



MICROBIAL, PHYSICOCHEMICAL, PROXIMATE AND SENSORY CHANGES DURING STORAGE AND SPOILAGE OF PUPURU BALLS, A CASSAVA BASED FERMENTED FOOD

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Abstract

Pupuru is a fermented molded ball of cassava (*Manihot esculenta*) staple food consumed by about 10 million people of the coastal part of the South-western Nigeria. In this study, microbial, physicochemical and proximate properties of *pupuru* balls were examined for a period of 12 days at ambient temperature (28 ± 2 °C). Microbiological analyses indicated that viable bacteria counts ranged from 3.5×10^4 cfu/g to 1.32×10^5 cfu/g. Lactic acid bacteria and fungi were not isolated from the fresh samples (0 day). Microorganisms associated with *pupuru* balls during storage and spoilage included bacterial such as *Bacillus subtilis*, *Lactobacillus* sp., and *Staphylococcus aureus*. Fungi isolates are *Candida krusei*, *Aspergillus flavus*, *A. niger*, *Fusarium* sp., *Rhizopus* sp. and *Penicillium* sp. *Bacillus subtilis* was found throughout the entire storage period. The isolated moulds are presumed to be environmental contaminants due to their sparse or irregular occurrence during storage. The pH of the *pupuru* balls ranged from 4.6 to 7.8 during storage. The results of the total titratable acidity showed a gradual increase within the first five days of production and decreased from the sixth day to the twelfth day of analysis. Proximate analysis was carried out to determine the nutrient composition of the fresh and spoilt *pupuru* (at 6th day) samples. The moisture content of the spoilt *pupuru* increased after spoilage from 33.84% to 35.56%; ash content from 1.11% to 1.24%; fat content 2.34% to 7.73% and crude protein from 4.82% to 5.15%. There were decreases in the crude fibre contents and the carbohydrate contents of *pupuru* when spoilt from 1.41% to 1.23% and from 57.32% to 49.09%, respectively. The *pupuru* balls were observed to deteriorate after four days becoming soft and moist at room temperatures. All the sensory parameters monitored were found to reduce significantly with storage period. There is need for new techniques of preserving this food in order to avoid the risk of food borne diseases.

Keywords: Cassava, Fermented food, Microorganisms, Organoleptic, Properties, Proximate, Physiochemical, Pupuru, Shelf-life, Spoilage

Introduction

Foods are complex mixtures of macro- and micro-nutrients which provide energy and contribute to human well-being (Adams and Moss, 2000). Microorganisms present in

food samples could be originated from the natural microflora of the raw materials while some are introduced during the course of harvesting, processing, storage and distribution (Adams and Moss, 2000).

Microorganisms, however, have significant influence on the quality, food safety and the shelf-life of the foods.

Pupuru is the traditional name given to a molded ball of processed cassava tuber (*Manihot esculenta*), which has gone through fermentation. More recently, it also refers to the fine white flour from it. *Pupuru* probable originated from the southern part of Ondo state, where it is largely being consumed with vegetables that are also peculiar to the people. *Pupuru* is particularly common amongst the *Ikale*, *Ilaje* and *Ijaw* ethnic groups of Ondo state including *Apoi* and *Arogo-Ijaw* communities all in coastal South-Western Nigeria. It has also been reported to be popular in some communities of riverine southern, eastern and the middle belt Nigeria where it is called *ikwurikwu* (Aboaba *et al.*, 1988; Shittu *et al.*, 2003). It is particularly used as both regular meal and, at times, weaning food to replace the more popular *ogi*.

The traditional processing of cassava into *pupuru* involves peeling of the tubers to remove the outer layer of the cassava, washing to remove dirt, cutting into smaller bits, steeping of the cassava tuber pieces in water or stream and, fermentation of the milled tubers for a period of 4-6 days, which also helps to remove the poisonous cyanide present in cassava tubers. The fermented cassava mash is then removed from the water into a jute bag to drain off the water in a process similar to what obtained for *fufu*, a more popular cassava fermented product (Oyewole, 1990). It is also molded between the two palms of the arms into balls and smoked-dried on a platform otherwise called *aka* on a local wooden stove. The ball has brownish to black outer coating arising from the firewood smoke. When it is ready for consumption it can be processed to *pupuru* flour by peeling the outer coating and sieving (Oyetayo, 2006). It is cooked into

a thick porridge consumed with protein-rich soup.

Cassava as the raw material is one of the most staple food plants in the world. About 54 million metric tons per annum of the world cassava produced are from Nigeria and its consumption exceeds those of other root crops (FAO, 2013). Cassava varieties (or cultivars) are classified according to morphological traits as well as taste, cyanide content, average yield, disease performance and pubescence (Chiwona-Karlton *et al.*, 2003; Gbadegesin *et al.*, 2013). The fleshy portion of cassava contains 62% moisture, 35% starch, 1% protein, 0.3% fat, 2% fiber and 1% ash. The fleshy roots contain 35mg/100g of vitamin C, trace amount of niacin and fat-soluble vitamins (Purseglove, 1991). Cassava utilization cuts across human consumption, animal feed stock, industrial or medicinal products, and valued market products (Asonye, 2001; Nweke *et al.*, 2002). The processing of cassava products using traditional techniques has helped to reduce the problem of carbohydrate deficiency in areas where cassava is extensively cultivated in Nigeria. In accordance to Olatidoye *et al.* (2010), the advantage of cassava over other starchy crops is the variety of uses to which its roots can be subjected to. In Nigeria, cassava, is consumed in boiled, baked and fried forms in addition to various other products that are gotten from fermenting the crop including *pupuru*. Nigeria is considered as one of the current leading cassava producing countries in the world (FAO, 2002). This work aims to study the microbial, physicochemical, proximate and sensory changes during the storage of *pupuru* at ambient temperature.

Materials and methods

Pupuru balls

Pupuru balls used for this study were purchased from a local producer at the main market in Okitipupa, Ondo state, Nigeria.

These were set up in triplicates and examined for a period of 12 days at ambient temperature. On daily bases, *pupuru* ball samples were assessed considering the microbiological, physicochemical, proximate and organoleptic properties (Oyewole, 1990; Collins *et al.*, 1995; AOAC, 2005; Teniola *et al.*, 2005). These analyses were carried out on the stored *pupuru* balls placed on the shelf for storage at room temperature (28 ± 2 °C) using standard methodologies.

Microbiological analysis

Microbial counts and other microbiological procedures were carried out as described by (Teniola and Odunfa, 2002; Teniola *et al.*, 2005). The media used for the study were, Nutrient Agar (Oxoid CM0309), deMan Rogosa Sharpe (MRS) (Oxoid CM0361), Potato Dextrose Agar (PDA) (Oxoid CM0139), and Plate Count Agar (PCA) (Oxoid CM0325) (Teniola *et al.*, 2005). The tests used for the identification of microorganisms are as indicated by (Collins *et al.*, 1995; Teniola and Odunfa, 2002; Teniola *et al.*, 2005; Chowdhury *et al.*, 2013; and Rahnama and Habibi, 2015).

Physicochemical analyses

pH

The changes in pH as the storage time increased were measured using pH meter (Uniscope pH meter, models PHS-3B, Surgifriend medicals, England), with reference glass electrode. The pH meter was calibrated prior to usage, with standard phosphate buffers pH4, pH7, and pH10 (from manufacturer). Method of pH evaluation according to Oyewole (1990) and AOAC (2005) were used.

Total titration acidity (TTA)

The TTA was determined by titrating 10 ml of distilled water extract of the inner crust of the dried *pupuru* balls, with 0.1 N NaOH using phenolphthalein solution as indicator. Determination were made in

triplicates (Oyewole, 1990; Teniola *et al.*, 2005).

Proximate analysis

Proximate analysis was carried out to determine the nutrient composition of the fresh and stored *pupuru* samples. The freshly produced *pupuru* balls (day 0) and the spoilt stored *pupuru* (day 6) were analysed by the standard procedures of AOAC (2005) for moisture, ash, crude-fiber, fat and crude-protein. The carbohydrate contents were estimated by the difference in values obtained when all the chemical composition values were subtracted from 100%. The analyses were carried out in triplicates.

Organoleptic (sensory) analysis

A 10-member trained panel was set up consisting of people familiar with *pupuru* who were also indigenes of Okitipupa and the *Ikaleland* where *pupuru* is very popular. Sensory attributes of the *pupuru* balls were observed on daily basis for twelve days by the panelists. The food samples were evaluated by the panelists in terms of appearance (colour), texture (hand-feel), aroma/smell and the taste of the product (Teniola and Odunfa, 2001) and their respective means were calculated. Results were statistically analysed by the analysis of variance (ANOVA) using the Duncan's Multiple Range Test (0.05 level).

Results and Discussion

The changes observed in the microbial population of the *pupuru* balls during storage are shown in Table I. The lowest total viable bacteria count was observed at 0 h with population of 3.50×10^4 cfu/g which continued to increase gradually up to 1.35×10^5 cfu/g on the 9th day after which it started to decline slightly to 1.08×10^5 cfu/g at the last day of storage (12th day). This pattern may imply that there was a major decline in nutrients available for this group of bacteria after the 9th day leading to reduced microbial bacteria growth observable on the 12th day.

Almost a similar pattern was observed for the lactic acid bacteria population changes that also peaked at the 9th day with the count of 1.26×10^5 cfu/g. Although, lactic acid bacteria were not isolated within the first three days of *pupuru* storage even though *pupuru* is a lactic acid fermented product. Fungal populations were also not isolated from the freshly stored sample until the 3rd day of storage.

The absence of lactic acid bacteria and fungal populations from the fresh *pupuru* balls may be as a result of the intense heat from the fire generated from the smoke-cooking process during *pupuru* ball drying and preservation process. However, the yeast population appeared on the 6th day of storage which later declined on the 9th day and almost stable till the end of the storage

period.

Although, the moulds were also not detected from the fresh *pupuru* samples, they became more established during the course of the storage period up to the last day of storage (12th day), reaching a population of up to 9.00×10^5 cfu/g. The increases in the microbial loads of *pupuru* balls over the storage period may be due to the proliferation of microorganisms in the samples utilizing the nutrients available within *pupuru* balls (Teniola and Odunfa, 2002).

The total viable bacteria count observed from fresh *pupuru* at 0 h must have been dominated by heat resistant, spore forming bacteria such as *Bacillus* species Amoa-Awua and Jakobsen (1995) had earlier reported the presence of *Bacillus* from freshly fermented and uncooked cassava.

Table I: Microbial count during the storage of *pupuru* on the shelf at room temperature

Storage Period (Days)	Total Viable count (cfu/g)	Mould count (cfu/g)	Yeast (cfu/g)	colony Lactic bacteria (cfu/g)	aci
0	3.50×10^4	0	0	0	
3	9.70×10^4	1.30×10^3	3.15×10^5	0	
6	1.02×10^5	6.00×10^3	7.20×10^5	7.00×10^3	
9	1.32×10^5	3.00×10^3	1.06×10^5	1.26×10^5	
12	1.08×10^5	9.00×10^4	NA	3.40×10^4	

*Results are mean of duplicate readings.

The bacteria isolates identified were *Bacillus subtilis*, *Lactobacillus* sp. and *Staphylococcus aureus*, while fungal isolates were *Candida krusei*, *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum*, *Fusarium* sp., *Neurospora* sp., *Penicillium* sp., *Mucor* sp. and *Rhizopus* sp. (Tables 2 and 3). According to Obadina *et al.* (2009), moulds such as *Aspergillus*, *Fusarium*, *Mucor* and *Rhizopus* have been isolated from some cassava fermented products like *lafun*, *fufu*, and *garri* during storage. However, most moulds isolated are soil inhabitants or plant pathogens hence their association with cassava, a tuber crop (Nelson *et al.*, 1994).

Mucor is dangerous and can affect human's respiratory system (Wang *et al.*, 2016). The spores of *Penicillium* and *Aspergillus flavus* spores are usually found in air as natural flora (Khan and Karuppayil, 2012). *Aspergillus flavus* and *Aspergillus parasiticus* are known aflatoxin producers while *Penicillium species* are ochratoxin producers and are often isolated from many foods including cassava (Bryan, 1988; Ismaiel and Papenbrock, 2015). Aflatoxins are toxic to a variety of animals and have been confirmed to be carcinogenic to a few animals such as turkey, rats, sheep and mice (Buszewska-Forajta, 2020).

Table 2: Morphological and biochemical features of bacteria isolates from stored pupuru balls

Microbial isolates	Bacteria isolates		
	PB1, PB11	PB2, PB4, PB7	PB3, PB9
Cell morphology	Positive rod with spore (centre)	Positive rod without spore	Positive cocci in irregular cluster
Colonial morphology	Irregular undulate form,	Convex with entire margin	Round and convex
Anaerobic growth	-	+	+
Growth at 60 °C	+		
Catalase	+	-	+
Starch hydrolysis	+	-	-
Oxidase	-	-	-
Indole	-	-	-
Voges Proskauer	+		+
Urease	-	-	+
Mannitol		+	+
Lactose	-	+	-
Xylose	+	-	
Glucose	+	+	
<i>Probable bacteria</i>	<i>Bacillus subtilis</i>	<i>Lactobacillus</i> sp.	<i>Staphylococcus aureus</i>

Table 3: Morphological characteristics of fungal isolates from stored pupuru samples

Fungal isolates	Colonial morphology	Microscopic appearance	Others	Probable organisms
YP4	Flat, smooth, moist and cream in colour Presence of wide films on upper sides of tubes/when grown on Sabouraud broth	Produces blastoconidia singly with pseudo-hyphae.	elongated with Cycloheximide and growth on Sabouraud dextrose agar at 37°C	Rapid growth, <i>Candida krusei</i>
MP2, MP4	Greenish yellow on surface and cream-yellow at the back	Hyphae are septate and hyaline, roughened wall conidiophores		<i>Aspergillus flavus</i>
MP3	Black on the surface and the Reverse in cream.	Hyphae are septate and Hyaline, roughened wall Conidiophores		<i>Aspergillus niger</i>
MP6	Cream colour on top and the reverse side is yellow	Sickle shape macroconidia		<i>Fusarium oxysporum</i>
MP7	Pure white cottony, Shaped macroconidia	Sickled Clamydospore macroconidia	and	<i>Fusarium</i> sp.
MP8	Peach cottony, growing at the edges of the plate to the center	Contains wild type hyphal cells		<i>Neurospora</i> sp.
MP10	Bluish green on the surface	Septate hyphae, branched Conidiophores with phialide In brush like clusters		<i>Penicillium</i> sp.
MP11	Soft white fluffy appearance White reverse is plain white	Long, unbranching sporangiophores.		<i>Rhizopus</i> sp.
MP13	Dark grey on the surface	Zygomycetes, wide hypae, long sporangiophores with branching.		<i>Mucor</i> sp.

Table 4 revealed the distribution of the isolated microflora throughout the period of *pupuru* storage. *Bacillus subtilis* was found throughout the entire storage period while *Staphylococcus aureus* was consistent and repeatedly isolated between 0 and day 9th days. Equally consistent is the 6 days isolations of *Candida krusei* between 3th day to 9th day and *Lactobacillus* sp. (6th and 12th) thereby reflecting their very significant roles as indigenous microorganism in *pupuru*. *Bacillus subtilis*, *Lactobacillus* sp. and *Candida krusei* had been implicated as starter cultures in *fufu* processing (Oyewole, 1990). Many other isolates that are more sparsely distributed and non-consistent during storage such as *A. flavus*, *A. niger*, *Fusarium* sp., *F. oxysporon*, *Neurospora* sp., *Penicillium* sp., *Mucor* sp. and *Rhizopus* sp. may be seen as contaminants and spoilage microorganisms coming in contact with *pupuru* from the environment (Susanti, et al., 2019, Garnier et al., 2017).

The pH of the freshly prepared *pupuru* was 4.6. There were more or less gradually

increases in the pH throughout the storage period until the final day (12th day) with the highest pH value of 7.8 (Figure 1). The increase in pH of the stored *pupuru* balls is in accordance with Shittu *et al.*, (2010), and might be partly due to further reduction of acid by the microflora, particularly the fungal populations which are known to degrade organic acids in related fermentations (Nuraida *et al.*, 1995; Teniola and Odunfa, 2002). Figure 2 showed the total titratable acidity (%TTA) change during storage of *pupuru* balls at room temperature. Fresh *pupuru* contained initial value of 0.16 %TTA that increased irregularly to its peak of 0.22% TTA observed between the 4th and 6th days. After the 6th day peak a regular decline in the %TTA value was observed until the last day (12th day) with 0.02 %TTA. It is difficult to relate the initial slight increase in the percentage titratable acidity and increase in pH within the first five days of storage. The pH meter used to measure pH changes in the stored samples is probably not sensitive enough to highlight the initial slight increases in acidity.

Table 4: Microbial species isolated from *pupuru* balls at different stages of storage

Storage period (days)					Microbial species
0	3	6	9	12	
-	-	-	+	-	<i>Aspergillus flavus</i>
-	-	+	+	-	<i>Aspergillus niger</i>
+	+	+	+	+	<i>Bacillus subtilis</i>
-	+	-	-	-	<i>Fusarium oxysporon</i>
-	+	-	-	-	<i>Fusarium</i> sp.
-	-	+	+	+	<i>Lactobacillus</i> sp.
-	-	-	-	+	<i>Mucor</i> sp.
-	+	-	+	-	<i>Neurospora</i> sp.
-	+	+	-	+	<i>Penicillium</i> sp.
-	+	-	-	-	<i>Rhizopus</i> sp.
-	+	+	+	-	<i>Candida krusei</i>
+	+	+	+	-	<i>Staphylococcus aureus</i>

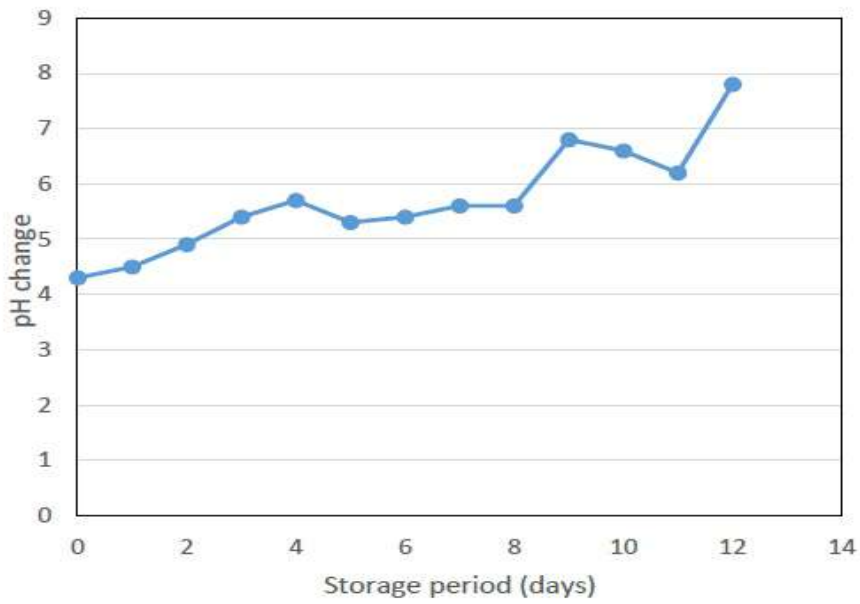


Figure 1: Changes in pH during storage of pupuru balls at room temperature (28 ± 2 °C)

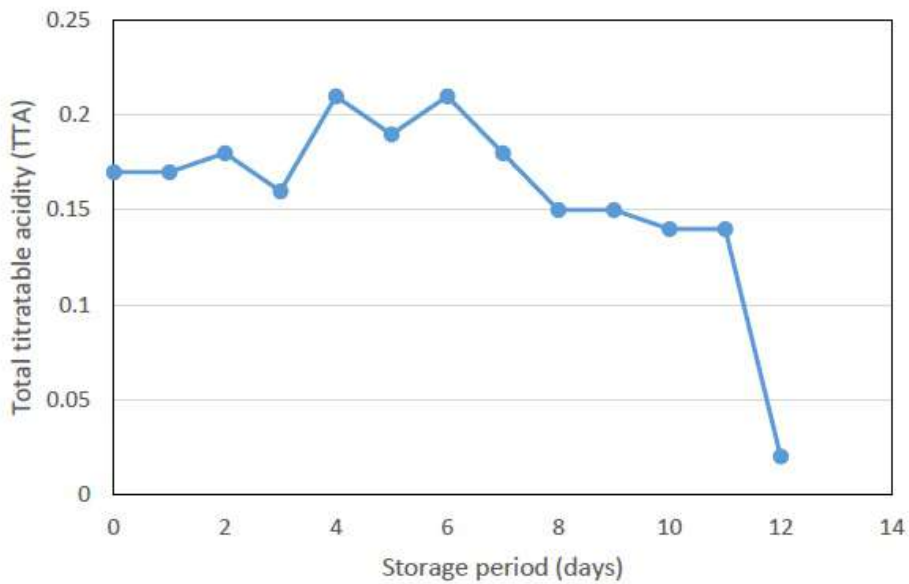


Figure 1: Changes in total titratable acidity (TTA) during storage of pupuru balls at room temperature (28 ± 2 °C)

Comparing the proximate composition of the fresh *pupuru* and the stored *pupuru* samples on the 6th day, there were increase in the moisture content of the spoilt *pupuru* after storage (6 days) from 33.84% to 35.56%, ash content from 1.11% to 1.24%; fat content 2.34% to 7.73% and crude protein from 4.82% to 5.15% (Table 5). There were decreases in the crude fibre contents of the spoilt *pupuru* from 1.41% to 1.23% and a decrease in the carbohydrate contents from 57.32% to 49.09%. The increase in the moisture content of *pupuru* indicates that there was re-adsorption of moisture from the environment and this

justifies the traditional practice of preferred storage of *pupuru* balls just above the local cooking fireplace. The increase in the percentage moisture content of stored flour can be attributed to the hygroscopic properties of the flour (Adejumo, 2013; Butt *et al.*, 2004) and might be due to the fact that at a high humidity (mean relative humidity $76.5 \pm 3\%$), the vapor pressure may have increased which aids water absorption into the samples (Akindahunsi and Oboh, 2000; de Araujo and Pena, 2020). Also, the decrease in the carbohydrate content may be explained by the constant utilization of the available nutrients for microbial growth during storage.

Table 5: Proximate analysis of fresh and 6 days stored *pupuru* balls (spoilt) after storage at room temperature

Parameters	‡Fresh <i>pupuru</i> balls (day 0)	‡Sixth day stored <i>pupuru</i> balls (spoilt)
Moisture content	33.9	35.65*
Ash content	1.11	1.24*
Fat content	2.3	7.73*
Crude fibre	1.41*	1.23
Crude protein	4.83	5.15*
Carbohydrate	57.32*	49.09

*Results are the mean of triplicate data. Values are significantly different (P=0.05) from their pairs in the same row.

Organoleptic or sensory feature during *pupuru* storage revealed that the appearance/colour of the ball remained the same during the first four days of storage. After this period, there was a significant change in the colour from white to off white. This further changed to pink and greyish colour by the tenth day of storage. The

pupuru ball was able to retain its distinct aroma within the first four (4) days, but developed a faint smell within days four (4) and five (5). An unpleasant smell developed during the remaining days of storage by which the product became spoilt. Also the *pupuru* developed a sour taste after the fifth (5) day (Table 6).

Table 6: Organoleptic evaluation of pupuru balls stored at room temperatures

*ATTRIBUTES	Storage period (days)											
	1	2	3	4	5	6	7	8	9	10	11	12
COLOUR	8.2 ^a	8.0 ^a	7.6 ^a	7.0 ^{ab}	4.2 ^c	4.2 ^c	4.0 ^c	4.0 ^c	4.2 ^c	3.8 ^c	2.8 ^d	2.0 ^d
TEXTURE	7.4 ^a	7.0 ^a	7.0 ^a	6.8 ^a	6.0 ^{ab}	4.8 ^{bc}	5.0 ^{bc}	3.6 ^c	3.6 ^c	3.6 ^c	3.0 ^c	3.0 ^c
AROMA	9.0 ^a	8.2 ^{ab}	7.6 ^{bc}	6.3 ^d	7.0 ^c	6.0 ^d	4.5 ^e	4.0 ^e	4.0 ^e	4.0 ^e	2.5 ^f	2.5 ^f
TASTE	7.6 ^a	7.3 ^a	7.3 ^a	7.0 ^a	4.0 ^b	4.0 ^b	4.0 ^b	3.0 ^c	3.0 ^c	3.0 ^c	0 ^d	0 ^d

^{a-f} values in the same row with different superscripts differ significantly (p=0.05).

*Result data are the mean score values from a 10-man panel.

This study therefore shows that the shelf life for *pupuru* balls stored under ambient temperature must not exceed four days after production and that moulds are the main spoilage microorganisms of *pupuru*.

In conclusion, *pupuru* balls spoil faster due to the residual moisture in the dried balls and sometimes the harsh weather they are been exposed to by the sellers. Shelf life for a freshly produced *pupuru* ball is four days under room temperatures. *Pupuru* is acidic in nature and moulds associated with these products during storage may use the organic acids present in the product for growth resulting in spoilage. In order to avoid economic loss and public health hazards, producers should always produce *pupuru* balls that can easily be sold, while sellers should provide dry storage condition for their goods. New research should be conducted in the area of developing a more advanced fermentation technique which would in turn increase the shelf life, taste, and safety of the product. Consumers should avoid the purchase of large quantity of *pupuru* for future consumption beyond four days. Some *pupuru* being sold in the markets are not always freshly produced, precautions should be taken to avoid eating spoiled *pupuru* because of public health hazards including food poisoning.

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