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Petit-Suisse Cheese Production with Addition of Probiotic *Lactobacillus casei*

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Abstract

Growing concern to increase life expectancy has promoted several studies in the field of nutrition, especially those on food and their effects on the human body. Assays have been performed to improve their nutritional quality. In fact, there is considerable interest in encouraging development of new ingredients, with the innovation in food products and the establishment of new market niches for these ingredients. The global market for functional foods is growing and always envisages new products with functional technological and physiological characteristics. The petit-suisse cheese is a product of high nutritional value, rich in calcium, phosphorus and fat-soluble vitamins, with a high percentage of milk proteins, coupled to excellent digestion and assimilation by the human body. Current assay describes the production of a petit-suisse cheese to which was added the probiotic *Lactobacillus casei* BGP 93, and determines the physical, chemical and microbiological viability of lactobacilli in cheese during storage period. Levels of protein, fat in the dry extract, moisture and pH were analyzed for physical and chemical parameters; likewise, detection of coliforms, *Staphylococcus*, *Salmonella*, *Listeria monocytogenes*, and yeasts and molds was performed for microbiological parameters; monitoring of the viability of *Lactobacillus casei* BGP 93 during its life time was conducted. The petit-suisse *cheese* proved to be a good vehicle for the addition of probiotic microorganism. *Lactobacillus casei* remained viable during shelf life even with decreasing pH, and may be considered a functional product during 30 days of storage.

Keywords

Cheese, Probiotic, *Lactobacillus casei*

1. Introduction

Growing concern in increasing the population's life expectancy has produced several studies on nutrition, espe-

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cially with regard to food and their effect on the human organism to improve nutrition and life quality. Food is the main factor for the prevention of disease and improvement of health. It avoids and controls several types of Non-Transmissible Chronic Diseases (NTCDs), such as diabetes, hypertension, neoplasias and heart conditions. Several studies have been undertaken to prove the beneficent activities of certain food when risks caused by the above-mentioned diseases are decreased and by diarrheas due to imbalanced microbiota, intestine inflammatory diseases, atopic eczemas and others. There is also a great interest in developing new ingredients with innovatory food products and the establishment of new market niches for these ingredients [1].

Functional food market is growing worldwide and new products with functional technological and physiological characteristics are constantly sought for. The dairy industry is actually in the limelight for the availability of its products, especially yoghurts and other types of fermented milk segments, in which functionality is performed by using probiotic cultures and/or the addition of prebiotic ingredients [2].

Petit-suisse cheese is a high nutrition product with a high percentage of lactic proteins (6% at the lowest) [3], rich in calcium, phosphorus and lipid-soluble vitamins, featuring excellent digestion and assimilation qualities by the human organism [4] [5].

Pasteurization, coagulation, fermentation stages are some of the parameters that affect the variety and quality of petit-suisse cheese [6]. Pre-heated milk prior to its acidification at a temperature and time higher than those for pasteurization provides higher cheese yield due to water from denatured proteins and thus greater water retention. In fact, a greater interaction between whey proteins and casein occurs [4] [6].

Current assay analyzes petit-suisse cheese with probiotic *Lactobacillus casei* BGP 93 and determines its physical, chemical, microbiological and viability of the lactobacillus during the storage period.

2. Materials and Methods

2.1. Production of the Petit-Suisse Cheese

The following ingredients were used in the production of the petit-suisse cheese:

a) Skimmed milk powder
b) Commercial coagulant—HA-LA (Christian Hansen®, Valinhos, Brazil)
c) Probiotic culture—*Lactobacillus casei* BGP-93 (Clerict-Sacco®)
d) Saccharose—refined sugar
e) Calcium chloride
f) Pasteurized milk cream and UHT milk
g) Full strawberry pulp (Borsato®)

2.2. Manufacture of Petit-Suisse Cheese with Probiotic Micro-Organisms

Three batches of petit-suisse cheese were manufactured, following methodology by Albuquerque [7]. The cheese was produced at the Laboratory for the Inspection and Technology of Milk and its Derivatives of the School of Veterinary Medicine and Animal Science of the Universidade Federal da Bahia. Milk was reconstituted, pasteurized at 65˚C for 30 minute and cooled at 35˚C. Calcium chloride at the proportion of 25 mL for every 100 L of reconstituted milk powder; curds at 10%, following manufacturer's instruction; lactic yeast; probiotic micro-organism *Lactobacillus casei* BGP 93 (Sacco®) were added. The mixture was placed in a sanitized plastic pail and incubated for 12 hours. Draining was undertaken after fermentation to obtain mass. Two rinsings were performed with 1 L of boiled water and cooled each time, till pH was close to 4.5. Milk cream to reduce fats at 9%, sugar at 16% and strawberry fruit pulp (Borsato®) at 5% were added. Cheese were disposed in polyethylene pots with lids and stored at a temperature not higher than 10˚C.

2.3. Analysis of the Manufactured Product

Samples of cheese stored at refrigeration temperature (up to 10˚C) were retrieved for the analyses of protein, humidity and pH rates, following Norm 68 [8]; total coliforms, coagulase-positive *Staphylococcus*, *Salmonella spp*. *Listeria monocytogenes*, filament fungi, counts of probiotic bacteria and yeasts, following Norm 62 [9]. Physical and chemical analysis of total coliforms, *Listeria monocytogenes*, filament fungi and yeasts and the monitoring of lactic bacteria during storage were made at the Laboratory for the Inspection and Technology of Milk and its Derivatives of the School of Veterinary Medicine and Animal Science of the Universidade Federal da Bahia (UFBA) Salvador BA Brazil. Analyses for coagulase-positive *Staphylococcus* positive and *Salmonella spp* were performed at the Laboratory of Applied Microbiology and Public health (LAMASP) of the Universidade Estadual de Feira de Santana (UEFS), Feira de Santana BA Brazil. All analyses were made in triplicate and results given in averages. The viability of probiotic bacteria in the product was analyzed as from the third day of production and repeated at every ten days up to a month of the product's production, corresponding to its commercial expiry date.

2.4. Analysis of the Results

A descriptive analysis of results was undertaken. Averages of batches were compared by analysis of variance (ANOVA); means of microbiological data were compared by Tukey's test at 5% probability; co-relationship between acidity and the counting of lactic bacteria was done by Pearson's coefficient of co-relation [10].

3. Results and Discussion

3.1. Physical and Chemical Analysis

Table 1 and **Figures 1-3** show mean rates of protein, fat in dry extract, humidity and acidity petit-suisse cheese plus probiotic. Protein and humidity rates meet the legislation demands. Instruction 46 of the Ministry of Agriculture and Livestock Supplies (MAPA) classifies petit suisse cheese as a high-humidity product, which should not be lower than 55.0% [11]. Legislation also rules on protein rates which should be at least 6.0% [3]. Although no standard ruling is given with regard to acidity, it is a highly relevant factor since it warrants the product's shelf life. Fat rates show that petit-suisse cheese is a non-fat cheese, within the 10.0 and 24.0 bracket of fatty matter stipulated by technical ruling [11].

Maruyama *et al*. [12] researched the instrumental textures of the potentially probiotic petit-suisse cheese and the influence of different combinations of gums up to 21 days storage at 4˚C. They reported higher rates than those of current research.

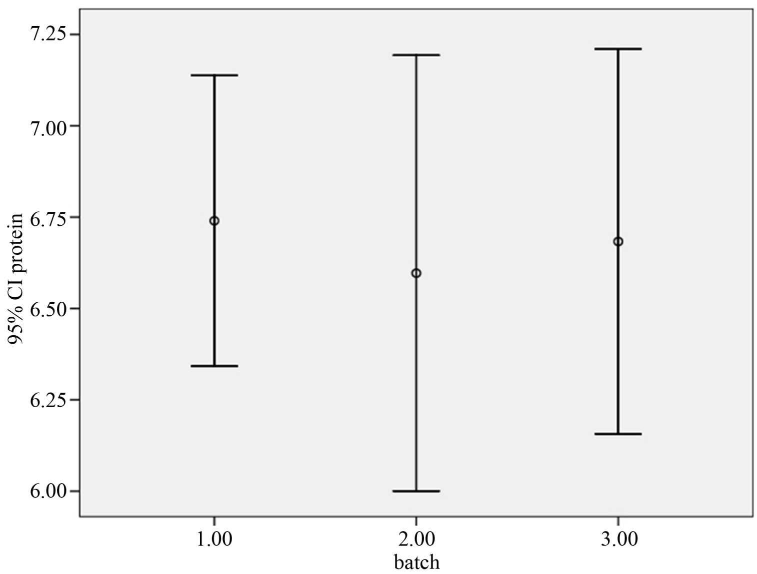

Figure 1. Mean rates of protein percentage in petit-suisse cheese plus probiotics.

Table 1. Mean results of physical and chemical analyses of petit-suisse cheese with probiotic micro-organisms.

Sample	Protein (%)	Fat in dry extract (%)	Humidity (%)	Acidity (pH)
Batch 1	6.74 +/− 0.16	19.36 +/− 0.30	61.44 +/− 1.14	4.17 +/− 0.20
Batch 2	6.59 +/− 0.24	19.03 +/− 0.40	63.62 +/− 0.65	4.21 +/− 0.19
Batch 3	6.68 +/− 0.21	19.50 +/− 0.29	63.80 +/− 0.77	4.20 +/− 0.23

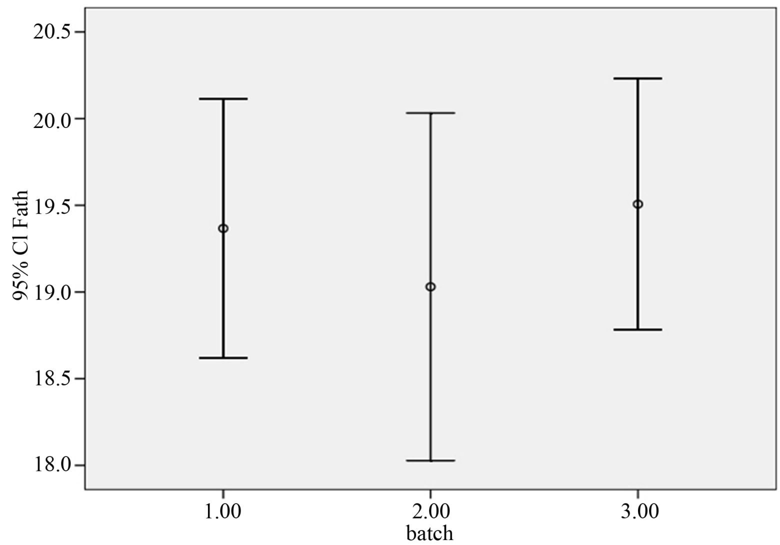

Figure 2. Mean rates of fat percentage in petit-suisse cheese plus.

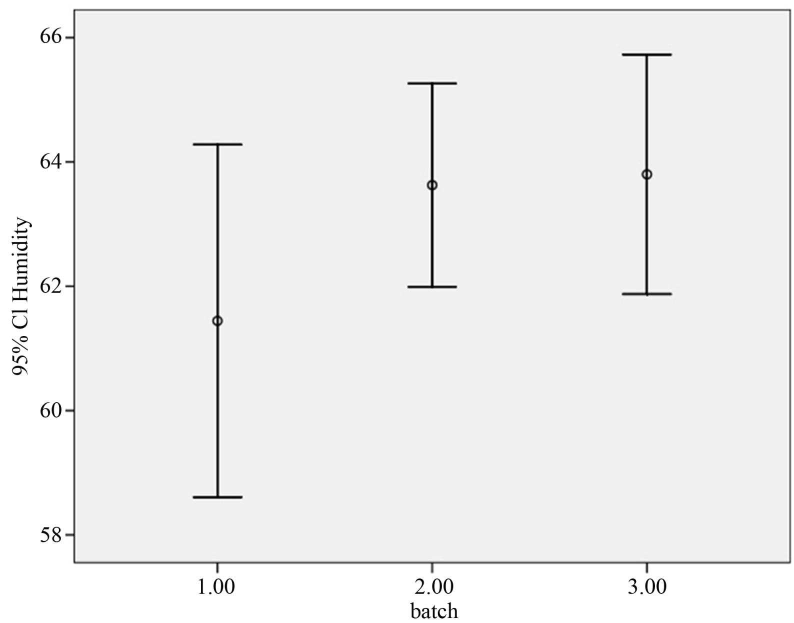

Figure 3. Mean rates of humidity percentage in petit suisse cheese plus probiotics.

Souza *et al*. [13] manufactured low calorie petit-suisse cheese with strawberry flavor from concentrations of saccharose and different sweeteners and reported humidity rates between 69.77% and 85.33%, whereas fat rates in all formulations reached 0.1%.

Alves [14] researched the behavior of *Escherichia coli* in Fresh Minas cheese with *Lactobacillus acidophilus* and direct acidification with lactic acid with several treatments and reported humidity percentages ranging between 60.74 and 64.61.

Veiga *et al*. [15] analyzed six petit-suisse cheese brands in Campinas SP Brazil and reported pH between 4.42 and 4.51; fatty matter percentage between 4.47 and 6.22; protein between 6.59 and 8.88%.

Veiga & Viotto [16] studied the effect of thermal treatment of milk in the manufacture of petit-suisse cheese by ultrafiltration of coagulated milk and the effect of thermal treatment of milk in the membrane's performance. They registered pH 4.4 and fatty matter 5.8%, with protein as the varying component, or rather, 8.95% with thermal treatment at 85˚C and 9.02% at 72˚C.

In Maringá PR Brazil, Boatto *et al*. [17] investigated the physical and chemical composition of soya petit-suisse cheese enriched with calcium. They used common soybeans and lipoxygenase-free soy. The following results were registered: pH 4.3 - 4.42; total acidity 6.26% - 6.02%; humidity 67.53% - 69.43%; crude protein 5.43% - 4.70%; fatty rates 4.27% and 2.92%, respectively.

In five assays with fresh creamy cheese plus *Lactobacillus paracasei* spp. *paracasei*, Buriti, Cardarelli and Saad [18] reported fat percentage in dry extract between 21.3% and 28.7% higher than those in current assay, after the first day of storage at 4˚C.

Ong, Henriksson and Shah [19] developed a probiotic cheese with *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus paracasei* and *Bifidobacterium* spp. and evaluated the effect of these bacteria on proteolytic activity and the production of organic acid. The following results were obtained for fatty matter: 31.4% - 31.03% - 31.89%; for protein rates: 27.46% - 27.70% - 28.07%; for pH: 5.40 - 5.14 - 5.12; for humidity: 39.00% - 39.27% - 39.33%, in three batches with different compositions. The authors report that the cheese's chemical composition did not change, although results demonstrate an increase in free amino-acids in secondary proteolytic activity.

Prudencio *et al*. [20] manufactured petit-suisse cheese with whey retentates and the application of betalain and anthocyanin extracts to verify its stability with regard to pH and temperature. Average humidity results average humidity results ranged between 75.53% and 76.22%; protein between 6.22 and 6.71; fats between 4.60% and 4.30%; acidity (lactic acid) between 10.41% and 10.79%; pH between 4.55 and 4.57. The same authors warrant that extracts are stable as stains and may be used in cheese since they do not modify the product's composition. Only a single sample had a decrease in protein contents.

Ong and Shah [21] manufactured a probiotic cheddar cheese and evaluated the influence of cure temperatures in the survival of probiotic micro-organisms, composition of the cheese and profile of organic acids after 24 weeks. They reported decrease in humidity rates and increase in protein and fat rates. In the case of organic acids, an increase occurred during curing process in all acids, especially in lactic acid.

Ong, Henriksson and Shah [22] researched the effect of the probiotic strains *Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Lactobacillus casei* or *Bifidobacterium* ssp. on the proteolytic activity and on the profiles of organic acids in probiotic cheddar cheese. Assay showed that the addition of probiotics failed to have any influence on fat, protein and pH rates, although the cheese's humidity rates increased.

3.2. Micro-Biological Analysis

Table 2 presents results on the microbiological quality of probiotic petit-suisse cheese. All microbiological parameters required by Resolution 146 of the 7th March 1996 were complied with.

Results are mostly due to the application of good manufacturing practices during the entire process where the hygiene of utensils, handling, prime matter and pH of the medium were under control. An acid medium impairs the development of undesirable micro-organisms. Filamentous fungi and yeasts derive from the environment but rates are so low that concern is unnecessary [11]. Buriti [23] researched three probiotic cheese formulations and reported large fungi counts on the 21st day of storage, whereas coliforms and *Staphylococcus* were present at all stages, from its elaboration to storage of the product.

Brazilian legislation determines that very high humidity cheese (>55%) with viable and abundant lactic bacteria, as in the case of petit-suisse cheese in current analysis, may have a maximum of 10^3 UFC/g total coliforms; 10^2 UFC/g thermotolerant coliforms, 10^2 UFC/g coagulase-positive *Staphylococcus*; 5×10^3 moulds and yeasts; and absence of *Salmonella* sp. and *Listeria monocytogenes* in 25 grams [11].

Table 2. Results on the microbiological quality of petit suisse cheese with probiotic micro-organisms.

Sample	Total coliforms (MPN)	*Coagulase-positive Staphylococcus*	*Salmonella* ssp.	*Listeria monocytogenes*	Filamentous fungi and yeasts log·UFC·g^{-1}
Batch 1	<0.3	<10	Absent	Absent	0.85 +/− 0.217
Batch 2	<0.3	<10	Absent	Absent	1.08 +/− 0.150
Batch 3	<0.3	<10	Absent	Absent	0.96 +/− 0.057

Dabiza and El Deib [24] evaluated soft cheese with probiotic micro-organisms and reported that total coliforms and *Staphylococcus* ssp. disappeared after 30 days maturation, whereas fungi disappeared after 15 days. However, Osman and Abbas [25] registered yeasts in probiotic cheese and coliform bacteria and fungi were not detected in probiotic cheese.

3.3. Monitoring the Viability of *Lactobacillus casei* BGP 93

Table 3 shows the viable population of lactobacilli added to petit-suisse cheese during storage under refrigeration.

Results in **Table 3** and illustrated in **Figure 4** show no significant difference ($p > 0.05$) in counts of *Lactobacillus casei* BGP 93 added to petit-suisse cheese during the entire storage period of 30 days, while actually **Figure 4** reflected the relationship of acidity and survival curve of *Lactobacillus casei* BGP 93. No significant difference ($p > 0.05$) among the batches was observed.

Table 3 and **Figure 4** show that after 30 days storage at 4˚C the *Lactobacillus casei* BGP 93 population in the three batches met the parameters recommended by Brazilian legislation although several researchers reported that a minimum concentration of 1×10^6 UFC·g^{-1} of the product is sufficient for probiotic effects [26]-[28]. Results in current study indicate that petit-suisse cheese is a good vehicle for *Lactobacillus casei* BGP 93. They continue viable in the cheese even with acidity decrease.

Buriti, Rocha and Saad [29] researched the survival of *L. acidophilus* in Minas Frescal cheese with the addition of mesophile bacteria and directly acidified with lactic acid (*Lactococcus lactis* subsp. *lactis* and *Lactococ-*

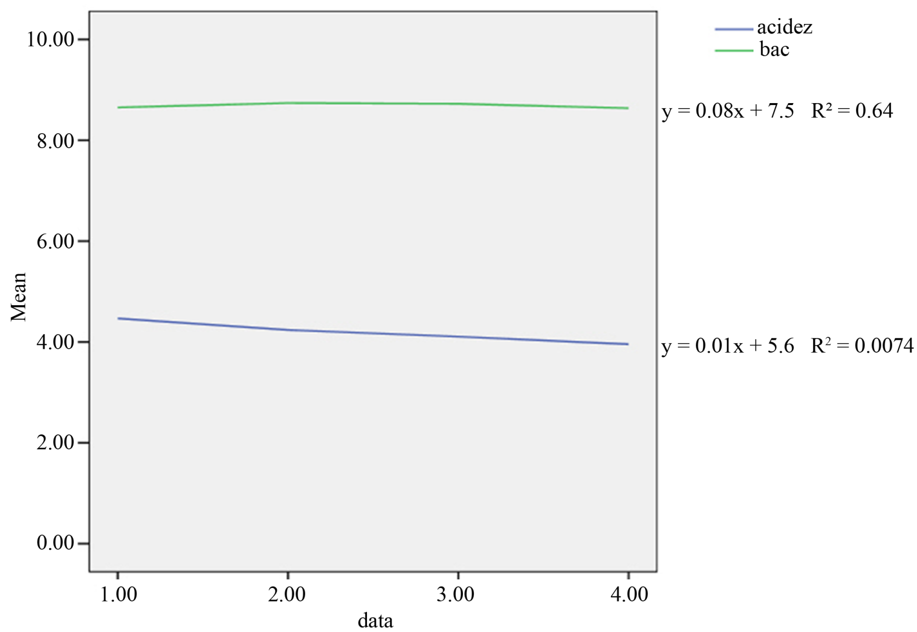

Figure 4. Relationship of acidity and survival curve of *Lactobacillus casei* BGP 93.

Table 3. Count results of viable lactobacilli population in petit-suisse cheese plus probiotic micro-organisms.

Sample	*Counts of *Lactobacillus casei* BGP 93 log·UFC·g^{-1} Storage days			
	3 days	13 days	23 days	33 days
Batch 1	8.66 +/− 0.05	8.75 +/− 0.03	8.76 +/− 0.04	8.65 +/− 0.03
Batch 1	8.63 +/− 0.01	8.72 +/− 0.02	8.69 +/− 0.01	8.61 +/− 0.05
Batch 3	0.66 +/− 0.02	8.74 +/− 0.03	8.69 +/− 0.03	8.61 +/− 0.04

cus lactis subsp. c*remoris*). Although probiotic culture in both cheese remained viable during 21 days of storage, counts of *Lactobacillus acidophilus* were slightly higher in cheese with mesophile culture, suggesting a synergism among the micro-organisms.

Ribeiro, Simões and Jurkiewicz [30] developed a Minas Frescal cheese with *Lactobacillus acidophilus*, produced from ultrafiltration retentates and inoculated 10^6, 10^7 and 10^8 UFC·g^{-1} *L. acidophilus*. Counting of probiotic bacteria was made at 7-day intervals during a 28-day storage and decrease in *L. acidophilus* population was not significant for the three evaluated cheeses.

Vinderola *et al*. [31] produced fresh cheese from ultrafiltration retentates with several *Streptococcus. thermophilus* and *Lactococcus lactis* combinations, with probiotic cultures of *Bifidobacterium*, *Lactobacillus acidophilus* and *Lactobacillus casei*. The authors reported a decrease in *Lactobacillus acidophilus* and *Bifidobacterium* populations, less than one logarithmic cycle. However, the population of *Lactobacillus casei* remained constant during 60 days storage under refrigeration.

Influence of probiotic cultures of *Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Lactobacillus casei* or *Bifidobacterium* sp. on proteolytic activity and on the profile of organic acid in probiotic cheddar cheese after six days maturation at 4˚C was evaluated and results show that all micro-organisms remained at the high level of >8.0 $\log_{10}$ UFC·g^{-1}. However, the concentration of acetic acid was higher in cheese with *Bifidobacterium longum* 1941, *Bifidobacterium lactis* LA FTI® B94, *Lactobacillus casei* 279 and *Lactobacillus paracasei* LAFTI® L26. No significant difference was detected at acetic acid level and in control when *Lactobacillus acidophilus* was added [32].

In the case of survival rate (**Figure 4**), it should be underscored that population remained stable from the start of production till the product's expiry date, with a viable population at 8.66 log·UFC/g (4.0×10^8 UFC/g).

The most recent recommendation for probiotic food [33] is based on the daily portion of viable micro-organisms that should be taken for functional effects, with a minimum between 10^8 and 10^9 UFC/day. It is thus suggested that 60 g of the product under analysis should be consumed so that a daily portion of 10^9 UFC of probiotic micro-organism could be taken. The quantity is sufficient to protect the organism from diseases caused by other micro-organisms. However, excessive consumption of probiotic micro-organisms may cause intestine disorders. Excess of calories may jeopardize efforts for weight decrease.

Table 4 shows mean pH rates of petit-suisse cheese plus probiotic micro-organisms during storage period.

Table 4 demonstrates no significant variation ($p > 0.05$) in pH in petit-suisse cheese with probiotico during storage. The same response for the viability of lactobacilli cells incorporated to the product is available.

Probiotic availability in a food matrix depends on several factors such as the type of culture added to the product, the interaction with other micro-organisms in the food, the production of hydrogen peroxide during bacterial metabolism and the product's final acidity [34].

The substrate's pH remained at 4.2 level due to the production of lactic acid from the proliferation of lactobacilli in the product. Acid production failed to reach higher levels probably because of the buffer capacity of nutrients in the milk (phosphates, citrates and peptones). Although acidity is a limiting factor for the development of many micro-organisms, the *Lactobacillus casei* BGP 93 was not affected by this factor due to it being an acid-tolerant strain and resisted to pH 3.0, as indicated by the manufacturer in the culture's technical specifications.

4. Conclusions

Petit-suisse cheese is a good vehicle for the addition of probiotic micro-organisms, especially *Lactobacillus casei*.

Table 4. Mean pH rates of probiotic petit suisse cheese during storage.

Sample	3 days	13 days	23 days	33 days
Batch 1	4.46 +/− 0.11	4.13 +/− 0.06	4.13 +/− 0.06	3.96 +/− 0.06
Batch 2	4.46 +/− 0.05	4.26 +/− 0.06	4.16 +/− 0.06	3.96 +/− 0.06
Batch 3	4.46 +/− 0.15	4.26 +/− 0.15	4.16 +/− 0.15	3.93 +/− 0.11

Since *Lactobacillos casei* remained viable during shelf life, even when there was a decrease in pH, the cheese may be a functional product during 30 days of storage.

References

[1] Raizel, R., Santini, E., Koppert, A.M. and Filho, A.D.R. (2011) Efeitos do consumo de probióticos, prebióticos e simbióticos para o organismo humano. *Revista Ciência & Saúde*, **4**, 66-74.

[2] Souza, J.C.B., Guergoletto, K.B., Garcia, S. and Sivieri, K. (2011) Viabilidade da adição de *Lactobacillus casei* (LC-1) protegido com trealose e goma acácia em sorvetes. *Alimentos e Nutrição*, **22**, 231-237.

[3] Brasil (2000) Ministry of Agriculture, Livestock and Supply. Instruction, Paragraph .53 of December 29, 2000. Brasilia.

[4] Gambelli, L., Manzi P., Panfili, V. and Pizzoferrato, L. (1999) Constituents of Nutritional Relevance in Fermentaded Milk Products Commercialised in Italy. *Food Chemistry*, **66**, 353-358. http://dx.doi.org/10.1016/S0308-8146(99)00075-8

[5] Van Dender, A.G.F. and Panfili, V. (1985) Adaptação de tecnologia de fabricação e termização do queijo quark. *Revista do Instituto de Laticínios Cândido Tostes*, **40**, 33-53.

[6] Kelly, A.L. and O'Donnell, H.J. (1998) Composition, Gel Properties and Microstruture of Quarg as Affected by Processing Parameters and Milk Quality. *International Dairy Journal*, **8**, 295-301. http://dx.doi.org/10.1016/S0958-6946(98)00084-3

[7] Albuquerque, L.C. (2002) *Queijos no mundo*: *Origem e Tecnologia*. Juiz de Fora: CT/ILCT/EPAMIG.

[8] Brasil (2006) Ministry of Agriculture, Livestock and Supply. Instruction, n.68 of December 12, 2006. Brasilia.

[9] Brasil (2003) Ministry of Agriculture, Livestock and Supply. Instruction n.62 of August 26, 2003. Brasilia.

[10] Banzatto, D.V. and Kronka, S. do N. (1992) Experimentação agrícola. Jaboticabal: FCAV/UNESP.

[11] Brazil (1996) Ministry of Agriculture, Livestock and Supply. Ordinance No. 146 of 07 Sea 1996. Official Gazette of the Federative Republic of Brazil, Brasilia.

[12] Maruyama, L.Y., Cardarelli, H.R., Buriti, F.C.A. and Saad, S.M.I. (2006) Textura instrumental de queijo *petit-suisse* potencialmente probiótico: Influencia de diferentes combinações de gomas. *Ciência e Tecnologia de Alimentos*, **26**, 386-393. http://dx.doi.org/10.1590/S0101-20612006000200022

[13] Souza, V.R., Carneiro, J.D.S., Pinheiro, A.C.M., Pinto, S.M., Carvalho, L.P. and Menezes, C.C. (2010) Elaboração de queijo *petit-suisse* sabor morango de baixo valor calórico. *Revista do Instituto de Laticínios Cândido Tostes*, **65**, 49-58.

[14] Alves, C.C.C. (2010) Comportamento da *Escherichia coli* em queijo minas frescal elaborado com utilização de *Lactobacillus acidophilus* e de acidificação direta com ácido lático. Master's Dissertation, Universidade Federal Fluminense, Rio de Janeiro.

[15] Veiga, P.G., Cunha, R.L., Viotto, W.H. and Petenate, A.J. (2000) Caracterização química, reológica e aceitação sensorial do queijo *petit-suisse* brasileiro. *Ciência e Tecnologia de Alimentos*, **20**. http://dx.doi.org/10.1590/S0101-20612000000300012

[16] Veiga, P.G. and Viotto, W.H. (2001) Fabricação de queijo *petit-suisse* por ultrafiltração do leite coagulado. Efeito do tratamento térmico do leite no desempenho da membrana. *Ciência e Tecnologia de Alimentos*, **21**, 267-272. http://dx.doi.org/10.1590/S0101-20612001000300003

[17] Boatto, D.A., Mesomo, M.C., Madrona, G.S., Branco, I.G. and Matumoto-Pintro, P.T. (2010) Desenvolvimento e caracterização de queijo tipo *petit-suisse* de soja comum e de soja livre de lipoxigenase, enriquecidos com cálcio. *Ciência e Tecnologia de Alimentos*, **30**, 766-770. http://dx.doi.org/10.1590/S0101-20612010000300031

[18] Buriti, F.C.A., Cardarelli, H.R. and Saad, S.N.I. (2008) Textura instrumental e avaliação sensorial de queijo fresco cremoso simbiótico: Implicações da adição de *Lactobacillus paracasei* e inulina. *Revista Brasileira de Ciências Farmacêuticas*, **44**, 75-84. http://dx.doi.org/10.1590/S1516-93322008000100009

[19] Ong, L., Henriksson, A. and Shah, N.P. (2007) Chemical Analysis and Sensory Evaluation of Cheddar Cheese Produced with *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus paracasei* or *Bifidobacterium* sp. *International Dairy Journal*, **17**, 937-945. http://dx.doi.org/10.1016/j.idairyj.2007.01.002

[20] Prudencio, I.D., Prudêncio, E.S., Gris, E.F., Tomazi, T., Marilde, T. and Bordignon-Luiz, T. (2008) *Petit-suisse* Manufactured with Cheese Whey Retentate and Application of Betalains and Anthocyanins. *LWT—Food Science and Technology*, **41**, 905-910.

[21] Ong, L. and Shah, N.P. (2009) Probiotic Cheddar Cheese: Influence of Ripening Temperatures on Survival of Probiotic Microorganisms, Cheese Composition and Organic Acid Profiles. *LWT—Food Science and Technology*, **42**, 1260-1268.

[22] Ong, L., Henriksson, A. and Shah, N.P. (2007) Proteolytic Pattern and Organic Acid Profiles of Probiotic Cheddar

Cheese as Influenced by Probiotic Strains of *Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Lactobacillus casei or Bifidobacterium* sp. *International Dairy Journal*, **17**, 67-78. http://dx.doi.org/10.1016/j.idairyj.2005.12.009

[23] Buriti, F.C.A. (2005) Desenvolvimento de queijo fresco cremoso simbiótico. Master's Dissertation, Universidade de São Paulo, Faculdade de Ciência Farmacêutica, São Paulo.

[24] Dabiza, N. and El-Deb, K. (2007) Biochemical Evaluation and Microbial Quality of Ras Cheese Supplemented with Probiotic Strains. *Polish Journal of Food and Nutrition Sciences*, **5**, 295-300.

[25] Osman, M.M. and Abbas, F.M. (2001) Fate of *Lactobacillus acidophilus* La-5 and *Bifidobacterium latis* Bb-12 in Probiotic Ras Cheese. *Proceeding of the* 8*th Egyptian Conference for Dairy Science & Technology*, **3**, 653-604.

[26] Ravila, R.R. and Shah, N.P. (1998) Selective Enumeration of *Lactobacillus casei* from Yoghurts and Fermented Milk. *Biotechnology Techniques*, **12**, 819-822. http://dx.doi.org/10.1023/A:1008829004888

[27] Shah, N.P. (2000) Probiotic Bacteria: Selective Enumeration and Survival in Dairy Foods. *Journal of Dairy Science*, **83**, 894-907. http://dx.doi.org/10.3168/jds.S0022-0302(00)74953-8

[28] Shah, N.P. (2001) Functional Foods, Probiotics and Prebiotics. *Food Technology*, **55**, 46-53.

[29] Buriti, F.C.A., Rocha, J.S. and Saad, S.M.I. (2005) Incorporation of Lactobacillus Acidophilus in Minas Fresh Cheese and Its Implications for Textural and Sensorial Properties during Storage. *International Dairy Journal*, **12**, 1279-1288. http://dx.doi.org/10.1016/j.idairyj.2004.12.011

[30] Ribeiro, E.P., Simões, L.G. and Jurkiewicz, C.H. (2009) Desenvolvimento de queijo minas frescal adicionado de *Lactobacillus acidophilus* produzido a partir de retentados de ultrafiltração. *Ciência e Tecnologia de Alimentos*, **29**, 19-23. http://dx.doi.org/10.1590/S0101-20612009000100004

[31] Vinderola, C.G., Prosello, W., Ghiberto, D. and Reinheimer, J.A. (2000) Viability of Probiotic (*Bifidobacterium*, *Lactobacillus acidophillus*, *Lactobacillus casei*) and Nonprobiotic Microflora in Argentinian Fresco Cheese. *Journal of Dairy Science*, **83**, 1905-1911. http://dx.doi.org/10.3168/jds.S0022-0302(00)75065-X

[32] Ong, L., Henriksson, A. and Shah, N.P. (2006) Development of Probiotic Cheddar Cheese Containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus paracasei* and *Bifidobacterium* spp. and the Influence of These Bacteria on Proteolytic Patterns and Production of Organic Acid. *International Dairy Journal*, **16**, 446-456. http://dx.doi.org/10.1016/j.idairyj.2005.05.008

[33] Brazil (2002) Ministry of Health National Health Surveillance Agency (ANVISA). RDC Resolution No. 2 of January 7, 2002.

[34] Vasiljevic, T. and Saha, N.P. (2008) Probióticss—From Metchnikoff to Bioactives. *International Dairy Journal*, **18**, 714-728. http://dx.doi.org/10.1016/j.idairyj.2008.03.004