Evaluation of quality indicators of traditional and "Zero Lactose" probiotic yogurts

Avaliação de indicadores de qualidade de jovens tradicionais e probióticos "Zero Lactose"

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ABSTRACT
It is assumed that the parameters related to food quality represent in the consumption the certification of a safe food. This experiment aimed to evaluate the physicochemical parameters (fat content, acidity, pH, dry extract, and viscosity); microbiological parameters (standard count of viable mesophilic and psychrotropics aerobic, molds and yeasts, Staphylococcus aureus count, enumeration of coliforms at 35°C and 45°C; Listeria monocytogenes and Salmonella spp. occurrence.) and, weekly, the growth of Lactic Bacteria (LB) up to 42 days of yogurts produced in six different treatments (I₁ to I₆). Two types of milk were used: whole Ultra High Temperature (UHT) milk and semi-skimmed zero lactose, and four probiotic cultures: Bifidobacterium, Lactobacillus acidophilus, Lactobacillus paracasei and Lactobacillus casei. The produced yogurts presented physicochemical characteristics of fat content, pH, and acidity according to the legislation. They were considered safe, supported by the results of microbiological surveys. The Lactic Bacteria counting showed that all yogurts had viable cells about at least 10⁶ CFU/mL, characterizing them as probiotics. The sensory analysis obtained better aroma and flavor evaluation with better purchase intention for yogurts I₄ and I₆, both zero lactose, added of the probiotics L. paracasei and L. casei, respectively.

Keywords: Milk; Dairy production; Lactic bacteria; Safe Food.

RESUMO
Supõe-se que os parâmetros relacionados à qualidade dos alimentos representem no consumo a certificação de um alimento seguro. Este experimento teve como objetivo avaliar os parâmetros físico-químicos (teor de gordura, acidez, pH, extrato seco e viscosidade); parâmetros microbiológicos (contagem padrão de mesófilos e psicotrópicos viáveis aeróbicos, fungos e leveduras, contagem de Staphylococcus aureus, contagem de coliformes a 35 °C e
45 °C; Listeria monocytogenes e ocorrência de Salmonella spp.) e, semanalmente, o crescimento de bactérias lácticas (LB) até 42 dias de iogurtes produzidos em seis tratamentos diferentes (I1 a I6). Foram utilizados dois tipos de leite: leite integral de Ultra Alta Temperatura (UHT) e lactose zero semidesnatada e quatro culturas probióticas: Bifidobacterium, Lactobacillus acidophilus, Lactobacillus paracasei e Lactobacillus casei. Os iogurtes produzidos apresentaram características físico-químicas de teor de gordura, pH e acidez de acordo com a legislação. Eles foram considerados seguros, apoiados pelos resultados de pesquisas microbiológicas. A contagem de bactérias lácticas mostrou que todos os iogurtes tinham células viáveis em torno de pelo menos 10^6 UFC / mL, caracterizando-os como probióticos. A análise sensorial obteve melhor avaliação do aroma e sabor com melhor intenção de compra dos iogurtes I4 e I6, ambos com lactose zero, adicionados dos probióticos L. paracasei e L. casei, respectivamente.

**Palavras-chave:** Milk; Produção leiteira; Bactérias lácticas; Alimento Seguro.

1. INTRODUCTION

Dairy field has fundamental importance for the development of Brazilian agribusiness, since its productive chain embraces a large number of people, directly and indirectly, involved in this activity, generating monthly income for farmers (Milkpoint, 2017). The per capita consumption of milk and dairy products has reached levels of 170 kg of product per year, with potential for expansion (Sistema Faeg, 2017).

According to the United States Department of Agriculture (USDA), milk production in Brazil is expected to increase by 1.8% in 2018 from 23.98 million tons forecast, driven by exports of dairy products (Canal Rural, 2018). In addition, the "Zero Lactose" dairy market has also grown due to the increase in consumers diagnosed with lactose intolerance (Dietrich, 2017). Analyzing this scenario, food industries have invested in new products and, allied to this idea, there is a strong investment in the production of probiotic fermented products. Lactic Bacteria have been used mainly as a tool for food safety and bio conservation since they produce in their metabolism compounds called bacteriocins, which have claims of antimicrobial action against pathogens and deteriorates (Padua, 2013).

According to Normative Instruction IN nº 46 (Brazil, 2007), it is understood by yogurt, products added or not of other food substances, obtained by coagulation and decrease of the pH of the milk, by lactic fermentation of cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, with or without other Lactic Bacteria (LB) in a complementary manner. These added LB influence significantly the physicochemical and microbiological characteristics of the final product. They must remain viable, active and abundant in the food during their commercial shelf life, in the minimum amount of 10^7 Colony Forming Units per
milligram (CFU/mg).

Sensory analysis provides technical subsidies for research, industry, marketing, and quality control. It can influence other actions such as new or reformulated products market test, evaluation of the effect on the final product caused by changes in the raw material or in technological processing, cost reduction, control of packaging effect on finished products and stability during storage (Oliveira, 2010).

This experiment aimed to carry out the study of the physicochemical and microbiological quality parameters of six different types of yogurts produced with whole and semi-skimmed zero lactose UHT milk, adding different probiotic cultures: *Bifidobacterium, Lactobacillus acidophilus, Lactobacillus paracasei* and *Lactobacillus casei*.

2. MATERIALS AND METHODS

All stages of yogurt production, as well as the evaluation of product quality control, were conducted at the Laboratory of Animal Origin (Milk) of the Chemical Engineering Department of the Federal University of Pernambuco.

2.1 YOGURT PROCESSING

Six different yogurts were developed, identified as I₁, I₂, I₃, I₄, I₅, and I₆. It was prepared a mixture with whole or semi-skimmed zero lactose UHT milk with 5% of skimmed milk powder and 10% of sucrose. Then, the mixture was homogenized and pasteurized, at 82°C ± 1°C per 15 minutes. After that, it was cooled down to 42°C ± 2°C, and the starter and probiotic cultures were inoculated, as shown in Table 1 for each yogurt formulated. After inoculation, the product was filled into polyethylene bottles, incubated at 42°C ± 2°C for 3-4 hours. After that, they were then maintained at a temperature of 4°C ± 1°C.

Table 1 - Probiotic cultures used in traditional and low lactose yogurts.

| Yogurt | Type of UHT Milk | Dairy Yeast | Probiotic Culture |
|--------|------------------|-------------|-------------------|
| I₁     | Traditional      | Lyophilized DVS (Direct Vat Set) * | *Bifidobacterium, L. acidophilus, L. bulgaricus* |
| I₂     | Zero lactose     | Lyophilized DVS (Direct Vat Set) | *Bifidobacterium, L. acidophilus, L. bulgaricus* |
| I₃     | Traditional      | Culture of work** | *L. paracasei* LBC 81 |
| I₄     | Zero lactose     | Culture of work | *L. paracasei* LBC 81 |
| I₅     | Traditional      | Commercial natural yogurt | *L. casei* commercial |
| I₆     | Zero lactose     | Commercial natural yogurt | *L. casei* commercial |

* S. thermophiles, *Bifidobacterium* BB-12 (1 x 10⁶ CFU/g) and *L. acidophilus* LA-5 (1 x 10⁶ CFU/g)
** S. thermophilus and *L. bulgaricus*
2.2 PHYSICOCHEMICAL, MICROBIOLOGICAL AND SENSORY ANALYSIS

The trials were realized following the IN nº 68 (Brazil, 2006). The evaluation of pH was performed with bench pH meters. In the acidity analysis, the Dornic solution was used, in which every 0.1mL of the solution corresponds to 0.1 g of lactic acid/100mL (1ºD). For fat content (%), the Gerber method was performed. The viscosity was determined using the analogical rotary viscometer (Quimis® brand), according to the standard procedure described in the instruction manual.

The microbiological tests were performed according to IN nº62 (Brazil, 2003). It was realized the evaluation of the pathogenic bacteria *Staphylococcus aureus* seeded in Baird-Parker agar and confirmation in the catalase test, the enumeration of total coliforms and thermotolerant coliforms in MPN (most probable number), the detection of *Listeria monocytogenes* and *Salmonella spp.* and the standard counts of viable mesophilic and psychotropics bacteria, molds and yeasts. Finally, it was performed weekly the LB count up to 42 days, according to the methodology described in Silva (2007).

For sensorial analysis, the samples were randomly offered to 100 untrained judges in an amount of approximately 5.00g. For the evaluation, the affective test method of acceptability by hedonic scale was used with 9 points (1 = extremely disliked to 9 = extremely liked). During the test, the judges answered the questionnaire, evaluating the yogurt with the attributes of appearance, color, aroma, texture, flavor, and overall impression, ending with the intention to purchase the products.

The Student's T-test was performed in the physicochemical analysis.

3. RESULTS AND DISCUSSION

The results of the physicochemical analysis, mean values, and standard deviation are described in Table 2.
Table 2 - Analytical results of samples from the whole and semi-skimmed yogurts produced experimentally.

| Yogurt | Fat Content (%) | Acidity (% lactic acid) | pH    | TDE (%) | Viscosity (cP) |
|--------|-----------------|-------------------------|-------|---------|----------------|
| I₁     | 3.05±0.07       | 1.07±0.01               | 4.59±0.05 | 21.3±  | 6500±1556       |
| I₂     | 0.70±0.00       | 1.05±0.01               | 4.66±0.00 | 21.5±  | 4950±1485       |
| I₃     | 2.95±0.21       | 0.92±0.01               | 4.82±0.02 | 21.5±  | 2750±636        |
| I₄     | 0.90±0.00       | 0.95±0.01               | 4.66±0.03 | 20.3±  | 2438±795        |
| I₅     | 2.20±0.14       | 0.99±0.01               | 4.57±0.04 | 21.6±  | 7800±2263       |
| I₆     | 0.45±0.07       | 0.88±0.00               | 4.64±0.01 | 20.5±  | 4250±1061       |

(*)The analysis were performed in duplicate, and the means were presented with their respective standard deviation; (**) I₁- [Traditional milk] + [Bifidobacterium e L. acidophilus]; I₂- [Zero lactose milk] + [Bifidobacterium e L. acidophilus]; I₃- [Traditional milk] + [L. paracasei]; I₄- [Zero lactose milk] + [L. paracasei]; I₅- [Traditional milk] + [L. casei] e I₆- [Zero lactose milk]+[ L. casei]. (***) Equal letters on the same line do not differ statistically (p> 0.05).

There was no significant difference (p> 0.05) with the Student T-Test, among the yogurts I₁ and I₂, I₃ and I₄, I₅ and I₆, on the physicochemical parameters. The results of centesimal fat content were satisfactory, according to IN n°46/2007 (Brazil, 2007). The yogurts I₁, I₃, and I₅ were produced with whole UHT milk, and they were classified for the fat content as integral (3.0 - 5.9%) for samples I₁ and I₃, and partially skimmed (0.6 – 2.9%) for sample I₅. Those yogurts produced with UHT semi-skimmed Zero lactose milk were classified as partially skimmed, for samples I₂ and I₄, and as skimmed (max. 0.5% of fat content), for sample I₆.

The results for acidity were in the range of 0.88 to 1.07% in lactic acid, according to IN n°46/2007 (Brazil, 2007). Capitani et al. (2014) found concordant results in probiotic yogurts, with acidity levels of 0.6 to 0.9%; although, the results were inferior to the values obtained by Feitosa et al. (2017), which presented acidity of 1.2%, being produced with 8% of sucrose.

The values found for pH are in agreement with Mathias et al. (2013), whose variation was similar, from 4.6 to 4.8. These acidity characteristics favor final products with low syneresis indices, mainly due to the existence of equilibrium of loads.

Values of 20.3 to 21.6% for defatted dry extract (DDE%) were satisfactory, promoting a more consistency to the product. Yogurt’s quality and acceptability characteristics are related directly to its consistency and texture. The viscosity range found experimentally varied from 2438.0 to 7800.0 cP (centipoise), in agreement with the values obtained by Martins et al.
(2012), which obtained variations from 2013.0 to 2451.0 cP in produced yogurts with low lactose contents. The viscosity is not a characteristic mentioned in the Brazilian legislation for yogurt and fermented milk, so, there are no reference values. However, it is closely related to the solids content in the yogurt (DDE%) and the high viscosity of a yogurt can enable the reduction (or extinction) of the syneresis during storage.

The results of microbiological tests are shown in Table 3.

Table 3 - Results of the microbiological evaluation for deteriorating microorganisms in the samples of whole and semi-skimmed yogurt produced experimentally.

| Samples | Molds and Yeasts (log_{10} CFU/mL) * | Total Coliforms (35°C) (MPN/mL) ** | Thermotolerant Coliforms (45°C) (MPN/mL) ** |
|---------|------------------------------------|------------------------------------|-----------------------------------------------|
| I₁      | Estimated < 1.0                    | <3.0 (-;9.5)                       | 9.4 (3.6; 38)                                 |
| I₂      | Estimated < 1.0                    | 3.6 (0.17;18)                     | 3.6 (0.17;18)                                 |
| I₃      | Estimated < 1.0                    | 38 (8.7;110)                      | 9.4 (3.6; 38)                                 |
| I₄      | Estimated < 1.0                    | <3.0 (-;9.5)                       | <3.0 (-;9.5)                                  |
| I₅      | Estimated < 1.0                    | 3.6 (0.17;18)                     | 9.4 (3.6; 38)                                 |
| I₆      | Estimated < 1.0                    | <3.0 (-;9.5)                       | <3.0 (-;9.5)                                  |
| Legislation *** | -                                 | -                                 | <10                                           |
| Legislation **** | 2.301                             | <100                              | <10                                           |

* Values expressed in Log of Colony Forming Units per milliliter (log_{10} CFU/mL); values expressed as <1.0 represent no growth.
** Values expressed in Most Probable Number per milliliter (M.P.N/mL) with their respective confidence intervals; values expressed as <3.0 represent no growth.
*** Resolution RDC n°12 of 2001 (BRASIL, 2002);
**** IN n° 46 de 2007 – Indicative samples (BRASIL, 2007).
***** I₁- [Traditional milk] + [Bifidobacterium e L. acidophilus]; I₂- [Zero lactose milk] + [Bifidobacterium e L. acidophilus]; I₃- [Traditional milk] + [L. paracasei]; I₄- [Zero lactose milk] + [L. paracasei]; I₅- [Traditional milk] + [L. casei] e I₆- [Zero lactose milk] + [L. casei].

The most probable number (MPN) of total coliforms (<3.0 to 38 MPN / g) and thermotolerant (<3.0 to 9.4 MPN/g) obtained in the analyzed samples are in accordance with the standards established by the legislation (Brazil, 2001; Brazil, 2007), as shown in Table 3. The presence of molds (filamentous fungi) and yeasts was not detected in the microbiological tests. Their presence in the yogurts would cause deterioration, besides representing a risk to the public health due to the production of mycotoxins (Franco & Landgraf, 2008; Silva et al., 2007). It was verified the absence of Salmonella spp. and Listeria monocytogenes, as well as...
typical coagulate-positive staphylococi colony in any of the yogurt samples. These results demonstrate excellent microbiological quality of the product.

The values obtained for mesophilic bacteria varied from: estimated <1.00 to estimated <1.85 log10 CFU/mL, the equivalent to 1.0x10^1 and 7.0x10^1 CFU/mL. For psychrotrophic bacteria, the values were slightly higher, ranging from: estimated <1.00 to 2.57 log10 CFU/mL, equivalent to 1.0x10^1 and 3.7x10^1 CFU/mL. There are no standards found in the legislation of minimum values for aerobes mesophilic and psychrotrophic in fermented milk, being these indicators of hygienic-sanitary quality of the foods. According to Franco & Landgraf (2008), the detection of sensory changes in food by these microorganisms is perceptible when the count is greater than 10^6 CFU/g or mL (7.0 log10 CFU/mL) and it is associated with the abuse of binomial time/temperature during storage.

The results for Lactic Bacteria count are shown in Table 4.

Table 4 – Lactic Bacteria counting (LB).

| Time (days) | I_1* | I_2* | I_3* | I_4* | I_5* | I_6* |
|------------|------|------|------|------|------|------|
| 1          | 1.4x10^8 | 4.6x10^9 | 3.3x10^8 | 2.1x10^8 | 4.6x10^9 | 1.0x10^10 |
| 7          | 1.9x10^10 | 9.5x10^9 | 3.0x10^8 | 3.9x10^10 | 3.7x10^9 | 2.7x10^8 |
| 14         | 3.2x10^9  | 4.3x10^8 | 2.9x10^9 | 5.1x10^8 | 1.0x10^9 | 2.5x10^7 |
| 21         | 5.4x10^10 | 9.6x10^8 | 4.8x10^9 | 3.9x10^8 | 3.0x10^10 | 3.1x10^9 |
| 28         | 6.0x10^7  | 1.5x10^7 | 1.6x10^9 | 2.0x10^9 | 6.9x10^9 | 8.3x10^9 |
| 35         | 1.1x10^6  | 8.0x10^8 | 6.2x10^8 | 5.6x10^8 | 1.0x10^9 | 1.6x10^9 |
| 42         | 2.7x10^6  | 7.0x10^7 | 4.0x10^9 | 1.7x10^9 | 1.4x10^9 | 1.8x10^9 |

(*) Result obtained in Colony Forming Units per milliliter (CFU/mL), (**) I_1- [Traditional milk] + [Bifidobacterium e L. acidophilus]; I_2- [Zero lactose milk] + [Bifidobacterium e L. acidophilus]; I_3- [Traditional milk] + [L. paracasei]; I_4- [Zero lactose milk] + [L. paracasei]; I_5- [Traditional milk] + [L. casei] e I_6- [Zero lactose milk]+[L. casei].

The yogurts I_4, I_5 and I_6, after 42 days presented satisfactory values according to ANVISA standards (Brazil, 2001), in which is said that the probiotic food must contain a minimum viable quantity in the range of 10^8 to 10^9 CFU/mL. MAPA IN n°46/2007 (Brazil, 2007) mentions that the minimum viable LB value for the food to be considered probiotic is 10^6 CFU/mg or mL, so, it may be concluded that all yogurts are in agreement and can be classified as functionals, as shown in Table 4.
At the end of the 42 days, yogurts produced with lactose-free milk, I2 (7.0x10^7), I4 (1.7x10^9) and I6 (1.8x10^9) presented LB counts higher than their corresponding product with traditional milk, I1 (2.7x10^6), I3 (4.0x10^6) and I5 (1.4x10^9). According to Longo (2006), lactose is not used directly in the fermentative process by lactic bacteria, which requires its hydrolysis by the lactase inside the bacteria, thus, the use of milk with hydrolyzed lactose for the production of lactose-free yogurts provides favorable conditions to the growth of LB and starter cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

Lactic bacteria dispersion occurred during the 42-day period, reaching the maximum growth at the first day, for the sample I6, in the 7-day time period, for the yogurts I2, I4 and I5, and in 21 days for I1 and I3. It is still possible to notice that the yogurts I1, I2, and I3 presented the highest drops.

In the sensory analysis of the yogurts produced, the results obtained for the acceptance index of traditional probiotic yogurt and zero lactose are shown in Figure 1.

The results of Figure 1 confirm that the samples I3, I4, I5, and I6 showed good acceptance in all the questions (above 70%). The samples I1 and I2 presented values below 70% in appearance (I1 and I2), texture (just I1) and color (just I2). This was explained by Buriti & Saad (2017), that used "yogurt type" DVS culture, in which the heterofermentative action of *Bifidobacterium* produced lactic acid and acetic acid (2:3) and interfered with the final characteristics of the product. The six formulations showed good acceptance for the aroma and flavor. The presence of probiotics did not affect the flavor of the foods. There was no difference between traditional and lactose-free probiotic yogurts with the same culture. The yogurts with probiotic incorporation, besides the increase of yogurt nutritional qualities, also promoted the commercial viability of the product (Cruz
et al., 2016).

Still, about the flavor, it was observed that the yogurt with low lactose content (I₂, I₄, and I₆) presented higher averages than its corresponding one, justified by the presence of galactose and glucose, which increase the sweetness of yogurt (Melo Filho & Vasconcelos, 2011). The samples I₃, I₄, and I₆ reached satisfactory averages, being above 7 in the attributes of appearance, color, and overall impression. Figure 2 shows the percentage of purchase intention of probiotic yogurts.

Figure 2 - Intention to purchase traditional and low lactose probiotic yogurts.

![Intention to purchase traditional and low lactose probiotic yogurts.](image)

(*) Columns given in percentages (%); (**) I₁ - [Traditional milk] + [Bifidobacterium, L. acidophilus]; I₂ - [Zero lactose milk] + [Bifidobacterium, L. acidophilus]; I₃ - [Traditional milk] + [L. paracasei]; I₄ - [Zero lactose milk] + [L. paracasei]; I₅ - [Traditional milk] + [L. casei] e I₆ - [Zero lactose milk] + [L. casei]

Regarding purchase intention, the averages ranged from 3 to 4, being "maybe would buy / maybe would not buy" and "possibly would buy." The yogurts I₁ and I₂ obtained a higher rejection of the judges, 44.07%, and 33.91%, respectively, opted for "certainly would not buy" and "possibly would not buy", while for the other samples, the sum did not reach 20%. The yogurt I₁ got the highest rejection, with a rating of 16.95% "certainly would not buy" rating. The rest of the samples presented with more than 50% in the sum of those that "certainly would buy" and "possibly would buy". The I₆ yogurt presented the highest percentage of judges who "certainly would buy" with 37.29% followed by I₄ with 35.60%.
4. CONCLUSIONS

The yogurts produced had physicochemical characteristics of fat content, pH, and acidity according to IN n°46 (Brazil, 2007), presented high viscosity, a parameter that is not foreseen in legislation. The yogurts were also classified as safe according to legislation with the microbiological research results. The LB count up to the 42-day period showed that all yogurts had viable cells about at least 10^6 CFU/mL, characterizing them as probiotics. The sensory analysis resulted in a better aroma and flavor evaluation, with a better purchase intention, to the yogurts I_4 and I_6, both with low lactose content and with the addition of probiotics L. paracasei and L. casei, respectively.

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