Symbiotic profile of petit suisse diet cheese with added brazilian nuts extract, *Bifidobacterium bifidum* and *Lactobacillus paracasei*

Perfil simbiótico do queijo petit suisse diet com adição de extrato de nozes brasileiras, *Bifidobacterium bifidum* e *Lactobacillus paracasei*

DOI: 10.55905/oelv22n1-016

Recebimento dos originais: 01/12/2023
Aceitação para publicação: 02/01/2024

**Jéssica de Medeiros Damaceno**  
Graduate in Food Engineering  
Institution: Universidade Tecnológica Federal do Paraná - campus de Medianeira  
Address: Av. Brasil, 4232, Parque Independência, Medianeira – PR,  
CEP: 85884-000  
E-mail: jessicadamaceno04@gmail.com

**Larissa de Oliveira Bispo**  
Graduate in Food Engineering  
Institution: Universidade Tecnológica Federal do Paraná - campus de Medianeira  
Address: Av. Brasil, 4232, Parque Independência, Medianeira – PR,  
CEP: 85884-000  
E-mail: larissa.obispo@gmail.com

**Cristiane de Carli**  
Graduate in Food Engineering  
Institution: Universidade Tecnológica Federal do Paraná - campus de Medianeira  
Address: Av. Brasil, 4232, Parque Independência, Medianeira – PR,  
CEP: 85884-000  
E-mail: cris_decarli@hotmail.com

**Lucas Vinícius Cavichi**  
Graduate in Food Engineering  
Institution: Universidade Tecnológica Federal do Paraná - campus de Medianeira  
Address: Av. Brasil, 4232, Parque Independência, Medianeira – PR,  
CEP: 85884-000  
E-mail: luscavichi@hotmail.com
ABSTRACT

Functional foods, which promise to help cure or prevent diseases, are the new trend in the powerful food market at the beginning of the 21st century. Probiotics are live microorganisms that can be added as supplements in the diet, beneficially affecting the development of the microbial flora in the gut. Prebiotics, on the other hand, are nondigestible but fermentable oligosaccharides whose function is to change the activity and composition of the intestinal microbiota with the perspective of promoting the health of the host. The Brazil nut is an oilseed, its lipid content is of good quality, with high levels of unsaturated fatty acids, components of vitamins B1, B2, B3 and selenium that have antioxidant potential. Currently, the consumption of low-calorie foods and sweeteners has increased a lot in recent years and the dairy industry, in an attempt to cater this public, has been introducing low-calorie dairy derivatives into the market, the so-called “light” and “diet” products, which use various types of sweeteners as substitutes for sucrose. Petit Suisse cheese is a cheese made with skimmed milk and cream, with very high humidity, light consistency, smooth, creamy and can have a sweet or salty flavor. The objective of this work was to produce formulations of Petit Suisse cheese added with acidifying...
bacteria, probiotic bacteria *Bifidobacterium bifidum* and *Lactobacillus paracasei*, Brazil nut extract, sweeteners and to verify the symbiotic potential during storage.

**Keywords:** functional food, light and diet, dairy derivative.

**RESUMO**

Alimentos funcionais, que prometem ajudar a curar ou prevenir doenças, são a nova tendência no poderoso mercado de alimentos no início do século 21. Os probióticos são microrganismos vivos que podem ser adicionados como suplementos na dieta, afetando de forma benéfica o desenvolvimento da flora microbiana no intestino. Os prebióticos, por outro lado, são oligossacarídeos não digeríveis, mas fermentáveis, cuja função é alterar a atividade e a composição da microbiota intestinal com a perspectiva de promover a saúde do hospedeiro. A castanha-do-brasil é uma semente oleaginosa, seu teor lipídico é de boa qualidade, com altos teores de ácidos graxos insaturados, componentes das vitaminas B1, B2, B3 e selênio que têm potencial antioxidante. Atualmente, o consumo de alimentos e adoçantes de baixa caloria tem aumentado muito nos últimos anos e a indústria de laticínios, na tentativa de atender esse público, vem introduzindo no mercado derivados de leite de baixa caloria, os chamados produtos "leves" e "dietéticos", que usam vários tipos de adoçantes como substitutos da sacarose. Petit Suisse é um queijo feito com leite desnatado e creme, com alta umidade, consistência leve, suave, cremoso e pode ter um sabor doce ou salgado. O objetivo deste trabalho foi produzir formulações de queijo Petit Suisse adicionadas com bactérias acidificantes, bactérias probióticas *Bifidobacterium bifidum* e *Lactobacillus paracasei*, extrato de castanha-do-pará, adoçantes e verificar o potencial simbiótico durante o armazenamento.

**Palavras-chave:** alimentos funcionais, luz e dieta, derivados de laticínios.

**1 INTRODUCTION**

Currently, consumers have seen new food products appear on supermarket shelves, with a commitment to contribute to the search for a healthier life. Functional foods are the new trend of the intense food market (HEASMAN; MELLENTIN, 2001). Yogurts, margarines, fermented milks, cereals, etc. promise to help cure or prevent diseases such as cardiovascular disease, certain types of cancer, allergies, intestinal problems, etc. We can consider functional foods those that, in addition to providing basic nutrition, promote health. These foods have the potential to promote health through mechanisms not foreseen by conventional nutrition, and we should emphasize that this effect is restricted to promoting health and not curing diseases (REIG; ANESTO, 2002). The currently accepted international definition is that probiotics are live microorganisms,
administered in adequate amounts that confer a health benefit on the host (SAAD, 2006). The species most used to obtain probiotic products with alleged functional properties are lactobacilli and bifidobacteria (FOOD INGREDIENTS BRASIL, 2011). Microorganisms of the genus Bifidobacterium help host health by promoting the balance of the intestinal microbiota, fermenting carbohydrates that are not digested and absorbed in the upper intestinal tract, and also by having adverse effects on pathogenic bacteria. Lactobacillus paracasei species are the main probiotics of the genus currently used in foods. These microorganisms are tolerant to the acid and bile present in the gastrointestinal tract and are capable of adhering to the cells of the intestinal epithelium (GOMES; MALCATA, 1999; GOKTEPE et al., 2006). Prebiotics are non-digestible but fermentable oligosaccharides which function is to change the activity and composition of the intestinal microbiota with a view to promoting host health. Prebiotics stimulate the growth of endogenous microbial population groups, such as Bifidobacteria and Lactobacilli, which are said to be beneficial to human health (BLAUT, 2002). The Brazil nut, belonging to the Lecithydaceae family, is a fruit native to the Amazon region. It is an oleaginous seed, its lipid content is of good quality, with high levels of unsaturated fatty acids, which help in the oxidative processes of fat fractions, harmful to the body organism, such as LDL cholesterol fractions (GLORIA; REGITANO-D’ÁRCE, 2000; ENRÍQUEZ; SILVA; CABRAL, 2003; SOUZA; MENEZES, 2004). This fruit has components of the B1, B2 and B3 complex vitamins, pro vitamins A and vitamin E, minerals such as calcium, magnesium, iron, potassium, sodium and the presence of selenium, a mineral that has been extensively studied, can be found. due to its qualities in helping antioxidant processes, being part of the antioxidant enzyme (Glutathione Peroxidase), which helps inside the cell, converting toxic compounds into non-toxic ones (hydrogen peroxide) resulting in the reduction of the chain production of free radicals (MAHAN; ESCOTT-STUMP, 2002; DUTRA-DE-OLIVEIRA; MARCHINI, 1998; KANNAMKUMARATH; WROBEL; WUILLOUD, 2004; TEODORO, 2006). Nowadays, the consumption of low-calorie foods and sweeteners has increased a lot in recent years. The dairy industry, in an attempt to cater to this public, has been introducing low-calorie dairy products to the market, the so-called “light” ones, which substitute sucrose and various types of
sweeteners (REIS; MINIM; DIAS; CHAVES; MINIM, 2009). Petit Suisse cheese is a cheese made with skimmed milk added with milk cream. It is a cheese with very high humidity, light, smooth, creamy consistency and can take on a sweet or salty flavor, depending on the ingredients added, and is consumed fresh according to the classification established in the Technical Regulation of Identity and Quality of Petit Suisse Cheese (SANDRAZ, 1989; BRASIL, 2002). The objective of this work was to produce formulations of Petit Suisse cheese added with acidifying bacteria, probiotic bacteria *Bifidobacterium bifidum* and *Lactobacillus paracasei*, Brazil nut extract, sweeteners, and to verify the symbiotic potential during storage.

2 MATERIAL AND METHODS

The pasteurized skimmed milk and other ingredients used for the elaboration of the Petit Suisse Diet cheese formulations were purchased in a supermarket in the city of Medianeira - PR, the Brazil nut was obtained in the State of Rondônia. To carry out the study, the laboratories of UTFPR, Campus Medianeira were used. The elaboration of the Petit Suisse was carried out according to the methodology proposed by Albuquerque (2002), and the obtaining of the Brazil nut extract was got through the methodology of (FELBERG et al., 2004) with adaptations. Four formulations of Petit-Suisse cheese were made, with the addition of 10, 15 and 20% of Brazil nut extract and standard formulation without addition of Brazil nut extract, 1.5% of acidifying bacteria *Lactococcus lactis ssp lactis* and *Lactococcus lactis ssp cremoris* L (yofast MO 032 - SACCO®) and probiotic bacteria *Bifidobacterium bifidum* (HOWARU™ Bifido LYO – DuPont™ Danisco® - Fermentec®) and *Lactobacillus paracasei* (LBC 81/82 Lyo 10 D - DuPont™ Danisco® Fermentec®) and 0.65% sweetener. Formulations 01, 02, 03 and 04 of Petit Suisse cheese were designated by F1, F2, F3 and F4 (Standard) and were analyzed in periods of 0, 10, 20 and 30 days of storage. In the elaboration of the Petit Suisse cheese, Brazil nut extract and the sweetener were added to the milk, being homogenized. The base mixture was pasteurized at 75°C for 15 seconds and cooled to 35°C for inoculation of the acidifying lactic ferments and probiotics that were previously prepared, the rennet was added, slowly homogenizing. Then, it was placed to clot until reaching the point of coagulation, the curd
was then broken and stirred slowly for 15 minutes, the curd was drained for a period of 24 hours, obtaining the base mass of the cheese. The mass was broken and added with strawberry flavor pulp and strawberry flavor with constant agitation, followed by cooling and storage at a temperature of 5 °C. Analyzes of coliforms at 35 °C and 45 °C, Salmonella spp and molds and yeasts were carried out according to the methodology (BRASIL, 2003) and analyzes of the probiotic lactic acid bacteria Bifidobacterium bifidum and Lactobacillus paracasei according to the methodology (VINDEROLA; REINHEIMER, 2000).

3 RESULTS AND DISCUSSION

The results of the microbiological analyzes of the F1, F2, F3 and F4 (Standard) formulations of Petit Suisse cheese at 0D, 15D and 30 days are shown in Table 1.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Analyzes</th>
<th>0 Days</th>
<th>15 Days</th>
<th>30 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Coliforms at 35 °C and 45 °C (MPN. mL⁻¹)</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td>Molds and Yeasts (CFU mL⁻¹)</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella spp</em>/25 mL</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>F2</td>
<td>Coliforms at 35 °C and 45 °C (MPN. mL⁻¹)</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td>Molds and Yeasts (CFU mL⁻¹)</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella spp</em>/25 mL</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>F3</td>
<td>Coliforms at 35 °C and 45 °C (MPN. mL⁻¹)</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td>Molds and Yeasts (CFU mL⁻¹)</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella spp</em>/25 mL</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>F4 (Standard)</td>
<td>Coliforms at 35 °C and 45 °C (MPN. mL⁻¹)</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td>Molds and Yeasts (CFU mL⁻¹)</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella spp</em>/25 mL</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Source: Own authorship.
It was observed that there was no growth of microorganisms, guaranteeing the hygienic-sanitary safety of the raw material and its conservation during the 30 days. The microbiological analyzes were in accordance with the standard required by the legislation of cheeses with high humidity, and all formulations were below the minimum required by (RDC nº 161 of (BRASIL, 2022). According to the Technical Regulation of Identity and Quality of cheese with high humidity (BRASIL, 2005), the cheese formulations with high symbiotic moisture developed are in accordance with the legislation regarding the absence of *Salmonella* spp. As for the analysis of coliforms at 35°C and 45°C and molds and yeasts, no presence was found in the four developed formulations. In this way, good manufacturing practices, quality of the raw material used, ideal pasteurization of the milk used in cheese formulations with high humidity and adequate product storage conditions, during 30 days of refrigerated storage, are in accordance with the standards of the current legislation (BRASIL, 2005). The results of the analysis of probiotic bacteria in the F1, F2, F3 and F4 (Standard) formulations of Petit Suisse cheese at 0D, 15D and 30 days are shown in Table 2.

Table 2. Counts of probiotic bacteria in Petit Suisse Diet Cheese formulations at different storage times.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Probiotic Bacteria</th>
<th>0 Days</th>
<th>15 Days</th>
<th>30 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Lactobacillus paracasei</em> (CFU mL⁻¹)</td>
<td>1.8 x 10¹⁰</td>
<td>6.4 x 10¹¹</td>
<td>7.0 x 10¹³</td>
</tr>
<tr>
<td>F1</td>
<td><em>Bifidobacterium bifidum</em> (CFU mL⁻¹)</td>
<td>1.8 x 10¹⁰</td>
<td>6.4 x 10¹¹</td>
<td>7.0 x 10¹³</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus paracasei</em> (CFU mL⁻¹)</td>
<td>2.2 x 10¹⁰</td>
<td>6.8 x 10¹¹</td>
<td>7.2 x 10¹³</td>
</tr>
<tr>
<td>F2</td>
<td><em>Bifidobacterium bifidum</em> (CFU mL⁻¹)</td>
<td>2.2 x 10¹⁰</td>
<td>6.8 x 10¹¹</td>
<td>7.2 x 10¹³</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus paracasei</em> (CFU mL⁻¹)</td>
<td>1.7 x 10¹⁰</td>
<td>6.7 x 10¹¹</td>
<td>9.1 x 10¹³</td>
</tr>
<tr>
<td>F3</td>
<td><em>Bifidobacterium bifidum</em> (CFU mL⁻¹)</td>
<td>1.7 x 10¹⁰</td>
<td>6.7 x 10¹¹</td>
<td>9.1 x 10¹³</td>
</tr>
<tr>
<td>F4 (Standard)</td>
<td><em>Lactobacillus paracasei</em> (CFU mL⁻¹)</td>
<td>1.4 x 10¹⁰</td>
<td>6.3 x 10¹¹</td>
<td>8.2 x 10¹³</td>
</tr>
<tr>
<td></td>
<td><em>Bifidobacterium bifidum</em> (CFU mL⁻¹)</td>
<td>1.4 x 10¹⁰</td>
<td>6.3 x 10¹¹</td>
<td>8.2 x 10¹³</td>
</tr>
</tbody>
</table>

Source: Own authorship.
According to current legislation, for a product to be considered functional, it must present, by the end of its validity period, at least between $10^8$ and $10^9$ CFU in the daily portion, which is equivalent to the consumption of 100 g of product containing between $10^6$ and $10^7$ CFU of probiotic microorganisms (BRASIL, 2002). Therefore, the results obtained in the analyzes of probiotic bacteria of all the developed formulations exceeded the minimum necessary in the 30 Days of storage, being in accordance with the Legislation. The results obtained in Table 2 demonstrate that formulation 4, without the addition of Brazil nut extract (Standard), showed lower counts of Lactobacillus paracasei bacteria around $1.4 \times 10^{10}$ and Bifidobacterium bifidum $1.4 \times 10^{10}$ times, during the 0 days of manufacture, $10^{10}$ at the beginning of storage in relation to formulations F1, F2 and F3, respectively added 10%, 15% and 20% of chestnut extract. This can be explained by the need of adaptation of probiotic microorganisms to the medium, which requires the production of nutrients available and acidity produced by lactic acid bacteria, since the metabolic activity of Lactobacillus delbrueckii subsp bulgaricus and Streptococcus thermophilus during storage results in the production of organic acids that may affect the viability of probiotic cells in the future (DONKOR et al., 2006). However, Shah et al., (1995) suggest that, for therapeutic effect, the product must contain $\geq 10^5$ cells/mL and for Vinderola et al., (2000) the consumption of $10^8$ - $10^{11}$ CFU/day is recommended to achieve the benefits proposed by probiotic microorganisms.

Thamer; Penna, 2006; Vinderola et al., 2000, in studies carried out, state that the viability of microorganisms, when used in the production of fermented foods, depends on the interaction between the species present, the strain used, fermentation time, storage and post-acidification conditions, nutrients available, among others. Martín-Diana et al. (2003) recommend that the amount of inoculated microorganisms are equal to that desired in the final product, especially with regard to bifidobacteria, which have low development at very acid pH.

Formulations F1, F2, and F3 showed high counts ranging from $1.8 \times 10^{10}$ to $9.1 \times 10^{13}$ during 30 days of storage at 10 °C. Gomes; Malcata (1999) report that fermented products at the time of consumption must contain at least $10^6$ CFU mL$^{-1}$ of viable probiotic cells, due to the minimum daily therapeutic dose being $10^8$ - $10^9$ viable cells in
100g of fermented product. The high counts obtained in this study show that Petit Suisse Diet cheese meets this requirement, and the addition of Brazil nut extract made it possible to maintain the viability of probiotic bacteria in the product, presenting a prebiotic effect and can be considered a functional food. According to Brasil (2002), for a food to be considered functional, the minimum viable amount for probiotics must be in the range of $10^8$ to $10^9$ CFU in the daily recommendation of the ready-to-eat product. It corresponds to the consumption of 100 g of product containing from $10^6$ to $10^7$ CFU/mL or g, that is, from 6 to 7 Log CFU/g.

Bren, Santos, Almeida (2010) in their study with the elaboration of a probiotic drink from soluble soy extract obtained similar results to this study in relation to bifidobacteria which was $1 \times 10^{10}$ CFU. The elaboration of foods containing probiotic cultures in appropriate concentrations of viable cells during shelf life is a technological challenge (KOURKOUTAS et al., 2005). In a work carried out by Gardiner et.al., (1998), the performance of *Lactobacillus paracasei* was confronted with that of *Lactobacillus salivarius*, isolated from the human small intestine, during the maturation of Cheddar cheese, in which the species *Lactobacillus paracasei* presented multiplication and maintenance of high viability, while *Lactobacillus salivarius* counts decreased. Roberfroid (2000) suggested that symbiotic products can improve the survival of bacteria, when passing through the upper part of the gastrointestinal tract, and thus produce greater effects in the large intestine. Mattila-Sandholm et al. (2002) stated that the interaction between the probiotic and prebiotic, in vivo, can be favored by an adaptation of the probiotic to the prebiotic substrate, prior to consumption, and in some cases, this can result in a competitive advantage for the probiotic, if it is consumed with the prebiotic. And according to Bielecka et al., (2002) it is not known whether the individual contributions are additive or synergistic, but there may be an increase in the beneficial effects of each one of them. The interaction between probiotic and prebiotic in vivo can be favored by an adaptation of the probiotic through prebiotic consumption. This should result in a competitive advantage for the probiotic if it is consumed with the prebiotic (PUUPPONEN-PIMIÄ et al., 2002).
4 CONCLUSION

Petit Suisse cheese added with Brazil nut extract and probiotic bacteria presented in all formulations results of microbiological analyzes within the standards required by current legislation for cheeses with high humidity, demonstrating good manufacturing practices during processing and adequate storage conditions under refrigeration during the 30 days. The analyses of probiotic bacteria showed counts higher than $10^8\text{ CFU mL}^{-1}$ in all formulations showing that Petit Suisse cheese had a symbiotic effect, and there may have had an interaction between the probiotic bacteria and the prebiotic (Brazil nut extract). That one may have favoring the adaptation of the probiotic to the prebiotic, contributing to the growth of probiotic bacteria, resulting in an increase in the beneficial effects of each one of them, inferring that Petit Suisse cheese is able to provide basic nutrition, promote health, and can be considered a functional food.

ACKNOWLEDGMENTS

We appreciate the financial support of Frimesa® industries; Sacco®; Danisco® - Fermentec®.

We thank the Federal Technological University of Paraná - Campus Medianeira (UTFPR).
REFERENCES


BRASIL. Instrução Normativa nº 161, de 1º de julho de 2022. Regulamento técnico sobre os padrões microbiológicos para alimentos. Diário Oficial da União, Brasília, DF, publicada no DOU nº 126, de 6 de julho de 2022.


