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# Nutritional Composition and Acceptability of Cookies Made from Wheat Flour and Germinated Sesame (*Sesamum indicum*) Flour Blends

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## Authors' contributions

*This work was carried out in collaboration between all authors. Author BOTI designed and supervised the study, author AIO managed the analyses, managed the literature search, performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

Research Article

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## ABSTRACT

**Aims:** To evaluate the nutritional and sensory attributes of wheat-sesame supplemented cookies.

**Study Design:** Multifactorial Design.

**Place and Duration of Study:** Food Science and Technology Department, Federal University Technology, Akure, Ondo State, Nigeria between March 2011-2012.

**Methodology:** Sesame seeds were subjected to four days germination under closely monitored conditions of temperature and relative humidity. The sprouts were derooted, oven dried at 60°C for 6hrs, milled, sifted, packaged and stored at 4°C. Flour was analysed for proximate, antinutrients and amino acid compositions. Germinated sesame flour was used to supplement wheat flour at 5, 10 and 15% levels to bake cookies which were assayed for proximate composition, physical attributes and sensory evaluation.

**Results:** Germination increased protein content of sesame from 26.23% to 32.91% and reduced fat content from 52.7% to 23.22%. It also yielded positive effect on the antinutrients. Phytic acid content reduced from 31.59mg/g in raw seed to 16.20mg/g in germinated seed. Sesame seeds are rich in both essential and non-essential amino

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acids, processing significantly increased the values with leucine, methionine, lysine, phenylalanine, threonine and valine values higher than the recommended daily allowance. Protein, fat and ash content of wheat-sesame cookies increased with increase in sesame supplementation, 5% sesame cookies had 17.27%, 21.73% and 2.35% respectively while 15% sesame cookies had 18.80%, 25.02% and 4.21% respectively. The carbohydrate content on the other hand decreased with increase in sesame supplementation from 53.26 to 48.26%. 95:5% wheat-sesame cookies compared favourably with the control in terms of overall acceptability.

**Conclusion:** Sensory evaluation result revealed that the cookies supplemented with 5% germinated sesame flour was well accepted and not significantly different from the control (100% wheat flour cookies) in terms of aroma, taste and overall acceptability.

*Keywords: Germinated sesame seed flour; cookies; proximate; amino acid; physical characteristics; sensory attributes.*

## ABBREVIATIONS

*ND: Not Determined; NA: Not Available; RDA Source: FAO/WHO (1991); RDA: Recommended Daily Allowance; WF: Wheat Flour; WFC: Wheat Flour Cookies; WSC1: 95:5% Wheat-Sesame Cookies; WSC2: 90:10% Wheat-Sesame Cookies; WSC3: 85:15% Wheat-Sesame Cookies.*

## 1. INTRODUCTION

Plant proteins play significant roles in human nutrition, particularly in developing countries where average protein intake is less than the required. Many plant proteins usually in the form of protein extracts or seed flours are being investigated and tested for new products such as low cost fabricated foods which are nutritious, attractive and acceptable to consumers just like conventional foods from meat, fish and dairy products [1]. Seeds have nutritive and calorific values which make them necessary in diets. Due to the inadequate supplies of food proteins, there has been a constant search for unconventional protein sources, for use as both functional food ingredients and nutritional supplements. The ultimate success of utilizing plant proteins as ingredients largely depends upon the beneficial qualities they impact to foods, which in turn depend largely on their nutritional and functional properties [2].

Sesame seeds (*Sesamum indicum*) are tiny, flat oval seeds with a nutty taste. It is an important oil seed believed to have originated from tropical Africa with the greatest diversity [3]. Sesame seed is a staple food among many ethnic groups in Nigeria and it is cultivated in most areas of the middle belt and some northern states of Nigeria [4]. Sesame is an important source of oil (44-52.5%), protein (18-23.5%), carbohydrate (13%) [5,6]. The seeds are consumed fresh, dried or blended with sugar. It is also used as a paste in some local soups. The nutritional value of foods depends on their nutrient content and the bioavailability of these nutrients. Antinutritional factors in foods are food components that interfere with the digestion, absorption, or some other aspect of metabolism of nutrients contained in the food. Raw sesame seeds contain antinutrients mainly oxalates and phytates usually found in the seed hulls which can adversely affect mineral bioavailability in human nutrition [7,8]. Enhancement of the nutritional quality of sesame seeds can be anticipated through processing (such as soaking, roasting and germination) prior to consumption.

Wheat (*Triticum aestivum* L) is one of the important cereal grains because of its use for the preparation of many baked products. Unfortunately, lysine is the first limiting amino acid in wheat flour and more than 10% of it is being destroyed during baking [9].

The consumption of cereal foods such as biscuits and bread has become very popular in Nigeria, especially among children. Most of these foods are however, poor sources of protein with poor nutritional quality [10]. Enrichment of cereal-based foods with other protein sources such as oilseeds and legumes has received considerable attention because oil seed and legume proteins are high in lysine, an essential limiting amino acid in most cereals [11]. Some other oilseeds such as bambara groundnut and fluted pumpkin have been germinated and the flours used in food formulation but there is a dearth of information on certain compositions of germinated sesame flour. The study aims at investigating the effect of germination on the nutritional composition of sesame seed. The study also made efforts to supplement wheat flour with germinated sesame flour to develop nutritionally protein-enriched cookies which may help to address malnutrition problem and reduce importation of wheat in Nigeria.

## **2. MATERIALS AND METHODS**

Sesame seeds, wheat flour, sugar, salt, margarine, sodium bicarbonate and vanilla flavour used for this study were purchased from an open market in Akure, Ondo State, Nigeria. The chemicals used were of analytical grade and purchased from Pascal Scientific Laboratory Alagbaka, Akure, Nigeria.

### **2.1 Preparation of Germinated Sesame Seed Flour**

Sesame seeds were germinated as described by [12]. The seeds were sorted to remove stones and other extraneous materials. It was thereafter soaked for 2 hrs to achieve hydration then rinsed, drained and spread thinly on jute sack for germination to take place. The germination process was closely monitored to prevent discontinuity of germination and mould growth which was achieved by constant wetting and intermittent uniform spreading of the germinating seedlings. Germination was carried out for four days. The germinated seedlings were thoroughly rinsed with water, drained, derooted, oven dried (Gallenkamp) at 60°C for 6 h and then milled using a laboratory blender.

### **2.2 Proximate Composition**

The moisture, crude fat and ash contents were determined according to the method described in [13]. Crude protein was estimated using micro-kjeldahl method as described by [14], Crude fibre was determined as described by [15]. Carbohydrate content was calculated by difference.

### **2.3 Antinutrient Composition**

Phytic acid and phytin phosphorus content was determined using the modified method of [16], Oxalate content was determined using the method of [17].

## 2.4 Amino Acid Composition

The amino acid profile in the sample was determined using modified method of [18].

## 2.5 Composite Flour Formulation

Three composite flour blends were formulated: WSF1, WSF2, WSF3 were prepared by mixing varying proportions of wheat flour and germinated sesame flour as shown in Table 1.

**Table 1. Percentage composition for wheat and sesame composite flours**

Composite	Wheat flour	Germinated sesame flour
WF	100	0
WSF1	95	5
WSF2	90	10
WSF3	85	15

*WF: Wheat flour; WSF: Wheat-sesame flour*

## 2.6 Production of Cookies from Germinated Sesame and Wheat Flour

Cookies were produced as described by [19]. The raw materials used include flour (wheat and sesame flour) (100g), sugar (10g), margarine (30g), salt (2g), sodium bicarbonate (1g), water (50mls), milk (10g), vanilla flavour (2mls). These were weighed appropriately and two stage creaming up method was used. All the ingredients except flours were mixed thoroughly in a kenwood mixer (a 3-speed hand mixer), it was then transferred to a bowl. The flours and sodium bicarbonate were added with continuous mixing for 15minutes until smooth dough was obtained. A piece of this dough was cut, placed on a clean platform then rolled out using rolling pin until the desired uniform texture and thickness of 0.44cm was obtained. Cookies cutter was used to cut the sheet of the dough into required shapes and sizes. These were transferred on to a greased (with margarine) baking tray. The baking was done at 200°C and baked for 15 – 20 minutes. After baking, the hot cookies were removed from the pan and placed on a clean tray to cool down. The biscuits were then packed after cooling in polyethylene satchets of appropriate thickness and permeability using an impulse sealing machine prior to further analysis.

## 2.7 Physical Characteristics of Biscuit

The weight, diameter, spread ratio and breakability of the baked cookies were determined as described by [20].

## 2.8 Sensory Evaluation

Cookies made from wheat and sesame seed composite flours were subjected to sensory evaluation using twenty (20) semi-trained panelists drawn within the University community. They were evaluated for taste, aroma, crispiness, colour and overall acceptability. The ratings were on a 9-point hedonic scale ranging from 9 (like extremely) to 1 (dislike extremely). The control was cookies made from 100% wheat flour. The mean scores were analysed using analysis of variance (ANOVA).

## 2.9 Statistical Analysis

The results obtained from the various analyses were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 16.0. Means were separated with Duncan Multiple Range Test (DMRT) at 95% confidence level ( $p \leq 0.05$ ).

## 3. RESULTS AND DISCUSSION

### 3.1 Proximate Composition

Table 2 shows the proximate composition of raw and germinated sesame seed. It was observed that the protein content increased from 26.23% to 32.91% at the end of the 4day germination period. This result corroborates earlier reports of increased protein content during germination of various cereals, legumes and oilseeds [21,22], also similar result was reported for germinated pumpkin seeds by [23]. This increase could be attributed to a net synthesis of enzymic protein (proteases) by germinating seeds. The fat content decreased as germination progressed from about 52.70% in the raw seed to 23.22% by the end of the 96hour germination period. This resulted in over 50% reduction in fat content and negates earlier report of increase in lipid content of fluted pumpkin seeds during germination [22]. The observed decrease in the fat content of the germinated seeds might be due to the increased activities of lipolytic enzymes during germination. These enzymes hydrolyze fats to simpler products which can be used as a source of energy for the developing embryo. The decreased fat content implies an increased shelf life for the germinated sesame flour compared to the ungerminated counterpart. The moisture content increased slightly although quite low, [24] reported that low moisture content enhances keeping quality of flours. Ash, crude fibre and carbohydrate also increased with significant differences. The ash content increased from 5.83% to 6.34% by 72hrs of germination. The increase in ash content may be due to endogenous enzyme hydrolysis of complex organic compounds to release more nutrients leaving the antinutrients to leach into the germination medium [25]. It may also be attributed to addition effect caused by germinated sesame flour since sesame flour contains higher amount of ash and crude fibre [26].

**Table 2. Proximate composition of germinated sesame seed**

Composition (%)	Raw sesame flour	Germinated sesame flour
Moisture	1.91±0.03 <sup>b</sup>	2.92±0.08 <sup>a</sup>
Protein	26.23±1.36 <sup>b</sup>	32.91±0.94 <sup>a</sup>
Fat	52.7±0.12 <sup>a</sup>	23.22±0.80 <sup>b</sup>
Ash	5.83±0.12 <sup>b</sup>	6.12±0.12 <sup>a</sup>
Crude fibre	3.56±0.13 <sup>b</sup>	6.77±0.11 <sup>a</sup>
Carbohydrate	9.77±0.63 <sup>b</sup>	28.06±3.02 <sup>a</sup>

*Values are mean ± SD from triplicate determinations; different superscripts in the same row are significantly different ( $P < 0.05$ )*

### 3.2 Antinutrient Composition

Table 3 reveals the antinutrient composition of sesame seed at the end of the 4days germination period. Phytic acid and oxalate content of sesame seed reduced from 31.59 and 1.05mg/g to 16.20 and 0.51mg/g (49% and 51% reduction respectively). The observed reduction in phytate content during germination of different legume seed is apparently as a

result of large increase in phytase activity. [27] stated that during germination of cereals and legumes, phytate was degraded by intrinsic phytase. Similarly, [28] reported a noticeable reduction (over 75%) in phytic acid of three kidney bean varieties after four days of germination. Phytic acid decreases the availability of some minerals as well as protein, when bound to protein, it induces a decrease of solubility and functionality of the protein. Plant seeds utilize phytate as a source of inorganic phosphate during germination and thus tend to increase palatability, nutritional value and the mineral composition. Reduction of phytates could therefore favor enhanced absorption of the proteins as germination would reduce the immobilization effects of phytic acid and other antinutritional factors.

**Table 3. Composition of antinutritional factors of germinated sesame seed**

<i>Sample description</i>	<i>Antinutrients(mg/g)</i>		
	<b>Phytic acid</b>	<b>Phytin phosphorus</b>	<b>Oxalate</b>
Raw sesame seed	31.59 ± 0.95 <sup>a</sup>	8.89±0.11 <sup>a</sup>	1.05 ± 0.10 <sup>a</sup>
Germinated sesame day 1	20.87 ± 0.47 <sup>b</sup>	5.88±0.00 <sup>b</sup>	0.90 ± 0.1 <sup>b</sup>
Germinated sesame day 2	19.22 ± 0.95 <sup>c</sup>	5.41±0.32 <sup>c</sup>	0.84 ± 0.052 <sup>b</sup>
Germinated sesame day 3	17.58 ± 0.95 <sup>d</sup>	4.95±0.19 <sup>d</sup>	0.69 ± 0.052 <sup>c</sup>
Germinated sesame day 4	16.20 ± 0.41 <sup>e</sup>	4.56±0.08 <sup>e</sup>	0.51 ± 0.04 <sup>d</sup>

*Values are mean ± SD from triplicate determinations; different superscripts in the same column are significantly different (P < 0.05)*

### 3.3 Amino Acid Composition

Table 4 presents the amino acid profile of raw and germinated sesame seed flours. Sesame seed is a rich source of both essential and non-essential amino acids. It is majorly rich in glycine (4.5g/100g), leucine (6.34g/100g), lysine (4.31/100g), methionine (2.91g/100g), arginine (6.07g/100g), aspartic acid (10.66g/100g) and glutamic acid (16.26g/100g). Sesame seed is comparably richer in some essential and non-essential amino acids than melon seed such as lysine, methionine, serine, leucine, glycine and valine [29]. Aspartic acid (10.0 and 10.66g/100g) and glutamic acid (17.04 and 16.26g/100g) were found to be the most abundant of all the amino acids in germinated sesame flour. The results were similar to the report of [30] that aspartic acid and glutamic acid were the most abundant amino acids in legumes and nuts. Comparing the essential amino acids of germinated sesame seed flour with the recommended daily allowances (RDA) it was observed that arginine, histidine, leucine, methionine, lysine, phenylalanine and threonine in sesame flour were higher than the recommended daily allowance (RDA), this supports the earlier report by [31] that stated that germination augments the level of free amino acid. The proportions of arginine, histidine and other essential amino acids that are present in these processed sesame seeds indicated that the seeds may be useful in formulating weaning food. The proportion of essential amino acids in the processed sesame seed in the present study is comparable with the amino acids in oil seeds such as melon, pumpkin and gourd seed reported by [32].

**Table 4. Amino Acid composition of raw and germinated sesame flours**

<b>Amino Acid (g/100g)</b>	<b>Raw sesame flour</b>	<b>Germinated sesame flour</b>	<b>FAO/WHO (1991)</b>
Valine	3.84	4.16	4.2
Lysine	3.38	4.31	4.2
Histidine	2.01	2.49	2.4
Methionine	2.25	2.91	2.2
Threonine	3.11	3.57	2.6
Leucine	5.99	6.34	4.8
Isoleucine	3.66	3.89	4.2
Phenylalanine	4.14	3.89	2.8
Glycine	4.11	4.50	NA
Alanine	2.93	3.28	NA
Serine	3.43	4.57	NA
Proline	3.0	3.66	NA
Arginine	5.79	6.07	2.0
Tyrosine	2.26	2.42	NA
Cystine	1.18	1.46	NA
Aspartic acid	9.35	10.66	NA
Glutamic acid	15.59	16.26	NA
Tryptophan	ND	ND	1.4

*ND: Not Determined; NA: Not Available; RDA Source: [33]*

*RDA: Recommended Daily Allowance*

### 3.4 Physical Characteristics of Cookies

Data on physical characteristics of cookies as affected by incorporation of germinated sesame flour are presented in Table 5. In general, incorporation of sesame flour affected diameter, thickness and weight of corresponding cookies. Cookies diameters were reduced as well as thickness with increase in substitution level. Diameter and thickness of control (100:0 wheat flour) was 4.27cm and 0.40cm respectively while it reduced gradually to 3.80cm and 0.30cm with 85:15 sesame flour incorporation level respectively. Weight of the control biscuit was 7.55g. Weight varied between 6.82g and 5.01g as incorporation increased. Cookies made from 100% wheat flour were heavier and had a larger diameter than those made from the blends. Diameters and weights of cookies were reduced as well as thickness with increasing level of replacement with sesame flour. Similar results were reported by [34] when wheat flour was substituted with Black gram flour and also similar to those reported for millet-sesame flour biscuit by [35]. The decrease in weight could be due to increase in fat content of the blended flour as fat is lighter in weight [20].

**Table 5. Physical characteristics of wheat-sesame cookies**

Characteristics	WFC	WSC1	WSC2	WSC3
Weight (g)	7.55±0.43 <sup>a</sup>	6.82±0.33 <sup>b</sup>	6.73±0.50 <sup>b</sup>	5.01±0.25 <sup>c</sup>
Diameter (cm)	4.27±0.06 <sup>a</sup>	4.33±0.06 <sup>a</sup>	4.03±0.25 <sup>ab</sup>	3.80±0.17 <sup>b</sup>
Height (cm)	0.40±0.00 <sup>a</sup>	0.40±0.00 <sup>a</sup>	0.33±0.06 <sup>b</sup>	0.30±0.00 <sup>b</sup>
Spread ratio	10.67±0.50 <sup>b</sup>	11.07±0.46 <sup>ab</sup>	12.21±0.63 <sup>a</sup>	12.67±0.60 <sup>a</sup>
Breakability (%)	30±0.00 <sup>b</sup>	40±0.00 <sup>a</sup>	46.67±5.77 <sup>a</sup>	46.67±5.77 <sup>a</sup>

Values are mean ± SD from triplicate determinations; different superscripts in the same row are significantly different ( $P < 0.05$ )

WFC: Wheat Flour Cookies; WSC1: 95:5% Wheat-Sesame Cookies; WSC2: 90:10% Wheat-Sesame Cookies; WSC3: 85:15% Wheat-Sesame Cookies

### 3.5 Proximate Composition of Wheat-Sesame Flour Cookies

Table 6 presents the proximate composition of wheat-sesame flour supplemented cookies. The moisture content ranged from 2.68 to 3.74%, this may be due to the consistency of baking temperature and time, since it was the same baking condition that was used for all. The moisture content is considerably low and within the safe limits for baked goods in order to ensure shelf stability and prevent microbial contamination. The protein content (15.18% in wheat flour biscuit is lower compared to 17.27%, 18.13% and 18.80% for wheat-sesame flour blends of 95:5, 90:10 and 85:15 respectively. The protein content increased with increase in the substitution level of sesame seed flour. This could be due to the addition of flours from sesame seed, an oil seed which is noted to be a good source of protein [36]. Sesame protein has a good balance of amino acids with a chemical score of 62%, and a net protein utilization of 54% [35]. Similarly, [37] reported an increase in protein content of biscuits by incorporation of pigeon pea flour with millet flour. [20] reported an increase in protein of acha-soy bean flour biscuit corroborated by the findings of [38] in plantain-sesame flour cookies. The ash content of the cookies increased from 2.21 to 4.21% with increase in the percentage (0 – 15%) substitution with sesame seed flour. The increase in the ash content could make the product a good source of minerals as observed by other researchers [39,40]. The seed is rich in manganese, copper and calcium. Fat content increased with increase in replacement percentage of wheat flour with sesame flour (20.19%) in wheat flour biscuit to 21.73%, 24.37% and 25.02% for wheat-sesame flour blends of 95:5, 90:10 and 85:15 respectively. This agrees with the findings of [35] in the effect of sesame flour on millet biscuit characteristics. The carbohydrate content decreased with increased sesame flour from 59.58% in wheat flour biscuit to 53.26%, 50.80% and 48.26% for wheat-sesame flour blends of 95:5, 90:10 and 85:15 respectively. These findings are in agreement with previous studies conducted by [41] on supplementation of bakery foods with high protein peanut flour and [42] on incorporation of defatted soyabean flour for preparation of biscuits.



**Table 6. Proximate composition of wheat-sesame cookies**

Composition (%)	WFC	WSC1	WSC2	WSC2
Moisture	2.68±0.03 <sup>d</sup>	3.25±0.04 <sup>b</sup>	3.74±0.05 <sup>a</sup>	2.93±0.03 <sup>c</sup>
Protein	15.18±0.11 <sup>d</sup>	17.27±0.07 <sup>c</sup>	18.13±0.11 <sup>b</sup>	18.80±0.33 <sup>a</sup>
Fat	20.19±0.02 <sup>d</sup>	21.73±0.17 <sup>c</sup>	24.37±0.08 <sup>b</sup>	25.02±0.07 <sup>a</sup>
Ash	2.21±0.02 <sup>d</sup>	2.35±0.05 <sup>c</sup>	3.08±0.04 <sup>b</sup>	4.21±0.04 <sup>a</sup>
Crude fibre	0.14±0.03 <sup>c</sup>	0.48±0.02 <sup>b</sup>	0.52±0.02 <sup>b</sup>	0.78±0.10 <sup>a</sup>
Carbohydrate	59.58±0.16 <sup>a</sup>	53.26±2.77 <sup>b</sup>	50.80±1.02 <sup>bc</sup>	48.26±0.36 <sup>c</sup>

Values are mean ± SD from triplicate determinations; different superscripts in the same row are significantly different ( $P < 0.05$ )

WFC: Wheat Flour Cookies; WSC1: 95:5% Wheat-Sesame Cookies; WSC2: 90:10% Wheat-Sesame Cookies; WSC3: 85:15% Wheat-Sesame Cookies

### 3.6 Sensory Attributes of Wheat-Sesame Cookies

Table 7 reveals the sensory attributes of cookies from various blends of wheat flour and germinated sesame flour. The addition of germinated sesame flour decreased the mean score of taste from 7.60 to 4.10 with increase in the percentages (0 – 15%) of substitution with germinated sesame seed flour. This could be due to the bitter taste of some inherent compounds in sesame flour particularly at high temperature. 5% replacement with sesame flour was well accepted and not significantly different from wheat flour cookies in aroma, taste and overall acceptability, 10% sesame flour replacement was also fairly acceptable while 15% sesame flour replacement was significantly different ( $P < 0.05$ ) from others in appearance, aroma and overall acceptability.

**Table 7. Sensory attributes of cookies**

Sensory parameters	WFC	WSC1	WSC2	WSC2
Taste	7.60±1.07 <sup>a</sup>	7.30±1.06 <sup>a</sup>	5.00±1.94 <sup>b</sup>	4.10±1.97 <sup>b</sup>
Aroma	7.50±1.18 <sup>a</sup>	6.70±1.77 <sup>ab</sup>	5.90±1.37 <sup>bc</sup>	4.70±1.49 <sup>c</sup>
Crispiness	7.40±1.26 <sup>a</sup>	7.20±1.23 <sup>ab</sup>	6.20±1.03 <sup>b</sup>	7.40±0.97 <sup>a</sup>
Appearance	7.90±0.57 <sup>a</sup>	6.60±2.22 <sup>ab</sup>	5.20±1.93 <sup>bc</sup>	4.60±1.71 <sup>c</sup>
Overall acceptability	7.80±0.79 <sup>a</sup>	7.20±1.03 <sup>a</sup>	7.20±1.03 <sup>a</sup>	3.40±1.71 <sup>c</sup>

Results in each column are the means of replicate determinations; different superscripts in the same row are significantly different ( $P < 0.05$ )

WFC: Wheat Flour Cookies; WSC1: 95:5% Wheat-Sesame Cookies; WSC2: 90:10% Wheat-Sesame Cookies; WSC3: 85:15% Wheat-Sesame Cookies

## 4. CONCLUSION

Germinated sesame flour supplemented cookies had better nutritional quality as a result of increased protein content and lysine content derived from sesame flour. The economic impact of using wheat-sesame composite flour in the manufacture of cookies is to achieve reduction in the volume of wheat flour importation likewise encourage sesame cultivation and nutritionally it could help to alleviate the problem of Protein Energy Malnutrition since consumption of cookies in Nigeria is high; it could also serve as a vehicle for increasing intake of protein and fat.

## **CONSENT**

Not applicable.

## **ETHICAL APPROVAL**

Not applicable.

## **ACKNOWLEDGEMENTS**

Not applicable.

## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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