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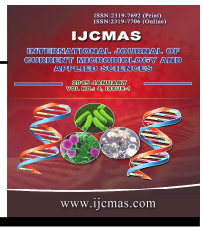

Original Research Article

Conservation tests of *Kluiklui* from Agonlin district in Republic of Benin: Study of the Microbiological stability

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Keywords

Kluiklui, Preservation, Sanitary quality, Packing, Agonlin, Benin

A B S T R A C T

The sanitary quality and the effect of the packaging on the preservation of "kluiklui" were determined during 4 months of preservation under different storage conditions. Samples were collected from saleswomen in the markets and directly from transformers after frying in the sterile plastic bags. Microbial analyses concerning determination of total aerobic mesophilic flora, coliforms flora, Staphylococcus flora, yeast and molds were determined every week up to four months. The results showed that The microbial flora level in the saleswomen'sample were respectively $4{,}8.10^3 \pm 14$ cfu/g; $4{,}9.10^2 \pm 83$ cfu/g; $5{,}1.10^2 \pm 74$ cfu/g; $5{,}3.10^2 \pm 25$ cfu/g for TAMF, CT, YM and *Staphylococcus sp*. All microbial flora were absent in transformers 'samples except the TAMF which was $1.7\ 10^1 \pm 4$ cfu/g. Only anaerobic flora was absent in both samples. Saleswomen'samples presented a high level of contamination. Storage conditions revealed that the packaging in plastic bag is better and the temperature of 4°C represents a good condition of preservation. A period of 9 weeks appears to be the maximum for a good preservation. The results indicated that good hygienic practices during processing of kluiklui, an airtight blister and a temperature of 4°C could contribute highly to a good sanitary quality of the product after frying for long duration of preservation.

Introduction

Peanut (*Arachis hypogea*), or groundnut belongs to the family of *Fabaceae* native from South and Central America (Dillehay and Tom, 2007). It is a leguminous plant highly adapted to tropical and subtropical climates. It is a key crop for small scale farmers especially in Africa and Asia where the crop serve as a valuable source of dietary

protein, oil, and fodder for livestock (Usman, 2013). In the West Africa'countries such as Senegal and Nigeria, it constitutes an important source of protein, fat, minerals and vitamins in the diet of rural people, especially children (Adjou et al., 2012).

In Benin, peanut is one of the most important leguminous consumed by the majority of the population (Elisha, 2004; Ediage et al, 2011; Egal et al, 2005; Honfo et al., 2010). Its processing into groundnut oil, peanut cake locally called *" Kluiklui "*, roasted peanut and peanut sauce are the main uses of this product.

Kluiklui is a popular food for local population. It is a peanut crab obtained from dough after oil' extraction. Afterwards, this dough is fried with oil to make *''kluiklui''*. This transformation is done traditionally according to a process based on ancestral knowledge. Kluiklui is an endogenous product with high geocultural identity. The product could be conserved for weeks by the producers or sellers before consumption. During selling in the market, food products are subjected to cross contamination because of the lack or non observance of hygienic practices when saleswomen handling food products. In this condition, kluklui is not under the lee of that contamination.

Adjou et al (2012) have shown that *''Kluiklui''* sold in most of Benin' markets had a high level of microbial contamination. This contamination is related to many factors as well during the processing as the storage conditions before selling. Indeed, Garba et al (2014) show that there is several critical points in the craft process of peanut. These problems could be avoid with the respect of good processing and preservation practices..

The present study aims to improve the preservation conditions of kluiklui in order to preserve both marketable and sanitary qualities of the product. Specifcally, the study was intended to :

- evaluate the impact of different packages on the product during the term of storage
- determine the effect of storage temperature of the product
- determine the effect of package and storage temperature on the microbial flora during the period of preservation. .

Materials and Methods

Material of study

The analysis was made on the one hand on samples collected from saleswomen on market and on the other on samples collected directly from local transformers. Ten saleswomen and three transformers using improved process for kluiklui production randomly chosen were involved in this study. Improved process included sterilization of tools of processing and decontamination of ingredients and spices added during peanut processing.

Three (03) lots containing each 20 kluiklui, were randomly chosen from every saleswoman. A total of 30 lots were collected. From transformers, a lot of one hundred and sixty (160) kluiklui were collected three times from each of them. Then nine (09) lots corresponding to a total of one thousand four hundred and forty (1440) of kluiklui were collected form transformers.

After collecting, samples were directly put in the plastic bag and then introduced in a cooler for transport in the laboratory.

Treatment of samples and preservation conditions

Each sample collected from transformers was separated into five different lots. Four lots out five of each sample were packed up in the plastic package and the fifth into the sterilized bottle for test preservation. Figure 1 showed the distribution of samples

Methods

Conservation test

Microbiological stability of peanut cake (kluiklui) had been followed for four (04) months under different conditions of storage. Lot 1 corresponding to the reference was directly analyzed to determined the initial level of microbial flora before storage. This sample was taken directly process after frying from transformers using improved. Characteristics and storage conditions of each lot were described below.

- Lot 2 constituted of samples packed in plastic bag and stored at room temperature (25°).
- Lot 3 represented samples packed in plastic bag and stored alternatively for 24h at room temperature and 24h at 4 ° C.
- Lot 4 was constituted by samples packed in plastic bag and stored at 4°C
- Lot 5 represented samples put in sterilized bottles and store at temperature.

Each lot was analyzed periodically every seven (07) days up to four months under storage conditions.

Microbiological analyzes

Microbiological analyses were performed according to the standard methods. 10 g of each sample were crushed and suspended in 90 ml of buffer solution (peptone water added with 0.03g/L of tween 80). The solution was then homogenated and keep on at room temperature for 45 minutes under aseptic conditions to stimulate revivification of germs. From this solution decimal dilutions have been performed for seeding on suitable media depending on the germs.

Determination of total aerobic mesophilic flora (TAMF)

TAMF) was determined according to the standard NF V08-051, using Plate Count Agar (PCA OXOID CM0463) medium. 1 mL of each decimal dilution chosen has been inoculated into Petri dish and 15 mL of the PCA medium have been added and mixed. After solidification od the medium, petri dish were incubated at 30°C for 72 hours.

Determination of Yeast and molds

Yeasts and molds (Y&M) were enumerated on Sabouraud medium supplemented with chloramphenicol following recommendations of the standard NF ISO 7954. 0.1 mL of each dilution was spread on the surface of the medium. Then, the petri dish were incubated at 25°C for five days.

Determination of coliforms

Total coliforms (TC) and Fecal Coliforms (FC) were analyzed according to the standard NF ISO 4831. The medium used for coliforms determination was Violet Red Bile lactose Agar (VRBL). 1 mL of each decimal dilution chosen had been inoculated into Petri dish and 15 mL of the medium were added and mixed. Incubation was done at 30°C and 44°C respectively for total coliform and fecal coliform during 24 to 48 hours.

Determination of Anaerobic sulfito-reducing bacteria (ASR)

As it has been described above, ASR flora was determined using the standard NF V08-061. used was Tryptone Sulfite neomycin (TSN) medium was used for determination. 1 mL of the dilution was introduced into the tube and 20 mL of TSN medium was added. The medium was mixed and incubated after solidification under strict anaerobic conditions

Determination of *Staphylococcus* flora

The *Staphylococcus* (Staph) flora was determined using the standard NF ISO 6888-1. The enumeration was done on Braid Parker (BP OXOID CM0275) medium supplemented with yolk and potassium telluride. 0.1 mL of the dilution has been spread on the surface of the precooled agar medium. The petri dish was then incubated at 37 ° C for 48h.

Determination of moisture

The moisture was determined using AOAC method (AOAC, 1995) by determining weight lost of the product when drying in an oven. Five (05) grams of each sample were weighed and placed in an oven at a temperature of 105 ° C for a period of up to 72 hours. After this, samples were took off and cooled in desiccators before and weighing.

Statistical analysis

For microbial flora determination triplicate agar plates with 15 and 300 colonies were counted. Data collected were reported as cfu/g. Statistical analysis was carried out by descriptive analysis using Minitab software version 16. ANOVA test was performed on data and a threshold of 5% ($P< 0,05$) has been defined for the values considered meaningful.

Result and Discussion

Quality of markets samples

Microbial flora of sample at the start date of conservation

To trace the level of microbial contamination during storage, the table 1 presented the results obtained from sample 1 and sample collected from saleswomen at the market. The numeration of microbial flora obtained from both samples showed that, the samples of the market presented higher values for all flora than those obtained with sample taken directly from transformers. All values obtained are significantly different for each flora. In the lot1, there is no specific flora such as coliforms, yeast and mould and Staphylococcus out of total aerobic mesophilic flora. Likewise, there is no anaerobic flora in the sample taken from saleswomen. However, values obtained for both samples are in accordance with those recommended for a good sanitary quality and marketable quality of food product.

Moisture evolution during storage

Because of their significant role in food alteration, moisture level was determined during the four months. Evolution of water content under different storage conditions and packing was presented on the figure 2. The results showed a significant increase of moisture for all types of packing except sample conserved in the fridge at 4°C in which the level remained the same during the whole time of conservation. This result could be explained by the effect of cold storage. Indeed, low temperature contributed to reduce availability of water.

The high level was observed with sample conserved in the sterilized bottle followed with those conserved in the plastic bag at ambient temperature. Indeed with the sample into the bottle, the percentage of moisture passed from 5.77% to 13.01% after 16 weeks. The same observation was done for samples stored in plastic bag at ambient temperature (from 5.82% to 10.31%). This result showed that the bottle' cork is not hermetic and allow circulation of ambient air inside the bottle. .

Difference between samples conserved in plastic bag at ambient temperature and those conserved alternatively at 4°C and ambient temperature was observed after 12 weeks of storage. The moisturel level was the same for both samples for 12weeks of storage.

Evolution of microbial flora during storage

The analyses have shown a total lack of CT, Staph and ASR.

The level of microbial flora was determined during the 16 weeks of storage. Only total aerobic mesophilic flora and fungi flora were determined in samples. Coliforms, anaerobic flora and *Staphylococcus sp* flora were absent from sample. Figure 3 and 4 presented respectively evolution of total aerobic mesophilic flora (TAMF) and yeast and molds flora. Concerning the TAMF, the microbial flora presents the same evolution for all samples. An initial determination was 18 cfu/g. Week 3 and week 10 are considering as the critical points where the level of microbial flora was very high. However, the level obtained after 3 weeks was higher than those obtained at week 11. Likewise, for all sample after 6 weeks of storage, the level of microbial flora is lower than those obtained before conservation. This low level of flora is maintained until the ninth week. Samples conserved into the bottle at ambient temperature remained which had the high level of aerobic mesophilic flora during storage, 72 cfu/g after 3 weeks and 33 cfu/g at the end of storage. Nevertheless, the number obtained are conformed with standard.

On the other hand, yeast and mold were absent in all samples except sample conserved in bottle at ambient temperature in which mold and yeast starting development after 9 weeks of storage. This results was in correlation with those obtained for moisture. Indeed, this sample was which had the high level of moisture, what predispose it to an attack of molds. A high moisture contributes to the development of yeast and molds.

However, from the 9th week there is mold growth in the samples stored into bottles at ambient temperature. These molds reach 2.1 10^1 CFU / g at the 16th week.

The level of initial contamination is important in the evolution of the microbial flora during storage. When comparing sample used, it was observed that samples collected directly from saleswomen in the market presented a high level of contamination. These results confirmed those obtained by Adjou et al (2012) who reported a high contamination of *''Kluiklui''* sold in Benin' markets. This high contamination could be resulted from a lack of good hygienic practices. According to (Collins, 1997; Barro et al., 2002a; 2002b, Mensah et al.,2002), microbial contamination of foods was attributed to traditional methods of production and packaging, poor personal hygiene of food handlers during processing and packaging. *Staohylococcus sp* is an ubiquist microorganism. Its presence in the sample could result from a bad handling of products

by sellers. A low microbial level in samples collected directly from transformers could be explained by sterilization of different ingredient involved in Kluiklui production, utensils used for processing, package and application of good hygienic practices for handling manipulation. An impact of these actions on reduction of microbial flora in food products had already reported by Sobel et al. (1998) the reduced by applying a good h

Total mesophilic aerobic flora is the first criterion for appreciation of food sanitary quality. The level determined for all samples was lower than that recommended by the International Commission of Microbiological Specifications for Food, which is 10^5 ufc/g. Thes e results showed that both kluiklui from saleswomen and transformers could be consumed without any health damage. The very low level of microbial flora obtained could also resulted from the effect of frying in which oil reached a temperature of 120°C. The flora determined after frying could probably resulted from bad handling during preservation.

The absence of coliforms in all samples indicates no fecal contamination. Indeed, coliforms are used as an indicator of microbial quality of foods because they may be indirectly associated with fecal pollution(Archibald, 2000; CEAEQ, 2000; Edberg et al., 2000;).. Likewise anaerobic sulfito-reducing bacteria (ASR) were absent. Concerning the development of fungi flora (yeast and molds), moisture level is one of the most important elements for mold growth (Boudra et al., 2002). The development of mold in the bottle'sample after 9 weeks could be explained by the fact that the bottle cork is not airtight. This contributed to the circulation of air on the one hand and to an increase of moisture level in kluiklui conserved in the bottle one the other hand. Besides, mold and yeast level remained constant for others samples. This could be related to the type of packing (plastic bag) which is impermeable to air, and, to the action of cold which is bacteriostatic.

The microorganisms present in food can cause organoleptic changes and alter the marketability of food products, or constitute a danger to public health because of their human pathogen power. Therefore the application of the improvements in the manufacture of *''kluiklui''* 'and their packaging for their storage has proven effective for controlling the growth of microorganisms. The plastic packaging remained the best package and storage at low temperature contributed also to inhibit microorganisms proliferation. According to the results obtained, kluiklui can be preserver in a good package for a period of up to 9 weeks.

Table.1 Numeration of microbial flora of Lot 1 and saleswomen' sample

Origin of samples	TAMF	CT	YM	SRA	Staph
Saleswomen	$4,8.10^3$±1414a	$4,9.10^2$±83a	$5,1.10^2$±74a	<1a	$5,3.10^2$±25a
Lot1	$1.7\ 10^1$±4b	<1b	<1b	<1a	<1b

The values are mean. Mean followed by the same letter in the same column are not significantly different ($p < 0.05$).

TAMF: total aerobic mesophilic flora; CT: total coliform flora; YM: Yeast and Mould flora; Staph: *Staphylococcus* sp. flora

Figure.1 Sampling and treatment for the evaluation of the microbiological stability of *''Kluiklui''*

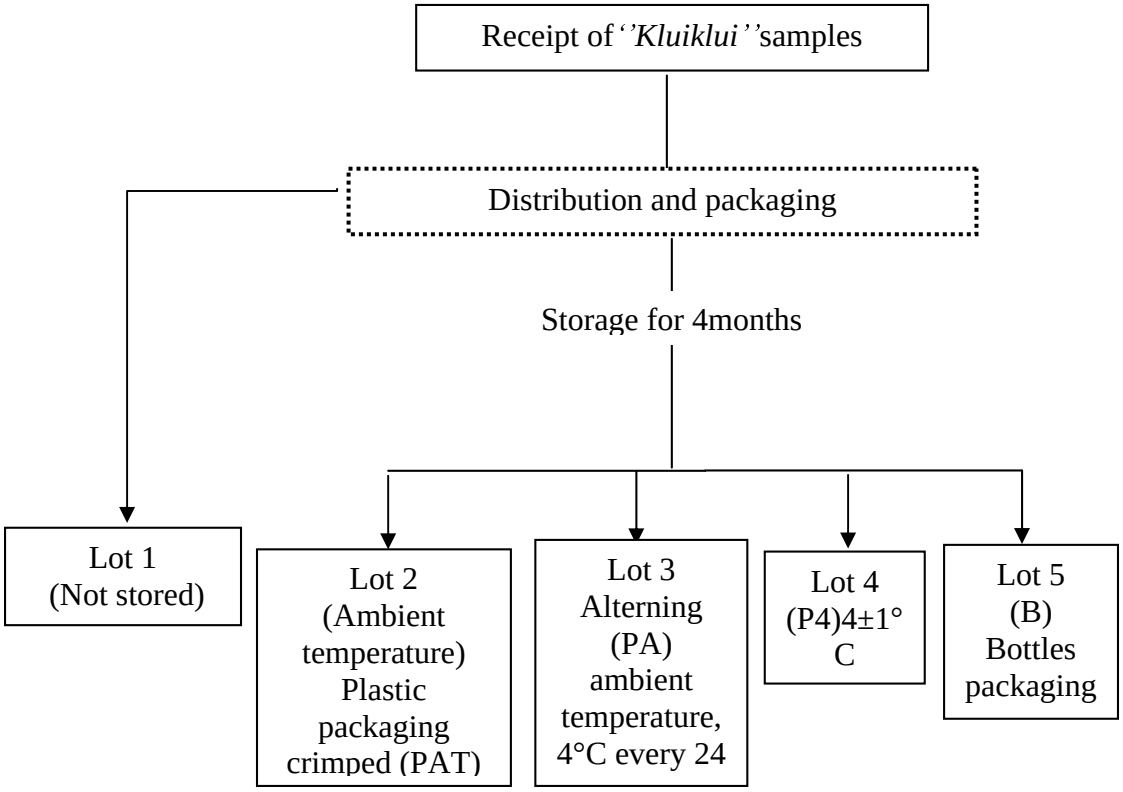


Figure.2 Evolution of the moisture level in the samples during the conservation
With PAT: Samples stored in plastic at ambient temperature; PA: Samples stored in plastic in alterning temperature; P4: Samples stored in plastic at 4°C; B: Samples Stored in bottles at ambient temperature

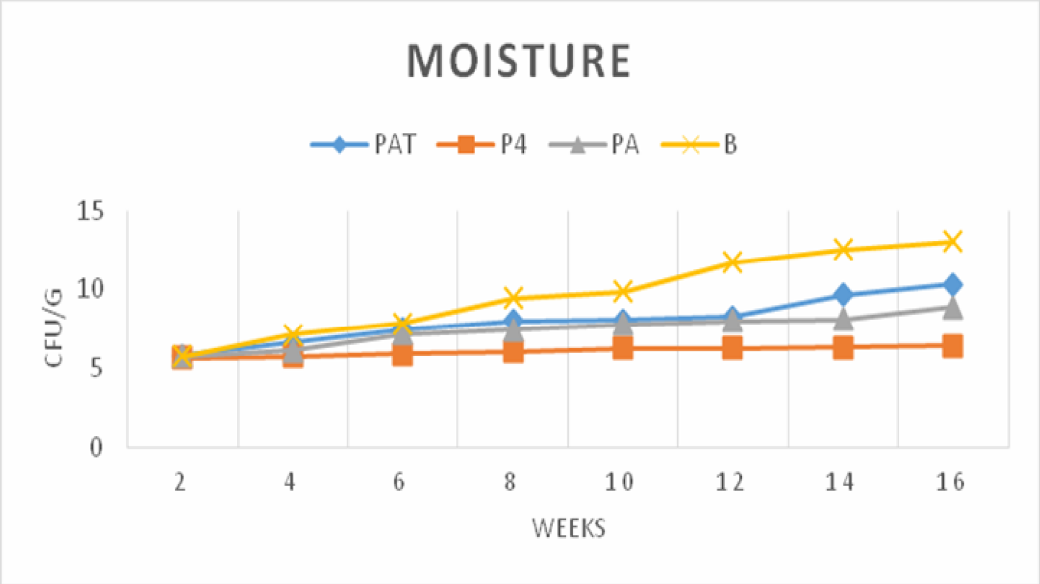


Figure.3 Evolution of the Total Mesophilic Flora in the samples during the conservation
With PAT: Samples stored in plastic at ambient temperature; PA: Samples stored in plastic in alterning temperature; P4: Samples stored in plastic at 4°C; B: Samples Stored in bottles at ambient temperature

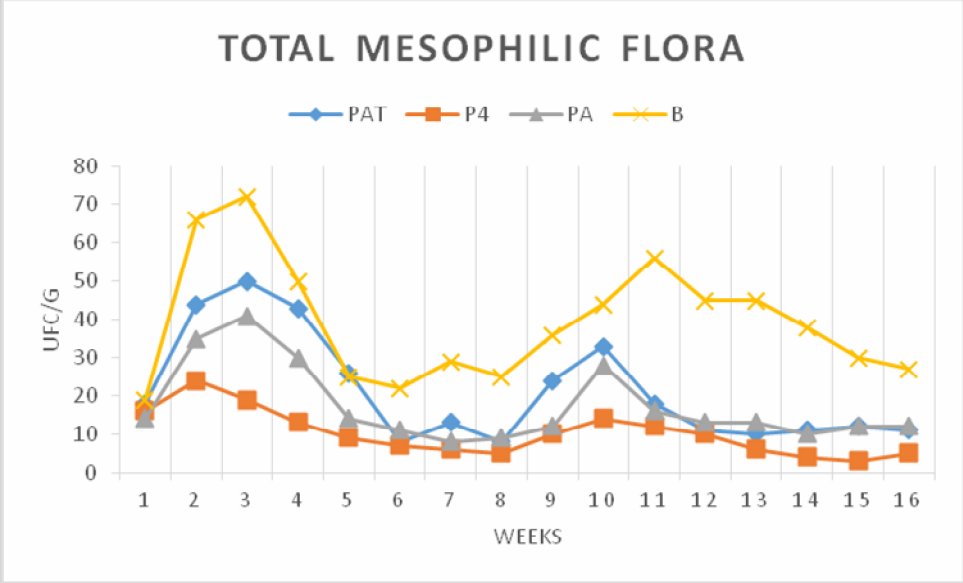
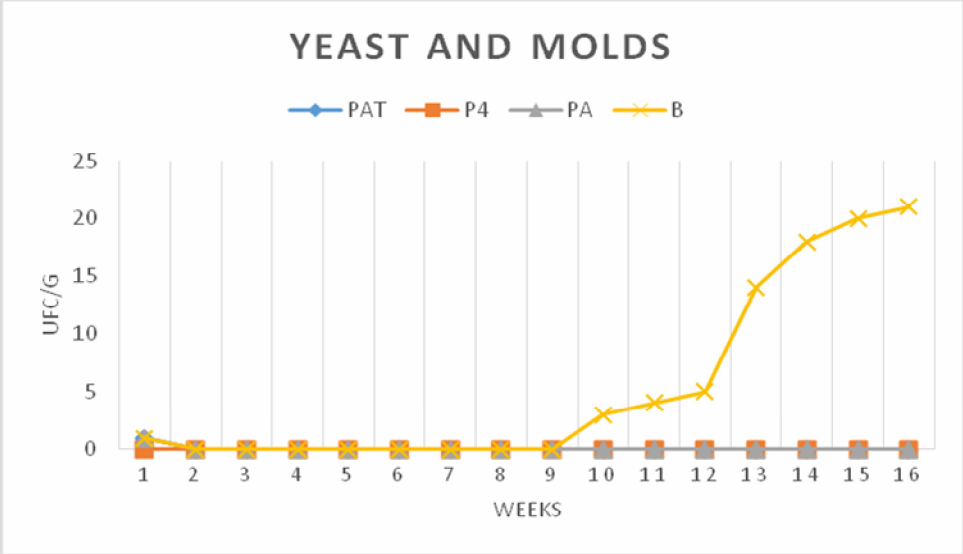


Figure.4 Evolution of yeasts and molds in the samples during storage
With PAT: Samples stored in plastic at ambient temperature; PA: Samples stored in plastic in alterning temperature; P4: Samples stored in plastic at 4°C; B: Samples Stored in bottles at ambient temperature



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