

Research Article

Chemical and Microbiological Quality Evaluation of Yoghurt Produced and Marketed in Chimoio, Mozambique

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Abstract

Background: Among several products derived from milk, yogurt is one of the food products elaborated in the dairy industry of Chimoio. Yogurt quality depends on good quality of the raw material and efficient control at all processing stages. The objective of this study was to analyse the physical-chemical and microbiological quality of the yogurt produced and marketed in Chimoio, Mozambique.

Material and Methods: This study was conducted in the Food and Water Laboratory in the Faculty of Engineering of the Catholic University of Mozambique. Twelve samples were purchased from the main Chimoio supermarket. Determination of pH, acidity, total soluble solids, proteins, lipids, and moisture was performed. Microbiological analyzes of yogurt were performed according to the manual of microbiological analysis for total bacteria counting, identification and quantification of *Staphylococcus aureus*, Yeast and Mold Count, total coliform enumeration and confirmation tests for total coliforms.

Results: The mean values were 4.3 Standard deviation (SD) = 0.04 for pH, 1.47 SD 0.08 for titratable acidity, 5.25% SD 0.05 for fat content, 3.11% SD 0.097 for protein and 86.2% SD 0.15 for humidity. Fifty percent of the samples presented aerobic mesophilic bacteria mean 1.8×10^5 CFU / ml. In terms of Molds and Yeasts, it was obtained 0.77×10^2 CFU / ml and in the *Staphylococcus aureus* count, 0.72×10^2 CFU / ml was obtained. Results of enumeration of total and fecal coliforms in yogurt samples indicate total coliform contamination of 28 NMP / g and 4 NMP / g for fecal coliforms.

Conclusion: From the findings, it can be concluded that the analyzed samples of the yoghurt produced and marketed in the city of Chimoio presents satisfactory quality for consumption. Improvement of the handling conditions during the production process should be observed

Background

The quality and safety of processed foods are decisive factors for the competitiveness in the industry and consumer market [1]. Among several products derived from milk, yogurt is one of the food products elaborated in the dairy industry of Chimoio. Yogurt quality depends on good quality of the raw material and efficient control at all processing stages [2]. Yogurt is a result of milk fermented by lactic acid bacteria, (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). These bacteria use part of lactose, the sugar found in the milk, and transform it into lactic acid and aromatic compounds that characterize the yogurt [3]. Yogurt changes, like any other food derived from the development of microorganisms and biochemical reactions [4].

Although the exact origin of yogurt is still a mystery to

researchers, there are some events that give clues to its emergence. Yogurt is impressively old and is believed to date back to the 3rd millennium B.C., when a shepherd in what is now Turkey fermented milk in sheep-skin bags to conserve it. Nowadays it is consumed worldwide, with historical prevalence in western countries but with a fast-growing foothold in emerging markets [5]. Formerly yogurt was considered a medicine as it was easy to digest and had benefits for the intestinal flora due to milk proteins that contain a high biological value and are partially pre-digested by the action of lactic bacteria, thus allowing better digestion [6]. Quality control of yogurt is of paramount importance for the consumers. Among the various parameters that indicate the quality and safety of yogurt, the most important are those that define the chemical and microbiological characteristics. The analyses are necessary to obtain information on the consumption aptitude,

nutritional value, the hygiene conditions during its production, processing, storage, distribution, on its shelf life and on the risk that can be posed to human health [7].

In Mozambique, due to lactose intolerance among the local communities, in the rural areas the villagers tend to consume fermented milk from their cows and in the cities, there is a growing demand for yogurt consumption. The increasing consumption of yogurt is not derived only from the lactose intolerance, but also due to its beneficial properties such as easy digestibility, high nutritional value and therapeutic effects [8]. Very few studies have been carried out in Mozambique in general and particularly in Chimoio on yogurt quality. The objective of this study was to analyse the physical-chemical and microbiological quality of the yogurt produced and marketed in Chimoio, Mozambique.

Material and Methods

Study Area

Chimoio is a municipality located in Manica Province in the central region of Mozambique. The population is estimated to be 324,816 in an area of 174 km² with altitude ranging from 513 to 786 meters. The major economic activities are: agriculture production, livestock, general trading, metallurgical industry, food industry, tourism, telecommunication, banking and insurance [9]. This study was conducted in the Food and Water Laboratory in the Faculty of Engineering of the Catholic University of Mozambique

Sample Collection

Twelve samples were purchased from the main Chimoio supermarket. The samples were randomly selected and immediately transported to the laboratory in isothermal boxes under ice bath and stored at controlled temperatures (4°C) in the refrigerator of the laboratory until the analysis were carried out.

Determination of chemical parameters of yoghurt

The samples were mixed and analyzed in triplicate for determination of pH, acidity, total soluble solids, proteins, lipids, and moisture according to the methodology described by Adolfo Luz Institute [10].

pH determination

To determine the pH, 10 g of the sample was weighed into a beaker, and diluted with 100 ml of distilled water. The pH was determined using a digital pH meter (Hanna Instruments).

Determination of titratable acidity (% citric acid)

The determination of total acidity was performed according to the method described by Nielsen [11]. Ten grams of the sample was weighed into a 50 ml beaker, then with the aid of a graduated pipette, 10 ml of distilled water was added and mixed with a magnetic stirrer. Then 5 drops of the phenolphthalein solution were

added and titrated with 0.1 N sodium hydroxide solution using a 25 ml burette until a pink color appeared.

Determination of moisture content

The moisture content was determined according to the analytical standards of the procedures described by Nielsen [11]. Aluminum capsules were heated in a series 2000 scientific oven, 5 g of the sample was weighed and placed in capsules and heated at 105°C for 5 hours. After this time, the sample was weighed and the procedure repeated until a constant weight was obtained.

Determination of proteins

The determination of proteins was performed according to the biuret method described in analytical standards by Carvalho [12]. A sample was prepared by weighing 1 g of the sample and mixing in 99 ml of distilled water in a 250 ml beaker.

Determination of lipids

The determination of lipids was done by the discontinuous method according to the procedures described by Nielsen [10].

Microbiological Analysis

Safety and preparation of culture media

All culture media were prepared according to the manufacturer's instructions for proportions and sterilization. For the quality assurance certification and absence of microbial contamination, a factor that could result in false-positive results, all materials, instruments and utensils were sterilized in the autoclave at 121°C for 15 min and kept immersed in the bath at 35°C until the moment of inoculation.

Microbiological analysis of yoghurt

Microbiological analyzes of yogurt were performed according to the manual of microbiological analysis methods described by Silva [13].

Total bacteria counting

The investigation of mesophilic bacteria was carried out in PCA culture medium by means of the deep seeding technique allowing the visualization of colony formation from viable plaques cells incubated at 37°C for 48 h. Plates were inoculated by the depth seeding method where aliquots of 1 ml of each dilution (10⁻⁴, 10⁻⁵, 10⁻⁶) were seeded in duplicate in previously sterilized petri dishes and 20 ml of PCA agar added. After the incubation period, typical colony forming units were counted, interpreted, multiplied by the dilution factor and expressed in CFU / ml.

Identification and quantification of *Staphylococcus aureus*

For the rapid counting of *S. aureus*, duplicate Petri film plates were inoculated with 1 ml of successive dilutions of 10⁻¹ to 10⁻³ and incubated at 37°C for 24 hours and then transferred

to a chamber at $62 \pm 2^\circ\text{C}$ and maintained for 4 hours. After this period, reactive thermos nuclease discs were placed on the plates and incubated again at 37°C for 1 hour. The counting was then performed considering blue coloration surrounded by a pink area as positive for staphylococcus.

Yeast and Mold Count

The standard counting of molds and yeasts was done according to Silva [13] and 1 ml aliquots of the first 3 successive dilutions (10^{-1} , 10^{-2} , 10^{-3}) were inoculated into previously sterilized plates of the medium of YEA (Yeast Extract Agar) with tartaric acid added to obtain the desirable pH of 3.55, then spread with the aid of a loop, waited for about 10 minutes for coagulation and drying of the medium and incubated at room temperature for 5 days. These analyses were performed in duplicate. Verification and estimation of colonies was performed by counting the number of creamy colonies for yeasts and large colonies with cotton or purulent characteristics for molds.

Total coliform enumeration (Presumptive test)

The enumeration of coliforms was performed using the Most Probable Number (MPN) technique according to Silva [13], which is the estimation of the density of viable microorganisms present in a sample under analysis. The inoculation was done by selecting the

successive dilutions (10^{-1} , 10^{-2} , 10^{-3}) of the samples and inoculating into 3 series of tubes containing 10 ml of 0.5% of MacConkey agar adding 1 ml of each dilution containing Durham tubes inverted. These tubes were incubated at $35 \pm 2^\circ\text{C}$ for 48 hours and the tubes showing gas production in the Durham tubes (resulting from lactose fermentation) were considered positive.

Confirmation tests for total coliforms.

Confirmation of the presence of total coliforms was done according to [13] by inoculating 1 ml of each dilution of the tubes considered positive in the presumptive test in 0.5% bright green broth and the tubes were inoculated in a bath. The presence of gas and turbidity of the medium (a result of lactose fermentation) in Durham tubes reveals the presence of coliforms and thus considered positives.

Statistical Analysis

Data were entered in an excel spreadsheet, means and standard deviation were calculated using the statistical package Bioestat 3.0.

Results

Table 1 presents the results of the chemical composition of the yogurt sold in Chimoio.

Sample	pH	Titrateable acidity (g/100 g)	Fats (%)	Proteins (%)	Humidity (%)
1	4.34	1.402	5.21	3.04	86.0
2	4.27	1.45	5.24	3.22	86.
3	4.28	1.549	5.31	3.08	86.3
Mean	4.30	1.47	5.25	3.11	86.2
SD	0.04	0.08	0.05	0.097	0.15
CV (%)	0.88	5.11	0.98	3.12	0.18

Table 1: Chemical composition of yogurt sold in Chimoio supermarkets.

The mean values were 4.3 Standard deviation (SD) = 0.04 for pH, 1.47 SD 0.08 for titrateable acidity, 5.25% SD 0.05 for fat content, 3.11% SD 0.097 for protein and 86.2% SD 0.15 for humidity.

Microbiological Results

Fifty percent of the samples presented aerobic mesophilic bacteria mean 1.8×10^5 CFU / ml. In terms of Molds and Yeasts, it was obtained 0.77×10^2 CFU / ml and in the *Staphylococcus aureus* count, 0.72×10^2 CFU / ml was obtained. Results of enumeration of total and fecal coliforms in yogurt samples indicate total coliform contamination of 28 NMP / g and 4 NMP / g for fecal coliforms,

Discussion

The pH is the parameter that determines the acidity and alkalinity of a product and, offers an indication of contamination

from bacteria or chemicals, while also providing a convenient method to estimate the acid development of a dairy product. [14]. The pH differences in the fermented milk may be related to the type and quantity of starter culture used for the manufacture [15].

In this study the pH was 4.3, and close values were found in Beira, Mozambique and in Brazil [16,17]. Lower values were reported in Para, Brazil [4]. The international minimum standard acceptable for pH is 4.4 [18]. There is a relationship between titrateable acidity and pH during lactic acid fermentation. In this study titrateable acidity was 1.47. The Codex standard for fermented milk [18] recommends a minimum of titrateable acidity of 0.6% while the Brazilian Quality and Identity Standard (PIQ) board [19] established values in the range of 0.6 to 1.5%. In Beira, Mozambique a value of 0.45% was reported [12] and Brazil [13] found values of titrateable acidity of 0.83 to 1.06%. The results of the titrateable acidity obtained in this study agreed with these

findings while [4] found values ranging from 1.08 to 1.33%.

The mean value for fat content in this study was 5.23%, similar to reported in Beira, Mozambique [12]. According to the Portuguese Association of Nutritionists [20], this type of yogurt is classified as fat yogurt. In Nigeria [21] the fat content of yogurt ranges from 1.5 to 2.5 while in Brazil yogurt ranges from 1.2 to 2.0% lower than the Chimoio yogurt.

The average protein content in this study was 3.11%, higher than the Codex Alimentarium minimum standard [18] of 2.7%. Results similar to this study were reported in Brazil [22,23]. This fact has a great importance since those foods are widely consumed by children that require a higher daily intake of protein in their diet.

Water content is one of the most important characteristics in consumer sensory perception of food. Change in moisture content will dramatically affect flavor and texture as well physical and chemical properties [24]. Water content in this study was 86.2%. In Beira, Mozambique [16] a study found 86.27% moisture content. Values of moisture content similar to this study were found in Brazil [25]. Higher moisture content can be an indication of fraud by water addition. The aerobic mesophilic bacterial count ranged from 1.9×10^2 to 2.2×10^5 CFU / ml which were higher than the value obtained in Nigeria [26,27].

The aerobic mesophilic bacteria mean was 1.8×10^5 CFU / ml in this study, higher than the FAO recommendation [28] that the bacterial count should not exceed 1.0×10^5 CFU / ml. This difference may probably be due to non-aseptic handling and inadequate heat treatment during the pasteurization process. Mesophilic bacteria when present at counts above 10^6 CFU / ml may cause deterioration processes and shorten the shelf life of foods.

In terms of Molds and Yeasts this study obtained 0.77×10^2 CFU / ml and this value is within the limits. The Normative Instruction of Brazil [29] establishes a maximum limit of 200 UFC / ml of molds and yeasts in yogurts. The results indicate that there was no contamination by molds and yeasts that usually originate from the environment, packaging or ingredients [15]. Counts above the values are related to the addition of sugar or fruit in the yogurt providing conditions appropriate for yeast multiplication [29] Leandro.

In this study the *Staphylococcus aureus* count was 0.72×10^2 CFU / ml. There is no legislation establishing the level of acceptable contamination of staphylococcus in yogurt. In other countries the allowed level is 1.0×10^2 UFC/ml for milks and fermented dairy drinks [30]. According to this standard, the result of this study is within the permitted range. The total coliform contamination was 28 NMP / ml and 4 NMP / g for fecal coliforms. According to Brazilian Normative Instruction establishes 100NMP / ml for total coliforms and 10NMP / ml for fecal coliforms. The values obtained in this study are within the limits. Considering that coliforms are

destroyed during the pasteurization process, the results can be considered as indicators of good hygienic-practices during the production process as well as efficient pasteurization, and careful and adequate handling during subsequent processes.

Conclusion

From the findings, it can be concluded that the analyzed samples of the yoghurt produced and marketed in the city of Chimoio presents satisfactory quality for consumption. Improvement of the handling conditions during the production process should be observed, since even in low contaminations the presence of *Staphylococcus aureus* was observed, which at high levels may constitute a danger to human health due to the formation of enterotoxins that can cause food intoxication. The presence of fecal coliforms which are a reflection of contamination during handling of the product is also a concern. The need to establish physical-chemical parameters for fermented milk beverages by the Mozambican authorities should be stressed.

Declarations

- a) **Ethic of approval and consent to participate:** Non-applicable
- b) **Consent for publication:** Not applicable
- c) **Availability of data and material**
- d) **Competing interests:** The authors declare that they have no competing interests.

Authors' contributions

The author JLF was involved in research design, and manuscript writing. GM was responsible for data collection and processing.

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