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FORTIFICATION OF BREAD WITH THE SCLEROTIA OF *PLEUROTUS TUBER-REGIUM*

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Abstract: *Bread is a staple food in most parts of the world. In Nigeria, it is the second most consumed food after rice. However, grains used in the production of bread, such as wheat, millet, maize, and others, are known to be poor in protein; therefore, bread is also low in protein content. This study aimed to supplement wheat flour with flour made from the sclerotium of Pleurotus tuber-regium to enhance the nutritional value of bread. Wheat flour was supplemented with different concentrations of Pleurotus tuber-regium sclerotium flour (5-25%), while 100% wheat and 100% flour served as positive and negative controls, respectively. Evaluations such as dough rising, product weight, sensory parameters, and proximate composition were carried out. Data were analysed using analysis of variance and the Spearman correlation coefficient. The supplementation negatively affected the rising of the dough from 1.2 (5% supplementation) to 0.4 (25% supplementation) after 25 minutes of incubation, but positively influenced the weight of the products from 348 g (non-supplemented sample) to 495 g (100% sclerotium flour). The bread with the lowest level of supplementation (5%) had the highest acceptability (5.31); however, further increases in supplementation reduced acceptability. The supplementation improved the protein, fat, and ash contents, and decreased the moisture, crude fibre, and carbohydrate contents of the bread. Overall, the supplementation appeared to improve the nutritional composition of the bread while also enhancing its shelf stability, as moisture content decreased with higher supplementation. Additionally, it suggests a viable use of the sclerotium in producing composite flour for bread making.*

Keywords: *bread supplementation, sclerotium, Pleurotus species, wheat, proximate composition*

1. Introduction

Human history cannot be accurately written without referencing the history of bread. Sets of archaeological evidence suggest that bread predates the practice of agriculture. It is estimated that the oldest known bread product dates back approximately 14,400 years [1]. Bread is a staple food made from dough, which includes flour from wheat (*Triticum aestivum* L.) and other ingredients such as sugar, salt, improver, margarine, milk, water, baker's yeast, and additional components depending on the type of bread or consumer preference [2]. In Nigeria, the most popular bread is yeast bread [3]. Although wheat is grown in Nigeria, it is not the high-gluten variety used to make bread – those varieties are not suited to tropical climatic conditions [4.5]. Nigeria produces only 600,000 tonnes out of its annual demand of 3.7 million metric tonnes [6]. *Pleurotus tuber-regium* is a species of oyster mushroom and the only member of its genus that produces a sclerotium [7]. The sclerotium is a compact mass of hardened mycelium that serves as a survival structure for the mushroom [8]. Nutritionally, the sclerotium is a rich source of protein, carbohydrates, dietary fibre, and minerals, making it an important dietary supplement in regions where protein deficiency is common [9]. In particular, the sclerotium contains polysaccharides with medicinal potential. Beyond its nutritional

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benefits, various studies highlight its bioactive properties, including antioxidant, antimicrobial, antidiabetic, and immunomodulatory activities [8,10]. Bread is a low source of protein, which the body requires for growth, renewal, and repair [11]. There is a strong need to explore ways to enhance its nutritional profile, particularly through supplementation using easily accessible local bioresources. Despite recent economic challenges, bread consumption in Nigeria remains significant, with per capita consumption projected at 18.7 kg in 2025 and market volume reaching 5.10 billion kg by 2030. The market is gradually shifting towards healthier and more diverse options, but faces considerable pressures from inflation and reliance on imports [12].

Due to the Russia/Ukraine war, wheat prices increased in Nigeria and other parts of the world that source their wheat from these two countries. The small wheat cultivated in Nigeria is said to have low gluten content, which is essential for bread baking. Therefore, it is important to consider supplementation or composite flours to reduce our overreliance on imported flour for bread production [13]. In this context, several composite flours have been developed for bread making, such as wheat and breadfruit composite flour [14]; composite flour of wheat, tiger nut, and defatted sesame composite flour [15]; wheat and fermented cashew kernel composite flour [16]; wheat with defatted and undefatted cashew kernel [17], and other combinations.

This study was thus designed to explore the sensory characteristics, nutritional content, and overall performance of *Pleurotus tuber-regium* sclerotium in bread making. This could enhance the utilisation of the sclerotium in Nigeria and improve the financial welfare of peasant farmers who are the primary suppliers of the sclerotium to markets across the country. Reducing the amount of wheat flour imported into Nigeria will lessen pressure on the naira, helping to maintain a stronger currency. Additionally, this effort will enable us to harness the rich nutritional and medicinal constituents of *Pleurotus tuber-regium* sclerotium, potentially enhancing the nutritional profile of bread and elevating it to a nutraceutical status.

2. Materials and methods

2.1 Sample collection

Materials for this work (sugar, salt, eggs, improver, butter, milk, yeast, and flour) were obtained from Ajegunle market, Oyo town, Nigeria.

2.2. Sample preparation

The sclerotium of *Pleurotus tuber-regium* was obtained from the Department of Microbiology and Biotechnology, Ajayi Crowther University, Oyo, Oyo State, Nigeria. The black/brown rind of the sclerotium was peeled off after drying. It was then ground into powder and stored aseptically until it was needed. The flour was mixed with the powdered sclerotium in different concentrations as shown in Table 1.

2.3 Baking of bread

Different bread doughs were made from wheat flour and sclerotium flour using the ratios and quantities in Table 1, and other ingredients such as sugar, salt, margarine, water, yeast, and eggs, using a modified method of Akubor and Obiegbuna [18]. Here, the dried ingredients were weighed as described in Table 1. Table 2 shows all the ingredients added to the flours of wheat and sclerotium to bake the bread samples. The same quantities of the ingredients (sugar, salt, butter, preservatives, ingredient improver, milk flavour, yeast, flavour, and egg) were used for all the samples, except for water, where the requirements of each of the samples were found to differ. Table 2 shows all the ingredients added to the flours of wheat and sclerotium to bake the bread samples. The same quantities of the ingredients (sugar, salt, butter, preservatives, ingredient improver, milk

Table 1.

Supplementation of wheat flour with sclerotium flour

S/N	Supplementation %	Quantity of wheat flour (g)	Quantity of sclerotium (g)	Sample codes
1	5	475	25	A
2	10	450	50	B
3	15	425	75	C
4	20	400	100	D
5	25	375	125	E
6	0	500	-	F
7	100	-	500	G

flavour, yeast, flavour, and egg) were used for all the samples, except for water, where the requirements of each of the samples were found to differ. The different water requirements of the dough were due to differing quantities of water needed to adequately mix the different ingredients of each sample to achieve a smooth, consistent, and elastic dough. The higher the sclerotium flour, the more water was required for a consistent texture of the dough. Sample G required between 3 and 4.5 times as much water as some samples did.

Table 2.

Quantities of ingredients used for baking

Samples	Ingredient									
	Sugar (g)	Salt (g)	Butter (g)	Preservatives (g)	Improver (g)	Milk flavour (g)	Yeast (g)	Water (cl)	Flavour (g)	Egg (number)
A	85	8	60	8	8	10	25	21	2	1
B	85	8	60	8	8	10	25	22	2	1
C	85	8	60	8	8	10	25	23	2	1
D	85	8	60	8	8	10	25	25	2	1
E	85	8	60	8	8	10	25	30	2	1
F	85	8	60	8	8	10	25	20	2	1
G	85	8	60	8	8	10	25	95	2	1

Keys: Sample A: 475 g of wheat flour + 25 g of sclerotium flour; Sample B: 450 g of wheat flour + 50 g of sclerotium flour; Sample C: 425 g of wheat flour + 75 g of sclerotium flour; Sample D: 400 g of wheat flour + 100 g of sclerotium flour; Sample E: 375 g of wheat flour + 125 g of sclerotium flour; Sample F: 500 g of wheat flour; and Sample G: 500 g of sclerotium flour

The yeast was creamed in a container with milk and water mixture. Then a hole was created in the centre of the mixture of flours, into which the creamed yeast was added and thoroughly mixed. This mixture was then fermented at 35 °C. The remaining ingredients were added and kneaded manually until a smooth dough free from stickiness was obtained (the quantities of each of the ingredients are shown in Table 2). Then the dough was further proofed at 35 °C for 10 minutes. It was further kneaded for proper mixing of ingredients. Then, the doughs were moulded and transferred into fat-greased pans and allowed to proof for about 2 hours, and then baked at 220 °C for 10 minutes in a baking oven. The flowchart of the processes involved in the making of bread is shown in Figure 1.

2.4 Determination of the height of the dough and the weight of the bread

The heights of each dough and the weights of each bread were measured using a ruler and a weighing balance, respectively, according to the method of Fashogbon et al. [19].

2.5 Sensory evaluation

The organoleptic assessment of the bread samples was conducted by 30 untrained panellists. Questionnaires with sensory scores based on a 7-point hedonic scale were distributed to each panellist, where 1 indicated dislike extremely and 7 indicated like extremely. They were instructed to taste each of the prepared bread samples and rinse their mouths after each tasting. Their perceptions of the samples were scored across the following attributes: taste, appearance, acceptability, aroma, comparability, and flavour [20].

Wheat flour + sclerotium flour + ingredients
↓
Weighing
↓
Mixing
↓
Proofing
↓
Kneading
↓
Moulding
↓
Second proofing
↓
Baking
↓
Cooling
↓
Bread
↓
Packaging

Source: [18]

Fig. 1 Flowchart of the process of bread baking

2.6 Proximate analysis

Proximate composition analysis of the bread samples was carried out to determine moisture, fat, crude fiber, protein, ash, and carbohydrate contents using standard AOAC methods. 3 g of bread was weighed into a pre-dried crucible and oven-dried at 105 °C for 2 h to a constant weight for moisture determination [21]. Fat content was determined by Soxhlet extraction using 200 mL of n-hexane for 10 h; the solvent was evaporated at 105 °C, and the flask was reweighed to obtain fat content. Crude fiber was analysed by sequential digestion of 3 g of defatted sample with 1 M H_2SO_4 and NaOH, followed by drying at 105 °C and ashing at 550 °C [22]. Protein was determined by the Kjeldahl method: 0.396 g of sample was digested with concentrated H_2SO_4 and catalyst at 70 °C for 2 h, distilled, and titrated with 0.1 N HCl; crude protein was calculated using a factor of 6.25 [23]. For ash content, 1 g of starch was ignited at 550 °C for 3 h in a muffle furnace, cooled, and reweighed [21]. Carbohydrate content was obtained by difference (eq. 1).

$$\%\,Carbohydrate = 100 - (\%\,Moisture + \%\,Protein + \%\,Fat + \%\,Ash + \%\,Fiber) \quad \text{(eq. 1)}$$

2.7 Statistical analysis

Statistical analyses were carried out using analysis of variance and the Spearman correlation coefficient at $p < 0.05$.

3. Results

Table 3 shows the height of flour doughs during 25 minutes of fermentation, measured at 5-minute intervals after the final kneading. An increase in the height of the dough is equivalent to dough rising. At the initial stage of fermentation (0 minutes), there was a variation in the height of doughs depending on the supplementation with sclerotium flour, with the highest (5.6 cm) in sample G (sclerotium flour only). An increase in the height of doughs of all samples with an increase in fermentation time was observed, except in sample G (sclerotium flour only), whose height (5.6 cm) remained the same throughout the fermentation period. The dough of sample A, which was supplemented with 5% of sclerotium flour, increased from 2.8 cm (at 0 minutes) to 4.0 cm (at 25 minutes) and had the highest increase in rise of 1.2 cm. The weight of bread after baking is shown in Fig. 2. The lowest weight (348 g) was recorded in the sample with only wheat flour, and the highest (495 g) was in the sample made from sclerotium flour only. There was a gradual increase in the weight of the products with an increase in supplementation with sclerotium flour.

Table 3.

Height of dough (cm)

Samples	**Time (minutes)**					
	0	**5**	**10**	**15**	**20**	**25**
A	2.8±0.03^{f}	3.2±0.06^{e}	3.6±0.04^{f}	3.9±0.04^{f}	4.0±0.01^{e}	4.0±0.03^{e}
B	3.2±0.07^{e}	4.0±0.04^{d}	4.2±0.03^{e}	4.2±0.01^{e}	4.2±0.04^{d}	4.2±0.04^{d}
C	3.8±0.03^{d}	4.0±0.03^{d}	4.3±0.03^{d}	4.3±0.03^{d}	4.5±0.03^{b}	4.5±0.01^{b}
D	4.0±0.01^{bc}	4.1±0.07^{cd}	4.2±0.03^{e}	4.2±0.06^{e}	4.2±0.01^{d}	4.2±0.03^{d}
E	4.1±0.07^{b}	4.3±0.03^{b}	4.5±0.01^{b}	4.5±0.03^{b}	4.5±0.03^{b}	4.5±0.04^{b}
F	3.9±0.04^{cd}	4.2±0.01^{bc}	4.4±0.03^{c}	4.4±0.01^{c}	4.4±0.04^{c}	4.4±0.01^{c}
G	5.6±0.01^{a}	5.6±0.03^{a}	5.6±0.07^{a}	5.6±0.03^{a}	5.6±0.04^{a}	5.6±0.01^{a}

Mean values with different alphabetical superscripts along the column are significantly different at $p < 0.05$
Keys: Sample A: 475 g of wheat flour + 25 g of sclerotium flour; Sample B: 450 g of wheat flour + 50 g of sclerotium flour; Sample C: 425 g of wheat flour + 75 g of sclerotium flour; Sample D: 400 g of wheat flour + 100 g of sclerotium flour; Sample E: 375 g of wheat flour + 125 g of sclerotium flour; Sample F: 500 g of wheat flour; and Sample G: 500 g of sclerotium flour

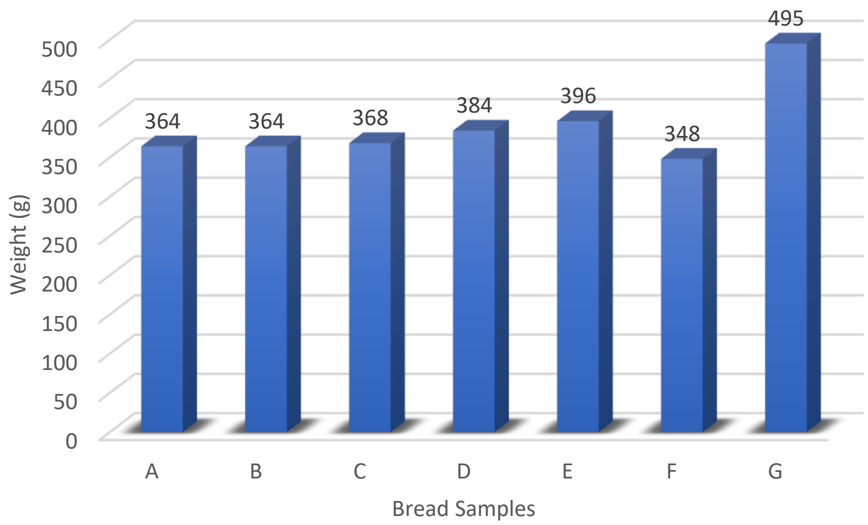

Fig. 2 Weights of products after baking

Table 4a shows the assessment of the panellists of each of the products. For appearance, sample C has the highest rating of 5.32±0.18 and was significantly different ($p < 0.05$) from the lowest (1.37±0.24), which was obtained in sample G. With respect to flavour, sample A had the highest score of 5.10±0.29, closely followed by sample B (4.89±0.27), and the lowest is in sample G (1.31±0.26). Sample A was also rated the best for taste (5.35±0.31), while sample D was rated the lowest at 3.61±0.35. A decrease in the value for mouthfeel was observed with an increase in supplementation with sclerotium flour, with the highest value (5.69±0.25) in sample A and the lowest (1.34±0.19) in sample G. With regards to aroma, bread made without supplementation (sample F) ranked the best (4.63±0.26), and values of aroma decreased with an increase in supplementation with sclerotium flours, with the lowest (1.65±0.30) in bread made from sclerotium flour only (sample G). The values of aroma in samples A, B, C, D, and F were not significantly different ($p > 0.05$). The sample without any supplementation (Sample F) was adjudged to be the most comparable (5.10±0.31) to normal bread; however, the values obtained for samples A (4.95±0.33), B (4.69±0.30), and C (4.45±0.24) were not significantly different

($p > 0.05$) from the value recorded in sample F.
Overall, bread made from wheat flour supplemented with 5% of sclerotium (sample A) was ranked highest in three parameters (flavour, taste and mouthfeel), and the values recorded in appearance, aroma and comparability of bread without supplementation (sample F) were not significantly different ($p > 0.05$) from what was obtained by sample A. Sample A has the highest degree of likeliness (5.31) and falls within the range of acceptability (like moderately), as shown in Table 4b.

Table 4a.
Sensory parameters of bread made from wheat and sclerotium flours

Sensory Parameters	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Friedman's Statistic
Appearance	4.10± 0.32^{b}	5.23± 0.20^{a}	5.32± 0.18^{a}	4.69± 0.23^{ab}	3.18± 0.33^{b}	4.11± 0.29^{b}	1.37± 0.24^{c}	83.37**
Flavor	5.10± 0.29^{a}	4.89± 0.27^{ab}	4.77± 0.21^{ab}	3.9± 0.31^{bc}	3.61± 0.24^{c}	4.42± 0.28^{abc}	1.31± 0.26^{d}	75.20**
Taste	5.35± 0.31^{a}	4.79± 0.26^{a}	4.45± 0.17^{ab}	3.61± 0.35^{b}	3.77± 0.23^{b}	4.66± 0.26^{b}	1.35± 0.24^{c}	75.36**
Mouth Feel	5.69± 0.25^{a}	4.97± 0.26^{a}	4.02± 0.22^{b}	3.71± 0.30^{b}	3.48± 0.23^{b}	4.79± 0.27^{a}	1.34± 0.19^{c}	86.91**
Aroma	4.44± 0.33^{a}	3.98± 0.27^{a}	3.82± 0.21^{a}	3.53± 0.27^{a}	3.58± 0.34^{a}	4.63± 0.26^{a}	1.65± 0.30^{b}	52.23**
Comparability	4.95± 0.33^{a}	4.69± 0.30^{a}	4.45± 0.24^{ab}	3.79± 0.37^{b}	3.52± 0.27^{b}	5.10± 0.31^{a}	1.50± 0.18^{c}	69.22**
Rating	5.31	5.11	4.61	3.95	3.08	4.71	1.23	
Degree of Likeliness	1st	2nd	4th	5th	6th	3rd	7th	

Mean values with different alphabetical superscripts across the row are significantly different at $p < 0.05$
Keys: Sample A: 475 g of wheat flour + 25 g of sclerotium flour; Sample B: 450 g of wheat flour + 50 g of sclerotium flour; Sample C: 425 g of wheat flour + 75 g of sclerotium flour; Sample D: 400 g of wheat flour + 100 g of sclerotium flour; Sample E: 375 g of wheat flour + 125 g of sclerotium flour; Sample F: 500 g of wheat flour; and Sample G 500 g of sclerotium flour

Table 4b.
Overall product assessment by taste panellists

Range of Scale	Rating
1.000 – 1.857	Dislike Extremely
1.857 – 2.714	Moderate Dislike
2.714 – 3.571	Slight Dislike
3.571 – 4.428	Undecided
4.428 – 5.285	Like Slightly
5.285 – 6.142	Like Moderately
6.142 – 7.000	Like Extremely

Table 5 shows the correlations existing among the attributes assessed. There were high positive correlations between appearance and comparability (0.923), appearance and flavour (0.884), appearance and taste (0.799), and appearance and mouthfeel (0.743). The proximate composition of bread made from different sclerotium flour supplementations is shown in Table 6. The highest moisture content (31.37%) was recorded in bread made from sclerotium flour only (sample G), and the lowest (29.43%) was obtained in bread supplemented with 25% sclerotium (sample E). The highest protein content (9.10%) was recorded in sample B, which was supplemented with 10% sclerotium. The fat content increased with an increase in supplementation with sclerotium, with sample G having the highest (7.40%), followed by sample E (7.13%). The evaluation of the ash content showed that the bread made from 25% sclerotium supplementation (sample E) had the highest (3.07%), and sample A with 5% supplementation had the lowest (2.47%). The crude fibre ranged from 0.57% in sample G to 0.93% in sample B. The highest carbohydrate content (52.53%) was observed in sample A, and the lowest (50.00%) in sample G. The carbohydrate, crude fibre, and protein contents in sample A (with 5% supplementation) were not significantly different ($p > 0.05$) from those obtained in sample F (without supplementation).

Table 5.

Spearman correlation coefficients of attributes of bread product

	Appearance	Flavor	Taste	Mouth-Feel	Aroma
Flavor	0.884**				
Taste	0.799**	0.797**			
Mouth-Feel	0.743**	0.782**	0.843**		
Aroma	0.636**	0.679**	0.644**	0.715**	
Comparability	0.923**	0.811**	0.835**	0.775**	0.698**

Table 6.

Proximate composition of supplemented bread products

Parameters	Samples						
	A	B	C	D	E	F	G
Moisture content %	31.27± 0.15^{a}	29.77± 0.15^{d}	30.53± 0.15^{c}	30.97± 0.15^{b}	29.43± 0.15^{e}	30.67± 0.15^{c}	31.37± 0.15^{a}
Protein %	8.37± 0.15^{cd}	9.10± 0.10^{a}	8.70± 0.10^{b}	8.53± 0.15^{bc}	8.93± 0.15^{a}	8.53± 0.15^{bc}	8.20± 0.10^{d}
Ether extract (Fat) %	4.77± 0.15^{f}	5.03± 0.15^{e}	6.17± 0.15^{d}	6.60± 0.10^{c}	7.13± 0.15^{b}	5.27± 0.15^{e}	7.40± 0.10^{a}
Ash %	2.47± 0.06^{d}	2.93± 0.15^{ab}	2.70± 0.10^{bcd}	2.57± 0.15^{cd}	3.07± 0.15^{a}	2.80± 0.10^{bc}	2.47± 0.15^{d}
Crude fibre %	0.60± 0.10^{b}	0.93± 0.06^{a}	0.70± 0.10^{b}	0.60± 0.10^{b}	0.70± 0.10^{b}	0.63± 0.06^{b}	0.57± 0.06^{b}
Carbohydrates (by difference) %	52.53± 0.25^{a}	52.23± 0.21^{a}	51.20± 0.20^{b}	50.73± 0.35^{c}	50.73± 0.21^{c}	52.10± 0.10^{a}	50.00± 0.26^{d}

Mean values with different alphabetical superscripts across the row are significantly different at $p < 0.05$
Keys: Sample A: 475 g of wheat flour + 25 g of sclerotium flour; Sample B: 450 g of wheat flour + 50 g of sclerotium flour; Sample C: 425 g of wheat flour + 75 g of sclerotium flour; Sample D: 400 g of wheat flour + 100 g of sclerotium flour; Sample E: 375 g of wheat flour + 125 g of sclerotium flour; Sample F: 500 g of wheat flour; and Sample G: 500 g of sclerotium flour

4. Discussion

The high-water requirement of sample G (sclerotium only) may be due to the abundance of non-starch polysaccharides in it, especially β-glucans and chitin. These components form a highly porous and highly branched structure that permits extensive water absorption [24]. Additionally, other studies have reported that the sclerotial powder can swell to over three times its original volume when hydrated, which is attributed to the hydrophilic nature of the polysaccharides and their capacity to form gels upon hydration [10]. The inability of sample G, the dough prepared with only sclerotium to rise, may result from its high dietary fibre (DF) content, particularly β-glucans and chitin, which make up over 80% of its dry weight. These compounds are highly branched, rigid polysaccharides which are resistant to the enzymatic actions of amylases or glucanases produced by *Saccharomyces cerevisiae*. *β*-glucans and chitin need specialised enzymes like *β*-1,3-1,6-glucanases or chitinases to hydrolyse these complex fibres into fermentable sugars like glucose [25,26]. It could also be due to a deficiency or low availability of essential nutrients such as amino acids, ammonium salts, vitamins, and minerals by *Saccharomyces cerevisiae* [27]. This may further be influenced by osmotic stress and water activity, which are linked to the substantial dietary fibre content of the sclerotium, potentially resulting in low water activity when incorporated into a dough-like matrix. Since *S. cerevisiae* requires a water activity level as high as 0.9 for optimal performance, reduced water activity induces osmotic stress, thereby hindering fermentation [27]. Another factor could be the structural barriers to enzyme access to sugars, which could be the hyper-branched *β*-glucans in the sclerotium, forming a gel-like matrix when hydrated; this matrix might physically trap sugars and prevent their accessibility to yeast enzymes. Fluctuation and deviation in pH may also be responsible for this occurrence, as the acidic pH under which *S. cerevisiae* functions well may not be guaranteed due to the innate pH or buffering capacity of the sclerotium, thereby negatively affecting the growth of the yeast [26]. Furthermore, since the sclerotium was not sterilised before use, chances exist that some bacterial and fungal contaminants may interfere and compete with the yeast, thereby inhibiting its growth and fermentation activities. Natural antimicrobials in the sclerotium may also be responsible for the non-rising of sample G dough, since one of the functions of the sclerotium is to resist microbial attack against the mushroom [25,26].

Moreover, it has been established that even amylase from the sclerotium of *P. tuber-regium* requires a unique pH of 5 at 70 °C to break down sclerotial starches, and *S. cerevisiae* lacks such adaptations [28]. The difference in the weight of the products with increased supplementation may not be unconnected with the increased water requirement of the samples with higher supplementation.

The decreased acceptance of the products with increased supplementation may be due to a less pleasant mouthfeel, which can be attributed to the poor fermentation performance of the yeast due to some of the reasons previously explained. It may also be due to the high presence of antinutrients such as terpenoids (34.77 mg/%) and cyanogenic glycosides (10.8 mg/%), which impart a bitter or astringent taste when consumed. The baking process may not be adequate to fully degrade them, thereby causing an unpalatable flavour. Also, the high presence of oxalates (7795.3 mg/%) in the sclerotium, which is capable of causing a harsh or bitter aftertaste, may have contributed to lower acceptance with higher supplementation [10].

The mushroom has been established to produce nematocidal and bacteriocidal substances, which may persist after the process of baking or cooking; these substances may have negative impacts on the taste and flavour of the products [26,29]. It has been established in previous works that the substrates used for cultivation can determine the chemical composition and antinutritional constituents of the sclerotium. Wild strains have been observed to have higher bitterness than cultivated ones. So, the antinutritional constituent and general chemical composition due to the cultivation process or materials may have contributed to the observed phenomenon [30].

The highest rating of sample F in aroma and comparability may be linked to its being closest to the experience the panellists have of bread. This is understandable since it is the only sample made entirely from wheat flour. The inability of samples D, E, and G to make the favourably accepted list of samples may be connected to the high level of the sclerotium they contain, which has been highlighted to contain compounds that can negatively affect the product's acceptance. This low acceptance with increased supplementation is in agreement with the works of Okpala and Akpu [31] and Obasi et al. [32], who reported low acceptance with increased supplementation with orange peel flour and soybean, respectively. The reduction in moisture content with higher supplementation, especially when the positive control is compared to the highest supplemented product [17,32], is an indication of the shelf stability of the products, as they will support microbial growth less. The supplementation also caused a significant increase in the protein contents of the samples, particularly those that were favourably accepted by the panellists. The supplementation also improved the fat content of the samples as the addition of the sclerotium increased. This trend agrees with the work of Afolabi et al. [33]. This may not be unconnected to the high fat content reported of the sclerotium in previous works [33]. The ash contents of the samples also followed the same pattern as the fat contents. The fibre contents of the samples decreased and slightly increased in one or two cases, contrary to the huge gap in the fibre content of the sclerotium compared to that of wheat. While wheat has about 14.70 g/100g of dietary fibre, which is predominantly made of Arabinoxylan (70%), cellulose, and insoluble fibre, the sclerotium contains approximately 80 g/100 g of dietary fibre made up of chitin, β-glucans, and hyperbranched polysaccharides [10,26,30].

5. Conclusion

Wheat flour supplemented with the sclerotium of *Pleurotus tuber-regium* in breadmaking gave better nutritional components and was more acceptable to consumers when compared with the non-supplemented one. Sclerotium cannot be used as a sole substrate for bread production, but it can be used to formulate a composite flour for high weight, which is preferred in the market by Nigerian consumers. The supplementation significantly improved the protein, fat, and ash contents of the samples, thereby making them more nutritious. It also reduced the moisture content of the products, pointing to a longer shelf life. Only wheat flour was supplemented with sclerotium flour in this study; further work can be carried out on supplementing other types of flour with sclerotium flour, and the shelf stability of the product can be evaluated.

Conflict of interest

The authors declare that they do not have conflict of interest.

6. References

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