Proximate Composition and Microbial Properties of Cookies produced from Blends of Wheat Flour, Roasted Bambara Nut Flour and Mushroom Flour

Victor-Aduloju A. T., Nnamuchi C. D. And Ojeyinka, O. D.

Department of food science and technology, Enugu State University of Science and Technology.

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***CORRESPONDING AUTHOR**

at.victoraduloju@unizik.edu.ng; 08162828236

ABSTRACT

Cookies produced from blends of wheat flour, roasted bambara nut flour and mushroom flour with 100% wheat cookies as control were evaluated for proximate (Moisture carbohydrates, crude fiber, fat, protein, and ash) and microbial properties (Bacterial count, fungal count and coliform count) using standard methods. The control had the highest moisture content of 10.58% and was significantly different from other samples. The fat ranged from 5.74 - 9.04, ash 3.10 - 5.71, fibre 3.11 - 6.08% and carbohydrate 55.38 - 64.90%. The sample with 65% wheat, 19.40% roasted bambara nut and 15.52% mushroom had the highest value for fat, protein, fiber and ash. The microbial analysis showed that the total viable bacterial count ranged from 2.29 - 4.54 CFU/g, fungal count ranged from 2.19 - 3.10 CFU/g. There was no coliform count in the control and sample 1(67.89 % wheat, 18.25 % roasted bambara nut, 13.86 % mushroom). The results of the cookies sample analyses demonstrate that the combination of wheat, roasted Bambara and mushroom yields a highly nutritious product. However, adherence to proper hygienic conditions during preparation is crucial to maintaining the cookies quality.

INTRODUCTION

Cookies are a beloved treat enjoyed worldwide by people of different ages. From classic chocolate chip cookies to melt-in-the-mouth shortbread varieties. (Taiwo *et al*. 2017). Traditionally, wheat flour forms the 'backbone' of cookie recipes, due to its gluten which provides structure, texture and contributes to the characteristic chewiness or crumbliness depending on the cookie type (Oluwafemi and Seidu, 2017). The basic composition of cookies, primarily consisting of wheat flour, sugar and fat may not align with evolving consumer preferences for healthier food options (Abioye *et al*. 2018).

As a result, there is growing interest in exploring alternative ingredients and formulations to enhance the nutritional profile and sensory properties of cookies. More and more people are looking to low-carb diets with slow-digesting foods and functional ingredients. This creates a challenge for food companies, on how to develop products that meet these needs, especially for people with health problems. In response, researchers are exploring ways to make cookies from blends of flours that don't come from wheat, while also adding beneficial ingredients like fiber and resistant starch. Alternative flours derived from legumes, nuts, and even vegetables offer potential benefit.

Wheat (*Triticum spp*.) remains a crucial cereal crop. As the world's leading cereal crop, wheat plays a major role in human diets, providing a substantial source of energy. Wheat flour is a powder created by grinding wheat kernels. One type, called hard flour, is particularly high in gluten (12-14%) (Nagi *et al*. 2012). This gluten gives hard flour dough a unique elasticity and strength, allowing it to hold its shape well (Chapman *et al.,* 2012). Its dependence on baking industries presents challenges, particularly in tropical regions where it struggles to thrive. This reliance on wheat flour imports creates financial burden and neglect of locally grown alternative flours like roasted Bambara nut flour and mushroom flour. A growing number of people experience adverse reactions to gluten, a protein found in wheat flour, leading to celiac disease or gluten sensitivity (Rubio-Tapia and Sousa, 2023). This has fuelled the demand for gluten-free alternative flour from locally grown crops to surmount the challenges of wheat importation and gluten-intolerance.

Bambara nuts, native to Africa, are gaining recognition for their well-balanced nutrient profile. They are a good source of protein (11-18%), dietary fiber (6-8%), essential minerals (phosphorus, iron, zinc, potassium and calcium), and unsaturated fatty acids like oleic and linoleic. (Adeyeye *et al.*, 2016). Its protein content makes it available as an ingredient for the fortification of baked goods. Bambara nut flour also introduces a subtle nutty flavour that would complement the taste profile of cookies.

Mushroom flour is obtained from dried and grounded mushrooms. It is an emerging functional ingredient with potential health benefits. Mushroom is rich in dietary fiber (β-glucans, chitin), vitamins (B vitamins like riboflavin, thiamine), minerals (potassium, selenium) and bioactive compounds with antioxidant and prebiotic properties (Peter, 2013). The addition of mushroom flour may elevate dietary fiber content, offer unique savoury or umami flavours depending on the mushroom variety, and potentially act as a prebiotic, promoting gut health by stimulating the growth of beneficial bacteria. Peter (2013) investigated the effect of oyster mushroom powder on cookies' nutritional profile, finding an increase in dietary fiber content (up to 7.8%) and a significant rise in potassium levels.

Conventional cookies rely heavily on wheat flour, a source of gluten and carbohydrates. However, there's a growing demand for gluten-free alternatives. This is one of the most challenging tasks in the development of gluten-free products, due to the shortage of alternative ingredients that can imitate the functional properties of wheat protein which are responsible for the viscoelasticity in the wheat dough (Roman *et al.*, 2019). Consumers seek bakery products with increased protein, fiber and other beneficial nutrients this is why using locally available flour to improve cookies nutritional and functional properties is vital in this study. However, as a result of the high cost of importation of wheat into the country and also high exchange rate, reducing reliance on wheat flour and exploring local options can address import costs and promote sustainability (Adebola *et al.*, 2021). Therefore, the challenge lies in developing cookies that minimize wheat flour content while maintaining desirable textural properties and incorporate nutritious flours like bambara nut flour (rich in protein and fiber), Utilize mushroom flour as a potential source of dietary fiber, vitamins, and minerals and to also identify if these cookies possess well-balanced functional and microbial properties.

MATERIALS AND METHODS

Source of raw materials

The wheat flour, bambara nut seed, mushroom (*Pleurotus ostreatus*), and other baking ingredients such as eggs, margarine, salt, sugar, and banking powder that was used for this study were purchased from the Eke Awka market in Awka South Local Government, Anambra State. All materials were carefully packed in clean, lightweight plastic bags to keep them fresh, enhance preservation and transported to the Food Processing Laboratory within the Department of Food Science and Technology at Nnamdi Azikiwe University in Awka, Anambra State, Nigeria. All equipments and reagents used for the experiment were sourced from the Department of Food Science and Technology and they were of analytical grade.

Production of flour

Roasted Bambara nut flour: The Bambara nut flour was produced using the method described by Okarfor *et al*. (2014). The Bambara nut seeds were carefully cleaned and sorted to remove unwanted material after which they were uniformly arranged on trays and roasted at 140°C for 40 minutes using an electric oven (Memmert GmBH, model KG 8540). Subsequently, they were left to cool, it was milled using an Apex mill AM-1, sifted through a 0.4 mm mesh packed into high-density polyethylene bags, sealed and stored in a refrigerator at 4°C until needed.

Mushroom (*Pleurotus ostreatus*) flour

Using the methods described by Mahmud *et al.* (2018), the mushroom was properly washed to remove insects and soil, after which it was dried in a Binatone dehydrator:BDHY-101 at a low temperature of 40°C. Subsequently, the dried samples were finely ground into a powder and sieved using a 2 mm sieve to eliminate clumps. It was then packed into a high-density polyethylene bag and stored at room temperature until needed.

Production of Cookies

The method described by Ndife *et al.* (2014) was used. The wheat flour was weighed according to their varying inclusion of Bambara nut flour and mushroom flour and labelled as samples 1, 2, 3, 4 , 5 and control respectively. The dry components were mixed together (composite flour, sugar, baking powder and salt). Then the wet components were also mixed separately (egg, butter, milk, and flavour). The wet component was then added to the dried component and kneaded, water (30 ml) was also added and the whole mixture was kneaded for 10 min into a consistent dough**.** The dough formed was made into small balls, rolled on a flat board, and cut into shapes, then placed in a greased tray. It was then baked at 160ºC for 15 minutes, cooled, and then packaged.

Method of Analyses

Proximate Analysis

The standard method of AOAC (2015) was used to determine the proximate composition of the samples.

Microbial Analysis

The cookies samples were analysed for bacterial, fungal and coliform count by methods described by Isong *et al.* (2013). The media used for the isolation and enumeration of the microorganisms are nutrient agar (NA), which is used for the isolation and counting of the total viable mesophilic bacterial count, potato dextrose agar (PDA), which is used for the isolation of fungi and eosine methylene blue agar for coliform count according to the manufacturer's instructions. Each medium was sterilised and prepared. 1g of each sample was taken aseptically and blended for 2 minutes in 9 ml of sterile, distilled water. Serial dilutions (1 ml of each dilution) were used to plate fungi and bacteria on potato dextrose agar and plat agar, respectively, in sterile petri dishes. Bacteria were incubated for 24 hours at 37^0C and fungi for 48 - 72 hours at 20^0C. Using a colony counter (Gallenkamp), visible colonies were counted and represented as log CFU/g of the cookies sample.

Statistical Analysis

Data generated from the respective analysis was compiled appropriately and subjected to analysis of variance (ANOVA). The mean of the data was separated using Duncan Multiple Range Test (Statistical Product and Service Solution package version 30)

RESULTS AND DISCUSSION

Proximate Composition of Cookies

The moisture content ranged from 7.72 - 10.58 %. Sample 5 (65.0 % wheat, 19.40 % roasted bambara nut, 15.52 % mushroom) had the least value of 7.72 % while the control sample had the highest value (10.58 %). Significant difference ($p<0.05$) was observed between the control and some of the cookie samples. The result showed that the moisture content was significantly high which can be attributed to the temperature-time combination used during processing. The values (4.53 - 8.97 %) recorded by Duruanyanwu *et al*. (2023) for cookies produced from wheat flour, unripe plantain and *Moringa oleifera* leaf is lower than the values obtained from the recent study. The values (13.45-20.93 %) recorded by Ejemole (2021) for cookies produced from wheat and African yam bean flour is higher than the values obtained from the recent study. Moisture content plays a crucial role in determining the shelf life of foods. Moisture content refers to the amount of water present in a food product, usually expressed as a percentage of the total weight. It is an essential factor because it directly impacts the growth of microorganisms, chemical reactions, and physical changes that occur during storage.

The fat content ranged from 5.79 - 9.04 %. The control sample had the least value (5.79 %) while sample 5 had the highest value (9.04). The result showed that the bambara nut may have contributed to the fat content of the cookie due to its high fat profile. The values (19.30-26.53 %) recorded by Duruanyanwu *et al*. (2023) for cookies produced from wheat flour, unripe plantain and *Moringa oleifera* leaf is higher than the values obtained in this research. Also, the value of fat content observed in cookies produced from wheat and differences ranged from 2.77 - 6.10 % according to Ejemole (2021). The differences can be attributed to the type and composition of raw materials used. Fat content in food refers to the amount of fat present in a particular food product, usually expressed as a percentage of the total weight of the food. Fat is one of the three main macronutrients essential for the human diet, alongside carbohydrates and proteins. It is a concentrated source of energy, providing 9 calories per gram, which is more than double the calories per gram provided by carbohydrates and proteins (4 calories per gram).

The protein content ranged from 12.53 - 17.09 %. The control sample had the least value (12.53 %) while sample 5 had the highest value (17.09 %). Significant difference ($p<0.05$) was observed amongst the analyzed samples. The result showed that the blend of wheat, Bambara nut and mushroom flour significantly increased the protein content of the cookies. The values (0.65-2.98 %) recorded by Ejemole (2021) for cookies produced from wheat and African yam bean flour is lower than the values obtained from this study. Protein is one of the three main macronutrients our bodies need to function properly, the others being fats and carbohydrates. It's vital for building and repairing tissues, including muscles and skin, and it also plays a role in producing enzymes and hormones (Preis *et al*., 2010). Proteins are needed for the growth and development of children, adolescents, and pregnant women.

The fiber content ranged from 3.11 - 6.08 %. The control sample had the least value of 3.11 % while sample 5 had the highest value (6.08 %). Significant difference was observed amongst some samples ($p<0.05$) while some samples did not significantly differ from each other ($p>0.05$). Bambara nut, wheat and mushroom may have contributed the significant increase in fiber content of the cookies. The values (0.65-2.98 %) recorded by Ejemole (2021) for cookies produced from wheat and African yam bean flour is lower than the values obtained from the recent study. The fiber content can be affected by the raw materials used and the combination ratio used for production. It plays a crucial role in maintaining digestive health and offers various other health benefits, such as promoting bowel regularity, managing weight, and supporting heart health (Adesina and Osobamiro, 2012).

The ash content ranged from 3.10 - 5.71 %. The control sample had the least value (3.10 %) while sample 5 had the highest value (5.71 %). There was a significant ($p<0.05$) difference between the analyzed samples. The result showed that blends of wheat, bambara nut and mushroom improved the ash content of the cookies. The result of the determination of the ash content of the different mushroom biscuit sample formulations by Ajayi and Oyetayo (2023) showed that the addition of mushroom to the biscuit composition generally increased the ash content of the resultant biscuit. Ash provides insights into the overall purity and mineral composition of the food product (Marinichi *et al*. 2023).

The carbohydrate content ranged from 55.38 - 64.90 %. Sample 5 had the least value (55.38 %) while the control sample had the highest value of 64.90 %. There was a significant difference between some of the samples ($p<0.05$) while some samples were not significantly different from each other. The results showed that the blends of wheat, bambara nut and mushroom flour increased the carbohydrate content of the cookies. The values (50.77 - 60.10 %) recorded by Ejemole (2021) for cookies produced from wheat and African yam bean flour is lower than the values obtained from the recent study.

Table 1: Proximate Composition of Cookies Samples

Sample	Moisture (%)	Fat (%)	Crude Protein (%)	Fiber (%)	Ash (%)	CHO (%)
Control	$10.58^{a} \pm 0.22$	$5.79^{e} \pm 0.04$	$12.53^{f} \pm 0.45$	$3.11^{d} \pm 0.04$	$3.10^{f} \pm 0.61$	$64.90^{b} \pm 0.33$
1	$8.94^{b} \pm 0.66$	$8.13^{b} \pm 0.12$	$16.11^{b} \pm 0.04$	$5.25^{b} \pm 0.02$	$4.94^{b} \pm 0.98$	$56.65^{e} \pm 0.69$
2	$8.54^{b} \pm 0.01$	$8.35^{b} \pm 0.10$	$15.41^{c} \pm 0.02$	$5.50^{b} \pm 0.41$	$4.44^{c} \pm 61$	$57.52^{d} \pm 0.12$
3	$9.86^{a} \pm 0.06$	$6.51^{d} \pm 0.23$	$14.35^{e} \pm 0.04$	$4.13^{c} \pm 0.04$	$3.68^{e} \pm 0.64$	$67.70^{a} \pm 0.14$
4	$8.72^{b} \pm 0.01$	$7.15^{c} \pm 0.10$	$15.11^{d} \pm 0.18$	$5.35^{b} \pm 0.03$	$3.92^{d} \pm 0.31$	$59.76^{c} \pm 0.14$
5	$7.72^{c} \pm 0.41$	$9.04^{a} \pm 0.04$	$17.09^{a} \pm 0.01$	$6.08^{a} \pm 0.03$	$5.71^{a} \pm 0.31$	$55.38^{e} \pm 1.75$

**Values of Table are mean ± standard deviation of three (3) replicates. Values in the same column bearing different superscript differed significantly ($p<0.05$)*

***Key:** Control sample: 100 % wheat, Sample 1: 67.89 % wheat, 18.25 % roasted bambara nut, 13.86 % mushroom, Sample 2: 75.41 % wheat, 17.47 % roasted bambara nut, 7.12 % mushroom, Sample 3: 81.48 % wheat, 6.10 % roasted bambara nut, 12.42 % mushroom, Sample 4: 71.51 % wheat 11.76 % roasted bambara nut, 16.73 % mushroom, Sample 5: 65.0 % wheat, 19.40 % roasted bambara nut, 15.52 % mushroom*

Microbial Analysis of Cookies

The microbial analysis of cookies produced from blends of wheat, bambara nut and mushroom flour are shown in Table 2. The parameters analyzed were the bacterial count, fungi and coliform count.

The bacterial count ranged from 2.29 - 4.48×10^5 CFU/g. The control sample had the least value (2.29×10^5 CFU/g) while sample 4 had the highest value (4.48×10^5 CFU/g). The bacterial count of cookies obtained from this study is lower than 1.8x10^7- 6.0x10^7CFU/g reported by Eluma *et al*. (2015) for microbial spoilage of meat pies sold in Jos metropolis.

The fungal count ranged from 2.19 - 3.10×10^5 CFU/g. The control sample had the least value (2.19×10^5 CFU/g) while sample 4 (71.51 % wheat 11.76 % roasted bambara nut, 16.73 % mushroom) had the highest value (3.10×10^5 CFU/g). There was a significant ($p<0.0\%$) difference between some of the samples while some samples were not significantly different ($p>0.05$). The result indicated the presence of fungi in all the samples which can be attributed to contamination through air, water, poor handling and poor hygiene during processing. The values (1.00 - 7.00×10^5 CFU/g) recorded by Duruanyanwu *et al*. (2023). For cookies produced from wheat flour, unripe plantain and *Moringa oleifera* leaf is higher than the values obtained from this study. The growth of fungi can occur over a wide range of temperatures and pH and some of these fungi can produce mycotoxins in as reported by Oluwadara *et al.* (2022). Extreme care should therefore be taken to prevent or curb the risk of contamination from any of these sources.

The total coliform count ranged from 1.26-2.22×10^5 CFU/g. Sample 3 and 5 are not significantly different. The result revealed that the control sample and sample 1 were not contaminated by the coliform. Coliform in food is an indication of fecal contamination. Coliform contamination is an indication of fecal matter present in food samples which might have been introduced through water and poor handling during processing.

Table 2: Microbial Analysis of Cookies

Sample	**Bacterial Count (10^5 CFU/g)**	**Fungal Count (10^5 CFU/g)**	**Coliform Count (10^5 CFU/g)**
Control	$2.29^d \pm 0.21$	$2.26^c \pm 0.17$	$0.00^d \pm 0.00$
1	$3.17^d \pm 0.14$	$2.19^c \pm 0.03$	$0.00^d \pm 0.00$
2	$4.23^b \pm 0.03$	$2.41^b \pm 0.04$	$1.26^c \pm 0.06$
3	$4.48^a \pm 0.06$	$2.53^b \pm 0.02$	$2.21^a \pm 0.08$
4	$4.54^a \pm 0.05$	$3.10^a \pm 0.02$	$1.35^b \pm 0.05$
5	$3.41^c \pm 0.04$	$2.25^c \pm 0.06$	$2.22^a \pm 0.03$

Values in the table are mean ± standard of three (3) replicates. Values in the same column bearing different superscripts differed significantly (P>0.05).

***Key:** Control sample: 100 % wheat, Sample 1: 67.89 % wheat, 18.25 % roasted bambara nut, 13.86 % mushroom, Sample 2: 75.41 % wheat, 17.47 % roasted bambara nut, 7.12 % mushroom, Sample 3: 81.48 % wheat, 6.10 % roasted bambara nut, 12.42 % mushroom, Sample 4: 71.51 % wheat 11.76 % roasted bambara nut, 16.73 % mushroom, Sample 5: 65.0 % wheat, 19.40 % roasted bambara nut, 15.52 % mushroom*

CONCLUSION

The study showed that blending wheat flour with bambara nut flour and mushroom flours resulted in cookies with enhanced nutritional value. The proximate analysis showed an increase in protein, fat, fiber, carbohydrate and ash which can be attributed the incorporation of wheat, roasted bambara nut and mushroom in the cookie. Microbial assessments confirmed the presence of spoilage microorganisms which could have been introduced into the food product through poor handling and hygiene, air and fecal matter during processing.

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