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An Evaluation of the Quality Characteristics of Probiotic-Influenced Cookies from Air Potato (Dioscorea Bulbifera)

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Abstract

In this study, a controlled fermentation process was initiated using pure strains of *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus acidophilus* for the development of air potato (*Dioscorea bulbifera*) flour and cookies. Flours from unfermented, spontaneous-fermented and probiotic-fermented air potatoes were formulated as RAPF1, UAPF1 and PAPF1 respectively; while cookies from flours of unfermented, spontaneous-fermented and probiotic-fermented air potatoes were formulated as RAPF2, UAPF2 and PAPF2 respectively. In order to investigate the effect of probiotic inclusion on the flours and cookies of air potato, the proximate, functional, mineral and sensory attributes as well as phytochemical constituents were evaluated using standard methods. For the flours, a significant reduction in carbohydrate was observed in PAPF1 (45.48 ± 0.89%), when compared with RAPF1 (55.55 ± 0.77%) and UAPF1 (63.94 ± 0.16%); thus, indicated its suitability as a low carbohydrate diet. Cookies from the flours showed the highest retention of protein and ash in PAPF2; while CHO were significantly decreased. The highest total phenolic and total flavonoid content was observed in PAPF2 at 0.22 ± 0.02 mgTAE/g and 0.48 ± 0.27 mgTAE/g respectively. The mineral constituents of the flour and cookies of fermented air potatoes were significantly ($p<0.05$) elevated when compared with the unfermented samples. Probiotic-influenced fermentation of air potato resulted in good physical properties in terms of bulk density, swelling index, water holding capacity, emulsification capacity and oil absorption capacity in both flour and cookies. The hedonic responses of sensory parameters showed significant differences ($p<0.05$) between the cookies, and PAPF2 was favorably comparable to conventional whole wheat cookies. According to the principal component analysis (PCA), the loading plots of correlations between parameters indicated that texture and aroma had greater influence on the acceptability of the cookies. The inclusion of probiotics for the fermentation of air potato could be a suitable application for the development of a functional cookies with potentials as a healthy snack for diet-specific individuals.

Keywords: Air potato, probiotic, fermentation, flour, cookies.
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1.0 INTRODUCTION

Air potato (*Dioscorea bulbifera*) is a specie of true yam in the yam family, and is native to Africa, Asia and northern Australia. It is widely used in traditional Indian and Chinese medicine in the treatment of sore throat, gastric cancer and carcinoma of rectum and goiter. Furthermore, various extracts of bulbs of the plant have been reported to have anti-hyperlipidemic, antitumor, antioxidant, anorexiant, analgesic, anti-inflammatory and anti-hyperglycemic properties. According to Afiukwa and Igwe (2015), air potato possesses high nutritional composition of protein, lipids, crude fibre and minerals.

A number of studies had reported the antioxidant and antidiabetic activities of several roots and tuber crops. In addition, the influence of processing methods on their bioactivities had also been extensively studied. Ji *et al.* (2012) reported the contents of phenolic compounds and glycoalkaloids of 20 potato clones and their antioxidant, cholesterol uptake and neuroprotective activities *in vitro*. In another study, diosgenin (a steroidal saponin) contained in Chinese yam showed a prebiotic effect and also had beneficial effects on the growth of enteric lactic acid bacteria (Huang, Cheng, Deng, Chou, & Jan, 2012). Recently, several researchers had focused on the optimization and utilization of bioactive compounds inherent in plant-based foods (such as fruits, vegetables, whole grains and legumes) with the sole aim of solving the problems associated with human health and nutrition such as several cardiovascular and metabolic disorders (Mena & Angelino, 2020) as well as curbing food insecurity.

A significant number of compounds found in plant-based foods (vitamins, minerals, etc.) and several bioactive compounds (phenolic, flavonoid, anthocyanin, etc.) with health promoting biological functions are known to be influenced by processing conditions such as fermentation, germination, freezing, etc (Orlien & Bolumar, 2019). Thus, food processing is ubiquitous and plays an important role in our daily diet. They can significantly influence the nutritional composition and chemical structures of food, and consequently enhance the health value and quality of their products. Food fermentation is one of the oldest food processing technique known to extend shelf life as well as to improve nutritional and sensory qualities of foods.

In food fermentation processes, various microbes such as lactic acid bacteria, molds, and yeasts considered as generally recognized as safe (GRAS) microbes are commonly used for food fermentation processes. Fermentation is a natural process that improves nutrients, appearance, flavor and aroma of foods. Hence, its health benefits could not be over-emphasized. Interestingly, lactic acid bacteria (LAB) is commonly used in food fermentation, and have been previously implicated in a diverse range of health-promoting effects such as protection against infectious diseases, immunoregulatory, anti-allergenic, anti-obesity and antioxidant effects as well as enhancing the bioavailability of vitamins and minerals (Linares *et al*., 2017).

Despite the health beneficial effects of the air potato, it is still underutilized; especially in Africa where it is mostly cultivated. A major limitation to its usage are common presumptions that it is not edible, attributed to its conventional bitter taste; hence, its poor organoleptic acceptability. Further, its limited utilization could be attributed to poor processing quality of its products. Consequently, food products that would encourage the awareness of consumers about the plant would create an awareness of its use and eventually encourage its wide cultivation worldwide. Therefore, this current study aimed to develop a novel food snack from fermented flour of air potato, as well as evaluate the effect of probiotic inclusion on its organoleptic, nutritional and functional qualities.

2.0 MATERIALS AND METHODS

2.1 Materials

Air potato tubers were freshly harvested from a local farm in Edo State, Nigeria. Probiotic starter cultures (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus*) were purchased from ATTC, J8H4G4 (500 Aeroparc, Quebec, Canada). All reagents used were analytical grade.

2.2 Preparation and formulation of air potato flour

For the preparation of air potato flour samples (figure 1), the method of Umar and Teresa (2019) was used with modifications. In brief, the outer flesh of the air potato tubers were aseptically peeled after washing with clean water. To a portion of the tubers steeped in ultra-pure water, controlled fermentation process was initiated using probiotic starter culture (pure strains of *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus acidophilus*) for 24 hrs. To another portion, a spontaneous fermentation process was allowed for 24 hrs after steeping in ultra-pure water. The fermented tubers were then drained, dried to a moisture content of ≤13% (Codex Standard for tuber flour (Codex Stan 176–1989)) and then blended to flour using an attrition mill (Munson's Model SK-30- SS food-grade attrition mill). The flour samples were stored in air-tight packages until further analysis. The formulation for the air potato flour are shown in table 1.

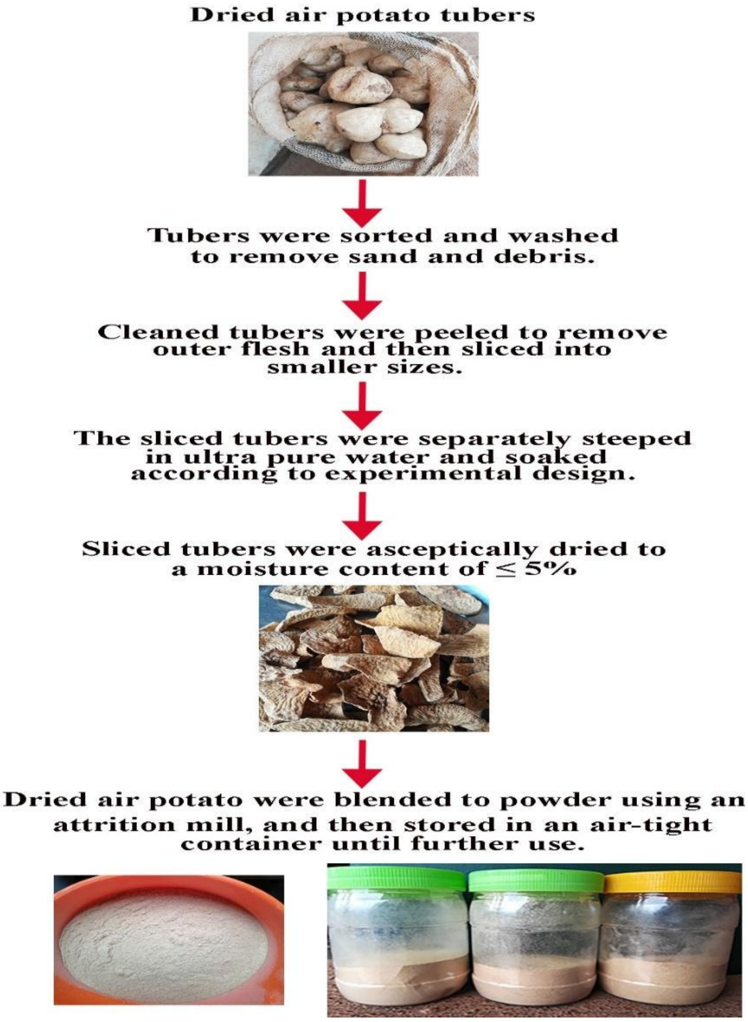

Figure 1: Flow chart for the preparation of air potato flour.

2.3 Preparation of air potato cookies

Flour from air potato tubers was used for the development of cookies according to the slightly modified method of Panghal, Chhikara and Khatkar (2018). Unfermented flour and cookies of air potatoes were used as control.

Table 1: Formulation of air potato flour and cookie samples

Sample code	Sample name
RAPF1	Flour from unfermented air potato
UAPF1	Flour from uncontrolled fermented air potato
PAPF1	Flour from probiotic-fermented air potato
RAPF2	Cookies from flour of unfermented air potato
UAPF2	Cookies from flour of uncontrolled fermented air potato
PAPF2	Cookies from flour of probiotic-fermented air potato.

2.4 Sensory evaluation

The cookies of air potato flours were assessed for colour, flavor, consistency, taste and overall acceptability using a 5-point hedonic scale according to previously described method of Obaroakpo *et al*. (2019). Principal component analysis (PCA) was performed on the sensory parameters of the cookies using Graph-Pad Prism v. 9.5.1 (San Diego, CA, USA) software package for Windows. PCA was applied to assess the relationships between the sensory attributes of a cookie sample. The loading plot showed the similarities and differences between measured variables. While close variables indicates direct correlations; opposed variables indicates indirect correlations between attributes.

2.5 Mineral analyses

The amount of minerals present in the sample was determined as described by AOAC (2010). The ash of the sample obtained was dissolved in 10 mL of 2 M HNO_3, boiled for 5 min. and then filtered into a volumetric flask through a Whitman no. 1 filter paper. The filtrate was made up to 50 mL with a distilled water. An atomic absorption spectrophotometer (Model 220 GF, Buck Scientific, and East Norwalk, CT, USA) was used for the determination of calcium, phosphorus and magnesium contents. Sodium and potassium content were determined using Jenway Flame Photometer PFP7 (Cole-Parmer Instrument Co., Ltd., UK).

2.6 Proximate analysis

According to the proceedure described by AOAC (2023), the protein content of the flours and cookies were determined using Micro-Kjeldahl method. The moisture, crude fiber, fat and ash contents of the samples were determined as described by AOAC (2023). Carbohydrate content was estimated by differences.

2.7 Quantitative phytochemical screening of air potato flour and cookies.

Total phenolic content of the extracts were determined by Folin Ciocalteu method of Ezeonu and Ejikeme (2016) with some modifications. The extracts (1 mL) were mixed with Ciocalteu reagent and allowed to stand for 15 min. Then 5 mL of saturated Na_2CO_3 were added. The mixtures were allowed to stand for 30 min at room temperature. Total phenol content was measured at 760 nm and was expressed in terms of garlic acid equivalent (mg g^{-1} of extracted compound). The aluminium chloride colorimetric assay was used to evaluate the total flavonoid content of according to the slightly modified method of Gulcin (2020), and the results were expressed as mg of gallic acid equivalent per g dry weight.

The method described by Ejikeme, Ezeonu and Eboatu (2014) was used for the determination of tannins. The tannin content were quantified by a tannic acid standard curve and expressed as milligrams of Tannic Acid Equivalence (TAE) per 100 g of dried sample and the soluble tannins calculated as:

$$\text{Soluble tannins} = \frac{\text{C (mg)} \times \text{extract volume (mL)}}{\text{DF} \times \text{aliquot (mL)} \times \text{sample weight (g)}}$$

Where: C = concentration extrapolated from standard calibration curve, DF = dilution factors.

The estimation of saponin content in the air potato flour and cookies were determined according to the method described by Ejikeme *et al.* (2014) based on vanillin-sulphuric acid colorimetric reaction and expressed as quercetin equivalents (mg QE/g sample) derived from a standard curve.

2.8 Functional analyses

2.8.1 Determination of bulk density

50 g of air potato sample was placed into a 100 mL measuring cylinder and tapped to a constant volume. The bulk density (g/cm3) was determined as the weight of dry sample (g) divided by volume of the slurry (cm^3) as reported by Adejuyitan, Otunola, Akande, Bolarinwa and Oladokun (2009).

2.8.2 Determination of water absorption capacity

Water absorption capacity of the sample was determined by placing 1g of flour into 10 mL of water. It was allowed to stand at room temperature for 1 hr and then centrifuged at 200 x g for 30 min. The volume of water in the sediment was measured. Water absorption capacity was calculated as mL of water absorbed per gram of cookies (Adejuyitan *et al*., 2009).

2.8.3 Determination of swelling power

The swelling capacity of the sample was determined using the method described by Onwuka (2005). 0.1 g of the sample was weighed into different beakers/test tubes as W_1. 5 mL of sugar solution at different concentration was added to each sample. The resulting slurry was heated at 30 °C for 15 mins in water bath followed by cooling at 28 °C and centrifuged at 2500 rpm for 15 mins. The supernatant was removed and poured in a dish for solubility determination. The weight of the swollen sediment was taken as W_2. The supernatant was dried to a constant weight in air-oven at 100 °C for 4 hrs.

$$\text{Swelling power (\%)} = \frac{W2-W1}{sample\ weight} \times 100$$

Where W_1 = weight of dry sample (g)
W_2 = dry weight of sample supernatant (g).

2.8.4 Oil absorption capacity

Oil absorption capacity was evaluated at room temperature in triplicates as described by Abbey and Ibeh (1988) using 1 g of sample and 10 mL of refined vegetable oil (Life Brand. Density, 0.89 gml^{-1}). The values were expressed as mL of oil absorbed by 1 g of sample.

2.8.5 Foam capacity

Foam capacity was determined by the method of Lawhon, Cater and Mattil (1972) with slight modifications. The sample (2 g) was suspended in distilled water (100 mL) and stirred at room temperature for 5 mins using a magnetic stirrer at 10 Ruhrer speed (Phywe, Gottigen, Germany). The content along with the foam were immediately poured into a 250 mL measuring cylinder. Volume of foam (mL) after mixing was expressed as the foam capacity for the time periods and expressed as percentage. Measurements were made in triplicates and averaged.

2.5.6 Emulsification capacity

Emulsification capacity (EC) was determined according to the procedure of Beuchat, Cherry and Quinn (1975) at room temperature. A 2 g of sample and 23 mL of distilled water or NaCl (0.2 - 1.0 M) solution were mixed for 30 s using a Phywe magnetic stirrer at 10 Ruhrer speed. After complete dispersion, refined vegetable oil (Life Brand, density 0.89 gmL^{-1}) was added continuously (in mL portions) from a burette and blending continued at room temperature until the emulsion breakpoint was reached. EC was determined in the pH range of 1-12 and the values are expressed as percentage of oil emulsified by 1 g of cookie samples.

2.5.7 Gelation capacity

Least gelation concentrations for raw and heat processed air potato cookies were determined using the method of Coffman and Garcia (1977) as modified by Abbey and Ibeh (1988). The sample were mixed with 5 ml of distilled water in test tubes to obtain suspensions of 2-20% (w/v) concentration. The test tubes were heated for 1 h in a boiling water bath, cooled rapidly under running tap water and further cooled for 2 h in the refrigerator at 4 °C. The least gelation concentration was regarded as that concentration at which the sample did not fall or slip from the inverted test tube.

2.5.8 Gelation temperature

For the determination of gelation temperature, the Differential Scanning Calorimetry (DSC) method as described by Nilsson *et al.* (2022) with slight modifications. In brief, gelatinisation characteristics of the thermal properties of air potato flour and cookies were measured using a Mettler Toledo DSC. To a 1. 3 ± 1 mg of sample, 12 ± 1 µL of distilled water was added and then the paste was placed in an aluminium pan and sealed hermetically. The sealed pan containing the paste was allowed to stand overnight at room temperature in order to achieve uniform distribution of water before DSC analysis. Then, the sample was scanned at a rate of 10 °C/min from 30 – 200 °C, and thermogram was recorded. During intermittent scans, sample chamber was flushed with inert gas (nitrogen, purge rate 50 mL/min). An empty aluminium pan was used as reference and the blank pan value was subtracted from all measurements.

3.0 STATISTICAL ANALYSIS

The data obtained from all analyses were statistically analyzed using SPSS 20.0 for Window (SPSS Inc. Chicago, IL, USA) correlation between parameters were assessed by Pearson's correlation test; while Duncan multiple range test was applied to determine the difference between means. The significance differences among samples were evaluated at probability levels of $p<0.05$ and $p<0.0001$.

4.0 RESULTS AND DISCUSSION

4.1 Effect of probiotic-influenced fermentation on proximate composition of air potato flour

In order to establish the nutritional and quality characteristics of cookies from air potato, the flour samples were first evaluated for their proximate composition and the results are depicted in figure 2. The ash content showed that the inclusion of probiotics resulted in a significant increase ($p<0.05$) as observed in PAPF1 (16.22 ± 0.45%). The slightly increased ash content observed in PAPF1 enhances its suitability as a functional flour. There was no significant difference ($p>0.05$) between the ash contents of UAPF1 and RAPF1 at mean values of 8.51 ± 0.51% and 10.35 ± 0.05% respectively.

Based on the required standard for acceptable moisture content in flour samples (≤13%) all samples in this study were considered suitable, as their moisture contents were at a range of 2.07 ± 0.01% - 4.79 ± 0.38%. However, among flour samples, the highest moisture content was observed in PAPF1 which could be possibly attributed to the microbial activities of the probiotic strains.

Compared with the unfermented potato flour (RAPF1), it was observed that fermentation processes increased the protein content of air potato flour. Although, there were no significant differences between the flour samples, mean values showed that the highest protein was recorded in PAPF1 (11.78 ± 0.81%). The enhancement of protein content could be attributed to the microbial synthesis of proteins from metabolic intermediates during their growth in fermentation (Song, Shin, & Baik, 2012). In another study (Baruah, Mamoni, & Rajeev, 2018), it was reported that the carbohydrate content in food samples could have been utilized by microorganisms during fermentation to synthesize amino acids needed for their growth and development; hence, resulted in an increased protein content.

The results of the crude fibre content of the flour samples showed no significant differences ($p>0.05$) between RAPF1, UAPF1 and PAPF1 (3.11 ± 0.03%, 3.01 ± 0.60%, 3.16 ± 0.03% respectively). The results of this study also showed that the crude fat content had no significant differences ($p>0.05$) among air potato flour samples.

Among air potato flours, significant differences ($p<0.05$) were recorded in carbohydrate content such that, the lowest content was observed in PAPF1 (45.48 ± 0.89%); while the highest content was recorded in UAPF1 (63.94 ± 0.16%). The low carbohydrate content observed in PAPF1 was remarkable. According to Nordmann *et al.* (2006), low carbohydrate diets possess the ability to induce short-term weight loss when matched calorie-for-calorie with other diets higher in carbohydrate content.

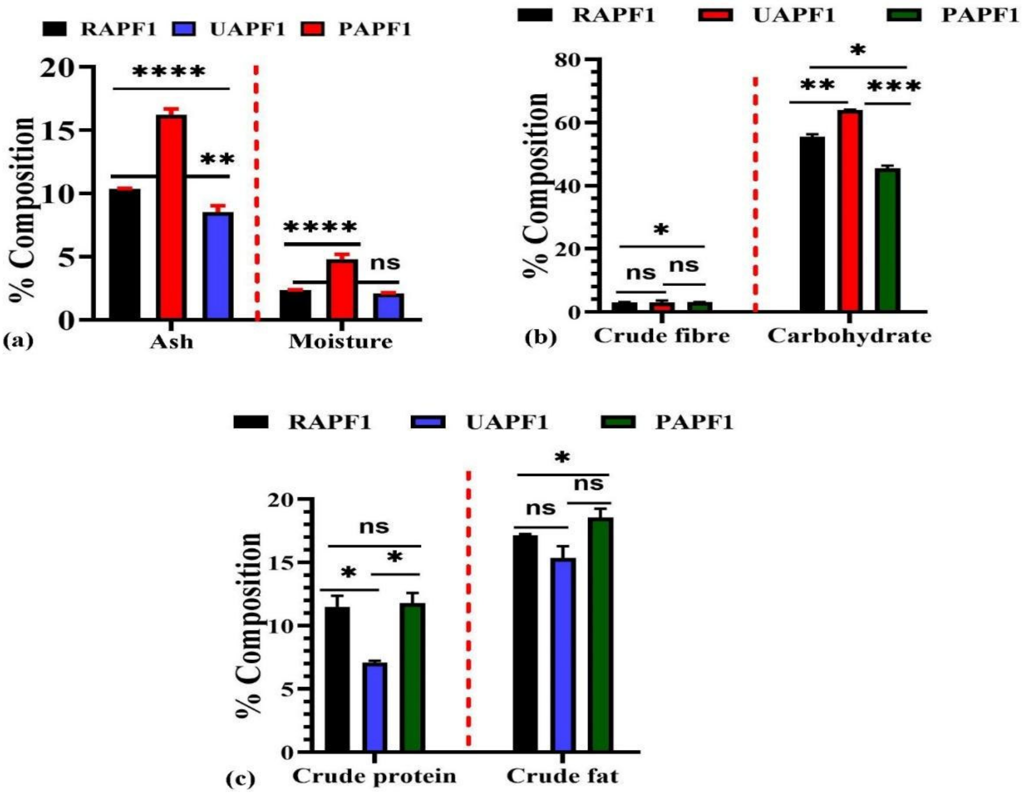

Figure 2: Percentage proximate composition of air potato flour samples. **(a)** Ash and moisture content **(b)** Crude fibre and carbohydrate content **(c)** Crude protein and fat content. Values are means ± standard deviation of triplicate determinations. **KEY: RAPF1** = Unfermented air potato flour; **UAPF1** = Uncontrolled fermented air potato flour; and **PAPF1**= Probiotic-fermented air potato flour. *, **, *** and **** indicates level of significance at $p<0.0001$ probability level, while "**ns**" indicates no level of significance among samples.

4.2 Effect of probiotic-influenced fermentation on functional characteristics of air potato flour

The functional characteristics of the air potato flour samples are shown in figure 3. The results of the water absorption capacity (WAC) of the flours of air potato showed highest capacity in RAPF1 (1.52 ± 0.02 g/mL) and PAPF1 (1.72 ± 0.03 g/mL) with no significant differences ($p>0.05$) between them (figure 3a). According to Omueti, Otegbayo, Jaiyeola and Afolabi (2009), water absorption capacity indicates the absorbing ability of a product immersed in water. Hence, it shows the ratio of the weight of water absorbed by the product in a saturated state and the weight of the product in a dry state (Chandra, Singh, & Kumari, 2015).

The results of the oil absorption capacity (OAC) of the flours showed the highest and lowest values in UAPF1 and PAPF1 at mean values of 2.26 ± 0.02 g/mL and 1.83 ± 0.03 g/mL respectively (figure 3a). It had been reported that flours with high OAC are potentially beneficial in their structural interactions; especially for the improvement of palatability, extension of shelf life as well as flavor retention (Suriya, Rajput, Reddy, Haripriya, & Bashir, 2017).

The gelation capacity of a flour is an index of its tendency to form gel. The highest gelation capacity of the flour samples was observed in RAPF1 (90.13 ± 0.15%). Fermentation did not affect significantly the gelation capacity of the flours, because it was observed that of RAPF1 was not significantly different ($p>0.05$) from PAPF1 (93.93 ± 2.76%). A similar observation on the effect of fermentation on gelation capacity was reported by Udensi, Ukozor and Ekwu (2005) who mentioned that fermentation did not affect significantly the gelation capacity of the cassava flours studied. For gelation temperature, the result showed that fermentation processes influenced the gelation temperature as observed in PAPF1 and UAPF1 (78.70 ± 1.21 ^{o}C and 81.27 ± 1.50 ^{o}C respectively).

The results of the emulsification capacity (figure 3c) showed no significant differences ($p>0.05$) among all flour samples of air potato. Hence, it was concluded that fermentation processes had no effect on the emulsification capacity of the flour samples. For foaming capacity of flour samples (figure 3c), the highest mean value was observed in UAPF1 (18.87 ± 0.99%), while the lowest capacity was observed in PAPF1 (12.20 ± 0.20%). El-Adawy and Taha (2001) reported that good foam capacity and stability are desired attributes for flours intended for use in the production of various baked products such as cookies.

Fermentation process significantly influenced the swelling index of the flour samples (figure 3d) as observed in PAPF1 and UAPF1 which had mean values of 2.13 ± 0.03% and 1.91 ± 0.01% respectively, when compared to RAPF1 (1.83 ± 0.03%). The swelling power indicates the ability of the flour to imbibe water and swell; hence, a higher swelling index indicates higher associative forces. A similar trend to the swelling index was observed in the bulk density of the flour samples (figure 3d), where the highest mean value was observed in PAPF1 (0.77 ± 0.01%). The high bulk density of PAPF1 flour suggests their suitability for use in food preparations.

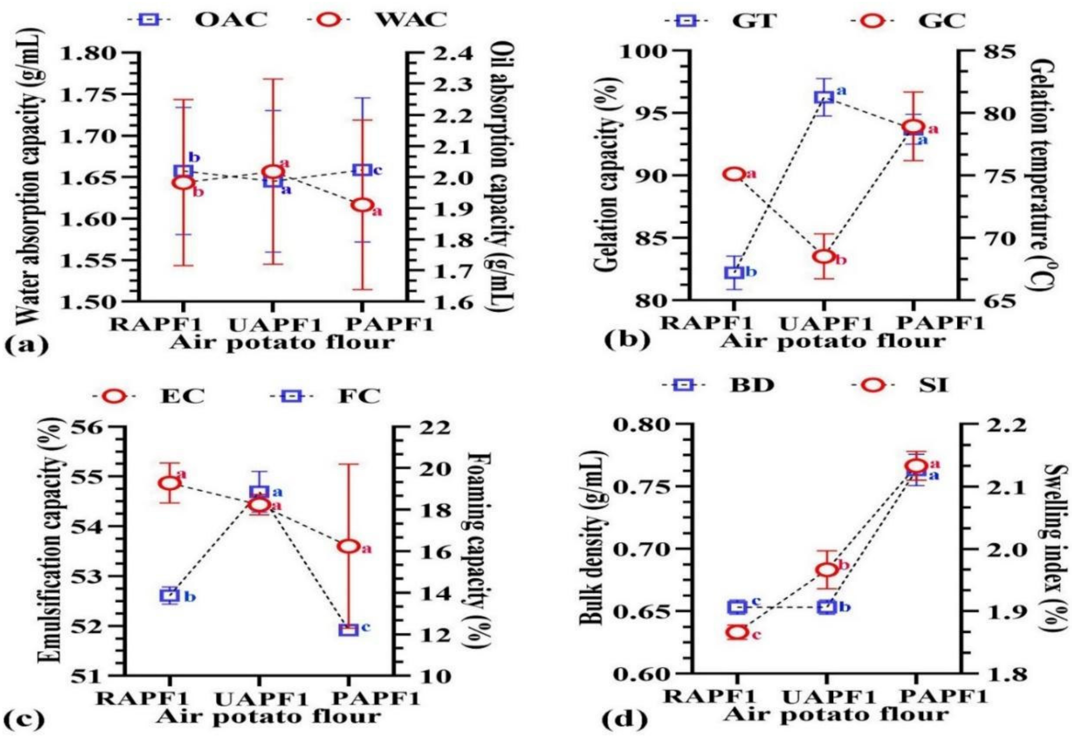

Figure 3: Functional characteristics of air potato flour samples. **(a)** Water absorption and oil absorption capacity **(b)** Gelation capacity and gelation temperature **(c)** Emulsification capacity and foaming capacity **(d)** Bulk density and swelling index. Values are means ± standard deviation of triplicate determinations. **KEY: RAPF1** = Unfermented air potato flour; **UAPF1** = Uncontrolled fermented air potato flour; and **PAPF1**= Probiotic-fermented air potato flour. Mean values on the same line on graph with the same superscript are not significantly different at $p<0.05$.

4.3 Effect of probiotic-influenced fermentation on phytochemical constituents in air potato flour and cookies

Recent researches had revealed the importance of phytonutrients to human health, owing to their antioxidant properties. Although, fermentation has significant effect on phytochemicals that are both beneficial and adverse, its ability to increase the antioxidant properties of foods can be explored as a cheap way to reduce oxidative stress and its associated disorders. The results of the phytochemical composition of air potato flours and cookies after qualitative and quantitative screening are shown in table 2. The results showed the presence of phenols, alkaloid, saponin, flavonoid and tannin in air potato flours and cookies. However quantitatively, it was observed that the total phenolic content in PAPF2 had the highest mean value (0.22 ± 0.02 mgTAE/g); while the lowest value was observed in RAPF1 (0.10 ± 0.02 mgTAE/g). This was expected because several studies had established the fact that fermentation enhances bioactive components in foods due to their stability in acidic conditions (Balli *et al.*, 2020).

For alkaloid content, it was observed in this study that the imbibement of fermentation processes for the production of air potato cookies resulted in a significant reduction when compared with the control (RAPF1). These reductions were observed in UAPF2 and PAPF2 at mean values of 0.34 ± 0.02 mgTAE/g and 0.74 ± 0.05 mgTAE/g respectively. The result of the saponin content was significantly increased by the fermentation processes. Hence, the highest saponin content was observed in UAPF2 (0.73 ± 0.03 mgQE/g) and was not significantly different (p>0.05) from PAPF2 (0.66 ± 0.05 mgQE/g).

The highest total flavonoid content was observed in PAPF2 (0.48 ± 0.27 mgQE/g). An uncontrolled fermented process for air potato flour resulted in the production of cookies with significantly lowered flavonoid content as observed in UAPF1 and UAPF2 at mean values of 0.10 ± 0.01 mgQE/g and 0.18 ± 0.02 mgQE/g respectively. The result of the tannin content showed lowest mean value in PAPF1 (0.10 ± 0.02 mgTAE/g) and was not significantly different (p>0.05) from RAPF2 (0.22 ± 0.02 mgTAE/g). The highest tannin content was observed in UAPF1 (0.34 ± 0.02 mgTAE/g), and was comparable to those of UAPF2 and PAPF2 (0.13 ± 0.00 mgTAE/g and 0.14 ± 0.01 mgTAE/g respectively). The result of this study confirmed the report of Pellati, Benvenuti, Magro, Melegari and Soragni (2004) who mentioned that fermentation is considered as one of the most suitable tool to exploit the biogenic and functional potential of plant matrices, as well as enrich them with bioactive compounds.

Table 2: Phytochemical constituents in air potato flour and cookies

Sample	Quantitative phytochemical composition				
	Total phenolic content (mgTAE/g)	Alkaloid content (mgTAE/g)	Saponin content (mgQE/g)	Total flavonoid content (mgQE/g)	Tannin content (mgTAE/g)
RAPF1	0.10 ± 0.02^{b}	0.12 ± 0.01^{e}	0.34 ± 0.02^{c}	0.31 ± 0.01^{ab}	0.11 ± 0.02^{b}
UAPF1	0.13 ± 0.00^{ab}	1.21 ± 0.02^{b}	0.73 ± 0.03^{a}	0.10 ± 0.01^{b}	0.22 ± 0.02^{a}
PAPF1	0.11 ± 0.02^{ab}	1.54 ± 0.04^{a}	0.52 ± 0.04^{b}	0.37 ± 0.03^{ab}	0.10 ± 0.02^{c}
RAPF2	0.12 ± 0.02^{ab}	1.61 ± 0.03^{a}	0.12 ± 0.03^{d}	0.28 ± 0.01^{ab}	0.12 ± 0.02^{c}
UAPF2	0.14 ± 0.01^{b}	0.34 ± 0.02^{d}	0.35 ± 0.02^{c}	0.18 ± 0.02^{b}	0.13 ± 0.00^{ab}
PAPF2	0.22 ± 0.02^{a}	0.74 ± 0.05^{c}	0.66 ± 0.05^{a}	0.48 ± 0.27^{a}	0.14 ± 0.01^{ab}

Values are means ± standard deviation of triplicate determinations. Mean values on the same column with the same superscript are not significantly different at p<0.05. **KEY**: **RAPF1** = Unfermented air potato flour; **UAPF1** = Uncontrolled fermented air potato flour; **PAPF1**= Probiotic-fermented air potato flour; **RAPF2** = Cookies from unfermented air potato flour; **UAPF2** = Cookies from uncontrolled fermented air potato flour; and **PAPF2** = Cookies from probiotic-fermented air potato flour.

4.4 Effect of probiotic-influenced fermentation on mineral composition of air potato flour and cookies

The mineral composition of air potato flour and cookies are shown in table 3. For the retention of essential mineral in food products, processing conditions are an integral aspects important to several researchers. In this study, it was observed that when compared with the control (RAPF1), a reduction of calcium content was observed in all samples. However, the retention of calcium was highest in UAPF1 at 0.70 ± 0.02%. For phosphorus, the results showed highest content in PAPF1 (0.35 ± 0.03%). Interestingly, the iron content was observed to be significantly increased in PAPF2 (2.88 ± 0.02%), when compared with RAPF1 (2.52 ± 0.44%). Similar to the report of Sukhikh *et al.* (2022), our result showed that magnesium content was drastically reduced during fermentation. The lowest mean value was observed in PAPF2 (0.08 ± 0.00%).

Table 3: Mineral composition of air potato flour samples and cookies

Sample	**Mineral composition (%)**			
	Magnesium	Calcium	Phosphorus	Iron
RAPF1	0.17 ± 0.00^{e}	0.20 ± 0.01^{d}	0.24 ± 0.01^{b}	2.52 ± 0.44^{ab}
UAPF1	0.48 ± 0.03^{c}	0.70 ± 0.02^{a}	0.14 ± 0.00^{c}	1.81 ± 0.21^{cd}
PAPF1	0.69 ± 0.01^{a}	0.33 ± 0.00^{c}	0.35 ± 0.03^{a}	2.17 ± 0.03^{bc}
RAPF2	1.62 ± 0.01^{a}	0.53 ± 0.00^{b}	0.23 ± 0.02^{b}	1.44 ± 0.04^{d}
UAPF2	0.42 ± 0.01^{d}	0.12 ± 0.00^{e}	0.31 ± 0.01^{a}	2.40 ± 0.05^{ab}
PAPF2	0.08 ± 0.00^{f}	0.21 ± 0.01^{d}	0.14 ± 0.02^{c}	2.88 ± 0.11^{a}

Values are means ± standard deviation of triplicate determinations. Mean values on the same column with the same superscript are not significantly different at p<0.05. **KEY**: **RAPF1** = Unfermented air potato flour; **UAPF1** = Uncontrolled fermented air potato flour; **PAPF1**= Probiotic-fermented air potato flour; **RAPF2** = Cookies from unfermented air potato flour; **UAPF2** = Cookies from uncontrolled fermented air potato flour; and **PAPF2** = Cookies from probiotic-fermented air potato flour.

4.5 Organoleptic characteristics of cookies developed from probiotic-influenced air potato flour.

Functional cookies were produced from air potato fortified with probiotic strains. Plate 1 showed the cookies produced from air potatoes in its unfermented state, uncontrolled fermented state, as well as in its probiotic-influenced fermented state. For pictorial comparison, cookies developed from whole wheat flour was used as control.

Plate 1: Cookies developed from air potato flour and conventional whole wheat flour. **KEY: RAPF1 =** cookies produced from unfermented air potato flour; **UAPF1 =** cookies produced from uncontrolled fermented air potato flour; and **PAPF1** = Cookies produced from probiotic-fermented air potato flour.

For PCA, only the first two principal components are represented and illustrates the correlations between the parameters of sensory analysis. The loading plot for whole wheat flour cookies (WWFC) (Figure 4a) depicted the liaison of these parameters with each other. The first two PCs explained 81.98% of the total variance (PC1 = 63.47%, PC2 = 18.51%) for the evaluated characteristics. Texture and aroma were closely located on the same side of the loading plot indicating that they are contributors of the organoleptic quality of WWFC. Although, colour parameter had no close relationship with texture and aroma, it was observed to have the highest impact on acceptability due to its high value on PC2. Figure 4b depicts the loading plot for RAPF2, whose acceptability was only influenced by texture. In fact, only PC1 with a variance of 46.68% was selected. For UAPF2 (figure 4c), the PCA also showed only selected PC1 which explained 56.39% of the total variance. However, it was observed that its acceptability by the taste panelists were mostly influenced by its taste, aroma and texture; hence indicates that they initiated the same effect. For PAPF2 (figure 4d), PC1 (35.56%) and PC2 (28.50%) were selected and accounted for a total variance of 64.06%. Both colour and taste parameters had high PCs, but at opposite sides of each other on the loading plots. Hence, it could be deduced that there was direct positive relation between.

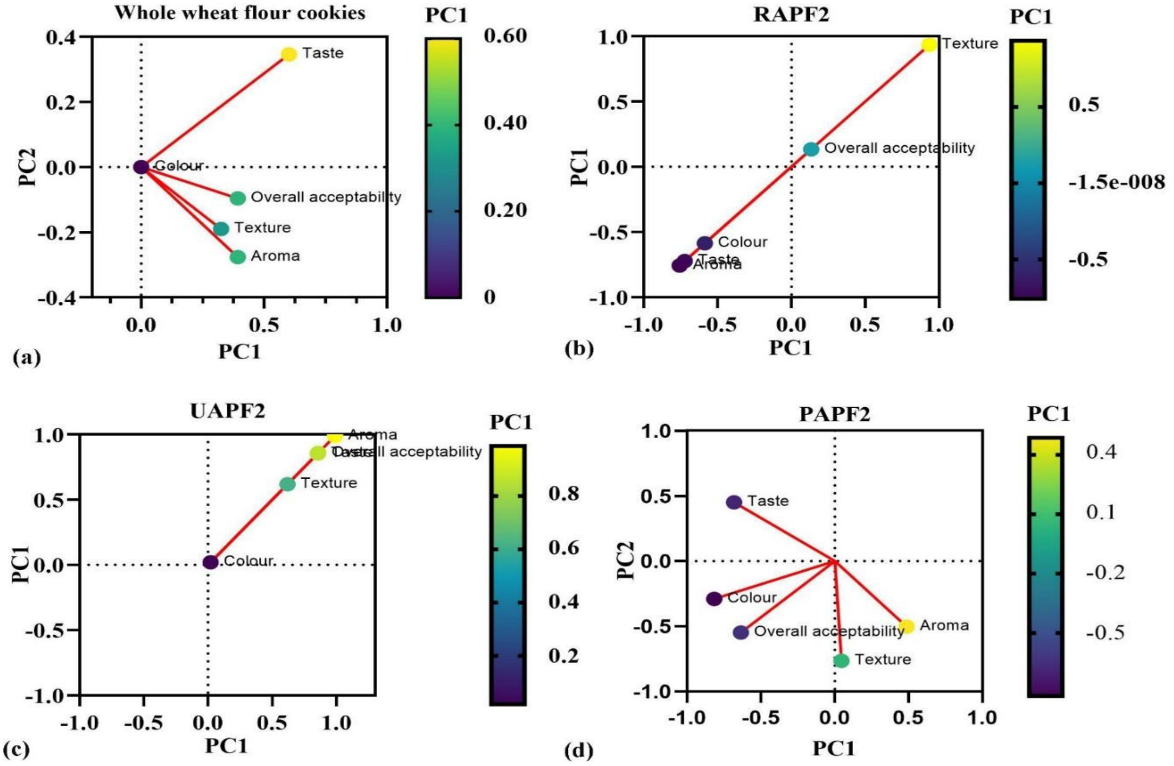

Figure 4: Principal component analysis loading of **(a)** WWFC = Whole wheat flour cookies; **(b)** RAPF2 = Cookies from unfermented air potato flour; **(c)** UAPF2 = Cookies from uncontrolled fermented air potato flour; and **(d)** PAPF2 = Cookies from probiotic-fermented air potato flour based on the sensory characteristics.

Among samples, a comparative evaluation of the sensory characteristics (table 4) showed that the acceptance of colour attributes was least in RAPF2 (3.00 ± 0.68), and was not significantly different ($p>0.05$) from UAPF2 (3.11 ± 0.57). As expected, colour acceptance was mostly preferred in WWFC (5.00 ± 0.00), and was closely comparable to that of PAPF2 at a mean value of 4.67 ± 0.48. In a similar trend, taste attribute was least preferred in RAPF2 (2.56 ± 0.69) and UAPF2 (2.78 ± 0.64); while favorably preferred in WWFC (4.56 ± 0.69).

For aroma, PAPF2 and WWFC were most acceptable without significant differences ($p>0.05$) at 4.44 ± 0.50 and 4.56 ± 0.50 respectively. It has been reported that the addition of probiotics may alter the taste and aroma of the final food product due to production of different metabolites such as organic acids during fermentation and extended storage (Terpou *et al.*, 2019). Hence, the incorporation of a probiotic culture in adequate quantities for the delivery of their health benefits is essential. In texture attribute, the result also showed the most preference in WWFC (4.56 ± 0.50), attributed to its crispiness. The lowest texture preference was observed in PAPF2 at a mean value of 2.44 ± 0.50. This was expected because the probiotic activities caused an increased moisture; hence a reduced crispiness acceptable for cookies. Overall, sensory preference was observed in WWFC (4.67 ± 0.48) and was favourably comparable to PAPF2 (4.67 ± 0.48).

Table 4: Sensory characteristics of air potato cookies

Cookies sample	**Sensory parameters**				
	Colour	**Taste**	**Aroma**	**Texture**	**Overall acceptability**
WWFC	5.00 ± 0.00^a	4.56 ± 0.69^a	4.56 ± 0.50^a	4.56 ± 0.50^a	4.67 ± 0.48^a
RAPF2	3.00 ± 0.68^c	2.56 ± 0.69^c	2.56 ± 0.97^c	3.00 ± 1.07^b	2.56 ± 0.69^b
UAPF2	3.11 ± 0.57^c	2.78 ± 0.64^c	3.00 ± 0.68^b	3.33 ± 0.48^b	2.78 ± 0.64^b
PAPF2	4.67 ± 0.48^b	4.11 ± 0.32^b	4.44 ± 0.50^a	2.44 ± 0.50^a	4.67 ± 0.48^a

Values are means ± standard deviation (n = 36). Mean values on the same row with the same superscript are not significantly different at $p<0.05$. **KEY**: **WWFC** = cookies from whole wheat flour (control); **RAPF2** = Cookies from unfermented air potato flour; **UAPF2** = Cookies from uncontrolled fermented air potato flour; and **PAPF2** = Cookies from probiotic-fermented air potato flour.

4.6 Effect of probiotic influence on proximate composition of air potato flour cookies

The proximate composition of the cookies developed from air potato flour samples are represented in figure 5. The result of the ash content showed that PAPF2 had the highest mean value of 15.68 ± 0.77% and was comparable to that of UAPF2 (14.79 ± 0.49%). Between RAPF1 and RAPF2, no significant differences ($p>0.0001$) were recorded in ash content. For moisture content, PAPF2 had the highest content of 5.67 ± 0.04%; hence, could have a shorter shelf-stability when compared with other cookies in this study. According to Vesterlund, Salminen and Salminen (2012), the regulation of water activity to a low value may offer possibilities for extending the shelf life of dry probiotic products.

Taking into cognizance the crude fibre content of the air potato flour samples, it was observed that the processing conditions of the flour to cookies resulted in the loss of fibre retention. The lowest fibre content was observed in UAPF2 (2.12 ± 0.03%) and was not significantly different ($p>0.05$) from that of PAPF2 (2.13 ± 0.03%). The decrease in the crude fibre content may be attributed to the ability of the probiotics to metabolize the available fibre broken down enzymatically during fermentation which is then utilized as a carbon source (Oboh & Akindahunsi, 2014).

The result of the crude fat content showed the lowest mean value in RAPF1 (17.15 ± 0.10). At varying levels, RAPF2, UAPF2, and PAPF2 were significantly different from the control (RAPF1). The results also showed no significant differences ($p>0.05$) between RAPF2, UAPF2, and PAPF2 (23.72 ± 1.28%, 25.70 ± 1.22% and 27.95 ± 0.48% respectively. Cookies are mostly made with the inclusion of fat. Hence, compared with the control, the increased fat content was expected.

The result of the CHO content showed the lowest mean value in PAPF2 (36.16 ± 2.06%); although, it was not significantly different ($p>0.05$) from those of RAPF2 and UAPF2 (42.13 ± 2.35% and 39.56 ± 1.26% respectively). In addition to CHO, the degradation of proteins is an important factor in the determination of the nutritional and quality characteristics of fermented foods. For crude protein content, no significant differences ($p>0.05$) were observed among all cookie samples. However, PAPF2 had the highest mean value of protein content of 13.65 ± 0.65%, while the lowest mean value (11.50 ± 0.87%) was observed in RAPF1.

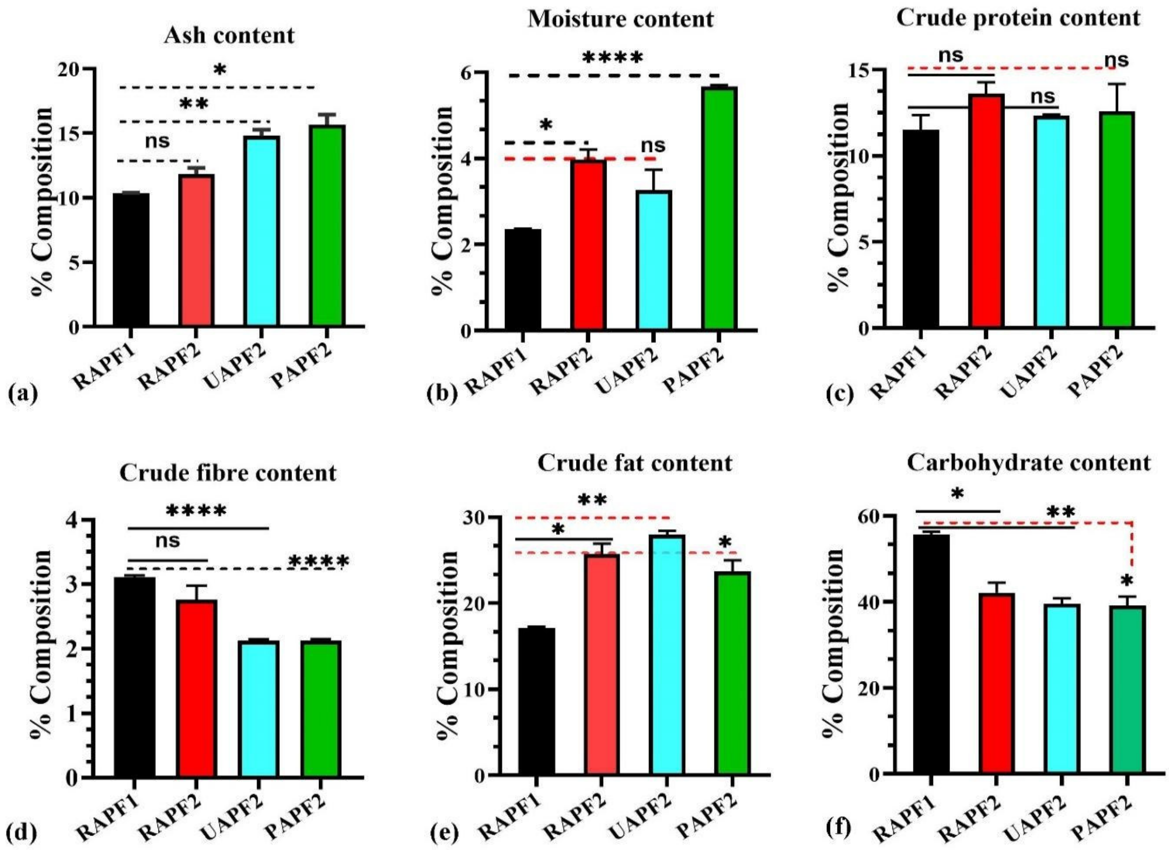

Figure 5: Percentage proximate composition of cookies developed from air potato flour. **(a)** Ash content **(b)** Moisture content **(c)** Crude protein content **(d)** Crude fibre content **(e)** Crude fat content and **(f)** Carbohydrate content. Values are means ± standard deviation of triplicate determinations. **KEY: RAPF1** = Unfermented air potato flour; **RAPF2 =** Cookies from unfermented air potato flour; **UAPF2 =** Cookies from uncontrolled fermented air potato flour; and **PAPF2 =** Cookies from probiotic-fermented air potato flour.. *, **, *** and **** indicates levels of significance at $p<0.0001$ and $p<0.05$, while "**ns**" indicates no level of significance compared with control (RAPF1).

4.7 Effect of probiotic influence on functional characteristics of air potato flour cookies

The functional characteristics of the cookies developed from air potato were evaluated for WAC (figure 6a). WAC is an important parameter to be considered in the preparation of snack and extruded foods as well as baked products. It was observed from the results that probiotic-influenced air potato cookies (PAPF2) had a reduced WAC of 1.54 ± 0.01 g/mL, and was not significantly different ($p>0.05$) from that of the control (1.52 ± 0.02 g/mL). The lowest WAC observed in PAPF2 correlated with the result of its moisture content where it recorded the highest content compared with other samples. According to Singh (2001), water absorption capacity represents the ability of a product to associate with limited water conditions.

The result of the OAC of the cookie samples showed that PAPF2 had the highest capacity of 2.44 ± 0.01 g/mL when compared with RAPF2 (2.04 ± 0.07 g/mL and UAPF2 (1.72 ± 0.02 g/mL). There are speculations that oil absorption capacity has indirect correlation with flavour retention (Omobolanle, Oloyede, Ocheme, Chiemela, & Akpa, 2016). Interestingly, the report of Omobolanle *et al.* (2016) agrees with the findings in this study where PAPF2 recorded the highest value of aroma content observed in organoleptic assessment.

Gelation characteristic is an important functional property of food components that is directly linked to its texture and its viscoelasticity. Among all cookie samples, PAPF2 recorded the highest gelation capacity of 97.10 ± 0.79%. This result does not agree with the report of Ammar *et al.* (2016) that suggests that good gelling characteristics are credited to the presence of carbohydrates and proteins. Here, the results showed that gelling capacity was highest in PAPF2 which also had the lowest carbohydrate content.

Gelation temperature (GT) is an important aspect of the functional qualities of food samples developed from flour that is appropriate for the measurement of the onset of gelation under static and flow conditions (Venkatesan, Singh, & Fogler, 2002). In this study, GT was highest in UAPF2 at a mean value of 77.20 ± 1.28 ^{o}C. A higher gelation temperature could be as a result of greater heat stability of double helices comprised by longer amylopectin chains (Roman, Campanella, & Martinez, 2019). We speculated that, the long amylopectin chains could have increased the heat resistance of microorganisms; hence, a higher gelation temperature. As expected, the lowest GT was observed in RAPF2 (54.73 ± 0.12 ºC). According to Xu, Yan and Feng (2016), raw starch-digesting glucoamylases are capable of directly hydrolyzing raw starch to glucose at low temperatures.

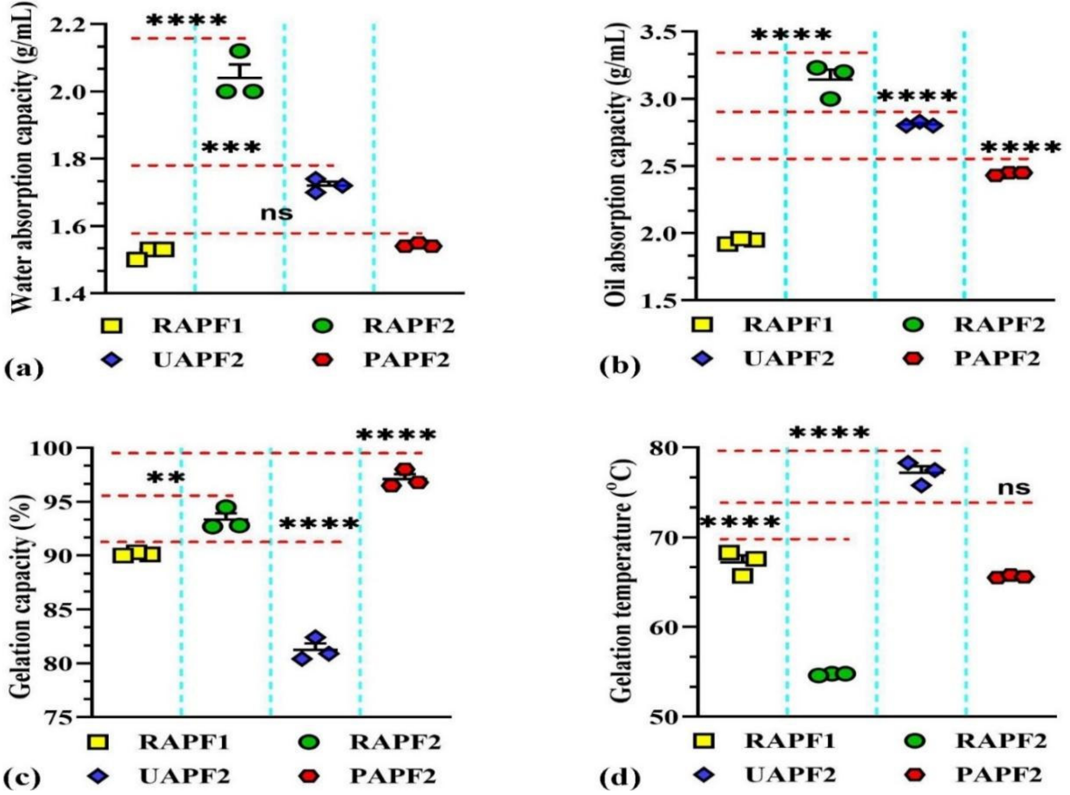

Figure 6: Functional characteristics of air potato cookies. **(a)** Water absorption **(b)** oil absorption capacity **(c)** Gelation capacity and **(d)** gelation temperature. Values are means ± standard deviation of triplicate determinations. **KEY: RAPF1** = Unfermented air potato flour; **RAPF2** = Cookies from unfermented air potato flour; **UAPF2** = Cookies from uncontrolled fermented air potato flour; and **PAPF2** = Cookies from probiotic-fermented air potato flour. *, **, *** and **** indicates level of significance at $p<0.0001$ and $p<0.05$, while "**ns**" indicates no level of significance compared with control (RAPF1).

In the production of pastries, emulsion capacity is importantly considered, since it indicates the maximum amount of oil that can be emulsified by protein dispersion (Enujiugha, Badejo, Iyiola, & Oluwamukomi, 2003; Omobolanle *et al.*, 2016). The emulsification capacity of the air potato cookies (figure 7a) showed no significant differences ($p>0.05$) between RAPF2, UAPF2 and PAPF2 (69.97 ± 0.51%, 69.67 ± 0.95% and 69.97 ± 0.51% respectively). However, the lowest mean value of emulsification capacity was recorded in RAPF1 (54.87 ± 0.40%).

Several researchers had demonstrated that the bulk density of any food depends on the combination of related factors such as intensity of attraction between inter-particle forces, individual particle size and their number of contact points (Khanam, Chikkegowda, & Swamylingappa, 2013). In this study, the result of bulk density of the air potato cookies showed that no significant difference ($p>0.05$) was observed between PAPF2 (0.67 ± 0.03 g/mL), UAPF2 (0.65 ± 0.02 g/mL) and RAPF1 (0.63 ± 0.01 g/mL). Contrary to the report of Baruah *et al.* (2018), our results was not altered by changes in the fermentation characteristic of the air potato flour prior to cookies production.

According to Zhang *et al.* (2022), increased swelling power in fermented samples could be attributed to the modification of starch granules during fermentation, which would consequently lead to higher water uptake by the granules. The result of the swelling index showed the highest mean value in PAPF2 (2.30 ± 0.02%), while the lowest mean value was observed in RAPF1 (1.83 ± 0.03%). Invariably, both spontaneous and controlled fermentation caused significant effects on swelling capacity of the cookies. However, a greater effect was observed caused by probiotic-influenced fermentation.

For foaming capacity, no significant differences were observed among samples in this study. However, the lowest foaming capacity was observed in RAPF2 (8.54 ± 0.03%), while the highest foaming capacity was observed in UAPF2 (16.13 ± 0.47%). In a study, El-Adawy and Taha (2001) reported that foaming capacity could perform as functional agents in many food formulations. Overall, the functional qualities of the air potato cookies were significantly influenced by the fermentation processes. Although, notable influences were observed during uncontrolled fermentation processes, more significant effect was observed in a controlled probiotic-influenced fermentation process.

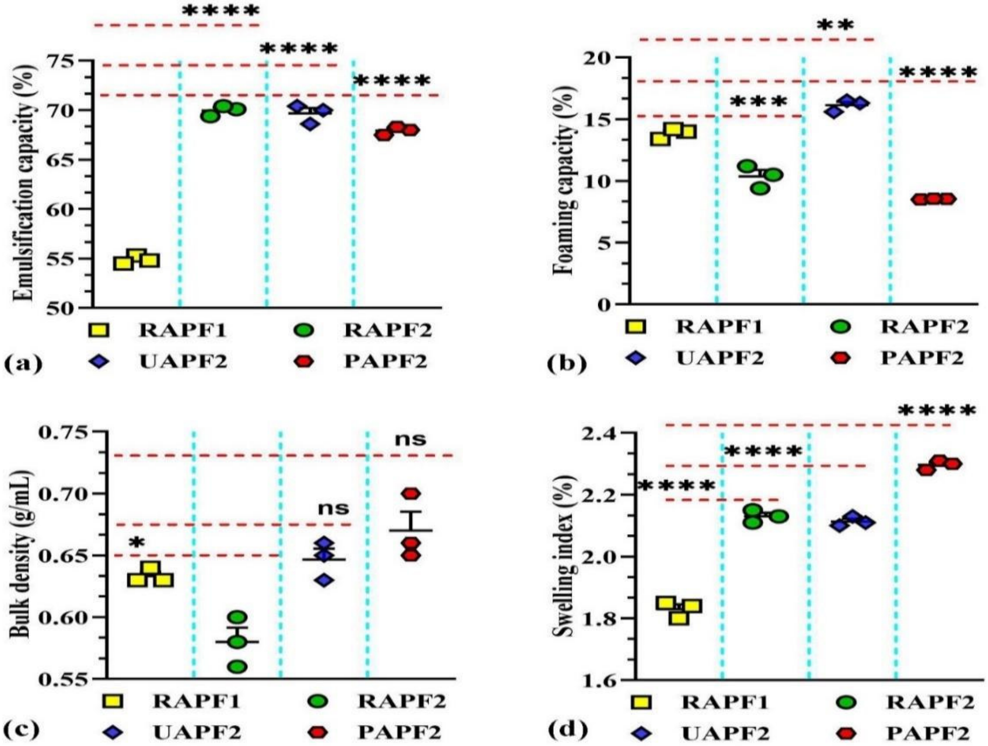

Figure 7: Functional characteristics of air potato cookies. **(a)** Emulsification capacity **(b)** foaming capacity **(c)** Bulk density and; **(d)** swelling index. Values are means ± standard deviation of triplicate determinations. **KEY: RAPF1** = Unfermented air potato flour; **RAPF2 =** Cookies from unfermented air potato flour; **UAPF2 =** Cookies from uncontrolled fermented air potato flour; and **PAPF2 =** Cookies from probiotic-fermented air potato flour. *, **, *** and **** indicates level of significance at $p<0.0001$ and $p<0.05$, while "**ns**" indicates no level of significance compared with control (RAPF1).

5.0 CONCLUSION

The development of probiotic food formulations has become a key research area for the future functional food market; with considerations to the fact that consumers are becoming more health conscious and concerned about the beneficial value of food. In addition, the concept of probiotics as fortifiers in food products of underutilized crops have been an effective tool in developing food products. In this research, it was concluded that cookies prepared from probiotic-influenced fermented air potato flour showed significant results as a result of the enhancement of its nutritional characteristics, organoleptic and functional qualities. However, the crucial point is for probiotics to be maintained in high counts during products shelf life. Hence, for a future aspect of this research, a study on the counts sustained during storage of the flour and cookie products would be necessary.

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Author Contributions

O. J. U., funding acquisition and project administration, investigation, writing-review and editing, data analysis and writing-original draft preparation; I. I., investigation, data analysis; O. A., investigation, data analysis. All authors have read and agreed to the published version of the manuscript.

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