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Microbiology of Lactic Acid Fermentation of Eggplant Fruits

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The microorganisms responsible for the lactic acid fermentation of eggplant fruits were cultured on the following media: Raka-ray no. 3 agar, orange-serum agar, MRS agar, blood serum agar and sabouraud 2% maltose broth. Pure cultures isolated on the various media were identified using various biochemical tests. The microorganisms which were responsible for the fermentation of the eggplant fruits were *Lactobacillus* and *Leuconostoc*. The lactic acid produced, preserved the eggplant fruits for four months when packaged with polyethylene bags.

Key Words: Lactic acid, Fermentation, Eggplant fruits.

INTRODUCTION

In many countries in the tropics, eggplant fruits are abundant soon after the early rains, but become scarce late in the rainy season and more so in the dry season. Therefore, the need for simple preservation methods such as lactic acid fermentation of eggplant fruits is very important. Eggplant fruits are usually preserved by drying, refrigeration and recently by canning[1].

The lactic acid fermentation of vegetables such as cabbage and cucumber have been well documented. However, little research has been done on eggplant fruits. The lactic acid fermentation of vegetables, *i.e.*, cabbage and cucumbers, shows similarities in the types of microorganisms that are responsible for fermentation and the flavour compounds produced[2].

The sequence of lactic acid bacteria in fermentation is determined by their acid tolerance. In vegetable fermentation, *Lactobacilli* are stronger acid producers than *Streptococci* of the four main groups of lactic acid bacteria[3]. Dworwschak[4] reported that the aroma of fermented vegetables was due to the presence of large amount of volatile compounds including amines, fatty acids, esters and ketones and products from the interaction of these compounds during fermentation.

During the lactic acid fermentation of cabbage, the salt (2–3%) plays several significant roles. It extracts moisture from the shredded cabbage by osmosis to form the brine in which fermentation takes place. It also helps to maintain the crisp texture of the cabbage by withdrawing water and inhibiting endogenous pectolytic enzymes, which cause the product to soften; it contributes to the flavour of the product and helps inhibit some micro flora of the cabbage such as *Pseudomonas,* which would otherwise cause spoilage and, it provides the environment and proper functioning of the lactic acid bacteria[5].

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During the lactic acid fermentation of cabbage, acidity rises in three stages: first to approximately 0.67%, then to 1.20–1.25% and finally to 1.50–1.70% corresponding to the successive populations of *Leuconostoc mesenteroides, Lactobacillus plantarum* and *Lactobacillus brevis*. The rapid fall in pH brought about by *Leuconostoc mesenteroides* discourages the growth of extraneous bacteria. The acetic acid, mannitol and ethanol production contribute to the flavour of the final product[6].

EXPERIMENTAL

Culturing of lactic acid bacteria

3% NaCl fermented eggplant fruit was blended. 1 mL of the sample was added to each Raka-Ray No. 3 agar plate aseptically. The plates were incubated at different conditions as follows: 37°C in the presence of CO_2 for 3 days and at 37°C without CO_2 for 3 days. The plates were observed for growth and colony morphology. The experiment was repeated for the 2nd to 7th day fermented eggplant fruits[7].

Lactobacillus: 20 mL of 3% NaCl day old fermented eggplant fruit sample was dissolved in 20 mL quarter strength Ringer solution. 1 mL of the sample was added to each sterile petri dish. Molten MRS agar (45°C) was poured into the dish and mixed thoroughly. Upon setting, another layer of the uninoculated MRS agar was poured over the surface to produce a layer-plate. The plates were incubated for 3 days at 37°C in the presence of CO_2 and without CO_2. The plates were observed for growth, colony morphology and further tests carried out. The experiment was repeated for the 2nd to 7th day fermented eggplant fruits[7].

Leuconostoc* and *Pediococcus: 3% NaCl fermented eggplant fruit was blended. 1 mL of the sample was added to each orange-serum agar plate aseptically. Half of the plates were incubated at 25°C in the presence of CO_2 for 2 days and the other half at 35°C in CO_2 for 2 days. Controls were incubated at 25°C and 35°C without CO_2. The plates were observed for growth and colony morphology. The experiment was repeated for the 2nd to 7th day fermented eggplant fruits[7].

***Streptococcus*:** 3% NaCl fermented eggplant fruit was blended. 1 mL of the sample was added to each blood serum agar plate aseptically. The plates were incubated at 35°C for 2 days. The plates were observed for growth and colony morphology. The experiment was repeated for the 2nd to 7th day fermented eggplant fruits[7].

Yeast (Control): 3% NaCl fermented eggplant fruit was blended. 1 mL of the sample was added to each sabouraud 2% maltose broth tubes aseptically and incubated at 25°C for 5 days. The tubes were observed for growth. The experiment was repeated for the 2nd to 7th day fermented eggplant fruits[7].

Tests for identification of bacteria: The bacteria present were identified using gram staining (Jensen's modification), catalase test and indole test[8].

Shelf life of packaged product: The fermented eggplant fruits were packaged using polyethylene. The packaged fermented eggplant fruits were pasteurised using steam. Microbial study was done on the packaged samples to determine contamination by microorganisms and hence the shelf life of the packaged product.

RESULTS AND DISCUSSION

Among the media studied, there was no growth on the orange-serum agar plates incubated at 35°C, blood serum agar plates incubated at 35°C and sabouraud-2% maltose broth. The orange-serum agar and blood serum agar at these conditions in the presence of CO_2 select for *Pediococcus* and *Streptococcus* respectively whereas the sabouraud-2% maltose broth selects for yeast. The lack of growth on these media means the absence of *Pediococcus* and *Streptococcus* and yeast.

The rapid fall in pH brought about by the lactic acid fermentation and hence the lactic acid bacteria, the anaerobic conditions and temperature of the fermentation (ambient temperature) decreased the growth of extraneous bacteria[7]. However, the growth was observed after the first day of fermentation on MRS agar, which is selective for *Lactobacillus.* Also, the orange-serum agar plates incubated at 25°C, which selects for *Leuconostoc*, had growth after the second day of fermentation. The bacteria on the MRS and orange-serum agar plates were present at the end of seven days of fermentation (Table-1).

TABLE-1
LOG COUNTS OF THE MAIN MICROBIAL GROUPS OF THE FERMENTED SAMPLES

Group of microorganisms	Fermented eggplant	Day growth was observed						
		1	2	3	4	5	6	7
Lactobacillus	8.12 ± 0.32	+	+	+	+	+	+	+
Leuconostoc	4.13 ± 10.41	–	+	+	+	+	+	+
Pediococcus	0	–	–	–	–	–	–	–
Streptococcus	0	–	–	–	–	–	–	–
Yeast	0	–	–	–	–	–	–	–

+ Growth; – No growth.
Values are means 1 SD for four samples.

Bacteria isolated on the MRS and orange-serum agar (cocci in pairs and chains) were all gram positive, catalase negative and indole negative. These isolates were identified based on the ability to develop at various temperatures (20–25 and 36°C) under anaerobic conditions. The identification was completed using the morphology of the bacteria to put them into their various genera.

The bacteria on the MRS agar plates, which were observed after the first day of fermentation, were slender rods to short coryneform cocci-bacilli—*Lactobacillus*[9]. On the other hand, the growth on the orange-serum agar (incubated at 25°C), which was observed after the second day of fermentation, were cocci in pairs and chains—*Leuconostoc*[7].

The bacteria identified, that is, *Lactobacillus* and *Leuconostoc* belonged to the heterofermentative acidifying and aroma producing species of the lactic acid producing bacteria. These play important roles in the acidification and aroma production in fermented eggplant fruits.

The *Lactobacillus* and *Leuconostoc,* which were responsible for the fermentation of the eggplant, are heterofermentative lactic acid bacteria. They produce

acetic acid, carbon dioxide, ethanol, mannitol and traces of other compounds in addition to lactic acid[10].

The results obtained from the lactic acid fermentation of eggplant fruits in this study when compared with the well studied lactic acid fermentations of cabbage and cucumber show a lot of similarities in the type of fermentative micro-organisms, flavour and aroma compounds. Three microorganisms are responsible for the lactic acid fermentation of cabbage to produce sauerkraut and these belong to two different genera: *Leuconostoc* and *Lactobacillus*. However, in the production of cucumber pickles, the microorganisms involved are in the following genera: *Leuconostoc, Lactobacillus* and *Pediococcus*[6].

The packaged fermented product should be wholesome and free from yeasts, contaminating pathogenic and lactic acid bacteria, which were responsible for the fermentation. Microbial analyses were carried out on the packaged refrigerated samples for a period of up to five months to determine the level of yeasts, pathogenic and lactic acid bacteria. The media used were: Raka-Ray No. 3 agar for isolating lactic acid bacteria (general), MacConkey agar for isolating *Salmonella, Shigella* (pathogenic bacteria) in foodstuffs, water etc. and Sabouraud 2% maltose broth for culturing yeasts[9].

Growth was absent in the pasteurised and packaged (processed) samples (Tables 2–4). The results are that the levels of bacteria (lactic acid and spoilage) and yeasts for the fermented eggplant fruit product were reduced to zero after processing.

TABLE-2
MICROBIAL STUDIES USING RAKA-RAY No. 3 AGAR

Month	CFU/mL				
	1	2	3	4	5
Fermented eggplant	0.0	0.0	0.0	0.0	0.0

TABLE- 3
MICROBIAL STUDIES USING MacCONKEY AGAR

Month	CFU/mL				
	1	2	3	4	5
Fermented eggplant	0.0	0.0	0.0	0.0	9.0

TABLE- 4
MICROBIAL STUDIES USING SABOURAUD-2% MALTOSE BROTH

Month	CFU/mL				
	1	2	3	4	5
Fermented eggplant	0.0	0.0	0.0	0.0	7.5

CPU = colony forming units

The zero level was maintained for at least four months after processing. However, counts were observed on MacConkey and Sabouraud-2% maltose broth after the fourth month. This shows that the pH, acidity, moisture, etc. in the fermented product did not support bacteria and yeast growth. The pasteurisation was very effective and as a result no counts were observed on the Raka-Ray No. 3 agar throughout the five-month period.

Presently, in Africa, eggplant fruits are extensively used; however, eggplant fruits are usually in short supply during the dry season because no suitable preservation techniques exist. From this study, the vegetable can be preserved for about four months using lactic acid fermentation and packaging with polyethylene.

Conclusions

Two main genera of microorganisms were responsible for the lactic acid fermentation of eggplant fruits. These were *Lactobacillus* and *Leuconostoc*. The fermented eggplant fruits were shelf stable for a maximum of four months when packaged in polyethylene and refrigerated.

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