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***Research article***

# MICROBIAL, PHYSICAL AND SENSORY ATTRIBUTE OF COOKIES PRODUCED FROM WHEAT FLOUR FORTIFIED WITH *Termitomyces robustus* AND SPICED WITH CURRY LEAVES (*Murraya koenigii*)

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**ABSTRACT :** Wheat flour was used to substitute mushroom flour at the ratio of 70:30 as category A, 50:50 as category B, 30:70 as category C, and each categories were substituted with spice (*Murraya koenigii)* at concentration ratio of 5g, 10g, and 15g respectively. The cookies prepared without wheat flour and also without mushroom flour serve as positive control. The spread ratio was determined with meter rule: Total *Staphylococcus*, *Bacillus, Coliform* and Fungi count were determined using standard microbiological procedure and the effect of the spice (*Murraya koenigii)* concentration was noted. Consumer preference or otherwise was also determine using a taste parnel list. The mean quality scores of microbial count, *Staphylococcal* count and Fungi count reduces significantly at $p<0.05$ as the spice concentration increases but the *Bacillus* proves resistant to the effect of the spice. The mean quality sensory scores of the cookies range from: colour (3.55 – 3.45), flavour (4.9 – 3.5), taste (4.9 – 3.0) and overall acceptability (5.0 – 3.0) for 70:30. Colour (4.9 – 3.0), flavour (4.0 – 3.5), taste (4.9 – 3.0) and overall acceptability (4.9 – 3.0) for 50:50. Colour (3.5 – 2.0), taste (2.5 – 2.0) and overall acceptability (3.0 – 2.0) for 30:70 respectively. 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 (*Murraya koenigii)* be encouraged to achieve and harvested the preservatives, potential of the spice (*Murraya koenigii)* and the other medical properties that has been recorded from literature review.

**Key Words:** *Murraya koenigii, Spice, Termitomyces robustus,* Wheat flour

## INTRODUCTION

A cookie is a "small flat or slightly raised cake". The sensitivity of these biscuits or cookies properties to deviations in the baking process and in dough ingredient levels has been investigated and it was found that dispersion in cookies weight is primarily due to variability in dough piece weight, which in turn is linked to spatial variations in the thickness of the dough sheet from which the pieces are cut (Kevin and Claudia, 2000). Cookies moisture content is sensitive to fluctuations in oven temperature and dough piece moisture content, though at a level that may not be commercially significant (Kevin and Claudia 2000). Aladekoyi and Shakpo (2011) suggested the appreciable quality of *Termitomyces robustus* due to their high protein content, ascorbic acid and mineral contents with low fat value. Mushroom (*Termitomyces robustus*) produce a wide range of secondary metabolites with high therapeutic value (Demain 1999). Health promoting properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immuno-stimulatory effect, have been reported for some species of mushroom (Barros *et al.*, 2007). Both fruiting body and the mycelium contain compounds with wide ranging antioxidant and antimicrobial activities (Ferreira *et al*., 2007). In an earlier study, Sua *et al.*, (2000) reported that mushroom may be a source of new anti-microbial capable of inhibiting microorganisms that are resistance to common antibiotics.

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*Murray koenigii* (curry leaves) belonging to the family of Rutaceae is used as a spice for its characteristics flavour and aroma. It is reported to have antioxidant, anti-hypoglycaemic and antimicrobial activities (Ningappa *et al.*, 2008). Arulselvan and Subramanian (2007); reported that, when man is increasing complexities for himself by adding to free radicals nature is helping him out by bestowing natural antioxidants which inactivate the free radicals and stop the detrimental action. These antioxidants stop unwanted oxidation in the body which involve the formation of free radicals and further deteriorate the condition of the body. Phytochemicals are also present in this leaves such as beta carotene, ascorbic acid (vitamin C), folic acid, and vitamin E (Gruenwald and Thomson 2004). *Muraya koenigii* is cultivated domestically for its aromatic leaves, popularly called curry leaves, which are extensively employed as spices for flavorings of curries and chutneys in southern India. The leaves are part of seasoning of almost every savory preparation as well as pickles. The leaves root and bark are considered to be a tonic, stomachic and carminative. Vegetables are importance sources dictary fiber carotenoids, vitamins and phenolics for both infant and adults. Increased in talic of vegetables is generally associated with a reduced risk of cancer and cardiovascular disease (De Azevedo and Rodriguez, 2005; Kris – Etherton *et al*., 2002). The novelty of our study lies in the comprehensive bioanalysis of exotic leaves in terms of their component nutrients so that they can be used as national and inexpensive treatment alternatives to synthetic antioxidant drugs. In addition, the study contributes to new information about this leaf being good sources of nutrients and as antimicrobials (FSIS/USDA, 2009).

The present research work aims and objectives are to produce cookies from wheat flour fortified with mushroom flour and spice with curry leaves (*Murraya koenigii)* to serve as control to microbial contamination of the fortified cookies and to explore the other organoleptic properties of the spice.

## MATERIAL AND METHOD

### Sample Collection

Fresh mushroom was collected from Oja Oba market, pulverish, oven-dried and grinded to fine flour. Fresh leaves of *Murraya koenigii* was collected from farm Owena in Ondo town and it was carefully washed in running tap water, then rinsed in sterile distilled water. The leaves was sun-dried and it was well dried in fumes cabinet before it was grinded into fine powder by using steriled mortar and pistil. These provided powders were stored in airtight glass container, which protected it from the sunlight.

### Sample collection for baked flour ingredient

Wheat flour, baking powder, sugar, eggs, butter, milk, salt etc were all gotten from the market, Oja Oba in Owo, Ondo-State.

### Preparation of composite flours

Flours were prepared from *Termitomyces robustus* (mushroom) and were mixed with wheat flour at various proportions. Biscuit cookies were produced from the composite flours according to the method of Giami *et al*, (2004); as shown in Figure 1 and Table 1 respectively. The flours were screened through a 0.25mm British standard sieve (model Bs 410).

### Preparation of Cookies

The ingredient will be carefully and accurately weighted (Butter 20g) (Sugar 5g) and (Eggs 10g) will be mixed together to form a mass. The composite flour and baking powder (0.1g), milk (25g) was sieved and added to the butter, sugar egg mass and mixed to get a homogenous mass. The butter, the cookies was cut out with aid of cookies cutter having diameter of 86mm and placed in trace. Baking was done at $225^0$C for 13minutes the biscuit was allowed to cool at room temperature for 15minutes (AACC International 2010, Ihekoronye, 1999). (Curry leave powder was added at ratio 5, 10, 15g/100g of flour of the experiment) and the control flour was devoid of the spice (curry leaves).

### Microbial Evaluation

The finished biscuit cookies sample was powdered and 1g was measured and dissolved in 10 ml of sterile distill water and centrifuged at 5000vpm for 5minutes.

The supernatant was decanted leaving 5ml in the test-tube. After, through mixing 10ml dilution of each sample, homogenate was made and 0.2 of dilution ($10^{-1}$and $10^{-4}$) was spread on selected media for the analysis of microbial load. (Uzuegbu and Eke, 2001), employed similar method. Umoh *et al*., (2004), Onuorah and Akijede (2004). All media was prepared according to manufacturer direction and autoclaved at 121$^0$C for 30minutes at 151b pressure.

**Sensory Evaluation**

Sensory evaluation was carried out according to Opawale *et al.*, (2011). Six common consumers participated in this study. Consumers were randomly selected from RUGIPO metropolis.

Each consumer evaluate the samples for usual puffiness, appearance/colour, odour /aroma, taste, overall texture/mouth feel and overall liking on 9 points. Hedonic scale (1=dislike extremely, 2=dislike, 3= neither like nor dislike, 4 = like, 5 = like extremely).

## The Mean Statistical Analysis

The value of result was obtained in duplicate and was used to calculate the mean ± standard deviation. The data obtained were statistically analyzed using analysis of variance (ANOVA).

**RESULTS AND DISCUSSION**

Table 1 above shows the main total viable count of the microbial isolates on general purpose media (nutrient agar) and selective media (manitol soy agar, Tryptose soy agar, Eosin methylene blue agar, bacterial count and potato dextrose agar for fungi count). For all the categories (A, B and C) of the cookies produced at a ratio of WF: MF, 70:30, 50:50 and 30:70 respectively, increase in the spice concentration induced a noticeable reduction in the microbial load.

**Table 1 Microbial evaluation of cookies produced from wheat flour fortified mushroom flour spiced with composite cookies**

| Wheat: Mushroom | Category | Spice | N.A | M.S.A | T.S | P.D.A | EMB |
|---|---|---|---|---|---|---|---|
| 70%WF:30%MF | A | 5g | 1.5x$10^3$ | 2.0x$10^3$ | 8.0x$10^2$ | 1.6x$10^3$ | Nil |
| | | 10g | 1.0x$10^3$ | 1.2x$10^3$ | 1.5x$10^3$ | 6.0x$10^2$ | ,, |
| | | 15g | 2.8x$10^2$ | 1.0x$10^3$ | 2.0x$10^3$ | 2.5x$10^2$ | ,, |
| 50%WF:50%MF | B | 5g | 1.6x$10^3$ | 2.5x$10^3$ | 1.8x$10^3$ | 1.7x$10^3$ | ,, |
| | | 10g | 1.2x$10^3$ | 1.8x$10^3$ | 1.0x$10^3$ | 6.0x$10^2$ | ,, |
| | | 15g | 7.0x$10^2$ | 9.0x$10^2$ | 8.0x$10^2$ | 4.0x$10^2$ | ,, |
| 30%WF:70%MF | C | 5g | 2.0x$10^3$ | 1.8x$10^3$ | 9.0x$10^2$ | 1.5x$10^3$ | ,, |
| | | 10g | 2.0x$10^3$ | 1.3x$10^3$ | 1.6x$10^3$ | 5.0x$10^2$ | ,, |
| | | 15g | 1.0x$10^3$ | 1.2x$10^3$ | 2.1x$10^3$ | 3.0x$10^2$ | ,, |
| Control | | | | | | | |
| 100:0 →0% | | W | 1.0x$10^3$ | 1.2x$10^3$ | 2.5x$10^3$ | 6.0x$10^2$ | ,, |
| 0:100 → 100% | | M | 8.0x$10^2$ | 1.0x$10^3$ | 9.0x$10^2$ | 4.0x$10^2$ | ,, |

Total viable counts on nutrient agar reflect the hygienic conditions in which the cookies were produced. The 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 product.

The total microbial load on nutrient agar ranges from $2.8x10^2$ to $1.5x10^3$ cfu/g compare with the microbiological standards of fortified blended goods, total viable counts < 100,000 cfu/g. The result is still within acceptable value. Significant reduction in the total viable count of the microbial isolates on nutrient agar was attributes to increase in the concentration of the spice *Murraya koenigii.* It has been recorded that, *Murraya koenigii* has antimicrobial properties (Ningappa *et al*., 2008) and (De Azevedo *et al.,* 2005).

**TABLE 2 Physical analyses of cookies produced from wheat flour fortified mushroom flour spiced with *Murraya koenigii.***

| WHEAT / MUSHROOM | Category | DIAMETER | THICKNESS | SPREAD FACTOR (SF) |
|---|---|---|---|---|
| 70:30% | A | | | |
| 5g | | 3.55±0.07 | 0.2±0.14 | 1.775x103 |
| 10g | | 3.55±0.07 | 0.25±0.07 | 1.420x103 |
| 15g | | 3.45±0.07 | 0.3±0 | 1.150x103 |
| 50:50% | | | | |
| 5g | B | 3.75±0.35 | 0.25±0.07 | 1.500x103 |
| 10g | | 3.65±0.07 | 0.35±0.07 | 1.043x103 |
| 15g | | 3.6±0.28 | 0.35±0.07 | 1.029x103 |
| 30:70% | C | 3.65±0.07 | 0.35±0.07 | 1.041x103 |
| | | 3.45±0.21 | 0.4±0 | 8.63x102 |
| | | 3.45±0.21 | 0.4±0 | 8.63x102 |
| Control | | | | |
| 100:0% | | 3.45±0.07 | 0.35±0.07 | 0.986x103 |
| 0:100% | | 3.40 ±0.14 | 0.4±0 | 0.925x103 |
| | | | | |

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 (*Murraya koenigii)* concentration also leads to corresponding decrease in *Staphylococci* counts at each category respectively. It has been recorded that the antimicrobial activity of *Murraya koenigii* leaf extract has been shown to be better compared to other leaves. The leaf extract can possibly used in place of synthetic chemicals. The antimicrobial nature of this leaf extract can also find applications in several food systems as an antimicrobial preservative which can also prolong shelf life stability (Onyeke, 2003). Staphylococci exist in air, dust, water food or on food equipment, environmental surface, human and animals. Human and animals are the primary reservoir although food handlers are usually the main source of food contamination. Staphylococcus counts ranges from $9.0x10^2$ to $2.0x10^3$ at the concentration of 5g, and 15g respectively. These values, compare with the standards of staphylococcus counts for fortified blended foods, whole wheat flour (<10cfu/g), shows that the work value is higher than the standard value, due to the contamination of the product during processing. The increase in spice *Murraya koenigii* leads to significant increase in total *Bacillus* count on tryptose soy agar. This shows that the spice *Murraya koenigii* has no significant antimicrobial effect on the organism. The presence of *Bacillus* species could also be attributed to the contamination of raw material. It is also a popular thermophile 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 *Bacillus* counts in all the A,B,C categories are all within the range of 10 – 20cfu/g, these shows that the cookies produced are acceptable compare with the standard. That is, the contamination level is still acceptable. *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 resulted in outbreaks of borne illness.

**Table 3 Sensory evaluation of cookies produced from wheat flour fortified mushroom flour spiced with *Murraya koenigii*.**

| Wheat flour/Mushroom | Category | Spice | Colour | Flavour | Taste | Overall acceptability |
|---|---|---|---|---|---|---|
| 70:30% | A | 5g | 5.0±0 | 4.9±0.71 | 4.9±0.71 | 5.0±0 |
| | | 10g | 4.9±0.71 | 4.0±0 | 4.9±0.71 | 4.9±0.71 |
| | | 15g | 3.5±0.71 | 3.5±0.71 | 3.0±0 | 3.0±0 |
| 50:50% | B | 5g | 4.9±0.71 | 4.0±0 | 4.9±0.71 | 4.9±0.71 |
| | | 10g | 4.0±0 | 4.0±0 | 4.9±0.71 | 4.0±0 |
| | | 15g | 3.0±0 | 3.5±0.71 | 3.0±0 | 3.0±0 |
| 30:70% | C | 5g | 3.5±0.71 | 3.5±0.71 | 2.5±0.70 | 3.0±0 |
| | | 10g | 2.5±0.70 | 3.0±0 | 2.5±0.7 | 2.0±0 |
| | | 15g | 2.0±0 | 2.0±0 | 2.0±0 | 2.0±0 |
| Control | | | | | | |
| 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 |

The total fungi count on P.D.A ranges from $1.7x10^3$ to $6.0x10^2$ cfu/g, these range is also acceptable has compared to the standard (1,000cfu/g). Both yeast 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 food depends on food type, organisms involved and degree of invasion. The contaminated food may be slightly blemished, severaly blemished, or completely decomposed, with the actual growth manifested by not spots of various sizes and colours, unsightly scabs, slime, white cotton mycelium, or highly coloured sporulating mold. Abnormal flavours and odours may also be produced. Diameter, thickness and spread ratio of the cookies produced from WF fortified with WF at different concentration of spice was used to analysed the physical properties. As the ratio of mushroom flour increase there is a corresponding decrease in spread factor of the cookies this show that starch polymer molecules are more highly bound with granues and swelling is limited in the cookies with wheat flour when heated (Oluwamokomi *et al.,* 2010). Considering category A, (WF:MF/70:30) there is a corresponding decrease in the spread factor as the spice concentration increase in the following order (5g, 10g, 15g) $1.775x10^3$, $1.420x10^3$ and $1.150x10^3$. Category B, (WF:MF/50:50) also showed a spread factor decrease from $1.500x10^3$ to $1.043x10^3$ and $1.029x10^3$ as the spice concentration increases, in the following order (5g, 10g, 15g) as well as category C, (WF:MF/30:70) which also follows similar pattern. As the spice concentration increase, the spread ratio decrease per each category. The decrease in the values of spread factor simply shows that the starch polymer molecules are more tightly bound with granules and swelling is limited in the cookies with wheat flour when heated. The above results support the findings of (Mridula *et al*; 2007) that spread ratio decrease significantly with increase in proportion of sorghum flour addictives to wheat flour. The sensory evaluation of the of the cookies with the following organoleptic property (colour, flavor, taste, and overall acceptability) produce in WF:MF/70:30, 50:50, 30:70 fortified with spice concentration of 5g, 10g, 15g reveals different significant variability. The increase in the concentration of the spice (5g, 10g, 15g), category A (70:30/WF : MF) leads to the significant different at probability level $p< 0.05$ whereas there is no significant different on the probability level $p<0.05$, as the spice concentration increase. This simply means that the increase in the concentration of the spice of category A, those not have any effect on the cookies produced at WF: MF/70:30%. Samples from the cookies prepared from WF: MF composite flour of category B (50:50) shows non-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 a significant change on the various organoleptic parameters but the various organoleptic parameters produced there effect in a significant different way.

The biscuit produced under this category were partially accepted at the 5g concentration of the spice (curry leaf) but, for 10g, and 15g there was neither like nor dislike and dislike respectively. Samples from the cookies prepared from WF: MF category C (30:70) showed a non-significant different at the probability level p< 0.05 using analysis of variance as a statistic tool. This showed that the increase in the concentration of the spice has a non-significant change on the various organoleptic parameters. The cookies produced under category C where out rightly rejected by the panel hedonic scales.

## 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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