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Review Article

Effect of Packaging Material and Storage Conditions on the Microbial Quality of Pearl Millet Extruded Snack

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ABSTRACT

Cereal grains are the most important source of the world's food and have a significant role in human diet throughout the world. The major cereals in Nigeria consist of maize, sorghum, millet and rice. It is the major functional food consumed in Nigeria due to its abundant availability and it is amenable to different processing technologies. Millets (Penisetum glaucum) are groups of small seeded species of cereal grains, widely grown around the world for food and staples. It is one of the most extensively cultivated cereals in the world, after rice, wheat and sorghum, particularly in arid to semi-arid regions. Millet is predominantly starchy and the bran layer of millet is a good source of b-complex vitamins. It also serves as a source of antioxidants in our diets. Thus, the presence of all the required nutrients in millets makes them suitable for large scale utilization in the manufacture of food products such as baby foods, snack foods and dietary food. Pearl millet also known as "bajra" is currently the world's sixth most important cereal grain and is grown extensively in Africa, Asia, India and the Near East as a food grain and is the staple source of nutrition for millions of people. The crop has a wide adaptability to local environment for its properties of been tolerant to drought and heat. For this reason, it is widely grown in tropical regions of the world including Africa and Asia. It has high level of calcium, iron, zinc, lipids amino acids and antioxidants but bioavailability is low, inherently due to the presence of antinutritional factors, such as phytic acid, polyphenols tannin and trypsin inhibitor etc. Processing methods like fermentation are required to improve the nutritional, sensory value and availability of macro and micronutrients and reduce the anti-nutrients.

Keywords: Packaging material; Storage conditions; Pearl millet

INTRODUCTION

Fermentation of foods has been practiced for improving the flavor, texture and palatability of foods. It is one of the processes known to reduce these anti-nutrients. It also leads to an increase in protein content, enhancement of carbohydrate accessibility, improvement in amino acid balance, decrease in antinutritional factors like tannin and phytic acid and also increases the bioavailability of various nutrients as well as imparts less nutrient loses as compared to cooking.

In the processing of food, extrusion is one of the commonly adopted processing techniques by food industries which employ mixing, forming, texturing and cooking to develop a novel food product. Extrusion cooking is one of the most efficient and versatile food processing technologies that can be used to produce pre-cooked and dehydrated food products such as snacks food, baby foods, breakfast cereals, noodles, pastas and cereals based blends [1]. Extrusion cooking of cereals is a very important process in food industry because it has a potential in generating quality snack products. It is capable of producing assorted products such as breakfast cereal, modified starch, pasta, and animal feed and extruded snacks. The bioavailability of nutrients during the processing of foods is always considered important when obtaining a nutritional snack product. The advantages of extrusion cooking with respect to the nutritional content of the final product are the inactivation of antinutrients, destruction of aflatoxins and increasing the digestibility of fiber.

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LITERATURE REVIEW

Ready to Eat (RTE) Breakfast cereals are highly favored by customers of all the ages as they offer variety, convenience and most of all high nutrient value. The consumption of Breakfast cereals and other related products is said to be increased due to lack of time for the preparation of meal during these modern times which provides us with a great opportunity to increase the Flakes and Breakfast cereals varieties in the market.

The burden of foodborne illness and the number of food recalls associated with microbial contamination of extruded snacks has risen in recent years. Microbes have the ability to multiply at high rates when favorable conditions are present. Microbial growth in snacks results in food spoilage with the development of undesirable sensory characteristics and in certain cases the food may become unsafe for consumption.

The sourdough technology is considered to be one of the oldest biotechnological processes used to obtain cereal-based foods predating the production of commercial yeast. It was initially obtained from the spontaneous fermentation of microorganisms present in the flour or in other raw material. Sourdough is defined as a dough of flour and water fermented by yeasts and lactic acid bacteria (LAB), used as a leavening agent for the production of bakery products. The rheology, flavor, nutritional and functional properties of sourdough based baked products greatly rely on the activity of these microorganisms. Lactic acid bacteria usually originate from the flour, dough ingredients or the environment [2]. Compared to products fermented by yeasts alone, the sourdough baked goods have longer shelf life, resulting in the antifungal production, as well as bacteriocins production and of higher acidity higher sensory quality through more elasticity, internal humidity, and volatile compound concentration and greater nutritional value due to the reduction of anti-nutritional compounds such as phytase enzyme.

MATERIALS AND METHODS

Pearl millet (Pennisetum glaucum), Packaging materials (High density polyethylene and Aluminum laminate packs) used in this study were purchased from Kuto, a local market outlets in Abeokuta, Ogun state, Nigeria

Preparation of millet flour

The millet grains were cleaned to remove extraneous materials and milled into flour using hammer mill with screen size 800 µm, then sieved with a 425 µm wire mesh to obtain finer flour [3]. The pearl millet flour was kept in air-tight polyethylene bags kept at room temperature for analysis.

Spontaneous fermentation of millet flours

Sourdoughs were prepared according to the method as described by Edema, Emmambux, & Taylor with few modifications. Approximately 200 g of flour were weighed into 500 ml glass jars and thoroughly mixed with 200 ml portable water (flour: water; ratio {1:1}). This preparation yielded a paste like dough, which was subjected to spontaneous fermentation at 28 ± 2 for 48 h. The pearl millet sourdough obtained was subsequently used as starter materials for sour dough extruded snacks.

Processing of pearl millet sourdough extruded snack

Millet sourdough extruded snack was prepared by adopting the method as described by Mohammed for maize sourdough with slight modification. The weights of ingredients per 200 g millet flours were 100 g sourdough, water 60-70 ml, egg 52g, milk flavour 25g, fat 10 g sugar 30 g and baking powder 4 g. These was transferred into a mixing bowl and mixed for 10 min at high speed using Kenwood KMX 50 series. The snack dough's were fitted into the extrusion machine to obtain sourdough extruded snacks. The sourdough extruded snacks were cooled at 28 ± 2 , packed in high density polyethylene bags and aluminum laminates and stored for further characterization [4].

Packaging treatments

The developed extruded snack (200 gm) were exposed to two different packaging treatments and kept under freezing, refrigeration and room temperature set at -50 ± 20 , 4 ± 20 , 6O ± 20 , 28 ± 2O and 37 ± 20 storage conditions. The packaging materials used for the study were aluminum laminate and high density polyethylene bags of 200 gauge thickness [5]. The stored samples were evaluated for their quality attributes i.e. microbial, intrinsic properties at regular interval of 7 days up to two months.

Sampling of sourdough extruded snack

Packaged pearl millet sourdough extruded snack were subjected to five storage conditions: freezing, refrigeration, room temperature and 370 , set at -50 ± 20 , 4 ± 20 ,6 ± 20 , 28 ± 2O and 37 ± 20 respectively; stored for two months and evaluated at an interval of seven days. The microbial and intrinsic properties of the pearl millet sourdough extruded snack were determined.

Microbiological analysis of sourdough extruded snack

All glassware's used were washed thoroughly, air-dried and sterilized at 160 for 1 h. The media used were sterilized in an autoclave at 121 for 15 min. The bench working areas were swabbed with ethanol before any microbiological analysis in order to avoid contamination.

Preparation of culture media

All culture media used for the enumeration and isolation of organisms were prepared according to standard protocol as described by Rocha and Malcata [6,7]. They include: De Mann, Rogosa and Sharpe agar (MRS) for Lactobacillus spp., Plate count agar (PCA) for total aerobic bacteria count and Potato Dextrose Agar (PDA) for yeast. Peptone water: preparation was done by dissolving 15 g of peptone tablet in 1000 ml of distilled water. All mixtures were heated gently to dissolve the medium completely and then sterilized in an autoclave at 121 for 15 min.

Total viable count

Pour plate method as described by Harrigan and McCance was used. One gram of the sample was macerated into 9ml of Ringers solution and mixed thoroughly by shaking. This was further diluted to obtain 10-2 and 10-3 concentration. Then 0.1ml dilution was transferred from each dilution bottle into the corresponding plate and 15ml of sterile nutrient agar medium

was poured and mixed thoroughly with the inoculum by rocking the plates [8]. The plates were incubated at 38 for 24 hours after which the colonies formed were counted and expressed as colony forming units per gram (cfu/g).

Mould count

The pour plate method as described by Harrigan and McCance was also used. The sample dilution weighing 0.1ml was transferred from each dilution into corresponding plates and 15ml of sterile Sabour and Dextrose Agar (SDA) medium was poured and mixed thoroughly with the inoculum by rocking the plates. The plates were incubated at ambient temperature for three days after which colonies formed were counted and expressed as colony forming units per gram (cfu/g).

Microbial enumeration of sourdough extruded snack

Quantification of viable organisms was carried out by plating sample/inoculum on appropriate selective media as described by Rocha and Malcata. One gram of samples was aseptically collected using standard protocols at 12 h intervals. The samples were gently homogenized in 9.00 ml sterile 0.10% (w/v) peptone water and appropriate serial dilutions prepared. Then, 0.1ml of each dilution was inoculated into selective and non-selective medium. PCA plates was incubated at 35 for 24 h, MRS plate was incubated anaerobically at 37 for 48 h, PDA at 27 for 3 to 4 days. Total viable count was determined by surface viable count and results expressed as log of colony forming unit per gram (Logcfu/g) of sample. Pure cultures of the isolates were obtained by sub-culturing on appropriate media plates and maintained in agar slants and stored at 4 for subsequent assays.

Identification of isolates

The bacteria isolates were identified based on standard methods. Fungal isolates were identified based on cultural and morphological characteristics with reference to standard atlas.

Determination of pH

The pH of the sourdough extruded snack during storage was monitored and recorded weekly using a pre-calibrated pH meter. The pH was determined as described by AOAC. 10 g of each sample was suspended in 90 ml sterile distilled water and homogenized. The mixtures were allowed to stand for 30 minutes before being filtered. The pH values of the filtrates were determined by a combined glass electrode probe and a pH meter (Mettler-Toledo, Essex M3509 Type 340). Three readings were taken per sample. The readings were taken by inserting the probe into the fermenting meals directly. The probe was sanitized by swabbing with 95% ethanol prior to each use. Triplicate determinations were made in all cases.

Determination of moisture content

The moisture content of sourdough extruded snack was determined using the method as described by Association of Official Analytical Chemist. Five grams of each sample was weighed into cleaned, dried and weighed glass petri dishes. The dishes with their content were placed inside the Gallemkamp hot air oven at a temperature of 105°C for 3 h. Thereafter the samples were cooled in the desiccators and weighed. The dishes were taken back to the oven and allowed to stay for 30 min. This process was continued until a constant weight was achieved. Therefore, the percentage moisture content was calculated using the formula:

Where:

W1=Weight of sample in dish before drying

W2=Weight of dried sample + dish after drying

W=Weight of sample

RESULTS AND DISCUSSION

The data presented reveals, how the packaging material and storage period influenced the microbial quality of pearl millet extruded snack. The microbiological quality assessment showed that no microbial growth was detected at the time of packaging which was due to the extrusion cooking temperature used. Growth appearances were however recorded in all the packages during the period of storage which was higher in fungi than bacteria as the storage period progressed. The differences in total bacteria counts (TBC) of snack stored at different storage temperatures may be attributed to the nature of the packaging materials themselves. One such nature factor is relative permeability to air. Polyethylene sachet is more permeable to air than aluminum laminate. Permeability to gases such as oxygen, carbon (IV) oxide and water vapor has been reported to affect the growth and survival of microorganisms in packaged foods. Permeability to air (oxygen) may be aiding the growth of fungi encountered in the present study. Low bacteria count in the packages may be due to low pH (acid) and high sugar content which do not favour the growth of most bacteria, since they act as hurdles against them. Similar findings have been reported. An increase in the total bacteria viable count of snack as observed in HDPE packages during the period of storage at room temperature and 37°C compared to those in ALLA suggest that HDPE packages may be of high microbial contamination compared to ALLA packages observed that during manufacture and handling electrostatic charges can occur on the plastic. These charges attract air-borne materials such as dust and microorganisms. Atasevar et al. reported higher viable count in cheese vacuum packaged in plastic compared to those packaged in tin containers. Increase in viable bacteria count observed in snack at room temperature during storage than at cold storage supported the report of El-Owni and Hamed who also reported an increase in total bacteria count of Jibna Beyda stored at room temperature. The low microbial count observed in PMSD snack as compared to control was attributed to starter culture used in snack manufacture. This presence of sourdough develops acidity in snack and increased the lactic acid bacteria which inhibited the growth of other organisms. The higher bacteria count as observed in HDPE than in ALLA packages is in agreement with result reported by Atasever et al,. The reason for the observed increase in mold count in snack packaged in HDPE than that in ALLA is because the pH tended to be more acidic in aluminum packages than in polyethylene packages. It is likely that the low pH was the inhibitory factor to the presence of bacteria in PMSD snack

observed as compared to control. Increase in mold count observed by snack packaged in HDPE as compared to ALLA is due to the packaging snack in anti-acid packaging closed by double seaming. The presence of Aspergillus and Penicillium could be attributed to the surrounding air and packaging materials. Yeast is important for good batter and leavening while LAB produce acids and other metabolites which inhibit the growth of spoilage organisms. Samples at cold storage had good storage stability as compared to samples stored at 28 and 37°C during the period of storage. The increase in Microbial population and frequency of occurrence of bacteria, mold and lactic acid bacteria observed in snack stored at 28 and 37°C that suggest that the population of indigenous LAB and mold increased drastically with storage time and temperature. This co-existence between LAB and mold confirmed the synergistic relationship between the organisms in a fermenting food matrix. According to Ogunsakin et al., the ability of LAB and mold to grow together is very crucial in fermented food. The increase in microbial load as the storage period lengthened might have been due to a corresponding increase in moisture content during storage. Microbial studies indicated that snack packaged in HDPE and laminate, stored at room temperature up to 2 months had better stability as the microbial load remained within the permissible limits. The microbial load of all extrudates stored at frozen, refrigeration, 28 and 37°C (as measured by the total plate count per gram) was generally low , based on the maximum permissible level of microbial loads in ready-to-eat foods (less than 106 cfu/g) which suggest that the product can stay longer than 2 months. Food Standards declares ready to eat foods with aerobic plate counts 104<105 as acceptable. The results obtained showed that the bacterial counts of all the samples stored did not exceed the maximum recommended standards by the International Commission on Microbiological Specification of Foods. According to this agency, the acceptable limit of mesophilic aerobic bacteria in dried food products should not exceed a maximum of 103 cfu/g.

The result of the pH content of extruded snack prepared from pearl millet as influenced by packaging material and storage period as observed in weeks. An increase in pH of snack packaged in HDPE as compared to Aluminum laminate might be attributed to the protective effect of foil laminate. Significant increase in pH was observed in snack stored at 28°C and 37°C as compared to frozen (-5°C) and refrigeration (4°C and 6°C) temperature. The accumulation of acids is normally accelerated by storage at high temperatures and in the presence of light or water. In the present study , there was higher accumulation of acids in the transparent polyethylene bags which do not only allow more light in the product but also moisture buildup when compared to aluminum laminate pack. Noteworthy increase in pH content was recorded with the lengthening of storage period. The increase in acids during storage might be due to the breakdown of long fatty acid chain into individual acid moieties and increased lipid hydrolysis at elevated temperature. Similar results were also reported by Bindu and Srinivas pH content of all the snack were within the range. A decrease in pH value observed in sourdough snack as compared to control might be due to the production of acid by fermentation of sugar. Singh et al. also reported gradual decrease in pH. Increase in pH with storage time as observed in millet snack could be attributed to the dispersion of different molecules during extrusion cooking after fermentation. The generally observed high microbial counts in this study could be attributed to differences in thickness of storage materials used which affect the moisture content of the snack and the microbial populations and the influence of environmental factors which have been shown to play a significant role in affecting the quality of food products. Increase in bacteria and fungi count as observed in millet snack might be attributed to increase in snack moisture content during storage which allow proliferation to occur. This is because the successful growth of these microorganisms depends upon them getting an adequate supply of moisture. With the highest recorded moisture increase of snack stored at 37°C of throughout the storage period, there is a good possibility of bacterial multiplication. The results of this work suggest that the storage medium, the method of storage and packaging material had significant effect on quality of the snack.

The result of the moisture content of extruded snack prepared from pearl millet as influenced by packaging material and storage period as observed in weeks. Moisture content is an important factor to analyse the microbial quality and shelf life of snack food products as it is a critical entity which support microbial growth & proliferation. The low moisture content observed in this study is a reflection of low water activity (aw) which in turn reduces microbial proliferation rate and a necessary factor in the extension of shelf life and effective storage of products. As storage progressed, a slight increase in moisture observed by lower mean values may be influenced by the packaging material used, the increase being more in HDPE packaged product as compared to aluminum laminate .This was due to higher water vapour permeability of HDPE than aluminum laminate. Adedeji et al., reported that laminates containing aluminium foil provided good protection against moisture losses because of superior moisture barrier properties of the foil. Moisture content is a determining factor for cohesiveness. The moisture content is affected significantly due to moisture increases, storage treatments, packaging and their interaction due to hygroscopic properties of flour. The low moisture content observed in the snack was within the range reported to have no adverse effect on quality attribute of the product. Tafa reported that the lower the moisture content of a product to be stored, the better the shelf stability of such product. The low moisture content would be anticipated to yield products with a high degree of crispness. The variation in moisture content of these products could be directly related to the water vapour transmission rate of the packaging materials. The aluminum foil packages kept in refrigerated condition were most effective as least moisture could migrate through them. This was mainly due to the fact that aluminum foil is considered to have low water vapour transmission rate (WVTR) under humid condition (80% RH). Charunuch et al. reported an increase in moisture content in Thai rice extruded snack supplemented with mulberry from 3.5 to 5 per cent during storage of 4 months observed an increase in moisture content in breakfast cereals during a storage period of six months. They also concluded that there was intermediate gain in moisture

through HDPE as compared to aluminium foil which has high moisture barrier properties. The packaging type and storage conditions applied affect the quality, shelf-life and safety of food products through their influences on moisture content, water activity and nutrient compositions of the food product. Reason for the moisture gain in sample stored at 37 °C as compared to 28°C could be due to the greater relative humidity found in the ambient storage conditions (100%) than in the retail storage conditions (75%). Increase in moisture content has been associated with increase in fiber content. The results of moisture content were all within the permissible limits indicating that RTE extruded products are safe for consumption after 2 months of storage at all temperatures. Moisture content of samples is presumed as one of the most important determinants of shelf stability and the values observed in this work suggests that extrusion process reduces the final moisture contents of extruded snack to a level that might support their shelf stability. High-moisture products (>12%) usually have shorter shelf stability compared with lower-moisture products (<12%). Higher moisture uptake in the HDPE might be due to their permeability to moisture and air had also mentioned that aluminium foil had less water vapour transmission rate as compared to polyethylene. The gain in moisture content during the storage period might be due to hygroscopic nature of dried products, storage environment (temperature, relative humidity) as well as the nature of packaging material. Increase in moisture content observed during the storage period in all samples was desirable for extruded snacks to maintain their crispness. Increase in moisture content of snack was due to absorption of moisture from the storage environment owing to their hygroscopic nature. Increase in moisture content was due to the hygroscopic nature of the products. Similar results for extruded snacks were reported by Kocherla et al. and Sumathi et al. also reported that increase in the moisture content was considerable for pearl millet based extruded samples packed in HDPE. Results obtained in the present investigation are well in agreement with the above-mentioned researchers. Charunuch et al. reported an increase in moisture content in Thai rice extruded snack supplemented with mulberry during storage of 4 months. Leelavathi and Rao stated that higher moisture pick up of biscuits containing bran during storage could be due to greater hygroscopicity of wheat bran. Snack packed in laminate absorbed less moisture during storage which might have been due to the impervious nature of aluminium foil in the laminate to air and water vapour. The results are in concurrence with the air spaces of the RTE extruded products in across sectional view. This indicates that the more the air spaces in the extruded products, the more the increase in moisture content of the RTE extruded products on storage. Moisture also impacts the rate of spoilage of the snack.

CONCLUSION

Aluminium laminate storage of snacks at lower temperatures is desirable to maintain the microbial quality of snacks .It could be concluded that packaging of snack in Aluminium laminate is better than in HDPE as low count of bacterial, mold and lab were observed in snack packed in Aluminium laminate as compared to HDPE. Cold temperature storage of snack resulted in better microbiological characteristics as compared to room temperature storage. In this study, Pearl millet sourdough extruded snack stored in aluminium laminate packs minimized the influence of moisture, light and air in comparison to transparent high density polyethylene. Based on the microbial quality, aluminium laminate would be more effective in long term storage of extruded snack to maintain microbial stability and the quality attributes essential for prolonged shelf life.

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