Fermentation dynamics during production of *ogi*, a Nigerian fermented cereal porridge.

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Abstract: Fermentation dynamics including growth kinetics and physico-chemical changes during fermentation of *ogi* was studied. The population of filamentous moulds declined significantly ($P < 0.05$) during fermentation from 6.8 $\log_{10}$ cfu/g at 0 h to 3.7$\log_{10}$ cfu/g at 12 h of steeping; thereafter no mould population was observed again throughout the fermentation period. The moulds isolated were *Aspergillus niger, Aspergillus flavus, Rhizopus nigricans, Fussarium subglutinans* and *Penicilium citrinum.* Continuous increase in yeast population was observed throughout the fermentation period. Similarly, LAB population increased significantly (P>0.05) from 4.65 $\log_{10}$ cfu/g at 0h of soaking to 7.0 $\log_{10}$ cfu/g at 48h soaking. The yeasts isolated during the fermentation period include *Saccharomyces cerevisiae, Rhodotorula graminis, C. krusei, C. tropicalis, Geotrichum candidum* and *Geotrichum fermentum.* The LAB isolates were identified as *Lactobacillus fermentum, Lactobacillus plantarum and Lactobacillus brevis.* The temperature of fermenting maize remained relatively constant between 28°C to 30°C throughout the fermentation. The pH decreased and acidity increased during fermentation. Reducing sugar increased gradually throughout the steeping period. During the souring stage however, there was a progressive decrease in the level of reducing sugar with the length of the souring period.
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Key words: Ash**,** Fermentation, Moulds, Lactic acid bacteria, Reducing sugars and Yeasts.

Introduction

Ogi- acid-fermented cereal gruel is a staple food of several communities in Nigeria. It is traditionally made from maize, sorghum or millet. Several reports had identified steeping and souring as the two fermentation stages involved in the traditional process of *ogi*. It is prepared by steeping clean grains in water at room temperature (25±2°C) for 48-72 h. The steep water is decanted and the fermented grain is washed with clean water and then wet-milled. The bran is removed by wet sieving and the sievate is allowed to settle for another 24-48 h, a process referred to as souring during which time fermentation also proceeds and the solid starchy matter, *ogi*, sediments (Akinrele, 1970; Banigo and Muller, 1972; Akingbala *et al.*, 1981). The wet *ogi* usually has a smooth texture, a sour flavor resembling that of yoghurt and a characteristic aroma that differentiate it from starch and flour. The color of *ogi* depends on the type of cereal used: cream for maize, light brown for sorghum and greenish to grey for millet (Banigo and Muller, 1972).

The wet o*gi* can be boiled at 8-10% total solids into a porridge or pap which serves as weaning food for infants, breakfast for children and convenient meal for the convalescence (Onyekwere *et. al.*, 1989).
Fermentation of *ogi* is by microorganisms from the environment and quality control is absent in the traditional method of preparation (Onyekwere *et. al.*, 1989; Halm *et. al.*, 1993). A lot of nutrient losses occur during processing of cereals for *ogi* manufacture hence, several attempts have been made to improve the nutritional status of *ogi* by fortifying it with protein rich substrates. However, nutritional improvements of these fermented cereal gruels with proteineous foods lowered their pasting viscosities and sometimes affected their sensory attributes adversely. These factors are likely to influence consumer acceptability (Osungbaro, 2009).

Microbiological and nutritional studies show that organisms responsible for fermentation of *ogi* could be majorly responsible for its nutritional improvement (Odunfa *et al.*, 1994)

The traditional fermentation systems of products like *ogi* can lead to microbial evolution of strains with unique technological and other beneficial properties. Fermented foods are consumed in every country of the

world and, there is growing scientific evidence that many fermented foods are good for health.

The purpose of the investigation presented in this paper was to study the fermentation dynamics during the processing of maize into *ogi* in selected processing centre, with the aim of providing a rational basis for the improvement of processing techniques and thus the nutritional quality of *ogi* for its use as complementary food for infants and young children.

Materials and methods

Sample collection and traditional fermentation of *ogi*

Different *ogi* processors were chosen within Abeokuta in South western Nigeria. Dried maize grains were soaked and fermented by each processor following similar approaches. Maize grains were soaked in water and allowed to ferment (steeping) for 48h. The softened grains were washed, wet milled and wet sieved. The wet *ogi* was the left in a covered container and left to ferment (souring) at ambient temperature (28± 2°C) for 48h.

At 12h interval during the fermentation, samples were collected aseptically and transported immediately to the laboratory for analyses. The fermentation trials and sampling were carried out in duplicate.

The samples were analysed for determination of aerobic plate count, Lactic acid bacteria (LAB), yeasts and moulds counts, pH, Total Titrable acidity and sugars. The temperature change during fermentation was recorded directly using Digitron thermometer model 2751-K. Triplicate determinations were made in all cases.

Acidity changes

Changes in the pH and total titratable acidity (TTA) were assessed at 12h interval throughout the fermentation period. The pH was determined using a Metrohm 620 pH meter (Metrohm Herisau, Switzerland) with a reference glass electrode. The pH meter was calibrated prior to each reading with standard buffers.

The TTA was determined by titrating 20ml of the supernatant against 0.1N NaOH until pH 8.30 was attained. The relative lactic acid equivalent is the amount of NaOH consumed in ml. Each ml of 1N NaOH is equivalent to 90.08 mg of lactic acid.

Total reducing sugars

Extracts were produced from the *ogi* samples by homogenizing 5g of the sample with 25ml distilled water. The mixed homogenates was centrifuged at 8,000 revolutions for 20 minutes to obtain the clear supernatant used for the analysis. The total reducing sugar contents of the extracts were determined by the dinitrosalicylic acid (DNSA) reagent method of Miller (1959).

Analysis of Proximate composition.

Moisture, ash, crude protein, crude fat and fibre were evaluated at different period during fermentation using the procedures of A.O.A.C. (1990) as follows: Moisture content was determined by weight loss of 2g of sample after heating in an oven (Gallenkamp Hotbox oven, size 1) at 105^{o}C for 3hrs. The ash content was measured by heating the sample at 550^{o}C until the difference between two successive weights was less than 1mg. Protein content was determined by multiplying total nitrogen, estimated by standard Kjeldahl method by 6.26. Fat content was determined by ether extraction method using a glass soxhlet. The crude fibre content was determined using fibretec extraction. The carbohydrate content was determined by differences: 100 - (%protein + % fat + % ash).

Microbiological analysis.

Ten gram of sample were homogenized with 90 ml of 0.85% (w/v) sterile physiological saline in a stomacher lab-blender (400, Seward, London, U.K.) for 1 min and serially diluted in the same diluent. One ml of these dilutions was prepared by pour-plate method in the respective media for moulds, yeasts and lactic acid bacteria (LAB). Aerobic bacteria were grown and counted on Plate Count Agar (PCA) (Oxoid CM 325, Unipath Ltd, Basingstoke, Hampshire, England) incubated at 30°C for 3 days. The morphological characteristics of colonies on PCA were examined and the number of colony forming units (CFU) for each morphotype was recorded separately. Enumeration of yeasts and moulds was on yeast dextrose peptone agar (YEDPA) and Sabouraud dextrose agar (SDA, Oxoid CM 41) respectively containing 50 mg/L chloramphenicol and 50mg/L chlortetracycline to inhibit bacterial growth. Incubation was at 25^{0}C for 3 to 5 days. LAB were isolated on deMan Rogosa and Sharpe (MRS) agar (Oxoid CM 361) after incubation under anaerobic conditions in an Anaerobic Gas-Pack system at 30°C for 48–72 h. Colonies were either selected randomly or all sampled if the plate contained less than 10 colonies. Purity of the isolates was checked by streaking again on fresh agar plates of the isolation media, followed by microscopic examinations. Microbiological data were transformed into logarithms of the numbers of colony forming unit (cfu) per g.

Identification of isolates

Isolates on PCA were examined for Gram reaction, catalase production and sporulation (7 days incubation in Nutrient Broth added 50 mg/l MnCl2). Presumptive LAB isolates on MRS agar were examined for Gram

reaction, catalase production, gas production from MRS-broth, with citrate omitted, containing inverted Durham tubes (Schillinger and Lücke, 1987) and growth at 15°C and 45°C in MRS broth. Cell morphology and motility were examined by microscopy observation of cells grown in broth for 24 h.

Identification of filamentous moulds was carried out following the taxonomical keys of Schipper (1976, 1984) and Hesseltine (1991).

Presumptive yeasts colonies grown on MYGP were examined for cell morphology. Catalase production was determined by adding to a colony on a glass slide a drop of H_2O_2 solution (30%).

Fermentation and assimilation of carbon compounds were determined using API 50 CHL kits for LAB, API 50 CHB kits for aerobic bacteria and API 32 ID kits for yeast according to the manufacturer's instructions (BioMerieux, Marcy-l'Etoile, France). The results were recorded visually and analysed by APILAB Plus V3.2.2 software (BioMerieux).

Results

Population of moulds which was originally from the dried maize grains decreased significantly ($P < 0.05$) during fermentation from 6.8 $\log_{10}$ cfu/g at 0 h to 3.7$\log_{10}$ cfu/g at 12 h of steeping; thereafter no mould population was observed again throughout the steeping period (Fig 1). Moulds were not isolated at all during the souring period. The moulds isolated during soaking were *Aspergillus niger, Aspergillus flavus, Rhizopus nigricans, Fussarium subglutinans* and *Penicilium citrinum.*

Throughout the fermentation process, the predominant microorganisms were presumptive lactic acid bacteria and yeasts increasing in numbers at the onset of fermentation to the end of fermentation. Continuous increase in yeast population was observed throughout the 48h steeping period and the counts ranged from 2.9 $\log_{10}$ cfu/g at 0 h of soaking to 6.6 $\log_{10}$ cfu/g at the end of steeping (48h). Similarly, LAB population increased significantly (P>0.05) from 4.65 $\log_{10}$ cfu/g at 0h of soaking to 7.0 $\log_{10}$ cfu/g at 48h soaking (Fig 1).

A general reduction in yeasts and LAB population was observed at the beginning of souring (after washing, wet milling and sieving). Subsequently, continuous increase in yeast and LAB population was observed till the end of the souring period. Yeasts counts during this period ranged from 4.6$\log_{10}$ cfu/g at 0h to 6.9$\log_{10}$ cfu/g at 48h. During the same period, LAB population increased from 4.3$\log_{10}$ cfu/g to 7.9$\log_{10}$ cfu/g (Fig 2). The yeasts isolated during the fermentation period include *Saccharomyces cerevisiae, Rhodotorula graminis, C. krusei, C. tropicalis, Geotrichum candidum* and *Geotrichum fermentum.* The LAB isolates were Gram positive, catalase negative, non-motile, rod, elongated cocci or cocci shaped and were identified as *Lactobacillus fermentum, Lactobacillus plantarum and Lactobacillus brevis.*

The temperature of fermenting maize remained relatively constant between 28°C to 30°C throughout the fermentation.

Kinetics of acidity changes during the steeping period showed a gradual reduction in the pH from 6.1 $\log_{10}$ cfu/g at 0h to 5.8 $\log_{10}$ cfu/g at 24h. Thereafter the pH decreased significantly ($P < 0.05$) to 4.1 at the end of steeping (Fig 3). During the 48h souring period, the pH decreased significantly ($P < 0.05$) from 6.1 $\log_{10}$ cfu/g at the beginning of souring to 4.1 $\log_{10}$ cfu/g at 48h (Fig 4). At the same period, the titratable acidity increased significantly ($P < 0.05$) from 0.01% to 0.4%. Changes in acidity with time during fermentation were significant at 95% confidence interval. High negative correlations exist between fermentation time and pH during steeping and souring.

An initial drastic decline in the reducing sugar level from 6.67 $\pm$ 0.04 at 0 h to 2.70 $\pm$ 0.36 at 24h was recorded, thereafter, the reducing sugar increased gradually throughout the steeping period. During the souring stage however, there was a progressive decrease in the level of reducing sugar with the length of the souring period

Changes in proximate analysis of samples during the spontaneous fermentation of maize are presented in Table 1. Results showed that while increases were observed for the carbohydrate content of the *ogi* as compared to the maize grains, there were reduction in the ash content, fat and fibre of the maize grains when processed into *ogi.* Least significance difference (LSD) used to compare means showed that with the exception of ash, the mean difference between the proximate composition of the raw maize and those of the processing steps is significant ($p \leq 0.05$).

Discussion

The moulds isolated during the initial stage of fermentation were identified as *Aspergillus niger, Aspergillus flavus, Rhizopus nigricans, Rhizopus stolonifer, Mucor circinelloides, Fussarium subglutinans,* and *Penicilium citrinum.* The presence of moulds at the initial stage of fermentation of maize for *ogi* production and the subsequent elimination had been reported previously (Jespersen *et al.,* 1994; Omemu *et al.,* 2007a). Mucoraceae fungi have roles in initial phase of fermentation mostly in saccharification of the substrates (Thapa and Tamang, 2004).

The study also confirmed the major involvement of LAB in *ogi* fermentation. They were obligatory or

facultative heterofermentative rods and cocci which were present throughout the process. From the beginning to the end of the fermentation, their number increased 2–4 log-units. Their growth was followed by the acidification of the product. Studies on other types of fermented products reported that LAB were the predominant microorganisms involved in the fermentation of products like *kenkey, Gari, Agbelima,* with *L. plantarum* being identified most often (Amoa-Awua *et al.*, 1996; Kostinek *et al.*, 2005).

The yeast counts also increased during the fermentation of maize for *ogi* production. The coexistence and symbiotic association between lactic acid bacteria and yeasts in African traditional fermented products have been reported by several authors (Jespersen *et al.*, 1994; Hounhouigan *et al.*, 1993; Omemu *et al.*, 2007b). Besides their role in the build up of typical flavour of fermented products, some yeast has been reported to show amylolytic, protease and phytase activities. This enzymatic ability may contribute to breaking down maize starch and also allow better access to nutritionally essential minerals (Amoa-Awua *et al.*, 1996; Omemu *et al.*, 2007b).

A large spectrum of yeasts was found to be involved in the maize fermentation for *ogi* production. The yeasts identified include *Saccharomyces cerevisae, Candida krusei, C. tropicalis, Geotrichum fermentans, G. candidum* and *Rhodotorula graminis.* Of the yeasts identified, *Saccharomyces cerevisae, C. krusei* and *C. tropicalis* were the dominant species associated with this fermentation especially during the souring period. Jespersen (2003) considered *S. cerevisiae* as the yeast species most often reported in African indigenous fermented foods and beverages.

The cause of the increase in acidity and consequent drop in pH during fermentation of cereal was likely due to utilization of free sugars by yeasts and LAB (Efiuvwevwere and Akona, 1995; Zvauya *et al.*, 1997). The total sugar contents decreased significantly ($P < 0.05$) throughout the fermentation. This is due to a maximum break down of starch substrates to reducing sugars by amylolytic enzymes produced by some of the fermenting organisms (Nout and Aidoo, 2002).

In conclusion, the study of the fermentation dynamics of *ogi* will enhance the use of starter culture and also help in optimizing the process for improved *ogi*.

Table 1: Changes in proximate composition of maize during fermentation

Parameters	Raw maize ± SD*	ST_{12} ± SD	ST_{24} ± SD	SU_0 ± SD	SU_{24} ± SD
Moisture contents	11.9 ± 0.2	32.2 ± 12	33.2 ± 1.0	ND	ND
Crude protein	12.0 ± 0.2	11.1 ± 1.0	11.2 ± 0.0	11.2 ± 0.2	11.6 ± 1.0
Crude Fat	4.9 ± 0.4	3.7 ± 1.0	3.6 ± 0.1	3.6 ± 0.1	3.6± 0.1
Crude fibre	1.5 ± 0.3	1.0 ± 0.2	0.9 ± 1.0	0.8 ± 0.1	0.6± 0.0
Ash content	1.4 ± 0.2	1.2 ± 1.0	1.2 ± 0.2	1.2 ± 0.0	0.9 ± 1.0
Carbohydrate	80.1 ± 0.3	83.0 ± 0.2	83.5 ± 0.6	82.9 ± 0.1	82.5± 0.0

*Mean of triplicate values ± Standard deviation.
ST_{12} - 12h Steeping
ST_{24} - 24h Steeping
ST_{48} - 24h Steeping
SU_0 - freshly prepared *ogi*
SU_{24} - 24h souring
ND- Not determined.

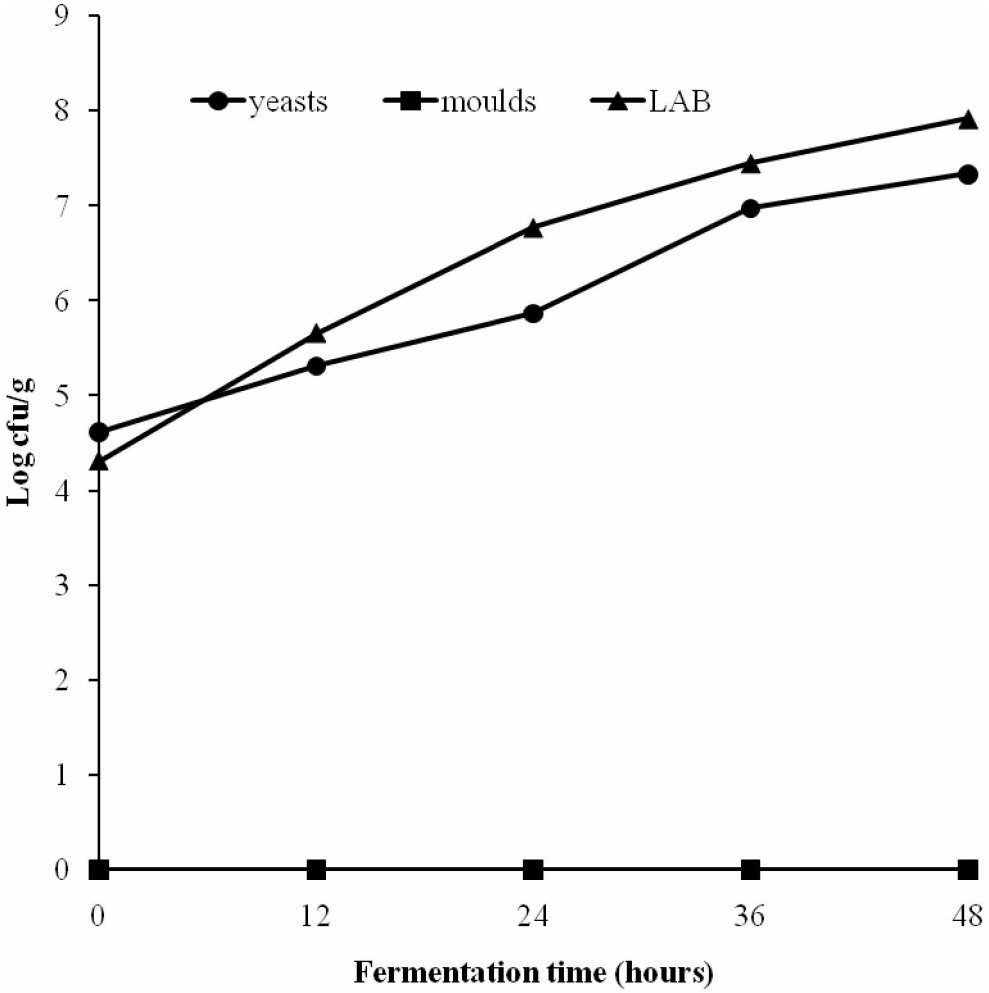

Fig 1: Growth kinetics during the steeping of maize for *ogi* production.

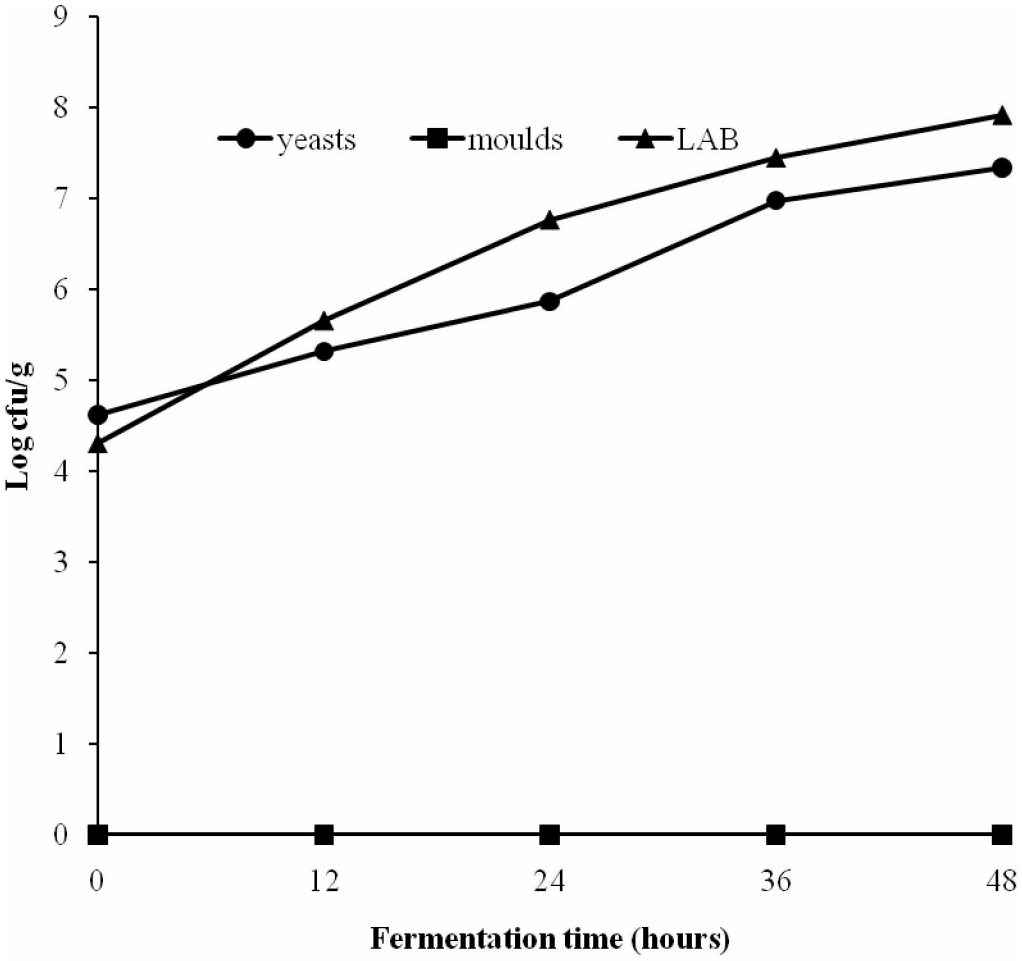

Fig 2: Growth kinetics during the souring period.

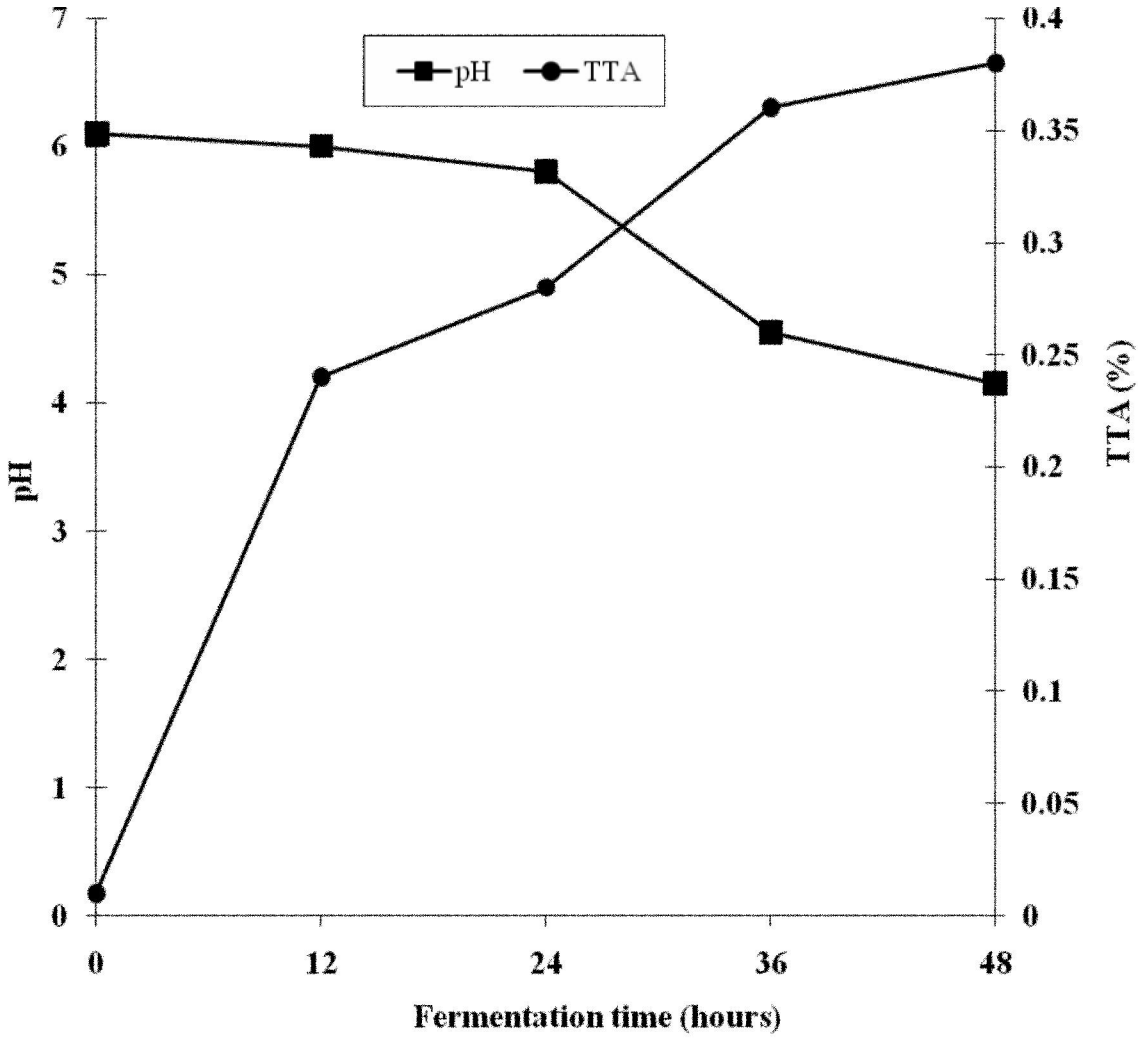

Fig 3: Acidity changes during steeping of maize for *ogi* production

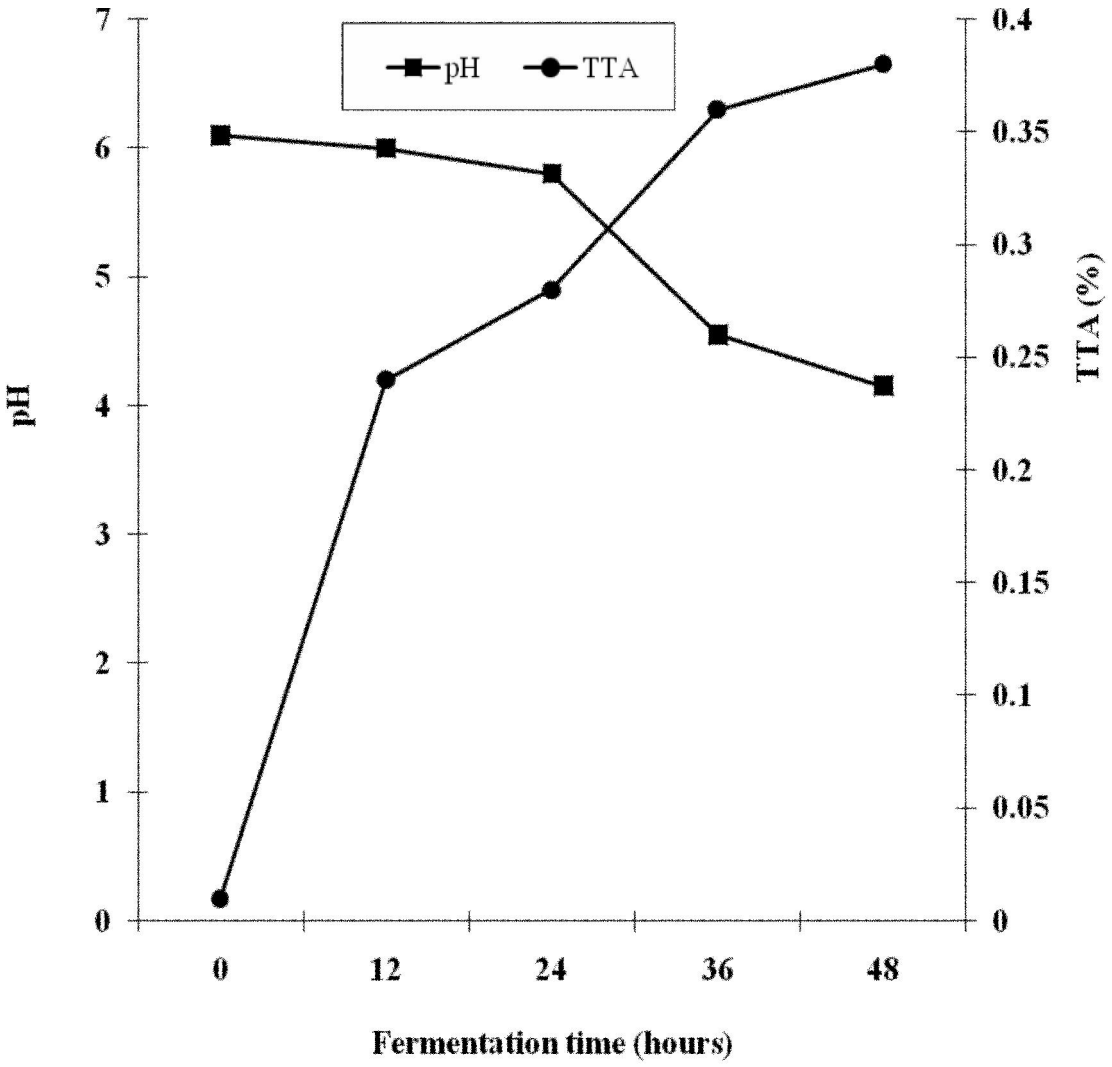

Fig 4: Acidity changes during the souring period.

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