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Microbiological Changes During Storage of Extruded Snacks Produced from Yellow Cassava Substituted with Processed Sesame Seeds Flours

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Abstract: As the need for healthy snack consumption is rising on a daily basis, evaluating its storability is also needful. This study was done to examine the storability of extruded snacks developed from yellow cassava substituted with sesame seed flour blends. Germinated and fermented sesame seed flours were blended with yellow cassava flour differently at 0, 15 and 30% levels of substitution. The flours were appropriately mixed for the production of extruded snacks in using single screw extruder before frying in a deep fryer. The snacks were packaged in polythene and stored at room temperature. Microbial evaluation was carried out on the snacks on weekly basis for four weeks to determine their storability. The initial counts of total aerobic, fungi (yeast and mould) and coliform counts ranged from $0.48x10^4$ to $2.55x10^4$, $1.06x10^4$ to $2.56x10^4$ and $8.00x10^2$ to $1.20x10^3$cfu/g respectively. The samples showed significant differences ($p<0.05$). The values increased as the length of day increases with samples containing germinated sesame seeds flour having the highest while the extruded snacks with 0% sesame seeds flour had the lowest counts. The samples with fermented sesame seeds flours were observed to contain lower microbial loads compare to those with germinated sesame seeds flour. The results of this study nevertheless indicated that the level of contamination of the snacks were within acceptable/specified limits.

Keywords: Germinated Sesame Seed, Yellow Cassava, Extruder, Yeast Count, Fermented Sesame Seed

1. Introduction

Cassava (*Manihot esculenta crantz*), is a member of the *Euphorbiaceae* family and is virtually cultivated in the tropical region [1]. The cassava plant is a perennial shrub cultivated for its edibility. It makes up over half of the root and tuber crops cultivated across African countries and are among the utmost food crops grown in Nigeria, Brazil, Democratic Republic of Congo and Thailand. Yellow Cassava is bio-fortified with micronutrients majorly Vitamin A which could be a sustainable approach to control micronutrient malnutrition and hence resulting in proper performance of visual organs, development and conservation of the integrity of the epithelial cells. This product could reach the public especially in the tropics through some of the key cassava-based food products.

In Nigeria, there is a wide spread diversification of oil crops in many parts of the country which ranges from commonly used ones like soyabean, palm kernel and groundnut, to the unexploited ones like walnut, locust bean, African oil bean and sesame seed.

Sesame (*Sesamum indicum* L.) belongs to the group of oil crops having high nutritional value in relation to high amount of proteins rich in sulfur-amino acids, fatty acids, vitamins and minerals [2]. Sesame seeds are great sources of vitamins B complex such as niacin, folic acid, thiamin, pyridoxine and riboflavin. They are also rich in health benefit phenolic compounds such as sesamol, sesaminol, furyl-methanthiol etc. Phenolic compounds show strong antioxidant and antimicrobial properties which make them of great value in nutraceuticals in formulating healthy foods [3]. Consequently, sesame rich diets have the capacity to reduce the vulnerability of contracting cancers and cardiovascular diseases [4]. Makinde and Akinoso [5] reported that processes applied to sesame seeds such as roasting, fermentation and germination further enhanced the nutrient quality thereby decreasing anti-nutritional contents of the sesame seeds.

Nigerian researchers, being faced with the challenges of the multifarious economic policies made by the Federal Government of Nigeria to increase the usage of local sources of flour for partial replacement of wheat flour and the issues with the massive importation of cereal grains as raw materials by manufacturing companies has led them to seeking indigenous replacements which will be applicable as much as the imported variety [6]. It is presumed that this move would cause the reliance on the importation of wheat to decrease drastically and also elevate the standard of living and income for the local farmers that produce crops used in composite flours. Composite flour has been widely used in food, feed and chemical industries [7]. The production of composite flours using various crops to substitute wheat flour completely or partly for confectionary and bakery goods has been carried out and reported by researchers [8, 9]. Consumption of snacks has gradually gained global popularity in recent years. According to Vasanthakaalam and Dusingizimana [10], the daily energy intake from snacks should be maximum 10% as recommended by the food consumption guidelines for consumers. Supplementing required energy and nutrient could be enhanced by consuming health snacks appropriately [11]. Extruded snack produced from extrusion cooking is an example of such snacks. Extrusion cooking is among the germane mechanization that have shown excellent prospects for the evolution of novel snack product [12, 13]. However, [14] stated that carbohydrates are the main nutrients in extruded snack ingredient and thus making the snacks energy short of protein and other essential nutrient such as vitamin A whereas consumers are in constant need of more nutritious snacks limited in fat but rich in protein, fibre and vitamins. Yellow cassava with high pro-vitamin A and sesame seeds with high protein, fibre and other nutrients can be used to produce ready-to-eat extruded snacks as a means of meeting the consumer's need. Meanwhile, there might be some changes and decrease in quality of the extruded snacks during storage on shelf or other medium which might be due to some factors such as microorganisms, moisture, temperature light and lipid oxidation. However, very little information is available on the use of yellow cassava substituted with processed sesame seed flours for extruded snacks production most especially the storability of this product.

Therefore, the objective of this investigation was to investigate the storability of extruded snacks from yellow cassava flour substituted with processed sesame seed flour

2. Materials and Methods

2.1. Source of Materials

Yellow Cassava roots were sourced from locally trained farmers at the Federal University of Agriculture Abeokuta. Other material such as sesame seeds, wheat flour, sugar, salt, margarine and xanthan gum were sourced from Kuto open market in Abeokuta, Ogun State, Nigeria. Extruder used was the locally fabricated extruder of the Department of Food Technology Moshood Abiola Polytechnic, Abeokuta, Ogun State.

2.2. Methods

2.2.1. Processing of High quality Yellow Cassava Flour

High quality yellow cassava flour was produced using the method described by [15, 16]. The fleshly harvested yellow cassava roots were weighed, washed, peeled, sliced and blanched at 80°C for 4minutes. The roots undergo drying and milling operations and then sieved before packaging in a polyethylene film prior to further utilization.

2.2.2. Processing of Germinated Sesame Seed Flour

The method used in producing the black sesame seed flour was as described by [17]. At room temperatures (25±2°C), dehulled seeds of black sesame seeds were sprouted for five days in which they were kept in trays lined with wet filter paper. The sprouted seeds were exposed to hot air oven to be dried at 40°C to a constant weight. Milling operation was done on the samples using an attrition mill so as to pass through a 0.5mm sieve and stored in zip sealed packages until required for further investigation.

2.2.3. Processing of Fermented Sesame Seed Flour

The method of [5] was adopted for the production of the black sesame seeds flour. It was prepared by cooking dehulled seeds of black sesame seeds in boiling water for 6hours and cooling gradually. The cooked seeds were placed in a well-covered plastic container after which fermentation took place at temperatures (35±2°C) for a period of one week i.e. 7days. The fermented seeds were oven dried at 105°C for 12 hours in order to end the fermentation process. The samples were then milled in an attrition mill to pass through a 0.5mm sieve and stored in polyethylene bags until set for further evaluation.

2.2.4. Sample Preparation

Each of the Germinated and Fermented sesame seed flour was mixed with Yellow cassava flour at levels of 0, 15 and 30%. The flour blends were modified to suitable moisture content by sprinkling quantified proportion of purified water and thoroughly mixed for 15 minutes. The blends were packaged in polythene bags and refrigerated overnight to keep the moisture at equilibrium. The samples were brought down to room temperature before extrusion cooking.

2.2.5. Extrusion Process

An entire line of fabricated extruder was used to carry out this process, which has a motor coupled with a speed reducer (extrusion by mechanical friction), besides a single extrusion screw, with 130mm of barrel diameter, 440mm of extruder length, a hydraulic cooling system for the control of temperature, variable speed, and 50kgh–1 capacity. In the cause of extruding a proportion of the next test material was used to clean the extruder. The extruded samples were fried in a deep fryer into extruded snacks. Each fried sample was portioned into two and tightly sealed until subsequent investigation.

2.3. Microbiological Analysis of Extruded Snacks

The samples were evaluated for bacteria and fungi counts using pour plate techniques as described by [18, 19]. A gram of the sample was dispersed into 9ml of sterile distilled water in a test tube as aliquots. The mixture was shaken to homogenize. Serial dilution were carried out using 10–fold dilutions and appropriate aliquots used to determine the total aerobic count on Plate count agar (PCA) and Potato Dextrose agar (PDA) for fungi count. MacConkey and Eosin methylene blue agars (Oxoid) were used for coliform counts. One milliliter of each extruded snacks flour sample was pipetted in an aseptic condition into a test tube containing 9ml sterile 0.1% buffered peptone water (Merck) solution and appropriate serial dilutions were made. MacConkey and eosin methylene blue agars (Oxoid) agar plates were incubated at 25°C for 0week, 1week, 2weeks, 3weeks and 4weeks.

2.4. Statistical Analysis

All the data obtained were subjected to one way analysis of variance (ANOVA). Duncan Multiple Range Test (DMRT) was used to determine the significant difference. All tests were conducted at 5% significance level using Statistical Package of Social Science (SPSS 21.0).

3. Results and Discussion

The total viable counts on the extruded snacks produced from yellow cassava flour substituted with sesame seed flour is represented in Tables 1, 2 and 3 showing total aerobic count, yeast and mould count and coliform counts respectively as observed from 0 (initial) to four weeks. The number and type of microbes present in a food substance under microbial investigation is a function of the quality of the food and the extent to which the consumer is endangered [20]. Total viable count according to [21] has been described as a means of predicting the shelf life of a food. Total counts have been said to be functional indicators in processing of food products and may however indicate poor handling or storage at retail level [22]. From the results, it can be inferred that significance difference ($p<0.05$) occurred among the extruded snack samples for all the microbial counts determined. The total aerobic count (Table 1) of the initial count ranged from $0.48x10^4$ to $2.55x10^4$ cfu/g and it increases gradually as the days progresses up to the range $1.98x10^5$ to $4.22x10^5$cfu/g. Extruded snacks produced from 100% yellow cassava had the lowest while snacks substituted with 30% germinated sesame seed ($4.22x10^5$cfu/g) had the highest. Table 2, which represents the yeast and mould count indicates that extruded snacks produced with 100% yellow cassava flour had the least ($1.06x10^4$cfu/g) yeast and mould count while it was highest ($2.56x10^4$cfu/g) in extrudates containing 30% germinated sesame seed flour at the initial stage. The yeast and mould count also increases as the period of storage at room temperature on shelf increases but minimally ($3.80x10^4$ to $5.40x10^4$cfu/g). Coliform counts (table 3) of the extruded snacks shows that it ranged between 1.0×10^2cfu/g and 2.5×10^3cfu/g after four weeks of storage at room temperature. It was observed from the results that inclusion of both fermented and germinated sesame seed flour at 15 and 30% levels steadily increased the microbial load of the extruded snack samples. The increase in the microbial loads of extrudate with addition of sesame seed at all levels might be attributed to the protein and fat contents in the sesame seeds flours. The increase was more in samples containing germinated sesame seeds flour this is in line with the report from scientific studies that during germination, carbohydrate are mobilized to synthesis amino acids of the growing seedling which encourage proliferation of microorganisms [23, 24]. It was also reported by Noah and Banjo [25] in a similar study carried out that the increase in microbial loads observed in the cookies fortified with red kidney beans was due to red kidney beans with high protein content. The protein usually encourages microbial action on food. So therefore, the result in this present study with addition of proteinous sesame seeds flour to yellow cassava flour corroborate this findings/significance of protein in microbial action. The rise in microbial load as the time of storage increased may be attributed to a commensurate increase in moisture contents during storage and availability of nutrient in the samples as suggested by [26, 27]. The present results are in line with findings of Oluwole *et al.* [28] and Uzoaga and Kanu [21] for a similar study on extruded white yam (*Dioscorea rotundata)* and Bambara groundnut (*Vigna subterranean*) blends and extruded snacks made from orange fleshed sweet potato, cassava, plantain fortified with *Moringa oleifera* powder respectively. However, the utmost authorized level of total aerobic colony of ready-to-eat foods such as extruded snacks as extracted from the manual of [29] ranged from $1x10^4$ to $<1x10^6$cfu/g for ready-to-eat food products. In this study, the level of contamination of the extruded snacks is minimal and within this standard range of $<10^6$cfu/g. Furthermore, according to International Commission on Microbiological Specification for Food [30], yeast and mould count should not exceed $1x10^5$cfu/g [31]. Based on PHLSG [29] and ICMSF [30] specifications, the microbial load of the stored extruded snacks in this present study were within the recommended safe limits. This thus indicates that the extruded samples were stable under the storage condition (room temperature) examined in this study. Microbial count of any food substance is a qualitative functional indicator in the extruded foods as well as exposing the prospective level of the extruded food products from the consumers stand point and product storability. Mould and yeast have however been linked as the causative organisms of spoilage in extruded food products and the total plate count must also be reduced to the nearest minimum so as to avert the chances of preformed toxins being formed [29] which were evidence in this present study. Although the presence of coliform in this study is an indication of unhygienic production process but the coliform counts (x10^{3}cfu/g) in this study did not exceed but lower than the 1x10^{4}cfu/g specified as tolerable limit for food by [30].

Table 1. *Total Aerobic count (cfu/g) of extruded snacks at room temperature*

Sample	Zero week ($\times10^{4}$)	Week one ($\times10^{4}$)	Week two ($\times10^{4}$)	Week three ($\times10^{5}$)	Week four ($\times10^{5}$)
A	0.48^{a}	0.73^{a}	1.74^{a}	0.98^{a}	1.98^{a}
B	0.74^{b}	1.49^{b}	1.96^{b}	1.82^{c}	2.12^{b}
C	1.64^{c}	2.42^{c}	3.22^{c}	1.25^{b}	4.08^{d}
D	2.55^{e}	3.76^{e}	4.86^{e}	2.17^{e}	4.22^{e}
E	2.19^{d}	3.45^{d}	3.86^{d}	1.92^{d}	2.72^{c}

Values in a column with different superscripts are significantly different ($p\leq0.05$) from one another
Keys
A-Extruded snacks from 100% yellow cassava flour
B- Extruded snacks from 85% yellow cassava flour, 15% fermented sesame seed flour
C-Extruded snacks from 85% yellow cassava flour, 15% germinated sesame seed flour
D-Extruded snacks from 70% yellow cassava flour, 30% germinated sesame seed flour
E-Extruded snacks from 70% yellow cassava flour, 30% fermented sesame seed flour

Table 2. *Yeast and mould count (cfu/g) of extruded snacks at room temperature.*

Sample	Zero week ($\times10^{4}$)	Week one ($\times10^{4}$)	Week two ($\times10^{4}$)	Week three ($\times10^{4}$)	Week four ($\times10^{4'}$)
A	1.06^{a}	1.12^{a}	1.84^{a}	2.60^{a}	3.80^{a}
B	1.15^{b}	1.40^{b}	2.12^{b}	3.10^{b}	4.20^{b}
C	2.31^{d}	3.22^{d}	3.48^{d}	4.30^{d}	4.90^{d}
D	2.56^{e}	3.36^{e}	3.64^{e}	4.30^{d}	5.40^{e}
E	1.46^{c}	2.75^{c}	2.16^{c}	3.60^{c}	4.50^{c}

Values in a column with different superscripts are significantly different ($p\leq0.05$) from one another
Keys
A-Extruded snacks from 100% yellow cassava flour
B-Extruded snacks from 85% yellow cassava flour, 15% fermented sesame seed flour
C-Extruded snacks from 85% yellow cassava flour, 15% germinated sesame seed flour
D-Extruded snacks from 70% yellow cassava flour, 30% germinated sesame seed flour
E-Extruded snacks from 70% yellow cassava flour, 30% fermented sesame seed flour

Table 3. *Coliform count (cfu/g) of extruded snacks at room temperature*

Sample	Zero week	Week one	Week two	Week three	Week four
A	1.20×10^{3}	1.40×10^{3}	1.70×10^{3}	2.00×10^{3}	2.50×10^{3}
B	8.00×10^{2}	1.10×10^{3}	1.20×10^{3}	1.40×10^{3}	1.70×10^{3}
C	1.00×10^{3}	1.30×10^{3}	1.40×10^{3}	1.60×10^{3}	1.80×10^{3}
D	7.00×10^{2}	1.00×10^{3}	1.10×10^{3}	1.20×10^{3}	1.50×10^{3}
E	5.00×10^{2}	8.00×10^{2}	1.00×10^{3}	1.10×10^{3}	1.30×10^{3}

Values in a column with different superscripts are significantly different ($p\leq0.05$) from one another
Keys
A-Extruded snacks from 100% yellow cassava flour
B-Extruded snacks from 85% yellow cassava flour, 15% fermented sesame seed flour
C-Extruded snacks from 85% yellow cassava flour, 15% germinated sesame seed flour
D-Extruded snacks from 70% yellow cassava flour, 30% germinated sesame seed flour
E-Extruded snacks from 70% yellow cassava flour, 30% fermented sesame seed flour

4. Conclusion

This study has shown the successful development of shelf stable extruded snacks from yellow cassava flour substituted with processed sesame seed flour. The extruded snacks were discovered to be minimally contaminated as the microbial loads were within the standard range. The incorporation of processed sesame seed flour at high levels (30% levels) increased the microbial loads most especially incorporation of 30% germinated sesame seeds flour into the cassava flour for extruded snacks. However, the addition of fermented sesame seeds flour to yellow cassava flour at 15% level of incorporation was with minimal microbial loads. The loads generally were within specified acceptable limits for snacks.

Conflict of Interest

Authors declare no conflict of interest.

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