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In vitro Antioxidant and Anti-diabetic Properties of Cookies Produced from Wheat and Soybean Composite Flour

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Abstract

Composite flours are formulated from wheat and soybean, which were mixed together to formulate four blends WSY 1 (100%), WSY 2 (80%-20%), WSY 3 (70%-30%), WSY 4 (60%-40%), respectively. The flours and the cookies were investigated for their proximate composition, amino acid profiles, *in vitro* bioactivities (antioxidant and anti-diabetic properties) and the consumer acceptability of the cookies using standard methods. The proximate compositions of the composite flour were greatly improved during the baking process into cookie with the carbohydrate, protein and fat contents ranges of 69.64-71.44%, 9.13-23.26% and 2.99-9.82%, respectively. The amino acid profiles of the cookies were well established with high biological values (>70%) in terms of their essential, non-essential and hydrophobic amino acids while glutamic acid served as the most abundant amino acid (19.15 – 28.69%) present in each of the cookies. The cookie samples were able to scavenge free radicals, reduced and chelate metallic ions when compared with a standard ascorbic acid, a well-known antioxidant. However, the *in-vitro* anti-diabetic properties of the cookie samples were revealed when they inhibited the activities of α-amylase and α-glucosidase when compared with a standard acarbose, a well-known anti-diabetic drug. Overall, the cookie sample from the 60% wheat and 40% soybean composite flour was acceptable to the consumers as shown by their ratings and perceptions, which could be highly found useful as potential bioactive antioxidant and anti-diabetic agents in the management of diabetes mellitus.

Keywords: *Cookies; antioxidant; anti-diabetic; biological value; hydrophobic amino acid*

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1. Introduction

Diabetes is a metabolic disorder in which there are high level of sugar in the blood, a condition called hyperglycemia, which happened when the body did not make enough insulin or could not use it well. Thereby causing the level of glucose (blood sugar) to build up in the blood (Olugbuyi *et al*., 2022). Composite flour was generally considered to be blends of wheat flour with other flours for production (Arise *et al*., 2021). Thus, recent approach has shown the composite flour to contain two or more edible flours blended together at different ratios for novel food production, such as cookie, with one positive health benefit or the other (Olugbuyi *et al*., 2022). Thus, cookie is produced as nutritive snacks from unpalatable dough that is transformed into appetizing products through the application of heat in the oven (Malomo and Udeh, 2018).

Cookie is generally acceptable food product and could be used as an excellent and convenient food item for protein fortification to improve the nutritional well-being/health of the people, and in nutritional programs which would enhance reduction in protein malnutrition that is prevalent in Nigeria as well as other developing countries (Malomo and Udeh, 2018). Cookie is universally accepted as a very convenient form of food that is important for population. It is a good source of nutrients, such as macronutrients (carbohydrates, protein, and fat) and micronutrients (minerals and vitamins) that are essential for human health (Oluwajoba *et al.*, 2012).

Legumes are rich in protein and essential minerals but contained small quantities of fats that were mostly unsaturated. Attempts have been made to improve its utilization in human diet due to increasing need for cheaper and available plant proteins, especially amongst Nigerian populace. Soybean (*Glycine max*) is relatively cheap and contained a high amount of protein (23%) that is rich source of lysine but is usually deficient in sulphur-containing amino acids especially methionine and cystine. It is relatively high in protein and could be used to fortify cookies (Adeola *et al.,* 2011). Soybean contained complex phytochemicals, beta-carotene (a pro-vitamin A carotenoid), vitamins C, which were major and well-known antioxidants (Onabanjo and Ighere, 2014). It contained protein alongside with all essential amino acids (Onabanjo, 2014). In Nigeria, wheat production is limited and wheat flour is imported to meet local flour needs for bakery products. Thus, a huge amount of foreign exchange is used every year for importing wheat. Efforts have been made to promote the use of composite flours in which flour from locally grown crops and high protein seeds replaced a portion of wheat flour for the use in cookie, thereby helping in producing protein-enriched cookie (Arise *et al*., 2021).

Therefore, this study envisaged to utilize the flour blends of wheat and soybean flours in the production of enriched baked products with good balance of some of the essential amino acids meant to serve as potent antioxidant and anti-diabetic agents.

2. Materials and Methods

2.1 Materials

The commercial wheat flour, which have been commonly used for all baking processes, was obtained from a commercial baking ingredients store in Ado Ekiti, Nigeria. The soybean seeds were obtained from the King's market, Ado Ekiti, Nigeria and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. All chemicals used were of analytical grade and obtained from Sigma-Aldrich, London, United Kingdom.

2.2 Preparation of soybean flour and composite cookies from flour blends

The Soybean flour was obtained according to the described methods (Adeyanju *et al*., 2016). Briefly, the raw seeds were sorted and cleaned, roasted in an oven for 20 min at 180 °C, dehulled and milled using a Binatone kitchen blender (mode BLG 4O2, Zhongshan, Haishang) and the resultant flour sieved to obtain a uniform size of 400 µm. The individual flours were now formulated into composite flour blends as WSY 1 (100%), WSY 2 (80%-20%), WSY 3 (70%-30%), WSY 4 (60%-40%), respectively. Cookies were produced as previously described by Malomo and Udeh (2018) with the following ingredients, composite flour, margarine, baking powder, salt, beet, eggs and water. The dried ingredients were thoroughly mixed in a bowl for few minutes followed by adding the margarine and eggs and kneaded to form batter. The batter was then rolled on a rolling board sprinkled with flour for a uniform thickness and cut with a 50 mm-diameter cookie cutter. The cookies were placed in baking trays leaving a 25 mm space in between and baked at 180 ^{0}C for 10 min in the baking oven. After baking, the cookies were cooled at ambient temperature, packaged in polyethylene bags and stored prior to subsequent analysis.

2.3 Proximate composition analysis

Proximate compositions of cookies were determined as described by AOAC (2012). The carbohydrate content was determined by difference (100 - the sum of the content of protein, fat, ash and moisture) while energy value was calculated using Artwater factor (fat x 9 + carbohydrate x 4 + protein x 4 kcal/100 g).

2.4 Amino acid analysis

The amino acid profiles of the cookies were determined using the High-performance liquid chromatography (HPLC) method as previously described by Malomo *et al*. (2020). Briefly, food sample was placed in hydrolysis ampoule, then dried under vacuum using a Savant SpeedVac. Approximately 100 µL of 6 N HCL was placed in the lower part of the ampoule, freezed in a dry ice/ethanol bath, attached to a vacuum system via ¼" ID x 5/8" OD Tygon tubing, then slowly thawed and evacuated to <150 mtorr. Oxygen/methane flame was used to seal the neck of the tube at the constriction. After hydrolysis and acid removal, samples that contained 0.5-10 µg of protein were reconstituted with 60-200 µgof Na-S sample buffer and the amino acid composition then finally analyzed. The cysteine and methionine contents were determined after performic acid oxidation and the tryptophan content was determined after alkaline hydrolysis.

2.5 *In vitro* Antioxidant assays

The radical scavenging activities of the cookie samples were determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) as described (Malomo *et al*., 2020). The reaction of DPPH with an antioxidant compound which can donate hydrogen, leads to its reduction. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. To 1 ml of different concentrations of the extract or standard (ascorbic acid) in a test tube was added 1 ml of 0.3 mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30min after which the absorbance was read at 517 nm. Ferric reducing antioxidant power was determined as described (Malomo *et al*., 2020). Briefly, 100 µl of the extract were mixed with 2.5 ml of 200 mmol/l phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50 °C for 20 min. Then, 2.5 mlof 10% trichloroacetic acid was added, and the tubes were centrifuged at 10,000 rpm for 10 min. After this, 5 ml of the upper layer were mixed with 5.0 ml distilled water and 1 ml of 0.1% $FeCl_3$, and the absorbance of the reaction mixtures was measured at 700 nm.

The deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium according to the procedures previously described (Malomo *et al*., 2020). The reaction mixture containing $FeCl_3$ (100 µM), EDTA (104 µM), H_2O_2 (1 mM) and 2-deoxy- D-ribose (2.8 mM) at various concentrations of extracts in 1 ml final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1 hr at 37ºC. The mixture was heated at 95 ºC in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally, the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm. Metal chelating activity was measured as described (Malomo *et al*., 2020). To prepare the solution, 0.1 mM $FeSO_4$ (0.2 ml) and 0.25 mM ferrozine (0.4 ml) were subsequently added into 0.2 ml of flour sample and dough meal. After incubating at room temperature for 10 min, absorbance of mixture was recorded at 562 nm.

2.6 *In vitro α-amylase and α-glucosidase activity assay*

The *in-vitro* α-amylase activity could be measured by hydrolysis of starch in the presence of α-amylase enzyme. This process was quantified by using iodine, which gave blue colour with starch. The reduced intensity of blue colour indicated the enzyme-induced hydrolysis of starch in to monosaccharides. If the substance possessed α-amylase inhibitory activity, the intensity of blue colour would be more. In other words, the intensity of blue colour in test sample is directly proportional to α-amylase inhibitory activity (Olugbuyi *et al*, 2022). α-amylase activity was carried out by starch-iodine method. 10 µL of α- amylase solution (0.025 mg/ml) was mixed with 390 µl of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37 °C for 10 min, 100 µl of starch solution (1%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 ml of 1% iodine solution was added, and after adding 5 ml distilled water, the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were carried out under the same reaction conditions. Inhibition of enzyme activity was calculated as (%) = (A-C) X100/ (B-C), where, A= absorbance of the sample, B= absorbance of blank (without α-amylase), and C= absorbance of control (without starch).

The α-glucosidase inhibition activity was performed according to the slightly modified method (Olugbuyi *et al*, 2022). The α-glucosidase activity can be measured in-vitro by determination of the reducing sugar (glucose) arising from hydrolysis of sucrose by α-glucosidase enzyme. The final volume of the reaction mixture was 100 µl, which contained 70 µl of phosphate buffer saline (50 mM, pH 6.8), 10 µl of test extracts, and 10 µl (0.057 U) enzyme. The content was mixed, pre-incubated at 37 ºC for 10 min, and pre-read against the reagent blank value by spectrophotometry at 400 nm. The reaction was initiated using 10 µl of 0.5 mM substrate (i.e., p-nitrophenol glucopyranoside). Acarbose was used as a positive control. After incubation at 37ºC for 30 min, optical absorbance was measured against the reagent blank value by spectrophotometry at 400 nm.

2.7 Determination of physical properties of and Evaluation of sensory attributes cookies

The cookies were analyzed for weight, diameter, thickness (width) and spread factor (diameter/thickness) according to respective procedures previously described by Malomo and Udeh (2018). Cookie diameter (D) and thickness (T) were determined using a vernier caliper. Spread factor (SF) was also determined from the diameter and thickness, using a formula: SF = (D/T X CF) X 10 where CF is correction factor, at constant atmospheric pressure. The cookies were coded and presented to twenty (20) semi-trained panelists to be evaluated for their appearance, texture, taste, aroma, mouth feel, crumbling, overall acceptability using the Hedonic scale of 1 to 9, where 1 = dislike extremely and 9 = like extremely.

2.8 Statistical analysis

All determinations were carried out in triplicates. Data was subjected to analysis of variance (ANOVA) using SPSS (version 21, USA), while means was separated using New Duncan Multiple Range Test (NDMRT) at 5% level of significance ($p<0.05$).

Results and Discussion

3.1. Proximate composition of the cookies

Table 1 showed the approximate compositions of the cookie samples produced using wheat-soybean composite flours. There was a variation of 5.70 to 6.01% of moisture content. When compared to the other samples, sample WSY 1 had the highest moisture content that was statistically significant ($P<0.05$). The protein concentrations in the cookie samples varied from 9.13 to 23.26%, with the lowest and highest values seen in WSY 1 and WSY 3, respectively. These results corroborated those of Malomo and Udeh (2018) about the procedure of food fortification aimed at increasing protein content. The range of the crude fats in the cookie samples was 2.99 to 9.82%, with the greatest and lowest values seen in WSY2 and WSY1 respectively. Replacing the wheat flour with soybean flours produced food items with lower fat levels, inevitably increased the bioactivity (anti-obesity) of the finished goods. The total ash content of the cookie samples varied from 2.45 to 4.50%, with WSY 3 exhibiting the highest value. This indicates that soybeans include known macro elements including calcium, potassium, salt, and iron (Arise *et al*. 2021). The range of fiber contents in the cookie samples was 2.10 to 7.04% with WSY 2 having the lowest fiber content and WSY 3 having the highest, indicating that the addition of soybean flour increased the fiber contents of the composite flours. Crude fiber has reportedly been shown to help prevent diabetes, colon cancer, heart disease, and other conditions (Godswill, 2019). The cookie samples' carbohydrate contents varied from 66.69 to 71.44%, with WSY 3 and WSY 1 having the lowest and highest carbohydrate values, respectively. The consumption of samples WSY 3 and WSY 4 may be particularly efficient in controlling blood sugar because of their low carbohydrate contents, which may translate into an estimated low glycemic index.

3.2 Amino acid profile of cookie samples

Table 2 showed the amino acid profile of the cookie samples. The samples' ranges for total essential amino acids (TEAA) and non-essential amino acids (TNEAA) were 30.06 to 37.65 and 47.47 to 56.28%, respectively. Comparing the WSY 3 sample to the other samples, it was found that the total essential amino acids were considerably ($p>0.05$) greater in that sample. This could be explained by the ratio's 30% soybean flour content. The current findings' total essential amino acids, however, were significantly ($p<0.05$) greater than the FAO recommendations for adults and children, which are 26 and 39% of essential amino acids, respectively (Twinomuhwezi *et al*., 2020). Thus, it's possible that the essential amino acids in the cookies will be sufficient to promote healthy growth and development in infant, children, and adults. The body is unable to produce the important amino acids, including phenylalanine, tryptophan, and tyrosine, which make up the hydrophobic amino acids (HAA), hence diets are the only way to obtain them. As a result, compared to other samples (35.42-38.15%), the WSY 3 showed greater amounts of HAA (39.85%). The majority of these HAA are body-found serum aromatic amino acids, which have been connected to elevated insulin secretion in type-2 diabetic patients (Olugbuyi *et al*., 2022).

Table 1 - Proximate compositions of cookies (**%**)

Sample/ Parameters	Moisture	Crude protein	Crude fat	Ash	Crude fibre	Carbohydrate
WSY 1	6.01 ± 0.36[b]	9.13 ± 0.06[d]	9.82 ± 0.12[b]	4.06 ± 0.04[a]	7.04 ± 0.30[a]	71.44 ± 0.11[ab]
WSY 2	5.70±0.30[c]	21.40±0.40[b]	2.99±0.01[d]	4.05±0.00[a]	2.10±0.05[d]	70.78±0.20[b]
WSY 3	5.93±0.03[c]	23.26 ±0.40[a]	3.50±0.20[c]	4.50 ±0.12[a]	3.35±0.40[c]	66.69 ± 0.36[d]
WSY 4	5.92±0.10[c]	23.05 ±0.58[a]	3.60±0.16[c]	2.45±0.08[c]	3.34±0.17[c]	69.64±0.37[bc]
Commercial sample	8.27 ± 0.11[a]	10.02 ± 0.03[c]	10.91± 0.01[a]	3.61± 0.01[b]	4.72 ± 0.05[b]	72.69 ± 0.14[a]

Means (n=3) with different letter in the column are significantly different (p<0.05). **Key:** WSY 1 = 100% wheat flour; WSY 2 = 80% wheat flour + 20% soybean flour; WSY 3 = 70% wheat flour + 30% soybean flour; WSY 4 = 60% wheat flour + 40% soybean

Therefore, high value of aromatic amino acids obtained in the present study, as a result of high HAA and concise biological values (~90%) is found beneficial in prevention and management of type-2 diabetes. Past study had also reported that the plasma concentrations of branched chain amino acids (BCAA), the good examples of HAA, were prognostic for the onset and progress of Type 2 diabetes (Udeh *et al*., 2021). Hence, the BCAA recorded in present study is potentially advantageous to the management of type-2-diabetes mellitus.

Table 2 – Amino acid profiles of cookies samples (**%**)

Amino acids/ Samples	WSY 1	WSY 2	WSY 3	WSY 4	Average ±Std		#LSD (p<0.05)
Leucine	5.76	7.05	7.11	7.59	6.88	0.68	0.37
Lysine	2.88	2.96	3.12	2.98	2.99	0.09	1.00
Isoleucine	2.95	3.03	3.21	3.88	3.27	0.37	0.33
Phenylalanine	4.54	4.71	4.48	4.57	4.58	0.08	1.00
Valine	3.82	3.04	3.91	3.97	3.69	0.38	1.00
Methionine	1.90	1.96	1.99	1.71	1.89	0.11	0.15
Tryptophan	1.02	1.29	1.36	1.34	1.25	0.14	1.00
Threonine	3.28	3.65	3.01	3.30	3.31	0.23	1.00
Tyrosine	2.21	2.37	3.35	2.72	2.66	0.44	1.00
Cystine	1.67	1.90	2.21	2.37	2.04	0.27	1.00
Histidine	2.07	2.18	2.30	2.30	2.21	0.10	0.09
Alanine	3.22	3.62	3.29	5.21	3.84	0.81	1.00
Proline	8.33	8.69	7.94	7.79	8.19	0.35	1.00
Glutamic	19.15	20.19	23.87	28.69	21.98	3.74	1.00
Serine	4.33	4.94	3.66	3.41	4.09	0.60	1.00
Aspartic acid	5.17	5.24	5.45	5.30	5.29	0.10	1.00
Glycine	3.26	4.03	3.34	4.61	3.81	0.55	1.00
Arginine	4.01	4.20	4.85	5.19	4.56	0.48	1.00
TEAA	30.03	33.69	35.38	37.65	34.19	2.78	1.00
TNEAA	47.47	51.36	53.07	56.28	52.05	3.18	1.00
HAA	35.42	37.66	38.15	39.85	37.77	2.58	1.00
BV (%)	71.37	81.11	86.27	89.04	81.95	6.75	1.00

TEAA- Total Essential amino acids, **TNEAA**- Total Non-essential amino acid, **HAA**– Hydrophobic amino acids, **BV**- Biological value, **#LSD** = Least significant difference. **Key:** WSY 1 = 100% wheat flour; WSY 2 = 80% wheat flour + 20% soybean flour; WSY 3 = 70% wheat flour + 30% soybean flour; WSY 4 = 60% wheat flour + 40% soybean

3.3. Antioxidant properties of cookie samples

It is a known fact that antioxidants inhibited oxidation process by preventing the formation of free radicals, thereby played major roles in preventing several chronic diseases such as cardiovascular diseases, diabetes, obesity, etc. (Malomo *et al*., 2010). Therefore, the hydroxyl (OH) radical scavenging activity of the cookie samples is presented in Fig 1A. The properties of the samples were recorded in the ranges of 22 to 77% when compared to the common and well-known antioxidant, ascorbic acid (80%). It was observed that the sample WSY 4 had the significant (p<0.05) highest property when compared to other samples WSY 1, 2 and 3, respectively. The DPPH radical scavenging activity of the cookie samples followed the similar trend like that of OH radical scavenging abilities according to the results shown Fig 1B. For instance, a low property (28%) was reported for WSY 1 while a high ability (62%) was obtained for WSY 4, respectively. It was observed that the samples shown significant (p<0.05) difference between each other against DPPH activities and when compared to ascorbic acid (80%). The metal (Fe^{2+}) chelation antioxidant activities of the cookie samples presented in Fig 1C revealed a contrary and divergent pattern. For instance, the sample WSY 3 had the highest activity (80.89%) when compared to WSY 1 (28%) and WSY 4 (72%), unlike a situation in Figs 1 and 2 whereby the sample WSY 4 was having the highest OH and DPPH radical scavenging properties, respectively. The contrary observable differences in this result reported in Fig 1C when compared to those in Figs 1A and 1B could be as a result of the differences in the mechanisms of their antioxidative actions. The results presented in Fig 1D revealed that the sample WSY 4 also had the significant (p<0.05) highest ferric reducing antioxidative potentials (FRAP) when compared to WSY 1, 2 and 3, respectively. For instance, WSY 3 and WSY 4 had 0.48 and 0.63 mmol Fe^{2+}/mg when compared with the commonly known antioxidant, an ascorbic acid (0.88 mmol Fe^{2+}/mg). It was observed that the antioxidant activities of sample WSY 1, 2 and 3 (with respect to hydroxyl, DPPH radical scavenging and FRAP activities except the Fe^{2+} chelation activity) were significantly ($p<0.05$) lower than WSY 4. The implication of this is that the samples WSY 1, 2 and 3 exhibited less ability to scavenging free hydroxyl and DPPH radicals as well as having potentials to reduce Fe^{3+} to Fe^{2+}.

The relative higher antioxidant potentials (66-78%) of the composite cookie samples may be attributed to their higher protein contents (~25 mg/100 g), hydrophobic amino acids (~40 mg/100 g) and biological values (~90%). This observation agreed with other findings that reported the efficacy of protein and amino acids profiles to enhance the antioxidant capacity, which was related to the release of bioactive peptides (Lobo *et al.*, 2010). The current observations also agreed with past work on the contributions of antioxidants in the management of diabetes and its complications (Adefegha *et al.*, 2016). Besides, studies (Adefegha *et al.*, 2016; Oluwajuyitan *et al.*, 2020) have demonstrated a positive significant decrease in the pathogenesis of cardiovascular disease such as diabetes and hypertension with consumption of antioxidant-rich foods.

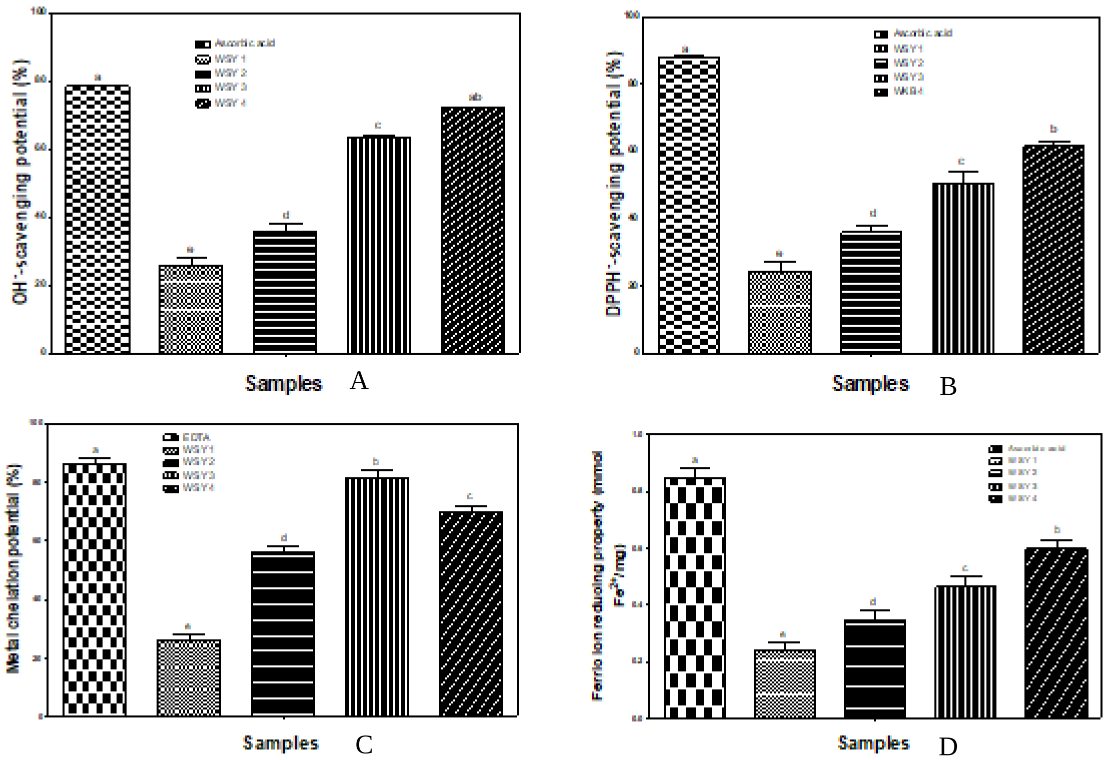


Figure 1A: Hydroxyl (OH); **1B:** DPPH radical scavenging **1C:** Metal chelation; **1D:** Ferric reducing properties of different cookies samples.

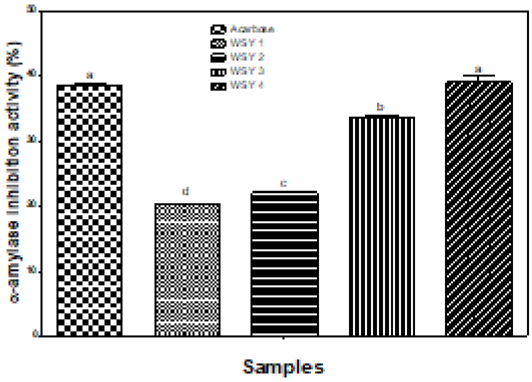
Bars (n=3) with different letter are significantly different ($p<0.05$). **Key:** WSY 1 = 100% wheat flour; WSY 2 = 80% wheat flour + 20% soybean flour; WSY 3 = 70% wheat flour + 30% soybean flour; WSY 4 = 60% wheat flour + 40% soybean.

3.4. In vitro anti-diabetic properties (α-amylase and α-glucosidase inhibition potentials)

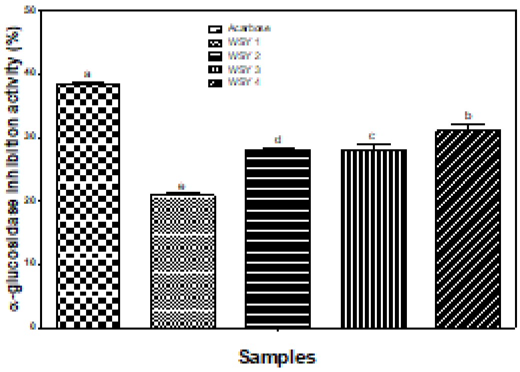
Fig 2A showed the α-amylase inhibition potentials of the composite cookies when compared with acarbose, a common anti-diabetic drug. The inhibition ranged from 20 (WSY 1) to 32% (WSY 4). However, the cookie WSY 4 had significant ($p<0.05$) higher α–amylase inhibition than the other samples but unfortunately lower than the 38% reported for acarbose, a common anti-diabetic drug. Notably, the higher hydrophobic acid obtained for WSY 4 (Table 2) might be accountable for its higher α–amylase inhibition (Fuentes-Zaragoza *et al.*, 2010). Thus, this could enhance the utilization of WSY 4 as a potential non-insulin dependent anti-diabetic agent with no negative side-effect. Fig 2B showed the potential of the cookie samples to inhibit α- glucosidase activities. The sample WSY 4 had higher inhibitions that the other composite cookie samples (WSY 1, 2 and 3). However, the result (Fig 2B) showed that the samples WSY 2 and 3 almost have similar inhibition power against the α-glucosidase. Hence, these samples have potentials to modulate the type 2 diabetes in human body. The cookie sample WSY 4 obtained from 40% soybean flour has improved inhibition of α-glucosidase and this may be due to its higher protein contents as well as its better hydrophobic amino acids, when compared to other samples (Fuentes-Zaragoza *et al.*, 2010). Interestingly, the α-amylase is a prominent enzyme found in the pancreatic juice and saliva that hydrolyzed complex starches to oligosaccharides while α-glucosidase is the enzyme found in the mucosal brush border of the small intestine that hydrolyzed oligosaccharides to glucose and other monosaccharides (Kazeem *et al.*, 2013; Rege & Chowdhary, 2014).

3.6 Physical property of cookies

The result of the physical properties of cookie samples was shown in Table 3. Some cookie samples from 100% wheat flour only and some purchased from commercial stores served as both negative and positive controls, respectively. The weights of the composite cookie samples were obtained in the ranges of 5.28-5.40 g, which is significantly ($p<0.05$) much more comparable to those (WSY 1) from 100% wheat only (4.08 g) and those bought from commercial stores (5.51 g). The widths of the cookie samples were almost similar to one another with ~30 mm. Meanwhile, an increase in width was noticed with increasing levels of soybean flour substitution and similar trend was recorded on thickness of the cookie samples. The spread ratio of the cookies decreased with increasing level of soybean substitution from 0 (WSY 1) to 40% (WSY 4).



A



B

Figure 2A: α-amylase; **2B:** α-glucosidase inhibition activities of different cookies samples

Bars (n=3) with different letter are significantly different ($p<0.05$). **Key:** WSY 1 = 100% wheat flour; WSY 2 = 80% wheat flour + 20% soybean flour; WSY 3 = 70% wheat flour + 30% soybean flour; WSY 4 = 60% wheat flour + 40% soybean

Table 3 - Physical properties of the cookies

Samples/ Parameters	WSY 1	WSY 2	WSY 3	WSY 4	Commercial sample
Weight (g)	4.08 ± 0.06^{c}	5.34 ± 0.01^{ab}	5.28 ± 0.01^{ab}	5.40 ± 0.03^{ab}	5.51 ± 0.01^{a}
Width (mm)	30.04 ± 0.08^{bc}	31.56 ± 0.04^{bc}	32.58 ± 0.06^{b}	32.72 ± 0.21^{b}	35.03 ± 0.58^{a}
Thickness (mm)	3.00 ± 0.02^{bc}	3.21 ± 0.05^{ab}	3.24 ± 0.07^{ab}	3.30 ± 0.04^{a}	3.10 ± 0.01^{bc}
Spread ratio	10.01 ± 0.07^{b}	9.83 ± 0.13^{c}	$10.06.\pm0.01^{b}$	9.91 ± 0.01^{c}	11.30 ± 0.04^{a}

Means (n=3) with different letter in the row are significantly different ($p<0.05$). **Key:** WSY 1 = 100% wheat flour; WSY 2 = 80% wheat flour + 20% soybean flour; WSY 3 = 70% wheat flour + 30% soybean flour; WSY 4 = 60% wheat flour + 40% soybean

Spread ratio was affected by the competition of ingredients for the available water (Malomo and Udeh, 2018). Other functional properties such as, proteins and fat might have also affected the spread ratio. It can therefore be deduced that both protein and fat content in the soybean flour had effect on the spread ratio of the cookie samples. The spread ratio here seemed not to be due to competition over available water by ingredients as both wheat and soybean flours absorbed water during dough mixing. Hence, the observed noticeable differences may be traced to the protein and fat contents of the blends and thus affected the rise in the cookie samples during the baking process. In all, sample WSY 4 with the composition of 60% wheat flour: 40% defatted soybean flour is physically preferred to others.

3.7. Sensory attributes of cookies

Sensory analysis is carried out using untrained panelists to evaluate sensory characteristics, like appearance, aroma, taste, texture and overall acceptability of the food product. Mean score for sensory evaluation of cookie given in Table 4 revealed that there are significant differences ($p\leq0.05$) between the samples in terms of their attributes like taste, mouth feel crumbling, appearance, texture and overall acceptability. Sensory rating of cookie for appearance showed that sample WSY 2 was ranked high (7.60) when compared to the commercial cookie (8.00).

Table 4: Sensory attributes of the cookies

Samples	Appearance	Taste	Texture	Mouthfeel	Crumblings	Aroma	Overall accept.
WSY 1	7.10^{bc}	7.16^{b}	6.50^{c}	7.91^{a}	7.10^{bc}	7.40^{c}	7.80^{c}
WSY 2	7.60^{ab}	7.23^{b}	7.81^{b}	7.80^{a}	7.64^{b}	8.70^{a}	7.72^{c}
WSY 3	7.35^{b}	7.34^{b}	7.64^{b}	7.10^{ab}	7.38^{b}	7.15^{cd}	7.76^{c}
WSY 4	7.05^{bc}	7.12^{b}	8.05^{a}	6.65^{c}	6.85^{d}	8.15^{ab}	8.14^{a}
Mkt sample	8.00^{a}	8.00^{a}	8.00^{a}	7.00^{ab}	8.00^{a}	9.00^{a}	9.00^{a}

Means (n=50) with different letter in the column are significantly different ($p<0.05$). **Key:** WSY 1 = 100% wheat flour; WSY 2 = 80% wheat flour + 20% soybean flour; WSY 3 = 70% wheat flour + 30% soybean flour; WSY 4 = 60% wheat flour + 40% soybean

This result agreed with the past report that baked goods using soybean at minimal level as ingredient, provided one of the most attractive possibilities because it increased dough yield and contributed to attractive crumb and crust (Bunde *et al.*, 2010). Moreso, noticeable change in colour from light brown to darker shades of brown could be associated to non-enzymatic browning reactions (Maillard reactions) between reducing sugar molecules and lysine. Soybeans is reported to be rich in lysine, which produces darker shades of brown colours (Bunde *et al.*, 2010). There was no significant difference in the taste of the composite cookies, mainly might be as a result of presence of soybean because it has a characteristic bland flavour that is neither bitter nor sweet. In contrast, the mouthfeel characteristics decreased with increasing levels of soybean flour (from 0 to 40%), which could also be due to the difference in particle size of the composite flours as observed in the higher substitution. The remarked feeling grits in cookie sample WSY 2 could be associated to its small particle size. The overall acceptability of the cookie revealed that the sample WSY 4 (60% wheat: 30% soybean) had higher acceptability than the others and corresponds closely to the commonly known commercial samples.

Conclusion

The present findings revealed the wheat and soybean composite flours as potential ingredients for the production of acceptable quality cookies. It was thus concluded that cookie sample produced from mixtures of 60% wheat flour: 40% soybean flour gave the best products due to its high protein, hydrophobic amino acid, improved antioxidant properties, enhanced inhibition of carbohydrate hydrolytic enzymes as well as their acceptability to the consumers. These properties and bioactivities of the cookie showed that it would be helpful and potentially useful in reducing insulin demand, improving satiety, improving blood glucose control in diabetic people, and numerous age-associated diseases such as CVD and certain cancers.

In any case, adoption of this technology of cookie production will result in production of better protein and fibre-enriched cookie to the ever-increasing number of consumers. Moreover, consumption of functional cookie samples made from composite flour blends could be said to be nutritionally more superior to those from whole-wheat flour in terms of improving the nutritional status of the consumers. This can be used as a vehicle for protein fortification and other nutritional improvement as cookie is widely accepted bakery products highly consumed in Nigeria. Moreso, the fact that soybean flour is relatively cheaper in Nigerian market than wheat flour would make the cookie formulation done at the much-reduced cost of production.

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Author ICA performed the experiment, collected and analyzed the data as well as prepared the draft of the manuscript. Author SAM designed the experiment, supervised the study, analyzed the data, thoroughly read and edited the manuscript.

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References

Arise, A.K., Sarafa A. Akeem, Omotola F. Olagunju, Oluyemisi D. Opaleke, Deborah T. Adeyemi, Development and Quality Evaluation of Wheat Cookies Enriched with Bambara Groundnut Protein Isolate alone or in Combination with Ripe Banana Mash, Applied Food Research, **1(1),** 2021, 100003, https://doi.org/10.1016/j.afres.2021.100003

Adefegha, S. A., Oboh, G., Oyeleye, S. I., & Ejakpovi, I. (2016). Erectogenic, antihypertensive, antidiabetic, antioxidative properties and phenolic compositions of Almond Fruit (*Terminalia catappa* L.) Parts (Hull and Drupe) – in vitro. *Food Biochemistry*, **41**, e12309.

Adeola SS, Foloranso ST, Gama EN, Amodu MY and Owolabi JO, 2011. Productivity and Profitability analysis of cowpea production in Kaduna state. Pelagia research library, pp. 72-73. Retrieved on 17/2/2013 from www.pelagiare search library.com

Adeyanju, B.E.; Enujiugha, V.N.; Bolade, M.K. Effects of addition of kidney bean (Phaseolus vulgaris) and alligator pepper (Aframomum melegueta) on some properties of aadun (a popular local maize snack). *J. Sustainable Tech*. **2016,** *7*(1), 45-58.

Association of Official Analytical Chemist, AOAC. *Official Methods of Analysis. Association of Official Analytical Chemists*. 18th edn. Washington D.C., USA. **2012,** 170 -184.

Bunde MC, Osundahunsi FO and Akinoso, R. (2010). Supplementation of biscuit using rice bran and soyabean flour. AJFAND.10 (9):47-59.

Fuentes-Zaragoza, E., Riquelme-Navarrete, Sánchez-Zapata, M.J., E., Pérez-Álvarez, J.A., Resistant starch as functional ingredient: A review, Food Research International, Volume 43, Issue 4, 2010, Pages 931-942, ISSN 0963-9969, https://doi.org/10.1016/j.foodres.2010.02.004

Godswill, A. C. (2019). Proximate composition and functional properties of different grain flour composites for industrial applications. *International Journal of Food Sciences*, *2*(1), 43–64. https://doi.org/10.47604/ijf.1010

Kazeem MI, Raimi, OG, Balogun RM, potential of different extracts of Blighia sapida Koenig. American Journal of Research Communication. 2013a; 1(7):178-192

Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010 Jul; 4(8):118-26. doi: 10.4103/0973-7847.70902.

Malomo, S.A., and Udeh, C.C. (2018). Quality and *In Vitro* Estimated Glycemic Index of Cookies from Unripe Plantain-Crayfish-Wheat Composite Flour. *Applied Tropical Agriculture*, 23(2), 82-89

Malomo, S.A., Nwachukwu, I.D., Girgih, A.T., Idowu, A.O., Aluko, R.E., and Fagbemi, T.N. 2020. Antioxidant and renin-angiotensin system (RAS) inhibitory properties of cashew nut and fluted-pumpkin protein hydrolysates. *Polish Journal of Food and Nutrition Science.* 70(3), 275-289.

Olugbuyi, A.O., Oladipo, G.O., Malomo, S.A., Ijarotimi, S.O., and Fagbemi, T.N. 2022. Biochemical ameliorating potential of optimized dough meal from plantain (*Musa AAB*), soycake (*Glycine max*) and rice bran (*Oryza sativa*) flour blends in Streptozotocin-induced diabetic rats. *Applied Food Research*, 1-11. https://doi.org/10.1016/j.afres.2022.100097

Oluwajoba, S. O., Malomo, O., Ogunmoyela, O. A. B., O., D. O. E., & Odeyemi, A. (2012). Microbiological and nutritional quality of warankashi enriched bread. *Journal of Microbiology, Biotechnology and Food Sciences*, *2*(1), 42–68.

Oluwajuyitan, T.D., Ijarotimi, O.S., Fagbemi, T.N., Nutritional, biochemical and organoleptic properties of high protein-fibre functional foods developed from plantain, defatted soybean, rice-bran and oat-bran four, Nutr. Food Sci. (2020), https://doi.org/10.1108/NFS-06-2020-0225.

Onabanjo, O.O. and Ighere, D.A. (2014) Nutritional, Functional and Sensory Properties of Biscuit Produced from Wheat-Sweet Potato

Composite. Journal of Food Technology Research, 1, 111-121. https://doi.org/10.18488/journal.58/2014.1.2/58.2.111.121

Rege AAA, Chowdhary AS. (2014). Evaluation of Alpha-Amylase and Alpha-Glucosidase Inhibitory Activities of Shilajit. International Journal of Advanced Research. 2(2): 735–40

Twinomuhwezi, H. Awuchi, C.G., & Rachael, M. (2020). Comparative Study of the Proximate Composition and Functional Properties of Composite Flours of Amaranth, Rice, Millet, and Soybean. American Journal of Food Science and Nutrition, 6(1), 6–19

Udeh, C., Ifie, I., Job, A., and Malomo, S.A. 2021. Kidney bean protein products as potential antioxidative and antihypertensive alternatives for non-pharmacological inhibition of angiotensin-converting enzymes. *Journal of Scientific African*, 11, e00693, 1-16, https://doi.org/10.1016/j.sciaf.2021.e00693.

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