

## EFFECT OF MAIZE VARIETY AND BACTERIA STARTER CULTURE ON MAIZE FERMENTATION PROCESS

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### ABSTRACT

The effect of different maize variety and bacteria starter culture on maize fermentation process was evaluated. Eleven varieties of maize were subjected to fermentation both naturally and in the presence of bacteria starter culture (bactocell). One variety was further fermented with different bacteria starter cultures. The cultures used were; *Pediococcus acidilactici* (Bactocell), *Lactobacilli amylophilus*, *L. reuteri*, *L. caesi* and *L. plantarum*. The pH levels, amount of lactic acid produced and its effect on pathogenic *E. coli* were determined for all the fermented products. The results showed that there was a significant difference ( $p < 0.05$ ) in the amount of lactic acid produced by the different varieties of maize. Furthermore, maize fermented with bactocell had a pH of 3.59 – 3.84 after 24 h and this was significantly lower than the naturally fermented maize (pH 4.53 – 4.83). The reduction in pH was attributed to the production of lactic acid which is a by-product of the fermentation process. *L. plantarum* fermented maize produced the highest amount of lactic acid (81.81mM) and also produced a higher inhibition in the growth of *E. coli* compared to the other bacteria starter cultures tested. The maize variety did not affect their fermentation process but influenced the amount of lactic acid produced. Furthermore, maize fermented with bacteria starter cultures were faster than the natural process and resulted in increased lactic acid production.

**Keywords:** Lactic acid; bacteria; maize variety; fermentation; starter cultures

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### 1. INTRODUCTION

Fermentation is the process of subjecting foods or suitable materials to the action of microorganisms or enzymes so that desirable biochemical changes can cause significant modification of the food materials. This usually results in the food becoming more nutritious, digestible, safer and better flavoured<sup>1</sup>. Fermentation is relatively low-energy preservation technique that can increase product shelf-life and reduce the need for refrigeration or other energy-intensive food preservation techniques<sup>2</sup>.

It is one of the oldest known methods for preparing and preserving foods. A product for which fermentation is of great relevance is cereal which can be fermented as foods for infants and adult as well as animals<sup>2, 3</sup>. In West Africa, pap (locally made maize starch mucilage) is a staple food prepared from fermented maize, sorghum or millet. The consumer preference for pap might be due to the organoleptic properties of the resulting product after fermentation. It has also been reported that fermentation can enrich food substrate biologically with protein, essential amino acids, fatty

acids and vitamins. Other roles include imparting a diversity of flavours, aromas and texture and decrease cooking time<sup>1,2</sup>. Microbiological and nutritional studies have showed that the lactic acid bacterium *Lactobacillus plantarum*, the aerobic bacteria *Corynebacterium* and *Aerobacter*, the yeasts *Candidamycoderma*, *Saccharomyces cerevisiae* and *Rhodotorula* and moulds *Cephalosporium*, *Fusarium*, *Aspergillus* and *Penicillium* are the major organisms responsible for the fermentation and nutritional improvement of pap ('ogi' by the Yoruba speaking part of Nigeria)<sup>3,4,6,7</sup>. *L. plantarum* have also been reported to be predominantly responsible for the production of lactic acid during fermentation process, *Corynebacterium* hydrolyses maize starch to organic acids while *S. cerevisiae* and *Candida mycoderma* contributes to flavour development<sup>4,6,7</sup>. In general, natural fermentation of maize leads to increase in the availability of digestible carbohydrates as well as some non-digestible poly and oligosaccharides. Certain amino acids may be synthesised and the availability of 'B' group vitamins may be improved<sup>1, 2</sup>. The main goal of fermentation is to generate sufficient

lactic acid and low pH levels that could prevent both spoilage and pathogenic organisms.

There are however several varieties of maize and different bacteria starters responsible for the fermentation process. While so much has reported about the nutritional benefits of fermentation. The objective of this study was to investigate the effect of differences in maize varieties and starter cultures on maize fermentation process.

## 2. MATERIALS AND METHOD

**2.1 Materials:** Maize grains of different varieties were obtained from the Institute of International Tropical Agriculture (IITA), Ibadan, Nigeria. The eleven varieties obtained were code labelled 'A' to 'K' and their physical properties are listed in table 1.

The microorganisms and bacteria starter cultures used for this study are listed in table 2 and coded 'i' to 'v'. They were obtained as pure cultures from the Biological Science Laboratory of the School of Biomedical and Biological sciences, University of Plymouth, UK. All other chemicals used were of reagent grade and were used as received.

**Table 1: The different maize varieties**

CODE	Maize variety	Colour	Shape
a	Quality protein maize	white	Flint
b	ACR87T2MSRW	white	Dent
c	TZBR.SYN-Y	yellow	Flint and dent
d	TZESR-W.SE	white	Dent
e	9021-18STR	white	Flint
f	TZ Sweet corn	yellow	Flint
g	ACR.20TZOL COMP.4C3	white	Dent
h	Pop Corn	yellow	Flint
i	9044-24STR	yellow	Dent
j	AK94 DMR-ESRY	Yellow	Flint
k	T2 SR-Y-1.C4	yellow	Dent and flint

Table 2: Showing different bacteria starter cultures

Codes	Bacteria starter
I	Bactocell ( <i>Pediococcus acidilactici</i> )
II	<i>Lactobacilli amylophilus</i>
III	<i>Lactobacilli reuteri</i>
IV	<i>Lactobacilli caesi</i>
V	<i>Lactobacilli plantarum</i>

## 2.2. METHODS

**2.2.1 Fermentation process:** The maize grains (10 g) of each variety was washed with distilled water and wet milled. Distilled water was added to make up to 100 ml and allowed to ferment in appropriate fermentation tanks. The pH of the different maize samples was determined at the beginning of the experiment and after 24 h intervals up to 48 h using a pH meter (Hanna-pH 213, Germany). This above procedure was repeated with the further addition of 10 ml of 1.0 g/ml of a starter culture (bactocell) in each of the maize variety. In another experiment, 10 g each of the 'B' maize variety was further subjected to fermentation process under the influence of different bacteria starter cultures listed in table 2 above. Freeze dried (0.1g) starter culture were reconstituted in 100 ul of sterile water. Each of the starter culture (10 ml) was added to 30 g of each maize sample in a sterile container and was made up to 100 ml with distilled water, incubated at 30oC and the pH taken at the beginning and after 24 and 48 h of the experiment. All the samples analysed for pH were stored at 30oC in an incubator (Labmark-VSL, Czech).

**2.2.2 Lactic Acid Analysis:** The naturally fermented sample and the sample fermented with different bacteria starter culture were assessed both qualitatively and quantitatively for lactic

acid using high performance liquid chromatography (HPLC) (Gynkoteck, Germany) after 24 h. To each of 0.5 ml samples, 20 µl of 7% (v/v) Sulphuric acid solution was added to denature the protein in the sample and to fully protonate the organic acid under investigation. The samples were diluted with 50 µl distilled water, mixed in a vortex mixer and centrifuged at 13000 rpm for 20 mins. The subsequent supernatant was carefully removed and retained for HPLC analysis.

### 2.2.3 Challenge studies

The fermented samples were inoculated each with 1ml of overnight broth culture of *E. coli* and properly mixed using a vortex mixer for 30 secs. The *E.coli* was enumerated by adding 10 ml of the *E. coli*/maize mixture to 90 ml of sterile maximum recovery diluent (MRD) (Oxoid Ltd, Basingstoke, UK) and was mixed on a vortex mixer for 30 secs. A serial dilution was made on a nutrient broth from which it was plated on MaConkey agar at the beginning of experiment and after 4h and incubated at 37oC overnight and the colonies formed were counted.

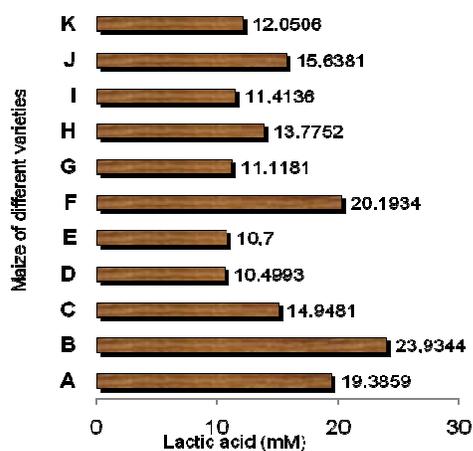
### 2.3 Statistical analysis

All statistical analysis was conducted using Minitab version 13.0, General linear model and One-Way Anova were carried out and p values greater than 0.05 was considered significant.

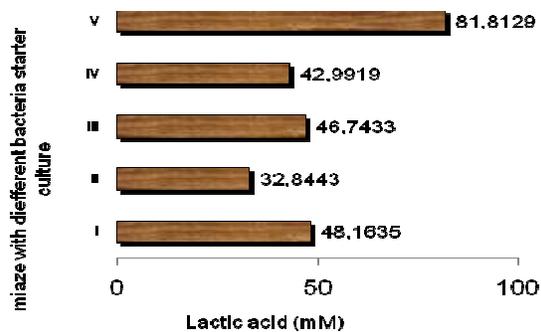
### 3. RESULT

**Table 3: Mean pH values of naturally fermented and bacto-cell fermented maize of different varieties.**

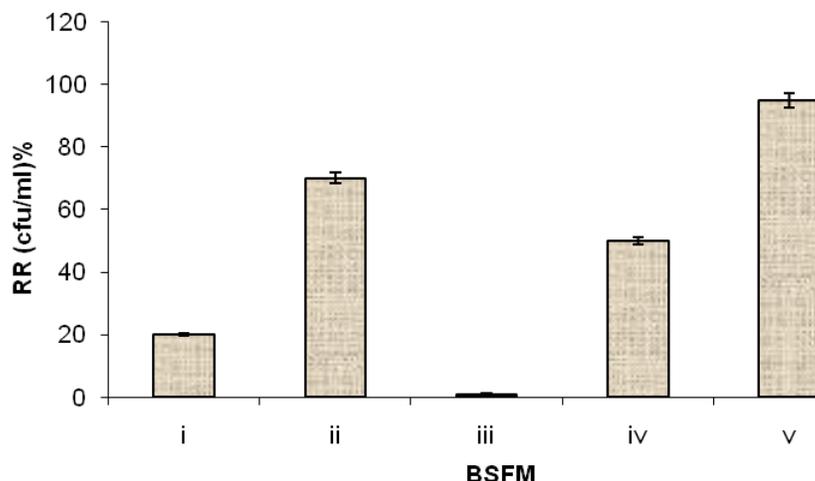
codes	Natural fermentation			Bactocell assisted fermentation		
	0 h	24 h	48 h	0 h	24 h	48 h
A	6.7	4.80	3.73	6.7	3.68	3.46
B	6.7	4.73	3.67	6.7	3.59	3.43
C	6.6	4.57	3.50	6.6	3.67	3.47
D	6.6	4.77	3.67	6.6	3.64	3.46
E	6.6	4.80	4.13	6.6	3.58	3.43
F	6.4	4.87	3.93	6.4	3.84	3.69
G	6.4	4.70	4.13	6.4	3.69	3.46
H	6.6	4.83	4.43	6.6	3.63	3.44
I	6.4	4.53	3.87	6.4	3.59	3.40
J	6.4	4.73	4.07	6.4	3.71	3.52
K	6.4	4.73	3.40	6.4	3.66	3.46



**Figure 1: Lactic acid produced by natural fermentation of different maize varieties**



**Figure 2: Lactic acid produced by different bacteria starter cultures.**



**Figure 3: Rate of reduction in the population of *E. coli* after 4 h inoculation (RR= reduction rate; BSFM= bacteria starter fermented maize)**

#### 4. DISCUSSION

The difference between the mean pH of the natural and Bactocell assisted fermentation is shown in table 3. In the first hour, the pH was essentially that of distilled or deionised water ranging from 6.4 to 6.7. After 24 h, there was a reduction in the pH for all samples. However, the reduction in the pH of the Bactocell assisted fermentation was significantly greater ( $p < 0.05$ ) than that of the naturally fermented. This finding agrees with earlier studies that the presence of bacteria starter cultures enhance fermentation processes<sup>4, 8</sup>. Furthermore, the reduction in the pH of the bactocell fermented samples after 48 h was not significantly different from that obtained after 24 h as well as that obtained for the naturally fermented ( $p > 0.05$ ). This is probably because that the fermentation was already completed for the bactocell fermented maize within 24 h. The pH values for all maize varieties fermented by natural process were higher than those by bactocell assisted. It is possible that the bacteria starter also facilitated a more complete fermentation process which did not seem to have been completed in the natural process even at

48 h. The reduction in pH has been associated with the production of lactic acid which is one of the by-products of maize fermentation<sup>6,7,8</sup>. Finally, there was no significant difference ( $P > 0.05$ ) in the pH values within the different varieties of maize used. This suggests that the difference in maize variety did not affect the fermentation time and process.

The effect of maize variety on lactic acid production during fermentation by natural process is shown in figure 1. The results revealed a significant variation in the amount of lactic acid produced by the different varieties of maize. A, B and F varieties produced the highest amounts of lactic acid of 19.38, 23.93 and 20.19 mM, respectively. While K, I, G and E had the least, with each producing 12, 11, 11 and 10 mM of lactic acid, respectively. It is evident from this study that while the maize variety did not influence the fermentation time or process, it influenced the amount of lactic acid production. However, figure 2 further revealed that when bacteria starter cultures were used for the 'B' variety, a further increase in the amount of lactic acid production was obtained.

This increase was significantly greater than that obtained by the natural fermentation process ( $p < 0.05$ ). The Bactocell starter produced up to 81.8 mM of lactic acid which was significantly high ( $p < 0.0001$ ) when compared to that produced by *L. planterus* 48.2 mM which was the next highest.

The effect of fermentation on *E. coli* after 4 h is reported in figure 3. From the result, there is a significant difference ( $P < 0.001$ ) in the rate of reduction of microbial load from fermented maize by bacteria starter cultures which was as a result of the large amount of lactic acid and low pH produced. The reduction rate (RR) was however not proportional to the amount of lactic acid in the medium. This would mean that other factors could have contributed to the antibiosis observed. The antibiosis mediated by lactic acid bacteria (LAB) has been attributed to the production of hydrogen peroxide and antibiotics<sup>9</sup>. The production of organic acids reduced the pH to below 4.0 making it difficult for some spoilage organisms that are present in maize to survive as well as pathogenic organisms<sup>9, 10</sup>.

The health importance of fermentation is of relevance because studies have shown that the sour taste of lactic acid fermentation has an enhancing effect on the appetite of sick infants<sup>11</sup> and that fermentation could enhance the activity of the infant gut micro flora and in the process suppress colonisation by enteropathogenic bacteria. However, studies have also indicated that fermentation often goes wrong with excessive gas and off flavours being produced which reduces the palatability of the food. One way to solving this problem will be by inoculating the cereal (maize) by specific lactic acid bacteria<sup>3</sup>.

## CONCLUSION

The difference in maize variety did not influence the fermentation rate and time but had a significant effect on the lactic acid production. Furthermore, the inclusion of bacteria starter cultures resulted in faster and a more complete fermentation process as well as a significant increase in lactic acid production when compared to the naturally fermented maize. Finally, the antimicrobial effect must have been mediated by factors other than the lactic acid produced during the fermentation process. The findings of this study can be used to further improve the outcome of maize fermentation process.

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