



Consumer Perceptions and *In Vitro* Anti-Inflammatory Activities of Cookies Produced from Wheat and Soybean Composite Flour Blends

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Abstract

Cookies offered several important advantages including; wide consumption, relatively long shelf life, good eating quality, highly palatable and acceptable in most countries. This study thus, investigated the quality of cookies produced from wheat-soybean composite flour blends at different ratios. The soybean seeds were thoroughly processed, milled, sieved and mixed with the commercial wheat flour. Four blends namely: WSY 1 (100:0), WSY 2 (30:70%), WSY 3 (40:60%) and WSY 4 (50:50%) (wheat:soybean), respectively were prepared and later used to bake cookies. The proximate, anti-inflammatory (protein denaturation and cyclooxygenase inhibition) activities of the composite cookies were examined while panelists were assigned to assess the cookie samples. The results of the proximate composition showed that the composite flour technology had significant ($p < 0.05$) positive effects on the cookies. The anti-inflammatory results showed the inhibitory potentials of the cookies against the cyclo-oxygenase activities at low concentration (IC_{50} ; 12.85 $\mu\text{g/ml}$, respectively) when compared to sodium diclofenac (18.60 $\mu\text{g/ml}$), a well-known and common non-steroidal anti-inflammatory drug. No panelist showed a total dislike for the taste of any of the samples. The added kidney bean seeds flours showed no significant ($p < 0.05$) effect on the acceptability and preference of the samples. Hence, it is possible to produce cookies from wheat-soybean composite flours loaded with high protein contents to serve as effective anti-inflammatory agents (low IC_{50}).

Keywords: Wheat–soybean composite flour, Cookies, Proximate composition, Anti-inflammatory activity, Sensory evaluation

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1. Introduction

Legumes are good sources of cheap and widely available proteins for human consumption (Arise et al., 2022). They are staple foods for many people in different parts of the world. They ranged between the highly utilized legumes such as soybeans, cowpeas to the lesser-known ones like African yam beans (Sudanic, 2020). The known legumes together with other conventional legumes could be used for combating protein energy malnutrition (PEM) and other inflammatory diseases, like cancer, bowel disruption, arthritis, etc. Soybeans contained different food fractions and proteins that were comparable to other food legumes. The seed is a highly priced food legume in every part of Nigeria (Olugbuyi et al., 2022) owing to high crude protein content. Soybeans had its origin in Ethiopia but was now widely cultivated in tropical Africa, especially West Africa, in Cameroon, Ghana, and Nigeria. It was grown for seeds to be used as food since it tolerated an annual rainfall of < 1000 mm (Alawode et al., 2017). The consumption of soybean is known to contribute to daily nutrition, food availability, and diet diversification to communities utilizing it; this dated back to the Nigerian civil

war of 1967–1970, where the crop's food and nutritional potentials were efficiently utilized in fighting malnutrition and hunger. Some studies have examined various objectives on soybean flour from seed sourced from open markets and communities (Arise et al., 2022; Olugbuyi et al., 2022; Okorie, 2018; Alawode et al., 2017; Uchegbu and Amulu, 2015). There was presence of complex protein, carbohydrates and dietary fibre, which have been proved to be associated to reduce the risk of heart disease, diabetes and obesity. (Anderson et al, 2009). Raw soybeans also contained large amount of anti-nutritional factors such as phytic acid, hemagglutinins, trypsin inhibitors, saponins and tannins which can affect absorption of protein, carbohydrates and certain minerals (Olugbuyi et al., 2022). Traditional processing methods such as hydration, germination, thermal processing (cooking and autoclaving) and fermentation have been proved to be effective in eliminating the antinutritional factors present in legumes (Khattab et al, 2009; Nergiz and Gokgoz 2007; Khalil 2001). Soybean is an excellent source of high-quality protein, low in saturated fat, and free of cholesterol. In addition, it contained a high dietary fiber, the second largest component, which has

been reported to reduce the risk of colon cancer and other diseases (Anderson et al., 1999; Mateos-Aparicio et al., 2008). Soybean, with its 40% protein, is richer than any other food crop or even livestock. It was also made up of 20% oil, and contained all the eight essential amino acids, making it the healthiest legume crop. Soybean contributed significantly to overall human nutrition in terms of both calorie and protein intake. The crop appeared to be well placed to meet the fast-growing demand for vegetable oil and animal feed in developing countries (Shaw and Rajcan, 2017).

Cookie is a conventional wheat flour-based food product produced from unpalatable dough with the application of heat and transformed into an appetizing product (Ikuomola et al., 2017). It formed part of the bakery products that were consumed extensively all over the world by all ages (Sengev et al., 2015). Cookies are ready to eat, convenient and inexpensive snack food products containing digestive and dietary principles of vital importance (Adeyeye & Akingbala, 2015). The principal ingredients are wheat flour, fat, sugar and water and optional ingredients include milk, salt, flavouring agent, aerating agent, food additives and others. Soft wheat flour has been the major ingredient used in the production of cookies but imported by countries with unfavorable climatic conditions such as Nigeria. The increase in the importation could lead to economic drain, high prices of these bakery products and a threat to food security. This necessitated a need for strategic development in the use of inexpensive local resources in the production of cookies in African Countries (Moyib et al., 2008). It is a good source of protein, carbohydrate, minerals and vitamins. The primary challenge to wide consumption of soybean included presence of oligosaccharides, such as verbascose, raffinose and stachyose, which resulted into production of flatulence after eating (Aremu and Ibirinde, 2012; Abioye et al., 2015). It is, thus very important to find alternative utilization methods apart from cooking to prevent further neglect and promote its cultivation. Moreso, an increase in urbanization coupled with availability and easiness of ready -to-eat foods and growing number of working mothers, have profoundly contributed to the popularity and increased consumption of snack foods in Nigeria. The escalating cost of wheat importation and difficulty in cultivating wheat in the tropics has focused attention on the need to explore the use of alternative local

flours as supplements or substitutes for wheat flour in the baking industry (Arise et al., 2022). There is also an increasing interest in the consumption of functional foods where the consumers are not only interested in the nutritional benefits of foods but also in the health benefits (Azzura and Paola, 2009).

Inflammation is caused by injury to a living tissue. It has four primary indicators i.e pain, redness, heat or warmth and swelling (Sarveswaran, et al., 2017). When there is an injury to any part of the human body, the arterioles dilated thereby producing redness by increasing the blood circulation towards the injured tissue (Eshwarappa et al., 2016). Moreso, the use of synthetic drugs, are accompanied with series of negative health side effects and exorbitant cost price caused more havoc and pocket-draining for most health-challenged consumers as well (Aparicio-Saguilan et al., 2007). Hence the need for alternative but cheaper sources of materials to serve as non-steriodial anti-inflammatory agents (NSAIDs), such as common plant materials or legume crops. The main objective of this study is to examine the nutritional qualities and *in vitro* anti-inflammatory properties of cookies produced from wheat-soybean composite flours

2. Materials and Methods

2.1 Materials

The commercial wheat flour, which have been commonly used for all baking processes, were obtained from a commercial baking ingredients store in Ondo city, Nigeria. The soybean seeds were obtained from the market at Ondo city, 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. The soybean flour was made from the seeds after subjecting it to the pre-heat treatment, dried, milled and sieved through 0.4 mm wire mesh to obtain the final flour for further analysis according to the previously described method (Alawode et al., 2017).

2.2 Formulation of composite flour blends

The composite flour blends were formulated from wheat and soybean flours as stipulated in the Table 1 *viz*:

Table 1: Flour blends formulations

Samples	Formulations	Wheat	Soybean	Total (%)
WSY 1	Wheat (commercial flour)	100	0	100
WSY 2	Wheat + Soybean flour	30	70	100
WSY 3	Wheat + Soybean flour	40	60	100
WSY 4	Wheat + Soybean flour	50	50	100

2.3 Production of cookies

Cookies were produced as previously described by Malomo & Udeh (2018) with the following ingredients: composite flour, margarine, baking powder, salt, sugar, eggs and water. The dry 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 °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.

2.4 Proximate composition analysis

The proximate composition (moisture content, crude fiber, crude fat, total ash, and crude protein contents of the flour blends and cookies was determined as described by Association of Official Analytical Chemist (2012). The total carbohydrate content was obtained by difference. The crude

protein contents were determined by micro-Kjedahl method to obtain the nitrogen content. The crude protein was then calculated as (gN x 6.25) while the crude fat was obtained using Soxhlet apparatus.

2.5 Determination of anti-inflammatory properties

Inhibition of protein denaturation

The inhibition of protein denaturation was evaluated by the method of Sakat *et al.* (2010) with slight modification. 500 µL of 1% bovine serum albumin was added to 100 µL of plant extract. This mixture was kept at room temperature for 10 min, followed by heating at 51 °C for 20 min. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Acetyl salicylic acid was taken as a positive control. The experiment was carried out in triplicates and percentage inhibition for protein denaturation was calculated using:

$$\% \text{Inhibition} = 100 - ((A1 - A2) / A0) * 100$$

Where A1 = absorbance of the sample; A2 = absorbance of the product control; A0 = absorbance of the positive control.

Anti-cyclooxygenase activity

The anti-cyclooxygenase activity was measured using the assay mixture containing Tris- HCl buffer, glutathione, hemoglobin & enzyme. The assay started by the addition of arachidonic acid and terminated after 20 min incubation at 37 °C by addition of 0.2 ml of 10% TCA in 1N HCl, mixed and 0.2 ml of TBA was added and contents heated in a boiling water bath for 20 min, cooled and centrifuged at 1000 rpm for 3 min. The supernatant was measured at 632 nm for COX activity.

2.6 Evaluation of sensory attributes

The cookies were coded and presented to fifty (50) semi-trained panelists to be evaluated for their appearance, texture, taste, aroma, mouth feel, crumbings, overall acceptability using the Hedonic scale of 1 to 9, where 1 = dislike extremely

and 9 = like extremely as previously described by Malomo and Udeh (2018).

2.7 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 ($p < 0.05$).

3. Results and Discussion

3.1 Proximate composition of flour blends and cookies

The proximate composition of cookies produced from wheat and soybean composite flours are presented in Table 2. Moisture content ranged from 5.19 to 8.01%. It was observed that all the samples had low moisture content especially WSY 4. There was significant difference ($p < 0.05$) observed in all the samples. Past report (Anhwange *et al.*, 2009) suggested that moisture content can be used to determine the shelf life, physical, chemical and even microbiological changes during storage. This low moisture observed in the entire cookies sample corroborated the findings previously reported (Edema *et al.*, 2005) to increase the shelf life of food products. The protein content ranged from 7.13 to 22.21%. It was observed that all the samples had high protein content more than the control sample with significant difference ($p < 0.05$) between them all., which might be due to the addition or inclusion of soybean flour to WSY 2, 3 and 4, respectively. The highest protein content was observed in the sample WSY 3 and this was significantly higher than 10.02% obtained for the commercial cookie sample. The high protein content is associated to effective repairing of worn-out tissues in the body system (Olaoye *et al.*, 2006). The carbohydrates content ranged between 55.33 to 71.44%. It was observed that all the samples had high carbohydrate content with the highest observed in the control sample. Their carbohydrate contents were significantly ($p < 0.05$) different from one another.

Table 2: Proximate compositions of cookies (%)

Sample/ Parameters	Moisture	Crude protein	Crude fat	Ash	Crude fibre	Carbohydrate
WSY 1	8.01 ± 0.36 ^a	7.13 ± 0.06 ^e	11.82 ± 0.12 ^a	4.06 ± 0.04 ^c	7.04 ± 0.30 ^d	71.44 ± 0.11 ^{ab}
WSY 2	5.74 ± 0.06 ^b	21.51 ± 0.05 ^b	4.64 ± 0.07 ^d	5.65 ± 0.14 ^{ab}	15.49 ± 0.05 ^b	55.33 ± 0.09 ^c
WSY 3	5.89 ± 0.05 ^b	22.21 ± 0.05 ^a	5.59 ± 0.01 ^c	5.72 ± 0.05 ^a	17.54 ± 0.05 ^a	55.89 ± 0.08 ^c
WSY 4	5.19 ± 0.11 ^b	20.91 ± 0.01 ^c	5.74 ± 0.03 ^c	5.98 ± 0.01 ^a	14.73 ± 0.01 ^c	57.09 ± 0.14 ^c
Commercial sample	8.27 ± 0.11 ^a	10.02 ± 0.03 ^d	10.91 ± 0.01 ^b	3.61 ± 0.01 ^d	4.72 ± 0.05 ^c	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 = 30% wheat flour + 70% soybean flour; WSY 3 = 40% wheat flour + 60% soybean flour; WSY 4 = 50% wheat flour + 50% soybean flour

The fat content of the cookies ranged from 4.64 to 11.82% when compared with the commercial sample (10.91%). It was observed that all the samples had low fat content with the WSY 2 sample having the lowest (4.64%). There was significant difference ($p < 0.05$) observed in all the samples while the low fat signified that the samples could help the body by reducing obesity. The ash content ranged from 4.06 to 5.98%. It was observed that all the samples had higher ash content much more than the control sample WSY 1 (4.06%) but way higher than the commercial sample (3.61%). There was no significant difference observed between sample WSY 3 and WSY 4 but

was observed ($p < 0.05$) in control sample. The high ash content could be related to high quantity of mineral in the samples as previously (Dada *et al.*, 2012) reported. The fibre content ranged between 5.49 and 17.54%. It was observed that all the samples had higher fibre content than the control. The highest fibre content was observed in WSY 3. There was significant difference ($p < 0.05$) observed in all the samples. The high fibre helped in lowering blood cholesterol level while systematically slowing down the process of absorption of glucose, thereby helping in keeping blood glucose level in control (Hamza *et al.*, 2014).

3.2 Anti-inflammatory properties of cookies

The inhibition of protein denaturation of composite cookies at 50% inhibition (IC_{50}) is presented in Fig 1. It was observed that the composite cookie WSY 3 has low IC_{50} (22 $\mu\text{g}/\text{ml}$) when compared to the control sample (70 $\mu\text{g}/\text{ml}$), which is the 100% wheat cookies (WSY 1). The main cause of inflammation is denaturation of protein. Inflammation, which is generally

referred to as a complex biological response of vascular tissues to harmful stimuli, has been associated with pain, and it involved in an increase of protein denaturation, an increase of vascular permeability, and membrane alteration, proteinase, among others (Ferrero-Millani *et al.*, 2007). Therefore, this result showed that sample WSY 3 is able to inhibit denaturation of protein more, which caused inflammation, when compared to the control.

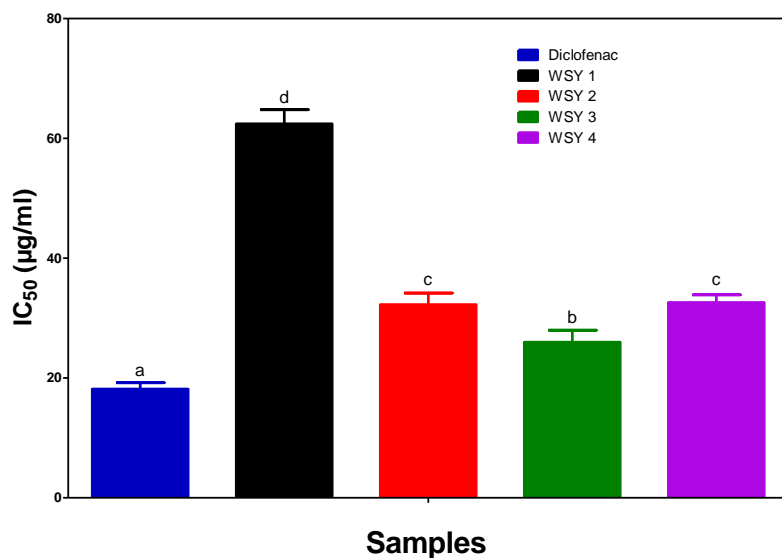


Figure 1: Inhibition of protein denaturation activity of different cookies at 50% level of inhibition concentration (IC_{50})

Bars (n=3) with different letter are significantly different ($p < 0.05$).

Key: WSY 1 = 100% wheat flour; WSY 2 = 30% wheat flour + 70% soybean flour WSY 3 = 40% wheat flour + 60% soybean flour; WSY 4 = 50% wheat flour + 50% soybean

Cyclo-oxygenase is an enzyme that catalyzed the primary oxidation of unsaturated fatty acids or unsaturated fats by oxygen, leading to inflammation in the body (Ferrero-Millani *et al.*, 2007; Patel *et al.*, 2013). It was observed from the results presented in Fig 2, that the activities of the cyclo-oxygenase were being able to be checkmated through the obtained significant ($p < 0.05$) low IC_{50} (12.85 $\mu\text{g}/\text{ml}$) when compared to

sodium diclofenac (18.60 $\mu\text{g}/\text{ml}$), a well-known and common non-steroidal anti-inflammatory drug and the samples WSY 1, 2 and 4 (45.54-46.01, 20.01-20.06 and 22-23 $\mu\text{g}/\text{ml}$), respectively. The present study reported in Fig 2 thus, showed that the cookies sample WSY 3 could be regarded as potential agents in the inhibition of activities of the enzyme implicated in the inflammatory reactions in the body (Patel *et al.*, 2013).

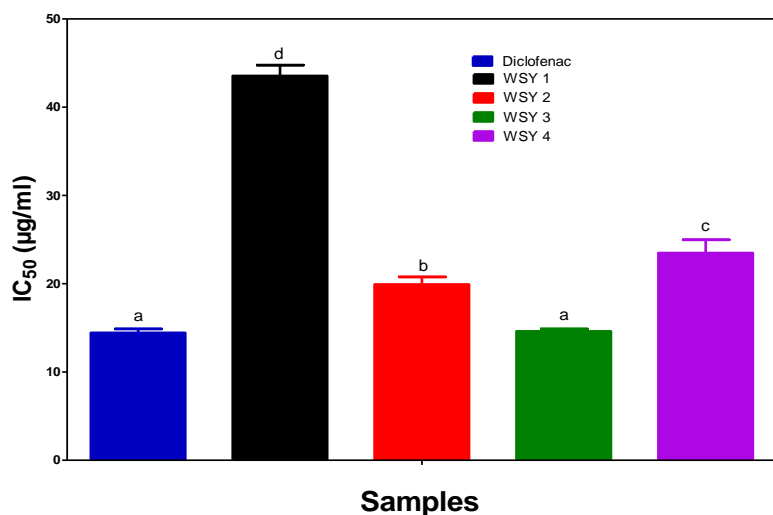


Figure 2: Anti-cyclooxygenase activity of different cookies at 50% level of inhibition concentration (IC_{50})

Bars (n=3) with different letter are significantly different ($p < 0.05$).

Key: WSY 1 = 100% wheat flour; WSY 2 = 30% wheat flour + 70% soybean flour WSY 3 = 40% wheat flour + 60% soybean flour; WSY 4 = 50% wheat flour + 50% soybean

Table 3: Consumer perceptions of the cookies

Samples	Appearance	Taste	Texture	Mouthfeel	Appearance	Aroma	Overall accept.
WSY 1	8.50 ^b	8.26 ^b	8.70 ^b	8.95 ^a	8.20 ^{cd}	8.70 ^b	8.90 ^{ab}
WSY 2	8.90 ^{ab}	8.80 ^b	8.85 ^b	8.90 ^{ab}	8.65 ^{bc}	8.70 ^b	8.70 ^b
WSY 3	8.55 ^b	8.30 ^b	8.60 ^b	8.00 ^b	8.30 ^{cd}	8.55 ^c	8.45 ^c
WSY 4	7.15 ^c	7.10 ^c	7.15 ^c	7.05 ^c	7.05 ^d	7.65 ^d	7.10 ^d
Commercial product	9.00 ^a	9.00 ^a	9.00 ^a	9.00 ^a	9.00 ^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 (**Negative control**); WSY 2 = 30% wheat flour +70% soybean flour; WSY 3 = 40% wheat flour + 60% soybean flour; WSY 4 = 50% wheat flour +50% soybean flour

3.3 Sensory attributes of the cookies

Mean scores for sensory attributes of cookies given were shown in the Table 3. The results revealed that there were significant differences (p<0.05) between sensory attributes like taste, colour, aroma, crispness and overall acceptability, of the experimental and commercial cookie samples. Cookies prepared from sample WSY 2 containing 30% wheat flour + 70% soybean flour was rated significantly (p<0.05) higher than the other level of substitution in all the attributes evaluated. The sensory rating of cookies for appearance showed that the sample WSY 2 cookies was high due to excellent colour, followed by WSY 3 while minimum colour was observed in WSY 4 cookie sample. Mean score of taste decreased from 8.80 to 7.10 when 100% wheat cookie sample WSY 1 was supplemented with the 50% level of soybean flour. Mean for mouthfeel shown in Table 6 revealed that sample WSY 1 had highest score (8.95) followed by WSY 2 (8.90). The aroma of cookies decreased from 8.70 to 7.65. Mean for aroma revealed that the panelists rated the samples WSY 1 (8.70) and WSY 4 (7.65) at top and bottom positions, respectively. Overall acceptability was determined on the basis of quality scores obtained from the evaluation of appearance, aroma, taste and mouthfeel of the cookies. The mean overall acceptability of cookies showed that the sample WSY 1 (8.90) was highly acceptable followed by WSY 2 (8.70), WSY 3 (8.45) and WSY 4 (7.10) has lowest acceptability when compared to the commercial cookies (9.00).

4. Conclusion

The use of wheat and soybean flours have been found to improve the proximate composition of the cookies in this study. Also, it has been found as remedy for inflammatory diseases by exhibiting anti-inflammatory activities such as in protein denaturation scavenging activity and anti-cyclooxygenase activities. Most especially from this study, cookies from WSY 3 composite flour, which is 40% wheat flour + 60% soybean flour, showed increase in protein and fibre values compared with the control sample (100% wheat flour). It also showed decrease moisture content, making it safe for a long period of time. Its sensory attributes such as taste, aroma, crispness, mouthfeel and appearance were significantly (p<0.05) comparable to the control and this made it totally acceptable as functional food to be utilized for its health-management (anti-inflammation) qualities.

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Authors contributions

All authors performed the experiments, wrote the manuscripts, read and approved the final manuscript.

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