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# Microbiological and Sensory Quality of Tigernut Composite Flour

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**ABSTRACT**

This study investigated microbial load and sensory quality of locally produced tigernut-based composite flour. Microbial quality such as total viable count (TVC), presence of yeast and mold, *E. coli*, *Salmonella* spp and *Staphylococcus aureus* were determined. These were compared to the microbiological specification for composite flours. The sensory quality of the composite powders such as colour, taste, aroma, mouthfeel and overall acceptability were also determined. Mean counts of the TVC and *Staphylococcus aureus* counts ranged between 3.31 to 3.22 and 2.21-2.58 $\log_{10}$ cfu/g respectively. *Salmonella*, *E. coli* and yeast and molds were not detected in any of the samples analyzed. Mean sensory scores ranged between 3.2±1.32-1.8±0.63 for colour, 3.1±1.10-2.4±0.52 for taste, 3.2±1.32-2.3±1.06 mouthfeel, 3.1±0.99-2.1±0.57 for aroma and 5.5±1.96-3.5±2.17 for preference. Significant differences were observed between the samples in terms of colour, aroma and preference. The microbial quality of the tigernut-based composite flour falls within the acceptable standard, hence guarantying the safety of the consumer. The use of composite flour would help to improve the nutritional value of food at presumably cheaper cost since all the raw materials are locally available and does not require special skills for the preparation.



## Introduction

*Cyperus esculentus* L. commonly called tigernut is a cosmopolitan, perennial crop of the same genus as the papyrus plant and belongs to the Division–Magnoliophyta, Class–Liliopsida, Order–cyperales and Family–Cyperaceae [1].The tubers are about the size of peanuts and are abundantly produced in West Africa including Ghana. It has many other names like Zulu nut, yellow nutgrass, ground almond, chufa, edible rush and rush nut**.** Tiger-nut has been cultivated since early times (chiefly in south Europe and West Africa) for its small tuberous rhizomes which are eaten raw or roasted, used as hog feed or pressed for its juice to make a beverage.

The colour is brown and has a sweet flavour when eaten. Tigernut has been used extensively mainly for human consumption in Spain [2]. Tigernuts are prepared and eaten cold as snacks. The milk can be extracted, treated and bottled. The flour is used to make cakes and biscuits and the oil is used for cooking [3]. In United Kingdom, tigernut is superb bait for carp fishing [3]. In Ghana, the utilization of tigernut is highly limited in spite of the fact that tigernut is cultivated widely in the certain parts of the country. Tigernuts may be eaten raw mainly as snacks or fried and eaten mixed with roasted groundnuts [4]. It has been reported [5] that sweetened tigernut extract are bottled and sold in Ghana. Tiger nut has been used extensively mainly for human consumption in Spain [2, 6] and eaten cold as drink.

Tiger-nuts are valued for their highly nutritious starch content, dietary fibre and carbohydrate (7) and are rich in sucrose (17.4- 20.0%), fat (25.5%), protein (8.0%) (8, 9). Tiger-nut is also rich in mineral content such as sodium, calcium, potassium, magnesium, zinc and traces of copper [10, 11]. The dietary fibre content of tiger-nut is effective in the treatment and prevention of diseases such colon cancer, coronary heart diseases, obesity, diabetes and gastro-intestinal disorders [12]. Tiger-nut tubers are diuretic and can be used as stimulant and tonic [13] and in the treatment of flatulence, indigestion, diarrhoea, dysentery and excessive thirst [14]. In addition, tigernut has been demonstrated to contain higher essential amino acids than those proposed in the protein standard by [15] for satisfying adult needs for protein [16].

Tigernut products are of high nutritional values and economic potentials, hence deserve greater attention than it is currently given. Since the crop is widely grown in parts of Ghana, its availability can be guaranteed. What is currently militating against the utilization of tigernut is the little awareness of the importance of this plant. The following are possible derivatives of tigernut: flour, milk, oil, cake, cream cheese, chocolate, biscuits, cookies etc. Composite flour technology refers to the process of mixing various flours from tubers and cereals or legumes with or without addition of wheat flour in proper proportions to make economic use of locally cultivated crops to produce high quality food products [17]. Composite flour is commonly used for porridge. Its composition varies from type to type, but the most common is a mixture of cereal flours in different ratios only or together with legumes and tubers.

Products developed from tigernut may be contaminated with microorganisms, both spoilage and pathogenic. Microbial quality of composite flour, powders and other food may be determined by significant total microbial count, coliform count and presence of pathogenic microorganisms.

Microbial quality determination is completely used to reflect hygienic practice in food production. Infant formulas/complementary foods can serve as a good medium

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for the growth of many microorganisms especially bacterial pathogens; therefore, its quality control is considered essential to the health and welfare of infants as well as adults. It has been reported [18] that the threat posed by diseases that spread through contaminated food is well known and the epidemiological impact of such diseases is considerable. The presence of these pathogenic microorganisms in composite flours developed from under-utilized crops has emerged as a major public health concern especially for consumers. Bacterial contamination of composite flours [18] can originate from different sources: farm, bad water, air, preparation equipment, handling, processing conditions, among others.

The consumer buys nutrition, convenience, image and functionality in food. Therefore, new products must provide all the responses like or near what the consumer expects. The objective of this research was to formulate a tigernut based composite flour and to evaluate the microbiological and sensory quality of the formulated composite flour.

## Materials and Methods

### Composite Formulation

### Preparation of tiger nut, soybean and maize flours

Tiger nut tubers (yellow variety), soybean and maize were purchased from a local market in Accra, Ghana. The method of (19) was used in the production of tiger nut flour. Tiger nut tubers were sorted to remove damaged and other extraneous materials and then washed with potable water mixed with NaCl. The sample was dried in an air oven at 65ºC for 24hrs and then milled to pass through 600µm sieve size to obtain tiger nut flour. The maize grains were sorted to remove unwanted materials like stones, pebbles and other foreign seeds. The maize sample were roasted in a hot air oven at 80o C till it turned golden brown and cooked. The roasted maize was milled into 25 um partilcle size using a hammer mill. After cleaning and removal of broken seeds and extraneous materials, the soybean were washed and soaked in 4-times its volume of potable water for 12hrs. The seeds were drained and some allowed to germinate. The germinated and ungerminated soybean seeds were blanched for 30 min with potable tap water twice its volume and then dehulled. The soybeans were dried in an air oven at 65ºC for 24hrs and then milled using a hammer mill and sieved through 600 µm aperture size to obtain soybean flour (20).The flour samples were stored in high density polyethylene pouches -4$^o$C until used for analysis.

### Composite flour Formulation

Seven diet formulations: $TMS_1$, $TMS_2$, $TMS_3$, $TMS_4$, $TMS_5$, $TMS_{5b}$ and $TMS_6$ were prepared by mixing varying proportions of the flours as shown in Table 1.

### Microbiological analysis

The different composite samples were microbiologically analyzed to determine the populations of indicator and pathogenic microorganisms. From each sample 10 g was weighed with an Electronic Balance (Mettler Toledo, Switzerland). The samples were blended with 90 ml diluents (0.1% peptone + 0.5 NaCl) for 90 min in a Waring Blender and stirred on a mechanical shaker (Junior Orbit Shaker, Lab-Line In-struments, United States of America) for 30 min. Total viable count was determined on Plate Count Agar (Oxoid, England). *Staphylococcus aureus* was estimated on Baird-Parker Agar (Oxoid, England) and *Escherichia coli* was estimated on Eosine Methlyene Blue Agar (Oxoid, England). The detection of *Salmonella spp*. was done using 25 g of sample on Xylose Lysine Deoxycholate Agar (Oxoid, England).Yeast and molds count was determined using Oxytetracycline Glucose Yeast Extract Agar. All samples were incubated at 37°C for 48 hours. Counts were carried out on plates containing 30-300 colonies using a colony counter and expressed as colony forming unit (cfu)/g.

### Sensory analysis

Sensory analysis was carried out according to the method described by (21). The samples were cooked into gruel with two times the quantity of water as the sample for ten minutes. The samples were coded and served to 10 semi-trained panelists. The panelists were asked to indicate their preference using a 5-point hedonic scale. Extremely good and extremely poor were ranked 5 and 1 respectively. The sensory attributes evaluated colour, mouth feel, taste, aroma and overall acceptability. The cooked samples were served to each panelist on a small cup in a random manner. With respect to taste, panelists were advised to rinse their mouth after tasting each sample and water was provided in plastic cups to each panelist for this purpose.

### Statistical analysis

All determinations were performed in triplicate. Statistical significance was established using One-Way Analysis of variance (ANOVA) and data were reported as mean ± standard deviation. Statistical analyses were carried out using SPSS for Windows, version 17.0 (SPSS Inc. Chicago, IL.USA). Significance was established at $p<0.05$.

**Table 1. Percentage Composition for formulation of tiger nut, maize and soybean infant food mix**

| Composite | Percentage (%) | | |
|---|---|---|---|
| | **Tiger nut flour** | **Maize flour** | **Soybean flour** |
| $TMS_1$ | 50 | 25 | 25 |
| $TMS_2$ | 60 | 25 | 15 |
| $TMS_3$ | 50 | 30 | 20 |
| $TMS_4$ | 70 | 20 | 10 |
| $TMS_5$ | 30 | 45 | 25 |
| $TMS_6$ | 30 | 45 | 25 |
| $TMS_7$ | 20 | 55 | 25 |

$TMS_6$: contains germinated/fermented soybean flour

## Result and Discussion

**Table 2. Microbiological quality of the various formulations expressed as $log_{10}$ cfu/g**

| Sample code | TVC | E. coli | Staph aureus | Salmonella typhi | Yeast and molds |
|---|---|---|---|---|---|
| TMS 1 | $3.31^{a}$ | ND | $2.34^{ab}$ | ND | 0.00 |
| TMS2 | $3.27^{a}$ | ND | $2.58^{a}$ | ND | 0.00 |
| TMS3 | $3.25^{a}$ | ND | $2.48^{ab}$ | ND | 0.00 |
| TMS4 | $3.25^{a}$ | ND | $2.41^{ab}$ | ND | 0.00 |
| TMS5 | $3.30^{a}$ | ND | $2.25^{b}$ | ND | 0.00 |
| TMS6 | $3.30^{a}$ | ND | $2.49^{ab}$ | ND | 0.00 |
| TMS7 | $3.22^{a}$ | ND | $2.21^{b}$ | ND | 0.00 |
| LSD | 0.3908 | - | 0.2790 | - | 0.00 |

a-e: Means in the same column with different letters are significantly different (P < 0.05) from each other. Values are mean ± SD of three replicates (n = 3).

### Microbiological quality

The microbiological quality of the various composite samples is presented in Table 2. The total viable count (TVC) ranged between 3.31 for TMS1 to 3.22 for TMS7. There were no significant differences between the composite samples. The TVC for this study compares favourably and are similar to values reported by [22] and are within the acceptable limits of $10^{7}$cfu/g [23] for flours. The TVC is normally used to assess the hygienic nature of the manufacturing or the processing conditions. A high bacteria count (TVC) alone does not make food unsafe but it does suggest non-hygienic handling, poor storage, inadequate general hygiene during processing and/or poor quality raw materials [24].

*Escherichia coli* can be an indicator of faecal contamination. *E. coli* was not detected in any of the samples analyzed. The absence of *E. coli* which is used as an indicator organism in the composite flour could indicate that the process of preparing the flour samples was done hygienically and was free from faecal contamination. It is also possible that the organism itself was absent in the raw materials used or might have been eliminated during processing of the composite flours. The values reported in this study are similar to [22].

*Staphylococcus aureus* continues to be major cause of community-acquired and health-care related infection throughout the world. Animals, humans, food and inanimate environment can provide a favourable environment for the transmission of S aureus [25, 26]. Staphylococcus aureus as an indicator of contamination of processed foods could come from the skin, mouth, or nose of handlers [27]. The count of *Staphylococcus aureus* in this study ranged between 2.21-2.58 $log_{10}$ cfu/g. Even though there were significant differences between the samples, they were within the acceptable microbial contamination levels as specified by the ICMSF [28, 29, 30]. The *Staphylococcus aureus* counts as reported in this study are comparable to values reported by [31] in a maize/sesame composite.

*Salmonella* sp. was not detected in any of the samples analyzed. This suggests a possible absence of *Salmonella* sp in the raw materials or destruction of the organisms during processing. The tigernut-based formulations meets the microbiological criteria of *Salmonella* being absent in the product [28, 29]. This compares with values reported by [32] in extra super maize meal.

Yeast and molds were also absent in the formulations. The level of contamination of flour by yeasts and moulds is of paramount importance when considering the quality and safety of food ICMSF [28]. The maximum legal limit for fungi in flour is 5 $log_{10}$ cfu/g (WFP). Yeasts and moulds from this study results were within recommended limits. Higher level of fungi more than the legal limits deteriorates the quality of food and causes food borne diseases. Food decomposition occurs due to the presence of moulds and many species of moulds are known to produce mycotoxins [33].

**Table 3. Sensory Evaluation of gruel prepared from tigernut-based composite flour**

| Sample code | Color | Taste | Mouth feel | Aroma | Preference |
|---|---|---|---|---|---|
| TMS 1 | $2.6±1.07^{abc}$ | $2.4±1.26^{a}$ | $2.5±1.17^{a}$ | $2.9±0.99^{ab}$ | $3.3±1.70^{b}$ |
| TMS 2 | $2.8±1.23^{abc}$ | $2.4±1.35^{a}$ | $3.0±1.25^{a}$ | $3.1±0.99^{a}$ | $3.5±2.06^{b}$ |
| TMS 3 | $2.1±1.37^{bc}$ | $2.6±1.07^{a}$ | $2.3±1.06^{a}$ | $2.5±1.17^{ab}$ | $3.9±2.23^{ab}$ |
| TMS 4 | $3.2±1.32^{a}$ | $2.4±1.51^{a}$ | $3.2±1.32^{a}$ | $2.9±1.29^{ab}$ | $4.2±2.04^{ab}$ |
| TMS 5 | $3.1±1.37^{ab}$ | $2.8±1.32^{a}$ | $2.8±1.23^{a}$ | $2.9±1.19^{ab}$ | $5.5±1.96^{a}$ |
| TMS 6 | $2.3±0.95^{abc}$ | $3.1±1.10^{a}$ | $2.6±1.07^{a}$ | $2.4±0.97^{ab}$ | $4.0±1.70^{ab}$ |
| TMS 7 | $1.8±0.63^{c}$ | $2.4±0.52^{a}$ | $2.3±0.68^{a}$ | $2.1±0.57^{b}$ | $3.5±2.17^{b}$ |
| LSD | 1.0387 | 1.0711 | 1.0089 | 0.9379 | 1.7806 |

a-e: Means in the same column with different letters are significantly different (P < 0.05) from each other. Values are mean ± SD of ten replicates (n = 3)

**Sensory quality**

The mean sensory scores for gruel from the composite flours are presented in Table 3. Sensory qualities such as the colour, taste, mouthfeel, aroma and overall preference were evaluated. Colour is a property of product visual appearance and greatly influences the market quality, consumer acceptability and preference of food products [35]. The mean scores for the colour of the gruel from the composite flour sample ranged between the lowest of 1.8±0.63 for TMS 7 to highest of 3.2±1.32 for TMS4. Sample TMS1, TMS 2, TMS 4, TMS 5 TMS 6 were not different from each other. Also samples TMS1, TMS2, TMS 3,TMS 5and TMS 6 were also not different from each other ($p<0.5$). The differences in colour as observed may be attributed to the different compositions of tigernut, maize and soybeans. Colour of food is an important sensory attribute, which enhances acceptability.

The sensory score values for taste ranged between 2.4±1.35 for TMS 2 and 3.1±1.10 for TMS 6. All the samples were not significantly different from each other ($p<0.5$). Taste is an important sensory attribute of any food [36]. Since the sensory scores were not statistically different, we assume that that the different composition did not influence the taste of the samples.

Sensory scores for mouth feel ranged between 2.3±1.06 for TMS 3 and 2.3±0.68 for TMS 7.The samples were not significantly different from each other ($p<0.5$).

Aroma is an important parameter of food [36]. 'Good' aroma from food excites the taste buds, making the system ready to accept the product. 'Poor' aroma may cause outright rejection of food before they are tasted. Significant differences were obtained between the samples in terms of the aroma. Samples TMS 2 and TMS 7 were statistically different from each other. All other samples were not significantly different from each. Overall preference scores ranged between 3.3±1.70 for TMS 1 and 5.5±1.96 for TMS 5 with significant differences ($p<0.5$) recorded among the samples.

**Conclusion**

This study has shown that tigernuts can be successfully introduced in the formulation of composite flours, which are low cost, easy to prepare and safe. This is a new method of valuing this very important crop in the diets of vulnerable people such as children in poor regions but which produce large quantities of tigernut. The microbial quality of the tigernut-based composite flour falls within the acceptable standard, hence guarantying the safety of the consumer. The use of composite flour would help to improve the nutritional value of food at presumably cheaper cost since all the raw materials are locally available and does not require special skill for the preparation. The stability of the obtained flours should be further studied.

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