

Effect of defatted Soya flour supplementation of wheat bread on blood chemistry and oxidative stress in Wistar rats

ABSTRACT

The present study was designed to ascertain the effect of supplementation of bread with defatted soy flour on blood chemistry and oxidative stress in Wistar rats. Wheat flour mixed with high quality defatted Soy flour at several ratios: 90:10, 80:20, 70:30, and 60:40. The 90:10, 80:20, 70:30, and 60:40 flour mixtures were used to prepare 10%, 20%, 30%, and 40% Soya bread, respectively. The control bread (100%) was prepared with 100% wheat flour. Bread produced with these blends compared with regular 100% wheat bread and was tested for chemical and organoleptic characteristics. Sixteen Wistar rats were randomly given codes and allocated to 2 different groups via tables with random numbers to feed on the 100% wheat blend and soy supplemented bread (90% wheat flour/10% soy flour) for 28 days. The weights of the rats and each day's feed were monitored on daily basis. Blood was collected for biochemical assays and liver was used for antioxidant assay. Antioxidant assessment showed that there was a significant increase ($p<0.05$) in GSH, SOD and Catalase enzymes in experimental rats liver in test group as compared to the control (wheat bread) and a significant decrease ($p<0.05$) in test group for lipid peroxidation markers (MDA) relative to control group. Analysis of hepatic enzymes and lipid markers (AST, ALT and ALP) and (LDL, CHOL and TG) showed a significant decrease ($p<0.05$) in liver experimental rats in test group as compared to the control. There was a significant decrease ($p<0.05$) in levels of renal function markers (T.Bilirubin, creatinine and

Urea) as well as protein markers (T. Protein and Albumin) of experimental rats in test group as compared to control. These findings establish the nutritional and health promoting benefits of soy supplemented bread.

INTRODUCTION

Bread is a convenience food made from wheat flour: which is derived from bread wheat and whose technology of which dates back to the ancient Egyptians. Wheat is not being made in Nigeria as it is not cultivated in the tropics for climatic reasons (Edema *et al.* 2005; Olaoye *et al.*, 2006). Only very few countries grow wheat resulting in importation paid for with scarce foreign exchange. It is therefore of economic advantage if wheat import can be reduced by substitution with other suitable materials. This led to the whole idea of composite flour. Composite flour is a mixture of wheat with other materials to form suitable flour for baking purposes. A lot of efforts has been made and still being 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 production, thereby decreasing the demand for imported wheat and producing protein-enriched bread (Giami *et al.*, 2004; Olaoye *et al.*, 2006).

White bread contains 8 to 9% protein and lacks an essential amino acid called lysine (Alabi *et al.* 2001). As this indicates, it is an issue and extensive work is being carried out for the past few decades, to fortify white bread with high protein and high lysine materials (Giami *et al.*, 2004). According to the previous studies, cereal and legume proteins are nutritionally complementary to each other in terms of sulphur containing amino acids namely, methionine and lysine (Singh

43 *et al.* 1999). At the same time, (Alabi *et al.* 2001) reported that “Addition of wheat flour with
44 non-wheat flours can cause dough and bread quality to diminish”.

45 Soybean (*Glycine max*) belongs to the family leguminosae and sub-family papillionnideae.
46 Soybean has long been recognized as an excellent source of high quality protein. It is a
47 remarkable source of protein for both animals and human consumption and is also a leading
48 source of edible oils and fats (Singh *et al.* 1999; Alabi *et al.* 2001).As an important component
49 crop, soybean the legume richest in nutrients and the one from which the most dietary
50 products are made is used in various traditional farming systems of various countries
51 (Pamplona, 2005).It contains valuable phytochemicals and has extraordinary capacity to
52 nourish and prevent diseases. Soya has the advantages of containing virtually no sodium, a
53 mineral that cause fluid retention in the tissues; this makes it very suitable in cases of
54 cardiovascular disease.The soybean also contains a wide variety of chemical compounds that
55 have potent bioavailability. Among these compounds are the isoflavones, which may also be of
56 benefit in the prevention of cardiovascular disease (Ng *et al.*2013) and diabetes (Esmailzadeh,
57 2012). Incorporation of soy-related ingredients into a staple food such as a bakery product may
58 be a feasible means of increasing daily soy intake in people's diets (Murphy *et al.*1999).Soya is
59 also known to be a good source of the trace elements copper, zinc and manganese and can be
60 said to contain all the nutrients needed in food (Ampofo,2009). Apart from the higher
61 nutritional content of soybeans, it is also very cheap compared to wheat flour. Soybean is the
62 only source that contains all the amino acids. Its use in the production of bread as composite
63 flour has been reported (Basman *et al.*, 2003).

Therefore, in the present study we investigated the best formulation of defatted soy-fortified bread on proximate and organoleptic properties. The purpose of this study is to supplement wheat flour with defatted Soya flour to develop a nutritionally and health rich and acceptable bakery product by consumer in comparison with the traditional bread without any undesirable effects on its sensory characteristics.

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MATERIAL AND METHOD

Raw material

The hard wheat flour was purchased from Flour Mills Plc Lagos, Nigeria and the defatted Soy flour used for baking of composite bread was purchased from Nutrichem West Africa Limited; a leading Soy flour manufacturing company in Lagos. The soy flour was incorporated into wheat flour at different levels of substitution and used to make bread.

Preparation of composite flour

Wheat flour was mixed with High quality Soy flour at several ratios: 90:10, 80:20, 70:30, and 60:40. The 90:10, 80:20, 70:30, and 60:40 flour mixtures were used to prepare 10%, 20%, 30%, and 40% Soya bread, respectively. The control bread (100%) was prepared with 100% wheat flour. Ten samples of bread were prepared per group.

Preparation of dough for composite bread

The 100% wheat flour dough was prepared according to the batch formulation below:

Table 1.1: Formulation for Soy/Wheat bread

S/N	INGREDIENT	WEIGHT (KG) 1 MIX				
1.	WHOLE WHEAT FLOUR(g)	100	90g	80g	70g	60g
2.	DEFATTED SOY FLOUR(g)	0	10g	20g	30g	40g
3.	SUGAR(g)	8	8g	8g	8g	8g
4.	YEAST(g)	2	2g	2g	2g	2g
5.	SALT(g)	2	2g	2g	2g	2g
6.	YEAST(g)	2	2g	2g	2g	2g
7.	BREAD IMPROVER (g)	1	1g	1g	1g	1g
8.	CALCIUM PROPIONATE(g)	1	1g	1g	1g	1g
9.	MARGARINE(g)	1	1g	1g	1g	1g
10.	WATER (mls)	54mls	54mls	54mls	54mls	54mls
	TOTAL DOUGH WEIGHT	172	172	172	172	172

Bread making

The whole wheat flour was mixed, with varying inclusions of 0, 10, 20, 30 and 40% of the defatted soy-flour. The composite flours were blended with other baking ingredients (Table 7) in a mixer, kneaded for 10 min into consistent dough. The resulting dough was molded and placed in a pre-oiled baking mould pan. The dough was proofed for 55 to 60 min at 37°C and 80% relative humidity and baked in a reel oven for 38 min at 210°C.

Chemical analysis of Hard wheat flour, soya flour and bread

The ash, crude fat, crude protein, carbohydrate and crude fiber contents were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 2000).

95 Animal Model

96 Sixteen male Wistar rats (80-90 g) were procured from the Animal House of the college of
97 Medicine of the University of Lagos. They were housed in cages made of plastics with a
98 prepared gauze cover to enable ventilation. The animals were fed rat chow and water for 7
99 days of acclimatization. The animals were weighed throughout the period of the experiment.
100 After acclimatization, the mean weights of the male Wistar rats (n = 16) at the beginning of the
101 experiment were taken. The rats were divided into two diet groups, each eight and named
102 Control (C) and Test (T). The rats were housed in metabolic cages. The rats of the Control group
103 during the 28 days of the experiment received the 100% Wheat bread while the rats of the Test
104 group received 10% soy wheat bread. All rats were fed once a day ad libitum, having
105 unrestricted access to drinking water. The feed intake and body gains were monitored and
106 recorded daily every week. At the end of the experiment after 24 h of starvation, the rats were
107 sacrificed using cervical dislocation.

108 Liver and plasma analysis

109 On day 28, animals were humanely sacrificed using cervical dislocation. Blood was collected
110 into heparinized tubes via ocular puncture using capillary tube then followed by centrifugation
111 at 3000rpm for 10mins. Clear serum was collected separately for each sample and subsequently
112 used for further analysis. Liver tissues were homogenized with 5 volumes of ice-cold 50 mM Tris
113 HCl (pH 7.4) containing 30 mM sucrose. The homogenate was centrifuged at 1500 x g for 10 min
114 to remove nuclei and cell debris. The resulting supernatant was then used for total protein
115 determination, glutathione (reduced), superoxide dismutase and catalase. Total liver protein
116 levels were determined according to Gonall *et al.*, 1949, using bovine serum albumin as

standard. The concentration of reduced glutathione (GSH) was determined using the method of Sedlak and Lindsay (1968). The superoxide dismutase activity was assayed by its ability to inhibit the autooxidation of epinephrine, determined by the increase in absorbance at 480 nm (Sun and Zigman, 1978). The catalase activity was determined using the method of Luck H. (1971). Serum total cholesterol was estimated using method of Zak (1959), plasma triglyceride estimated by method of Mendez *et al*, (1975), HDL-C by Lopez- Vitrella *et al*, (1975), LDL-C and VLDLtriglyceride values were calculated by a modification of the friedewald formular (Sandkapm, 1990). Assay of alkaline phosphatase was carried out using Randox commercial enzyme kits according to the method of King *et al.*, (1954). ALT and AST were measured calorimetrically using Randox reagent enzyme kits based on the method of Reitman *et al.*, (1957). The plasma creatinine was measured using a creatinine test kit (Sigma creatinine No 555 – A Kit). Albumin and total bilirubin was measured using a candidate reference method by Doumas *et.al*, (1985). Automated reaction rate method by Fabiny *et al.*, (1971) was used for the determination of creatinine while spectrophotometric method by With *et al.*, (1961) was used for the determination of plasma creatinine levels.

Statistical analysis

All results were expressed as mean $\pm$ standard error of measurement (SEM) using statistical significance (t-test: using Prism GraphPad statistical software (version 5.0). Differences at $P < 0.05$ were considered to be significant.

RESULTS

Table 1: The proximate composition of wheat flour and soya flour

S/N	PARAMETERS	WHEAT FLOUR	SOYA FLOUR
1.	Crude protein	11.73 $\pm$ 0.04	48.33 $\pm$ 0.20*
2.	Moisture (%)	12.11 $\pm$ 0.08	9.57 $\pm$ 0.10*
3.	Ash (%)	0.56 $\pm$ 0.02	6.27 $\pm$ 0.07*
4.	Fat (%)	1.83 $\pm$ 0.07	2.65 $\pm$ 0.04*
5	Carbohydrate	73.77 $\pm$ 0.02	29.12 $\pm$ 0.48*
6.	Fibre	2.04 $\pm$ 0.02	4.06 $\pm$ 0.03*
7.	Food Energy Value (Kcal)	358.43 $\pm$ 0.10	334.70 $\pm$ 0.68*

Results represented as mean $\pm$ S.E.M

*statistically significant at $p < 0.05$ compared with the control, n=3

Table 2: The proximate composition of whole wheat and functional breads

S/N	PARAMETERS	BREAD SAMPLES				
		A (90% wheat flour bread)	B (90% wheat flour/10% soya flour composite bread)	C (80% wheat flour/20% soya flour composite bread)	D (70% wheat flour/30% soya flour composite bread)	E (60% wheat flour/40% soya flour composite bread)
1.	Crude protein	9.17 $\pm$ 0.1	10.52 $\pm$ 0.1*	11.45 $\pm$ 0.1*	12.35 $\pm$ 0.1*	12.89 $\pm$ 0.1*
2.	Moisture (%)	34.12 $\pm$ 0.2	35.44 $\pm$ 0.1	37.33 $\pm$ 0.2	38.26 $\pm$ 0.2*	40.31 $\pm$ 0.2*
3.	Ash (%)	1.92 $\pm$ 0.1	2.05 $\pm$ 0.2	2.45 $\pm$ 0.1*	2.49 $\pm$ 0.2*	2.69 $\pm$ 0.2*
4.	Fat (%)	3.61 $\pm$ 0.2	3.96 $\pm$ 0.1	4.2 $\pm$ 0.2*	5.29 $\pm$ 0.3*	6.42 $\pm$ 0.5*
5.	Crude Fibre (%)	3.16 $\pm$ 0.2	4.38 $\pm$ 0.2*	4.63 $\pm$ 0.2*	5.06 $\pm$ 0.2*	5.58 $\pm$ 0.1*

6	Carbohydrate (%)	48.01 $\pm$ 0.1	43.73 $\pm$ 0.2	40.02 $\pm$ 0.2	36.54 $\pm$ 0.2	32.18 $\pm$ 0.2
7.	Food Energy Value (Kcal)	261.34 $\pm$ 0.1	252.65 $\pm$ 0.1	243.72 $\pm$ 0.2	243.26 $\pm$ 0.2	237.74 $\pm$ 0.3

Results represented as mean $\pm$ S.E.M

*statistically significant at $p<0.05$, all compared to sample A (The standard), $n=3$

Table 3: The sensory evaluation of whole wheat and functional breads

S/N	PARAMETERS	BREAD SAMPLES				
		A (90% wheat flour bread)	B (90% wheat flour/10% soya flour composite bread)	C (80% wheat flour/20% soya flour composite bread)	D (70% wheat flour/30% soya flour composite bread)	E (60% wheat flour/40% soya flour composite bread)
1.	TASTE	6.46 $\pm$ 0.3	6.80 $\pm$ 0.2	6.13 $\pm$ 0.3	6.33 $\pm$ 0.2	5.73 $\pm$ 0.1*
2.	CRUST COLOUR	6.20 $\pm$ 0.1	6.50 $\pm$ 0.4	6.00 $\pm$ 0.2	5.83 $\pm$ 0.1	5.80 $\pm$ 0.2
3.	TEXTURE	6.00 $\pm$ 0.2	6.43 $\pm$ 0.2	6.16 $\pm$ 0.2	6.90 $\pm$ 0.3	6.33 $\pm$ 0.3
4.	FLAVOUR	6.60 $\pm$ 0.1	5.73 $\pm$ 0.1	5.40 $\pm$ 0.2	5.13 $\pm$ 0.1*	4.93 $\pm$ 0.1*
5.	OVERALL ACCEPTABILITY	7.36 $\pm$ 0.2	7.00 $\pm$ 0.1	6.63 $\pm$ 0.3	6.37 $\pm$ 0.2	6.03 $\pm$ 0.2*

Results represented as mean $\pm$ S.E.M

*statistically significant at $p<0.05$, $n=30$

153 **Table 4: Concentration of oxidative stress markers in rats' liver after 4 weeks administration**
 154 **of 100% whole wheat flour bread and composite bread (90% wheat/10% soya flour).**

OXIDATIVE STRESS MARKER	CONTROL (GROUP A) (100% whole wheat bread)	TEST (GROUP B) (90% whole wheat/10% soya flour composite bread)
REDUCED GLUTATHIONE (GSH) ($\mu\text{mol/ml}$)	2.31 $\pm$ 0.1	6.5 $\pm$ 0.4*
SUPEROXIDE DISMUTASE (SOD) ($\mu\text{mol/ml}$)	88.77 $\pm$ 1.95	120.29 $\pm$ 2.69*
CATALASE ($\mu\text{mol/ml}$)	712.0 $\pm$ 3.2	731.8 $\pm$ 5.3*
MALONDIALDEHYDE (MDA) (Umol/ml)	1.49 $\pm$ 0.02	1.19 $\pm$ 0.02*

155 Results represented as mean $\pm$ S.E.M

156 *statistically significant at $p < 0.05$, $n=8$

157

158 **Table 5: Concentration of hepatic activity biomarkers in rats after 4 weeks administration of**
 159 **100% whole wheat flour bread and composite bread (90% wheat/10% soya flour).**

LIVER ENZYMES	CONTROL (GROUP A) (100% whole wheat bread)	TEST (GROUP B) (90% whole wheat/10% soya flour composite bread)

ASPARTATE TRANSAMINASE (U/L)	206.20 $\pm$ 6.06	150.10 $\pm$ 4.44*
ALANINE TRANSAMINASE (U/L)	70.66 $\pm$ 0.73	34.10 $\pm$ 0.73*
ALKALINE PHOSPHATASE (U/L)	288.1 $\pm$ 25.72	174.50 $\pm$ 13.40*

160 Results represented as Mean $\pm$ S.E.M

161 *statistically significant at p<0.05, n=8

162

163 Table 6: Renal function activity in rats' liver after 4 weeks administration of 100% whole
164 wheat flour bread and composite bread (90% wheat/10% soya flour).

LIVER ENZYMES	CONTROL (GROUP A) (100% whole wheat bread)	TEST (GROUP B) (90% whole wheat/10% soya flour composite bread)
UREA (μmol /L)	4.06 $\pm$ 0.47	2.18 $\pm$ 0.43*
CREATININE (μmol l)	32.76 $\pm$ 1.89	28.86 $\pm$ 0.66*
T.BILIRUBIN (μmol /L)	1.59 $\pm$ 0.21	0.68 $\pm$ 0.03*

165 Results represented as Mean $\pm$ S.E.M

166 *statistically significant at p<0.05, n=8

167 **Table 7: Protein profile in rats liver after 4 weeks administration of 100% whole wheat flour**
 168 **bread and composite bread (90% wheat/10% soya flour).**

PROTEIN MARKER	CONTROL (GROUP A)	TEST (GROUP B)
T.PROTEIN (g/L)	81.34 $\pm$ 0.85	62.69 $\pm$ 1.74*
ALBUMIN (g/L)	47.86 $\pm$ 0.97	28.39 $\pm$ 1.45*

169 Results represented as Mean $\pm$ S.E.M

170 *statistically significant at p<0.05, n=8

171

172 **Table 8: Lipid profile in rats' liver after 4 weeks administration of 100% whole wheat flour**
 173 **bread and composite bread (90% wheat/10% soya flour).**

LIPID MARKER	CONTROL (GROUP A)	TEST (GROUP B)
LDL (mmol/L)	0.73 $\pm$ 0.03	0.32 $\pm$ 0.01*
HDL (mmol/L)	0.93 $\pm$ 0.16	0.95 $\pm$ 0.13
CHOL (mmol/L)	1.95 $\pm$ 0.09	1.11 $\pm$ 0.11*
TG (mmol/L)	1.06 $\pm$ 0.09	0.66 $\pm$ 0.10*

174 Results represented as Mean $\pm$ S.E.M

175 *statistically significant at p<0.05, n=8

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DISCUSSION

The results obtained from the proximate analysis of the whole wheat flour and soybean flour were slightly different from that obtained from literatures (Potter *et al.*, 2006). The chemical composition of the composite flours have been shown to affect both physico-chemical properties and nutritional quality of their products (Mashayekh *et al.*, 2008). There was a significant increase ($p < 0.05$) in ash, protein, carbohydrate, fibre, moisture and food energy value of soya flour compared to hard wheat flour.

The increased supplementation of whole wheat flour with soy flour was found to have greatly affected the physico-chemical quality of the composite breads. The proximate values increased with increasing levels of soya bean substitutions except for carbohydrate content and energy values which showed the reverse. Carbohydrate decreased with increasing substitution of soya flour in all the bread samples. This trend supports the claim of (Akpapunam *et al.*, 1997). The carbohydrate content and energy values were highest in sample A (48.01% and 261.34 Kcal) and lowest in sample E (32.18% and 237.74 Kcal), respectively. The low carbohydrate and energy values were as result of the low fat content of the composite breads. Similar trends were reported by (Serem *et al.*, 2011) and (Islam *et al.*, 2007) in the fortification of wheat flours with defatted and non-defatted soy flour, respectively. The composite breads (B to D) contained energy values in the range of 243 to 252 Kcal, and hence conformed to the (FAO/WHO, 1994) except for sample E which contained energy value of 237.74 Kcal as well as sample A which recommended minimum energy content of 1674 kJ/ 100 g. The moisture contents of the composite breads increased with soy flour substitution by a range of 35.44 to 40.31%. Increase in moisture content has been associated with increase in fibre content (Akhtar

200 *et al.*,2008). High moisture content has been associated with short shelf life of composite
 201 breads as they encourage microbial proliferation that lead to spoilage (Ezeama, 2007).There
 202 was also an increase in the protein content of the composite breads with soy-flour substitution
 203 in the range of 10.52 to 12.89%. This increase is as a result of substitution of whole-wheat flour
 204 (11.73% protein) with soya bean flour of 48.33% protein content. Mashayekh *et al.* (2008) also
 205 reported increase in protein content of the bread as a result of the addition of soy flour. Other
 206 studies have also reported a similar increase of protein content in sorghum-soy composite
 207 flours (Awadelkareem *et al.*, 2008). The fat content also increased from 0.35 to 2.81% in the
 208 composite breads produced from soy-bean flour substitution. Soybean from which the soy-flour
 209 was produced from is an oil seed, must have contributed most of the oil content to the product.
 210 The high oil content of the composite bread ill affect the shelf stability (Potter *et al.*, 2006).The
 211 crude fibre content of the composite bread showed a percentage increase in the range of 1.22
 212 to 2.42% as the whole-wheat flour was substituted with soy bean flour. This could be attributed
 213 to the use of whole wheat flour and soy-flour, both of which had high crude fibre contents (2.04
 214 and 4.06%, respectively).The crude fibre most likely from the bran of the whole wheat flour and
 215 the hull of soy beans, represents variable fraction of dietary fibre and includes mostly the lignin,
 216 cellulose and hemicelluloses components (Islam *et al.*, 2007).The increased fibre and the lower
 217 carbohydrate content of composite breads have several health benefits, as it will aid in the
 218 digestion of the bread in the colon and reduce constipation often associated with bread
 219 produced from refined wheat flour (Jideani *et al.*, 2009). According to well documented studies,
 220 it is now accepted that dietary fibre plays a significant role in the prevention of several diseases

221 such as; cardiovascular diseases, diverticulosis, constipation, irritable colon, cancer and
222 diabetes (Elleuch *et al.*, 2011).

223
224 The taste of the bread refers to the sweet sensation caused in the mouth by contact with the
225 bread due to the sweetening agent (Ebuehi *et al.*, 2007). There seems, however, to be no
226 significant difference between the tastes of the whole wheat bread and the soy supplemented
227 bread up to 30% of soybean flour. However, there was significant reduction in the taste of the
228 bread with 40% soy flour substitution as compared to the bread with 100% Wheat flour. The
229 results of bread crust colour did not show a consistent pattern for all the bread samples, and
230 there was no significant difference in the bread samples and the control sample. The darker
231 color of the crumbs of whole wheat bread and fortified breads and biscuits have been reported
232 by several authors (Serrem *et al.*, 2011). The brownish bread appearance could be directly
233 related to the increase in fiber content (Hu *et al.*, 2007). Moreover browning of the breads
234 could also occur due to caramelization and maillard reactions, as the protein contributed by
235 soybean flour must have reacted with sugar during the baking process (Dhingra *et al.*, 2001).
236 Texture is the quality of the bread that can be decided by touch, the degree to which it is rough
237 or smooth, hard or soft. The scores for texture (softness and chewiness) of the composite bread
238 samples, increased with increase in soybean flour substitution, when compared to whole wheat
239 bread (control sample A). The bread with 30% soy flour substitution (sample D), had the best
240 texture score. Hard crumb texture, caused by increased fiber from wheat bran substitution was
241 reported by Eiman *et al.* (2008). The baking conditions (temperature and time variables); the
242 state of the bread components, such as fibres, starch, protein (gluten) weather damaged or
243 undamaged and the amounts of absorbed water during dough mixing, all contribute to the final

texture of the breads (Bakke *et al.*, 2007).The incorporation of soybean flour into whole-wheat bread resulted in poor flavour scores. The results showed a decrease in the scores as the whole-wheat flour was substituted with soy-flour. Sample E with 40% soy-flour recording the lowest value. Most of the panelist complained of beany flavour and aroma from the soyflour in the composite breads. Serrem *et al.*, (2011) reported that substitutions of defatted-soy-flour into wheat bread and biscuits were associated with the roasted soybean flavour, aroma and after taste. The beany flavour is commonly associated with food legumes (Okoye *et al.*, 2009). In soybeans, enzymatic breakdown by lipoxygenases or autoxidation of linoleic and linolenic acid produces hydroperoxides such as ketones, aldehydes and alcohols that may be responsible for the beany-flavour which discourages soy consumption (Awadelkareem *et al.*, 2008). The sensory evaluation also revealed that breads with soy-flour substitution up to 10% (sample B) were overall acceptable, even though normal bread was still preferred.The baking properties of composite flour are often impaired as well as the organoleptic attributes of the products, because of the dilution of the gluten content (Jideani *et al.*, 2009).Thus, different combinations of both synthetic and organic improvers such as malt flour, vital wheat gluten and ascorbic acid can be included in dough formulation to improve the baking and sensory qualities of the products (Elleuch *et al.*, 2011).

Chronic exposure to oxidative stress is known to exert deleterious effects on health with accumulating oxidative damage throughout a lifetime. Formation of reactive oxygen species occurs during normal physiological processes by both non-enzymatic and enzymatic sources, causing continuous damage to lipids, proteins, and nucleic acids. Oxidative modification of

these molecules by ROS plays a pivotal role in a wide range of common diseases and age-related degenerative conditions. Consequently, increases in antioxidative capacity are believed to be protective against this oxidative damage. Isoflavones in soy products have recently received attention as one of the phytochemicals with diverse properties. Isoflavones have been linked to decreased risk of cardiovascular disease, osteoporosis, endocrine-responsive cancer (eg breast, prostate, and colon cancer), and menopausal symptoms, due in part to their possible antioxidant activities (Yousef *et al.*, 2004).

The antioxidant enzymes: reduced glutathione, superoxide dismutase and catalase increased significantly ($p < 0.05$) in the experimental rats liver in group B (Test group) as compared to the control group (Group A) were also shown. However, lipid peroxidation marker; malondialdehyde decreased significantly ($p < 0.05$) in the experimental rats liver in group B (Test group) as compared to the control group (Group A). Research on the antioxidant action of isoflavones suggests free radical scavenging ability, ability to reduce low-density lipoprotein (LDL) and DNA susceptibility to oxidative stress and ability to boost the activity and expression of antioxidant enzymes. Isoflavones are found in vegetables and fruits in a biologically inactive glycoside form. The two major isoflavones, genistein and daidzein, are present in soy as β -D-glycosides, genistin and daidzin. These glycoside forms are biologically inactive. After ingestion, β -glucosidases in the intestinal wall hydrolyze the glycosides, resulting in conversion to their corresponding bioactive aglycones, genistein and daidzein. Only these aglycone forms are absorbed and are therefore biologically active. Genistein is further metabolized to p-ethyl phenol, and daidzein to equol and O-demethyangolensin (Cederroth *et al.*, 2009). The results presented here suggest

287 that supplementation with soy isoflavones enhances antioxidative function and prevents lipid
288 peroxidation, possibly through activation of the antioxidant enzymes.

289 Alanine transaminase (ALT) is an enzyme primarily localized to the liver but the aspartate
290 transaminase (AST) is present in a wide variety of tissues like the heart, skeletal muscle, kidney,
291 brain and liver. There serum levels is indicative of hepatic injury (Rosen *et al.*, 2000). Hence, they
292 are used as liver biomarkers. There was a significant decrease ($p < 0.05$) in liver function markers
293 (AST, ALT and ALP) of experimental rats in group B (Test group) as compared to the control
294 group (Group A).

295 Alkaline phosphatases are a family of zinc metalloenzymes, with a serine at the active center;
296 they release inorganic phosphate from various organic orthophosphates and are present in
297 nearly all tissues. In liver, alkaline phosphatase is found histochemically in the microvilli of bile
298 canaliculi and on the sinusoidal surface of hepatocytes. Alkaline phosphatase from the liver,
299 bone and kidney are thought to be from the same gene but that from intestine and placenta
300 are derived from different genes. The mechanism by which alkaline phosphatase reaches the
301 circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may result from
302 leaky tight junctions (Rosalki *et al.*, 1999) and the other hypothesis is that the damaged liver
303 fails to excrete alkaline phosphatase made in bone, intestine and liver (Daniel *et al.*, 1999).

304 There was a significant decrease ($p < 0.05$) in liver function markers (AST, ALT and ALP) of
305 experimental rats in group B (Test group) as compared to the control group (Group A). This
306 observation correlates with the results from similar works by (Eastham, 1985) which revealed
307 that a high protein diet may cause the serum alkaline phosphatase to fall into the lower range
308 of normal. The observed decrease in serum alkaline phosphatase may be ascribed to the protein

content of the high soy diets. This perhaps suggests that soybean confers protection on the liver tissues against injury, damage or disease, which are often the direct cause of elevation of the enzymes in the bloodstream (Sanjiv, 2002).

The levels of urea, bilirubin and creatinine levels as they are biomarkers for renal failure. Kidney maintains optimum chemical composition of body fluid by acidification of urine and removal of metabolic wastes such as urea, bilirubin, and creatinine. During renal diseases the concentration of these metabolites increases in blood (Corbett, 2008). Total bilirubin levels showed a significant decrease ($p < 0.05$) in the experimental rats in group B fed with soy supplemented bread (Test group) as compared to the control group (Group A). The increase in conjugated bilirubin makes the liver more effective because it is able to conjugate more bilirubin. Urea levels showed a significant decrease ($p < 0.05$) in the experimental rats in group B fed with soy supplemented bread (Test group) as compared to the control group fed with 100% wheat bread. Creatinine levels showed a significant decrease ($p < 0.05$) in the experimental rats in group B fed with soy supplemented bread (Test group) as compared to the control group fed with 100% wheat bread. This observation correlates with similar work by Aukema *et al.*, (1999) on soy protein effects on inherited polycystic kidney disease are influenced by gender and protein level. The increased creatinine level in soy-wheat bread has similar results as previous works by (Ogborn *et al.*, 1998) on Soy protein modification of rat polycystic kidney disease.

Albumin is quantitatively the most important protein in plasma synthesized by the liver and is a useful indicator of hepatic function. Albumin synthesis is affected not only in liver disease but also by nutritional status, hormonal balance and osmotic pressure. Liver is the only site of synthesis of albumin (Rosalki *et al.*, 1999). In the current study, it was observed that there was a significant increase ($p < 0.05$) in the levels of protein markers (T. Protein and Albumin) of experimental rats in group B (Test group) fed with wheat-soy bread as compared to the control group (Group A).

The lipid profile (which involves levels of total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and very-low density lipoprotein (VLDL)) serves as diagnostic indices in conditions such as chronic obstructive jaundice, hepatitis, coronary heart disease and atherosclerosis. Hyperlipidaemia is one of the risk factors for coronary heart disease while cholesterol is the major lipid constituent of atherosclerotic plaque. In support of previous findings (Balmir *et al.*, 1996). Cholesterol is a main sterol found in body tissues. There are various types of cholesterol, including LDL cholesterol which is an unhealthy form of cholesterol, HDL cholesterol which is considered as a healthy form of cholesterol and triglycerides, a form of fat carried through the bloodstream (James and Claude, 1998). There was a significant decrease ($p < 0.05$) in lipid markers (LDL, CHOL and TG) of experimental rats in group B (Test group) as compared to the control group (Group A). This correlates many authors' results. Recently, some human studies have revealed the association between dietary soy components with cardiovascular risk biomarkers in metabolic syndrome and diabetes (Ng *et al.* 2013). Some data are available concerning the favorable effects of soy products on

inflammatory biomarkers(Kimiagar *et al.*,2007).One study revealed that soy product consumption for 8 weeks could reduce malondialdehyde (MDA) levels in postmenopausal women with the metabolic disorder (Kimiagar *et al.*,2007).In a double-blind randomized clinical trial, 50 g/day soy protein containing 164 mg isoflavones for 10 weeks, reduced the cardiovascular disease risk in hypercholesterolemic postmenopausal women because of both modest reductions in serum lipoproteins and an increase in paraoxonase1 activity (Kimiagar *et al.*,2007). (Potter *et al.*, 1993) determined the relationship between soy product intake and serum total cholesterol concentration in 1242 men and 3596 women in Takayama City, Japan. The reported significant trend in decreasing total cholesterol concentration with an increasing intake of soy products in men and women was observed. Clinical trial data and the results of a meta-analysis suggested a hypocholesterolemic effect of soy protein. In meta-analysis of 38 controlled clinical trials, Anderson *et al.*, (1995) found that the consumption of soy protein rather than animal protein significantly decreased serum concentrations of total cholesterol, LDL cholesterol, and triglycerides. In a similar study, meta-analysis of 8 randomized controlled trials conducted by (Balmir *et al.*, 1996) demonstrated that the isoflavones exhibited LDL cholesterol-lowering effects independent of soy protein.The presence or absence of the soybean isoflavone fraction may be a confounding factor. This fraction, consisting primarily of genistein, daidzein and glycitein, has been shown to have a hypocholesterolemic effect in animals and humans. Potential mechanisms by which soy protein and/or isoflavones induce lowering of blood cholesterol concentrations include thyroid status, bile acid balance and the estrogenic effects of genistein and daidzein. There was no statistical significant difference ($p>0.05$) in HDL of experimental rats in group B (Test group) as compared to the control group

(Group A).This observation is in agreement with similar work by (Kurowska *et al.*, 1997) on effects of substituting dietary soybean protein and oil for milk protein and fat in subjects with hypercholesterolemia.

CONCLUSION

The results of this study suggest that high intake of soy supplemented bread may confer significant advantages on the health of the animal model used. Therefore the impact of this research results would go a long way in improvement of the nutritional status of Nigerians through consumption of functional foods like soy supplemented bread due to their rich nutritional content and health benefits. Therefore, it is hoped that relevant food and health agencies would help in championing the cause for the use of composite flour (wheat and soya) in the production of bread for human consumption.

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