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**ORIGINAL RESEARCH PAPER**

# AMINO ACID, PHYTOCHEMICALS, AND ANTIOXIDANT ACTIVITIES OF GLUTEN-FREE COOKIES FROM ORANGE-FLESHED SWEET POTATO AND *PLEUROTUS TUBER-REGIUM* SCLEROTIUM

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**Abstract**

This study investigated the amino acid composition, phytochemicals, and antioxidant activities of cookies prepared from blends of orange-fleshed sweet potato and *Pleurotus tuber-regium* sclerotium. Most of the amino acids of the cookies showed higher values than their corresponding flours. The highest contents of tannins, oxalate, and hydrogen cyanide were found in orange-fleshed sweet potato (OFSP) flour while sclerotium inclusion resulted in increased saponins and alkaloids. Antinutrients of the developed cookies were substantially lower than their corresponding flours. The OFSP flour and cookies showed the highest β-carotene, flavonoids, and phenolic contents. Developed cookies showed higher ($p<0.05$) phytochemicals and antioxidant activities than wheat cookies. However, phenolics and flavonoids increased in cookies because of baking while β-carotene and antioxidant activities decreased. This study showed the potentials of OFSP and *P. tuber-regium* sclerotium composite flour as an alternative food ingredient for protein-enriched functional cookies with potential health benefits.

**Keywords**: antioxidants, cookies, food security, orange-fleshed sweet potato, *pleurotus tuber-regium sclerotium*

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## Introduction

The emergence of various diseases over the years has encouraged the development of nutritious, safe, and wholesome foods with potential health benefits that could boost the immune system of consumers. To meet this demand, in the light of food and nutrition security and national development, there is a need to harness the potentials of locally available and underutilised crops with high nutritional values in the development of popularly consumed foods such as cookies. Further to this is the increased demand for convenience foods with improved health and nutritional benefits by consumers (Oladunjoye *et al.*, 2021).

Cookies are baked products commonly consumed as snack foods that are accepted worldwide among all age groups (Cheng and Bhat, 2016). They are convenient food items made from a combination of ingredients including baking agent, fat, egg, sugar, and wheat flour (Oyeyinka *et al.*, 2018), with wheat being the major ingredient (Kolawole *et al.*, 2020). The use of alternative flours either singly or in combination with wheat flour has been encouraged in recent times for several reasons including the need to further utilise traditional and indigenous crops such as sweet potato due to their high nutritional value, high cost of wheat importation (Adebayo and Ojo, 2012) and increased prevalence of celiac disease (Oyeyinka *et al.*, 2018; Sulieman *et al.*, 2019). For example, earlier reports documented the use of non-gluten flours for cookie production including cereals (Rao *et al.*, 2018), tubers (Kolawole *et al.*, 2020; Olatunde *et al.*, 2016; Oyeyinka *et al.*, 2018), and legumes (Oluwamukomi *et al.*, 2011). Tuber crops such as sweet potato, especially the OFSP is important because they can be potentially used to address vitamin A deficiency (Kurabachew, 2015) due to their high levels of beta-carotene (5091-16,456 μg/100 g), a precursor of vitamin A (Laurie *et al.*, 2012). Furthermore, the beta-carotene content of OFSP has health-promoting functions due to its high polyphenolics and antioxidant contents (Koala *et al.*, 2013; Kolawole *et al.*, 2018b). For this reason and other reasons stated above, the use of OFSP has been encouraged in cookie production (Bello *et al.*, 2020; de Sousa *et al.*, 2020; Kolawole *et al.*, 2020; Laelago *et al.*, 2015).

Although OFSP has shown potentials for use in cookie production, the protein content (1.51-5.83%) (Alam *et al.*, 2016; Senanayake *et al.*, 2013) is very low compared to wheat flour (13.6-14.2%) (Oyeyinka *et al.*, 2014; Oyeyinka *et al.*, 2020). In addition, the amino acid profile of OFSP is not well balanced, being deficient in sulphur-containing amino acids such as lysine (Ravindran *et al.*, 1995). Hence, there is a need to enrich OFSP flours with protein-rich crops to cater for the deficient amino acids as well as improve the overall nutritional profile of resulting products such as cookies.

Recently, Kolawole *et al.* (2020) reported that the sclerotium of *Pleurotus tubberegium* can be used to improve the nutritional value of OFSP-based cookies with acceptable sensory properties and as a food-based approach in addressing malnutrition in developing nations.

These authors found a substantial increase (approx. 95%) in the protein content of OFSP cookies enriched with 30% sclerotium and an appreciable amount of minerals and water-soluble vitamins than the wheat cookies (Kolawole *et al.*, 2020).

The sclerotium of *Pleurotus tubberegium* contains high protein which may vary between approximately 13 and 17% (Akindahunsi and Oyetayo, 2006; Fasidi and Ekuere, 1993) and bioactive polysaccharides with the potentials to treat diabetic complications (Huang *et al.*, 2014). The phytochemicals in the sclerotium also have anti-inflammatory, anti-lipidemic, antihypertensive, and anti-hyperglycemic activities (Ngai and Ng, 2006). The use of *Pleurotus tuber-regium* (Fr) sclerotia and OFSP in the production of cookies may be a way to further improve the use of these crops beyond their current level of usage. Hence, this study investigated the amino acid composition, antinutrients, phytochemicals, and antioxidant activities of cookies developed from OFSP and sclerotium of *Pleurotus tuber-regium.*

## Materials and methods

### *Materials*

The sclerotium of *P. tuber-regium* was sourced from the Aleshinloye market in Ibadan, Nigeria, while the OFSP sample was harvested from our experimental farm located at Agricultural Research Management Training Institute (ARMTI), Ilorin, Nigeria. Wheat flour and other ingredients which included fat, sugar, eggs, cooking oil, salt and baking powder were purchased from a shopping mall (Giant) in Serdang, Selangor, Malaysia. The chemicals, reagents, and solvents used were purchased from Merck Sdn. Bhd, Malaysia and were of analytical grade. The amino acid standards AAS-18 were purchased from IT Technology Research Sdn. Bhd.

### *OFSP and Sclerotium Flours' Preparation and Formulation*

The OFSP and sclerotium flours were prepared as previously reported by Kolawole *et al.* (2020). Flours were sieved using a sieve of an aperture size of 150 µm. Sclerotium flour was added to OFSP flour at 0, 10, 20, and 30% levels, while 100% wheat flour served as the control. The flours were vacuum sealed separately in opaque low-density polyethylene Ziploc bags and kept inside a tight-fitting plastic container which was stored until further usage.

### *Production of cookies*

Cookies were prepared using the different blends as shown above and as previously reported (Kolawole *et al.*, 2020). Briefly, flour (100 g), margarine (40 g), whole egg (20 g), sugar (40 g), and baking powder (1 g) were manually mixed to form a batter. The formed batter was thinly rolled on a wooden board with a rolling pin to a uniform thickness (2 mm) and was cut out using a dough cutter in different shapes of similar sizes. The cut-out dough pieces were baked in a hot oven at 160ºC for 15 min. The cookies were immediately cooled and used for different analyses.

### *Amino acid composition*

Amino acid composition of the cookies was determined according to the Pico-Tag method described by Bidlingmeyer *et al.* (1984). The samples were hydrolysed using 15 mL of 6 M HCl at 110ºC for 24 h in vacuum-sealed tubes flushed with nitrogen gas for 1 min to remove air. The hydrolysate was used to determine the amino acid profile using reverse-phase HPLC. A gradient system of buffer was used consisting of buffer A (100-0% after 60 min) and buffer B (0-100% after 60 min) with an operating temperature of 43ºC. The column used for analysis was a 5 µm Purospher Star RP-18 end-capped column from Merck (250 mm x 4.6 mm). Data were analysed using Borwin PDA software (version 1.5, JASCO CO. Ltd., Japan).

***Antinutritional Composition***

The spectrophotometric method of Brunner (1984) was used for saponin analysis. Tannin content was determined using the method of Makkar *et al.* (1993). Alkaloid and oxalate content was determined following the procedure described by Adeniyi *et al.* (2009), while the determination of hydrogen cyanide was done using the method of Essers *et al.* (1993).

***Antioxidant activity***

The β-carotene of the composite cookies was estimated following the procedure described by Hamadou *et al.* (2020). Total flavonoids content (TFC) was determined based on aluminium chloride colorimetric assay (Abdualrahman *et al.*, 2016), while the total phenolic content (TPC) and the DPPH (1, 1- diphenyl-2-picrylhydrazyl) free radical scavenging activity of the samples were determined using the Folin-Ciocalteu method (Klompong and Benjakul, 2015). A calibration curve was prepared from different concentrations of Gallic acid (Sigma Aldrich) for TPC and Catechin (Sigma Aldrich) for TFC and the results were expressed as mg gallic acid equivalents (GAE)/g sample and mg catechin equivalents (CE)/g sample, respectively.

***Statistical Analysis***

Samples were prepared in duplicate, and analyses were performed in triplicate. One-way Analysis of Variance (ANOVA) of Minitab V. 15 statistical package (Minitab Inc. Pennsylvania, USA) was employed in analyzing the experimental data which was carried out in triplicate. Means were separated using Fisher's least significant difference test at an acceptable significance level probability.

## Results and discussion

***Amino acid composition of cookies***

The amino acid composition, total essential amino acids, and total non-essential amino acids of the cookies generally increased with an increase in the level of sclerotium supplementation (Table 1). This could be due to the high amount of protein including amino acid in the sclerotium. Previous studies showed that *Pleurotus tuber-regium* sclerotium contains a significant quantity of protein (5.38-18.97%) (Alobo, 2003; Kolawole *et al.*, 2018a; Ude *et al.*, 2001; Wong *et al.*, 2003) and amino acid (Ikewuchi and Ikewuchi, 2011). The sclerotium flour used in this study also had significantly ($p \leq 0.05$) higher amino acid contents than the OFSP flour (data not shown). Aspartic acid (13.60-29.84 g/100 g protein) and glutamic acid (7.54-43.54 g/100 g protein) were the major amino acids in the OFSP cookies as well as the control wheat cookies (Table 1). The cookies produced from 100% OFSP and those from OFSP enriched cookies had significantly higher aspartic acid content than the control wheat cookie, but the wheat cookie showed substantially higher (approx. 2-6 times) quantities of glutamic acid than the OFSP cookies. These acidic amino acids play significant roles in energy metabolism, nutrition, and oxidative stress. For instance, Chen *et al.* (2021) found that low-protein diets supplemented with glutamic and aspartic acids protected against oxidative stress-induced intestinal dysfunction in piglets. Furthermore, both amino acids are required to synthesize proteins and other biologically active molecules, including arginine, glutamine, glutathione, purines, and pyrimidines (Blachier *et al*., 2009; Chen *et al*., 2021; Lane and Fan, 2015; Wu *et al*., 2013).

**Table 1.** Amino acid composition of OFSP-*Pleurotus tuber-regium* sclerotium cookies (g/100 g protein)*.

| Amino Acids | A | B | C | D | E |
|---|---|---|---|---|---|
| Isoleucine | 3.91±0.29$^{c}$ | 6.43±0.12$^{b}$ | 7.43±0.50$^{b}$ | 9.84±0.85$^{a}$ | 6.36±0.77$^{b}$ |
| Leucine | 3.83±0.65$^{c}$ | 12.68±1.39$^{b}$ | 15.04±0.64$^{b}$ | 20.03±1.31$^{a}$ | 18.85±1.11$^{a}$ |
| Methionine | 1.78±0.19$^{a}$ | 1.50±0.20$^{a}$ | 1.78±0.21$^{a}$ | 2.01±0.39$^{a}$ | 1.92±0.25$^{a}$ |
| Phenylalanine | 5.25±0.41$^{c}$ | 7.06±0.92$^{b}$ | 9.01±0.36$^{a}$ | 10.44±0.80$^{a}$ | 5.90±0.63$^{bc}$ |
| Threonine | 5.92±0.33$^{c}$ | 6.10±0.13$^{c}$ | 8.81±0.27$^{b}$ | 11.49±0.38$^{a}$ | 5.46±0.25$^{c}$ |
| Tryptophan | BDL | BDL | BDL | BDL | BDL |
| Valine | 3.81±0.30$^{d}$ | 7.85±0.73$^{b}$ | 10.11±1.07$^{a}$ | 11.89±0.30$^{a}$ | 5.69±0.63$^{c}$ |
| Histidine | 3.94±0.20$^{c}$ | 4.58±0.60$^{c}$ | 6.27±0.43$^{b}$ | 8.28±0.36$^{a}$ | 4.87±0.08$^{c}$ |
| Lysine | 2.86±0.53$^{b}$ | 3.54±0.39$^{ab}$ | 3.95±0.18$^{a}$ | 4.15±0.13$^{a}$ | 3.29±0.28$^{ab}$ |
| TEAA | 31.3 (36.46%) | 49.74 (41.74%) | 62.4 (43.48%) | 78.13 (43.65%) | 52.34 (34.47%) |
| Aspartic | 22.07±1.25$^{b}$ | 22.82±1.62$^{b}$ | 25.71±1.11$^{b}$ | 29.84±2.15$^{a}$ | 13.60±1.32$^{c}$ |
| Glutamic | 7.54±0.51$^{e}$ | 12.56±0.96$^{d}$ | 17.15±0.39$^{c}$ | 26.18±0.74$^{b}$ | 43.54±0.87$^{a}$ |
| Alanine | 4.25±0.24$^{c}$ | 6.11±0.74$^{bc}$ | 7.50±1.38$^{b}$ | 10.98±0.46$^{a}$ | 6.99±0.29$^{b}$ |
| Arginine | 5.90±0.48$^{c}$ | 7.45±0.58$^{b}$ | 7.86±0.10$^{ab}$ | 8.71±0.40$^{a}$ | 6.80±0.36$^{bc}$ |
| Cystine | 3.67±0.48$^{c}$ | 5.49±0.35$^{b}$ | 6.54±0.50$^{ab}$ | 7.49±0.35$^{a}$ | 5.87±0.27$^{b}$ |
| Tyrosine | 2.06±0.15$^{b}$ | 2.72±0.41$^{ab}$ | 3.09±0.18$^{a}$ | 3.15±0.27$^{a}$ | 2.61±0.33$^{ab}$ |
| Glycine | 2.71±0.40$^{a}$ | 2.73±0.47$^{a}$ | 2.97±0.37$^{a}$ | 3.53±0.79$^{a}$ | 3.41±0.28$^{a}$ |
| Proline | 2.78±0.47$^{b}$ | 2.92±0.43$^{b}$ | 3.25±0.40$^{b}$ | 3.50±0.35$^{b}$ | 7.51±0.29$^{a}$ |
| Serine | 3.57±0.34$^{c}$ | 6.62±0.55$^{b}$ | 7.04±0.33$^{b}$ | 7.49±0.32$^{b}$ | 9.18±0.37$^{a}$ |
| TNEAA | 54.55 | 69.42 | 81.11 | 100.87 | 99.51 |

Each value is a mean of triplicate determinations ± standard deviation; Means with different superscripts along the same row are significantly ($p$<0.05) different. BDL - Below Detectable Limit, TEAA - Total Essential Amino Acids, TNEAA - Total Non-Essential Amino Acids. A: 100% OFSP, B: 90% OFSP+ 10% Sclerotium, C: 80% OFSP+ 20% Sclerotium, D: 70% OFSP+ 30% Sclerotium, E: 100% Wheat, OFSP: Orange fleshed sweet potato, *Results reported on dry matter basis

The OFSP cookies enriched with 30% sclerotium flour and 100% wheat cookies had the highest and lowest values for isoleucine, leucine, methionine, cystine, tyrosine, phenylalanine, threonine, valine, lysine, aspartic acid, alanine, arginine, glycine, and histidine, respectively. These indicate that supplementing OFSP flour with sclerotium flour greatly improved the protein nutritional quality of the cookies.

***Anti-nutrient contents of OFSP-sclerotium composite flours and cookies***

The antinutrient composition of flour and cookies are shown in Table 2. Wheat flour and cookies had no saponins, tannin, alkaloids, hydrogen cyanide, and oxalate, but the 100% OFSP and the enriched flour and cookies showed varying levels of these antinutrients (Table 2). Sclerotium flour had the highest saponin (0.12 mg/g) and alkaloid (2.89 mg/g) contents while OFSP flour recorded the highest tannin (5.72 mg/g), hydrogen cyanide (9.22 mg/g) and oxalate (1.33 mg/g) contents. However, a decreasing trend was observed in the values obtained for tannin, hydrogen cyanide, and oxalate contents on supplementation of OFSP with sclerotium flour. An important observation is a substantial reduction in all anti-nutrients of the flours upon baking into cookies. Lower ranges of value of 0.01-0.02 mg/g, 1.23-1.51 mg/g, 0.29-0.49 mg/g, 1.12-2.72 mg/g, and 0.10–0.15 mg/g were obtained for the saponins, alkaloids, tannins, hydrogen cyanide and oxalate contents of the cookies, respectively. Iorgyer *et al.* (2009) reported that antinutrients such as hydrogen cyanide are heat-labile and hence, can volatilize or be destroyed during heat processing. This may explain the substantial reduction in antinutritional contents of the OFSP-sclerotium cookies compared to their corresponding flours. Importantly, the values recorded for saponins, alkaloids, and hydrogen cyanide contents of all the flours and cookies were below the safe limit of 0.485 mg/g, 0.61 mg/g and 50-200 mg/g, respectively, given as the maximum permissible limit for human consumption (FAO/WHO/UNU, 1991; WHO, 2003).

The low levels of oxalates obtained in this study are desirable since high levels have been implicated in the inefficient utilization of divalent minerals and subsequent rickets development (Aletor, 1995). The anti-nutritional function of saponins, tannins, alkaloids, and oxalate at high concentrations lie in their abilities to bind and/or precipitate certain macronutrients such as proteins and micronutrients such as calcium, iron, zinc, etc. while at low concentrations, they possess enormous health benefits (Akeem *et al.*, 2016). For example, it has been established that tannins have the potentials for therapeutic applications due to their anti-inflammatory (Ambreen and Mirza, 2020) and anti-ulcer activities (Demarque *et al.*, 2018). Valuable pharmaceutical and biological properties of mushrooms have also been suggestively attributed to the presence of bioactive compounds including alkaloids (Khan *et al.*, 2018).

***Total flavonoids and total phenol content of flours and cookies***

The total flavonoids (TFC) and total phenolic content (TPC) of the flours and cookies are presented in Table 3. The OFSP flour had significantly ($p \leq 0.05$) higher TFC and TPC than sclerotium flour, the blends, and the wheat flour control, which could be due to its higher tannin content (Table 2). Tannins are polyphenols found in many plants and have a history of antimicrobial properties (Ajiboye and Oyejobi, 2017; Scalbert, 1991; Udeh *et al.*, 2020). A similar trend of significantly ($p \leq 0.05$) higher TFC and TPC were observed in the OFSP cookies compared to cookies produced from OFSP-sclerotium flour blends and the wheat cookies (Table 3). However, there was an increase in the TFC and TPC of all the cookies compared with the flour from which they were prepared. Kolawole *et al.* (2018b) also found an increase in TFC and TPC of Irish, white- and orange-fleshed sweet potatoes and associated the increase to the possible breakdown and release of some free flavonoids and antioxidants following degradation of cellular constituents during heat treatment. The current study further confirms the high levels of TPC in OFSP products (Kourouma *et al.*, 2019).

**Table 2.** Anti-nutritional content of OFSP and *Pleurotus tuber-regium* sclerotium composite flours and cookies (mg/g)*.

| OFSP | PTS | Saponins | Tannins | Alkaloids | HCN | Oxalate |
|---|---|---|---|---|---|---|
| | | **Flours** | | | | |
| 0 | 100 | 0.12±0.01$^{a}$ | 1.33±0.02$^{d}$ | 2.89±0.19$^{a}$ | 5.90±0.48$^{d}$ | 1.04±0.35$^{c}$ |
| 100 | 0 | 0.05±0.01$^{c}$ | 5.72±0.03$^{a}$ | 1.11±0.34$^{c}$ | 9.22±0.44$^{a}$ | 1.33±0.04$^{a}$ |
| 90 | 10 | 0.07±0.01$^{b}$ | 5.09±0.05$^{b}$ | 1.45±0.10$^{b}$ | 9.01±1.64$^{a}$ | 1.29±0.09$^{a}$ |
| 80 | 20 | 0.08±0.01$^{ab}$ | 4.72±0.11$^{b}$ | 1.58±0.07$^{b}$ | 8.16±0.58$^{b}$ | 1.17±0.12$^{b}$ |
| 70 | 30 | 0.10±0.02$^{a}$ | 4.26±0.23$^{c}$ | 1.77±0.17$^{b}$ | 6.64±0.29$^{c}$ | 1.10±0.01$^{bc}$ |
| 100% Wheat | | ND | ND | ND | ND | ND |
| | | **Cookies** | | | | |
| **OFSP** | **PTS** | **Saponins** | **Tannins** | **Alkaloids** | **HCN** | **Oxalate** |
| | | - | - | - | - | - |
| 100 | 0 | 0.02±0.00$^{a}$ | 1.51±0.05$^{a}$ | 0.29±0.05$^{b}$ | 2.72±0.05$^{a}$ | 0.15±0.05$^{a}$ |
| 90 | 10 | 0.01±0.00$^{a}$ | 1.39±0.05$^{b}$ | 0.43±0.05$^{a}$ | 2.64±0.05$^{a}$ | 0.11±0.05$^{b}$ |
| 80 | 20 | 0.01±0.00$^{a}$ | 1.23±0.05$^{c}$ | 0.48±0.05$^{a}$ | 1.91±0.05$^{b}$ | 0.11±0.05$^{b}$ |
| 70 | 30 | 0.02±0.00$^{a}$ | 1.26±0.05$^{bc}$ | 0.49±0.05$^{a}$ | 1.12±0.05$^{c}$ | 0.10±0.05$^{b}$ |
| 100% Wheat | | ND | ND | ND | ND | ND |

Each value is a mean of triplicate determinations ± standard deviation; Mean values with different letters within the same column are significantly ($p$<0.05) different. ND: Not detected; HCN: Hydrogen cyanide, OFSP: Orange fleshed sweet potato, PTS: *Pleurotus tuber-regium sclerotium* *Results reported on dry matter basis

### *Beta carotene of flour and cookies*

As expected, the beta-carotene content of OFSP flour (8.64 mg/100 g) was substantially higher than that of sclerotium (2.03 mg/100 g) and wheat flour (1.25 mg/100 g) but reduced with increasing levels of sclerotium flour in the blends (Table 3). This is due to the dilution of the beta-carotene with the sclerotium flour. Previous studies similarly reported high levels of beta-carotene (5091-16,456 µg/100 g) in OFSP (Laurie *et al.*, 2012).

The developed cookies (4.70-5.96 mg/100 g) including the 100% OFSP cookie (7.11 mg/100 g) had high levels of beta-carotene compared with the wheat cookie (0.83 mg/100 g) and could therefore be regarded as functional foods. Baking resulted in losses of the beta-carotene with percentage losses ranging between 18 and 34% (66-82% retention) for cookies made from 100% OFSP and cookies from 100% wheat flour. The loss in beta-carotene following the baking process might be linked to the ability of temperature to influence β-carotene loss (Demasse *et al.*, 2007). Earlier studies also indicated that long-chain polyunsaturated carotenoids can undergo isomerisation from the trans to the cis form at high temperatures, resulting in the loss of carotenoids including β-carotene content (Tannenbaum, 1976).

**Table 3**. Total flavonoids, total phenolics and beta-carotene content of OFSP and *Pleurotus tuber-regium* sclerotium composite flours and cookies*.

| OFSP | PTS | Flours | | |
|---|---|---|---|---|
| | | β-carotene (mg /100g) | TFC (mg CE/g) | TPC (mg GAE/g) |
| 0 | 100 | 2.03±0.23$^{d}$ | 0.04±0.00$^{e}$ | 1.36±0.06$^{c}$ |
| 100 | 0 | 8.64±0.38$^{a}$ | 0.36±0.01$^{a}$ | 1.81±0.13$^{b}$ |
| 90 | 10 | 7.88±0.54$^{ab}$ | 0.30±0.02$^{b}$ | 1.69±0.08$^{bc}$ |
| 80 | 20 | 7.20±0.31$^{bc}$ | 0.27±0.01$^{bc}$ | 1.55±0.12$^{bc}$ |
| 70 | 30 | 6.48±0.15$^{d}$ | 0.25±0.01$^{c}$ | 1.40 ±0.09$^{c}$ |
| 100% Wheat | | 1.25±0.14$^{d}$ | 0.19±0.01$^{d}$ | 3.33±0.41$^{a}$ |
| | | Cookies | | |
| OFSP | PTS | β-carotene (mg /100g) | TFC (mg CE/g) | TPC (mg GAE/g) |
| | | - | - | - |
| 100 | 0 | 7.11±0.71$^{a}$ | 0.62±0.01$^{a}$ | 1.83±0.06$^{a}$ |
| 90 | 10 | 5.96±0.49$^{ab}$ | 0.56±0.01$^{b}$ | 1.80±0.02$^{ab}$ |
| 80 | 20 | 5.22±0.23$^{bc}$ | 0.42±0.01$^{c}$ | 1.70±0.02$^{bc}$ |
| 70 | 30 | 4.70±0.36$^{c}$ | 0.35±0.01$^{d}$ | 1.62±0.02$^{c}$ |
| 100% Wheat | | 0.23±0.03$^{d}$ | 0.20±0.00$^{e}$ | 0.40±0.04$^{d}$ |

Each value is a mean of triplicate determinations ± standard deviation; Mean values with different letters within the same column are significantly ($p$<0.05) different. TFC: Total Flavonoids Content, TFC: Total Phenolic Content, DPPH: 1, 1 - diphenyl-2-picrylhydrazyl, OFSP: Orange fleshed sweet potato, PTS: *Pleurotus tuberregium sclerotium* *Results reported on dry matter basis. CE: catechin equivalents; GAE; gallic acid equivalents

### *Antioxidant activity of cookies*

The antioxidant potentials of the developed cookies were assessed using the DPPH method and the result is presented in Figure 1. Cookies made from wheat flour, which was the control, had the lowest DPPH value of 6.27% compared to the 100% OFSP cookies (15.58%) and the OFSP enriched cookies (17.24-19.55%). The high antioxidant activity of the developed OFSP-sclerotium cookies, as shown by their high DPPH values, may be linked to their high level of phytochemical components such as total flavonoids, total phenolics, and beta-carotene (Table 3). The addition of sclerotium flour to OFSP enhanced the antioxidant of the resulting cookies and this could be linked to the pharmacological actions of its non-starch polysaccharides which are primarily made up of bioactive β-glucans (Cheung and Lee, 2000; Tao *et al.*, 2006; Zhang *et al.*, 2006). Other factors that may explain the increased antioxidant activity of the enriched cookies include the relatively higher contents of saponins and alkaloids in the sclerotium flour (Table 2). The increase in β-carotene, flavonoids, phenolics, and enhanced antioxidant properties of the cookies suggest the potential of the cookies in the prevention and management of various cardiovascular and degenerative diseases (Akeem *et al.*, 2016; Kolawole *et al.*, 2018b).

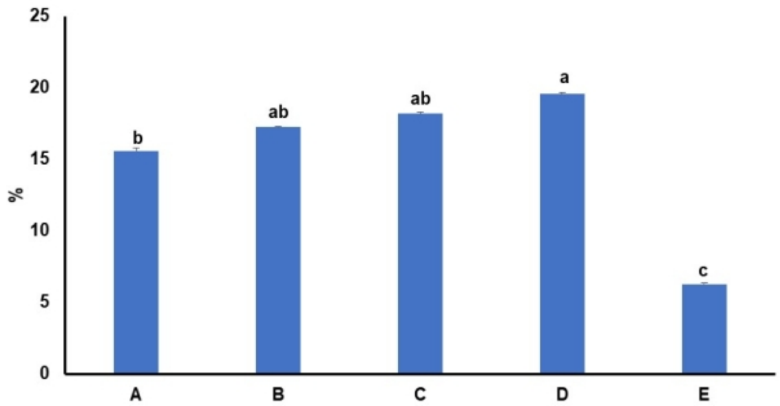


**Figure 1**. DPPH (1, 1- diphenyl-2-picrylhydrazyl) free radical scavenging activity of cookies A: 100% OFSP, B: 90% OFSP+ 10% Sclerotium, C: 80% OFSP+ 20% Sclerotium, D: 70% OFSP+ 30% Sclerotium, E: 100% Wheat, OFSP: Orange fleshed sweet potato, Error bars indicate standard deviation. Results are reported on dry matter basis.

## Conclusions

The developed OFSP-*Pleurotus tuber-regium* sclerotium cookies can be described as functional foods because of their enhanced amino acid composition and good amounts of β-carotene, flavonoids, and phenolics with high antioxidant activities. Enriched cookies exhibited low levels of antinutrients which are within the safe range for human consumption. This gives some level of assurance that the novel snacks would not be hazardous to health. However, further in vivo studies might be required to buttress this fact. Baking of the flours into cookies enhanced most of the amino acids, reduced all antinutrients, and increased the total phenolics and flavonoids of the samples. The developed gluten-free snack would not only help to address protein malnutrition, vitamin A deficiency, and improve consumers' health but would also enhance the utilization of OFSP and sclerotium of *Pleurotus tuer-regium*, thereby reducing wheat importation. It is recommended that cookies be produced from blends of OFSP and sclerotium flours at both household and commercial levels

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