International Journal of Biochemistry Research & Review
10(1): 1-14, 2016, Article no.IJBcRR.22912
ISSN: 2231-086X, NLM ID: 101654445

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Defatted Soya Flour Supplementation of Wheat Bread Confers Oxidative, Renal, Hepatic and Cardiovascular Protective Effects in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author OATE designed the study, wrote the protocol and supervised the work. Author HKO carried out all laboratories work and performed the statistical analysis. Author HKO wrote the first draft of the manuscript. Author HKO managed the literature searches and edited the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2016/22912
Editor(s):
(1) Mohamed Fawzy Ramadan Hassanien, Biochemistry Department, Zagazig University, Egypt.
Reviewers:
(1) Anthony Cemaluk C. Egbuonu, Michael Okpara University of Agriculture, Umudike, Nigeria.
(2) Iriani R. Maldonade, Brazilian Agricultural Corporation, Brazil.
(3) Nilgun Ertas, Necmettin Erbakan University, Turkey.
Complete Peer review History: http://sciencedomain.org/review-history/12975

Original Research Article

Received 4th November 2015
Accepted 26th December 2015
Published 14th January 2016

ABSTRACT

Background: The present study was designed to ascertain the effect of supplementation of bread with defatted soy flour on antioxidant, renal, hepatic and cardiovascular status in Wistar rats.
Methods: Hard wheat flour was mixed with high quality defatted soy flour at several ratios: 90:10(w/w), 80:20(w/w), 70:30(w/w), and 60:40 (w/w). The respective ratios of the flour mixtures were mixed with other ingredients and used to bake the wheat-soy bread. The 100% hard wheat flour baked bread was the control. All bread samples were tested for both chemical and sensory characteristics. Forty Wistar rats were randomly given codes and allocated to 5 different groups via tables with random numbers to feed on the 100% wheat breads and soy supplemented bread groups respectively for 28 days and sacrificed using cervical dislocation. Blood was collected through ocular puncture and used for biochemical assays while liver was used for antioxidant assay.

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Results: There was a significant ($p<0.05$) increase in the liver levels of antioxidant enzymes: reduced glutathione (GSH),superoxide dismutase (SOD) and catalase (CAT) enzymes in experimental rats liver of wheat-soy bread groups compared to the control group and a significant ($p<0.05$) reduction in wheat-soy bread groups for lipid peroxidation-malondialdehyde (MAL) compared to non-supplemented group. However, antioxidant enzymes and lipid peroxidation biomarker levels of the wheat-soy bread groups were not significantly ($p>0.05$) different from one another. There was a significant ($p<0.05$) reduction in the serum levels of liver enzymes namely: Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alanine Phosphatase (ALP) as well as serum levels of lipid biomarkers namely: Low Density Lipoproteins (LDL), Cholesterol (CHOL) and Triglycerides (TG) in wheat-soy bread groups as compared to the wheat bread group (control). However, the serum levels of these lipid biomarkers in all the four wheat-soy bread experimental rat groups were not significantly ($p>0.05$) different from one another. The serum levels of renal function markers (Total Bilirubin, Creatinine and Urea) as well as protein markers (Total Protein and Albumin) were significantly ($p<0.05$) lower in wheat-soy bread experimental rat groups compared to wheat bread group but were not significantly ($p>0.05$) different for the wheat-soy supplemented groups.
Conclusion: Our findings establish the nutritional and health promoting benefits of soy supplemented bread. In addition, it was also deduced that 10% wheat-soy bread gave the bread the best overall quality acceptability.

Keywords: Wheat-soy blends; proximate composition; antioxidant; sensory attributes; biochemical assay.

1. 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 [1,2]. 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. Much efforts have been put into promoting the use of composite flours in bread production substituting some portions of wheat flour with flour obtained from high protein seed which consequently has led to the decrease in the rate of importation of wheat [3].

White bread contains 8 to 9% protein and lacks an essential amino acid called lysine [1]. 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 [3]. 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 [4]. At the same time, [1] reported that "Addition of wheat flour with non-wheat flours can cause dough and bread quality to diminish".

Soybean (Glycine max) is a member of the family leguminosae with its sub-family being papillionnideae. Soybean has long been known to be a very good source of high quality protein and also a very well-known source of protein for both animals and human consumption as well as its being an important source of edible oils and fats [1,4]. With soybean being the legume richest in nutrients and also serving as the source from which the most dietary products are produced is used in various traditional agricultural systems in different countries [5]. Soybean contains very useful phytochemicals hence its capability of nourishing and preventing diseases. Soybean contains virtually no sodium, a mineral that can cause the tissues to retain fluids; this makes soybean very useful in the management of cardiovascular disease. In addition, soybean contains a lot of varied chemical compounds that have high bioavailability. One of these compounds is the isoflavone, which has potentials in the prevention of cardiovascular disease [6] including diabetes [7]. Inclusion of soy-related ingredients into a staple food such as a bakery product may be a successful method of increasing daily soy intake in people's diets [8]. Soybean has also been known to be an important source of the trace minerals like copper, zinc and manganese hence it can be said to contain all the nutrients needed in food [2]. Apart from the higher nutritional content

of soybeans, it is also very cheap compared to wheat flour. Soybean is the only source that contains all the amino acids. Its use in the production of bread as composite flour has been reported [7].

Therefore, in the present study we investigated the best formulation of defatted soy-fortified bread on proximate and organoleptic properties. In addition, we also carried *in vivo* experiments to determine the nutritional and health effects of soy-fortified bread. Consequently, purpose of this study is to develop a functional bakery food that is very nutritious, health-friendly and very acceptable to consumers in terms of quality attributes.

2. MATERIALS AND METHODS

2.1 Raw Materials

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 in Lagos, Nigeria. The margarine was from Smilde Foods BV, Netherlands. The salt and sugar used were purchased from Dangote salt refinery in Apapa, Lagos, Nigeria. Yeast was bought from AB Mauri group, Australia while the bread additives were purchased by Green gate specialties located in Lagos, Nigeria.

2.2 Preparation of Composite Flour

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

2.3 Preparation of Dough for Composite Bread

The 100% hard wheat flour dough and the 90:10(w/w), 80:20(w/w), 70:30(w/w) and 60:40(w/w) composite flour dough were prepared according to the batch formulation in Table 1.1.

2.4 Bread Making

The bread was prepared by using straight dough method which involved mixing all ingredients such as flour with extraction level of 59-60% w/w, refined salt free iodine (1-2% w/w), sugar (4-5% w/w), water (55-60% w/w), improver (1%), margarine (1%), preservative (1%) and bakery yeast (1-2% w/w) as leavening agent. The hard 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 1.1) in a mixer (spiral dough mixer-280V), kneaded for 10 min into consistent dough. The resulting dough was moulded and placed in a pre-oiled baking mould pan. The dough was proofed for 55 to 60 min at 37℃ and 80% relative humidity and baked in a reel oven for 38 min at 210℃.

Table 1.1. Formulation for 100% wheat and 90:10(w/w), 80:20(w/w), 70:30(w/w) and 60:40 (w/w) soy / wheat bread

S/N	Ingredient	Weight (KG) 1 mix				
		Group A (100%)	Group B 90:10% (w/w)	Group C 80:20% (w/w)	Group D 70:30% (w/w)	Group E 60:40% (w/w)
1.	Whole wheat flour (g)	100	90	80	70	60
2.	Defatted soy flour (g)	0	10	20	30	40
3.	Sugar (g)	8	8	8	8	8
4.	Yeast (g)	2	2	2	2	2
5.	Salt (g)	2	2	2	2	2
6.	Bread improver (Sebamyl Brand) (g)	1	1	1	1	1
7.	Margarine (g)	1	1	1	1	1
8.	Water (g)	54	54	54	54	54
	Total dough weight (g)	168	168	168	168	168

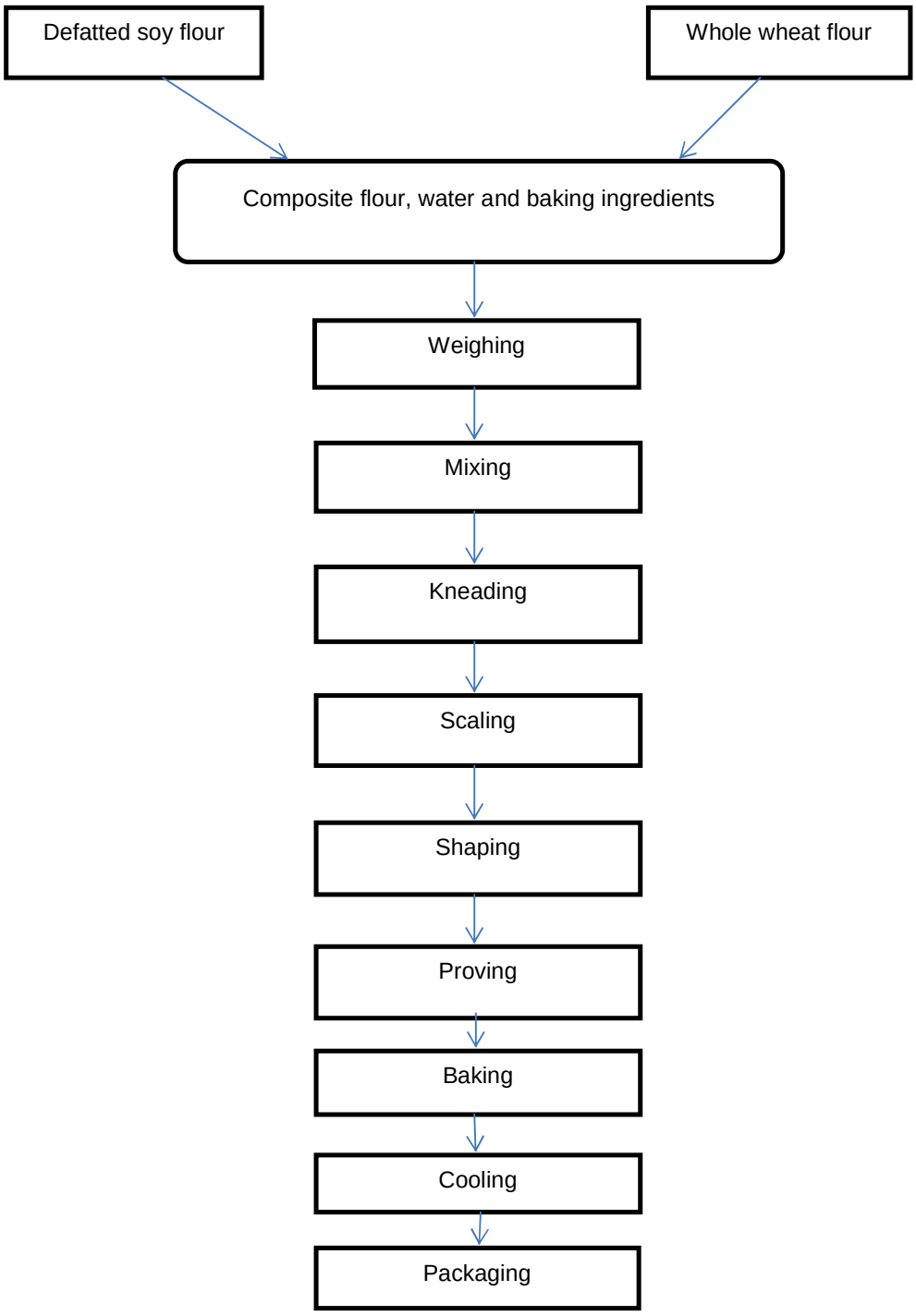

Fig. 1. Flowchart for bread production

2.5 Sensory Evaluation of Samples

The organoleptic characteristics of bread samples blends were determined determined by consumer panelist of 150 judges comprising of staff of Federal Institute of Industrial Research Oshodi (FIIRO) Nigeria, College of Medicine, University of Lagos, Nigeria students and bread customers from different bakeries in Lagos State Nigeria for taste and flavor, crust texture, fragrance and aroma, appearance, bendability (the property of being easily bent without

breaking), non-crumbliness and overall acceptability. The samples were served in dishes labeled with letters randomly. Each panelist received a rating form scored on a 1-9 hedonic scale. Breads were sliced into small pieces (15 × 15 cm) and were offered in distinct dishes at the same time. Water was provided for rinsing and conditioning the palate between samples.

2.6 Chemical Analysis of Hard Wheat Flour, Soy 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 [9].

2.7 Animal Model

Forty male Wistar rats (80-90 g) were procured from the animal house of the College of Medicine of the University of Lagos, Nigeria. They were housed in cages made of plastics with a prepared gauze cover to enable ventilation. The animals were fed rat chow and water for 7 days of acclimatization. The animals were weighed throughout the period of the experiment. After acclimatization, the mean weights of the male Wistar rats (n = 40) at the beginning of the experiment were taken. The rats were divided into five diet groups, each eight and named group A (control) and groups B, C, D and E (test). The rats were housed in metabolic cages. The rats of the group A (control) during the 28 days of the experiment received the 100% wheat bread while the rats of the test groups (B,C,D,E) received 10%, 20%, 30% and 40% soy bread respectively. All rats were fed once a day ad libitum, having unrestricted access to drinking water. At the end of the experiment after 24 hours of starvation, the rats were sacrificed using cervical dislocation.

2.8 Liver and Plasma Analysis

On day 28, animals were humanely sacrificed using cervical dislocation. Blood was collected into heparinized tubes via ocular puncture using capillary tube then followed by centrifugation at 3000 rpm for 10 mins. Clear serum was collected separately for each sample and subsequently used for further biochemical analysis. Liver tissues were homogenized with 5 volumes of ice-cold 50 mM Tris HCl (pH 7.4) containing 30 mM sucrose. The homogenate was centrifuged at 1500 x g for 10 min to remove nuclei and cell debris. The resulting supernatant was then used for total protein determination, glutathione (reduced), superoxide dismutase and catalase. Total liver protein levels were determined according to [10], using bovine serum albumin as standard. The concentration of reduced glutathione (GSH) was determined using the method of [11]. The superoxide dismutase activity was assayed by its ability to inhibit the auto oxidation of epinephrine, determined by the increase in absorbance at 480 nm [11]. The catalase activity was determined using the method of [12]. Serum total cholesterol was estimated using method of [13], plasma triglyceride estimated by method of [14], HDL-C by [15], LDL-C and VLDL triglyceride values were calculated by a modification of the friedewald formular [15]. Assay of alkaline phosphatase was carried out using Randox commercial enzyme kits according to the method of [16]. Alanine Transaminase (ALT) and Aspartate Transaminase (AST) were measured calorimetrically using Randox reagent enzyme kits based on the method of [17]. Albumin and total bilirubin levels were measured using a candidate reference method by [18]. Automated reaction rate method by [19] was used for the determination of creatinine while spectrophoto-metric method by [20] was used for the determination of plasma urea levels.

2.9 Statistical Analysis

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

3. RESULTS

Table 1 shows the proximate analysis of the hard wheat flour and soy which were used for the preparation of wheat and wheat-soy bread diets. There was no significant ($p>0.05$) difference in the various components of the flour.

Table 2 shows the proximate composition of hard wheat bread and wheat-soy functional bread diets. Crude protein levels increased significantly ($p<0.05$) of wheat-soy bread groups (groups B, C, D and E) compared to control (Group A). Moisture levels increased ($p<0.05$) significantly in only wheat-soy groups D and E compared to group A. There was no significant ($p>0.05$) difference in moisture levels of wheat-soy groups B and C compared to group A (Control). Ash levels increased ($p<0.05$) significantly in wheat-soy groups C, D and E as compared to the

control (group A). There was no significant ($p>0.05$) difference in ash levels of wheat-soy bread group B compared to control group. Fat levels in wheat-soy bread groups C, D and E increased ($p<0.05$) significantly when compared to control group. There was no significant ($p>0.05$) difference in fat levels in wheat-soy group B as compared with the control group. There was no significant ($p>0.05$) difference in carbohydrate and food energy levels of wheat-soy bread groups B, C, D and E compared to the control (group A).

Fig. 2. Pictorial representation of the top crust showing crust colour and appearance of bread baked with 100% hard wheat flour

Fig. 3. Pictorial representation of the top crust showing crust colour of (90:10 w/w) soy bread

The taste quality decreased ($p<0.05$) significantly in wheat-soy bread group E compared to Control (Group A). There was no significant ($p>0.05$) difference in taste quality in wheat-soy bread groups B, C and D compared to control (group A). There was no significant ($p>0.05$) difference in crust colour quality in wheat-soy bread groups B, C, D and E compared to the control (group A). There was no significant ($p>0.05$) difference in texture quality in wheat-soy bread groups B, C, D and E compared to control (group A). Flavour quality decreased significantly ($p<0.05$) in groups D and E as compared to control (Group A). There was no significant difference ($p>0.05$) in flavor quality in wheat-soy bread groups B and C compared to group A. Overall acceptability quality decreased ($p<0.05$) significantly in wheat-soy bread group E compared to control (group A). There was no significant ($p>0.05$) difference in overall acceptability quality in wheat-soy bread groups B, C and D compared to group A (Control).

Fig. 4. Pictorial representation of the top crust showing crust colour of (80:20 w/w) soy bread

Fig. 5. Pictorial representation of the top crust showing crust colour of (70:30 w/w) soy bread

Fig. 6. Pictorial representation of the top crust showing crust colour of (60:30 w/w) soy bread

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

S/N	Parameters	Hard wheat flour	Soy flour
1.	Crude protein	11.73±0.1[a]	48.33±0.2[b]
2.	Moisture (%)	12.11±0.1[a]	9.57±0.1[b]
3.	Ash (%)	0.56±0.1[a]	6.27±0.1[b]
4.	Fat (%)	1.83±0.1[a]	2.65±0.1[b]
5	Carbohydrate	73.77±0.1[a]	29.12±0.5[b]
6.	Fibre	2.04±0.1[a]	4.06±0.1[b]
7.	Food energy value (Kcal)	358.43±0.1[a]	334.70±0.7[b]

Results represented as mean±S.E.M;
Values with the same superscripts across the same row are not significantly different (P>0.05)

Table 2. The proximate composition of hard wheat and wheat-soy functional breads

S/N	Parameters	Bread samples				
		A (100%)	B 90:10% (w/w)	C 80:20% (w/w)	D 70:30% (w/w)	E 60:40% (w/w)
1.	Crude protein	9.17±0.1[a]	10.52±0.1[b]	11.45±0.1[b]	12.35±0.1[b]	12.89±0.1[b]
2.	Moisture (%)	34.12±0.2[a]	35.44±0.1[a]	37.33±0.2[a]	38.26±0.2[b]	40.31±0.2[b]
3.	Ash (%)	1.92±0.1[a]	2.05±0.2[a]	2.45±0.1[b]	2.49±0.2[b]	2.69±0.2[b]
4.	Fat (%)	3.61±0.2[a]	3.96±0.1[a]	4.2±0.2[b]	5.29±0.3[b]	6.42±0.5[b]
5.	Crude Fibre (%)	3.16±0.2[a]	4.38±0.2[b]	4.63±0.2[b]	5.06±0.2[b]	5.58±0.1[b]
6	Carbohydrate (%)	48.01±0.1[a]	43.73±0.2[a]	40.02±0.2[a]	36.54±0.2[a]	32.18±0.2[a]
7.	Food energy value (Kcal)	261.34±0.1[a]	252.65±0.1[a]	243.72±0.2[a]	243.26±0.2[a]	237.74±0.3[a]

Results represented as mean±S.E.M;
Values with the same superscripts across the same row are not significantly different (P>0.05)

Table 3. The sensory evaluation of hard wheat and wheat-soy functional breads

S/N	Parameters	Bread samples				
		A (100%)	B 90:10% (w/w)	C 80:20% (w/w)	D 70:30% (w/w)	E 60:40% (w/w)
1.	Taste	6.46±0.3[a]	6.80±0.2[a]	6.13±0.3[a]	6.33±0.2[a]	5.73±0.1[b]
2.	Crust colour	6.20±0.1[a]	6.50±0.4[a]	6.00±0.2[a]	5.83±0.1[a]	5.80±0.2[a]
3.	Texture	6.00±0.2[a]	6.43±0.2[a]	6.16±0.2[a]	6.90±0.3[a]	6.33±0.3[a]
4.	Flavour	6.60±0.1[a]	5.73±0.1[a]	5.40±0.2[a]	5.13±0.1[b]	4.93±0.1[b]
5.	Overall acceptability	7.36±0.2[a]	7.00±0.1[a]	6.63±0.3[a]	6.37±0.2[a]	6.03±0.2[b]

Results represented as mean±S.E.M
Values with the same superscripts across the same row are not significantly different (P>0.05)

Table 4 shows the concentration of oxidative stress markers in rats' liver after 4 weeks administration of wheat bread and wheat-soy bread. There was a significant ($p<0.05$) increase in levels of reduced glutathione, superoxide dismutase and catalase levels of experimental rats liver in all wheat-soy bread group (B,C,D,E) compared to the control group (Group A). However, there was no significant ($p>0.05$) difference in the levels of reduced glutathione, superoxide dismutase and catalase levels of experimental rats liver in all wheat-soy bread group (B,C,D,E) compared to one another.

There was a significant decrease ($p<0.05$) in the level of lipid peroxidation biomarker (MDA) level of experimental rats liver in all wheat-soy bread group (B,C,D,E) compared to the control group (Group A). However, there was no significant ($p>0.05$) difference in the level of lipid peroxidation biomarker (MDA) of experimental rats' liver in all wheat-soy bread group (B,C,D,E) compared to one another.

Table 5 shows the serum levels of liver biomarkers in experimental rats after 4 weeks administration of hard wheat bread and wheat-soy breads. There was a significant decrease ($p<0.05$) in serum level of liver biomarkers levels (AST, ALT and ALP) of experimental rats in wheat-soy bread groups B,C,D,E compared to the control group (Group A). However, there was no significant ($p>0.05$) difference in the serum level of hepatic biomarkers (AST, ALT and ALP) of experimental rats in all wheat-soy bread group (B, C, D, E) compared to one another.

Table 6 shows the serum levels renal biomarkers in rats after 4 weeks administration of hard wheat bread and wheat-soy breads. There was a significant ($p<0.05$) decrease in serum level of renal function markers levels (Urea, Creatinine and Total Bilirubin) of experimental rats in wheat-soy bread groups B,C,D,E compared to the control group (Group A). However, there was no significant ($p>0.05$) difference in the serum level of renal biomarkers (Urea, Creatinine and Total Bilirubin) of experimental rats' liver in all wheat-soy bread group (B,C,D,E) compared to one another.

Table 7 shows the serum levels of protein biomarkers of experimental rats after 4 weeks administration of hard wheat bread and wheat-soy breads. There was a significant ($p<0.05$) decrease in serum levels of protein biomarkers (Total protein and Albumin) of experimental rats in wheat-soy bread groups B,C,D,E compared to the control group (Group A). However, there was no significant ($p>0.05$) difference in the serum level of protein biomarkers (Total protein and Albumin) of experimental rats in all wheat-soy bread group (B,C,D,E) compared to one another.

Table 8 shows the serum levels of lipid biomarkers of experimental rats after 4 weeks administration of hard wheat bread and wheat-soy breads. There was a significant ($p<0.05$) decrease in serum levels of lipid biomarkers (Low Density Lipoprotein(LDL), High Density Lipoprotein (HDL), Cholesterol (CHOL) and Triglycerides (TG) of experimental rats in wheat-soy bread groups B,C,D,E compared to the control group (Group A). However, there was no significant ($p>0.05$) difference in the serum level of lipid biomarkers (Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Cholesterol (CHOL) and Triglycerides (TG)) of experimental rats in all wheat-soy bread group (B,C,D,E) compared to one another.

Table 4. Concentration of oxidative stress markers in rats' liver after 4 weeks administration of wheat bread and wheat-soy bread

Oxidative stress marker	A (100%)	B 90:10% (w/w)	C 80:20% (w/w)	D 70:30% (w/w)	E 60:40% (w/w)
Reduced glutathione (GSH) (µmol/ml)	2.31±0.1[a]	6.5±0.4[b]	6.8±0.4[b]	6.1±0.4[b]	6.9±0.4[b]
Superoxide dismutase (SOD) (µmol/ml)	88.77±2.0[a]	120.29±2.6[b]	121.5±0.7[b]	118.5±0.8[b]	119.5±0.4[b]
Catalase (µmol/ml)	712.0±3.2[a]	731.8±5.3[b]	729.5±0.4[b]	726.5±0.4[b]	738.5±0.4[b]
Malondialdehyde (MDA) (Umol/ml)	1.49±0.02[a]	1.19±0.02[b]	1.15±0.4[b]	1.12±0.4[b]	1.14±0.4[b]

Results represented as mean±S.E.M
Values with the same superscripts across the same row are not significantly different (P>0.05)

Table 5. Concentration of hepatic activity biomarkers in rats after 4 weeks administration of wheat bread and soy bread

Liver enzymes	A (100%)	B 90:10% (w/w)	C 80:20% (w/w)	D 70:30% (w/w)	E 60:40% (w/w)
Aspartate transminase (U/L)	206.20±6.1[a]	150.10±14.4[b]	152.5±0.5[b]	154.5±12.7[b]	148.5±13.4[b]
Alanine transaminase (U/L)	70.66±0.7[a]	34.10±27.6[b]	36.5±20.8[b]	35.5±15.1[b]	37.5±14.3[b]
Alkaline phosphatase (U/L)	288.1±25.7[a]	174.50±13.4[b]	176.5±10.8[b]	173.5±13.6[b]	172.5±12.4[b]

Results represented as Mean±S.E.M
Values with the same superscripts across the same row are not significantly different (P>0.05)

Table 6. Serum renal function activity in experimental rats after 4 weeks administration of wheat bread and soy bread

Liver enzymes	A (100%)	B 90:10% (w/w)	C 80:20% (w/w)	D 70:30% (w/w)	E 60:40% (w/w)
Urea (µmol /L)	4.06±0.5[a]	2.18±0.1[b]	2.20±0.3[b]	2.12±0.6[b]	2.10±0.5[b]
Creatinine (µmol /L)	32.76±1.9[a]	28.86±0.7[b]	26.74±0.4[b]	26.78±0.7[b]	25.18±0.4[b]
Total bilirubin (µmol /L)	1.59±0.2[a]	0.68±0.1[b]	0.61±0.4[b]	0.63±0.4[b]	0.64±0.4[b]

Results represented as Mean±S.E.M; Values with the same superscripts across the same row are not significantly different (P>0.05)

4. DISCUSSION

The proximate analytical results of the hard wheat flour and soybean flour differed slightly from that obtained from literatures [21]. The chemical composition of the wheat-soy composite flours have been found to affect both physico-chemical properties and nutritional quality of their products [22]. In the current study, there was a significant ($p<0.05$) increase in the levels of ash, protein, carbohydrate, fibre, moisture and food energy value of soy flour compared to hard wheat flour. This shows the inherent positive effects of the soy flour supplementation of wheat flour on the overall nutritional composition of wheat-soy bread.

The supplementation of hard wheat flour with soy flour was found to have positively improved the physico-chemical quality of the composite breads in the order of increasing supplementation ratios. The proximate values increased with increasing levels of soya bean substitutions except for carbohydrate content and energy values which showed the reverse. Carbohydrate content was observed to have decreased with increasing substitution of soya flour in all the bread samples. This trend supports the claim of [3]. The carbohydrate content and energy values were observed to have the highest amount in sample A (48.01% and 261.34 Kcal) and least in sample E (32.18% and 237.74Kcal), respectively. The low carbohydrate and energy values were attributable to the low fat content of the wheat-soy functional breads. Similar experimental trends of results were reported by [23] and [8] in their studies on the fortification of wheat flours with defatted and non-defatted soy flour respectively. The wheat-soy functional breads (B to D) were found to have energy values in the range of 243 to 252 Kcal, and hence conformed to the [24] with the exclusion of sample E whose energy value was 237.74 Kcal as well as sample A which recommended minimum energy content of 1674 kJ/ 100 g. The moisture levels of the wheat-soy functional breads increased with soy flour substitution by a range of 35.44 to 40.31%. Increase in moisture content has been reported to correlate with increase in fibre content [24]. It has also been reported that high moisture levels negatively affects short shelf life of composite breads as they encourage microbial proliferation that lead to spoilage [25]. There was an increase in the protein levels of the wheat-soy functional breads with soy-flour supplementation in the range of 10.52 to 12.89%. This increase is as a result of substitution of hard-wheat flour (11.73% protein) with soya bean flour of 48.33% protein content. This result correlates with the studies of [22] who also reported increase in protein content of the bread as a result of the addition of soy flour. Similar studies have also reported a similar trend of increase of protein content in sorghum-soy composite flours [26]. The fat content also increased from 0.35 to 2.81% in the composite breads produced from soy-bean flour supplementation. The increased fat content in wheat-soy bread as compared with the 100% hard wheat bread is as a result of soybean flour being produced from which soybean is an oil seed, must have contributed most of the oil content to the product. The high oil level of the composite bread has also been reported to affect the shelf stability [21]. The crude fibre content of the wheat-soy bread revealed a percentage increase in the range of 1.22 to 2.42% as the hard-wheat flour was replaced with soy bean flour. This could be ascribed to the use of hard wheat flour and soy-flour, both of which had high crude fibre levels of 2.04 and 4.06% respectively. The crude fibre which are contained mostly in the bran of the hard wheat flour and the hull of soy beans, represents variable fraction of dietary fibre and includes mostly the lignin, cellulose and hemicelluloses components [8]. The increased levels of fibre and the lower carbohydrate of wheat-soy functional breads have several health benefits, as it will improve digestion of the bread in the colon and lowers constipation which is usually associated with bread made from refined wheat flour [27]. The results of different studies have shown that that dietary fibre plays a significant role in the prevention of several diseases such as; constipation, cardiovascular diseases, irritable colon, cancer, diverticulosis and diabetes [28].

Table 7. Serum protein profile in rats after 4 weeks administration of wheat bread and soy bread

Protein marker	A (100%)	B 90:10% (w/w)	C 80:20% (w/w)	D 70:30% (w/w)	E 60:40% (w/w)
Total protein (g/L)	81.34±0.8[a]	62.69±1.7[b]	62.69±1.2[b]	62.69±1.1[b]	62.69±1.9[b]
Albumin (g/L)	47.86±1.0[a]	28.39±1.4[b]	25.69±1.5[b]	26.39±1.5[b]	27.19±1.4[b]

Results represented as Mean±S.E.M

Values with the same superscripts across the same row are not significantly different (P>0.05)

Table 8. Lipid profile in rats' liver after 4 weeks administration of wheat bread and soy bread

Lipid marker	A (100%)	B 90:10% (w/w)	C 80:20% (w/w)	D 70:30% (w/w)	E 60:40% (w/w)
LDL (mmol/L)	0.73±0.5[a]	0.32±0.1[b]	0.37±0.3[b]	0.32±0.1[b]	0.32±0.1[b]
HDL (mmol/L)	0.93±0.1[a]	0.95±0.3 [a]	0.92±0.1[a]	0.90±0.2[a]	0.95±0.4[a]
CHOL (mmol/L)	1.95±0.9[a]	1.11±0.1[b]	1.17±0.1[b]	1.13±0.1[b]	1.15±0.1[b]
TG (mmol/L)	1.06±0.4[a]	0.66±0.1[b]	0.62±0.1[b]	0.65±0.5[b]	0.60±0.4[b]

Results represented as Mean±S.E.M

Values with the same superscripts across the same row are not significantly different (P>0.05)

The taste of the bread is defined as the sweet sensation caused in the mouth by contact with the bread as a result of the sweetening agent [29].In the current study, it was observed that there was no significant difference between the tastes of the hard wheat bread and the soy supplemented bread up to 30% of soybean flour. However, there was significant reduction in the taste of the bread with 40% soy flour supplementation compared to the bread with 100% hard wheat flour. The results of bread crust colour were observed to show an irregular pattern for all the bread samples and there was no significant difference in the crust colour of the bread samples and the control sample. The darker colour of the crumbs of hard wheat bread and supplemented breads has been reported by several authors [23]. The brownish bread appearance of the wheat-soy functional breads is attributable to their increased fiber content [30]. Furthermore, browning of the breads could also occur due to caramelization and maillard reactions, as the protein in soybean flour could have possibly reacted with sugar during the baking process [31]. Texture is the quality of the bread that can be decided by touch, the degree to which it is rough or smooth, hard or soft [29]. In the current study, it was observed that the scores for texture (softness and chewiness) of the wheat-soy functional bread samples, increased with increase in soybean flour substitution when compared to hard wheat bread (control sample A). The 30% soy flour supplemented bread (sample D), had the best texture score. Hard crumb texture, caused by increased fiber from wheat bran replacement by soy flour was reported by [32]. Studies have also shown that the final texture of the hard-wheat bread is influeneced by many factors such as: the state of the bread components, such as fibres, starch, protein (gluten) whether damaged or undamaged, baking conditions (temperature and time variables); and the amounts of absorbed water during dough mixing [33]. The inclusion of soybean flour into hard-wheat bread led to poor flavour scores. The results revealed a decrease in the scores as the whole-wheat flour was replaced with soy-flour. Sample E with 40% soy-flour recorded the least score. Many of the panelist complained of how the beany flavour and aroma from the soy flour was noticed in the wheat-soy composite breads. [23] reported that inclusion of defatted-soy-flour into wheat bread and biscuits were associated with the roasted soybean flavour, after taste and aroma. The beany flavour is noticeable commonly among food legumes [34]. The possible mechanism for the production of the beany flavor is through the enzymatic breakdown linoleic and linolenic acid by lipoxygenases or autoxidation producing hydroperoxides such as ketones, aldehydes which dissuades people from soy consumption [26]. The organoleptic evaluation results showed that breads with soy-flour supplementation up to 10% (sample B) had the highest overall acceptability notwithstanding the normal bread being the most preferred. The baking characteristics of composite flour as well as the organoleptic attributes of the products are usually affected because of the dilution of the gluten content [27]. Thus for commercial purposes, different combinations of both synthetic and organic improvers like malt flour and ascorbic acid can be incorporated in bread formulation to improve the baking and sensory qualities of the products [28].

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 [35].

The antioxidant enzymes: reduced glutathione, superoxide dismutase and catalase serum levels increased ($p<0.05$) significantly in the experimental rats liver in groups B,C,D and E (Test group) compared to the control group (Group A) but there was no significant ($p>0.05$) difference in their respective levels amongst the soy supplemented groups. However, the level of lipid peroxidation biomarker; malondialdehyde decreased significantly ($p<0.05$) in the rats liver in all the soy bread treated grouped compared to the non-soy bread treatment group but there were also no significant ($p>0.05$) difference in the level of malondialdehyde amongst the soy bread treatment groups. 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 [36]. The results presented here suggest that supplementation with soy isoflavones enhances antioxidative function and prevents lipid peroxidation. The possible mechanism which we propose for this action is through activation of the antioxidant enzymes.

Alanine transaminase (ALT) is an enzyme primarily localized to the liver but the aspartate transaminase (AST) is present in a wide variety of tissues like the heart, skeletal muscle, kidney, brain and liver. There serum levels is indicative of hepatic injury [37]. Hence, they are used as liver biomarkers.

Alkaline phosphatases are a family of zinc metaloenzymes having a serine at their active site; releasing inorganic phosphate from various organic orthophosphates and are found in almost all the tissues [38]. Alkaline phosphatase is found histochemically in the microvilli of bile canaliculi as well the sinusoidal surface of hepatocytes of the liver. The mechanism by which alkaline phosphatase reaches the circulation has not yet been fully elucidated; leakage from the bile canaliculi into hepatic sinusoids possibly could be from leaky tight junctions [39] and the other hypothesis is that the damaged liver fails to excrete alkaline phosphatase made in bone, intestine and liver [38].

There was a significant decrease ($p<0.05$) in serum levels of liver biomarkers (Aspartate Transaminase, Alanine Transaminase and Alkaline Phosphatase) of wheat-soy bread treated groups compared to the control group without any significant ($p>0.05$) difference in the serum levels of these three enzymes in all the wheat-soy bread treated groups compared to one another. This observation correlates with the results from similar works by [40] which revealed that a high protein diet may cause the serum alkaline phosphatase to fall into the lower range 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 [41].

The serum levels of urea, bilirubin and creatinine levels are biomarkers for renal failure [42]. 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 [42]. Total bilirubin levels showed a significant decrease ($p<0.05$) in the soy bread treated groups compared to the control group. The increase in conjugated bilirubin makes the liver more effective because it is able to conjugate more bilirubin. Urea and creatinine levels showed also a significant ($p<0.05$) decrease in the soy bread treated rat groups compared to the control group. This observation correlates with similar work by [43] on soy protein effects on inherited polycystic kidney disease are influenced by gender and protein level. The increased creatinine level in soy bread has similar results as previous works by [44] on soy protein modification of rat polycystic kidney disease.

Albumin is the most important protein in plasma manufactured by the liver and is a useful

biomarker of liver function [38]. Apart from the liver, albumin synthesis is also affected by hormonal balance, nutritional status and osmotic pressure. Liver is the only site of synthesis of albumin [39]. In the current study, it was observed that there was a significant ($p<0.05$) increase in the serum levels of protein biomarkers (Total Protein and Albumin) of all wheat-soy bread treated rat groups compared to the control group. However, there was no significant ($p>0.05$) difference in the serum levels of the two protein biomarkers in all soy bread treated groups compared to one another.

The lipid profile which includes the levels of total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and very-low density lipoprotein (VLDL) serves as biomarkers for cardiovascular diseases [38]. Coronary heart disease is one of the possible risk factors for hyperlipidemia as well as cholesterol being the major lipid constituent of atherosclerotic plague as established by previous findings [45]. Cholesterol is a main sterol found in body tissues. There are various types of cholesterol, including low density lipoprotein (LDL) cholesterol which is an unhealthy form of cholesterol, high density lipoprotein (HDL) cholesterol which is considered as a healthy form of cholesterol and triglycerides (TG), a form of fat carried through the bloodstream [38]. Of recent, some human studies have showed the relationship between dietary soy components with cardiovascular risk biomarkers in diabetes and metabolic syndrome [6]. Another study revealed the positive effects of soy products on inflammatory biomarkers [46]. One study showed that consumption of soy product for 8 weeks could lower malondialdehyde (MDA) levels in postmenopausal women suffering from the metabolic disorder [46]. In a double-blind randomized clinical trial, consumption of 50 g/day soy protein containing 164 mg isoflavones for 10 weeks lowered the risk of cardiovascular disease in hypercholesterolemic postmenopausal women as a result of significant decreases in the levels of serum lipoproteins and an increase in paraoxonase activity [46]. [40] 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, [47] 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 [45] 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 glycetein, has been shown to have a hypocholesterolemic effect in animals and humans. We propose the possible 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. In this current study, there was observed significant ($p<0.05$) reduction in the levels of high density lipoprotein (HDL), Low Density Lipoprotein (LDL), Cholesterol and Tryglycerides in all wheat-soy bread treated groups compared to the control (group A). Furthermore, there was no observed significant ($p>0.05$) differences in the levels of high density lipoprotein (HDL), Low Density Lipoprotein (LDL), Cholesterol and Tryglycerides in all wheat-soy bread treated groups compared to one another. This observation is in agreement with similar work by [21] on effects of substituting dietary soybean protein and oil for milk protein and fat in subjects with hypercholesterolemia.

5. CONCLUSION

The results of this study reveal that high intake of soy supplemented bread confered significant advantages on the health of the animal model used. Therefore the impact of this research results would go a long way in the improvement of the nutritional and health status of people through regular consumption of functional foods like soy supplemented bread. Therefore, it is hoped that relevant food and health agencies would help in championing the cause for the use of composite flour (wheat and soy) in the production of bread for human consumption.

ACKNOWLEDGEMENT

The authors would like to acknowledge the assistance of the staff of food analytical unit of Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria as well as Messrs. Duncan and Dada of Physiology department, College of Medicine, University of Lagos, Nigeria for their assistance in *in vivo* studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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