EFFECT OF WRAPPING MATERIALS ON MYCO FLORA GROWTH, PROXIMATE COMPOSITION AND SHELF LIFE OF SOLID PAP SOLD IN LAPAI, NIGER STATE, NIGERIA

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Abstract
Solid pap a gel-like traditional fermented starchy food produced from maize (Zea mays), is a popular food across the various multi-ethnic groups and socio-economic classes in Nigeria. However, packaging, an integral part of food processing that provides the proper environmental conditions for long shelf life and protects the products against microbiological, chemical or physical deterioration is a major problem. This study, therefore investigated the effects of wrapping materials on mycoflora growth, and proximate composition of the food. Proximate and microbial analysis of freshly prepared pap was done before storage for 10 days. The samples were wrapped in banana leaves (Musa paradiosiaca) and nylon. The proximate and microbial analyses were conducted during storage at day 3, 5 and 10. The results showed that fresh pap have percentage moisture (9.03%), ash (2.08%), crude fibre (3.04%), crude fat (0.85%), protein (3.99%), and carbohydrate (82.00%). The moisture content increased with period of storage from day 0 to 10 day (from 10.03% to 16.50% in leaf and from 10.03% to 12.20%in nylon). The proximate compositions of pap wrapped with nylon were significantly ($p<0.05$) higher than pap wrapped with leaf except for carbohydrate content which was a bit higher in pap wrapped with leaf than nylon at day 3, 5, and 10 of storage. The isolated fungi in pap included Mucor species, Aspergillus niger, A. flavus, Penicillium notatum. The occurrence of these fungi was observed to be significantly ($p>0.05$) increased from day 3 to day 10, the end of storage period in both wrapping materials. However, this study revealed that the pap is less susceptible to microbial attack, and nutrients are best retained when nylon are used to wrap the pap than leaf. Therefore, the use of banana leaves in wrapping the pap must be discouraged as it made it liable to easy attack by the fungi and invariably leads to its deterioration.

Keywords: *Proximate, Pap, Nylon, Leaf, Mycoflora*

Introduction
Solid pap is a gel-like traditional fermented starchy food item produced in Nigeria from maize, millet and sorghum (Plate 1). Its colour depends on the cereal used. It is cream to glossy white from maize, light brown from sorghum and grey to greenish colour from millet. This food had undergone a desirable change due to the action of the invading microorganisms or their metabolic products (Patience, 2013). Solid pap is known by different names in different localities such as eko (Yoruba), akasan (Benin), kamu (Hausa) and agidi (Ibo). It is becoming very popular, with acceptability cutting across the various multi-ethnic groups and socioeconomic classes. The ease of consumption, alone or with soup, stew, beans cake (akara), moi-moi, as light meal especially amongst post operative patients and other patients in the hospitals makes it very popular. (Ogiehor *et al*., 2005). The traditional production process involves soaking of maize grains in cold water for 1-3 days after which the water is decanted.

The soaked grains are wet milled and sieved and the filtrate is fermented for 2-3 days to yield wet 'ogi', which is sour, white starchy sediment and then boiled into a thick porridge, solid pap (Ujabadeniyi & Adebolu, 2005). The production varies from one locality to another resulting in a non-uniform product, non-specified quality indices, unknown shelf life and lack of safety indices, thus limiting product acceptability to immediate locality. Furthermore, solid pap deteriorates rapidly in storage (2-3 days), warranting repetition of the cumbersome and time consuming production cycles in order to keep product available (Ogiehor *et al.*, 2005).

Packaging is an integral part of food processing. It provides the proper environmental conditions for long shelf life. It protects the products against microbiological, chemical or physical deterioration (Komolafe, 2005). Processed foods can be preserved for extended periods by an aseptic packaging to exclude microbes and oxygen as well as to maintain a moderate temperature (Patience, 2013). However Packaging materials have also been known to be possible source of microbial contamination of this food (Wasiu *et al.*, 2013).

The role of packaging in the food industry which includes protection, containments, transportation, preservation and advertisement are not achieved in all of the packaging method used in Nigeria. This in turn results in a huge loss of the food product not only during packaging processes but also during transportation and sales (Enyisi *et al.*, 2014). The only regulatory body in Nigeria, "National Agency for Food and Drug Administration Control" (NAFDAC) has made tremendous progress in controlling the safety aspect in some of the food industry in Nigeria, such as in the confectionaries, sachet water industry and pharmaceutical industry. However, little or no efforts are made on the local food product which is the most common in the country (Adegunloye *et al.*, 2013).

Solid pap is traditionally wrapped in leaves or transparent polythene bags and marketed. These wrapping materials are poorly handled and transported. They are often dirty and are kept in the open with little or no provision for washing before use. These may therefore be a source of microbial contamination of the food (Adejumo & Ola, 2008).

Over the years few work has been done to try and investigate the effect of the commonly use packaging materials on the nutrient composition and microbial attacked on pap as a general local food especially in Niger State particular in Lapai.

The aim of this study was to investigate the effect of some wrapping materials on mycoflora growth, shelf life and proximate composition of solid pap sold in Lapai, Niger state Nigeria. The result will be used to establish the best hygienic wrapping materials for solid pap.

A B C D E

PLATE 1: A. Maize cob B. Maize grain C. prepared pap D. Pap E. wrapping leaves wrapped with leaves

Source: (http://www.coextra.eu/images/image1233.html)

Materials and Methods

Collection of Materials

Matured wholesome, disease free maize (*Zea mays)* grains, banana leaves and nylon (Plate 1) were bought from market in Lapai Niger State, Nigeria between June and July 2014.

Traditional Preparation of Pap

Three kg of maize grains were steeped in 6L of distilled water for 2days at room temperature. The steep water was decanted and grain washed in fresh distilled water followed by wet milling and wet sieving through 450um sieve screen. The resulting fines grains from traditional and modified process were allowed to settle and ferment (24hrs) to slurries and the sediment was used to prepare the pap using hot water to simulate the traditional preparation (Oyarekua & Eleyinmi, 2004). A mole of pap was made by using beaker to dispense 25cm^3 of maize paste on the leaf or nylon and wrapped immediately. 20moles of leaf wrapped pap and 20 moles of nylon wrapped pap were made. All samples were stored under ambient temperature for 10 days in the Biological Sciences laboratory Ibrahim Badamasi Babangida University, Lapai. Proximate composition of the pap and microbial growth were monitored at day 3, 5 and 10 of storage.

Proximate Analysis

Determination of Moisture Content

Two gram of the sample was placed in the crucible and heated at 105^{o}C until a constant weight was attained. The moisture content was calculated as loss in weight of the original sample and expressed as percentage moisture content (A.O.A.C., 2005).

$$\% \text{ Moisture} = (W2 - W3)/(W2 - W1)$$

Where: W1 = weight of empty crucible
W2 = weight of crucible +sample before drying
W3= final weight of crucible + sample after drying

Determination of Crude Protein

The sample (0.5g) was digested with 5ml of concentrated sulphuric acid in the presence of Kjeldahl catalyst. The nitrogen from the protein in the sample was converted to ammonium sulphate that reacted with 2.5ml of 2.5 % Brucine reagent, 5ml of 98 % sulphuric acid to give a coloured derivative and the absorbance read at 470nm. The percentage nitrogen is calculated and multiplied by 6.25 to obtain the value of the crude protein (A.O.A.C., 2005).

$$\% \text{ Nitrogen} = (Vs - Vb \times Nacid \times 0.01401)/W \times 100$$

Where: Vs =titre value of the sample
Vb = acid required to titrate
N acid = normality of acid
W = weight of sample in grams

Estimation of Crude Lipid

This estimation was performed using the Soxhlet extraction method of AOAC, (2005). Ten (10)g of the sample was weighed and wrapped with a No 1 Whatman filter paper and placed in a thimble. The thimble was covered with cotton wool and placed in the extraction column that was connected to a condenser. Two hundred (200) ml of n – Hexane was used to extract the lipid.

$$\%\text{Fat} = (W2 - W3)/(Weight\ of\ sample) \times 10$$

Where: W_2 = wt of filter paper and sample before extraction

W_3 = wt of filter paper and sample after extraction

Determination of Crude Fibre

Five (5)g of the sample and 200ml of 1.25 % H_2SO4 was heated for 30minutes and filtered with a Buchner funnel. The residue was washed with distilled water until it was acid free. Two hundred (200)ml of 1.25% NaOH was used to boil the residue for 30minutes, and it was filtered and washed several times with distilled water until it was alkaline free. It was then rinsed once with 10% HCl and twice with ethanol. Finally, it was rinsed with petroleum ether three times. The residue was put in a crucible and dried at 105^{o}C in an oven overnight. After cooling in desiccators, it was then ignited in a muffle furnace at 550^{o}C for 90 minutes to obtain the weight of the ash.
% fibre content = the loss in weight after incineration × 100.

Determination of Ash Content

The ash content of the sample was determined using AOAC standard method (2005). Five (5)g of the sample was weighed into a crucible of known weight and was dried in an oven for about 4hrs at 105^{0}C.The sample in the crucible was ashed in a muffle furnace at 5000^{o}C, until white was obtained. It was allowed to cooled in a desiccators and was then reweighed.
%Ash content = $(w3 - w1)/(w2 - w1) \times 100$

Where: W1= weight of empty crucible
W2= weigh of sample +weigh of crucible before aching
W3= weigh of sample + weigh of crucible after aching.

Carbohydrate Determination

The carbohydrate content was determined by subtracting the summed up percentage compositions of moisture, protein, lipid, fibre, and ash contents from 100% (Otitoju, 2009).
%Carbohydrate = 100 – (%Protein + %Moisture + %Ash + %Fibre)

Isolation of Fungi

Serial dilution technique was used, one gram of each samples was crushed and aseptically transferred into 9ml of sterile distilled water in test tubes. It was then shaken properly to allow for even distribution of microorganisms present in the sample. The dilution factors 10^{-1} and 10^{-2} were used as stock solution. One (1) ml of each dilution was aseptically taken from the suspension and transferred into sterile Petri dishes. Ten (10) ml of Potato Dextrose Agar (PDA) was poured into the Petri dish with 1ml of chloramphenicol. The plates were swirled gently to allow even distribution of the sample. Incubation was done at room temperature 28± 2ºC for 24 hours. Subcultures were made from the mixed cultures. Fungal isolates were identified using fungal family of the World Mycological Monographs (Cannon & Kirk, 2007; Amadi & Adebola, 2008).

Statistical Analysis

The experimental data generated at days 3, 5 and 10 of storage were statistically analyzed using Analysis of variance (ANOVA) using completely randomized design of SPSS statistical package computer software (2009 version), Turkey's test of the same package was used to compare the significant ($p<0.05$) differences among individual.

Results

Proximate composition of fresh pap

The results of the percentage proximate composition of the cold pap are presented in Table 1. The fresh pap at day 0 was found to contain 9.03±0.04 % moisture content, ash 2.08±0.01%, crude fat 0.85±0.05%, crude fibre 3.04±0.01%, crude protein 3.99±0.01% and carbohydrate 82.00±0.01%. The proximate composition of pap wrapped in banana leaf and nylon decreased continuously from days 3, 5 to day 10 of storage. The protein content was found to significantly decreased from day 0 to day 10 (from 3.99±0.01% to 2.35±0.11% and 3.05±0.12% in leaf and nylon respectively). However, there was no significant difference ($p<0.05$) in the decrease observed in nylon as with the leaf. The moisture content increased with period of storage from day 0 to 10 day. That was from 9.03±0.04% to 16.50±0.21% in leaf and from 9.03±0.04% to 12.20±0.6%1in nylon. The increase in moisture content was significantly different ($P<0.05$) in leaf while in nylon, there was no significantly different ($P<0.05$) between days 5 and 10. The Carbohydrate content at day0 (82.00±0.01%) was significantly higher ($P<0.05$) than the other storage days and decreased to 79.12±0.01% and 79.81±0.9% at day 10 in leaf and nylon respectively. The decrease was not significantly different ($P<0.05$) between days 3, 5 and 10 of storage in both the leaf and nylon. The crude fat was generally low compared to other food constituents. It was 0.85±0.05% at day 0 and decreased to 0.74±0.22% and 0.80±0.33% in leaf and nylon respectively. However, there was no significantly difference ($p<0.05$) between days 0, 3, 5and 10 in both leaf and nylon. This trend was also observed in crude fibre.

Table 1: Percentage Proximate composition of stored pap wrapped in leaf and nylon at day 0, 3, 5 and 10 of storage

	Control (%)	Pap wrapped with leaf (%)			Pap wrapped with nylon (%)		
Fungi isolated	Day 0	Day 3	Day 5	Day 10	Day 3	Day 5	Day 10
Moisture content	9.03±0.04^{a}	12.70±0.01^{b}	14.68±0.01^{c}	16.50±0.21^{d}	12.20±0.0^{b}	12.05±0.01^{c}	12.20±0.61^{c}
Ash content	2.08±0.01^{a}	0.81±0.04^{b}	0.80±0.01^{b}	0.78±0.11^{c}	2.00±0.01^{b}	1.49±0.05^{b}	1.35±0.01^{c}
Crude fat	0.85±0.05^{a}	0.79±0.10^{b}	0.75±0.11^{c}	0.74±0.22^{c}	0.83±0.00^{a}	0.83±0.01^{a}	0.80±0.33^{a}
Crude fibre	3.04±0.01^{a}	2.71±0.01^{b}	2.70±0.03^{b}	2.61±0.21^{c}	3.03±0.01^{a}	3.03±0.11^{a}	3.02±0.91^{b}
Crude protein	3.99±0.01^{a}	3.08±0.23^{b}	3.02±0.77^{b}	2.35±0.11^{c}	3.10±0.21^{a}	3.08±0.06^{a}	3.05±0.12^{c}
Carbohydrate	82.00±0.01^{a}	81.91±0.01^{b}	80.05±0.00^{b}	79.12±0.01^{b}	80.11±0.11^{b}	80.11±0.11^{b}	79.81±0.9^{b}

Values follows by the same superscript in same treatment and row compared with control are not differ significantly at $p<0.05$, values are Mean $\pm$ SEM of triplicate determination.

Fungal Contamination

A total of four fungi species (*Mucor* species*, Aspergillus niger, A. flavus, Penicillium notatum)* from three genera were isolated (Table 2). The incidence of fungal contamination started from day0 (freshly prepared pap). At this period the *Mucor* sp. has the highest population (2.00±1.99) of occurrence, while *P. notatum* was the least (0.28±0.12%). Generally speaking, the occurrence of the fungi isolated was higher in pap wrapped with banana leaves throughout the period of storage. The occurrence of these fungi was observed to be significantly ($p>0.05$) increased from day 3 to day 10, the end of storage period in both wrapping materials. At the end of storage period *A. flavus* has the highest occurrence from 0.86±0.35 in day 0 to 64.94±2.0 in day 10 followed by *A. niger* 1.86±1.90 day 0 to 62.05±1.70% at day 10 in pap wrapped with leaves, while *P. notatum* has the highest occurrence (31.25$\pm$ 0.11) followed by *A.*

niger (28.75±1.09) in pap wrapped with nylon. However, by the end of storage period, the occurrence of *Mucor* sp. was the least in both wrapping materials.

Table 2: Fungal population in pap samples on the day 0, 3, 5 and 10 using different wrappers

	Control (%)	Pap wrapped with leaf (%)			Pap wrapped with nylon (%)		
Fungi isolated	Day 0	Day 3	Day 5	Day 10	Day 3	Day 5	Day 10
Aspergillus flavus	0.86±0.35^{a}	26.13±1.70^{b}	43.48±2.00^{c}	64.94±2.00^{a}	23.08±1.03^{b}	11.80±0.33^{c}	24.38±1.03^{a}
Aspergillus niger	1.86±1.90^{a}	26.13±1.70^{b}	50.87±1.70^{a}	62.05±1.70^{a}	25.38±1.09^{b}	18.18±1.20^{c}	28.75±1.09^{b}
Penicillium notatum	0.28±0.12^{a}	22.36±0.33^{a}	32.61±0.13^{a}	44.58±0.13^{c}	20.00±0.11^{c}	15.45±0.00^{b}	31.25±0.11^{a}
Mucor species	2.00±1.99^{a}	7.90±2.74^{b}	23.04±0.74^{b}	38.43±0.74^{a}	11.54±1.22^{a}	14.55±1.85^{a}	15,62±1.22^{c}

Values follows by the same superscript in same treatment and row compared with control are not differ significantly at $p<0.05$, values are Mean $\pm$ SEM of triplicate determination.

Discussion

The present study revealed the nutritional composition of solid pap and how they are affected by different types of wrapping materials.

Packaging is an integral part of food processing, it provides the proper environmental conditions for long shelf life. This was in evidence from the results obtained from cold pap wrapped with two different materials (nylon and banana leaf) and stored for the period of ten days.

The results of the proximate composition before and after storage period showed that cold pap contain crude fat, crude flbre, crude protein, carbohydrate and ash as earlier reported by Enyisi *et al*., (2014) in maize grain and maize products. Pikuila and Ilelaboye, (2013) and Oyarekua and Eleyinmi, (2004) also made similar reports on the proximate and chemical composition of 'ogi' prepared from maize grain. However, the modification of traditional process of maize to 'ogi' and then to pap have been reported to significantly affect their proximate composition (Oyarekua & Eleyinmi, 2004).

The results on the moisture content revealed that moisture content which was at minimal percent atday 0 is an indication of stable self life if properly packaged and stored, because low moisture is necessary in food for good keeping quality and longer shelf life (Amadi & Adebola, 2008). The moisture increased with the period of storage in both wrapping materials. However, the moisture content of pap wrapped with leaves was found to be on the high side before the end of storage period probably due to high porosity of the leaf which may allow seapage of moisture from the environment thus triggering the activities of micro-organisms. This might be disadvantageous to the shelf life of pap as lower moisture content is important for long storage by maintaining fungal contamination and spoilage (Enyisi *et al*., 2014).

Moisture content is also an index of water activity and is used as a measure of the stability and susceptibility to microbial contamination. The high moisture content in pap wrapped with leaves showed that it might have short shelf life (Okerulu *et al*., 2015). Jonathan *et al*. (2010) also reported an increase in moisture content of stored onion from one month to 12 months and attributed it to probably high humidity of the

environment where onion was stored. Nylon wrapper could maintain the moisture level of the pap from initial 10.03± 0.04 to 12.05 ±0.061 at the end of day 10. However, the loss of nutrients is more pronounced in solid pap wrapped with banana leaf when compared with pap wrapped with nylon. Probably because nylon is much less permeable to water vapour and gases than leaves and are chemically inactive with food (Adejumo & Ola, 2008) and thus prevent absorption of moisture from the environment by the pap.

The ash content was found to be generally low probably due to leaching of soluble inorganic salts during steeping, fermentation and disposal of steep water prior to milling as reported by Oyarekua and Eleyinmi (2004). The ash reduced from day 0 from 2.08± 0.01 to 0.78±0.011 in pap wrapped with leaf and 1.35±0.01 in nylon. But this finding was not in agreement with Faleye *et al*., (2012) who reported increase in ash content of stored food and attributed it to probably the condiments added. But agreed with findings of Fagbohun (2012) who reported depletion in ash content of non-infected cocoa seed during storage. Aziz *et al*., (2000) also reported that *Aspergillus flavus* depleted zinc and iron from infected crushed corn. Also, Pikuda and Ilelaboye, (2013) reported reduction in ash content of 'ogi' probably due to the large surface area of the substrate which hasten leaching of minerals into steep water during processing.

The crude fat composition was also found to decrease with period of storage. The decrease in nylon wrapped pap was not as high as pap wrapped with leaves. Probably the decrease might be because of fungi infestation that produced enzyme lipase which hydrolyzed the fat for their use (Braid *et al*., 2012). But this agreed with Onifade and Jeff-Agboola (2003) who reported the decrease in fat content of stored infected *Cocos nucifera.*

There was no significant change in crude fiber of pap wrapped in nylon between day 0 up to day 10 of storage but significantly different from pap wrapped with leaves. The slight reduction may be due to enzymatic degradation of the fibrous material during storage as reported by Oyarekua and Eleyinmi (2004). The initial value of the fiber content obtained from freshly prepared pap at day 0 agreed with report of Ujabadenyi and Adebolu (2005).

Crude protein content at day 0 (4.99 ±0.01) was comparable with4.12, 5.93%, 4.8% and 5.4% values reported by Oyarekua and Eleyinmi (2004). The decrease with the days of storage may probably be as a result of the microbial attack which might secret enzymes to hydrolyse the protein for their use as reported by Braide *et al*. (2012). The finding was not in agreement with Pikuda *et al.* (2000) who reported an increase in protein content of samples on which fungi grow and that the increase could be from slight protein synthesis by proliferation of mico-organisms and synthesized enzyme protein. However, the protein content of nylon wrapped pap was higher than that of leaf at the end of storage.

Carbohydrate content of the pap was slightly decreased in both wrapping materials from day 0 to day 10. The initial high carbohydrate content at day 0 was higher than 65.63%to 70.23% reported by Ujabadenyi and Adebolu (2005). The little reduction may be due to the fact that the carbohydrate was used for metabolic activities during storage (Jonathan *et al*., 2010). The processing operations involving steaming, fermentation and pressure cooking may increase the digestibility of starch, rendering it

more susceptible to enzymatic digestion and hence the reduction (Oyarekua & Eleyinmi, 2004).

It is well known that fungi may cause a lot of deterioration and thus constitute hazards to the life of animals and man. The fungi isolated from stored pap in this study include the mesophilic fungi; *Aspergillus flavus*, *Aspergillus niger, Penicillium notatum* and thermophilic fungi; *Mucor* species. They have been implicated in the deterioration of food substances by the earlier reports of Amadi and Adebola (2008), Fadhunsi *et al*. (2011), Braide *et al*. (2012), Faleye *et al*. (2012) and Jonathan *et al*. (2010). These four fungi were isolated right from day 0, meaning that the pap has been contaminated by the spores of these fungi probably during processing from air or utensils used (Abbey, 2007). The occurrence of the fungi was observed to increase with days of storage probably because of the increase in moisture content and digested food substances which support the growth.

The results showed that the pap wrapped in nylon was safe for consumption than leaves even after day 10 of storage with little deterioration. Therefore, the use of leaves to wrapped pap should not be encouraged because it encourages fungi growth that in turn may produce aflatoxin which are secondary metabolites that are highly mutagenic and toxic for human and also animal as earlier reported in bean pudding, pounded yam and pap wrapped with *Banana* leaves by Adegunloye *et al.,* (2012).

Conclusion

In this study, the extensive microbial growth and the associated activities led to the decrease or increase observed in the proximate content of the pap during the period of storage. However, it was observed that these nutrients were best retained when nylon was used in wrapping the pap and also made the pap less susceptible to microbial attacked. Therefore, the use of banana leaves in wrapping the pap must be discouraged as it led to its quick deterioration.

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