MICROENCAPSULATION OF CALCIUM LACTOBIONATE FOR PROTECTION FROM MICROORGANISMS IN A SOLID PHASE FOOD

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ABSTRACT

Prebiotic compounds may be consumed during storage by the foodstuff's own microflora, so that new processes to protect these bioactive compounds before they are released in the gastrointestinal tract need to be developed. With this aim, lyophilized calcium lactobionate microparticles were designed, employing different coat materials (caseinate, gelatine, gum arabic, maltodextrin and mixtures of gum arabic and maltodextrin), some of which had previously been treated with transglutaminase enzyme. The microparticles were introduced into cottage cheese, as an example of a matrix with low water activity, and were found to exert a successful protective effect against consumption of lactobionate by lactic acid bacteria present in the cheese. Diffusion of the calcium lactobionate to the surface of the microparticles and diffusion of the lactic bacteria into the microparticles were negligible during the tested period. A model was developed to simulate the lactobionate consumption in the microparticles-cheese system. All the microparticles tested had good protective characteristics and no important improvement was observed when transglutaminase was added. Microparticles of sodium caseinate were chosen for the final digestibility analyses. Subsequently, in the digestibility tests, the calcium

lactobionate was released from sodium caseinate microparticles in the cottage cheese under both gastric and intestinal conditions. Therefore, this study presents a protective microparticle process for use in functional food products.

Keywords: prebiotic, calcium lactobionate, microencapsulation, cottage cheese, functional dairy product.

1. Introduction

Nowadays, there is a great demand for high quality food products with no risk of any ill effects and which, besides satisfying the consumers' nutritional demands, have the capacity to improve their health. To fulfil this perceived need, what has been called "functional food" has received growing attention recently. According the International Life Sciences Institute (ILSI), functional food can be defined as "that which beneficially affects one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease" [1].

The functional food market is currently growing, and Japan is one of the main producers and consumers. About 45% of Japanese functional foodstuffs aim to help with the maintenance of good gastrointestinal tract (GIT) conditions [2]. The GIT microbiota is arranged in a complex ecosystem which directly affects human nutrition and health. Thus, it is of interest to increase the number of beneficial bacteria to the point where they represent more than half the population. There are two ways to achieve this goal: using living bacteria, known as probiotics, and/or employing prebiotic compounds [1].

Prebiotics constitute a vast variety of compounds. Within this group, lactose derivates such as lactobionic acid are of great interest. This compound has received attention from

the food industry due to its properties as an antioxidant, stabilizer and gelling agent [3], but also for its prebiotic effect. CaLb consumption in the colon by the human microflora is capable of improving the growth of some probiotics of the *Bifidobacterium* genus [4] [5]. Lactobionic acid has been approved for use as calcium lactobionate (CaLb) by the FDA (FDA, 2017), although it is still being studied by European Committees [3].

Functional foods incorporate bioactive compounds which are usually sensitive to chemical and physical factors [7]. Specifically, for prebiotics, introducing them directly into food products leads to the risk of their being consumed by the foodstuff's own microflora. If that happens, the prebiotics will not be able to reach the consumers' GIT and so promote the growth of beneficial probiotics. To deal with the problems of the degradation of bioactive-compounds, the food industry has developed several tools, such as microencapsulation ([7], [8]). There are many microencapsulation techniques, but that of lyophilization allows the creation of microparticles that are stable over time [9]. There have been several studies on the microencapsulation of bioactive compounds, the vast majority of which do not include experimental investigation of the incorporation of microparticles into food matrices. The most usual food matrices employed to test microparticles are dairy products ([10], [11], [7]). The use of low water activity (a_w) food matrices such as bread is also common [12], as is the use of cereals.

As far as is known, there are few prebiotic microencapsulated products on the market. In this respect, the purpose of the present research was to develop and characterize CaLb microparticles, employing different coat materials and transglutaminase enzyme (TGase), with the objective of improving the microparticle properties. After analysing the microparticles, they were tested in cottage cheeses as an example of a food matrix with low a_w. The aim was to determine the protective effect of microparticles against the consumption of lactobionic acid by the cottage cheese's own microflora and to generate

an innovative functional dairy product that could be attractive for both consumers and the food industry.

2. Materials and methods

2.1.Materials

Several coat materials with different concentrations (w/w) were employed as described by other authors ([9], [13], [14], [15]). Sodium caseinate (NaCas), maltodextrin (MD) and gelatine (Gel) were purchased at Sigma-Aldrich Chemical Co. (Steinheim, Germany). Gum arabic (GA) was provided by Panreac (Panreac S.A., Barcelona Spain). CaLb (≥ 98% purity) was supplied by Sigma-Aldrich.

2.2.Microparticle preparation

Mixtures of 0.05 g mL $^{-1}$ CaLb and the different coat agent solutions were prepared at a ratio of 1:3 and they were frozen at -80 ºC for 12 hours. Microparticles were produced using the lyophilisation method (Tesltar Cryodos, 0.1 mBar, -70 ºC for 24 hours). After lyophilisation, microparticles were ground and sieved with a pore-size of 355 µm and stored in a dry atmosphere until use. The final concentration of CaLb for each microparticle type after preparation is indicated in Table 1.

Mixtures of NaCas and Gel with the active material were also treated with transglutaminase (TGase), in an attempt to improve their properties. In this case, pH was adjusted to 7.0 with 1M NaOH (Sigma-Aldrich) and TGase was added (0.33 g L^{-1}, 50 U g^{-1} protein) (Probind TX, BDF ingredients, Spain). The reaction was conducted in an oven at 45 ºC for 90 min. After that time, the temperature was increased to 70 ºC for 10 min to deactivate the enzyme. As in the case of the other microparticles, they were produced by the lyophilization method.

2.3.Microparticle characterization

2.3.1. *Encapsulation efficiency*

Encapsulation efficiency (EE) was calculated according to Cilek *et al.* (2012). EE is defined as the ratio of encapsulated active compound content (EAC) to total active compound (TAC). EAC is determined by calculating the difference between TAC and the surface-active compound content (SAC) (Equation 1).

$$EE=[(TAC-SAC)/TAC]x100 \quad (1)$$

To determine SAC, 1 mL of distilled water was added to 0.005 g of microparticles and they were gently shaken for 10 minutes. Samples were centrifuged at 13,200 rpm (Centrifuge 5415D, Sigma-Aldrich) for 5 minutes. The supernatant was used to measure SAC as described in section 2.5.

2.3.2. *Solubility*

This test investigates which kind of microparticle prevents further release of CaLb. 1 mL of distilled water was added to 0.005 g of each type of microparticle. Samples were incubated at 20 ºC and 300 rpm for 30 min (Thermomixer, Eppendorf, Hamburg, Germany). After centrifugation (13,200 rpm for 5 min), the supernatant was collected and analysed as described in section 2.5.

2.3.3. *Microstructure characterization*

The morphology of the microparticles was observed using scanning electron microscopy (SEM) (JSM-6610LV, JEOL, USA). Microparticles were mounted on stubs and coated with gold. The surface morphology of the microparticles was observed with magnifications of 100x.

2.4.Functionalization of cheese with calcium lactobionate

2.4.1. *Functional cottage cheese manufacture*

Milk was obtained from a herd of Murciano-Granadino goats from a local farm in San Martín del Rey Aurelio (Asturias, Spain). Cottage cheeses were made with low temperature-long time (LTLT) pasteurised milk, heated at 60 ºC for 25 minutes. This gentle pasteurization process allows some lactic acid bacteria (LAB) to survive, so it was not necessary to use a starter culture. Subsequently, milk was cooled to 34 ºC and rennet (Chy-Max®, CHR-Hansen, Denmark) was added (0.0225 g L^{-1}). After 40 minutes, the curd was cut several times to stimulate syneresis. Approximately 0.005 g of each microparticle type was mixed carefully with approximately 40 g of curd. The concentration of CaLb in the global cheese-microparticles mix is shown in Table 1 for all the tests. Cheeses were stored at 21 ºC for a day and then at 4 ºC during the maturation period for further analysis.

Analysis of microparticles inside functional cottage cheeses was carried out using 5 g samples of each cheese. 20 mL of H_2SO_4 (0.013N) (Sigma-Aldrich) [16] was added and samples were homogenised using a Stomacher®80 (Seward, United Kingdom) for 90 seconds at maximum speed. Afterwards, samples were heated at 64 ºC for 30 minutes. Finally, samples were centrifuged for 10 minutes at 13,200 rpm and the supernatant was stored until analysis as described in section 2.5.

2.4.2. *Textural analysis*

For textural characterisation, a TA.XTplus Texture Analyzer (Stable Systems, Godalming, Surrey, United Kingdom) was employed. Cottage cheese samples were subjected to a penetration test, at room temperature, using the spherical probe SMS P/0.5S with a test speed of 2.0 mm s^{-1} and a load cell of 5 kg. Results are expressed in terms of

firmness and stickiness values (grams). Experiments were triplicated and reported results correspond to the mean value.

2.4.3. *Digestibility test*

Gastric and intestinal conditions were simulated according to Minekus et al. (2014), with some modifications. The composition of the simulated gastric fluid (SGF) employed was 0.517 g L^{-1} KCl, 0.123 g L^{-1} KH_2PO_4, 2.106 g L^{-1} $NaHCO_3$ and 2.75 g L^{-1} NaCl. Simulated intestinal fluid (SIF) was made by mixing 0.509 g L^{-1} KCl, 0.110 g L^{-1} KH_2PO_4, 11.68 g L^{-1} $NaHCO_3$ and 2.24 g L^{-1} NaCl.

For the gastric simulation, 5 grams of cottage cheese were mixed with 7.5 ml SGF, 1.6 mL of pepsin (863 U mg^{-1} protein, CAS 9001-75-6, Sigma-Aldrich), with a concentration of 15.15 g L^{-1}, using SGF as solvent, 5 µL of $CaCl_2$ 0.3M (Sigma-Aldrich) and 0.696 µL of distilled water. The pH was adjusted to 3.0 with 1M HCl (Sigma-Aldrich). The mixtures were shaken at 300 rpm and 37 ºC for 90 minutes and samples were collected at 45 and 90 minutes.

After gastric simulation, intestinal simulation was carried out. 4.95 mL of SIF, bovine chymotrypsin (0.3% w/v) (60 U mg^{-1} protein, EC 232-671-2, Sigma-Aldrich), porcine pancreatin (0.1% w/v) (Sigma-Aldrich), 40 µL of 0.3M $CaCl_2$ and 1.3 mL of distilled water were added to the previous mixes. The pH was adjusted to 7.0 using 1M NaOH (Sigma-Aldrich). The mixtures were shaken at 300 rpm and 37 ºC for 2 hours and samples were collected at 15, 30, 45, 60 and 120 minutes. All samples taken at different times were centrifuged (13,200 rpm for 5 minutes) and filtered with a 0.45 µL syringe filter (Whatman, Sigma-Aldrich) before analysis.

2.4.4. *Morphological analysis*

Structural analysis was carried out to search for any changes in the cottage cheese structure when microparticles were added. Cottage cheeses were frozen at -80 ºC o/n and then were lyophilised (0.1 mBar, -70 ºC for 24 hours). Morphology was studied using SEM as described in section 2.3.3.

2.5.Analytical methods

CaLb was measured as lactobionic acid using High Performance Liquid Chromatography (HPLC). The system of liquid chromatography employed (Agilent 1200, Agilent Technologies Inc., Santa Clara, CA, USA) was equipped with a Coregel ION 300 column (Teknokroma, Barcelona, Spain) coupled to a refractive index detector. The mobile phase was a sulphuric acid solution (0.450 mmol L^{-1}, pH 3.1) with a flow rate of 0.3 mL min^{-1} and a column temperature of 75 ºC. Data acquisition and analysis were performed with ChemStation software (Agilent).

3. Results and discussion

3.1.Characterization of microparticles

3.1.1. *Encapsulation efficiency (EE)*

EE indicates the amount of active compound encapsulated with respect to the initial quantity used. Experiments were performed in triplicate and results are shown as the mean value with the standard deviation (Table 2).

The NaCas and Gel microparticles treated with TGase showed an improvement in the EE, NaCas being the better core material (Table 2). TGase catalyses the acetyltransferase reaction (intra or inter chain) between the γ-carboxamide groups of glutamic residues and the ε-amine groups of the lysine residues [18], which creates crosslinking. It has been shown that treatment with this enzyme improves the microparticle characteristics of

different kinds of core material, leading to a higher EE [19]. It is also capable of enhancing water retention [20] and as CaLb is a hydrophilic compound, core material treated with TGase achieved a better EE.

In the case of MD and GA, the EE values increased when more core material was employed. GA 8% showed an EE of 88.4±1.8%, while at 4% the EE decreased to 64.8±2.8%. Something similar happened with MD microparticles, where MD 12% showed a reduction of 10% in its EE value with respect to MD 16%. So, the greater the amount of core material, the higher the encapsulation efficiency. Other authors explain it as a faster precipitation of the core material [21].

Mixtures of MD and GA had the highest amount encapsulated and it can be seen that the EE rose when there was a higher proportion of GA. This can be explained by the properties of GA as a stabilizing and emulsifying agent [13] and by the fact that GA has a higher molecular weight, a characteristic that enables it to retain more active material [22].

3.1.2. *Microparticle solubility*

Solubility experiments were performed in triplicate and results are shown as the mean value with the standard deviation (Table 2). Excepting GA:MD mixtures, the rest of the microparticles have a CaLb release of 60%. It is a high percentage, as expected, because most coating materials employed were hydrosoluble.

GA:MD mixtures were extremely water-resistant, but GA and MD separately were not. The two polysaccharides together form water-resistant microparticles, possibly as a result of some interaction that leads to longer polysaccharide chain length, which implies lower solubility [8]. Gel was also soluble even after being treated with TGase and was even less water resistant. TGase activity may form some new bonds but others might be destroyed

due to physical and chemical factors like pH or temperature [18] changing the gelatine structure and thus affecting parameters like solubility. NaCas and NaCas-TGase had a mean solubility value of 69.1%. Caseins had a low solubility in an acid environment [23] but the salt form increases solubility.

Excluding GA:MD mixtures, the results demonstrated that these microparticles were not appropriate for addition to food matrixes with high $a_{w,}$ such as milk, but that they could be effective in food products with a low $a_{w,}$ like cheeses or bread. This is why microparticles have been used more frequently in such foods. There are several examples of the use of microparticles in such foods; for instance, casein microparticles were used with citric acid for chewing gums [24] or whey and MD microparticles with fruit extract for bread [12].

3.1.3. *Microcapsule morphology*

SEM was employed to study surface morphology (Figure 1). The visual appearance of all the microparticles was similar: a white powder with a particle size ≤ 355 μm.

There was no morphological difference between the microparticles treated with TGase and those that had not been treated. Only the Gel microparticles had a different appearance, with a less uniform surface and less compact structure (Figure 1C and D), while the others had a generally uniform appearance, despite having irregular shapes, and were smooth and with no pores (Figure 1E-K). Microparticle morphology is related to the active compound and the method employed to produce the microparticles. In this study, after lyophilisation, the microparticles were crushed and sifted and, as a result, all microparticles had a uniform and irregular shape despite the core material. Similar surface morphologies were obtained when biopolymers, such as chitosan, xanthan or β-

cyclodextrin, were employed as coat material with phenolics as active compounds in lyophilised microparticles developed by other authors [25] [26].

3.2.Functionalization of cheese with microparticles

Milk and dairy products have been the preferred food matrices for the incorporation of microparticles [7]. Some active compounds added to dairy products are plant-derived [11], vitamins [27] and organic acids [10]. There are very few studies investigating prebiotic microencapsulation. Employing dairy products in which probiotics are present together with a prebiotic that is protected against consumption by the food probiotic allows the production of synbiotic products [28] with interesting health properties and that are appealing to consumers.

3.2.1. *Evolution of CaLb regarding time*

After being characterized, the microparticles were added to the cheese matrix. CaLb was measured at initial time (t 0) and after 5 and 12 days. Experiments were performed in triplicate and results of the evolution with time of the concentration of CaLb for NaCas, NaCasTG, Gel, GA 4%, MD 16 % and the positive control (free CaLb without encapsulation in microparticles) are presented in the Figure 2 as the points at 0, 5 and 12 days.

Due to the LTLT process, the concentration of CaLb decreased over time because some LAB were still alive inside the cheese matrix. CaLb is used as substrate by some LAB [4] but microparticles provided protective capacity against bacterial consumption, as can be seen. The high consumption of CaLb by the LAB can be observed in the positive control. In the case of the Gelatine (Gel), the protective effect was low and therefore the consumption was relatively high. These results agree with the low EE of the Gel shown in Table 2. The concentrations of CaLb contained in NaCas, GA and MD microparticles

underwent only very low variations after 12 days, indicating a very low consumption by the LAB, and therefore a good level of protection. A comparison of the results for NaCas and NaCasTG indicate a small increase in the degree of protection against consumption when the core material was treated with TGase. For the mixtures GA:MD tested (data not shown) the behaviour was similar to GA and MD alone, no significant variations were found.

The real amount of active compound, CaLb, inside the microparticles was between 0.5 and 2.8 mg of CaLb g^{-1} of cottage cheese. This may seem a very large variation in CaLb content, but what is really significant is that even the lowest value is much greater than the amount found in the only CaLb-containing food product marketed today. This is Caucasian yogurt ("Caspian Sea yogurt"), which is sold in Japan, where FOSHU ("Food for specified health use") products have been extensively developed. This is the only yogurt that contains CaLb and its concentration is approximately 0.45 mg of CaLb g^{-1} of yogurt [29]. Protecting CaLb against LAB consumption by employing microparticles allowed an increase around 60% in the concentration of CaLb respect the commercial product. The FDA and committees are investigating suitable concentrations for consumption (FDA, 2017) but there is no specific regulation at the moment. There are several studies and some authors have concluded that amounts of up to 24 g of CaLb per day were well tolerated [4]. So, developing innovative new products with high concentrations of CaLb could be particularly interesting from a technological point of view. Specifically, NaCas and NaCas-TGase microparticles had a concentration of 1.14±0.03 and 1.19±0.04 mg of CaLb g^{-1} of cottage cheese respectively. Casein is found naturally in dairy products as the major protein and therefore, employing NaCas microparticles for developing functional dairy products will be accepted more readily by consumers, as it does not involve the use of foreign proteins or sugars.

3.2.2 *Modelling of CaLb transport and consumption in cheese*

A schematic description of the experimental work considers two phases, the nanoparticles phase and the cheese phase. The CaLb is present initially in the nanoparticles phase; there will be diffusion, J_1, to the surface, where there could be some biodegradation, and then diffusion to the bulk cheese phase, J2. The biodegradation reactions will take place in the neighbourhood of the interphase and in the cheese bulk. The concentrations of CaLb in the nanoparticles, interphase and cheese bulk will be c_p, c_s, and c_b, while the concentration of the bacterial population will be assumed as uniform in the cheese phase (Figure 3).

A general mass balance for the cheese phase will consider the accumulation of CaLb to be equal to its mass transfer ($J_1 = k_s(c_p - c_s)$) to the surface, minus its consumption at the surface $\left(Ak_s c_s X_s Y^s_{x/s}\right)$ and in the cheese bulk ($Vk_x X_s Y^s_{x/s}$), which would give

$$V_b \frac{dc_b}{dt} = J_1 - \sum r = J_1 - \left[\left(Ak_s c_s X_s Y^s_{x/s}\right) + \left(V_b k_x X_b Y^b_{x/s}\right)\right]. \qquad (2)$$

A being the interphase surface, V_b the volume of the cheese bulk, K_s and K_x the kinetic constants, Xs the biomass in the interphase and $Y_{x/s}$ the biomass/substrate stoichiometric coefficient.

A global mass balance for the cheese-microparticles mix could also be of interest in order to obtain experimental data (Figure 2) for the global concentration *c*. Experiments carried out to investigate the biodegradation of CaLb homogeneously distributed in the cheese have indicated a rapid degradation process, so the reaction would seem to occur near the interphase. Assuming a fast reaction which could take place in the cheese and in the nanoparticles film, and clustering the parameters, simplified equations can be obtained for the analysis of the experimental results.

A local mass balance for the nanoparticles phase will give $\frac{dC_p}{dt} = k'(c_p - c_s)$

(3)

and if c_s @0 we will obtain $\frac{dC_p}{dt} = k'c_p$

A global mass balance over time gives rise to

$$V_p c_{po} = Vc + \int_0^t V_p \frac{dc_p}{dt} dt = Vc + \int_0^t V_p k' c_p \, dt \quad (4)$$

The term c_{po} being the initial concentration of CaLb in the microparticles and V_p the volume of the microparticles.

Consequently, $\left(\frac{V_p}{V}\right) c_{po} = c + \int_o^t k'cdt$ (5)

This equation can be used to obtain the values of k´ from the experimental data of *c* vs. *t*. When NaCas and NaCasTG microparticles were introduced into the cheese, the values obtained for k' were 4.43 x 10^{-5} s^{-1} and 4.40 x 10^{-5} s^{-1} respectively, indicating that the use of transglutaminase did not exert an important effect on the protection process. When gum arabic (GA) was used, k' was found to be 4.61 x 10^{-5} s^{-1}, while the value of k' was 3.03 x 10^{-5} s^{-1} with maltodextrin (MD), and if gelatine (Gel) was used for making the microparticles, the value of k' was 3.1 x 10^{-5} s^{-1}. The values of the fitting of the model to the experimental results are shown in Figure 2, with the simulated lines.

As a comparison, the consumption in the positive control, using the same equation (5) gave k' as 4.66 x 10^{-5} s^{-1} when the lactobionate was not encapsulated in the microparticles. Taking into account the concentration of lactobionate in the cheese after 12 days, it can be seen that the microparticles exerted a protective effect, impeding the consumption of this compound by the lactic acid bacteria present in the cheese.

3.2.3 Textural characterization of functional cheeses

To produce attractive food products for consumers, it is particularly important to understand their texture and structure [30] [31]. Textural characterization allows the measurement of the firmness and stickiness of food products. Experiments were performed in triplicate and results are shown as the mean value with the standard deviation (Figure 4A and B).

In terms of firmness, the negative control (cheese without microparticles) had a mean value of 249.18±26.55 g (Figure 4A). All the cheeses had a similar value, except the positive control, Gel and Gel-TGase and GA cheeses. In the case of the positive control, the firmness value was 48% higher than the negative control due to the increase in free calcium in the matrix. $CaCl_2$ is one of the salts which is added to improve the curdling process. CaLb was added free to positive control cheeses, so calcium was able to enhance the precipitation of casein and the strength of its bonds, leading to a greater value of firmness [32]. Firmness of cheeses with GA microparticles showed an increase of 59% with respect to the negative control. GA has useful properties in the food sector due to its stabilising capacity [13]. It may stabilize casein-casein bonds, thus increasing the firmness of the matrix. The opposite happens with Gel microparticles where cheese firmness was 32% lower. The pH of cheese was between 3.5 and 4.5. At this pH, the number of basic amino acid residues available to participate in the TGase bond with caseins decreased rapidly and therefore firmness also decreased [33].

Referring to stickiness, the values obtained differed notably from the negative control (3.498± 0.474 g) (Figure 4B). The highest values were obtained with MD microparticles

and the mixtures of MD and GA. As there was no difference between the GA microparticle cheeses and the control, it can be assumed that MD was responsible for the increase in stickiness values. The presence of MD in the cheeses raised their moisture content [34] and that could affect the structure of the protein matrix, increasing stickiness. An increase in the stickiness value was also observed with TGase microparticles, particularly with Gel-TGase. In the case of casein microparticles, TGase catalyses bonds between different residues in caseins [18]. The increase in this kind of interaction could have as a result a higher stickiness in the final product.

As there was no great difference between the protective effect exerted by the different microparticles, and in all of them the amount of CaLb encapsulated was acceptable, as were their textural properties, NaCas and NaCas-TGase microparticles were the ones chosen for digestibility and cheese morphology analysis.

3.2.4 Functionalized cheese morphology

The visual appearance of the functional cheeses was similar to the cottage cheese without microparticles in its structure. Functionalised cottage cheeses were examined for matrix differences with SEM (Figure 5).

There was no difference between the negative control matrix and cheeses functionalised with NaCas and NaCas-TGase (Figure 5A, C and D, respectively). The morphology results corresponded with textural analysis, since there was no difference in firmness and stickiness values between the negative control and cheese functionalized with NaCas and NaCas-TGase microparticles.

The matrix of the positive control showed evident changes (Figure 5B). It was more homogeneous, rounded and less rough. This could be due to the presence of free calcium. Calcium promotes the curdling process, helping and improving casein precipitation [32].

Free calcium could interact with casein, and so change the matrix structure and textural properties, resulting in a firmer and more compact cheese.

3.3 Digestibility of microparticles and cheeses

The digestibility test showed the degree of degradation of microparticles with the aim of discovering whether CaLb can be released in the GIT to enhance the growth of its microflora. The digestibility test was carried out employing NaCas and NaCas-TGase after characterization of these microparticles and their functionalized cheeses.

NaCas and NaCas-TGase powder was first tested to ensure microparticle degradation. Experiments were performed in triplicate. NaCas-TGase microparticles were more resistant to the acid stomach environment, with a CaLb release of 84.8±1.2% (w/w), while NaCas had a liberation of 99.2±0.8% (w/w). Casein microparticles can resist low pH but they are rapidly digested by pepsin [23]. But nevertheless, when casein is treated with TGase, the microparticles are more resistant to pH and enzymes due to the cross-linking between proteins [35]. In the intestinal simulation, CaLb release with NaCas microparticles was similar to that seen in the stomach, while NaCas-TGase showed a higher degree of liberation (85.7±0.3%). A modification in the pH from acid (3.0) to neutral (7.0) might encourage a change in the protein structure by breaking bonds formed by TGase and increasing CaLb liberation.

Once it had been proved that the microparticles were degraded, functional cheeses were tested in triplicate. As occurred with microparticle powder, NaCas showed more release than NaCas-TGase (75.6±0.7% (w/w) versus 63.0±0.7% (w/w)) in the stomach. In the intestine, the liberation was similar, although NaCas-TGase functionalised cheeses had a greater degree of release (78.8±2.0% (w/w)). CaLb release in the cheese digestibility

experiments was lower than that observed in the microparticles in suspension. This could be due to the complexity of the food matrix.

4. Conclusions

Microparticles made with the different coat materials possessed good EE and morphological properties, although their high solubility in water limits their use to food products with a low a_w. All the microparticles exerted a successful protective effect against LAB consumption when they were added to cottage cheese. NaCas and NaCas-TGase were selected for further analysis as the coat material is formed by casein, which is the major protein present in milk. No great differences were observed when TGase was employed. The functional dairy product developed revealed good texture and digestibility capacity. Thus, an innovative functional product with an increase in the quantity of CaLb of 61.4% with respect to the only such product currently on the market has been developed and could potentially be improved by adding a specific beneficial probiotic CaLb consumer in order to produce a new symbiotic product.

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Figures Caption

Figure 1. SEM images of (A) NaCas, (B) NaCas-TGase, (C) Gel, (D) Gel-TGase, (E) GA 4%, (F) GA 8%, (G) MD 12%, (H) MD 16%, (I) GA:MD 75:25, (J) GA:MD 50:50 and (K) GA:MD 25:75 microparticles; scale bars correspond to 100 µm.

Figure 2. Experimental and simulated evolution of CaLb percentage in cottage cheese-microparticles mixes with time along 12 days. Points indicate experimental results, (♦) Positive control (free calcium lactobionate), (●) NaCas, (○) NaCas-TGase, (■) Gel (●) GA 8%, (■) MD 16%. Lines show simulated evolution using the proposed model.

Figure 3. Flows/reactions and concentration profiles assumed in the experimental system for modelling.

Figure 4. Textural analysis of functionalised cottage cheeses; (A) firmness analysis and (B) stickiness analysis. Both are expressed in force units (g).

Figure 5. SEM images of cheese (A) without microparticles in its structure, (B) with free calcium lactobionate, (C) with CasNa and (D) with NaCas-TGase microparticles; scale bars correspond to 50 µm.