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EFFECT OF GERMINATED SOY FLOUR ON THE SENSORY ACCEPTABILITY OF SOY-WHEAT COMPOSITE BREAD

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ABSTRACT

Germination is widely claimed as a means of correcting nutrient deficiencies of particular seeds, especially through alterations in the amino-acid balance of the proteins and enhancement of the content of vitamins Sprouts from soybean for example are actually described "Perfect", because all the life-giving proteins, carbohydrates, oils, vitamins and minerals necessary to support life are stored within the seeds. This work showed that sprouting of soybean flour added to wheat flour not only enhances the aesthetic acceptability but also improves the mouth feel of the bread.

Keywords: Organoleptically, Fluorodinitrobenzene, Methionine, Valine, essential amino acid index (EAAI), requirement index (RI), biological values (BV)

INTRODUCTION

One of the most important goals of any country is to give her citizens a diet which is adequate nutritionally (Malomo, 1982). Protein-energy malnutrition (PEM), a natural ramification of poverty, continues to be a perennial source of concern to a large segment of the world's population (Chopra and Sharma, 1992). In many developing counties, such as Nigeria, malnutrition is a common dietary problem (Mbaeyi and Ani, 2005). Infants and children from developing countries and elderly people from all around the world are the two main groups suffering from protein-energy malnutrition (Lesourd and Mazari**,** 1997). Animals are rich sources of protein for human consumption, but unfortunately the animal protein is becoming unaffordable for low income earners with the result that protein-energy malnutrition is becoming a household norm in Nigeria (Dandago, 1995).

Grain legumes or pulses (including soybeans) are rich and low-cost sources of dietary proteins and nutrients for a large part of the world's population (Egounlety and Aworh, 2003). These grain legumes contribute significantly towards protein, mineral and B-complex vitamin needs of people in developing countries (Dhingra and Jood, 2002) and play an important role in the traditional diets of many regions throughout the world (Messina, 1995). Soybeans is an excellent source of protein (about 35-40%), hence the seed is the richest in food value of all plant foods consumed in the world (Kure *et al.,* 1998). It is a very rich source of vegetable protein for all including growing children (Dandago and Igwe, 2006); and it has been identified as a suitable protein rich crop that could improve the nutritional and economic status of the general population in developing countries (Babajide *et al.,* 2003). Soybeans have great potential in overcoming protein-calorie malnutrition. Although soybean is not indigenous to Africa, it has received tremendous popularity as a cheap protein source in Nigeria (Nwabueze, 2007).

Soybean is currently widely and easily grown crop all over the world, hence it now finds application in diverse forms of processed foods (Otunola *et al.,* 2006).). It is therefore now intensively studied resulting in development of novel foods from it (Otunola *et al.,* 1998). The reasonable price and steady supply are also favorable factors in soybeans emerging as an important source of protein in nutrition (Olguin *et al.,* 2003). Furthermore, soybean products play an important role in health (Messina and Barnes, 1991; Messina, 1995; Sirtori *et al.,* 1995).

These reasons have promoted the recent appearance of numerous food products derived from soybean such as soybean flour, textured soybean, soybean dairy-like products, meat, bakery products prepared with soybean (Ladodo and Borovik, 1992). Its use in the production of bread as composite flour has been reported (Kure *et al.,* 1998; Dhingra and Jood, 2004; Basman *et al.,* 2003).

Bread is consumed worldwide in relatively large amounts (Bakke and Vickers, 2007).It is a popular food produced in Nigeria from wheat flour, yeast, sugar, water, sodium chloride and fat (Onigbogi and Ogundele, 2005).

It is however, relatively expensive, being made from imported wheat that is not cultivated in the tropics for climatic reasons (Edema *et al*., 2005). Efforts have been made to promote the use of composite flours in which flour from locally grown crops and high protein seeds replace a portion of wheat flour for use in bread, thereby decreasing the demand for imported wheat and producing protein enriched bread (Giami *et al.,* 2004).

Different levels of success have been recorded with the use of flours from legume in baked goods (Kure *et al.,* 1998; Dhingra and Jood, 2002; Basman *et al.,* 2003).

Although, Soybean has potentials for incorporation and utilization in human diets ((Iwe and Ngoddy, 1998); their use and nutritive value is limited by the presence of several anti-nutritional and toxic substances including oligosaccharides, enzymatic inhibitors, phytates, polyphenols and lectins (Egounlety and Aworh, 2003; Ahia, 2003). Since soybeans contain reasonable amounts of these anti-nutritional factors, an appropriate and convenient method of processing should be adopted in order to render them safe, palatable, digestible and nutritious (Lasekan *et al*., 2004).

Germination processes have been developed to overcome these disadvantages of soybean seed used in food products (Zhu *et al.,* 2005). Germination is a complex metabolic process during which the lipids, carbohydrates, and storage proteins within the seed are broken down in order to obtain the energy and amino acids necessary for the plant's development (Jachmanian *et al.,* 1995).Germination affects the anti -nutritional factors of the legume, although there is some disagreement as to the ultimate consequences, because the effect depends on the type of legume and on the conditions and duration of the germinating process (Zhu *et al.,* 2005).

Some studies have been carried out on the effects of germination on the chemical composition, biochemical constituents, anti-nutritional factors as well as functional properties of soybeans flour. Despite the studies on the effect of these processes on soybeans, information regarding the use of germinated soybean flour in bread making is scarce.

OBJECTIVES

Therefore, the main objective of this research was to obtain composite flour with improved nutritional value for bread baking.

The specific objectives are:

1. To determine the effect of germination on the nutritional quality of soy flour.
2. To determine the effect of germinated soy flour on the sensory acceptability of soy-wheat composite bread.

MATERIALS AND METHODS

Procurement of Material

The samples of commercially grown varieties of soybean were obtained from local market in Ota. All the grains were graded, sorted, and cleaned to remove defective seeds, dirt, debris and other foreign materials. Wheat flour (Honeywell), instant dry yeast, margarine, salt sugar, non-fat dry milk, vegetable oil, baking powder were also obtained from a local supermarket in Ota, Nigeria.

Preparation of Non-Germinated Soy Flour

The cleaned, sorted and graded raw soybean grains were milled into coarse particles, winnowed to remove hull, milled into flour and the obtained flour was sieved in a standard sieve to obtain flour of 500µm particle size (Fig 1).

Germination of Soybeans

The germination procedure for seeds was as follows: the seeds were washed with 0.7% sodium hypochlorite, soaked in distilled water at room temperature for 6 hours, and shaken every 30 min. The water was then drained off, and the seeds were then transferred and spread on trays which were covered by muslin clothes. The muslin clothes allowed oxygen to enter for the germinating seed while minimizing contamination. The *soy*beans were germinated at 25 °C ± 3, for 72 hours and seeds were sprayed daily with distilled water in order to maintain an adequate hydration level. The seeds were weighed prior to soaking, and after soaking before the germination operation.

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Preparation of Germinated Flour Samples

Flours from germinated beans were produced by drying the bean seeds in a hot air oven at 65^{o}C to about 10 percent moisture content. The dried beans were then dehulled, winnowed and milled using a locally fabricated attrition mill. The obtained flour was also sieved in a standard sieve to obtain flour of 500µm particle size (Fig 2).

Preparation of Plain and Blended Flours

Non-germinated and germinated soy flour were blended with wheat flour at different levels (5, 10 and 15% respectively).

The soy/wheat blends were called NGSW 1, 2, and 3; and GSW 1, 2, and 3 as follows:

NGSW1

95:5 wheat flour: non-germinated soybean flour

NGSW2

90:10 wheat flour: non-germinated soybean flour

NGSW3

85:15 wheat flour: non-germinated soybean flour

GSW 1

95:5 wheat flour: germinated soybean flour

GSW 2

90:10 wheat flour: soybean flour

GSW 3

85:15 wheat flour: germinated soybean flour. (Fig. 3)

The control was 100% wheat-bread flour. Three replicate blends, for flour testing and baking, were prepared for each treatment and analyzed independently.

STORAGE OF SAMPLES

All flour samples were stored in Ziploc bags in a deep freezer prior to analysis.

ANALYTICAL PROCEDURES

Proximate Composition

The proximate chemical composition analyses were performed in the Food Chemistry Laboratory, and Central Research laboratory of The Bells University of Technology, Ota. Moisture content of the samples was determined by oven-drying at 105 ± 1 °C for 4 hours (AOAC, 1995). Fat content was measured by extraction with diethyl ether in a Soxhlet system (Foss soxtec^{Tm} 2055 fat extractor) using 2g of the sample. Proteins were analysed as total nitrogen content by the Kjeldahl procedure (AOAC, 1995) using a Foss Tecator^{Tm} protein digestor and KJECTEC 2200 distillation apparatus, and the conversion factor used to transform nitrogen into protein was 5.71 for soybean flour, 5.70 for wheat flour and 5.705 for flour blends. Crude fibre was determined using the method described by Pearson (1973). Finally, ash content was determined by incineration of samples at 550 °C in a muffle furnace for 1 hour. (AOAC, 1995) and total available carbohydrates were estimated by difference.

All the determinations were made in triplicates in accordance with the established procedures (AOAC, 1985).

Determination of Trypsin Inhibitor

Trypsin inhibitors were extracted by the methods reported by Kakade *et al.* (1969).Samples were ground in a mini grinder and extracted with 10 volume of petroleum ether at room temperature (28±2 °C). One gram of the milled sample was suspended in 19 ml water and the pH was adjusted to 8.6 with 0.005 N NaOH. After mechanical shaking for 1 h, the suspension was centrifuged. One millilitre of the supernatant was diluted to 50 ml with phosphate buffer (pH 8.4).

Then, a trypsin standard curve obtained as 0.02–1.0 ml of the stock trypsin solution was pipetted into a triplicate set of test tubes (one set for each level of trypsin) and the final volume of each adjusted to 2 ml with the phosphate buffer. The tubes were set in a water bath at 37 °C. To one of the triplicate tubes was added 6 ml 5 g/l (w/v) trichloroacetic acid (TCA), (this served as blank). Two millilitres of casein solution previously brought to 37 °C was added to each tube. The tubes were allowed to remain at 37 °C for exactly 20 min at which time the reaction was stopped by adding 6 ml of 5 g/l TCA to the experimental tubes. After standing for 1 h at room temperature the suspension was filtered, and the absorbance of the filtrate was measured at 280 μm against the blank.

The results were expressed as milligram of inhibited trypsin/gram of protein extract. The trypsin inhibitor activity was calculated as follows: mg of inhibited trypsin/g of extract protein = $(A \times B)/C \times 1000 \times P$; being A the difference between the enzyme control and sample absorbance, B is the sample dilution factor, C is the trypsin factor (0.019) and P is the extract protein concentration in g/ml.

Determination of Oligosaccharides

The oligosaccharides were separated by thin-layer chromatography according to the method of Odunfa (1983). 50µl of ethnanol extract were spotted on precoated silica gel plates at 2 cm intervals along with 20 µl of reference standard mixture containing sucrose, raffinose, stachyose and melibiose. The solvent used was n-propanol, ethylacetate and water (6:1:3 v/v).

After 4 h development of the plates, the oligosaccharides were quantified by the guidestrip technique of Sugimoto and Van Buren (1970). The content of sugars was estimated according to the phenol–sulphuric acid method of Dubois *et al.* (1956).

BAKING PROCEDURE

Ingredients such as sugar, fat, salt and yeast were then added in appropriate proportions to each of the flour blends and the control flour (table 1).The amount of water added to each was determined based on the water absorption values obtained from the farinograph to obtain dough of good consistency. The dough obtained in each case was scaled into baking pan, and left to proof for at 38°C in a proofing cabinet (MIWE GR D-97450). Proofing was considered satisfactory when dough height had risen to 1cm above the top of the pan. Bread loaves were subsequently baked at 180 °C for 30 minutes in an electric oven (MIWE Oven CO 11206). The baked loaves were later depanned, cooled and used for subsequent analysis

Bread Firmness

Bread firmness were measured on freshly baked bread loaves using a TVT-300XP texture analyzer (First Blends Ltd., Ikeja, Lagos State) which has a cylinder probe with a 1 kg load cell. The weighted probe which was positioned vertically over the surface of the test sample (six centre slices from the bread loaves) was allowed to fall unto the sample and the depth of penetration after a fixed period of time was determined. The bread macro software provided by the texture analyzer was used to collect the data and the results were presented in terms of hardness.

Physical Measurements on Bread

The loaf weight, volume, specific volume, density and height were determined with Tex-vol instrument BVM-L370 (First Blends Ltd., Ikeja, and Lagos State).

SENSORY EVALUATION

The multiple comparison test method was selected for sensory evaluation. The sensory evaluation of samples of depanned and cooled bread was carried out by a panel of twelve judges. Evaluations of organoleptic characteristic were made on a six-point scale rating with respect to crumb texture, crust texture, crust colour, appearance, flavour, taste and overall acceptability with score "6" having excellent attributes, point "1" indicating extreme dislike and whole wheat bread as the reference sample-R(Larmond, 1977). Samples were coded with three-digit random numbers and presented in monadic and random order. Samples were kept in Ziploc bags for freshness.

STATISTICAL ANALYSIS

The flour and bread samples data were statistically analyzed using SPSS (Statistical Package for the Social Sciences) Version 15 for PC Windows. Data was presented as mean ± SE. One-way Analysis of Variance (ANOVA) was used for the proximate analysis data and two-way Analysis of Variance was used for sensory evaluation data. Comparison between groups were made using Duncan multiple range test for proximate analysis data while Least Significant Difference(LSD) test was used to separate the means of the sensory evaluation data. Differences were considered significant if Probability is less than 5% ($P < 0.05$) for both sets of data.

Organoleptic Characteristics

The blending of wheat flour with non-germinated and germinated soybean flour at different levels altered the organoleptic properties of different blended breads. Data on crust colour, appearance, flavour, crust texture, crumb texture, taste and overall acceptability are presented in Appendix 1

As the level of soybean flour in blends was increased, a darkening of crust colour of the breads was observed. However, there was no significant difference in crust colour up to 15% blending with flour from non-germinated soybeans. Also, no significant difference was observed with 5% blending with flour from germinated soybeans. However, beyond 5% blending with germinated soybean flour, a significant difference was observed. It was also observed that bread made from wheat-germinated soybean flour at 10% and 15% substitution levels received the lowest ratings by the judges. It can therefore be inferred that substitution with germinated soybean flour (beyond 5% level) has detrimental effects on the acceptability of soy-composite bread in terms of crust color. The darker crust colour may be because of the greater amount of the maillard reaction between reducing sugars and proteins (Raidi & Klein, 1983).

Despite the highest appearance score recorded by germinated soybean flour blended bread at 5% level by judges, there was no significant difference between this product and bread made from 5% to 15% non-germinated soybean flour. However, there was a significant difference between this product and other germinated soybean products i.e. 10% and 15% germinated soy composite bread. Gayle et al. (1986) and Hoseney (1994) suggested that the appearance of bread was an important sensory characteristic on which the acceptability of bread depends.

The sensory evaluation result for flavor showed that there was a significant difference between 5% germinated and 15% germinated soy composite bread. However, no significant difference was recorded between any of the other bread samples. 5% had the highest flavor score. The lack of discrimination amongst other samples depicts the inability of flavor perception by the judges.

Taste evaluation suggested that both types (germinated and non-germinated) of soy composite

Breads had highest taste scores at the 5% level. Results indicated that the taste score generally decreased with increased level of substitution with soy flour as compared with control bread However, all the bread samples had satisfactory taste scores. Bread from both 5% germinated and 5% non-germinated soybean(which recorded the highest scores) differed significantly from 15% non-germinated soybean bread but there was no significant difference between the other products. This indicates that the judges have low tolerance for bread made from 15% germinated soybean flour which was rated poorest in taste possibly because of the beany flavour of soyflour (Rastogi & Singh, 1989).

Overall acceptability scores showed that all the samples were acceptable by the judges as they were all rated above average. However, bread made from 5% to 15% non-germinated flour and 5% germinated flour were the most preferred. Bread made from 5% germinated flour scored highest and that made from 15% germinated flour received the lowest score. This is in agreement with significant difference in overall acceptability observed between bread made from 15% germinated soybean flour and bread made from 5%, 10%, 15% non-germinated soybean flour and 5% germinated soybean flour.

CONCLUSION AND RECOMMENDATIONS

The results obtained from this study indicated that more nutritious bread, in which anti-nutritional factors have been reduced, can be produced from germinated soy-wheat composite flour. However, since non-germinated flour gave better rheological properties than germinated flour (especially above 5% substitution level of germinated soybean flour) and bread produced from the different non-germinated flour showed better textural properties and greater loaf volume; it can be inferred that non-germinated soybean flour would be more desirable by bakers.

In addition, the overall acceptability of bread loaves made from wheat-germinated soybean flour at 10% and 15% substitution levels received the lowest ratings.

From the ongoing, it can be concluded that germinated soybean flour was not desirable to be used as composite flour in bread making beyond 5% substitution levels for consumer acceptability.

It can therefore be inferred that substitution with germinated soybean flour (beyond 5% level) has detrimental effects on the acceptability of soy-composite bread in terms of crust color. The darker crust colour may be because of the greater amount of the maillard reaction between reducing sugars and proteins (Raidi & Klein, 1983).

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It is recommended that the organoleptic qualities which were lower in bread made from germinated soybean and wheat blends than in un-substituted wheat bread could be improved upon by the addition of appropriate additives. These potentials, if properly harnessed could help in improving the nutritional quality of bread, decrease the cost of production and consequently contribute in ensuring food security.

Table 1: Formulation for control and composite bread

	Soybean Flour %	**Soybean** Flour g	**Wheat** Flour g	**Yeast** g	**Fat** g	**Sugar** g	**Salt** g
NGSWB1	5	40	760	10	32	108.8	14.4
NGSWB2	10	80	720	10	32	108.8	14.4
NGSWB3	15	120	680	10	32	108.8	14.4
GSWB1	5	40	760	10	32	108.8	14.4
GSWB2	10	80	720	10	32	108.8	14.4
GSWB3	15	120	680	10	32	108.8	14.4
WB	0	0	800	10	32	108.8	14.4

The amount of water added was determined based on the water absorption values obtained from the farinograph.

LEGENDS: GSWB=Germinated Soy-Wheat Bread;
NGSFWB= Non-germinated Soy-Wheat Bread and;
WB=100% Wheat Bread

Table 2: Physical and rheological measurements on bread loaves

	WEIGHT	**VOLUME**	**SPECIFIC VOLUME**	**DENSITY**	**HEIGHT**	**TOTAL HARDNESS**
NGSWB1	605	2875.4	4.8	0.2	217.2	220.00
NGSWB2	608	2709.9	4.5	0.2	218.8	229.75
NGSWB3	607	2364.6	3.9	0.3	226.1	370.00
GSWB1	604	2992.4	5.0	0.2	219.8	246.00
GSWB2	615	2921.3	4.8	0.2	216.2	232.75
GSWB3	615	2641.2	4.3	0.2	223.6	391.25
WB	606	2937.1	4.9	0.2	220.0	222.25

LEGEND: NGSWB1 (95:5 wheat flour: non-germinated soybean bread);
NGSWB2 (90:10 wheat flour: non-germinated soybean bread);
NGSWB3 (85:15 wheat flour: non-germinated soybean bread);
GSWB 1 (95:5 wheat flour: germinated soybean bread);
GSWB 2 (90:10 wheat flour: soybean bread);
GSWB 3 (85:15 wheat flour: germinated soybean bread)
WB (Wheat Bread)

Table 3: Levels of trypsin inhibitor and oligossacharides in non-germinated and germinated soybean flour

	Non-germinated Soyflour	Germinated Soyflour
Trypsin inhibitor	29.47±0.04	20.68±0.05
Raffinose	2.13±0.02	0.16±0.12
Stachyose	16.14±0.01	0.19±0.01
Verbascose	3.41±0.02	0.14±0.01

Mean of triplicate determinations ± standard error

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Appendix-I

SENSORY EVALUATION QUESTIONAIRE (MULTIPLE COMPARISON TEST)

NAME: ____.

DATE: __________________.

You are receiving 6 coded samples of bread to compare for crust color, appearance, flavor, crust texture, taste and overall acceptability. You have been given a reference sample marked R with which you are to compare each of the samples. Observe each sample and assign numerical scores of 1 to 6 to each of the samples (with score"6"having excellent attributes, score "1" indicating extreme dislike).

Sample no	4BX	W45	7R9	J8T	Y5F	P3Z
Crust color						
Appearance						
Flavor/Aroma						
Crust texture						
Crumb texture						
Taste						
Overall acceptability						

COMMENTS: ____