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MICROBIOLOGICAL AND PHYSICOCHEMICAL ANALYSIS OF AFRICAN YAM BEAN MOIMOI STORED AT ROOM AND REFRIGERATION TEMPERATURES.

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ABSTRACT

The microbiological and physicochemical properties of African yam bean moimoi stored at room (29 ±3°C) and refrigeration (7±3°C) temperatures were examined. At room temperature, the product kept for 12-16 hours. The shelf life at refrigeration temperature was about 3 days. The spoilage of the product was accompanied by an increase in microbial counts, total volatile nitrogen (TVN), Moisture content and titratable acidity (TTA.)., pH and firmness of the product decreased as spoilage progressed. The species of bacteria isolated include, Alcaligens spp., Lactobacillus spp., Bacillus spp. and Streptococcus spp. The fungal isolates included, yeast identified as Saccharomyces cerevisae, and two moulds Penicillium spp. and Aspergillus niger. These microorganisms are normal airborne microbes that are also saprophytic microorganisms.

Keywords: Microbiological, physicochemical, African yam bean moimoi, Storage, Shelf life.

INTRODUCTION

Moimoi is a popular Nigerian food produced from cowpea with small amounts of vegetable oil, salt, tomato, shrimp, vegetables, spices and water blended together to form a homogeneous paste. On heating, the paste solidifies into a gel (Okechukwu et al, 1992). Moimoi is eaten by all segments of the population except infants (Adeniji and Potter, 1980). Traditional diets ,in developing countries often lack variety and consist of large quantities of staple foods (cassava, yam or maize) with supplements of plantain, cocoyam, rice and beans depending on availability and season. The need to improve nutrition in developing countries through conventional processing of indigenous crops and familiar recipes has led to the discovery of the African yam bean (AYB). The African yam bean, nutritionally, compares favourably with the well-utilized cowpea (Evans and Boulter, 1974). However, this bean is underutilized due to its long cooking time (Njoku et al, 1989). In the present study, we report the microbiological and physicochemical characteristics of African yam bean moimoi stored at room and refrigeration temperatures.

MATERIALS AND METHODS

African yam bean moimoi was produced from the marble variety seed. At 24hrs intervals, 10g of the moimoi sample were taken from each pack. 90ml of normal saline solution was added and the sample macerated for about 2 minutes using a stomacher laboratory blender 400 (Seward, London). Further dilutions were prepared using the same diluent. Microbial population was assessed, using the spread plate technique. 0.1ml of the suitable dilution was spread onto prepared and dried petri dishes of suitable media for the enumeration of different organisms. Nutrient agar was used for total viable aerobic counts and enumeration of psychrotropic organisms. A liquid media repair method was adopted for the enumeration of heat-injured coliforms (Speck, 1976). Inoculums from these tubes were surface plated on MacConkey agar. For E. coli, the dilutions were plated on Eosine methylene blue agar. Enumeration of sporeformers and Staphylococci were

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carried out in molten nutrient agar and Baird Parker agar, respectively. Nutrient agar plus 1% skimmed milk (Angels et al, 1985) and nutrient agar plus 10% sterile fat were used to enumerate proteolytic and lipolytic organisms, respectively. For the enumeration of yeasts and moulds, appropriate dilutions of homogenates were plated onto potato dextrose agar. Colonies were grouped according to their morphology and cell characteristics. The colonies were then isolated in pure culture using the medium on which they were grown. The biochemical tests adopted for the characterization of the isolates were as described by Skerman, (1967)., Harrigan and McCance,(1976)and Collins and Lyne, (1984). The probable identities of the isolates was made as described by Buchanan and Gibbons, (1974). The texture of AYB moimoi was evaluated by using "the extent of sample resistance to penetration" method (Rowland & Soulides, 1942). For pH determination, 10g of the sample was homogenised in 100ml of deionised water. This was shaken and the pH measured using a, Meltler Delton 340 pH meter. The instrument was switched on and allowed to stabilise for 15 minutes. The instrument was standardized using freshly prepared buffers. Titratable acidity (TTA) was determined using a method similar to that of Grover, et al, 1983, and expressed as acidity /g. Total volatile Nitrogen (TVN) was determined as described by Pearson (1976).

RESULTS

AYB moimoi stored at room temperature (29 ± 3°C) started sliming and producing off-odour, as well as showing general changes in appearance such as growth of moulds and fungi after 16 hours. These observations reached peak, after 24 hours. For the product stored at refrigeration temperature (7±3°C), the product kept for 3 days before it started showing signs of spoilage (Tables 1 and 2).

TABLE 1: DESCRIPTIVE CHANGES IN AYB MOIMOI DURING STORAGE AT ROOM TEMPERATURE.

HOURS OF STORAGE	APPEARANCE	ODOUR	TEXTURE
0	Reddish yellow and smooth	Typical fresh	Firm to touch
12	Reddish yellow with little moisture	Typical fresh	Slightly soft
24	Moderately slimy and turning greenish	Slightly repugnant	Slightly marshy
36	Surface covered with moulds and fungi	Repugnant and offensive	Marshy
48	Surface completely covered with moulds	repugnant and offensive	Marshy

TABLE 2: DESCRIPTIVE CHANGES IN AYB MOIMOI DURING STORAGE AT REFRIGERATION TEMPERATURE

Day of storage	*Appearance*	*Odour*	*Texture*
0	Reddish yellow and smooth	Fresh	Firm to touch
1	Reddish yellow and smooth	Fresh	Firm to touch
2	More yellowish with little moisture	Less fresh	Slightly soft
3	Yellowish	Not fresh	Soft
4	Yellowish green	Offensive	Slightly marshy
5	Greenish yellow	Offensive and repugnant	marshy

Microbiological assessment of AYB moimoi stored at room temperature and refrigeration conditions are presented in Tables 3 and 4.

Microbial load on the samples increased with storage time. For samples stored at room temperature, initial total count was 3.0×10^3 cfu/g and increased rapidly to 3.3×10^7 cfu/g after 48 hours.

Proteolytic counts increased from 2.0×10^3 cfu/g to 3.5×10^6 cfu/g. Lipolytic counts rose from 1.6×10^3 cfu/g to 2.0×10^6 cfu/g during storage period. The sporeformers increased from 2.7×10^3 cfu/g to 1.8×10^7 cfu/g after a storage period of 48 hours. Yeast and mould count increased from 1.2×10^2 cfu/g to 3.0×10^6 cfu/g. For the coliforms, the trend was downward from 2.0×10^2 cfu/g to 4.0×10^1 cfu/g. For samples stored at refrigeration temperature, there was also an upward trend in microbial load with storage time. Total counts increased from 4.0×10^3 cfu/g to 3.0×10^5 cfu/g after 48hours of storage. Proteolytic counts and lypolytic counts

rose from 2.3×10^3 cfu/g to 6.0×10^4 cfu/g and 1.4×10^3 cfu/g to 3.0×10^4 cfu/g respectively after 48hours of storage. Sporefomers increased from 2.8×10^3 cfu/g to 2.8×10^5 cfu/g and yeasts and moulds from 1.5×10^2 cfu/g to 1.5×10^4 cfu/g. The coliforms decreased from 3.0×10^2 cfu/g to 2.0×10^1 cfu/g after 48 hours. The spectrum of bacteria isolated include *Alcaligens* spp, *Lactobacillus* spp, *Bacillus* spp and *Streptoccocus* spp. Moulds and fungi isolated include *Penicillium*, *Saccharomyces cerevisae* and *Aspergilus* (Tables 5).

TABLE 3: MICROBIAL COUNTS FROM AYB MOIMOI DURING STORAGE AT ROOM TEMPERATURE.

Hours of Storage	Total counts (cfu/g)	Proteolytic counts (cfu/g)	Lipolytic counts (cfu/g)	Spore counts (cfu/g)	Psychrohitic count (cfu/g)	Yeast and Moulds (cfu/g)	*Coliforms* (cfu/g)	*Staphylococcus* (cfu/g)	*Escherichai coli* (cfu/g)
0-6	3.0×10^3	2.0×10^3	1.6×10^3	2.7×10^3	NG	1.2×10^2	2.0×10^2	NG	NG
24	4.0×10^5	3.5×10^4	2.0×10^4	5.0×10^5	NG	2.0×10^5	8.0×10^1	NG	NG
48'	3×10^7	3.5×10^6	2.0×10^6	1.8×10^7	NG	3.0×10^6	4.0×10^1	NG	NG

NG ⇒ No growth

TABLE 4: MICROBIAL COUNTS FROM AYB MOIMOI DURING STORAGE AT REFRIGERATION TEMPERATURE

Hours of Storage	Total counts (cfu/g)	*Proteolytic counts (cfu/g)*	*Lipolytic* counts (cfu/g)	Spore counts (cfu/g)	Psychrohitic count (cfu/g)	Yeast and Moulds (cfu/g)	*Coliforms* (cfu/g)	Staphylococcus (cfu/g)	*Escherichai coli* (cfu/g)
0-6	4.0×10^3	2.5×10^3	1.4×10^3	2.8×10^3	NG	1.5×10^2	3.0×10^2	NG	NG
24	6.0×10^3	2.6×10^3	1.8×10^3	5.0×10^3	NG	2.0×10^3	5.0×10^1	NG	NG
48	3.0×10^5	6.0×10^4	3.0×10^4	2.8×10^5	NG	1.5×10^4	2.0×10^1	NG	NG

NG⇒ No growth

TABLE 5: CULTURAL CHARACTERISTICS OF MOULDS ISOLATED FROM AYB MOIMOI

Sample Code	Cultural Characteristics	Morphological characteristics	Inference
G	Grey large colonies, yellowish colonies with short mycelia, yellowish green mycelium	Conidia in long chains, branched cells, have stalk like conidiophores	Penicillium sp.
H	Black and Rhizoid colonies, yellow fluffy colonies	Conidia in chains, Columna head	Aspergillus niger
I	White or cream colonies with yeasty odour	Cells are ellipsoidal, cylindrical or elongated	Sacchromyces cervisae

TABLE 6: CHANGES IN THE PHYSICOCHEMICAL PARAMETERS OF AYB MOIMOI DURING STORAGE AT ROOM TEMPERATURE

Hours of Storage	TVN (MgH/100g)	pH	Moisture (%)	Texture (g/cm^2)	TTA
0	8.4	6.19	74	120	0.23
24	9.8	6.11	81	100	0.48
48	16	5.12	88	85	2.10

TABLE 7: CHANGES IN THE PHYSICOCHEMICAL PARAMETERS OF AYB MOIMOI DURING STORAGE AT REFRIGERATION TEMPERATURE

Hours of Storage	TVN (MgH/100g)	pH	Moisture (%)	Texture (g/cm^2)	TTA
0	8.4	6.19	74	120	0.23
24	8.2	6.16	76	110	0.36
48	9.10	6.17	80	105	1.60

For samples stored at both temperatures, no growth was recorded for psychrophiles, *Straphylococcus* spp and *Escherichia* coli. The results of physical and chemical parameters monitored during storage are as summarised in Tables 6 & 7. The results showed that, for the product stored under room condition, TVN increased from 8.4mgN/100g at 0 hour to 16mgN/100g at 48 hours.

Moisture content increased from 74% at 0 hour to 88% at 48hours. TTA increased from 0.23ml to 2.10ml within 48hours. pH decreased from pH 6.19 to 5.12 at 48hours. The force required to crush the product decreased from 120g/cm^3 at 0 hours of storage to 85g/cm^2 at 48hours.

For the product stored at refrigeration temperature, the trend was the same but at a slower rate. TVN increased from 8.4mgN/100g at 0 hour to 9.10mgN/100g at 48hours. Moisture content and titratable acidity increased in value from 74% to 80% and 0.23ml to 1.60ml, respectively. pH value decreased from 6.19 to 6.17 within the storage period of 48hours. The force required to crush the product decreased from 120g/cm^2 to 105g/cm^2 in a period of 48hours.

DISCUSSION.

Microbiological and physicochemical parameters are known and acceptable indices for measuring shelf life. Shelf life is the period of time between productions to the time when the product develops characteristics (e.g. changes in texture, colour, odours) that makes it unacceptable to the consumers. African yam bean due to it's high protein content (21-29%) and

other nutritive qualities has been gaining interest in recent times (Evans and Boulter, 1974., Obizoba and Soyzey, 1985). In this study we report the microbiological and physicochemical characters of AYB moimoi stored at room temperature (29±3°C) and refrigeration temperature (7±3°C). The rate of spoilage was faster at room temperature than refrigeration temperature. Low temperatures are used to retard chemical reactions and actions of food enzymes. It is also used to slow down or stop entirely, the growth and activities of microorganisms in food (Frazier and Westhoff, 1978; George, 1989). The changes associated with spoilage can be attributed to microbial activities (Omonigho and Ugbor, 1998, Shamshad et al, 1990). Total aerobic count, fungal count, proteolytic, lipolytic and spore counts all showed an upward trend at both temperatures studied with the rate being higher at the room environment. The readily available nutrients (sugars, amino acids, fatty acids vitamins and mineral salts) present in the AYB moimoi can be utilized by these microorganisms for rapid proliferation (Akpapunan, 1985). Some of the microorganisms especially the spore formers (Bacillus spp) are also capable of producing hydrolytic enzymes for the breakdown of polymers (carbohydrates, crude protein and fats/oil) as reported by Ensari et al (1995). The low microbial counts (bacteria and fungi) obtained in the freshly prepared moimoi can be attributed to the heat treatment (steaming) employed in the processing of the dish. The heat would kill most of the vegetative cells (Omonigho and Ugbor, 1998). Most spoilage organisms get into food as a result of post processing contamination from the handlers, air, during storage and other post processing operations. The trend for the coliforms was downwards at both temperatures. This could have resulted from a decrease in pH and an increase in TTA. Zamora and Fields (1979), observed a similar trend in fermented cowpea. Since the total aerobic count continued to increase during storage, it appears that the relatively low pH exerted an inhibitory effect on the coliforms while allowing more acid-resistant microorganisms e.g. lactic acid bacteria to propagate (Bulgarelli et al, 1988). Spores, from the spore formers might have reached the moimoi paste from the seeds used (Omonigho and Ugbor, 1998). No growth was recorded for psychrophiles, E. coli and *Staphylococcus* species. This might be due to their inability to compete well with a number of other bacteria (Trolley and Frazier, 1963, Rehberger et al , 1984 and Bulgarelli et al, 1988) and /or adequate heat treatment , good sanitary condition and proper handling during and after preparation of "AYB moimoi". In this study, spoilage corresponded with a decrease in pH and firmness and an increase in TVN and TTA. Similar changes were observed by Shamshad et al,(1990) in their shelf life studies of shrimps stored at different temperatures. The decrease in pH with an increase in TTA might be due to increased microbial activities resulting in the production or organic acids from available reducing sugars (Omonigho and Ugbor, 1998). The decrease in firmness could be as a result of increased metabolic activities which increase moisture content and reduced dry mass (Omonigho and Ikenebomeh, 1996). TVN is used widely to estimate amines which have ammonia as a major component. Ammonia has been used extensively as a decomposition criteria (Burnett, 1965). The increase in TVN in this study can be attributed to the proliferation of spoilage organisms especially Proteolytes, Sawaya and Abu-Ruwaida,(1989a and 1989b) established the relationship between the production of volatile nitrogen compounds and spoilage organisms. This study has shown that microorganisms, both fungi and bacteria were present in the moimoi stored at refrigeration and room temperatures. Microbial load and spoilage, both increased as the period of storage was increased. However, spoilage was faster at room temperature than refrigeration temperature.

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