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Probiotic viability of requeijão cremoso processed cheese formulations

Viabilidade probiótica de formulações de requeijão cremoso

Viabilidad probiótica de formulaciones de requeijão cremoso queso procesado

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Abstract
The increased demand for foods that provide nutritional and health benefits increased research in probiotic microorganisms applied to dairy products. Probiotics are affected by several factors that decrease their action on the gastrointestinal system, making it vital to protect them using encapsulation techniques that allow their incorporation into various food products. The study herein aimed to evaluate the best way to incorporate *Lactobacillus acidophilus* (free and encapsulated) to Requeijão cremoso processed cheese varieties. The microorganism was applied to different curd formulations using green banana biomass as a partial fat substitute. The pH, probiotic viability during shelf life and throughout the simulated gastrointestinal conditions, and capsules produced were assessed. While applying free *Lactobacillus acidophilus* to Requeijão cremoso varieties proved functionally ineffective, the encapsulated formulations presented satisfactory probiotic counts in all treatments (8–10 log CFU g^{-1}).
Keywords: *Lactobacillus acidophilus*; Encapsulation; Dairy derivative; Probiotic viability; Health teaching.

Resumo
O aumento da procura pelos consumidores por alimentos que tragam benefícios tanto nutricionais quanto à promoção da saúde, impulsionou as pesquisas com microrganismos probióticos aplicados a produtos lácteos. Os probióticos são afetados por diversos fatores diminuindo sua ação no sistema gastrointestinal, portanto, é necessário protegê-los utilizando técnicas de encapsulamento para incorporar em vários produtos alimentares. O presente estudo teve como objetivo avaliar a melhor forma de incorporação de *Lactobacillus acidophilus* (livre e encapsulado) às variedades de requeijão cremoso. O microrganismo foi aplicado em diferentes formulações de requeijão utilizando biomassa de banana verde como substituto parcial de gordura. Foram avaliados pH, viabilidade probiótica durante a vida de prateleira e nas condições gastrointestinais simuladas das formulações e caracterização da cápsula probiótica produzida. A aplicação de *Lactobacillus acidophillus* livres em requeijão cremoso apresentou resultado insatisfatório para ser considerado funcional, já as formulações que foram adicionadas de microrganismo na forma encapsulada, apresentaram contagem probiótica satisfatória em todos os tratamentos (8 a 10 log UFC.g^{-1}).
Palavras-chave: *Lactobacillus acidophilus*; Encapsulação; Derivados lácteos; Viabilidade probiótica; Ensino em saúde.

Resumen
El aumento de la demanda de alimentos que aporten beneficios nutricionales y para la salud incrementó la investigación en microorganismos probióticos aplicados a los productos lácteos. Los probióticos se ven afectados por varios factores que disminuyen su acción sobre el sistema gastrointestinal, por lo que es de vital importancia protegerlos mediante técnicas de encapsulación que permitan su incorporación en diversos productos alimenticios. El

presente estudio tuvo como objetivo evaluar la mejor manera de incorporar *Lactobacillus acidophilus* (libre y encapsulado) a las variedades de queso procesado Requeijão cremoso. El microorganismo se aplicó a diferentes formulaciones de cuajada usando biomasa de banano verde como sustituto parcial de la grasa. Se evaluaron el pH, la viabilidad del probiótico durante la vida útil y durante las condiciones gastrointestinales simuladas y las cápsulas producidas. Mientras que la aplicación de *Lactobacillus acidophilus* libre a las variedades de Requeijão cremoso resultó funcionalmente ineficaz, las formulaciones encapsuladas presentaron conteos de probióticos satisfactorios en todos los tratamientos (8–10 log UFC g^{-1}).
Palabras clave: *Lactobacillus acidophilus*; Encapsulación; Derivado lácteo; Viabilidad probiótica; Enseñanza de la salud.

1. Introduction

Functional foods are "natural or processed foods containing known biologically active compounds which, when dosed in quantitatively and qualitatively defined quantities, provide clinically proven and documented health benefits and are an important source of prevention, management, and treatment of chronic diseases of the modern age" (Di Bartolomeo, et al., 2013). Currently, the most studied functional components are antioxidants, unsaturated fatty acids, prebiotics, and probiotics (Yasmin, et al., 2015).

The World Health Organization (WHO) defines probiotics as "living microorganisms that, when consumed in adequate amounts, produce healthy benefits to the host" (FAO/WHO, 2006). The main probiotic species belong to the genus *Lactobacillus* and *Bifidobacterium*, which are part of the human intestinal microbiota and beneficially affect human health by improving the properties of the native microbiota (Cook, et al., 2012).

The numerous advantages of probiotics include relief of symptoms caused by lactose intolerance, diarrhea treatment, reduced serum cholesterol, immune response modulation, and infectious disease prevention (Kale-Pradham, et al., 2010).

However, incorporating probiotic cells into food matrices poses the challenge of preserving their viability during processing, storage, and passage through the gastrointestinal tract, where food is subjected to variations in temperature, pH, oxygen uptake, contact with bile salts, and antibacterial agents, among other conditions (López-Rubio, et al., 2009). The microencapsulation technique improves probiotic microorganism stability in products and increases their survival throughout the gastrointestinal tract (Pinto, et al., 2015). Microencapsulation by extrusion is the most popular one, since it is cheap, simple, and does not require high temperatures (Fávaro-Trindade, et al., 2011).

Another way to bolster product functionality is by adding prebiotics to the formulations, since they can stimulate the beneficial bacteria present in the intestine, which also results in low-calorie foods that may have their fat and sugar contents partially or totally replaced (Oliveira, et al., 2009). Numerous ingredients may suit this purpose, including green banana biomass, a component that can be applied to a wide variety of industrialized foods because it does not interfere with the sensory attributes of other ingredients. Additionally, due to having resistant starch, it presents prebiotic functional properties (Oi, et al., 2013).

Currently, most probiotic products available are dairy products, which are rich in lipids and proteins, protect the microorganisms and aid the probiotic to withstand the adverse conditions of the gastrointestinal tract (Kumar, et al., 2015). Requeijão cremoso falls into the refrigerated cheese category, which is renowned for probiotic incorporation (Buriti, et al., 2008). Nonetheless, the literature shows little research on applying probiotics, prebiotics, and fat substitutes to Requeijão cremoso.

Given the sweeping changes of dietary habits of the population and the important overview of functional products, this study aimed to develop Requeijão cremoso processed cheese with *Lactobacillus acidophilus* (La-14) using green banana biomass as a partial fat substitute and evaluate the pH, probiotic viability during shelf life and throughout the simulated gastrointestinal conditions, and produced-capsule characteristics.

2. Methodology

This study consisted of two steps. The first, a preliminary test, consisted of evaluating the probiotic viability during the shelf life of Requeijão cremoso coupled with encapsulated *Lactobacillus acidophilus* (F5) and Requeijão Cremoso processed cheese with the addition of free *Lactobacillus acidophilus* (F6). The second step consisted of applying encapsulated (La-14) to different Requeijão cremoso formulations employing green banana biomass as a partial fat substitute (F1, F2, F3, and F4) and evaluating pH and probiotic viability throughout the shelf life and under gastrointestinal conditions.

2.1 Capsule elaboration

The probiotic microorganism encapsulation consisted of two stages: inoculum preparation and capsule elaboration. To prepare the inoculum, La-14 from Danisco® was activated in MRS broth (1 g of La-14 culture for 100 mL of broth) and incubated in an oven (37 °C, 15 h). Afterward, the sediment was processed in a refrigerated centrifuge at 4670 x g for 15 min and washed with 0.85% sterile saline solution. The microorganism suspension obtained was encapsulated according to Kanmani, et al. (2011), with adaptations.

Probiotic capsule preparation using extrusion method was done by adding 1% microorganism suspension to 1% sodium alginate solution and dripping it into 0.1 mol. L^{-1} of calcium chloride solution kept under stirring until 30 min after the end of the encapsulation. Next, the capsules were separated from the calcium chloride solution and washed with distilled and filtered water, according to Boscarioli (2010), with adaptations.

2.2 Requeijão cremoso elaboration

Requeijão cremoso elaboration was carried out in two stages. First, the curd mass was obtained by using the enzymatic milk coagulation method, according to Rodrigues (2006). Subsequently, the ingredients were weighed according to the six formulations described in Table 1 and defined by preliminary tests performed on the product. Then, the processing of Requeijão cremoso was done according to Van Dender (2014). Upon completing the processing, the formulations were cooled to 40 °C under aseptic conditions to add encapsulated or free *Lactobacillus acidophilus*. Finally, the formulations were packed in aseptic plastic containers and stored under refrigeration.

Table 1. Composition of Requeijão cremoso formulations.

	Treatments/formulations					
Ingredients (%)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Curd mass	60	60	60	60	60	60
Cream	10	10	5	5	20	20
Green banana biomass[a]	10	5	10	5	-	-
Salt	1	1	1	1	1	1
Emulsifying salt	2	2	2	2	2	2
Water	20	20	20	20	20	20
L. acidophilus (encapsulated)[b]	10	10	10	10	10	-
L. acidophilus (free, activated)[c]	-	-	-	-	-	1

[a] Water (1 mL) was added to each gram of green banana biomass. [b,c] The percentages of these ingredients were calculated from the finished product. Source: Authors.

2.3 Probiotic capsule analysis

The elaborated probiotic capsule composition was evaluated for moisture content, proteins, ashes, lipids, carbohydrates and pH, according to the Adolfo Lutz Institute (2008). The water activity (Aw) was measured directly utilizing the AquaLab water activity analyzer (model 4TEV) at constant temperature (25 °C). The capsule size was measured using a Mastersizer® 3000E equipment (Malvern Instruments, UK). The encapsulation efficiency (EE) was employed to assess the survival rate of the encapsulated bacteria. It was calculated following the method suggested by Annan, et al. (2008), which considers the number of viable cells (log CFU g^{-1}) released from the microcapsules and the number of viable cells (log CFU g^{-1}) in the cell concentrate before the microencapsulation.

2.4 Requeijão cremoso analysis

The pH of Requeijão cremoso was analyzed on days 1, 15, 30, and 45 according to Normative Instruction 68/2006 (Brazil, 2006). The viable cells of *Lactobacillus acidophilus* were counted during the storage period on days 0, 15, 30, and 45, and gastrointestinal simulation of the samples and probiotic viability counts were performed according to Vinderola AND Reinheimer (2000), employing MRS agar.

The simulated gastrointestinal conditions were performed according to Madureira, et al. (2011), with adaptations. The probiotic capsule viability, free activated probiotic, and Requeijão cremoso formulations developed were evaluated sequentially in a medium that simulated different gastrointestinal tract sections (esophagus-stomach, duodenum, and ileum). Aliquots of moist capsules (1 g), free activated probiotic (1 g), and each treatment of Requeijão cremoso (1 g) were used.

For the esophagus-stomach section, pepsin (25 mg mL^{-1}, Sigma Aldrich) was employed and prepared in 0.1 N HCl. This solution was added throughout the gastric section in equal aliquots (0.05 mL mL^{-1} concentration, 90 min), with pH being adjusted (2) using 1 mol L^{-1} HCl. In the duodenum section (0.25 mL mL^{-1} concentration), a solution containing pancreatin (2 gL^{-1}, Sigma Aldrich) and bovine bile salts (12 gL^{-1}, Sigma Aldrich) was prepared in NaHCO3 0,1 mol L^{-1}. The ileum section was simulated by raising pH (6.5) using 0.1 mol L^{-1} NaHCO3. All solutions were prepared at the time of use and sterilized with a 0.20 μm pore membrane (Minisart, Sartorius Stedim Biotech, Germany).

The analysis was conducted in a refrigerated shaker incubator (TE-421, Tecnal, Brazil) kept at 37 °C to simulate human body temperature. Mechanical agitation was employed to simulate intestinal peristaltic movements, with intensities matching those reached in each digestive tract section. At the end of each section, aliquots were taken to count viable probiotic cells.

2.5 Statistical analysis

The results were analyzed employing analysis of variance (ANOVA), and the means were compared by Tukey's test at a significance level of 5% using SAS version 9.0.

3. Results and Discussion

The probiotic capsules developed were made up of protein (0.41%), humidity (96.82%), ashes (0.25%), fat (0%), and carbohydrates (2.52%), with a 0.98 Aw and 4.47 pH, presenting diameters averaging at 1.738 μm. These features may be attributed to the encapsulating agents and extrusion method used, which produces particles with diameters normally ranging from 500 μm to 3 mm (Krasaekoopt, et al., 2003). The literature reports that capsule sizes may be influenced by needle diameter, syringe pressure, calcium chloride concentration, stirring rate in the solution where the alginate is discarded, alginate–or other compounds used for encapsulation–concentration, and the presence of insoluble calcium carbonate particles suspended in alginate (Valero-Cases & Frutos, 2015).

The mean EE of *Lactobacillus acidophilus* capsules was 98.34%, revealing that the encapsulation was adequate. This high efficiency is due to the extrusion method carried out at room temperature and without solvents.

Preliminary tests were performed to verify *Lactobacillus acidophilus* viability under diverse manipulation conditions (free and encapsulated) and during storage (Table 2). The encapsulated *L. acidophilus* maintained optimal viability for over 45 days, evidencing that encapsulation is an effective way to prolong viability. When *L. acidophilus* was added to Requeijão cremoso in free form, its viability drastically decreased over time, presenting an unsatisfactory end count. Therefore, the need to employ encapsulation in Requeijão cremoso is evident, since the free additives did not provide good viability over time. According to Brazilian legislation, to be considered a probiotic, a food must present a minimum concentration of 8–9 log CFU g^{-1} in the daily portion of the ready-to-eat product, as indicated by the manufacturer (Brazil, 2008).

Table 2. *L. acidophilus* viability results (free and encapsulated) in Requeijão cremoso. Results expressed in log CFU g^{-1}.

Sample	Storage Days			
	0	15	30	45
Requeijão cremoso with encapsulated *L. acidophilus* (F5)	10.85 ± 0.09^{aA}	9.71 ± 0.08^{bA}	8.78 ± 0.14^{cA}	8.41 ± 0.15^{cA}
Requeijão cremoso with free *L. acidophilus* (F6)	9.23 ± 0.07^{aB}	5.78 ± 0.03^{bB}	4.10 ± 0.23^{cB}	1.39 ± 0.03^{dB}

The same lowercase letters in rows and uppercase letters in columns denote no statistical difference by Tukey's test at 5% probability. The values are the average of triplicate analyses. Source: Authors.

Due to the defensible viability of the encapsulated *L. acidophilus*, it was tested in innovative Requeijão cremoso formulations employing green banana biomass. The formulations were submitted to pH analysis, probiotic viability, and gastrointestinal simulation. The pH levels of the formulations during the 45-day storage period at 5 °C are listed in Table 3. All the formulations presented ~6 pH, which is superior to the values found by Van Dender (2014), who stated that typical Requeijão cremoso varieties must contain 38–40% of total dry extract, 60–62% of fat in the dry extract, 30–33% of total nitrogen, 1–1.5% of sodium chloride, and 5.2–5.7 pH.

Table 3. Mean pH values for the treatments throughout the storage period of the Requeijão Cremoso formulations.

Time	Treatments				
(days)	F1	F2	F3	F4	F5
1	6.65 ± 0.018^{cA}	6.73 ± 0.18^{aA}	6.71 ± 0.1^{bA}	6.62 ± 0.14^{dA}	6.64 ± 0.12^{cA}
15	6.50 ± 0.09^{aB}	6.58 ± 0.03^{aB}	6.57 ± 0.05^{aB}	6.52 ± 0.02^{aAB}	6.56 ± 0.04^{aB}
30	6.44 ± 0.17^{bC}	6.44 ± 0.12^{bC}	6.47 ± 0.13^{abC}	6.45 ± 0.18^{bB}	6.51 ± 0.16^{aB}
45	6.39 ± 0.1^{aC}	6.40 ± 0.09^{aC}	6.40 ± 0.09^{aC}	6.20 ± 0.13^{bC}	6.20 ± 0.15^{bC}

The same lowercase letters in rows and uppercase letters in columns denote no statistical difference by Tukey's test at 5% probability. F1: 10% pasteurized cream and 10% green banana biomass; F2: 10% pasteurized cream and 5% green banana biomass; F3: 5% pasteurized cream and 10% green banana biomass; F4: 5% pasteurized cream and 5% green banana biomass; F5-control: 20% pasteurized cream. The values are the average of triplicate analyses. Source: Authors.

There was a significant pH decline over time in every formulation, which is expected given the reactions in the food itself. Several factors may cause pH lowering during storage, including moisture reduction, hydrolysis of polyphosphates, and interactions among proteins that regulate ionic balance (Van Dender, 2014). Regarding probiotic viability, a decrease in

Lactobacillus acidophilus count over the shelf life in all formulations was noted (Table 4), and this is explainable by microorganisms being metabolically active within the capsules, producing metabolic acids, bacteriocins, and substrate loss (Pedroso, et al., 2012). The decrease was approximately 1 log CFU g^{-1} in the probiotic counts, lower than that found by Ramírez, et al. (2007), where they developed Requeijão cremoso with free *Lactobacillus casei* and observed a decrease of 3 log CFU g^{-1} in 15 days.

Table 4. *L. acidophilus* viability (log CFU g^{-1}) in Requeijão Cremoso formulations stored for 45 days under refrigeration.

Time (days)	Treatments				
	F1	**F2**	**F3**	**F4**	**F5**
0	9.86 ± 0.02^{aA}	9.44 ± 0.28^{aA}	9.69 ± 0.06^{aA}	9.71 ± 0.08^{aA}	9.61 ± 0.09^{aA}
15	9.25 ± 0.11^{aB}	9.36 ± 0.22^{aA}	9.54 ± 0.08^{aA}	9.55 ± 0.11^{aA}	9.43 ± 0.02^{aA}
30	8.90 ± 0.02^{aC}	8.96 ± 0.05^{aA}	8.95 ± 0.03^{aB}	8.97 ± 0.03^{aB}	8.91 ± 0.03^{aB}
45	8.08 ± 0.06^{aD}	8.18 ± 0.04^{aB}	8.24 ± 0.09^{aC}	8.20 ± 0.01^{aC}	8.14 ± 0.04^{aC}

The same lowercase letters in rows and uppercase letters in columns denote no statistical difference by Tukey's test at 5% probability. F1: 10% pasteurized cream and 10% green banana biomass; F2: 10% pasteurized cream and 5% green banana biomass; F3: 5% pasteurized cream and 10% green banana biomass; F4: 5% pasteurized cream and 5% green banana biomass; F5-control: 20% pasteurized cream. The values are the average of triplicate analyses. Source: Authors.

At the end of the 45-day shelf life, all Requeijão cremoso formulations had concentrations of ~8 log CFU g^{-1}, thus being considered probiotics. The International Dairy Federation recommends that the minimum probiotic cell concentration be 6–7 log CFU g^{-1} at the end of shelf life (Tripathi & Giri, 2014). Therefore, the shelf life of 45 days proves to be suitable for this type of product. According to Trabulsi & Sampaio (2000), probiotic-containing foods should have a half-life of 15–30 days with the number of viable cells > 6 log CFU g^{-1}.

There was no significant difference in microorganism viability among the treatments, implying that the diverse Requeijão cremoso formulations did not interfere with La-14 probiotic activity. Buriti, et al. (2010) found divergent data in their study using mousses containing free probiotics and noted that the different compositions positively altered the probiotic viability throughout the shelf life. The mismatch between the findings may be due to the microorganism encapsulation performed for Requeijão cremoso, whereas the mousses had the bacteria in their free form interacting favorably with the food matrix.

Although probiotic culture viability is crucial throughout the shelf life of the product, resistance to the gastrointestinal tract is equally essential, since probiotics must proliferate and colonize their specific location to provide benefits to hosts (Saad, et al., 2013). Therefore, in the gastrointestinal simulation (Table 5), capsules protected the microorganisms during the esophagus-stomach section, rupturing only in the duodenum and ileum sections. The treatments presented a significant difference in *Lactobacillus acidophilus* count at each stage, revealing that their different compositions interfered with the gastrointestinal simulation, albeit all treatments achieved adequate end counts (~8 log CFU g^{-1}).

Table 5. Gastrointestinal simulation test mean results for the elaborated Requeijão Cremoso formulations. Results expressed in log CFU g^{-1}.

Condition	Treatments				
	F1	**F2**	**F3**	**F4**	**F5**
Initial	9.49 ± 0.04^{bcA}	9.37 ± 0.02^{cA}	9.8 ± 0.01^{aA}	9.35 ± 0.03^{cA}	9.71 ± 0.13^{abA}
Stomach	3.17 ± 0.04^{aC}	3.07 ± 0.04^{aD}	3.23 ± 0.02^{aD}	3.06 ± 0.08^{aD}	3.18 ± 0.05^{aC}
Duodenum	7.92 ± 0.01^{abB}	8.01 ± 0.02^{aC}	7.83 ± 0.07^{bC}	7.92 ± 0.03^{abC}	7.99 ± 0.06^{abB}
Ileum	8.05 ± 0.01^{bB}	8.12 ± 0.03^{abB}	8.23 ± 0.02^{abB}	8.26 ± 0.05^{aB}	8.25 ± 0.07^{aB}

The same lowercase letters in rows and uppercase letters in columns denote no statistical difference by Tukey's test at 5% probability. F1: 10% pasteurized cream and 10% green banana biomass; F2: 10% pasteurized cream and 5% green banana biomass; F3: (5% of pasteurized cream e 10% of green banana biomass) F4 (5% of pasteurized cream e 5% of green banana biomass), F5-control (20%pasteurized cream). The analysis was carried out in triplicate. Source: Authors.

Oliveira, et al. (2014) observed similar findings when evaluating the gastrointestinal simulation of goat cheese with *L. acidophillus*, where a probiotic count decrease (~1 logarithmic cycle) was observed at the end of the digestion process. Verruck, et al. (2015) developed Frescal fresh cheese from buffalo probiotic, subjected it to gastrointestinal conditions, and reported a reduction (~2 logarithmic cycles) in the probiotic count at the end of the simulation.

4. Conclusion

Lactobacillus acidophilus encapsulation by extrusion produced capsules that protected the microorganism during shelf life. The proposed innovative formulations resulted in Requeijão cremoso varieties with probiotic viability ranging from 9.86 to 8.08 log CFU g^{-1}. The microorganism count in the gastrointestinal simulation ranged from 9.8 (initial) to 8.05 log CFU g^{-1} (ileum), proving satisfactory and conferring functionality to the product. The compositional difference of the Requeijão cremoso varieties did not interfere with the probiotic viability.

Further studies that explore other probiotics must be developed. Nevertheless, the findings herein revealed that Requeijão cremoso is an acceptable food matrix to receive encapsulated *Lactobacillus acidophilus*.

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References

Annan, N. T., Borza, A. D. & Hansen, L. T. (2008). Encapsulation in alginate-coated gelatin microspheres improves surviral of Bifidobacteriun adolescentis 15703T during exposure to simulated gastrointestinal conditions. *Food Research International*, 41, 184-193. 10.1016/j.foodres.2007.11.001

Boscarioli, M. P. M. (2010) *Influence of prebiotics on the encapsulation of probiotics added in ice cream.* Master Thesis, School of Engineering Mauá-CEUNEEM, São Paulo, Brazil.

Brazil (2006). Normative Instruction Nº. 68, of December 12, 2006, Official Physical-Chemical Analytical Methods for Control of Milk and Dairy Products. Ministry of Agriculture, Livestock and Supply.

Brazil (2008). RDC nº 2, January 7, 2008. Technical Regulation of Bioactive and Probiotic Substances Isolated with Allegations of Functional or Health Properties. Ministry of Health - National Agency of Sanitary Surveillance.

Buriti, F. C. A., Cardarelli, H. R. & Saad, S. M. I. (2008). Instrumental texture and sensory evaluation of fresh cheese Symbiotic cream: implications of the addition of *Lactobacillus paracasei* and inulin. *Brazilian Journal of Pharmaceutical Sciences*, 44, 75-84. 10.1590/S1516-93322008000100009

Buriti, F. C. A., Castro, I. A. & Saad, S. M. I. (2010). Viability of *Lactobacillus acidophilus* in synbiotic guava mousses and its survival under in vitro simulated gastrointestinal conditions. *International Journal of Food Microbiology*, 137, 121-129. 10.1016/j.ijfoodmicro.2009.11.030

Di Bartolomeo, F., Startek, J. B. & Van den Ende, W. (2013). Prebiotics to fight diseases: Reality or fiction? *Phytotherapy Research*, 27, 1457 – 1473. doi:10.1002/ptr.4901

FAO/OMS (2006). Probiotics in Food. Health and Nutritional Properties and Guidelines for Evaluation. In: FAO Food and Nutrition Paper 85, Roma.

IAL (2008). Physicochemical methods for food analysis. 1st digital edition, Adolfo Lutz Institute.

Kale-Pradhan, P. B., Jassal, H. K. & Wilhelm, S. M. (2010). Role of *Lactobacillus* in the prevention of antibiotic-associated diarrhea: a meta-analysis. *Pharmacotherapy*, 30, 119- 126. 10.1592/phco.30.2.119

Kanmani, P; Kumar, RS; Yuvaraj, N; Paari, K; Pattukumar, V & Arul, V (2011). Effect of cryopreservation and microencapsulation of lactic acid bacterium *Enterococcus faecium* MC13 for long-term storage. *Biochemical Engineering Journal*, 58, 140-147. 10.1016/j.bej.2011.09.006

Krasaekoopt, W., Bhandari, B. & Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal*, 13, 3-13. doi:10.1016/S0958-6946(02)00155-3

Kumar, B. V., Vijayendra, S. V. N. & Reddy, O. V. S. (2015). Trends in dairy and non-dairy probiotic products - a review. *Journal of Food Science and Technology*, 52, 6112–6124. doi:10.1007/s13197-015-1795-2

López-Rubio, A., Sánchez, E., Sanz, Y. & Lagaron, J. M. (2009). Encapsulation of living bifidobacteria in ultrathin PVOH electrospun fibers. *Biomacromolecules*, 10, 2823-2829. doi:10.1021/bm900660b

Madureira, A. R., Amorim, M., Gomes, A. M., Pintado, M. E. & Malcata, F. X. (2011). Protective effect of whey cheese matrix on probiotic strains exposed to simulated gastrointestinal conditions. *Food Research International*, 44, 465–470. 10.1016/j.foodres.2010.09.010

Oi, R. K., Moraes Junior, D. & Tambourgi, E. B. (2013). Feasibility Study for Production of Green Banana Flour in a Spray Dryer. *Chemical Engineering Transactions*, 32, 1825-1830. 10.3303/CET1332305

Oliveira, M. E. G., Garcia, E. F., Oliveira, C. E. V., Gomes, A. M. P., Pintado, M. M. E., Madureira, A. R. M. F., Da Conceição, M. L, Queiroga, R. C. R. E. & De Souza, E. L. (2014). Addition of probiotic bactéria in a semi-hard goat cheese (coalho): Surviral to simulated gastrointestinal conditions and inhibitory effect agains pathogenic bactéria. Food Research. International, 64, 241-247. 10.1016/j.foodres.2014.06.032

Oliveira, R. P. S., Perego, P., Converti, A. & Oliveira, M. N. (2009). Effect of inulin on growth and acidification performance of different prebiotic bacteria in co-cultures and mixed culture with Streptococcus thermophylus. *Journal of Food Engineering*, 91, 133-138. 10.1016/j.jfoodeng.2008.08.013

Pedroso, D. L., Thomazini, M., Heinemann, R. J. B. & Favaro-Trindade, C. S. (2012). Protection of *Bifidobacterium lactis* and *Lactobacillus acidophilus* by microencapsulation using spray-chilling. *International Dairy Journal*, 26, 127-132. 10.1016/j.idairyj.2012.04.008

Pinto, S. S., Fritzen-Freire, C., Benedetti, S., Murakami, F. S., Pe, J. C. C., Prudêncio, E. S. & Amboni, R. D. M. C. (2015). Potential use of whey concentrate and prebiotics as carrier agents to protect *Bifidobacterium*-BB-12 microencapsulated by spray drying. *Food Research International*, 27, 400 -408. 10.1016/j.foodres.2014.11.038

Ramírez, L. A. G., Ospina, A. J. G., Jamarillo, L. M. A. & Patino, B. T. (2007). Evaluation of the viability of a native probiotic strain of *Lactobacillus casei* in cream cheese. *Revista Lasallista de Investigación*, 4, 37-42. http://hdl.handle.net/10567/474

Rodrigues, F. (2006). *Requeijão, Fondue, Specialty, Processed cheese*. Do Autor, Juiz de Fora, MG.

Saad, N., Delattre, C., Urdaci, M., Schmitter, J. M. & Bressollier, P. (2013). An overview of the last advances in probiotic and prebiotic field. *LWT- Food Science and Technology*, 50, 1-16. 10.1016/j.lwt.2012.05.014

Trabulsi, L. R. & Sampaio, M. M. S. C. (2000). *Probiotics and Child Health*. *Nestlé*.

Tripathi, M. K. & Giri, S. K. (2014.) Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods*, 9, 225 – 241. 10.1016/j.jff.2014.04.030

Valero-Cases, E. & Frutos, M. J. (2015). Effect of different encapsulation types on the survival of *Lactobacillus plantarum* during storage with inulin and in vitro digestion. *LWT-Food Science and Technology*, 64, 824-828. 10.1016/j.lwt.2015.06.049

Van Dender, A. G. F. (2014). *Requeijão Cremoso and other processed cheeses: Manufacturing technology, process control and market aspects*. Setembro publishing company, São Paulo.

Verruck, S., Prudêncio, E. S., Vieira, C. R. W., Amante, E. R. & Amboni, R. D. M. C. (2015). The buffalo Minas Frescal cheese as a protective matrix of *Bifidobacterium* BB-12 under in vitro simulated gastrointestinal conditions. *LWT-Food Science and Technology*, 63, 1179-1183. 10.1016/j.lwt.2015.04.014

Vinderola, C. G. & Reinheimer, J. A. (2000). Enumeration of *Lactobacillus casei* in the presence of *L. acidophilus*, *Bifidobacteria* and lactic starter bacteria in fermented dairy products. *International Dairy Journal*, 10, 271-275. 10.1016/S0958-6946(00)00045-5

Yasmin, A., Butt, M. S., Afzaal, M., Van Baak, M., Nadeem, M. T. & Shahid, M. S. (2015). Prebiotics, gut microbiota and metabolic risks: Unveiling the relationship. *Journal of Functional Foods*, 17, 189-201. 10.1016/j.jff.2015.05.004