PROBIOTICS IN MILK POWDER: VIABILITY OF MICRO-ORGANISM, PHYSICOCHEMICAL CHARACTERIZATION AND SENSORY EVALUATION OF FOOD MATRIX

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RESUMO – Os efeitos benéficos de alimentos com adição de micro-organismos probióticos na saúde humana e, em particular, em produtos lácteos sobre as populações de alto risco, estão sendo cada vez mais reconhecidos pelos profissionais de saúde. Tem sido relatado que os probióticos podem desempenhar um papel importante no sistema imunológico, digestivo e funções respiratórias e podem ter um efeito significativo no alívio de doenças infecciosas em seres humanos. A indústria tem buscado alternativas para a adição de probióticos de maneira a elevar a qualidade de seus produtos, portanto, novas matrizes de alimentos e processos simples, porém eficazes, de produção precisam ser testados, uma vez que a sobrevivência no produto é considerada um requisito para os efeitos benéficos dos probiótico. Esta pesquisa avaliou a viabilidade de micro-organismos probióticos adicionados em leite em pó integral. O produto foi caracterizado físico-quimica e sensorialmente. Após 30 dias da adição houve decréscimo na contagem de probióticos entre (1,22 e 1,48 log UFC.g⁻¹) para Lactobacillus e Bifidobacterium porém não houve alterações significativas nas características físico-químicas e a equipe semi-treinada não constatou diferenças entre as amostras do dia zero e no final da vida útil do produto.

PALAVRAS-CHAVE: alternativas, Lactobacillus, Bifidobacterium, matrizes alimentícias

ABSTRACT – The beneficial effects of food with added live probiotic microorganisms on human health and, in particular, of milk products on high-risk populations, are being increasingly promoted by health professionals. It has been reported that these probiotics can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating infectious disease in humans. The industry has sought alternatives to the addition of probiotics in order to improve the quality of their products, therefore, new food matrices and simple but effective production processes need to be tested because the survival in the product is considered a prerequisite to the beneficial effects of probiotics. This research evaluated the viability of probiotic microorganisms added to whole milk powder. The product was physicochemical and sensory characterized. Thirty days after the addition there was a decrease in the probiotic count between (1.48 and 1.22 CFU.g⁻¹ log) for Lactobacillus and Bifidobacterium however, there were no significant changes in physical and chemical characteristics and semi-trained staff found no differences between zero-day samples and those at the end of shelf life.

PALAVRAS-CHAVE: alternativas, Lactobacillus, Bifidobacterium, food matrix

KEYWORDS: alternatives, Lactobacillus, Bifidobacterium, food matrix
1. INTRODUCTION

Milk drying to obtain the powder product is one of the various technologies for its use. In this type of process, nutrients are preserved almost completely. Another advantage of this technology is that the powder product enables a more economical transportation, production planning, distribution and manufacture of various products, having a longer shelf life and does not need refrigeration due to its low moisture content and water activity (Garrigues & Guardia, 2012).

Viable bacteria that have beneficial health effects based on intestinal improvement is the most common definition of probiotics, but the diversity of mechanisms of action of microorganisms, including immune regulation, with scientific evidence that specific strains of probiotic microorganisms, when administered in adequate amounts, confer health benefits to the host and are safe for human use has made the market of probiotics expand as consumers are hungry for health claims based on these and make their choices (Nomoto, 2005). Regular consumption of certain dairy products has a beneficial effect in preventing disease (Bakr, 2015).

There are also commercial interests in the concept of probiotic food, constituting a considerable part of the functional foods market, as evidenced by the variety of probiotic products available in supermarkets and specialty stores (Senok et al., 2005). Several authors have shown that regular consumption of viable probiotic microorganisms can be effective in improving the tolerance to lactose (Hove et al., 1999; Jankovic et al, 2010; Singh et al, 2011), reducing cholesterol levels (Meurman, 2005; Nguyen et al, 2007) and controlling gastrointestinal infections caused by viruses or bacteria (Gonet-Surowka et al., 2007; Shah, 2007). Moreover, it has been reported that the probiotics might influence intestines, directly or indirectly, by modulating the endogenous microflora or intestinal immune system physiology since the colonization of some strains can reduce the severity of acute diarrhea in children (Klayraung et al., 2012).

The beneficial effect of probiotics on gut microbiota includes factors such as antagonistic effects, immunological effects competition, and result in an increased resistance against pathogens. Thus, the use of probiotic bacterial cultures stimulates the proliferation of beneficial bacteria at the expense of potentially harmful bacteria proliferation, enhancing natural defense mechanisms of the host (Cook et al., 2002).

The use of probiotic bacteria in fermented milk products has been widely studied due to maintenance difficulties that affect the viability of these organisms during refrigerated storage. Factors such as acidity and dissolved oxygen, and interactions between species, inoculation practices and storage conditions can influence the survival of probiotic microorganisms in fermented dairy products. The number of viable bacteria that enter the intestinal tract and low pH in the stomach are limiting for the survival of probiotic microorganisms (Jankovic et al., 2010). In addition, many problems still exist with respect to low viability of probiotic bacteria in dairy foods. Several factors that affect the viability of probiotics have been reported in fermented milk products, including titratable acidity, pH and hydrogen peroxide, dissolved oxygen concentration, storage temperature, interaction with other microorganisms in products, the concentration of lactic and acetic acid, and the concentration of proteins (Bakr, 2015).

The competition between industries for market shares leads to a need to improve the quality of their products, so new food matrices and simple but effective production processes need to be tested, since survival in the product is considered a requirement for the beneficial effects of probiotics.

This work proposes a new food matrix for probiotic microorganisms, analyzing the viability of the probiotics and characterizing physicochemical and sensory the food matrix (milk powder).

2. MATERIAL AND METHODS

The study was conducted from April to June 2016, at the Department of Food Science and Technology of the Federal University of Santa Maria.
For the development of probiotic milk powder, whole instant milk powder was acquired and 1.0% of the crops of lactic yeast cultures containing freeze-dried DVS (Direct Vat Set) *Lactobacillus LA5*, *Bifidobacterium BB-12* and *S. thermophilus* (BioRich® - Chr's Hansen) were added, aseptically. With the help of a sterile glass rod the mixture was homogenized. Subsequently it was packed and stored at room temperature. After 24 hours, microbiological and physico-chemical analysis (A1) was performed, and repeated 30 days after the first analysis (A2), since it is the manufacturer's recommendation to use the product within 30 days. Sensory analysis were carried out at the end of the shelf life established for the product, by a semi-trained sensory team, consisting of 11 judges, at laboratory of Milk & Dairy Products, UFSM, previously submitted to, and approved by, the UFSM Ethics Committee in Research with Human Beings (56769116.9.0000.5346). The samples used were one with added probiotics and one without addition, both opened on the same day. The test used was the double duo-trio (presented twice, balanced), due to the fact that it is a discriminatory test with forced choice, where the intention is to know whether or not there are perceptible differences between the milk samples with and without probiotic microorganisms (IAL, 2008). The sensory test samples were diluted as indicated by the manufacturer (150 g of milk powder for one liter of water).

The physicochemical parameters determined were moisture, ash, protein by micro-Kjeldahl, fat by butyrometer and carbohydrates by difference, and all analyzes were performed in triplicate in both samples. Water activity was also measured. The methods followed the recommendations of Instruction No. 68 and/or the Adolfo Lutz Institute (Brasil, 2006; IAL, 2008).

For the quantification of *Lactobacillus*, acidified MRS agar was used, incubated at 37± 1 °C for 72 hours under anaerobic conditions. The microorganisms of the genus *Bifidobacterium* were measured in MRS agar supplemented with lithium chloride (0.1%), cysteine- HCl (0.05%) and dicloxacillin (0.5 mg / L), with incubation at 37± 1 °C for 72 hours under anaerobiosis (APHA, 2001).

Data was analyzed using analysis of variance (ANOVA) and the differences between the sample means were considered significant when the P value was <0.05. Bacterial counts were transformed to log 10 values.

### 3. RESULTS AND DISCUSSION

Table 1 shows the physical and chemical parameters of whole milk powder that were in accordance with the recommendations set by the Brazilian legislation (Brasil, 1996).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Humidity (g/100 g)</th>
<th>Ashes (g/100 g)</th>
<th>Protein (g/100 g)</th>
<th>Fat (g/100 g)</th>
<th>Lactose (g/100 g)</th>
<th>Water activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>2.49±0.01°</td>
<td>5.97±0.01°</td>
<td>24.97±0.08°</td>
<td>28.20±0.10°</td>
<td>37.37±0.15°</td>
<td>0.3135±0.01°</td>
</tr>
<tr>
<td>A2</td>
<td>3.81±0.01°</td>
<td>5.97±0.01°</td>
<td>25.04±0.01°</td>
<td>28.23±0.06°</td>
<td>37.50±0.10°</td>
<td>0.3331±0.01°</td>
</tr>
</tbody>
</table>

Legend: same letters in the same column indicate that there was no statistically significant difference at 5%. A1: whole milk powder time zero days; A2: whole milk powder time 30 days

Source: Authors

As can be seen in Table 1 the only parameter that showed a significant difference was humidity, which was expected, since in the milk powder packing process the oxygen in the atmosphere is replaced by nitrogen and, when the can is opened, nitrogen is lost and, because of the fact that the package has a plastic cover for closing, it is not hermetically sealed and is susceptible to moisture gain, depending on the external environment. A slight increase in water activity was noticed, but was not statistically significant.

According to Kontominas (2010), the permeability to water vapour of the packaging material is important in milk powder so that the product has an extended shelf life, and moisture content and water
activity should correspond to the minimum lipid oxidation rate. Values obtained were 3.5% and 0.24, respectively.

In new product development, sensory analysis is needed to provide useful information on the perception of consumers about the acceptance or rejection of the new product and, therefore, it is critical in improving the quality and shelf life of food, allowing these innovative products to be placed on the market without losses and risks for companies (Chapman, 2010).

In the double duo-trio sensory evaluation test, 90% of the panelists did not perceive the different sample, indicating that the addition of probiotics does not alter the perception of the product.

For consumers, the sensory characteristics directly influence the acceptability and repurchase of food, responding only if the product does or does not taste good. Trained panelists can be used to generate objective data analogous to the instrumental data (Illupapalayam et al., 2014).

Table 2 shows the results of microbiological analysis of microorganisms added into milk powder.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Lactobacillus (log CFU. g⁻¹)</th>
<th>Bifidobacterium (log CFU. g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>7.60</td>
<td>8.62</td>
</tr>
<tr>
<td>A2</td>
<td>6.90</td>
<td>4.08</td>
</tr>
</tbody>
</table>

A1: whole milk powder time zero days; A2: whole milk powder time 30 days
Source: Authors

Most probiotic products on the market are mostly in liquid or semi-solid form, where the products show low cell viability after oral administration, since these microorganisms can not survive the harsh conditions in the stomach (Klayraung et al., 2012). The development of dry products, such as milk powder probably allows for a greater survival of these microorganisms and, consequently, can act as an alternative to a market avid for functional products. The freeze-dried culture, added to milk powder, with low moisture content and water activity will preserve the viability of microorganisms.

In this study there was a decrease in log (CFU.g⁻¹) of microorganisms of 1.48 for the Lactobacillus and of 1.22 for Bifidobacterium, however, the sum of the counts reached values indicated by Brazilian legislation and recommended by FAO/WHO.

Food marketed with the intention of offering health benefits to the consumers through the addition of probiotics, should present a number of viable cells of probiotic cultures of, at least, \(10^6-10^7\) CFU.g⁻¹ (6 to 7 log CFU.g⁻¹) (FAO/WHO, 2006; Tripathi & Giri, 2014; Bakr, 2015). However, the National Health Surveillance Agency (ANVISA) (Brazil, 2008) determines that the product defined as probiotic has to present a minimum concentration of \(10^8-10^9\) CFU.g⁻¹ (8 to 9 log CFU.g⁻¹), proven at the end of the products shelf life.

There is scientific evidence that specific strains of probiotics microorganisms confer health benefits to the host and are safe for human use (Senok et al., 2005; Mizock, 2015; Fuchs-Tarlovsky et al 2016). The food matrix has great impact on the viability and shelf life of the product. The stability and viability of the probiotic microorganisms in dry state are affected by room temperature and water activity, wherein the water activity is a function of temperature. It is possible to maintain the viability of probiotics, slow down chemical and enzymatic reactions that can lead to cell death and preserving the integrity of the microbial cell membrane by maintaining the product at low temperatures and low water activity (Forssten et al., 2011).

For the industry, the viability of probiotics in foods is a challenge, especially in the development of foods with adequate doses when consumed, since various factors during processing and storage may affect the viability (Reid, 2015). The presence of probiotic microorganisms may also adversely affect their quality and sensory properties (Sahni et al, 2014; Tripathi & Giri, 2014). For the
Brazilian consumer demand for innovative, practical, safe and healthier products, allied with the consolidation of products in the market contributes to the development of probiotic foods.

4. CONCLUSIONS

The milk powder with added probiotics showed satisfactory results from the microbiological point of view, in accordance with parameters established by FAO and WHO to be considered a product with probiotic viability.

There is a growing awareness among consumers about the relationship between nutrition and health and functional foods are, more and more, available as conventional food in supermarkets. With awareness of personal nutritional needs, there is an increasing demand for functional milk and its by-products, since probiotic dairy products are promising and show, as in the product of this study (milk powder), excellent conditions for maintaining viability of probiotic bacteria, demonstrating potential advantage and valuable alternative to the dairy industry.

The use of a new food matrix such as milk powder and the addition, in a simple process, of freeze-dried probiotic microorganisms, is an innovative alternative to the dairy industry, eager for differentiated products with low production cost. Studies need to be deepened to establish functional requirements of probiotic microorganisms using in vitro methods for the results to be reflected in vivo studies.

5. REFERENCES


