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Microbial succession pattern in Ogi fermentation

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Abstract

Pap (ogi) is a common weaning food in sub-Saharan Africa. It is usually produced from the fermentation of maize or other cereals by the traditional fermentation method. This work studied the microbial changes which took place during the fermentation of pap (Ogi) using the pour plate method and identification of resulting organisms by biochemical tests and microscopy aided with lactophenol-cotton-blue. The steep liquor had a pH of 6.4 on the first day, which latter reduced to 3.4 at the end of fermentation. The bacterial count recorded for the steep liquor ranged from $3.3x10^5$ to $1.38x10^9$cfu/ml while the fungal count ranged from $9.0x10^4$ to $5.7x10^8$cfu/ml. The bacteria isolated include *Lactobacillus* species, *Bacillus* species, *Corynebacterium* species, *Streptococcus* species and *Clostridium* species. The isolated fungi genera include; *Aspergillus* species, *Fusarium* species, *Pencillium* species, *Saccharomyces* species and *Candida* species. A succession pattern of microorganism involved in the fermentation process was observed.The starter organisms include *Streptococcus* species, *Clostridium* species and *Fusarium* species. After the third day, these organisms were succeeded by *Penicillium* species, *Bacillus* species *Candida* species and *Aspergillus* species. After five days of fermentation, the fermenting microbial genera has been reduced to *Lactobacillus* species, *Corynebacterium* species and *Saccharomyces* species only. This study has highlighted the microbial succession pattern in ogi fermentation which can be utilized in improving the organoleptic qualities and fermentation process in the industrial production of ogi.

Keywords: Pap(ogi), microbial succession, fermentation.

Introduction

Pap is a fermented non-alcoholic starchy food and is a major staple food widely consumed in West Africa. It is a sour fine past beverage which when cooked produces a thin semi solid porridge (Nwosu and Oyeka, 1998). Pap (Ogi) porridge has a smooth texture and a sour taste resembling that of yoghurt. Ogi is a staple cereal fermentation product found predominantly in southern Nigeria and is the first native food given to babies at weaning. Pap is widely used as the first native food given to babies at weaning to supplement breast milk and is a major breakfast cereal for pre-school children and adults. It is consumed as a main meal for convalescing patients because it can easily be digested. As a weaning food, it is utilized mainly by low income earners category, it is estimated that about 25 million or more adults eat it about 4-5 days weekly (Banigo and Muller 1972). Milk and sugar may be added to improve the taste and nutritional quality. Pap can be cooked and turned into a stiff gel called Agidi which is similar to kenkey, a fermented Ghanaian product (Muller, 1988). Pap is produced generally by soaking/steeping maize grains in warm water for 2-3 days followed by wet milling and sieving through a screen mesh. The sieved material is allowed to sediment and ferment and is marketed as wet cakes wrapped in leaves (Jay, 2004).

Traditional weaning foods in West Africa are of low nutritive value and are characterized by low protein, low energy density and high bulk. Ogi has been implicated as one of the causes of protein-energy malnutrition in children during weaning period (Naismith, 1973) and if severe, it can result in kwashiorkor and maramus. The maize grain is the commonest cereal used to produce pap. It is the major raw materials used in producing of weaning food both conventionally and traditionally. It is used to wean children locally. It is usually produced from the fermentation of maize or other cereals and the colour depends on the cereal used. The traditional method of producing "Pap" involves the wild fermentation process, where indigenous microorganisms undertake the fermentation process. The use of cereals in weaning foods production especially maize is probably due to its availability and it is widely consumed when fermented. In producing pap traditionally, the following steps are followed. The grains are sorted, steeped in water for 1-3 days and wet milled, sieved, allowed to settle and the sediment put in sac or dough bag for the water to be drained before being marketed as wet cakes (Nwosu and Oyeka, 1998). Various microorganisms have been associated with the fermentation of pap (Ogi) as described by Akinerele (1970). They include *Cephalosporium*, *Aspergillus*, *Penicillium*, *Corynebacterium* spp, *Aerobactercloacee* and *Lactobacillus plantarum* among others. Okafor (1987) isolated *Pediococcus, Pentosaceus and Candida specie* from pap (Ogi). In a related study on fermented corn meal, Fields *et al* (1981) identified two species of *Lactobacillus*(*L. fermentum* and *L. cellobiosus*) and *Pediococcus* in fermented corn meal mixed with water at 37^0c.

The shelf life of pap (Ogi) is about 30 hours and can be extended through refrigeration. The problems associated with pap (ogi) prepared using the traditional methods are irregularity in flavour and loss of nutrients (Van veen and Sterinkrans, 1970). These may be attributed to the activities of undesirable microorganisms and differences in the processing conditions utilized.

Ogi is usually produced at small scale using traditional fermentation technologies. The microorganisms associated with the fermentation process are not strictly controlled neither is the succession pattern and the contribution of each of the microbial genera to the fermentation process clearly understood. The aim of this study is to determine the microorganisms associated with the fermentation of ogi, determination of the microbial load at each stage of the fermentation process and the succession pattern of the associated microorganisms.

Materials and Methods

Sample of maize grains was purchased from Opolo market Yenagoa, Bayelsa state and was transported in a sterile container to the laboratory for analysis. All the glassware utilized was sterilized in a hot air oven maintained at 160^OC for one hour, while other materials were sterilized in an autoclave maintained at 121^OC for 15 minutes. The pap (Ogi) was prepared using the traditional method described by Banigo *et al* (1974). Maize grains were picked to remove dirt and was transferred into a container. The maize was steeped in distilled water for 72 hours. The softened grain was wet milled using a manual grinder. The ground material was then slurred with water and then passed through a sieve. The chaff was discarded while the filtrate was allowed to sediment to obtain the starch paste (pap). An aliquot of the fermentate was collected daily for analysis. pH of the fermentation liquor was determined using a pH meter. The pH meter was first calibrated with a buffer solution before it was used to determine the pH of the liquor. For microbial analysis, a ten-fold serial dilution was undertaken for the enumeration of microorganism present by the spread plate method. Potato dextrose agar (PDA) supplemented with tetracycline antibiotics to inhibit the growth of commensal bacteria was utilized to enumerate fungi while nutrient agar was utilized for bacteria growth. After incubation, the plates were examined for colonies that appeared different in their cultural characteristics. These colonies were sub-cultured onto fresh media by the streaking method using a wire loop to obtain pure cultures. The identity of bacteria species obtained from the fermentate was confirmed by Gram staining and other biochemical tests as described by Harrigan (2000).After incubation, the fungal count was recorded using a colony counter. The different colonial morphologies obtained were recorded by visual observation before being subcultured to a new PDA plate. After subculturing, a small portion of each of the sub-cultured colony was cut using a sterile scalpel and placed on a sterile glass slide using a sterile forceps. This was covered with a cover slip and placed in a petri dish containing moistened cotton wool swabs and covered. This was allowed to stand for 3 days at 25^OC. afterwards, the cover slips were removed with a forcep and placed on glass slides containing lactophenol-cotton blue stain.

This was observed under the microscope to identify the shape, structure of conidia, pigmentation etc.

Results and Discussion

The fermentation of Ogi brought about significant changes in the pH and titratable acidity of the steeped liquor. The pH at day 1 of steeping was 6.4 and then rapidly reduced to 5.8 by day 2. At the end of the steeping period of 5 days, the pH was 3.4. This reduction in the pH of the fermentation liquor can be attributed to the fermentation of sugars in the corn mash used for the fermentation to acids by Lactic acid bacteria as reported by Odunfa (1984).

Table 1. pH changes in Ogi fermentation

Steeping time (Days)	pH
1	6.4
2	5.8
3	4.4
4	4.0
5	3.4

The total heterotrophic bacteria and fungi count of the steep liquor showed an increase as the fermentation progressed as shown in Table 2. The fungal count for the steeped water at day 1 was 9.0 x 10^4 cfu/ml. This count increased steadily and at the completion of steeping, the count reached 5.7 x10^8cfu/ml. For the bacterial count, there was a steady rise in the colony forming units of steeped water. It increased from a value of 3.3 x 10^5 cfu/ml from day 1 of steeping to 1.38x10^9 cfu/ml at the completion of steeping, showing a dramatic increase in the bacterial load of the steep liquor. This result is comparable to those obtained by Ijabadeniyi (2007).During the steeping, the bacterial counts were higher than the fungal counts. This indicates the predominance of bacteria in maize fermentation. The increase could be as a result of increase in acidity of the medium which favours the growth and proliferation of lactic acid bacteria.

Table 2. Microbial load of steep liquor during the fermentation of maize for pap production

Steeping time (days)	Fungal Count	Bacterial count
1	9.0x10^4	3.3x10^5
2	2.4x10^6	9.6x10^5
3	4.7x10^7	1.45x10^7
4	2.3X10^7	1.66x10^8
5	5.7x10^8	1.38x10^9

A total of 5 bacterial isolates were obtained from the steeped water including *Corynebacterium* species, *Bacillus* species, *Streptococcus* species, *Clostridium* specie and *Lactobacillus* species. Five fungi genera were isolated from the steeped water including: *Aspergillus* sp., *Fusarium* sp., *Pennicillium* sp., *Saccharomyes* sp. and *Candida albicans.* During the fermentation process, different microbial species occurred at different time intervals. Table 3. below shows the succession pattern of microorganisms in Ogi fermentation.

Table 3. Occurrence of microorganisms during the fermentation process

Isolates	Day 1 (24 hrs)	Day 2 (48 hrs)	Day 3 (72hrs)	Day 4 (96 hrs)	Day 5 (120 hrs)
Streptococcus species	+	+	-	-	-
Clostridium species	+	+	-	-	-
Fusarium species	+	+	-	-	-
Penicillium species	+	+	+	-	-
Bacillus species	+	+	+	-	-
Candida species	+	+	+	-	-
Aspergillus species	+	+	+	-	-
Lactobacillus species	-	+	+	+	+
Corynebacterium spp.	+	+	+	+	+
Saccharomyces species	-	+	+	+	+

Key: + present - absent

Microorganisms associated with the fermentation of ogi showed a successional pattern with some organisms occurring early on during the fermentation and being replaced by another genera as the fermentation process proceeded. On the first day of fermentation, the bacteria species: *Streptococcus, Clostridium, Bacillus* and *Corynebacterium* occurred while the fungi species of *Fusarium, Penicillium, Candida* and *Aspergillus* occurred as starter organisms. The bacteria *Lactobacillus* specie and yeast *Saccharomyces* specie are late fermenters, occurring only after the second day of fermentation. By day 3 of fermentation, most of the early bacteria fermenters including *Streptococcus, Clostridium* and *Bacillus* have ceased to grow, possibly due to increase in the acidity of the steep liquor. They were succeeded by the fungi species of *Fusarium, Penicillium, Candida* and *Aspergillus* which continued the fermentation up Day 3, thus concluding the primary fermentation process before being replaced by *Lactobacillus* sp., *Corynebacterium* sp. and *Saccharomyces* sp. only. These three organisms concluded the fermentation process up to the fifth day of fermentation and are responsible for the secondary fermentation of the steep liquor. These findings corresponds to the report of Nwosu and Oyeka (1998) who reported similar succession patterns in ogi fermented at 27^{O}C.

Conclusion

Understanding the different types of microorganisms associated with the fermentation of ogi and their succession pattern will aid in the improvement of the fermentation process for the industrial scale production of ogi and also to improve the organoleptic quality of the product such as taste, colour, aroma etc. This study is significant in characterizing the organisms involved in pap fermentation and at what stages so that a controlled industrial fermentation can be undertaken for large scale production.

It is recommended that further studies be undertaken to understand the role each of the microbial genera involved in the fermentation process plays and its contribution to the organoleptic quality of the finished product. Also, synergism between the fermenting organisms should be investigated to improve the industrial scale production of pap ogi.

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