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MICROBIOLOGICAL-PHYSICOCHEMICAL ASSESSMENT AND GASTROINTESTINAL SIMULATION OF FUNCTIONAL (PROBIOTIC AND SYMBIOTIC) GOUDA-TYPE CHEESES DURING RIPENING

EVALUACIÓN MICROBIOLÓGICA, FISICOQUÍMICA Y SIMULACIÓN GASTROINTESTINAL DE QUESOS FUNCIONALES (PROBIÓTICO Y SIMBIÓTICO) TIPO GOUDA DURANTE LA MADURACIÓN

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Abstract

The objective of this research was to obtain a ripened gouda-type cheese added with probiotic bacteria (*Bifidobacterium lactis*) and a prebiotic ingredient (Nutriose®FB), evaluating its potential as a functional food. The probiotic bacteria were encapsulated by spray drying using reconstituted skim milk (RSM) and a mixture of β-cyclodextrin-arabic gum (BC-AG) like encapsulant agents. Latterly, these ingredients were added during the production of gouda-type cheeses and microbiological as well as physicochemical evolution were assessed during ripening. At the end of this process, cheeses were subjected to gastrointestinal simulated conditions to evaluate probiotic survival. Results showed that the survival of microencapsulated cells was above 10^{10} CFU·g^{-1} in the probiotic powder and it is maintained above 10^{8} CFU·g^{-1} in the cheese, during all the ripening process (40 days). Furthermore, tests carried out on the final product indicate that the probiotic bacteria resist the gastrointestinal conditions, remaining viable at a high concentration ($> 10^{7}$ CFU·g^{-1}). An important aspect is that probiotic bacteria (*Bifidobacterium lactis*) and soluble fiber (Nutriose®FB) do not affected the physicochemical parameters of the gouda-type cheese. In conclusion, symbiotic gouda-type cheese obtained guarantees a beneficial effect to consumer, while keeps intrinsic physicochemical properties of the original cheese.

Keywords: ripened cheese, symbiotic food, microencapsulation, spray drying.

Resumen

Esta investigación tuvo como objetivo obtener un queso madurado tipo gouda adicionado con bacterias probióticas (*Bifidobacterium lactis*) y un prebiótico (Nutriose®FB) evaluando su potencial como alimento funcional. Las bacterias probióticas se encapsularon mediante secado por aspersión, empleando leche descremada reconstituida y una mezcla de β-ciclodextrina-goma arábiga como agentes encapsulantes. Posteriormente, se adicionaron a un queso tipo gouda y se evaluó el comportamiento microbiológico y fisicoquímico durante la maduración. Al final de este proceso, se realizó una simulación gastrointestinal para evaluar la supervivencia de los probióticos. Los resultados mostraron que la supervivencia de las células microencapsuladas es superior a 10^{10} UFC·g^{-1} en el polvo probiótico y se mantiene superior a 10^{8} UFC·g^{-1} en el queso, durante los 40 días del proceso de maduración. Además, pruebas realizadas en el producto final, indican que el probiótico resiste las condiciones grastrointestinales permaneciendo viable en una alta concentración ($>10^{7}$ UFC·g^{-1}). Un aspecto relevante es que las bacterias probióticas (*Bifidobacterium lactis*) y la fibra soluble (Nutriose®FB) no modificaron los parámetros fisicoquímicos del queso tipo gouda. En conclusión, se obtuvo un queso madurado simbiótico que garantiza un efecto benéfico al consumidor sin modificar las propiedades fisicoquímicas del queso original.

Palabras clave: queso madurado, alimento simbiótico, microencapsulado, secado por aspersión.

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1 Introduction

Several authors have shown the importance of achieving a high probiotic microorganism count at the beginning of the shelf life of the functional food. These counts must remain above 10^7 CFU·g^{-1} throughout all its shelf life to exert the expected probiotic effect, once this has been ingested (Stanton *et al*., 2001; Kailasapathy, 2006; Masuda *et al*., 2005; Mendoza-Madrigal *et al*., 2017).

The use of dairy products, like cheese as a functional food, enriched with probiotic microorganisms, is considered an attractive option to diversify functional foodstuff, with a great potential to improve consumers health (Lollo *et al*., 2015; Dantas *et al*., 2016). Cheese is a good alternative for probiotics delivery into the intestine and, as a result, has been the subject of marketing and research studies in recent years (Gomes da Cruz *et al*., 2009; Karimi *et al*., 2012b). Indeed, the addition of probiotic bacteria to cheese represents an added value to a product that provides health benefits, such as improvements in the immune system, oral and intestinal health in the elderly and reinforcement of intestinal immunity (Lollo *et al*., 2012; Albenzio *et al*., 2013).

Cheese has certain advantages as a carrier of probiotics compared to more acidic fermented dairy products such as yogurt. It creates a buffer against the high acidic environment in the gastrointestinal tract (GIT) and thus generates a more available environment for probiotic survival throughout gastric transit (Karimi *et al*., 2012a; Ortakci *et al*., 2012). As already reported by several authors, cheese is a promising food matrix for probiotics. Nevertheless, strain selection and possible process modifications should be carefully evaluated to maximize probiotic cell survival during cheese manufacture and ripening (Takanashi *et al*., 2014; Karimi *et al*., 2015).

In addition to the probiotic bacteria survival in cheese, it is important that the incorporation of these complementary bacteria does not affect the sensorial (flavor, texture, and appearance) or physicochemical characteristics of the conventional non-probiotic cheeses. This is particularly important for foodstuffs registered under a scheme of protected designation of origin (PDO), like several ripened cheeses all over the world. Therefore, it is necessary to compare the final characteristics of the functional products, as well as their evolution over time, when a new product is elaborated (Souza *et al*., 2008).

Despite the advantages of dairy products as vehicles of probiotics, usually their survival in this type of food has shown high variability. Even with numerous favorable results, it has been observed some limitations in the viability of probiotic bacteria due to the microenvironmental conditions. Indeed, the degree of survival of the microorganisms is mainly due to its sensitivity to stress caused by the characteristics of the food and environmental conditions during storage as well as preservation (Boylston *et al*., 2004).

In this context, the immobilization of probiotics using microencapsulation may improve the survival of these microorganisms in products, both during processing and storage, as well as during digestion process. The availability of spray-dried powders, protecting high numbers of viable probiotic microorganisms, is desirable for commercial applications. This process provides stability during shelf life storage and transportation of these cultures and their subsequent application in functional food development (Dianawati *et al*., 2016; Martín *et al*., 2015).

Ortakci *et al*. (2012) evaluated microencapsulation by spray drying as a method to supply probiotics to Mozzarella cheese and noted that probiotic survival improved, when the processing and storage conditions of the cheese are extremely stressful. However, this approach did not show an increase in the survival of probiotic bacteria in traditional feta cheese (Kailasapathy & Masondole, 2005). According to authors, this is probably due to open texture of cheese and dissolution of microcapsules. So, it is important to test another kind of encapsulant agents and to integrate microcapsules in suitable-texture ripened cheeses.

Besides, prebiotics incorporation into cheese could maintain or even increase the cell survival into the intestine. Indeed, prebiotics composed by non-digestible dietary components pass through the colon and selectively stimulate the proliferation and/or activity of desirable probiotic bacteria in-situ (Mattila-Sandholm *et al*., 2002). The addition of prebiotics, generally dietary fibers as inulin, resistant starch, pectin and beta-glucans, has induced the growth of probiotic bacteria. In this sense, Nutriose®FB, which is a dextrin obtained from wheat starch, represents a good alternative. In contrast to maltodextrin, Nutriose®FB is partially hydrolyzed and is not absorbed due to many α-1-6 linkages as well as the presence of non-digestible glucoside linkages (e. g. α-1.2 and α-1.3) and is a predilect substrate to *Bifidobacteria* strains (Lefranc-Millot *et al*., 2009).

Unexpectedly, only few studies relate the potential of ripening cheese as a functional food, especially as a food matrix carrier for probiotic bacteria and prebiotic ingredients, characterizing them as a symbiotic food. In this context, this research has aimed to obtain a ripened gouda-type cheese as a functional food added with encapsulated probiotic bacteria (*Bifidobacterium lactis*) and a prebiotic ingredient (Nutriose®FB), evaluating its potential as probiotic and symbiotic food.

2 Materials and methods

2.1 Encapsulation of Bifidobacterium lactis by spray drying

2.1.1 Probiotic strain and culture preparations

Fresh cultures of *Bifidobacterium lactis* BLC1 (Sacco System Company, Italy) were obtained after activation by two successive transfers in Man-Rogosa-Sharpe broth (MRS)(DIFCO, Becton Dickinson and Co., USA) supplemented with 0.05% w/v of L-cysteine (Sigma-Aldrich) to generate anaerobic conditions. Cellular cultures in late log phase were centrifugated (Sigma 3-18K ASPELAB) at 3900 rpm, for 10 min and at 4 °C and washed (buffer phosphate water, pH 7.2). This process was repeated three times and the cell biomasses obtained were mixed with the carrier material in sterile conditions.

2.1.2 Preparation of solutions with carrier material

According to Table 1, a pre-established quantity of encapsulant agent was mixed and balanced to 100 g with sterile distilled water. The reconstituted skim milk (RSM) (DIFCO®, Becton Dickinson and Co., USA) was heat treated at 90 °C for 10 min to destroy pathogens. Prior to dissolution in water, β-cyclodextrin (BC) (Sigma®, Sigma-Aldrich Inc., USA) was mixed with arabic gum (AG) (EMPROVE®, Merck KGaA, Germany) in a ratio of 9:1 (w/w). Stirring was performed using a homogenizer (PRO260, Laboratory Homogenizer with Digital Speed Control, America Pro Scientific Company, USA) at 10000 rpm for 5 min. For each experiment, 93.8 mL of solution was mixed with 6 mL of biomass and 0.2 mL of Tween 60 was added, followed by a second stirring cycle at 5000 rpm for 10 min.

2.1.3 Encapsulation of Bifidobacterium by spray drying

Encapsulant solutions containing *Bifidobacteria* were dried using a laboratory spray dryer (BUCHI B-290, Switzerland) operated at set conditions (Table 1). The outlet temperature was 76±2 °C and the carrier solution was constantly agitated using a magnetic stirrer. The dry probiotic microcapsules were collected, placed in sealed glass vials, and stored at 25 °C.

2.1.4 Probiotic powder yield

The Probiotic Powder Yield (*PPY*) was determined considering the solids introduced into the fed solutions before spray drying treatment and the total powder recovered in the cyclone dryer. *PPY* was calculated using the next formula:

$$PPY = (P/P_i) \times 100 \quad (1)$$

where, P_i is the weight of dry solids fed in the spray dryer and P is the weight of final spray-dried powder obtained.

2.1.5 Morphology and particles size

The microcapsules were analyzed in a Jeol® scanning electron microscope SEM model JSM 6390 LV (Tokyo, Japan) at an accelerating voltage of 10 and 15 kV. Before introducing in SEM, the samples were placed on a piece of adhesive paper and were coated with gold with a vacuum sputtering coater Leica® model EM SCD 500 (Wetlar®, Germany) as described by Lian *et al*. (2002).

2.2 Pilot-scale functional cheese processing

To produce cheeses, a single batch of cow milk was pasteurized in a pilot plant at 72.5 °C during 30 min, cooled to 37 °C and transferred in vats for the cheese-making. Five batches of cheese-making were produced and were inoculated with a commercial starter culture mix (Lyofast MS 064 CP; Sacco System Company, Italy). All batches were inoculated with the commercial starter mix culture composed of *Streptococcus thermophilus*, *Lactococcus lactis* biovar *diacetylactis* and *Lactococcus lactis* subsp. *lactis* (counter ratio 100:1) incubated after 30 minutes and rennet (Chr Hansen, Cuamex®) were added into the milk at a strength of 1:10000 (75% chymosin, 25% pepsin). After 30 minutes, the curd was cut, stirred for 10 minutes, drained from the whey and placed

into molds. The obtained cheeses (250 g) were drained under pressure during 24 h (25 °C, 90% relative humidity RH) and then brine salted (21% w/w NaCl aqueous solution at 9 °C during 24 h). Cheese ripening was carried out through 40 days at 9 °C and 75% RH into a cooling chamber. Cheese samples were collected at 1, 10, 20, 30, 40 days for further analysis and sampling was monitored by duplicate.

To investigate the effect of encapsulate agent as well as the presence of a prebiotic over the probiotic bacteria in the gouda-type cheese, different combinations were prepared. In the case of probiotic (batches 2 and 4) and symbiotic cheeses (batches 3 and 5), additional ingredients were incorporated before molding, as follows: probiotic cheeses were enriched with 1% w/w of BC-GA and RSM probiotic powders, respectively; in the case of symbiotic cheeses, 1% w/w of probiotic powder and 1% w/w of prebiotic ingredient (Nutriose®FB) were added. Finally, for the control cheese (batch 1) none additional ingredient was incorporated.

2.3 *Cell survival extraction and counting*

Cheese samples (10 g) were suspended in phosphate buffer solution (90 mL, pH 7.2)) and were shaken at room temperature to ensure a complete dispersion. The use of a disperser-homogenizer (Polytron®PT1200 Carl Roth, Germany) at 15000 rpm for 2 min ensures total release of immobilized probiotic bacteria from microcapsules, as reported in literature (Doherty *et al*., 2010). Control cheese samples were subject to the same conditions to keep consistent treatment conditions. Serial decimal dilutions were prepared from the initial suspension and following the Miles-Misra technique 20 μL drops were inoculated by triplicate on the surface of MRS agar, added with 0.05% L-cysteine Sigma-Aldrich® (Picot & Lacroix, 2004). Plates were incubated at 37 °C for 48 h under anaerobic conditions and subsequently the number of colonies was counted. Results are expressed as a logarithm of colony-forming unit per gram ($\log_{10}$CFU·g^{-1}).

2.4 *Survival of encapsulated and free bacteria during in-vitro digestion*

The ability for *in-vitro* survival of encapsulated and free cells was compared under digestion conditions, simulating the human gastric and intestinal environments. The method was based on a previous published procedure (Picot & Lacroix, 2004). The transit tolerance of the probiotic cultures was determined by exposing the microorganisms at 37 °C to simulated gastric juice for 30 min (pH 1.9) and simulated small intestinal juice for 6 h (pH 7.5), successively. Then, changes in total viable counts in the digestion mixture were periodically monitored.

Simulated gastric juice was prepared by dispersing pepsin 1:60000 (porcine gastric mucosa P7012, Sigma Aldrich®) in HCl 0.1N (Hycel®) and adjusting the pH to 1.9 with NaOH 1N (Hycel®), in order to achieve a final pepsin concentration in the digestion mixture of 0.26 g·L^{-1}. Simulated pancreatic juice was prepared by dispersing pancreatin (Hycel®) in sterile sodium phosphate buffer (0.02M, pH 7.5) and adjusting the pH to 7.5 with NaOH 1N in order to achieve a final pancreatin concentration in the digestion mixture of 1.95 g·L^{-1}. Concentrated bile salt solution (150 g·L^{-1}) was prepared by dissolving a bile extract powder (bile bovine B3883, Sigma Aldrich®) in distilled water. The resulting suspension was filtered and sterilized. The simulated gastric juice, bile salt solution and pancreatic juice were prepared just before use to avoid eventual degradation.

A cheese sample of 1.0 g was mixed with a phosphate aqueous solution (9 mL) and transferred aseptically to a 125 mL Erlenmeyer flask; temperature was maintained at 37 °C using a stirred water bath. Then, 5 mL of pepsin solution was added and after 30 min the reaction was stopped by increasing the pH to 7.5 using NaOH 1N. A sample of 1 mL was withdrawn and kept in ice before quantification of viable cells. Next, 5 mL of concentrated sodium phosphate buffer (0.5 M, pH 7.5) and 2.0 mL of bile salt solution were added. After adjusting the pH to 7.5 and the volume to 20 mL with sterile distilled water, 10 mL of the pancreatic juice was added for a final volume of 25 mL. At different times (1, 2, 3, and 6 h), 1 mL aliquots were removed, and the reaction was stopped by placing the samples in ice for 5 min. Formerly to bacteria enumeration, the sample was submitted to a process of releasing cells from microcapsules, as described in section 2.3. Survival at each stage is expressed on $\log_{10}$CFU·g^{-1} of cheese. During all experiments the pH was monitored, and no changes were observed.

2.5 *Compositional analysis*

Milk samples were analyzed for lactose, protein and fat contents by using a Milko Scan™ FT 120 (Foss®, Padova, Italy). As explained before, cheese samples were collected at 1, 10, 20, 30 and 40 days for both

microbiological and chemical analysis. Furthermore, one entire cheese was used for each sample. Cheese samples during manufacturing and ripening were analyzed for moisture using the oven drying method at 102 °C (IDF, 1982), salt content using a titration method with $AgNO_3$ (IDF, 1988), total protein and pH 4.6-soluble nitrogen using the Kjeldhal method (IDF, 1993), and fat content using the FIL-IDF Standard 5A method (IDF, 1997). The pH was determined with a Portamess pHmeter (Knick®, Berlin, Germany), placing the measurement electrode into the sample bulk. All analyses were performed by triplicate.

The Nutriose®FB concentration in the final product was obtained using the AOAC method 991.43 "Total, soluble, and insoluble dietary fiber in foods" and the AOAC method 32-07.01 "Determination of soluble, insoluble, and total dietary fiber in foods and food products" (AOAC, 2000).

2.6 *Sanitary quality of functional cheeses*

The sanitary quality of cheeses was evaluated. Molds and yeasts were determined according to the methodology proposed in Mexican Official Norms (NOM-111-SSA-1-1994), total coliform organisms and *E. coli* were carried out using a Petrifilm E.C. method (3M, approved AOAC official Method No. 2003.07), *Salmonella spp.* was determined by 1-2 test (Biocontrol Systems, approved AOAC official method No. 989.13) and *S. aureus* was determined by the Staph Express Count Plate (3M, approved AOAC official method No. 989.13).

2.7 *Statistical analysis*

Cell counts were converted to $\log_{10}$CFU·g^{-1} or $\log_{10}$CFU·mL^{-1} for statistical analysis. Moreover, to compare the survival of *Bifidobacterium* population after the simulated gastrointestinal digestion, microbiological and chemical analyses results were expressed as mean values of two independent experiments and standard deviations were calculated. Statistical analyses were performed with Statgraphics 7.0 (StatSoft Inc., Tulsa, OK, USA), using the Tukey's honest significant difference test with a significance level of $p = 0.05$ in all assays.

3 Results and discussion

Table 1 shows the results obtained for the probiotic microencapsulation. *Bifidobacterium lactis* was encapsulated using BC-AG and RSM, achieving viabilities of $1.89\pm0.25\times10^{11}$ CFU·g^{-1} and $1.98\pm0.38\times10^{10}$ CFU·g^{-1} as well as powder percentage yield of 77.53±1.2 and 63.26±1.8, respectively. Additionally, the microcapsules exhibited water activity (a_w) values below 0.25 in both cases (0.215±0.004 and 0.190±0.007, respectively). Moreover, low a_w values and low residual moisture contents ($< 4-5\%$ w/w) are prerequisites for the commercial production of spray-dried powders with good handling characteristics such as high flow ability, low stickiness and agglomeration as well as maximum probiotics survival (Barbosa-Canovas, 2005; Borges *et al.*, 2017). At conditions of low a_w, the matrix moves from the rubbery state toward the glassy state and water mobility is reduced consequently. This phenomenon inhibits cell metabolic activity of the bacterial cells, leading to extended shelf-life.

Figure 1 shows the SEM micrographs of the *Bifidobacterium* microcapsules obtained using RSM and BC-AG as encapsulant agent.

Table 1. Experimental results of encapsulation by spray drying of *Bifidobacterium lactis* using β-cyclodextrin-arabic gum (BC-AG) and reconstituted skim milk (RMS).

Exp.	AIT (°C)	AIF (mL·min^{-1})	CM	CMC (%)	LAB	CFU·g$^{-1}_{dm}$ before spray drying	CFU·g$^{-1}_{dm}$ after spray drying	a_w	PPY (%)
1	160	5	BC-AG	15	BL	$3.79\pm0.15\times10^{11}$	$1.89\pm0.25\times10^{11}$	0.215±0.004	77.53±1.2
2	160	5	RSM	20	BL	$5.33\pm0.43\times10^{12}$	$1.98\pm0.38\times10^{10}$	0.190±0.007	63.26±1.8

AIT=air inlet temperature; AIF=air inlet flow rate; CM=core material; CMC=core material concentration;
(LAB=lactic acid bacteria, BL=*B. lactis*); PPY=powder percentage yield; dm=dry material.

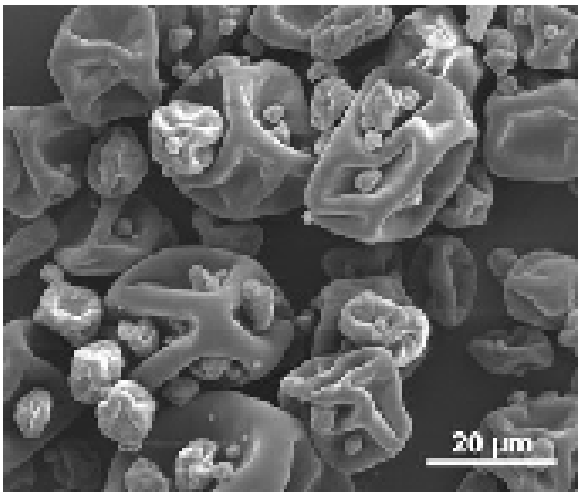

Fig. 1. Micrographs of microcapsules produced: A) *B. lactis* encapsulated with reconstituted skim milk (RSM), B) released *B. lactis* after sonication and C) *B. lactis* encapsulated with β-cyclodextrin arabic gum mixture (BC-AG).

Figure 1A reveals the absence of free bacteria, confirming the formation of probiotic microcapsules. The particles showed a pseudo-spherical shape and dispersed sizes, with typical concavities of microcapsules produced by spray drying. Sáenz *et al*. (2009) reported that the formation of concavities in the surface of atomized particles can be attributed to the rapid evaporation of liquid drops. The size of the microcapsules obtained is in a range from 7 to 15 μm. Such values are accepted for microcapsules obtained through spray drying, which vary from 10 to 100 μm, according to Fang and Bhandari (2010). Similar results were obtained for microcapsules obtained using BC-AG mixture (Figure 1C).

Figure 1B shows the *Bifidobacterium lactis* bacteria trapped into the capsules, released after microcapsule rupture by sonication. As expected, *Bifidobacterium lactis* exhibits a size between 1.7 and 2.2 μm.

The average composition characteristics of raw cow's milk used in making cheese were: density 1.029 g·mL^{-1}, fat content 4.3%, lactose content 4.5% and total protein content 3.8%. These compositional results are typical for cow milk (Muehlhoff *et al*., 2013).

The initial survival and evolution during ripening of *B. lactis* obtained for cheese batches are shown in Figure 2. The results of this study, corresponding to the counts of *B. lactis*, showed in all cases a decrease (~1 log-cycle), during the first 15 days. Later, probiotics cells survival remains almost constant until the end of cheese ripening process. Besides considering standard deviations, no significant differences were observed for different treatments at the end of the ripening process.

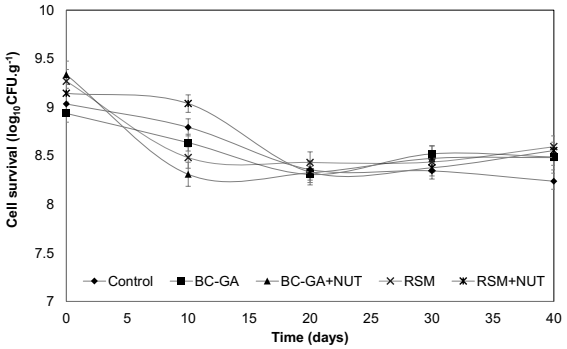

Fig. 2. Survival evolution of starter cultures (control) and *B. lactis* Bb-12 (probiotic and symbiotic) during ripening, for different treatments of gouda-type cheese stored at 9 °C (BC-AG=β-cyclodextrin arabic gum mixture; RSM=reconstituted skim milk; NUT=Nutriose®FB).

Table 2. Physicochemical properties of gouda-type cheese during ripening. (BC-AG=β-cyclodextrin arabic gum mixture; RSM=reconstituted skim milk; NUT=Nutriose®FB).

Analysis	Treatment	Time				
		Initial	10 days	20 days	30 days	40 days
pH	Control	$5.85\pm0.33^{ab,A}$	5.78 ± 0.01^{A}	5.90 ± 0.04^{A}	5.80 ± 0.01^{A}	5.89 ± 0.04^{A}
	BC-AG	$5.79\pm0.04^{b,A}$	5.75 ± 0.04^{A}	5.79 ± 0.07^{A}	5.79 ± 0.07^{A}	5.76 ± 0.07^{A}
	BC-AG+NUT	$5.12\pm0.07^{a,A}$	5.14 ± 0.03^{A}	5.44 ± 0.05^{A}	5.21 ± 0.10^{A}	5.37 ± 0.07^{A}
	RSM	$5.49\pm0.12^{ab,A}$	5.47 ± 0.07^{A}	5.75 ± 0.04^{A}	5.59 ± 0.05^{A}	5.52 ± 0.40^{A}
	RSM+NUT	$5.53\pm0.03^{ab,A}$	5.46 ± 0.02^{A}	5.71 ± 0.02^{A}	5.48 ± 0.02^{A}	5.69 ± 0.03^{A}
Moisture (%)	Control	$4.45\pm0.93^{a,A}$	5.80 ± 0.13^{A}	6.36 ± 0.04^{A}	6.63 ± 0.09^{A}	6.25 ± 0.12^{A}
	Control	$4.45\pm0.93^{a,A}$	5.80 ± 0.13^{A}	6.36 ± 0.04^{A}	6.63 ± 0.09^{A}	6.25 ± 0.12^{A}
	BC-AG+NUT	$57.78\pm0.90^{a,A}$	61.65 ± 0.42^{A}	62.81 ± 0.32^{A}	62.59 ± 1.04^{A}	66.01 ± 0.74^{A}
	RSM	$56.32\pm1.31^{a,A}$	58.11 ± 1.39^{A}	59.41 ± 0.67^{A}	59.14 ± 2.81^{A}	64.81 ± 1.71^{A}
	RSM+NUT	$57.97\pm2.17^{a,A}$	59.82 ± 0.38^{A}	62.16 ± 0.75^{A}	62.98 ± 0.72^{A}	66.72 ± 0.38^{A}
Ash (%)	Control	$4.45\pm0.93^{a,A}$	5.80 ± 0.13^{A}	6.36 ± 0.04^{A}	6.63 ± 0.09^{A}	6.25 ± 0.12^{A}
	BC-AG	$4.25\pm0.21^{a,A}$	5.60 ± 0.02^{A}	5.34 ± 0.06^{A}	5.49 ± 0.12^{A}	5.76 ± 0.05^{A}
	BC-AG+NUT	$3.82\pm0.02^{a,A}$	4.31 ± 0.04^{A}	4.04 ± 0.15^{A}	4.09 ± 0.10^{A}	4.47 ± 0.08^{A}
	RSM	$3.60\pm0.08^{a,A}$	5.37 ± 0.07^{A}	5.17 ± 0.07^{A}	5.62 ± 0.18^{A}	5.57 ± 0.17^{A}
	RSM+NUT	$4.85\pm0.15^{a,A}$	5.03 ± 0.06^{A}	5.84 ± 0.06^{A}	5.12 ± 0.06^{A}	5.83 ± 0.17^{A}
Fat (%)	Control	$22.55\pm0.71^{a,A}$	23.00 ± 0.50^{A}	22.50 ± 0.71^{A}	23.00 ± 1.41^{A}	23.00 ± 0.75^{A}
	BC-AG	$21.25\pm1.60^{a,A}$	23.25 ± 0.35^{A}	23.50 ± 0.70^{A}	24.00 ± 0.50^{A}	24.37 ± 1.19^{A}
	BC-AG+NUT	$21.75\pm0.35^{a,A}$	22.00 ± 0.50^{A}	22.00 ± 1.41^{A}	24.50 ± 1.71^{A}	23.75 ± 0.45^{A}
	RSM	$22.25\pm0.35^{a,A}$	22.25 ± 1.06^{A}	23.00 ± 0.70^{A}	22.00 ± 1.41^{A}	22.50 ± 1.10^{A}
	RSM+NUT	$22.00\pm1.41^{a,A}$	21.50 ± 1.12^{A}	22.50 ± 0.71^{A}	22.50 ± 0.05^{A}	23.00 ± 0.80^{A}
Proteins (%)	Control	$21.05\pm1.20^{ab,A}$	19.08 ± 1.00^{A}	20.05 ± 1.40^{A}	19.55 ± 1.00^{A}	21.50 ± 1.30^{A}
	BC-AG	$20.15\pm1.50^{ab,A}$	21.15 ± 1.50^{A}	20.81 ± 1.30^{A}	21.50 ± 1.60^{A}	22.40 ± 1.20^{A}
	BC-AG+NUT	$18.27\pm1.30^{a,A}$	19.72 ± 1.30^{A}	20.81 ± 1.50^{A}	19.97 ± 1.30^{A}	21.00 ± 1.40^{A}
	RSM	$20.83\pm1.60^{b,A}$	19.81 ± 1.80^{A}	21.38 ± 1.90^{A}	22.10 ± 1.40^{A}	21.95 ± 1.60^{A}
	RSM+NUT	$22.05\pm1.50^{b,A}$	23.50 ± 1.40^{A}	24.50 ± 1.50^{A}	24.00 ± 1.40^{A}	24.50 ± 1.20^{A}

Results are averages of three replicates.
At initial time, the mean values denoted by the same lowercase letters in each column (within investigated formulation) are not significantly different ($p < 0.05$; $n = 4$).
In each row, the mean values denoted by the same capital letters (within investigated ripening time evolution) are not significantly different ($p < 0.05$; $n = 4$).

According to several reports (Stanton *et al*., 2001; Kailasapathy, 2006; Masuda *et al*., 2005), the count of probiotic cells in a foodstuff should be approximately 10^7 CFU·g^{-1}, to provide a health beneficial effect. So, a daily ingestion of 10^8-10^9 CFU for each portion is recommended. In this study cheese exhibit an initial survival nearby 10^9 CFU·g^{-1} and no significant differences ($p < 0.05$) were found for samples with or without Nutriose®FB, during the ripening process.

Özer *et al*. (2008) observed the positive effects of microencapsulation, on viable counts of *B. bifidum* Bb-12 in Kasar-type cheese, these results show

that addition of encapsulated probiotics, into the cheese, enhances probiotic survival during ripening and storage. Similar results are shown by Hernández-Rodriguez *et al*. (2017), who demonstrated that entrapped *L. plantarum* cells within coacervates, incorporated in yogurt, did not suffer loss of survival during 21 days of refrigeration storage time, contrasting with high survival losses of free cells.

In addition to probiotic survival in the product until its consumption, survival after exposure to gastro intestinal tract (GIT) conditions is also crucial. Food matrices possess significant effects in successful delivery of probiotics into the intestine compared to delivery of these microorganisms via a liquid carrier or suspension (Abadía-García *et al*., 2013). Figure 3 shows results obtained under GIT simulated conditions for all treatments. Results for the control cheese represent the counting for starter mix culture, while for the probiotic and symbiotic cheeses represent only the counting for *Bifidobacterium lactis* added in microcapsules. For it, colonies were discriminated during the counting on behold of their appearance. As it can be seen, free starter mix culture do not resist under GIT conditions, obtaining concentration below 10^6 CFU·g^{-1} at the first stage of GIT simulation and even below 10^5 CFU·g^{-1} at the end of all GIT phases. In contrast, the cheeses enriched with encapsulated *B. lactis* using different encapsulant agents (BC-GA and MRS) and Nutriose®FB presented an increase of resistance under GIT conditions, with a final concentration from 10^7 to 10^9 CFU·g^{-1}. These results confirm, on the one hand, the effectiveness of the microencapsulation and, on the other hand, the almost complete release of the encapsulated bacteria.

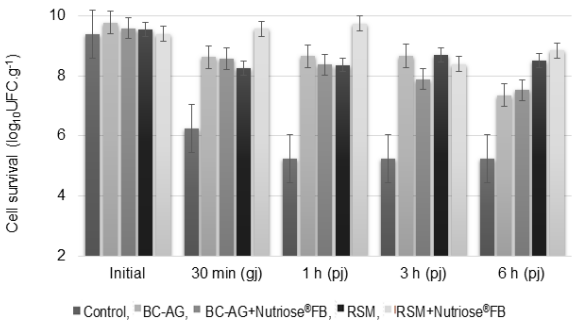

Fig. 3. Survival of free bacteria (control) and probiotic bacteria in formulated cheeses under gastrointestinal simulated conditions (gj=gastric juice at pH 1.9; pj=pancreatic juice at pH 7.5; RSM=reconstituted skim milk; BC-AG= β-cyclodextrin arabic gum mixture).

In fact, the orders of magnitude obtained in the systems enriched with probiotic powder correspond to the quantities added at the beginning of the process (~10^8-10^9 UFC·g^{-1}). It is important to note that the final concentration of viable free cells in the control cheese, at the end of the GIT simulation, is negligible compared to that of encapsulated cells (10^5 vs. $10^{7\sim9}$ UFC·g^{-1}). Furthermore, RSM microcapsules showed more stability until the end of GIT conditions, while BC-GA showed a reduction of 2 logarithmic-cycles. Nevertheless, all enriched cheeses keep their probiotic character throughout the process. As probiotic and symbiotic cheeses showed similar results for each encapsulant agent, it can be established that prebiotic Nutriose®FB only acts as an ingredient and do not promote probiotic proliferation during the ripening process.

Table 3. Fiber analysis obtained at the end of ripening period for different formulated cheeses (BC-AG=β-cyclodextrin arabic gum mixture; RSM=reconstituted skim milk).

Treatment	Insoluble fiber	Soluble fiber	Total fiber
		g·100 g$^{-1}_{cheese}$	
Control	0.05±0.01^a	2.08±0.16^a	2.13±0.17^a
BC-GA	0.48±0.08^b	3.42±0.04^a	3.91±0.05^a
RSM	0.20±0.11^a	3.63±0.05^a	3.83±0.17^a
BC-GA+Nutriose®FB	0.30±0.26^b	8.81±0.82^b	9.11±1.08^b
RSM+Nutriose®FB	0.05±0.04^a	8.31±0.41^b	8.36±0.37^b

The mean values denoted by the same letters in each column (within investigated formulation) are not significantly different ($p < 0.05$; $n = 4$).

Table 4. Initial and final microbiological analysis for different formulated gouda-type cheeses during ripening (BC-AG=β-cyclodextrin arabic gum mixture; RSM=reconstituted skim milk).

Treatment	*E. coli* (CFU/g)	Molds (CFU/g)	Yeasts (CFU/g)	*Coagulase-Positive Staphylococcus* (CFU/g)	*Salmonella spp.* (In 25 g)
Initial (day 1)					
Control	<10	48^a	10^a	<10	Absent
BC-AG	<10	47^a	10^a	<10	Absent
BC-GA+Nutriose®FB	<10	51^a	13^a	<10	Absent
RSM	<10	45^a	11^a	<10	Absent
RSM+Nutriose®FB	<10	47^a	12^a	<10	Absent
Final (40 days)					
Control	<10	26^a	4^a	<10	Absent
BC-AG	<10	17^a	4^a	<10	Absent
BC-GA+Nutriose®FB	<10	29^a	4^a	<10	Absent
RSM	<10	21^a	4^a	<10	Absent
RSM+Nutriose®FB	<10	19^a	4^a	<10	Absent

The mean values denoted by the same letters in each column (within investigated formulation) are not significantly different ($p < 0.05$; $n = 4$).

Similar results were obtained by Nejati *et al*., (2011) for a symbiotic ultra-filtrated (UF) cheese, enriched with encapsulated *Bifidobacterium lactis* Bb-12 and inulin as prebiotic. Their results showed that encapsulation can significantly increase the survival rate of probiotic bacteria during the cheese shelf life as well as *in-vitro* GIT simulated conditions, while the addition of inulin had no effect on these parameters. In Table 2, the comparison of the results of the statistical analysis applied to the initial time of the different treatments shows a significant difference in some parameters (pH, acidity and proteins). However, they can be attributed to the inherent variability of the manufacturing process since lots of cheeses were produced for each treatment.

In addition, the small differences found are within the ranges of variability found in commercial Gouda cheeses (Bazaes, 2004; Ameerally, 2015). Based on this, to analyze the evolution during the ripening, the results for each physicochemical property were contrasted as a function of time in each treatment. In this way, no significant differences were observed at the different times analyzed.

3.1 Determination of fiber content (Nutriose®FB)

The addition of Nutriose®FB did not show significant effect ($p < 0.05$) on the physicochemical properties of symbiotic cheeses as shown in Table 2. However, comparing the results of the total fiber content, as expected, the addition of prebiotic Nutriose®FB had a significant effect ($p < 0.05$) (Table 3). These symbiotic cheeses showed the highest content of soluble and total fiber. So, it can be hypothesized that prebiotic was not metabolized during the ripening process. Hence, it will be available for probiotic bacteria during the gastrointestinal process, fulfilling in this way its prebiotic character.

3.2 Sanitary quality of functional cheese

The microbiological analysis results for *Salmonella spp.*, *Escherichia coli*, *Staphylococcus aureus* as well as molds and yeast are shown in table 4. The functional cheeses (probiotic and symbiotic) were analyzed at the beginning and end of the ripening process. The analysis showed a low microbiological contamination.

In the industrial dairy products, the pathogens are generally contained in raw milk. Then, they may colonize the environment as well as equipment or final products (Kousta *et al*., 2010). The concentration of all pathogen microorganisms in the different cheeses (Table 5), was below the maximum allowable limits considering the official Mexican regulations for dairy products (NOM-243-SSA1-2010) for each microorganism evaluated, which means that the milk was pasteurized properly and the good manufacturing practices (GMP) were respected. Similar results were obtained by Zamora-Vega *et al*. (2012), they developed and characterized a functional food (fresh cheese) incorporating a probiotic microorganism (*Sacharomyces boulardii*) and a prebiotic agent (inulin), both encapsulated with sodium alginate and cactus mucilage. The results obtained showed that sanitary quality of symbiotic cheese is not modified by the addition of probiotic bacteria or symbiotic compounds, if the GMP are respected.

Conclusions

Functional matured cheeses with physicochemical and sanitary characteristics similar to a conventional gouda-type cheese were obtained. Spray drying by microencapsulation using different encapsulant agents (RSM, BC-AG) allowed to obtain a high probiotic survival ($>10^8$ CFU·g^{-1}_{cheese}) after the ripening period as well as all the stages of gastrointestinal simulated conditions ($>10^7$ CFU·g^{-1}_{cheese}). In addition, enriched cheeses with Nutriose®FB (symbiotic cheeses) exhibited the highest concentration of soluble fiber, characteristic greatly desirable in functional foods. Therefore, this research proved the stability of the elaborated microcapsules, which effectively protected the probiotic microorganism but also do not affect the main characteristics of this food, which establishes the possibility of producing functional cheeses without changes that affect the acceptance by the consumer.

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References

Abadía-García, L., Cardador A., del Campo S. T. M. (2013). Influence of probiotic strains added to cottage cheese on generation of potentially antioxidant peptides, anti-listeria activity, and survival of probiotic microorganisms in simulated gastrointestinal conditions. *International Dairy Journal 33*, 191-197. http://dx.doi.org/10.1016/j.idairyj.2013.04.005

Albenzio, M., Santillo, A., Caroprese, M., Ruggieri, D., Napolitano, F., Sevi, A. (2013). Physicochemical properties of Scamorza ewe milk cheese manufactured with different probiotic cultures. *Journal of Dairy Science 96*, 2781-2791. http://dx.doi.org/10.3168/jds.2012-6218

Ameerally, A.D. (2015). Sensory and Chemical Properties of Gouda Cheese. M.Sc. Thesis. Raleigh, North Carolina State University. Pp. 98. Available on https://pdfsecret.com/download/under-the-direction-of-maryanne-drake_5a3268e9d64ab2123392948c_pdf

AOAC. (2000). *Official Methods of Analysis*. 17th Edition, Association of Official Analytical Chemists, Arlington, VA.

Barbosa-Cánovas, G. V., Ortega-Rivas, E., Juliano, P., Yan, H. (2005). Drying. In *Food Powders-Physical Properties, Processing and Functionality*. First Edition, Kluwer Academic/Plenum Publishers, New York, 271-304.

Bazaes M.E. (2004). Características de calidad química y sensorial de queso Gauda. B.Sc. Thesis. Valdivia, Univ. Austral de Chile. Fac. Cien. Agr. Pp. 95. Available on http://cybertesis.uach.cl/tesis/uach/2004/fab362c/pdf/fab362c.pdf

Borges, M.V., Alves, M.E., Chavez M.A., Egito, A.S., Gross E., Ferrao S.P.B. (2017). Chemical, structural and proteomic profile of Buffalo milk powder produced in mini spray dryer. *Revista Mexicana de Ingeniería Química 16*, 64-67. Available on http://www.redalyc.org/articulo.oa?id=62049878008

Boylston, T.D., Vinderola, C.G., Ghoddusi, H.B., Reinheimer, J.A. (2004). Incorporation of bifidobacteria into cheeses: challenges and rewards. *International Dairy Journal 14*, 375-387. https://dx.doi.org/10.1016/j.idairyj.2003.08.008

Dianawati, D., Mishra, V., Shah, N.P. (2016). Survival of microencapsulated probiotic bacteria after processing and during storage: a review. *Critical Reviews in Food Science and Nutrition 10*, 1685-1716. http://doi.org/10.1080/10408398.2013.798779

Gomes da Cruz, G., Alonso Buriti, F.C, Batista de Souza, C.H., Fonseca Faria, J.A., Isay Saad, S.M. (2009). Probiotic cheese: health benefits, technological and stability aspects. *Trends in Food Science & Technology 20*, 344-355. http://dx.doi.org/10.1016/j.tifs.2009.05.001

Dantas, A. B., Jesús, V. F., Silva, R., Almada, C. N., Esmerino, E. A., Cappato, L. P., Silva, M. C., Raices, R. S. L., Cavalcanti, R. N., Carvalho, C. C., Sant'a, A. S., Bolini, H. M. A., Freitas, M. Q., Cruz, A. G. (2016). Manufacture of probiotic Minas Frescal cheese with *Lactobacillus casei*. *Journal of Dairy Science 99*, 18-30. http://dx.doi.org/10.3168/jds.2015-9880

Doherty, S. B., Wang, L., Ross, R. P., Stanton, C., Fitzgerald, G. F., Brodkorb, A. (2010). Use of viability staining in combination with flow cytometry for rapid viability assessment of *Lactobacillus rhamnosus* GG in complex protein matrices. *Journal of Microbiological Methods 82*, 301-310. http://dx.doi.org/doi:10.1016/j.mimet.2010.07.003

Fang, Z., Bhandari B. (2010). Encapsulation of polyphenols - a review. *Trends Food Science Technology 21*, 510-523. http://dx.doi.org/10.1016/j.tifs.2010.08.003

Fu, N., Chen, X. D. (2011). Towards a maximal cell survival in convective thermal drying processes. *Food Research International 44*, 1127-1149. http://dx.doi.org/10.1016/j.foodres.2011.03.053

Hernández-Rodríguez L., Lobato-Calleros, C., Ramírez-Santiago, C., Rodríguez-Huezo, M.E., Meraz, M. (2017). Microstructure and rheology of yogurt added with protein-*L. plantarum*-polysaccharide coacervate and stevia in substitution of milk-fat and sucrose. *Revista Mexicana de Ingeniería Química 16*, 77-89. Available on http://www.redalyc.org/articulo.oa?id=62049878009

IDF. (1982). *International Dairy Federation*. Cheese and processed cheese, determination of the total solids content. International Dairy Federation IDF Standard 4A. International Dairy Federation, Brussels, Belgium.

IDF. (1988). *International Dairy Federation*. Cheese and cheese products, determination of chloride content, potentiometric titration method. International Dairy Federation IDF Standard 88A. International Dairy Federation, Brussels, Belgium.

IDF. (1993). *International Dairy Federation*. Milk-Determination of nitrogen content. International Dairy Federation IDF Standard 20B. International Dairy Federation, Brussels, Belgium.

IDF. (1997). *International Dairy Federation*. Milk and milk products: Determination of fat content (general guidance on the use of butyrometric methods). International Dairy Federation IDF Standard 152A. International Dairy Federation, Brussels, Belgium.

Kailasapathy, K., Masondole, L. (2005). Survival of free and microencapsulated *Lactobacillus acidophilus* and *Bifidobacterium lactis* and their effect on texture of feta cheese. *The Australian Journal of Dairy Technology 60*, 252-258.

Kailasapathy, K. (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yogurt. *LWT-Food Science Technology 39*, 1221-1227. https://doi.org/10.1016/j.lwt.2005.07.013

Karimi, R., Mortazavian, A. M., Karimi, M. (2012a). Incorporation of *Lactobacillus casei* in Iranian ultrafiltered Feta cheese made by partial replacement of NaCl with KCl. *Journal of Dairy Science 95*, 4209-4222. https://doi.org/10.3168/jds.2011-4872

Karimi, R., Sohrabvandi, S., Mortazavian M. (2012b). Sensory characteristics of

probiotic cheese. *Comprehensive Reviews in Food Science and Food Safety 11*, 437-452. http://dx.doi.org/10.1 111/j.1541-4337.2012.00194.x

Karimi, R., Azizi, M. H., GhasemLou, M., Vaziri, M. (2015). Application of inulin in cheese as prebiotic, fat replacer and texturizer: a review. *Carbohydrate Polymers 119*, 85-100. https://doi.org/10.1016/j.carbpol.2014.11.029

Kousta, M., Mataragas, M., Skandamis, O., Drosinos, E.H. (2010). Prevalence and sources of cheese contamination with pathogens at farm and processing levels. *Food Control 21*, 805-815. https://doi.org/10.1016/j.foodcont.2009.11.015

Lefranc-Millot, C., Wils, D., Roturier, J.M. (2009). NUTRIOSE® soluble fiber. In: *Fiber Ingredients-Food Applications and Health Benefits* (Cho SS, Samuel P, eds). New York: CRC Press, 19-40.

Lian, W.C., Hsiao, H.C., Chou, C.C. (2002). Survival of bifidobacteria after spray-drying. *International Journal Food Microbiology 74*, 79-86.

Lollo, P. C., Cruz, A. G., Morato, P. N., Moura, C. S., Carvalho, L. B., Oliveira, C. A., Amaya-Farfan, J. (2012). Probiotic cheese attenuates exercise-induced immune suppression in Wistar rats. *Journal of Dairy Science 95*, 3549-3558. http://dx.doi.org/10.3168/jds.2011-5124

Lollo, P. C. B., Morato, P. N., Moura, C. S., Almada, C. N., Felicio, T. L., Esmerino, E. A., Cruz, A. G. (2015). Hypertension parameters are attenuated by the continuous consumption of probiotic minas cheese. *Food Research International 76*, 611-617. https://doi.org/10.1016/j.foodres.2015.07.015

Martín, J.M., Lara-Villoslada, F., Ruíz, M.A., Morales, M.E. (2015). Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. *Innovative Food Science & Emerging Technologies 27*, 15-25. https://doi.org/10.1016/j.ifset.2014.09.010

Mattila-Sandholm, T., Myllärinen, P., Crittenden, R., Mogensen, G., Fondén, R., Saarela, M. (2002). Technological challenges for future probiotic foods. *International Dairy Journal 12*, 173-182. https://doi.org/10.1016/S0958-6946(01)00099-1

Masuda T, Yamanari R, Itoh T (2005). The trial for the production of fresh cheese incorporated probiotic *Lactobacillus acidophilus* group lactic acid bacteria. *Milchwissenschaft 60*, 167-171.

Mendoza-Madrigal, A.G., Durán-Páramo E., Valencia del Toro G., Chanona-Pérez J.J., Celeste Martínez-Ramírez O., Arzate-Vázquez, I. (2017). Survival kinetics of free and immobilized *Bifidobacterium bifidum* in presence of food samples under gastrointestinal in vitro conditions. *Revista Mexicana de Ingeniería Química 16*, 159-168. Available on http://www.redalyc.org/articulo.oa?id=62049878016

Muehlhoff, E., Bennett, A., McMahon, D. (2013). Milk and diary products in human nutrition, First Edition, FAO, Rome, 43-63. Available on http://www.fao.org/docrep/018/i3396e/i3396e.pdf

Nuñez, M., Bautista, L., Medina, M., Gaya, P. (1988). *Staphylococcus aureus*, thermostable nuclease and staphylococcal enterotoxins in raw ewes' milk Manchego cheese. *Journal of Applied Bacteriology 65*, 29-34.

Ortakci, F., Broadbent, J. R., Mcmanus, W. R., Mchahon, D. J. (2012). Survival of microencapsulated probiotic *Lactobacillus paracasei* LBC-1e during manufacture of Mozzarella cheese and simulated gastric digestion. *Journal of Dairy Science 95*, 6274-6281. https://doi.org/10.3168/jds.2012-5476

Özer, B., Kirmaci, H.A., Şenel, E., Atamer, M., Hayaloğlu, A. (2008). Improving the survival of *Bifidobacterium bifidum* BB-12 and *Lactobacillus acidophilus* LA-5 in White-brined cheese by microencapsulation. *International Dairy Journal 19*, 22-29. https://doi.org/10.1016/j.idairyj.2008.07.001

Picot, A., Lacroix, C. (2004). Encapsulation of Bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yogurt. *International Dairy Journal 14*, 505-515. https://doi.org/10.1016/j.idairyj.2003.10.008

Nejati, R., Gheisari, H., Hosseinzadeh, S., Amin, H. (2011). Viability of encapsulated *Bifidobacterium lactis* (BB-12) in symbiotic UF cheese and its survival under *in-vitro* simulated

gastrointestinal conditions. *International Journal of Probiotics & Prebiotics 6*, 197-203.

Saénz, C., Tapia, S., Chávez, J., Robert, P. (2009). Microencapsulation by spray drying of bioactive compounds from cactus pear (*Opuntia ficus-indica*). *Food Chemistry 114*, 616-622. https://doi.org/10.1016/j.foodchem.2008.09.095

Stanton, C., Gardiner, G., Meehan, H., Collins, K., Fitzgerald, G., Lynch, P.B., Ross R.P. (2001). Market potential for probiotics. *American. Journal Clinical Nutrition 73*, 476-483.

Souza, M. W., Ferreira, T. B., & Vieira, I. F. (2008). Composição centesimal e propiedades funcionais tecnológicas da farinha da casca do maracujá. *Alimentos e Nutrição 19*, 33-36. Avalaible on http://serv-bib.fcfar.unesp.br/seer/index.php/alimentos/article/view/197/202

Takanashi, S., Miura, A., Abe, K., Uchida, J., Itoi, S., Sugita, H. (2014). Variations in bile tolerance among *Lactococcus lactis* strains derived from different sources. *Folia Microbiologica 59*, 289-293. https://doi.org/10.1007/s12223-013-0297-8

Tornadijo, M.E., Fresno, J.M., Bernardo, A., Martin Sarmiento, R., Carballo, J., (1995). Microbiological changes throughout the manufacturing and ripening of Spanish goat's raw milk cheese (Armada Variety). *Le Lait 75*, 551-570.

Zamora-Vega, R., Montañez-Soto, J.L., Martínez-Flores, H.E., Flores-Magallón, R., Muñoz-Ruíz, C.V., Venegas-González, J., Ariza-Ortega, T.J. (2012). Effect of incorporating prebiotics in coating materials for the microencapsulation of *Sacharomyces boulardii*. *International Journal of Food Science Nutrition 63*, 930-935. https://doi.org/10.3109/09637486.2012.687364