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Impact of Packaging Materials on the Microbial Stability and Moisture Content of Instant Pounded Yam Flour During Long-Term Storage

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Abstract

This study evaluated the microbial stability and moisture content of instant pounded yam flour stored in various packaging materials over a year at the Nigerian Stored Products Research Institute(NSPRI). Flour was packaged in 25kg and 50kg polyethylene bags and placed in secondary packaging materials: Multiply paper, calico, carton, and Plastic containers. Microbial analysis was conducted quarterly, with bacteria cultured at 37°C for48hours and fungi at 25°C for 72hours. The total colony counts of bacteria and coliform count increased overtime, with the highest counts in flour stored in calico. Fungal load also increased; particularly in multiply paper and calico. Moisture content decreased during the dry season and increased during the wet season. It was concluded that plastic containers provided the best protection against microbial proliferation, followed by multiply paper, carton, and calico. The findings suggest that packaging material permeability and storage duration significantly affect the microbial quality of stored pounded yam flour.

Keywords: Flour, Microbial, Packaging, Quality, Storage,

INTRODUCTION

Yam, belonging to the Dioscoreaceae family, is a staple food of significant importance in West Africa, second only to cereals in consumption (Obidiegwu*et al.*, 2020). It boasts a rich content of essential dietary nutrients, positioning it nutritionally above other tropical root crops (Shajeela*et al.*, 2011). Nigeria, contributing to approximately 94% of the global yam production, stands as the continent's leading producer (Adams and Nkoro, 2021).

The prominent yam species cultivated in Nigeria include *Dioscorearotundata* (white yam), *Dioscoreaalata* (wateryam), *Dioscoreacayenensis* (yellowyam), *Dioscoreabulbifera* (aerialyam or air potato), *Dioscoreaesculenta* (Chinese yam), and *Dioscoreadumentorum* (trifoliate yam) (Obidegwu*et al.*, 2020). Among these, traditional foods such as chips, amala, and particularly pounded yam are derived (Umoh, 2020). Pounded yam enjoys widespread popularity across Nigeria's major tribes and is traditionally prepared during yam festivals to celebrate the new harvest (Thompson and Oduro, 2021).

The conventional practice of manually pounding yam is quite strenuous and demands considerable time. To address this, advancements in food science have led to the creation of instant pounded yam flour (Olu*et al.*, 2012). This innovative method involves peeling,

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cleansing, chopping, blanching (which deactivates enzymes and diminishes microbial presence), desiccating, and grinding the yam tubers into a delicate flour (Olaoye and Oyewole, 2012). This product streamlines the preparation process to just mixing with boiling water.

Of all Dioscorea species, *Dioscorearotundata* is the most extensively cultivated and consumed in Nigeria (Alamu*et al.,* 2014). Unfortunately, despite the potential for storage up to six months without refrigeration, a significant portion of annual production succumbs to spoilage due to inadequate postharvest facilities (Adejo, 2017)..The influence of deteriorating activities during food processing, combined with subsequent storage condition differences, affects the storage stability of processed foods. Flour products' degradation is often associated with packaging materials and microbial spoilage (Ilouno*et al.*, 2016).This research is centered on the large-scale packaging of instant pounded yam flour, a trend that is increasingly popular among middle-class households due to heightened awareness of health and shifts in lifestyle. Packaging not only ensures cleanliness but also acts as a protective shield against pollutants (Kamboj*et al.*, 2020). The study delves into the effectiveness of different packaging materials in preserving the microbiological integrity of instant pounded yam flour during storage.

MATERIALS AND METHODS

Collection of sample

Freshly prepared instant yam flourweighing 640kg was procured from the processing unit of was procured from the processing unit of Nigerian Stored Products Research Institute Ilorin, KwaraState, Nigeria. Aluminium foil, cotton wool, test tubes and pipettes used for this study were purchased from Labtradecompany, Ilorin. All chemicals and reagents used were of analytical standard.

Packaging of Instant yam flour

The instant yam flour was packed in sizes of 25kg and 50kg in a food grade medium density (guage 0.35mm) polyethylene. The packaged products were then put in a secondary packaging materials made of multiplypaper, calico, carton and covered plastic containers.

Storage of Instant yam flour

The instant pounded yam flour weighing 25kg and 50kg was stored in polythene in calico, polythene in brown paper bags, polythene in covered plastic container and polythene in carton respectively at a moisture content it was obtained from the processing unit, they were then stored at ambient temperature (28±3°C). Thermo-hygrometer was placed in the storage room to record the temperature and relative humidity of the atmosphere. The instant yam flour was stored for one year and sample was taken at three-month interval for moisture and microbiological (bacteria, fungi and coliform) analysis.

Preparation of media

The materials used such as glass wares were properly sterilized in the oven at 160°C for 1h. The preparations of the Nutrient agar, Mac Conkey agar and Potato dextrose agar media were done according to the manufacturer's instructions, then autoclaved at 121°C for 15min.

Isolation and characterization of pure cultures of microorganisms

Accurately, 1g of sample was weighed and then placed in a sterile test tube and dissolved with 9ml of distilled water to make the stock. The suspension was filtered through sterile glass wool. Serial dilution was done to the necessary dilution factors and pour-plated. The plates were left to gel and then incubated. The bacteria plates were incubated at 37^{o}C for 48h while the fungal plates were incubated at 25^{o}C for 72h. At the end of each incubation period, the colonies were counted and expressed as colony forming unit per millilitre (CFU/ml). Distinct colonies were sub-cultured unto fresh media maintained on slants and preserved at 4^{o}C in the refrigerator (Fawole and Oso 2004).

The bacteria were identified using Koneman Chart (Procop*et al.*, 2017). Fungal load determination were carried out according to the method described by Feroz*et al.* (2016).

Moisture content determination
Moisture content of each blended samples were determined according to AOAC (2019),
A 5.0g each of all the samples was weighed into the pre-set oven and the drying was performed at 105°C for 4hrs to constant weight. Then they were removed from the oven and placed in the desiccators to cool and then weighed. The difference in weight was used to obtain the moisture content. All analyses were carried out in duplicate. The percentage moisture content was then calculated as:

$$\text{Moisture content MC \%} = \frac{\text{Weight loss } \times 100\%}{\text{Original weight}}$$

RESULTS AND DISCUSSION

Table1: Total Colony Count (TCC) of bacteria in instant pounded yam flour stored in different packaging material over storage period(CFU/ml)

Period of Storage (Month)	Packaging Material							
	Carton		Calico		Multiply Paper		Plastic	
	25kg	50kg	25kg	50kg	25kg	50kg	25kg	50kg
0	0.25±0.01^{a}	0.25±0.01^{a}	0.25±0.01^{a}	0.25±0.01^{a}	0.25±0.01^{a}	0.25±0.01^{a}	0.25±0.01^{a}	0.25±0.01^{a}
3	1.80±0.01^{c}	1.82±0.01^{c}	1.95±0.02^{d}	1.98±0.01^{d}	0.89±0.02^{a}	0.92±0.01^{a}	0.99±0.02^{b}	1.09±0.05^{b}
6	2.27±0.02^{c}	2.47±0.01^{c}	3.0±0.02^{d}	3.0±0.02^{d}	1.02±0.02^{a}	1.58±0.02^{b}	1.33±0.01^{b}	1.73±0.01^{b}
9	3.48±0.02^{b}	3.84±0.02^{a}	5.5±0.06^{d}	6.66±0.03^{c}	4.08±0.03^{c}	6.30±0.17^{b}	3.33±0.02^{a}	3.61±0.14^{a}
12	4.35±0.01^{a}	4.42±0.01^{a}	6.24±0.02^{c}	7.48±0.0^{c}	5.65±0.03^{b}	6.98±0.05^{b}	4.31±0.014^{a}	4.41±0.02^{a}

Table shows Mean±Standard Error (SE) of triplicate readings/values. Means with
Unshared superscripts are significantly different (p<0.05).

A significant difference (p≤0.05) was observed across various packages and units for all time points, excluding Month 0.

The total colony counts (TCC) of bacteria in instant yam flour stored in different packaging material over storage period as presented in Table 1.There is significant difference(p≤0.05) in all the months in the various packages and units except Month 0. Notably, 50kg carton and plastic packs at Months 9 and 12 exhibited comparable results, distinct from calico and multiple plastic packs, which demonstrated significant variability.The initial sample of instant yam flour had 0.25x10^{4}cfu/g bacteria colonies per gram. It increased appreciably; with the 50kg instant pounded yam flour stored in polythene in Calico having the highest population of 7.48x10^{4}cfu/g and 25kg instant pounded yam flour stored in polythene in covered plastic had the least population of bacteria (0.99x10^{4}cfu/g).

Table2: Total coliform count (TCC) in instant pounded yam flour stored in different packaging material over storage period

Period of Storage (Month)	Packaging Material							
	Carton		Calico		Multiply Paper		Plastic	
	25kg	50kg	25kg	50kg	25kg	50kg	25kg	50kg
0	0.13±0.1[a]	0.13±0.1[a]	0.13±0.1[a]	0.13±0.1[a]	0.13±0.1[a]	0.13±0.1[a]	0.13±0.1[a]	0.13±0.1[a]
3	0.52±0.01[b]	0.49±0.11[c]	0.94±0.02[c]	0.94±0.02[d]	0.17±0.02[a]	0.17±0.03[a]	0.15±0[a]	0.26±0.01[b]
6	1.07±0.03[c]	1.02±0.01[b]	2.39±0.02[d]	2.35±0.03[c]	0.71±0.03[b]	0.70±0.02[b]	0.50±0.04[a]	0.63±0.01[a]
9	7.43±0.02[d]	5.35±0.03[b]	7.13±0.02[c]	7.40±0.01[c]	2.92±0.01[b]	2.39±0.03[a]	2.29±0.01[a]	2.38±0.02[a]
12	8.35±0.02[c]	6.29±0.02[c]	8.33±0.01[d]	8.35±0.01[d]	4.35±0.02[b]	4.25±0.02[b]	3.77±0.01[a]	3.82±0.01[a]

Mean ± Standard Error (SE) of triplicate readings/values. Means with Unshared superscripts are significantly different ($p<0.05$).

From Table2,there is a significant difference ($p\leq0.05$) in total Coliform in all the months except the first month 0. However, in the third month, in 25kg and ninth month 50kg, multiple paper and plastic are the same and different from carton and calico which are different from each other.The coliform count of 50kg instant yam flour stored in polythene in calico was $8.35\text{x}10^2$cfu/g and it happens to be the highest at the 12th month, while that of 25kg instant pounded yam flour stored in polythene in plastic was $3.79\text{x}10^2$cfu/g and the least at the 12th month. The initial fungi load was $0.13\text{x}10^2$cfu/g. The total coliform group has been used sometime as an "indicator organism". An indicator organism by itself is considered to cause no diseases in manor animals, but its presence usually indicates the presence of pathogenic or disease-causing organisms. By measuring the number of total coliform present in a sample a judgment can be made as to the water's usability for a given purpose.

Table3: Total colony count (TCC) of fungi in instant pounded yam flour stored in different packaging material over storage period

Period of Storage (Month)	Packaging Material							
	Carton		Calico		Multiply Paper		Plastic	
	25kg	50kg	25kg	50kg	25kg	50kg	25kg	50kg
0	0.17±0.02[a]	0.17±0.02[a]	0.17±0.02[a]	0.17±0.02[a]	0.17±0.02[a]	0.17±0.02[a]	0.17±0.02[a]	0.17±0.02[a]
3	0.52±0.01[a]	0.49±0.02[ab]	0.94±0.02[c]	0.93±0.17[c]	0.57±0.02[a]	0.47±0.02[a]	0.65±0.03[b]	0.55±0.01[b]
6	1.05±0.03[c]	2.04±0.02[d]	1.05±0.02[c]	1.53±0.02[c]	0.63±0.02[a]	0.60±0.02[a]	0.73±0.02[b]	0.83±0.02[b]
9	1.18±0.11[c]	2.4±0.02[d]	1.64±0.02[d]	2.04±0.23[c]	0.77±0.01[a]	0.94±0.03[a]	1.05±0.03[b]	1.17±0.01[b]
12	2.95±0.02[d]	3.39±0.04[c]	3.03±0.02[c]	3.53±0.02[d]	1.15±0.02[a]	1.95±0.02[a]	2.77±0.01[b]	2.92±0.01[b]

Table shows Mean±Standard Error (SE) of triplicate readings/values. Means with Unshared superscripts are significantly different ($p<0.05$).

Also, Table 3 showed that there is significant difference ($p\leq0.05$) in all the total colony counts of fungi in all the months in various packaging categories except from the Month 0 that all the packages are not significantly different from each other. Meanwhile, In Month 3, in 25kg group, Carton and Multiply paper are the same but different from Calico and Plastic that are also different from each other. In same vain in the 50kg pack Carton, Multiple Paper and Plastic are the same but different from carton. In Month 6 in the 25kg pack, Carton and Calico are the same but different from Multiple Paper and Plastic which are also different from each other. But there is different in 50kg category in all the package type. Subsequently in Month 9 and 12 there was significant difference in all the package type in all units.The initial fungal load was 0.17×10^4cfu/g. At the end of the 12th weeks, polythene in brown multiply paper had a fungi population of 1.15×10^4cfu/g while polythene in calico had 3.53×10^4cfu/g. This high level of mould count gives an insight into the type of handling the instant yam flour had received at the source of yam purchased, through processing into instant yam flour before it is stored.

Table 4: The probable microorganisms isolated from the four packaging materials of the instant yam flour samples after storage.

Moulds	Bacteria
Acremonium spp.	*Bacillus spp.*
Aspergillus spp.	*Erwinia spp.*
Aspergillus spp.	*Escherichia spp.*
Fusarium spp.	*Lactobacillus spp.*
Mucor spp.	*Micrococcus spp.*
Penicillum spp.	*Lactobacillus spp.*
Rhizopus spp.	*Proteus spp.*
Fusarium spp.	*Staphylococcus spp.*
Rhizopus spp.	

The probable isolates from the instant yam flour in the twelve months of study are listed in Table 4. Ten fungi species were isolated in all, they are; *Acremoniumspp, Aspergillus spp., Aspergillus spp., Fusarium spp., Mucor spp., Penicillum spp., Rhizopusspp, Fusarium spp.* and *Rhizopus spp.*

Also, nine probable bacterial species were isolated, and they are *Bacillus spp, Erwinia spp., Escherichia spp., Lactobacillus spp., Micrococcus spp., Lactobacillus spp., Proteus spp.*and *Staphylococcus spp.* The presence of spore former especially *Bacilus spp*. and *Aspergillus spp*. were not surprising because they were isolated and identified from the market micro-environment where they were purchased due to exposure. The presence of Lactic acid bacteria on the instant pounded yam flour might be a contributing factor to its flavour and odour. It was reported that lactic acid bacteria on garri sample had been known to contribute to its initial flavour and odour (Bamidele *et al* 1988). From this work it is observed that instant pounded yam flour stored in polythene in Calico had the highest population of microorganisms (Mould, coliform and Bacteria). Hence, it is better to store instant yam flour in covered plastic, follow by multiply paper, carton and lastly in Calico

Table5: Moisture content (%)of instant pounded yam flour stored in different packaging materials

Period of Storage (Month)	Packaging Material							
	Carton		Calico		Multiply Paper		Plastic	
	25kg	50kg	25kg	50kg	25kg	50kg	25kg	50kg
0	8.40 ± 0.1^a	8.40 ± 0.1^a	8.40 ± 0.1^a	8.40 ± 0.1^a	8.40 ± 0.1^a	8.40 ± 0.1^a	8.40 ± 0.1^a	8.40 ± 0.1^a
3	6.83 ± 0.06^a	6.25 ± 0.03^c	7.0 ± 0.12^a	7.10 ± 0.05^a	7.33 ± 0.02^b	6.83 ± 0.02^b	6.98 ± 0.09^a	6.86 ± 0.01^b
6	6.13 ± 0.02^b	6.20 ± 0.12^b	6.03 ± 0.02^a	6.23 ± 0.02^b	6.53 ± 0.02^d	6.86 ± 0.08^c	6.23 ± 0.02^c	5.56 ± 0.02^a
9	7.20 ± 0.12^b	7.30 ± 0.12^a	7.55 ± 0.03^d	7.20 ± 0.23^a	7.45 ± 0.03^a	7.50 ± 0.57^a	6.70 ± 0.03^c	7.4 ± 0.12^a
12	7.52 ± 0.01^a	7.4 ± 0.03^a	7.80 ± 0.03^b	7.60 ± 0.05^a	7.70 ± 0.02^b	7.60 ± 0.12^a	7.77 ± 0.03^b	7.40 ± 0.01^a

Mean ± Standard Error (SE) of triplicate readings/values. Means with Unshared superscripts are significantly different ($p<0.05$)

The result of the moisture content determination in Table 5,showedthat there is no significant difference ($p \leq 0.05$)in the moisture content in the Month 0 in 25kg and 50kg Packs and in Month 9 and Month 12 in the 50kg pack. But there is significant difference ($p \leq 0.05$) in moisture content in other Months. However, in Month 3, in the 25kg pack, Carton, Calico and Plastic are the same and different from Multiple Paper. Similarly, in the 50kg pack in the third month, Multiple paper and plastic are the same and different from Carton and Calico which are also different from each other. In Month 6,25kg pack and in Month 9, 50kg pack there is significance different ($p \leq 0.05$)in all the packages. The instant pounded yam flour had initial moisture content of 8.40% before storage. During storage, the instant pounded yam flour stored in different packaging material generally showed a decreased in value of moisture content up to the sixth month before it increased again. The moisture content reduction maybe connected to the period of year in which the product was stored (September, 2022 to August, 2023). The first six months falls between September to February (dry season of the year), the other six months was between March to August (wet season). The moisture content increased appreciably from sixth week to the twelfth week in all the storage materials except that of 50kg polythene in covered plastic which remain stable at the ninth and twelfth week of storage.

Looking at the results, the microbial load (bacteria, fungi and coliform) of instant pounded yam flour stored in polythene in calico was higher than that of the other storage materials. The difference in total viable counts in instant pounded yam flour in different packaging materials may be attributed to the nature of the packaging materials themselves. According to Nwafor and Ikenebomeh (2009), one of such nature factor is relative permeability to air. Calico bag is more permeable to air than the other packaging materials, which facilitate movement of air both in and out of the bag. The period of year of storage also, has effect on the proliferation of organisms.

The effectiveness of the packaging materials in preventing proliferation of microbiological agent in the storage materials is in the orders, for bacteria: Covered Plastic < Carton < Multiply Paper < Calico; for coliform: Covered Plastic < Multiply Paper < Carton < Calico and for fungi: Multiply Paper < Covered Plastic < Carton < Calico. Also, the microbial load of the 50kg packed instant pounded yam flour regard less of the packaging material was higher than that of the 25kg packages. This must have been as a result of the fact that the 50kg packs have more nutrients, space and surface area than that of the 25kg packs.

CONCLUSION AND RECOMMENDATION

The storage materials used in this study are effective packaging materials, the covered plastic containers had the best protection against microbial proliferation, followed by multiply paper, then carton, and then calico,the findings suggest that packaging material permeability and storage duration significantly affect the microbial quality of stored pounded yam flour.

Covered plastic is recommended due to its consistent performance in keeping microbial counts and moisture content low, thus prolonging the product's shelf life and ensuring its safety. Future investigations should aim to refine packaging techniques to further stabilise yam storage and minimise food loss, contributing to regional food security.

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