c ±0.03	2.88 ^c ±0.01	11.44 ^d ±0.11	43.24 ^a ±1.01	1.04 ^e ±0.00
F	301.28 ^e ±1.22	3.72 ^b ±0.01	1.49 ^f ±0.00	6.86 ^e ±0.21	33.99 ^f ±2.00	1.44 ^c ±0.12
G	410.23 ^c ±2.01	2.64 ^f ±0.02	5.09 ^a ±0.01	16.32 ^a ±0.09	41.95 ^b ±1.32	1.37 ^f ±0.13
SEM	0.003	0.002	0.002	0.002	0.002	0.002

Explanations and abbreviations as in Table 1

Phosphorus is essential for bone mineralization and development of structure of cellular membranes, nucleic acids and nucleotides, including adenosine triphosphate (Vitabase, 2009).

Potassium maintains electrolyte balance in humans (NTBG, 2009) and its presence in the food is very useful. Morgan (1999) indicated that reducing intake of sodium reduces the development of hypertension. The high K:Na ratios of the soy powders imply that they could be useful in ameliorating sodium-related health risks among consumers (Arbeit *et al.*, 1992; CIHFI, 2008). Magnesium (mg 100 g⁻¹) contents ranged from 2.64 in C and G to 3.76 in B; and iron (mg 100 g⁻¹) content from 1.00 in A to 5.09 in G. Magnesium is essential in enzyme system and helps maintain electrical potential in nerves (Ferrao *et al.*, 1987). Fermenting in neutral medium for 16 h without changing the water (B) produced powder with highest calcium (440.44 mg 100 g⁻¹) and magnesium (3.76 mg 100 g⁻¹) contents among the samples. The soybean powder produced from soybean fermented for 16 h with changing of 1% citric acid solution at 4 h intervals had the highest levels of most minerals among the various soybean powders.

Effect of Fermentation Time and Soaking Media on Phytochemical Composition of the Soybean Powders

Table 3 shows phytochemical composition of the soybean powders/flours. Fermentation significantly ($p < 0.05$) reduced the phytochemical contents of the soy powders; and fermenting for 16 h was more effective than fermenting for 4 h. Soy flour sample fermented for 16 h in neutral medium was more effective in reducing tannin and phenol contents of the flour samples than samples fermented in alkaline or acidic medium at the same time interval

(16 h). On the other hand, samples fermented in alkaline medium with 4 h fermentation in neutral medium was the most effective in retaining β -carotene content (0.56 mg 100 g⁻¹) of the soy flour but 16 h fermentation in neutral medium without changing the water, produced powder with the lowest phytate content (sample B with 2.11 mg 100 g⁻¹). The soy powders were relatively high in phenol and phytate but low in tannin and β -carotene. Phenol content (mg 100 g⁻¹) ranged from 5.63 in soy flour fermented for 4 h (A) to 3.61 in soy flour fermented for 16 h without changing the medium (B) while phytate content (mg 100 g⁻¹) ranged from 6.75 in soy flour from soybean fermented for 4 h to 2.11 in soy flour from soybean fermented for 16 h in neutral medium with 4 h intermittent changing of medium. Fermenting for 16 h was better than fermenting 4 h soaking in detoxifying phytochemicals. Also, fermenting in neutral water was the best for detoxifying tannin and phenol. The 16 h fermentation without changing the water produced better soybean powder than the 16 h with intermittent changing the water.

Effect of Fermentation Time and Soaking Media on Functional Properties of the Soybean Powders

Table 4 shows the functional properties of the soybean powders. Water absorption capacity of the soy flours ranged from 36.7 in C (fermented 16 h in alkaline medium without changing of water) and F (fermented 16 h in alkaline medium with changing of water at 4 h intervals) to 23.3 in B (fermented 16 h in neutral medium without changing of water) and E (fermented 16 h in neutral medium with changing of water at 4 h intervals). The unfermented sample that was soaked for 4 h for easy dehulling had water absorption capacity of 30.0, a value lower

Table 3: Phytochemical composition of the soybean powders

Samples	Tannin (mg 100-g ⁻¹)	Phenol (mg 100-g ⁻¹)	β - carotene (mg 100-g ⁻¹)	Phytate (mg 100-g ⁻¹)
A	1.50 ^a ±0.04	5.63 ^a ±0.10	0.56 ^a ±0.00	6.75 ^a ±0.10
B	1.19 ^g ±0.02	3.61 ^g ±0.02	0.24 ^f ±0.01	2.14 ^f ±0.07
C	1.39 ^e ±0.03	5.49 ^b ±0.03	0.47 ^b ±0.00	4.28 ^c ±0.06
D	1.46 ^e ±0.00	5.44 ^c ±0.00	0.44 ^c ±0.01	5.65 ^b ±0.08
E	1.48 ^b ±0.01	4.77 ^c ±0.01	0.39 ^e ±0.00	2.11 ^g ±0.02
F	1.33 ^f ±0.03	4.89 ^d ±0.05	0.11 ^g ±0.02	5.45 ^c ±0.04
G	1.42 ^d ±0.05	4.68 ^f ±0.01	0.43 ^d ±0.00	5.07 ^d ±0.06
SEM	0.002	0.251	0.002	0.002

Explanations and abbreviations as in Tables 1 and 2

Table 4: Functional properties of the soybean powders

Samples	Water absorption capacity	Least gelation concentration	Apparent viscosity
A	30.00 ^b ±2.33	19.33 ^b ±1.32	0.89 ^b ±0.01
B	23.30 ^d ±2.22	18.00 ^e ±1.22	0.97 ^a ±0.01
C	36.70 ^a ±1.21	16.33 ^d ±1.01	0.89 ^b ±0.02
D	30.00 ^b ±1.19	18.00 ^e ±0.99	0.93 ^a ±0.03
E	23.30 ^d ±2.01	16.67 ^d ±1.23	0.90 ^{ab} ±0.01
F	36.70 ^a ±2.31	18.00 ^e ±1.11	0.89 ^b ±0.03
G	26.70 ^c ±1.12	20.67 ^a ±1.08	0.90 ^{ab} ±0.02
SEM	3.81	0.48	0.08

Explanations and abbreviations as in Tables 1, 2 and 3

than the acid-fermented but higher than the alkaline-fermented flours. Changing of media at intervals during the 16-h fermentation did not affect water absorption capacity of the flours. Also fermenting in alkaline and acidic media improved water absorption capacity of flours but this was higher in alkaline medium than in acidic medium. This could be due to hydrolysis of some insoluble polysaccharides and protein complexes during the adjusted alkaline and acidic pH conditions; and dissolution and leaching out of some neutral soluble fibre and protein in the neutral medium. Water absorption capacity of powder is the differences in weight of the powder before and after water absorption under sufficient water supply (Abbey and Ibeh, 1988). Imbibition of water is an important functional trait such as hydration, swelling, solubility and gelation in foods such as sausages, custards and dough (Adebowale *et al.*, 2005). Depending on a protein side chain (number of charged and polar group), a protein may bind varying amount of water (Vaclavik and Christian, 2003). Water absorption capacity is also specific for each type of starch, and it depends on several factors such as amylase-amylopectin ratio, intra and inter molecular forces and size of granules (Rahman *et al.*, 1999). The smaller the granules of the soybean powder, the higher the water absorption capacity of the powder (Singh *et al.*, 2010). Water absorption capacity varies with protein source, composition, processing methods such as heating and alkali processing (Ikegwu *et al.*, 2010). It is a function of ionic strength, pH, temperature, size and shape of the protein molecules. The least gelation concentration ranged from 16.33 in C (fermented 16 h in alkaline medium without changing of water) to 20.67 in G (fermented 16 h in acidic medium with changing of water at 4 h intervals). The unfermented sample had least gelation concentration of 19.33%, a value significantly higher than every other sample except for G. Least gelation concentration was highest in

flour fermented at acidic pH with changing of water and in flour from unfermented soybean. Apparent viscosity was 0.89 in A (unfermented soy flour), C and F; 0.90 in E and G; 0.93 in D (fermented 16 h in acidic medium without changing of water) and 0.97 in B. Apparent viscosity was low in all the samples. Several changes may occur upon heating a starch-water system, including enormous swelling, increased viscosity, translucency and solubility. These changes are defined as gelatinization (Ikegwu *et al.*, 2010). Huang *et al.* (2007), however, reported that amylopectin molecules are involved. Gelation of protein also occurs in flour and pastes and is very important for the preparation of puddings, jams and sauces that require thickening and jelling. Some kinds of proteins form gels through interactions with polysaccharide gelling agents such as starch and gelatin (Nunoo, 2009).

Effect of Fermentation Time and Soaking Media on Sensory Properties of the Soybean Powders

Table 5 shows sensory scores for the powdered soybeans samples on a 9-point Hedonic scale. The sensory scores were high for all the seven samples and ranged from 7.28 for sample G for colour to 8.50 in overall acceptability. Soaking, fermentation, pH of fermenting medium and changing of medium during fermentation significantly ($p < 0.05$) affected sensory attributes of soy powders. Soy powder produced from 4 h soaking was the most acceptable by the sensory panelists. Fermenting for 16 h in alkaline medium with intervals of 4 hourly changing of the medium (F) resulted to be the most acceptable colour (8.44) and flavour (8.44) while soaking for 4 h in neutral medium (A) resulted in the most acceptable mouth feel (8.44) and over-all acceptability (8.50). Thus, sample A was the most acceptable, but the best in quality was sample C. However, all the soy flours were acceptable to consumers; none of the sensory attributes was scored below 4.5 – the mid-mark of the 9-points hedonic scale used.

Table 5: Sensory properties of the soybean powders

Samples	Colour	Texture	Flavour	Mouth feel	Overall acceptability
A	8.33 ^b ±1.28	8.22 ^a ±0.94	8.33 ^b ±0.91	8.44 ^a ±0.98	8.50 ^a ±0.86
B	7.78 ^f ±1.52	7.83 ^d ±0.92	7.61 ^f ±1.75	7.61 ^f ±1.54	7.56 ^e ±1.65
C	7.94 ^e ±1.16	7.50 ^e ±1.30	7.94 ^d ±1.21	7.22 ^g ±2.18	7.67 ^f ±1.41
D	8.17 ^c ±0.99	8.11 ^b ±0.90	7.72 ^e ±1.84	7.89 ^d ±1.94	8.22 ^b ±0.73
E	8.06 ^d ±1.00	8.22 ^a ±0.73	8.33 ^b ±0.69	8.06 ^b ±1.00	8.00 ^c ±0.84
F	8.44 ^a ±0.62	7.89 ^e ±1.84	8.44 ^a ±0.71	7.94 ^d ±1.89	7.94 ^d ±1.92
G	7.28 ^g ±1.93	7.56 ^e ±1.04	8.17 ^g ±0.79	7.67 ^c ±1.19	7.89 ^e ±1.08
SEM	0.29	0.27	0.27	0.37	0.30

Footnote as with Tables 1-4

CONCLUSION

Fermentation time and pH of fermentation media affected nutrient composition, functional and sensory properties of powdered soybeans. The soybean powders were rich in both macro and micro nutrients, had good functional properties and were acceptable to the sensory panelists. Fermentation reduced phytochemical composition of the soy powders; and fermenting in neutral medium for 16 h produced the best soy powder. Fermenting soybean seeds in neutral medium for soy powder production is recommended.

REFERENCES

- AACC (2000). *Method 46-30; Approved Methods of American Association of Cereal Chemists*, (10th ed.), AACC International, St. Paul, MN
- Abbey B.W. and Ibeh S.O. (1988). Functional properties of raw and heat processed cowpea (*Vigna unguiculata*, walp) flour. *J. Food Sci.*, **53** (6), 1775-1791
- Adebowale K.O., Olu-Owolabi B.I., Olawumi E.K. and Lawal O.S. (2005). Functional properties of native, physically and chemically modified breadfruit (*Artocarpus altilis*) starch. *Ind. Crop Prod.*, **21**, 343-351
- Akande K.E. and Fabiyi E.F. (2010). Effect of processing methods on some anti-nutritional factors in legume seeds for poultry feeding. *Int. J. Poult. Sci.*, **9**, 996-1000
- AOAC (2000). Association of Official Analytical Chemists: Official Method of Analytical Chemists. 18th ed. Washington D.C pp. 18-62
- Arbeit M.L., Nicklas T.A. and Berenson G.S. (1992). Considerations of dietary sodium/potassium/energy ratios of selected foods. *J. Am. College Nutr.*, **11** (2), 210-22
- CIHFI (2008). Wichita USA. The Center for the Improvement of Human Functioning International (CIHFI). <http://biocenterlab.org/tests/urine/kna.shtml>, Accessed 14/08/ 2010
- De Villota E.D.D., Ruiz C.M.T., Rubio J.J. and De Andre's S. (1981). Equality of the in vivo and in vitro oxygen binding capacity of haemoglobin in patients with severe respiratory disease. *Brit. J. Anaesth.*, **53** (12), 1325-1328. DOI: 10.1093/bja/53.12.1325 PMID:7317251
- FAO (2009). Technology of products from soybeans. FAO Agricultural Science Bulletin 2. Rome, Italy
- Ferrao J.E.M., Ferrao A.M.B.C. and Anatures A.M.G. (1987). Garcia deorta, serieda estudes. *Agronomicos*, **14** (1-2), 35-39
- Giampietrol P.G., Bruno G.F., Casati A.G., Brunneti E.S., Spardoni C.L. and Galli E.D. (2004). Soy protein in long term feeding. *J. Pediatric Endocrinol. Metab.*, **5**, 240-248
- Huang J., Schols H.A., van Soest J.J.G., Jin Z., Sulmann E. and Voragen A.G.J. (2007). Physicochemical properties and amylopectin chain profiles of cowpea, chickpea and yellow pea starches. *Food Chem.*, **101** (4), 1338-1345
- Ikegwu O.J., Okechikwu P.E. and Ekumankana E.O. (2010). Physico-chemical and pasting characteristics of flour and starch from *Brachystegia eurycoma* seed. *J. Food Technol.*, **8** (2), 58-66
- Mellgaard M., Civille C.V. and Carr B.T. (1999). *Sensory Valuation Techniques*, 3rd ed., CRC Press Inc. Boca Raton
- Morgan T.O. (1999). Restriction of salt intake is needed to ameliorate the cardiovascular disease epidemic. *The Medical J. Aust.*, **170**, 176-178
- National Academy of Sciences (2004). Proceedings of the National Academy of Sciences, **101** (52), 118-124
- NTBG (2009). Hunger initiatives, Breadfruit Institute. National Tropical Botanical Garden (NTBG). <http://www.ntbg.org/breadfruit/breadfruit>. Accessed 26/08/ 2010
- Nunoo P. (2009). *Pasting of G-Irradiated Proteins from Vigna subterranean in Native Starch Models and the Surface Functional Properties of the Proteins*. M.Sc. Thesis Submitted to the Department of Food Science & Technology, Kwame Nkrumah University of Science & Technology, Kumasi, Ghana, p. 72
- Prestamo G.D., Lesmes M.F., Otero I.F. and Arroyo G.G. (2000). Soybean vegetable protein (Tofu preserved with high pressure). *J. Agric. Food Chem.*, **48** (7), 2943-7
- Price M.L. and Butler, L. G. (1977). A spectrophotometric method of determination of tannin content of sorghum grain. *J. Agric. Food Chem.* **25** (6), 1268-1273 <https://pubs.acs.org>
- Prodanov M.A., Vierra I.H. and Vidal V.A. (2004). Influence of soaking and cooking on thiamin, riboflavin and niacin contents in legumes. *Food Chem.*, **84**, 271-277
- Rahman M.A., Nahar N., Jabbar M.A. and Mosihuzzaman M. (1999). Variation of carbohydrate composition of two forms of fruit from jack tree (*Arthocarpus heterophyllus* L.) with different maturity and climatic conditions. *Food Chem.*, **65**, 91-97
- Roesler R., Malta G.M., Carrasco L.C. and Pastore, G. (2006). Evaluation of the antioxidant properties of the Brazilian cerrado fruit *Annona crassiflora* (Araticum). *J. Food Sci.*, **71** (2), C102-C107.
- Singh S., Singh N. and MacRitchie F. (2010). Relationship of polymeric proteins with pasting, gel dynamic and dough empirical-rheology in different Indian Wheat varieties. *Food Hydrocolloids*, **25** (1), 19-24
- Singh S.R., Rachie K.O. and Dashiell K.E. (2000). *Soybean for the Tropics Research Production and Utilization*. John Wiley & Sons Ltd., pp. 1-5, 167-170
- Singleton V.L., Orthofer R. and Lamuela-Raventos R.M. (1999). Analysis of Total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. *Method in Ezymology*, **299**, 152-178. [http://dx.doi.org/10.1016/S0076-6879\(99\)99017-1](http://dx.doi.org/10.1016/S0076-6879(99)99017-1)
- Sneller C.H. (2003). Impact of transgenic genotypes and subdivision on diversity within elite North America soybean germplasm. *Yong Wright*, pp. 117-208
- Ugwuona F.U. (2009). Chemical and sensory evaluation of soy-fortified cassava wheat biscuit. *Agro-Sci.*, **8** (1), 55-59
- Vaclavik V.A. and Christian E.W. (2003). *Essentials of Food Science* (2nd ed.), Elizabeth Christian Publishing, England, Part III, Chapter 8
- Vitabase C. (2009). Vitamin and health guide. [<http://www.vitamins-supplements.org/dietary-minerals/phosphorus.php>]