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The effects of baobab pulp powder on the micro flora involved in tempe fermentation

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Abstract Locally prepared tempe that underwent natural fermentation was characterized by the growth of *Lactobacillus plantarum*, *Streptococcus lactis*, *Bacillus* sp., *Salmonella* sp., *Klebsiella* sp., *Lactococcus lactis*, *Rhizopus* sp. and *Staphylococcus* sp., while fermentation carried out with the addition of varying levels of baobab pulp powder had mainly lactic acid bacteria (LAB)—*Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus acidophilus* and *Rhizopus* sp. dominating. Increasing concentrations of baobab pulp powder led to an increase in the population of lactic acid bacteria (LAB) from 2.3×10^2 to 3.3×10^4 while it reduced the population of inoculated *Rhizopus* from 10^2 to only six colonies on malt extract agar (MEA).

Keywords Fermentation · Tempe · Baobab

Introduction

Tempe is a nutritious fermented food obtained by the fermentation of soybeans using the fungus *Rhizopus oligosporus*. Although its consumption was initially confined to the Asian countries, recently its consumption has spread to other parts of the world, particularly developing countries in Asia and Africa, including Nigeria, where it plays an important role as a complementary food [1]. In Nigeria, it is fast becoming popular as a dietary protein supplement since animal protein is unaffordable by the majority of the populace. The production of tempe varies from one locality to another. In Indonesia and other parts of Southeast Asia, tempe is prepared without the addition of baobab pulp, but in Nigeria, where its consumption is still limited, it is fermented with baobab pulp powder in order to give the characteristic aroma and acidic taste preferred by the local people. Baobab (*Adansonia digitata*) pulp is rich in ascorbic acid, calcium, tartaric acid and potassium bitartrate [2]. Its usage in food fermentation is a common practice in Nigeria, especially in the northern part of the country where the Fulani Kraals use it in the fermentation of cow milk for "nono" production. The pulp is pounded gently into a powder by using a pestle and mortar. It is then sieved to separate the seeds from the powder. This is done to hasten the curdling process as well as to improve the quality and quantity of the product, especially during the dry season when cow lactation is low and "nono" demand is high.

Production of tempe as it is done in Asia by fermentation of soybeans with *Rhizopus oligosporus* brings about changes in texture, aroma, and flavor, as well as reducing anti-nutritional factors. It improves the nutritional quality and produces an antibiotic effective against some gram-positive bacteria including *Staphylococcus aureus* [3, 4]. Local communities in Nigeria are of the belief that the addition of extracts of baobab to the fermentation medium assist in achieving the improved sensory qualities desired.

Information on the process, microbiology and biochemistry involved in tempe fermentation has been extensively reviewed [5, 6, 7, 8, 9]. Many pathogenic microorganisms such as *Bacillus* sp. [10, 11, 12], lactic acid bacteria [13, 12] and yeasts [12] have been found in tempe fermentation. The presence of these microorganisms caused Tanaka et al. [14] and Nout et al. [16, 14] to question the microbiological safety of tempe. They demonstrated the ability of experimentally inoculated species of *Staphylococcus aureus*, *Clostridium botulinum*, *Salmonella* sp., *Yersinia enterolitica* and *Bacillus cereus* to exhibit strong growth in the non-acidified beans during fungal fermentation. These authors emphasized the importance of acidification of the beans prior to fungal fermentation for controlling the growth of these pathogens, if they are present. Apart from the slight acidity that occurred during the fermentation of tempe, little information is available on the possible ways of controlling the undesirable microbes that grow during the soaking period. Thus, the objective of this research is to determine the

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effect of the addition of baobab pulp on the pathogens that develop in the fermentation medium during tempe production.

Materials and methods

Collection of soybean samples

Soybean (*Glycine max*. (L) Merr.) seeds used for the experiments were obtained from the Department of Agronomy, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The seeds were sorted to remove extraneous materials and kept in a clean polythene bag in the laboratory until used.

Preparation of tempe

Fifty grams of soybean seeds were weighed into six 1-l conical flasks. The flasks were labeled A–F. Tempe was prepared from the soybeans in each of the flasks according to the method of Robert et al. [16]. The soybeans in each flask were boiled separately for 30 min, dehulled, and soaked in water. In each of the flasks (A–E), 5, 10, 15, 20 and 25 g of baobab pulp powder was added, respectively. Baobab pulp powder was not added to flask F, however, as this was to serve as the control experiment. Each flask was then inoculated with 1 ml of *Rhizopus oligosporus*, obtained from the University of Ibadan, Nigeria, to give 10^3 cfu/g. The flasks were incubated overnight at room temperature (25 °C) in a Mini/30/CLAD/vis incubator.

Isolation procedure

The total viable counts of the microorganisms were determined on plate count agar (PCA) (Oxoid) while lactic acid bacteria (LAB) were isolated on MRS agar (pH 5.5) [17]. Yeasts and molds were enumerated and isolated on malt extract agar (MEA) (Oxoid). The *Klebsiella* sp. count was conducted on plates of MacConkey-Inositol-Potassium tellurite agar as described by Thomas et al. [18]. All the plates were duplicated. Bacteria incubation was done using a Mini/30/CLAD/vis incubator at 35 °C, while fungi and mold were incubated at 25 °C in a separate incubator of the same model.

Determination of pH and titratable acidity

The pH of each tempe sample was determined using a combined glass-calomel electrode and a pH meter (pHM61 Radiometer, Copenhagen, Demark). Titratable acidity was done by titrating 25 ml of fermenting filtrate with 0.1 M NaOH. Three drops of 1% phenolphthalein indicator were added. The titratable acidity present in the sample was calculated based on the method of Nout et al. [13]

Identification procedure

Systematic, morphological and biochemical tests were conducted according to Cowan and Steel [19] with reference to Bergey's Manual of Systemic Bacteriology [20, 21]. Lactic acid bacteria (LAB) were identified using the conventional method of Kandler and Weiss [17] with complementary fermentation tests on API 50 CH gallery and CH medium (API system, Motalieu-Vercieu, France).

Data analysis

The data generated from the pH and titratable acidity readings were subjected to statistical analysis. The linear model procedure method was used to find out which of the concentrations of baobab pulp power has a maximum effect on the acidity of the medium during tempe fermentation at $p \geq 0.05$.

Results and discussion

The dominant microorganisms isolated from the fermentation medium apart from the *Rhizopus oligosporus* inoculum were mainly lactic acid bacteria (LAB). These were identified as *Lactobaccillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus acidophilus*, and *Lactococcus lactis* (Table 1). As the concentration of baobab pulp powder increased, the acidic medium present in tempe increased as well. This trend continued until the mold (*Rhizopus oligosporus*) could no longer survive in the medium. The mold was eliminated from the medium in the flask containing 15 g of baobab powder. Hence, it

Table 1 Microorganisms isolated from tempe samples with baobab pulp

Sample	Population (cfu/g)				Microbial species
	PCA	MRS	MEA	MCIK	
A	3.4×10^3	2.3×10^2	25	1.5×10^1	*Streptococeus* sp., *Lactobaccillus plantarum*, *Klebsiella* sp., *Bacillus* sp. *Staphylococcus* sp., *Rhizopus* sp., *Lactococcus lactis*
B	2.9×10^2	2.3×10^3	15	-	*Streptococcus* sp., *Lactobacillus plantarum*, *Lactococcus lactis*, *Lactobacillus plantarum* , *Rhizopus* sp.
C	2.0×10^2	2.8×10^3	10	-	*Lactococcus lactis*, *Lactobaccillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Rhizopus* sp.
D	7.9×10^1	3.1×10^3	6	-	*Lactococcus lactis*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Rhizopus* sp.
E	6.8×10^1	3.3×10^4	-	-	*Lactococcus lactis*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*
F	4.9×10^5	1.2×10^1	30	1.2×10^3	*Streptococcus* sp., *Bacillus* sp., *Staphylococcus* sp., *Lactococcus lactis*, *Rhizopus* sp., *Salmonella* sp., *Lactobacillus plantarum*

MCIK MacConkey-Inositol-potassium tellurite
MEA Malt extract agar
PCA Plate count agar
MRS De Man Rogosa Sharpe agar

Table 2 pH and titratable acidity of tempe sample

Sample	Concentration of Baobab pulp powder (g)	pH	Titratable acidity expressed as lactic acid %
A	5	3.8±1.4[a]	0.30±0.02[a]
B	10	3.5±0.80[a]	0.33±0.07[a]
C	15	3.3±0.30[a]	0.35±0.15[a]
D	20	2.8±1.2[b]	0.40±0.20[b]
E	25	2.35±1.1[b]	0.43±0.18[b]
F	0 (control)	4.6±0.80[a]	0.28±0.12[a]

Values represent the mean scores (n=3); Scores followed by the same letter in a column are not significantly different ($p \geq 0.5$)

could be assumed that the medium containing 15 g of baobab pulp powder and 50 g of soybeans or a ratio of 3:10, baobab powder and soybean seeds could be ideal for tempe production involving the addition of baobab powder. A concentration of the pulp powder above this value would prevent fungal fermentation, while the concentration below it may not control the growth of pathogenic microbes that colonize the soybeans during the fermentation.

Presently, the preparation of tempe involving the use of baobab pulp powder is still a traditional art. There is no form of quantification of the amount of pulp powder or the soybean seeds. The quantity of the powder used usually depends on the arbitrary judgment of the consumers, thus the pH of the final product varies.

The results of the investigation on the isolated microbes from soybeans in flask F (control) agree with the work of Nout et al. [13]. These authors implicated *L plantarum* (along with other microbes) as the dominant lactic acid bacteria species in tempe fermentation. In addition to this organism, other species of lactic acid bacteria such as *Lactobaccillus fermentum*, *Lactobaccillus acidophilus* , and *Lactococcus lactic* were also isolated form baobab pulp fermented tempe in this study. This could be due to the acidic environment created by the baobab powder, which favors their rapid proliferation. This is beneficial to consumers since most of the lactic acid bacteria species are nontoxic and have been reported to produce an enzyme that breaks the oligosaccharides in soybeans down to their mono and disaccharide constituents [22, 23]. The presence of lactic acid bacteria in tempe prepared as it's being done locally in Nigeria will not only improve the digestibility of tempe, but will also extend the shelf life of the product because of the preservative attributes of lactic acid bacteria.

Although the possible source of the lactic acid bacteria encountered in this study was not investigated, the involvement of lactic acid bacteria in a diverse range of fermentation processes have been reported [24]. The possibility of baobab pulp powder as the source cannot be completely ignored. Investigations into this aspect of the study are on-going.

Titratable acidity (expressed as a percentage of lactic acid) increased throughout the process of fermentation, resulting in a gradual decline in pH. However, the pH of tempe in the control flask indicated the presence of little acid created by the natural fermentation process, hence, the highest pH value of 4.6. The pH and titratable acidity of samples D and E were significantly different from the pH and titratable acidity of the control. Other treatments were not significantly different at $p \geq 0.05$ (Table 2).

In conclusion, this study established that an acidic medium, created by the addition of baobab pulp powder to tempe fermentation could prevent the growth of pathogenic bacteria such as *Salmonella* sp., *Bacillus* sp., and *Streptococcus* sp. Although this process is being done in the local production of tempe in Nigeria, there seems to be good scientific basis for this practice, particularly when aspects of microbiological safety are considered.

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