

Microbial, Physical and Sensory Attribute Of Cookies Produced From Wheat Flour Fortified with *Termitomyces robustus* and Spiced with Curry Leaves (*Xylopiya aethiopiya*)

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Abstract

Wheat flour was used to substitute mushroom flour at the ratio of 70:30, 50:50, and 30:70. And each category were substituted with spice (*Xylopiya aethiopiya*) which concentration ratio from 5g, 10g, 15g respectively. The cookies prepared without wheat flour and also without mushroom flour serve as positive control. The parameters, thickness, diameter and spread factors were determined with meter rule. Total staphylococcus, bacillus and coliform count were determined using standard form and the effect of spiced (*Xylopiya aethiopiya*) concentration was noted. Consumer preference or otherwise was also determine using a taste panel list. The main quality scores of bacteria count on the cookies ranges at 70:30 (1.5×10^3 , 1.0×10^3 and 9.2×10^2) ranges from highest to lowest for total viable count on nutrient agar, and also (1.0×10^2 , 0.9×10^2 , 0.7×10^1) for fungi count on PDA, (1.1×10^3 , 9.0×10^2 , 6.8×10^2) for *Staphylococcus aureus* count on manitol salt agar, and (1.2×10^3 , 1.1×10^3 , 9.2×10^2) for *Bacillus* count on tryptose soy agar. The physical quality parameters indicate a range of thickness (0.35-0.45), diameter (4.2-4.1) for 70:30, (0.35-0.4), (3.95-3.85) for 50:50, (0.35-0.4), (3.75-3.75) for 30:70. As the spice concentration increases from 5g, 10g, 15g respectively. The mean quality sensory scores of the cookies ranges for 70:30 from: colour (4.0-3.0), flavour (4.0-3.0), taste (3.5-3.0), overall acceptability (4.0-3.5), for 50:50 colour (3.0-3.0), flavour (3.5-3.0), taste (2.5-2.0), overall acceptability (3.0-3.0) and for 30:70 colour (2.0-1.75), flavour (2.0-2.0), taste (2.0-1.75), overall acceptability (2.5-2.0). The result shows a significant difference at probability level $P < 0.05$ as the spice concentration increases for category A and B but C display no significant difference. the production of cookies from wheat flour fortified with (*Xylopiya aethiopiya*) be encouraged to achieve and harvested the preservatives, potential of the spice (*Xylopiya aethiopiya*) and the other medical properties that has been recorded from literature review.

Key Words: *Xylopiya aethiopiya*, Spice, *Termitomyces robustus*, Wheat flour

1. Introduction

Mushrooms are saprophytes. They include members of the Basidiomycota and some members of the Ascomycota. Mushrooms have been a food supplement in various cultures and they are cultivated and eaten for their edibility and delicacy. They fall between the best vegetables and animal protein source (Manjunathan *et al.*, 2011). Mushrooms are considered as source of proteins, vitamins, fats, carbohydrates, amino acids and minerals (Jiskani, 2001). Essential amino acids such as lysine and cytosine are also present as well as water soluble vitamins and minerals in mushroom. The energy value of mushroom varies according to species (Buigut, 2002). Edible mushrooms are used extensively in cooking, because they are highly nutritious, they are rich in protein, vitamins as well as some mineral such as calcium, potassium, magnesium and iron (Jiskani, 2001). Mushroom are good sources of vitamins like riboflavin, biotin and thiamine (Chang and Buswell, 1996). Ogundana & Fagade (1981) indicated that mushroom is about 16.5% dry matter out of which 7.4% is crude fibre, 14.6% is crude protein and 4.48% is fat and oil.

They are also recommended to diabetic and anemic persons, owing to their low carbohydrate and high folic acid content. Some mushrooms are reputed to possess anti-allergic, anti-cholesterol, anti-tumor and anti-cancer (Jiskani, 2001). Mushrooms are now marketed along major highways and urban centers. They are also relatively much cheaper than beef, pork and chicken that contain similar nutrients.

Various parts of the plant have been traditionally employed in different therapeutic preparations. Sometimes, a combination of *Xylopiya aethiopiya* with other plant types or a combination of different parts of *Xylopiya aethiopiya* is used to achieve the desired effects (Fall *et al.*, 2003; Ogunkunle & Ladejobi, 2006).

Preliminary studies have shown that *Xylopiya aethiopiya* fruits contain pharmaceutical constituents such as alkaloids, tannins and flavonoids. The essential oil from various parts of *Xylopiya aethiopiya* has also been well characterized (Kouninki *et al.*, 2007). Several plant lipids have been reported to enhance healing from diverse ailments due to their anti-oxidant and anti-inflammatory properties (Azeb *et al.*, 2004; Motrin, 2005). Essential

oils or their constituents are odoriferous substances from plants and are extensively used as medicinal products, in the food industry as flavors and in the cosmetic industry as fragrances (Evans, 2003). Many of these oils have been shown to exert broad spectrum anti microbial activities (Schelz *et al.*, 2006; Hammer *et al.*, 1996).

Cookies are important food products used in snacks by children and adults in Nigeria. However, school growing children who need more protein for body weight than adults do most commonly relish them. Cookies hold an important position in snack food due to variety in tastes, crispiness and digestibility (Opawale *et al.*, 2011). Cookies are one of the popular cereals foods, apart from bread, consumed in Nigeria. They are ready to eat, convenient and inexpensive food products, containing digestive and dietary principles of vital importance (Kulkarni, 1997). They are nutritive snacks produced from unpalatable dough that is transformed into appetizing product through the application of heat in the oven (Olaoye *et al.*, 2007). In Nigeria, ready-to-eat baked products (snacks) consumption is continually growing and there has been increasing reliance on imported wheat (Akpapunam *et al.*, 1999).

The aim of this work is to produce cookies from wheat fortified with mushroom flour and spiced with *Xylopiya aethiopicia* in order to control the microbial load of the cookies and also to exploit the potential recorded by researchers on the selected mushroom (*Termitomyces robustus*) and the spiced.

The objective of this work is to produce cookies from wheat fortified with mushroom flour and to introduce spice (*Xylopiya aethiopicia*) as a control to microbial contamination of the fortified cookies. To carry out the sensory analysis of the cookies produce from wheat flour fortified with mushroom flour and spiced with *Xylopiya aethiopicia*.

2.0 Materials and Methods

2.1 Materials collection

Fresh mushroom was collected from a commercial seller in Ondo State at Oba market, Owo. Pulverized and oven dry. This was further grinded into powder to make flour. Erinje (*Xylopiya aethiopicia*) was also collected from Oja Ikoko market, sundry and grinded into powder. An egg, butter, sucrose, baking powder e.t.c. was gotten from the market.

2.2 Preparation of composite flours

Flour was prepared from the *Termitomyces robustus* (Mushroom) and was mixed with wheat flour at various proportions. Cookies was produced from the composite flours according to the method of Giami *et al.*, (2004) as shown in the figure 1 and table 1 respectively. The flour was screened through a 0.25mm British Standard sieve (Model B8410).

2.3 Preparation of cookies

The ingredient was carefully weighed butter 20g, sugar 5g, egg 10g, fat 25g and salt 0.1g was mixed together to form a mass. The composite flour and baking powder 0.1g was sieved and added to the sugar-butter-egg-fat-salt mass and was mixed to get a homogenous mass. The cookies were cut out with the aid of cookies cutter having diameter of 36mm and was placed in trays. Baking was done at 225°C for 13minutes. The cookies were allowed to cool at room temperature for 15 minutes (AACC International 2010). *Xylopiya aethiopicia* was added at 5g, 10g and 15g/ 100 g of flour and control was devoid of the spice (*Xylopiya aethiopicia*).

2.4 Physical Analysis

The weight of the cookies was measured by weighing on a weighing balance (Model Mettler PE 1600, Mettler Instruments Corporation, Greifensee, Zurich, Switzerland) with an accuracy of 0.1mg. The diameter was measured with a calibrated ruler as described by Ayo *et al.*, (2007). The spread factor was determined using Ayo *et al.*, (2007) method. Three rows of the two well-formed cookies were made and the thickness was measured as well as arranging the same cookies horizontally edge and the sum of the diameter was measured.

2.5 Sensory Evaluation of the cookies

Sensory evaluation of this research was reported according to Opawale *et al.*, (2011). The organoleptic evaluation of the cookies samples was carried out for consumer acceptance and preference using six untrained common consumers participated in the study. Consumer was randomly selected from Rufus Giwa Polytechnic, Owo metropolis. They were to evaluate the sensory properties based on colour, flavour, taste and overall acceptability using a five point Hedonic scale where one represents “extremely dislike”, two represents “dislike”. Three represents “neither like nor dislike”, four represents “like” and five represents “extremely like”. Means and standard errors of the means (SEM) of replicate scores were determined and subjected to analysis of variance (ANOVA) using the statistical package for social statistics (SPSS version 12).

2.6 Microbial Evaluation of the cookies

The finished cookies sample was powered and 1g was measured and was dissolved in 10ml of sterile water and was centrifuge at 5000rpm for 5minutes. The supernatant was decanted leaving 5ml in the test tube. After thorough mixing 10 fold dilution of each sample homogenate was made and 0.2ml of the dilution (10^{-1} and

10^4) was spread on selected media for the analysis of microbial load Uzuegbu and Eke (2001) employ similar method Umoh *et al.*, (2004); Onuorah and Akinjede (2004). All media was prepared according to manufacturer direction and autoclave at the temperature of 121°C for 30minutes at 15Ib pressure.

3.0 Results and Discussion

Table 1 above shows the main total viable count of the microbial isolates on general purpose media and selective media. For all the categories A, B and C of the cookies produced at a ratio of wheat flour: mushroom flour/ 70:30, 50: 50 and 30:70 respectively, increase in the spice concentration induced a noticeable reduction in the microbial load.

Total viable count as the name suggest it is a count of the total number of living bacteria in a sample, however it should be correctly referred to as on aerobic colony count at 30°C. Any anaerobic organisms (that is bacteria that will not grow in the presence of oxygen) will not be recovered and likewise 30°C incubation temperature may be too warm or too cold for certain bacteria to grow. Despite these limitations, in normal food samples it is used as a measure of microbiological quality with respect to levels of general bacteriological contamination.

Total viable count reflects the conditions in which the food was produced, stored or abused with experience, this count can be used to predict the shelf life or keeping quality of the product. The spoilage of many foods may be imminent when the total viable count reaches 10-100 million per gram of the product.

The reduction in the total viable count of the microbial isolates on nutrient agar was attributed to increase in the concentration of the spice *Xylopi aethiopica* and it has been recorded that *Xylopi aethiopica* has antimicrobial properties (Fleischer *et al.*, 2008).

The total microbial load on nutrient agar range from 9.0×10^2 to 1.5×10^3 cfu/g compare with the microbiological standards of fortified blended foods, total viable count TVC < 100,100 cfu/g. The result is still within acceptable value.

Manitol salt agar was used as selective media to support the growth of *Staphylococcus aureus*. The salt in the manitol salt agar is known to inhibit the growth of any other organisms apart from *Staphylococcus aureus*. The corresponding increase in the spice concentration also lead to corresponding decrease in *Staphylococcal* count at each category. It has been recorded that the ethanolic crude extracts of the selected spice (*Xylopi aethiopica*) has a significant antimicrobial effect on *Staphylococcus aureus* (Fleischer *et al.*, 2008). *Staphylococci* exist in air, dust, water, food or on food equipment, environmental surface, humans and animals. Human and animals are the primary reservoirs. Although food handlers are usually the main source of food contamination (Chris *et al* 1997). Spice reduction in *Staphylococcus* counts ranges from 6.8×10^2 to 1.8×10^3 at the concentration of 5g, 10g and 15g respectively. These values, compare with the microbiological standard of staphylococcus count for fortified blended foods, whole wheat flour (TVC <10 cfu/g). Are not within the range of acceptability

The total *Bacillus* counts in all the three categories are more than 10cfu/g. These shows that the cookies produced are not acceptable compared with the standard i.e. the contamination level is not within acceptable value. *Bacillus cereus* has ubiquitous distribution in the environment and can be isolated from a variety of processed and raw foods. However, its presence in foods is not a significant health threat unless it is able to grow. Consumption of food containing more than 10^5 viable cells/g has resulted in outbreaks of food borne illness. Tryptose soy agar was used as a selective media for bacillus count. The increase in spice concentration lead to significant decrease in total *Bacillus* count. These show that the spice (*Xylopi aethiopica*) has an antimicrobial effect on the organism. The presence of *Bacillus* species could also be attributed to the contamination of raw material from the farm. It is also a popular thermophilic organism that could be brought with raw materials into the factory. The slighted industrial faults would permit them into the final stage of production since they are thermostable (Giwa *et al.*, 2011).

The total fungi count on PDA ranges from 3.1×10^2 to 1.0×10^3 cfu/g these range is also acceptable has compared to the standard (1,000 cfu/g). Both yeasts and molds cause various degrees of deterioration and decomposition of foods. They can invade and grow on virtually any type of food at any time; they invade crops such as grains, nuts, beans and fruits in fields before harvesting and during storage. They also grow on processed foods and food mixtures. Their detectability in or on foods depends on food type, organisms involved and degree of invasion, the contaminated food may be slightly blemished, severely blemished or completely decomposed, with the actual growth manifested by rot spots of various sizes and colors unsightly scabs, slime, white cottony mycelium, or highly colored sporulating mold. Abnormal flavors and odors may also be produced.

The total coliform count on EMB in all the three categories is nil. These shows that the cookies produced is acceptable and it reflect hygiene standards adopted in the food preparation improper processing, handling and storage can allow the level to increase. Coliforms are also found on many types of plant material since the organisms are usually found at high levels in soil.

Coliforms are often referred to as “indicator organism”. In and of themselves they are not pathogenic; however their presence in the environment can indicate that conditions are favorable for pathogens to be present. Generic

E. coli is also a coliform; but of fecal origin. The presence of generic *E. coli* in a sample indicates fecal contamination. The total coliform *E. coli* test is a fast inexpensive way to assess the cleanliness of an environment or food, and can also be used to glean information regarding the potential for other contamination.

As the ratio of mushroom flour increases, there is a corresponding increase in spread factor. Decrease in spread factor with increase in wheat flour shows that starch polymer molecules are more tightly bound with granules and swelling is limited in the cookies with wheat flour when heated (Oluwanukomi *et al.*, 2010).

Considering category A (Wf:Mf/70:30) there is a decrease in spread factor from 1.2×10^3 to 8.7×10^2 and later increase to 9.1×10^2 in the order of 5g, 10g, 15g concentration of the spices. Considering category B (Wf:Mf/50:50) there is a stationary state decrease in the spread factor with spice concentration 5g and 10g having 1.1×10^3 and later decrease to 9.6×10^2 in the order of 15g concentration of the spices. Category C (Wf:Mf/30:70), there is a corresponding decrease in the spread factor as the spice concentration increases in the following order (5g,10g,15g) from 1.1×10^3 , 1.0×10^3 and 9.4×10^2 . The decrease in the spread factor value for category A, B, C simply shows that the starch polymer molecules are tightly bound with granules as the spice concentration increases. The above result is similar to the findings of Mridula *et al.*, (2007) that spread factor decreased significantly with increase in proportion of sorghum flour

The increase sensory scores are presented in table 3. The increase in the concentration of the spice (5g, 10g, 15g) in category A (70:30/Wf: Mf) leads to the significant different at probability level $p < 0.05$ using analysis of variance as a statistical tool. The cookies produce in this category A were acceptable by the panel using Hedonic scales

Sample from the cookies prepared from wheat flour: mushroom flour composite of category B (50:50) show significant different at probability level $P < 0.05$ using analysis of variance as a statistical tool. The increase in the concentration of the spice has significant changes on the various organoleptic parameters but the various organoleptic parameters produced there effect in a significantly different way. The cookies produce under this category were partially accepted at the concentration of 5g, 10g, and 15g of the spice.

Samples from the cookies prepared from wheat flour: mushroom flour of category C (30:70) show a non-significant difference at the probability level $p < 0.05$ using analysis of variance as a statistical tool. This shows that the increase in the concentration of the spice has non-significant changes on the various organoleptic parameters. The cookies produce under category C was out rightly rejected by the panel using Hedonic scale.

4 Conclusion

It can be concluded that the spice has a positive effect on the microbial attribute of the produced cookies as well as on the physical and sensory parameters measured. The introduction of the spice in the production of cookie should be encourage as it could improve the shelf life of the product without negative effect on the physical and sensory attribute.

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Table 1: Microbial evaluation of wheat flour: mushroom flour composite cookies (cfu/g)

WF:MF	Category	Spice Quantity	Nutrient Agar	Potatoes Agar	Dextrose	Eosine Blue	Methylene	Manitol Agar	Salt	Tryptose Agar	Soy
70:30	A	5g	1.5x10 ³	7.0x10 ²		Nil		1.1x10 ³		1.2x10 ³	
		10g	1.0x10 ³	4.0x10 ²		Nil		9.0x10 ²		1.1x10 ³	
		15g	9.2 x10 ²	3.1x10 ²		Nil		6.8x10 ²		9.2x10 ²	
50:50	B	5g	1.8x 10 ³	8.4x10 ²		Nil		1.4x10 ³		1.5x10 ³	
		10g	1.2x10 ³	5.0x10 ²		Nil		1.2x10 ³		1.2x10 ³	

		15g	9.0×10^2	4.0×10^2	Nil	8.5×10^2	8.5×10^2
30:70	C	5g	2.1×10^3	1.0×10^3	Nil	1.8×10^3	1.7×10^3
		10g	1.3×10^3	8.3×10^2	Nil	1.4×10^3	9.0×10^2
		15g	1.0×10^3	5.5×10^2	Nil	1.2×10^3	5.4×10^2
100:0	M		1.0×10^3	6.0×10^2	Nil	1.2×10^3	2.5×10^3
0:100	W		8.0×10^2	4.0×10^2	Nil	1.0×10^3	9.0×10^2

Table 2: Physical analysis of wheat flour: mushroom flour composite cookies

	Spice quantity	Diameter	Thickness	Spread Factor (sf)
WF:MF				
	5g	4.1 ± 0.28	0.35 ± 0.07	1.2×10^3
70:30	10g	3.9 ± 3.37	0.45 ± 0.70	8.7×10^2
	15g	4.1 ± 0.14	0.45 ± 0.70	9.1×10^2
	5g	3.95 ± 0.21	0.35 ± 0.07	1.1×10^3
50:50	10g	4.2 ± 0.28	0.4 ± 0.14	1.1×10^3
	15g	3.85 ± 0.21	0.4 ± 0.14	9.6×10^2
	5g	3.75 ± 0.21	0.35 ± 0.21	1.1×10^3
30:70	10g	3.55 ± 0.07	0.35 ± 0.70	1.0×10^3
	15g	3.75 ± 0.07	0.4 ± 0.14	9.4×10^2

100:0	M	3.55±0.07	3.0±0	1.1x10 ²
0:100	W	3.65±0.07	3.0±0	1.2x10 ²

Table 3: Sensory evaluation of wheat flour: mushroom flour composite cookies

	Spice quantity	Colour	Flavour	Taste	Overall acceptability
WF:MF					
70:30	5g	4.0±0	4.0±0	3.5±0.71	4.0±0
	10g	4.0±0	4.0±0	3.5±0.17	4.0±0
	15g	3.0±0	3.0±0	3.0±0	3.5±0.71
50:50	5g	3.0±0	3.5±0.17	2.5±0.17	3.0±0
	10g	3.0±0	3.5±0.71	2.5±0.71	3.0±0
	15g	3.0±0	3.0±0	2.0±0	3.0±0
30:70	5g	2.0±0	2.0±0	2.0±0	2.5 ±0.71
	10g	2.0±0	2.0±0	1.75,±0.35	2.0±0
	15g	2.0±0	2.0±0	1.75±0.35	2.0±0
100:0	W	5.0±0	5.0±0	5.0±0	5.0±0
0:100	M	1.5±0.71	1.5±0.71	1.5±0.71	1.5±0.71

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