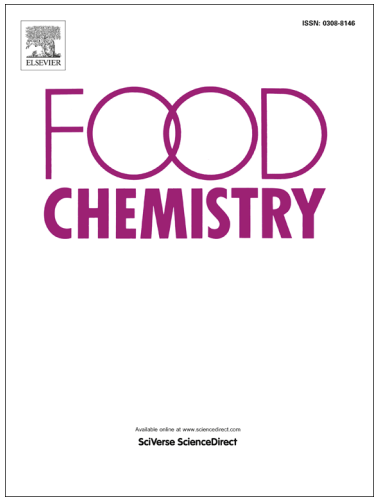

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Designing dairy desserts for weight management: structure, physical properties and *in vitro* gastric digestion

Running title: Designing dairy desserts for weight management

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ABSTRACT

The first aim of this study was to observe the effect of adding dairy proteins and reducing the cream content in order to obtain healthier dairy desserts for use in weight management. The extra-whey protein low-cream sample had the densest, firmest matrix, which is related to increased satiety. The second aim was to investigate the *in vitro* gastric digestion behavior of whey and casein proteins in a heat-treated semisolid real food. The extra-casein protein sample matrix broke down more slowly than the others because the caseins clotted at the gastric pH. Despite being heated, the whey proteins in the panna cottas were more resistant to pepsin digestion than caseins; this is related with a higher satiety capacity. These findings suggest that the combination of reducing fat content (to obtain a reduced energy density product) and adding whey protein (to increase satiety capacity) allows obtaining dairy desserts for weight management.

Keywords: dairy dessert, dairy proteins, *in vitro* digestion, microstructure, SDS-PAGE, texture

1. Introduction

Excess weight and obesity represent an increasing health problem worldwide that seriously raises the risk of developing severe metabolic disorders and cardiovascular diseases (Munsters & Saris, 2014). The contribution of energy-dense high-fat sugary foods to weight gain is well recognized (Halford & Harrold, 2012). Energy-dense and high-fat foods are associated with high palatability, and vice versa. However, a significant inverse correlation between palatability ratings and satiety index scores has been found, so the more palatable foods are generally less satiating (Holt, Brand Miller, Petocz, & Farmakalidis, 1995). Moreover, although all types of fat contain almost the same amount of energy, increases in animal fat — rich in saturated fatty acids (SFA) — and in trans-fat have a stronger association with weight gain than increases in vegetable oils — rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Field, Willett, Lissner, & Colditz, 2007). Consequently, designing healthy foods with low energy density, by reducing its animal fat content, and high satiating capacity, through adding extra protein, which is the most effective food macronutrient in providing a satiating effect (Geraedts, Troost, & Saris, 2011; Lundin, Golding, & Wooster, 2008; Morell, Hernando, Llorca, & Fiszman, 2015; Solah et al., 2010), could be an important way to fight excess weight and obesity.

Ingested food evokes satiation through two primary effects on the gastrointestinal (GI) tract: gastric distention (mechanical stimulation) and the release of gut peptides, which are hormones from the intestine (chemical stimulation) (Cummings & Overduin, 2007; Geraedts et al., 2011). Both effects

slow down gastric emptying. Skim milk powder (SMP) is widely used to fortify dairy desserts, but the new milk and whey fractionation technologies produce a large diversity of dairy ingredients, such as caseinates and whey protein concentrates (WPC). These ingredients have different properties and can be used separately or blended to replace SMP in dairy desserts (Remeuf, Mohammed, Sodini, & Tissier, 2003). Some authors have found differences between whey and casein in satiating terms. Casein, unlike whey, coagulates in the stomach, due to precipitation by gastric acid. Therefore, casein has a longer gastric emptying time than whey, which is not subject to acid precipitation and empties rapidly into the duodenum (Lundin et al., 2008). The concept of 'fast' and 'slow' proteins has been introduced to describe these differences in the digestion and absorption of proteins. According to this concept, a fast protein such as whey is more satiating than a slow protein such as casein (Mahé et al., 1996). Although the behavior of these proteins (whey and casein) has been extensively studied in simplified model systems or liquid preloads (Bendtsen, Lorenzen, Bendsen, Rasmussen, & Astrup, 2013; Hoad et al., 2004; Lacroix et al., 2006; Solah et al., 2010; Zhang & Vardhanabhuti, 2014a, 2014b; Zhang, Zhang, & Vardhanabhuti, 2014), few satiety-related studies have incorporated both proteins into semi-solid or solid real food (Kopf-Bolanz, Schwander, Gijs, Vergères, Portmann, & Egger, 2014; Morell, Hernando, et al., 2015; Morell, Piqueras-Fiszman, Hernando, & Fiszman, 2015).

The structure of food and its physical properties, such as texture or volume, are also important when designing reduced-fat and satiating foods. On the one hand, the structure and textural characteristics are altered when formulating reduced-fat or low-fat foods, affecting product acceptance by the consumer

(Lobato-Calleros, Reyes-Hernández, Beristain, Hornelas-Uribe, Sánchez-García, & Vernon-Carter, 2007). Therefore, most authors have investigated the effect of using dairy proteins (whey proteins principally) as fat replacers in order to improve the texture of low-fat dairy foods such as yogurt and cheese and maintain their sensory characteristics. According to Damin, Alcântra, Nunes, & Oliveira (2009), the nature and proportions of the different proteins (skim milk, whey protein concentrates, caseinates) in the formulation significantly affect the texture of yogurts. Equally, the incorporation of a high level of dairy proteins plays a key role in satiety (Zhang & Vardhanabhuti, 2014b). The reason is, firstly, that protein is recognized as the macronutrient with the highest satiating ability, and secondly, as mentioned in the previous paragraph, that adding proteins can enhance texture, and several authors have reported that a solid meal has a greater effect on satiety than a liquid meal of equivalent size and energy content (Chambers, McCrickerd, & Yeomans, 2015; Hoad et al., 2004; Solah et al., 2010).

The microstructure and physicochemical properties of foods also have significant effects on digestibility (Zhang & Vardhanabhuti, 2014a). Studies have demonstrated that food disintegration and gastric emptying are a complex process involving numerous variables, including particle size, meal volume, calories and composition of the meal, viscosity, and physical properties such as density, texture and microstructure (Kong & Singh, 2008). Therefore, a better understanding of how protein structures relate to degradation properties under gastric conditions might help to provide complementary information on gastric emptying, and thus also on their influence on satiety in the GI tract, and can

assist food manufacturers in developing the next generation of structured food for health (Zhang & Vardhanabhuti, 2014a).

The primary objective of this study was to reformulate a dairy dessert, panna cotta, in order to obtain a healthier product which could be used in weight management. Two different approaches were used: reducing the fat to diminish the calorie content and adding extra milk protein from different sources to increase satiety. The second aim of this study was to investigate whether the digestion behavior of whey and casein proteins incorporated into a heat-treated semi-solid real food was similar to their behavior in the liquid preloads or model systems studied until now. Accordingly, their microstructural (confocal laser scanning microscopy), textural (puncture test) and electrophoretic (SDS-PAGE) properties were assayed before and after *in vitro* oral plus gastric digestion.

2. Materials and methods

2.1. Panna cotta formulations

A control panna cotta (sample P) was prepared with skim milk powder (Central Lechera Asturiana, Corporación Alimentaria Peñsasanta S.A., Siero, Spain) reconstituted in distilled water, liquid cream (Hacendado, Esnelat S.L., Urnieta, Spain) with a 35% fat content, and κ-carrageenan (Satiagel^{TM} ME5, Cargill France SAS, Saint-Germain-en-Laye, France) (Table 1). Nine panna cotta samples were formulated with reduced amounts of cream (medium, low or zero) and the addition of different dairy proteins in order to obtain panna cottas with higher protein contents than the control. The proteins added to the formulation were skim milk powder (M); whey protein concentrate (W) (Avonlac^{TM} 482 IP, Glanbia Nutritionals Ltd., Kilkenny, Ireland), or calcium

caseinate (C) (Fonterra Co-operative Group Ltd, Reference 385, Palmerston North, New Zealand) (Table 1).

2.2. Sample preparation

To avoid phase separation in samples C_M, C_L and C_0, the calcium caseinate powder was pre-dissolved in 100 mL of distilled water and heated at 80 ºC for 10 min before adding it to the mixtures.

The different ingredients were placed in a cooking device (Thermomix TM 31, Wuppertal, Germany) where they were heated to 90 ºC with continuous stirring (700 rpm). After reaching this temperature, the mixtures were maintained under the same conditions for 6 min, then placed in silicone molds and cooled to ambient temperature. The samples were stored at 4-5 ºC in a refrigerator until they were analyzed.

2.3. Texture analysis

The firmness of the panna cottas was determined with a puncture test, using a TA.XT-Plus Texture analyzer (Stable Microsystems, Godalming, U.K.) equipped with a 30 kg load cell and a 12 mm diameter flat-ended cylindrical plastic probe. The crosshead speed was set at 10 $mm \cdot s^{-1}$ and the penetration distance at 10 mm. The firmness of the panna cotta was defined as the maximum force (N) attained during sample penetration (Salvador & Fiszman, 2004). Six replications were performed for each sample.

2.4. *In vitro* oral plus gastric digestion

To simulate oral digestion, samples P, M_0, W_0 and C_0 were mixed with artificial saliva in a hand blender (Ufesa, model BP4566, Barcelona, Spain) for 15 s. The ratio of saliva to sample was 1:4 on a weight basis. The artificial saliva was prepared according to the method described by Morell, Fiszman, Valera, & Hernando (2014). To simulate gastric digestion, an adaptation of the *in vitro* digestion model proposed by Abdel-Aal (2008) was used. It consisted of a jacketed glass reactor (1 L capacity) with continuous magnetic stirring, maintained at 37 ºC in a temperature-controlled circulating water bath throughout the test. Each sample was mixed with simulated gastric fluid (SGF, a solution containing 0.034 M NaCl) in a proportion of 50 mL SGF/ 100 g panna cotta, and digested. The pH value was reduced to 1.9 with HCl 10 N, and pepsin (P7125, pepsin from porcine gastric mucosa, ≥ 400 units/mg protein, Sigma-Aldrich) was added at a pepsin to protein ratio of 1:4 on a weight basis. The mix was maintained at 37 ºC with continuous stirring for 120 min. Digestion was stopped by raising the pH to 7 with NaOH 1 N. Sampling was carried out at 0, 30, 60 and 120 min.

2.5. Confocal Laser Scanning Microscopy (CLSM)

A Nikon C1 confocal microscope unit fitted on a Nikon Eclipse E800 V-PS100E microscope (Nikon, Tokyo, Japan) was used. An argon laser line (488 nm) was employed as the light source to excite Rhodamine B and Nile Red fluorescent dyes. Rhodamine B (Fluka, Sigma-Aldrich, Missouri, USA) with λex max=488 nm and λem max=580 nm was solubilized in distilled water at 0.2%. This dye was used to stain proteins and carbohydrates. Nile Red (Fluka, Sigma-Aldrich, Missouri, USA) with λex max=488 nm and λem max=515 nm was

solubilized in PEG 200 at 0.1 g/L. This dye was used to stain fat. A 40×/1.0/Oil/ Plan Apo Nikon objective lens was used. Twenty microliters of the sample were placed in the central microscope slide. The Rhodamine B and Nile Red solutions were added, and the cover slide was carefully positioned to exclude air pockets. The observations were made 10 min after diffusion of the dyes into the sample. The images were observed and stored with a 1024×1024 pixel resolution using the microscope software (EZ-C1 v.3.40, Nikon, Tokyo, Japan).

2.6. Protein electrophoresis

2.6.1. Extraction of proteins

Samples P, M_0, W_0, C_0 were freeze-dried (TELSTAR Lioalfa-6, Terrassa, Spain) for 48 h at 1kPA and -45 ºC. Sample P was previously defatted using the Soxhlet method. Each sample was mixed with Laemmli buffer (4.8% (w/v) sodium dodecyl sulfate, 0.1 M dithiothreitol, 0.001 M ethylenediaminetetraacetic acid, 20% (v/v) glycerol, 0.05% (w/v) bromophenol blue and 0.125 M Tris-HCl; pH 6.8) (Borreani, Llorca, Larrea, & Hernando, 2016), adjusting the protein concentration of the samples to 8 mg/mL.

2.6.2. SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a Multiphor II Electrophoresis System (Pharmacia Biotech, Piscataway, USA), using ExcelGel SDS Homogeneous 15% polyacrylamide gels at 600 V, 20 mA, 20 W and 15 ℃ (Borreani et al., 2016). The samples were loaded at a protein concentration of 64 micrograms of protein/well.

The protein bands were stained with Coomassie Brilliant Blue tablets (Phastgel Blue R., Pharmacia). De-staining was performed in an aqueous solution of 25% ethanol and 8% acetic acid. The samples were kept in a solution of 10% glycerol and 7.2% acetic acid.

A high molecular weight calibration kit consisting of: phosphorylase b (97 kDa), bovine serum albumin (66 kDa), egg albumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α- lactalbumin (14.4 kDa) (Amersham GE Healthcare, UK) was used as standard.

The gels were scanned with an ImageScanner III LabScan 6.0 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and analyzed with the ImageQuant TL Image analysis Software v7.0 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

2.7. Statistical analysis

Analysis of variance (ANOVA) was performed on the data using the Statgraphics Centurion VI.II software package (Statistical Graph Co., Rockville, USA). Fisher's Least Significant Difference (*LSD*) test was used to assess the differences in mean values ($P < 0.05$).

3. Results and discussion

3.1. Texture analysis

The firmness values obtained by the puncture test are shown in Table 2. The control (P) and M_M panna cottas showed the lowest values, without significant differences ($P > 0.05$) between them. The firmness values of samples M_L and M_0 were higher than those obtained in samples P and M_M ($P < 0.05$). Therefore,

it seems that as the amount of cream increased, the firmness values decreased. The panna cottas formulated with added whey protein (W_M, W_L, W_0) showed the highest firmness values and significant differences between them ($P < 0.05$). The samples formulated with caseinate (C_M, C_L, C_0) showed significantly higher firmness values than those with added milk (M_M, M_L, M_0) or the control (P), but significantly lower values than the samples with added whey protein (W_M, W_L, W_0). As in the milk samples (M_M, M_L, M_0), there were no significant differences between the samples with a low cream content (C_L) and without cream (C_0), but there were significant differences between these and the sample with a medium cream content (C_M).

In general, the firmness values decreased as the cream content rose. The samples with a medium cream content exhibited significantly lower firmness values ($P < 0.05$) than those with a low or zero cream content, regardless of the type of dairy protein added. As the fat content is reduced, a high degree of cross-linking of protein molecules may occur, resulting in three-dimensional networks that exhibit high resistance to deformation as observed by Lobato-Calleros et al. (2007). Moreover, as observed in CLSM section, the panna cottas prepared with the medium cream content (M_M, W_M, C_M) showed more and larger fat globules than the samples with the low cream content (M_L, W_L, C_L), which could lead to softer, smoother and creamier textures. Softer cheese pies when cream was added to the formulation was also reported by Marcano, Morales, Vélez-Ruiz, & Fiszman (2015). On the other hand, although the increase in firmness values could also be due to the different carrageenan concentration among the samples, the firmness values of control (P) and M_M samples, which exhibited differences in carrageenan concentration (0.40 and

0.46%, respectively), were not significantly different ($P > 0.05$). Moreover, the firmness values between the samples with low and without cream content (0.48 and 0.50% carrageenan, respectively) in M and C samples (M_L, M_0, C_L, C_0), neither were significantly different. Therefore, it seems not to be a direct relationship between the carrageenan concentration and firmness values. Regarding the type of protein added, the firmness values increased significantly ($P < 0.05$) as follows: extra milk protein < caseinate < whey protein. Similarly, Morell, Piqueras-Fiszman, et al. (2015) found that the dairy ingredient source significantly affected the yogurt's firmness, as the samples with extra caseinate and the samples with extra milk protein showed significantly lower firmness values than the samples with extra whey protein concentrate, but higher values than the control sample. Therefore, the increase in firmness values seems to be more related to the addition of the different dairy proteins and the reduction of cream content than to the increase in carrageenan concentration.

3.2. Confocal Laser Scanning Microscopy (CLSM)

3.2.1. CLSM before oral plus gastric digestion

The microstructure of the panna cottas stained with Nile Red and Rhodamine B is shown in Figure 1. Fat is observed in green-orange, stained with Nile Red, and protein and carrageenan are observed in red, stained with Rhodamine B. The control panna cotta (P) exhibited an open protein matrix interspersed with fat globules in the intervening spaces. Some large fat globules could be observed, owing to emulsion destabilization processes involving coalescence. Coalescence occurs when two or more droplets merge to form a bigger droplet (losing their individual interfacial films) and their contents mix (Mao & Miao,

2015). As expected, the panna cottas prepared with the medium cream content (M_M, W_M, C_M) showed more and larger fat globules and more coalescence than the samples with the low cream content (M_L, W_L, C_L). Nevertheless, regardless of the protein used (M, W or C), no visual differences in the structure of the protein matrix were observed between the samples formulated with a medium, low or zero amount of cream. Therefore, the presence or absence of cream did not appear to influence the protein structure.

In contrast, the M_0 panna cotta, devoid of fat content, showed a continuous homogeneous network. This became more aggregated when part of the milk protein was replaced by whey protein in sample W_0 or by caseinate in sample C_0, leading to a more heterogeneous structure (Figure 1). In consequence, these samples (W_0 and C_0) exhibited higher resistance to deformation than sample M_0 in the texture analysis assay. Similarly, Lobato-Calleros et al. (2007) found that incorporating whey protein concentrate (WPC) into matrices yielded a higher protein/fat ratio that gave rise to a relatively large area of compact and continuous protein matrix. Moreover, the protein network of sample W_0 seemed to be more heterogeneous, showing a higher degree of aggregation, which resulted in higher firmness values than for sample C_0. This is probably because the cross-linking capacity of denatured whey proteins plays a key role in the structure after heating by contributing to increased bridging between protein particles (Remeuf et al., 2003). In contrast, Damin et al. (2009) reported that casein-based (sodium caseinate) products tended to produce firmer gels than yogurts supplemented with WPC.

In general, the protein network became denser and more aggregated in panna cottas made with extra protein and no cream, as follows: sample W_0

showed the highest aggregation, followed by sample C_0, while sample M_0 exhibited the lowest aggregation.

3.2.2. CLSM during oral plus gastric digestion

During *in vitro* gastric digestion (Figure 2), the control panna cotta (P) exhibited protein network degradation and fat globule coalescence. These were more pronounced at min 120. In the same way, Guo, Ye, Lad, Dalgleish, & Singh (2014) observed a continuous protein network with coalescence of oil droplets in the emulsions during *in vitro* gastric digestion, which they attributed to flocculation of the released oil droplets and mechanical shearing as the likely cause. Ye, Cui, & Singh (2011) observed that fat globules in raw milk flocculated during incubation in SGF at 10 min and that this flocculation was enhanced at longer incubation times. The flocculation may have arisen from hydrolysis of charged milk fat globule membrane (MFGM) proteins, resulting in a decrease in electrostatic repulsion (through the fat globules' linking together) or from aggregation of the casein micelles in the serum phase, induced by a combination of low pH and pepsin hydrolysis, trapping the fat globules in the casein aggregates (Ye et al., 2011).

In sample M_0, protein network degradation was appreciable at 60 min and even more at 120 min. This degradation could be observed as the loss of continuity of the protein network, giving place to a discontinuous matrix that forms little "islands". In contrast, sample W_0 had the highest protein matrix degradation compared to samples M_0 and C_0, as the protein network was degraded at 30 min and the degradation continued at 60 and 120 min. Sample C_0 was the least degraded compared with the other samples (P, M_0 and W_0). In

particular, its protein network was slightly degraded at 30 min and this degradation was more pronounced at 60 min but remained almost unchanged at 120 min. The lower protein degradation of sample C_0 was probably due to the fact that caseins coagulate at gastric pH, so large aggregates could be seen practically unchanged afterwards.

3.3. Electrophoresis

The *in vitro* oral plus gastric digestion patterns of the control sample and the samples prepared with added protein and without fat (P, M_0, W_0 and C_0) were examined using the SDS-PAGE technique under reducing conditions. Figure 3 presents the electrophoregram, showing the protein profiles before (0 min) and after different simulated *in vitro* gastric digestion times (30, 60 and 120 min).

In the control (P) sample before digestion (0 min), the bovine serum albumin (BSA) band was observed at approximately 66.0 kDa; α_s-casein (α_s-CN), β-casein (β-CN) and κ-casein (κ-CN) at approximately 33.6, 27.2 and 21.3 kDa respectively; and β-lactoglobulin (β-lg) and α-lactalbumin (α-la) at approximately 17.5 and 14.4 kDa. After 30 min of gastric digestion, the BSA band disappeared almost completely and the casein band (α_s-CN and β-CN) lost much of its intensity (falling from 32.25% to 7.74% and from 29.39% to 22.06% respectively), whereas the κ-CN, β-lg and α-la bands remained practically unchanged. Moreover, peptide bands appeared below the α-la band (14.4 kDa), due to the degradation of high molecular weight proteins into proteinaceous molecules with a low molecular weight. After 60 and 120 min of pepsin digestion, the casein bands (α_s-CN, β-CN and κ-CN) disappeared, but β-lg and

α-la remained almost unchanged and the peptide bands were slightly more intense.

In the M_0 sample, as in the control sample, the BSA, α_s-CN and β-CN bands lost much of their intensity between 0 and 30 min of gastric digestion (from 10.81%, 45.14% and 34.83% to 2.89%, 18.05% and 7.8%; respectively), k-CN remained practically unchanged and several peptide bands were visible. Kim et al. (2007) found similar results: high molecular weight bands, such as BSA, were completely eliminated from SDS-PAGE electrophoregrams when studying native and heated dairy hydrolysates produced by pepsin after a 30 min incubation period. After 60 min, α_s-CN and β-CN lost more intensity (1.1% and 1.39% respectively), and at 120 min they disappeared completely, together with k-CN. This result was in accordance with Barbé et al. (2013), who observed that caseins are rapidly cleaved by pepsin, so rapid hydrolysis was expected. Indeed, Tunick et al. (2016) and Kopf-Bolanz et al. (2014) found that the degradation of casein bands was very rapid during gastric digestion. Nevertheless, the electrophoretic bands corresponding to β-lg and α-la remained intense throughout the digestion period. Although Kopf-Bolanz et al. (2014) observed β-lg in decreasing amounts depending on the heat treatment of the milk, the β-lg band was still present in differently heated milk. In fact, with regard to β-lg, several studies have shown insignificant differences among milk samples subjected to heat treatments throughout gastric digestion (Inglingstad et al., 2010; Tunick et al., 2016; Wada & Lönnerdal, 2014).

In the W_0 sample, the β-lg dimer (indicated by an arrow in Figure 3) and β-lg bands lost intensity at 30 min of gastric digestion, whereas the α-la band, which had a poor intensity at min 0 (6.48%) did not seem to change. Similarly,

Nguyen, Bhandari, Cichero, & Prakash (2015) and Inglingstad et al. (2010) found that α-la was the most resistant protein to human digestive enzymes and therefore underwent limited digestion. As in the other samples, at min 30 the peptide bands were slightly visible (26.45%). At min 60, the β-lg dimer, β-lg and α-la bands had disappeared.

In the C_0 sample, as in the control (P) and M_0 samples, from 0 to 30 min the BSA band disappeared, the α_s-CN and β-CN bands lost most of their intensity (from 18% and 67.9% to 9.71% and 6.48% respectively) and some slight peptide bands appeared. From 30 to 120 min of gastric digestion no major changes were found, and only the casein bands (α_s-CN, β-CN and k-CN) had disappeared at min 120.

According to the results, although the panna cottas were heated to 90 ºC — which can denature whey proteins (Matignon et al., 2014), resulting in increased susceptibility to pepsin proteolysis (Lundin et al., 2008) — it seemed that in the first 30 minutes of gastric digestion the whey proteins (β-lg and α-la) were more resistant to pepsin attack than the casein proteins (α_s-CN and β-CN), regardless of the panna cotta to which they were added. This finding suggests that despite the whey proteins' losing some resistance to hydrolysis, the behavior of the dairy proteins was the same when incorporated into real food as when incorporated into simple formulations without heating.

In the present study, the addition of whey proteins and elimination of cream obtained the highest firmness values. This is probably due to a major cross-linking of proteins, which exhibited high resistance to deformation leading to high firmness values, as discussed in section 3.1. These results are in

accordance with the microstructure results, where the samples formulated with whey proteins exhibited a denser or more aggregated protein matrix than the caseinate, milk and control samples. Moreover, Marcano et al. (2015), Morell et al. (2014), Morell, Hernando, et al. (2015) and Morell, Piqueras-Fiszman, et al. (2015) found that texture had a positive effect on expected satiating capacity, as the expected satiating capacity scores were completely aligned with the TPA firmness and penetration instrumental texture assessment (Marcano et al., 2015). It is worth noting that density, thickness, and compactness/firmness are attributes that consumers generally tend to associate with a product's satiating ability (Morell, Piqueras-Fiszman, et al., 2015). Therefore, the samples with added whey protein and a low fat content, which obtained the highest firmness values and showed the densest protein network, probably would have the highest satiating capacity.

At gastric level, in conjunction with gastric distension (involved in the mechanical effect on satiation), the release of peptides and resulting concentration of amino acids play a key role in satiation (Cummings & Overduin, 2007) and in satiety (Geraedts et al., 2011). The present study focused on the release of peptides by protein-degrading pepsins attacking at gastric level. The SDS-PAGE results showed that in the panna cottas, the whey proteins were more resistant than the casein proteins to gastric digestion by pepsin in the first 30 min. Although caseins coagulate in the stomach and empty more slowly from the stomach into the intestine than whey proteins, they are more exposed to gastric peptic hydrolysis, as the open structure of the caseinate proteins allows enzymes greater access to target residues, resulting in rapid proteolysis (Lundin et al., 2008). In this regard, some authors (Bowen,

Noakes, Trenerry, & Clifton, 2006; Veldhorst et al., 2008) have found that whey proteins exhibit a faster rate of gastric emptying, resulting in a stronger increase in the postprandial plasma amino acid concentration than casein proteins, which would increase satiety. Moreover, gastrointestinal satiety hormones are secreted in response to protein ingestion and they exhibit a greater plasma concentration with the ingestion of whey proteins than with that of casein proteins (Veldhorst et al., 2008). Therefore, whey protein from panna cottas could be emptied from the stomach into the intestine faster and in a more unaltered form than casein, giving rise to a faster and higher release of plasma amino acids and satiety-related hormones.

4. Conclusions

This study demonstrates that the addition of extra dairy proteins and the reduction of cream content in panna cottas leads to a denser and more aggregated matrix than in the control sample, and therefore increases the firmness values of the panna cottas, which can be related with increased satiety. In particular, the samples with whey protein and a low fat content exhibited the highest firmness values and the densest protein network. Therefore, this panna cotta could be expected to have the highest satiating capacity.

The digestion behavior of dairy proteins in a heat-treated complex food matrix (panna cotta) during *in vitro* gastric digestion was also studied. Although caseins clotted at gastric pH, and thus showed a dense, aggregated matrix which broke down more slowly than the other samples over the digestion time, they were very susceptible to proteases being rapidly cleaved by pepsin.

Regardless of the panna cotta formulation and despite the heating process, the whey proteins were more resistant to pepsin digestion than the caseins. Therefore, although the heat treatment reduced their resistance to pepsin attack, these proteins behaved in the same way as in simple model systems. The resistance of whey proteins to pepsin digestion can also be related to a high satiating capacity.

Considering the results as a whole, panna cottas with whey protein added and a low fat content would exhibit the highest satiation and satiety effects.

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References

Abdel-Aal, E. S. M. (2008). Effects of baking on protein digestibility of organic spelt products determined by two in vitro digestion methods. *LWT - Food Science and Technology*, *41*(7), 1282–1288.

Barbé, F., Ménard, O., Le Gouar, Y., Buffière, C., Famelart, M. H., Laroche, B., Le Feunteun, S., Dupont, D., & Rémond, D. (2013). The heat treatment and the gelation are strong determinants of the kinetics of milk proteins digestion and of the peripheral availability of amino acids. *Food Chemistry*, *136*(3-4), 1203–1212.

Bendtsen, L. Q., Lorenzen, J. K., Bendsen, N. T., Rasmussen, C., & Astrup, A. (2013). Effect of dairy proteins on appetite, energy expenditure, body weight, and composition: a review of the evidence from controlled clinical

trials. *Advances in Nutrition: An International Review Journal*, *4*(4), 418–438.

Borreani, J., Llorca, E., Larrea, V., & Hernando, I. (2016). Adding neutral or anionic hydrocolloids to dairy proteins under in vitro gastric digestion conditions. *Food Hydrocolloids*, *57*, 169–177.

Bowen, J., Noakes, M., Trenerry, C., & Clifton, P. M. (2006). Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. *The Journal of Clinical Endocrinology and Metabolism*, *91*(4), 1477–1483.

Chambers, L., McCrickerd, K., & Yeomans, M. R. (2015). Optimising foods for satiety. *Trends in Food Science and Technology*, *41*(2), 149–160.

Cummings, D. E., & Overduin, J. (2007). Gastrointestinal regulation of food intake. *Journal of Clinical Investigation*, *117*(1), 13–23.

Damin, M. R., Alcântara, M. R., Nunes, A. P., & Oliveira, M. N. (2009). Effects of milk supplementation with skim milk powder, whey protein concentrate and sodium caseinate on acidification kinetics, rheological properties and structure of nonfat stirred yogurt. *LWT - Food Science and Technology*, *42*(10), 1744–1750.

Field, A. E., Willett, W. C., Lissner, L., & Colditz, G. A. (2007). Dietary fat and weight gain among women in the Nurses' Health Study. *Obesity*, *15*(4), 967–976.

Geraedts, M. C. P., Troost, F. J., & Saris, W. H. M. (2011). Gastrointestinal targets to modulate satiety and food intake. *Obesity Reviews*, *12*(6), 470–477.

Guo, Q., Ye, A., Lad, M., Dalgleish, D., & Singh, H. (2014). Effect of gel

structure on the gastric digestion of whey protein emulsion gels. *Soft Matter*, *10*(8), 1214–1223.

Halford, J. C. G., & Harrold, J. A. (2012). Satiety-enhancing products for appetite control: science and regulation of functional foods for weight management. *The Proceedings of the Nutrition Society*, *71*(2), 350–362.

Hoad, C. L., Rayment, P., Spiller, R. C., Marciani, L., De Celis Alonso, B., Traynor, C., Mela, D. J., Peters, H. P. F., & Gowland, P. A. (2004). In vivo imaging of intragastric gelation and its effect on satiety in humans. *Journal of Nutrition*, *134*(9), 2293–2300.

Holt, S. H. A., Brand Miller, J. C., Petocz, P., & Farmakalidis, E. (1995). A satiety index of common foods. *European Journal of Clinical Nutrition*, *49*(9), 675–690.

Inglingstad, R. A., Devold, T. G., Eriksen, E. K., Holm, H., Jacobsen, M., Liland, K. H., Rukke, E. O., & Vegarud, G. E. (2010). Comparison of the digestion of caseins and whey proteins in equine, bovine, caprine and human milks by human gastrointestinal enzymes. *Dairy Science & Technology*, *90*(5), 549–563.

Kim, S. B., Ki, K. S., Khan, M. A., Lee, W. S., Lee, H. J., Ahn, B. S., & Kim, H. S. (2007). Peptic and tryptic hydrolysis of native and heated whey protein to reduce its antigenicity. *Journal of Dairy Science*, *90*(9), 4043–4050.

Kong, F., & Singh, R. P. (2008). Disintegration of solid foods in human stomach. *Journal of Food Science*, *73*(5), 67–80.

Kopf-Bolanz, K. A., Schwander, F., Gijs, M., Vergères, G., Portmann, R., & Egger, L. (2014). Impact of milk processing on the generation of peptides during digestion. *International Dairy Journal*, *35*(2), 130–138.

Lacroix, M., Bos, C., Léonil, J., Airinei, G., Luengo, C., Daré, S., Benamouzig, R., Fouillet, H., Fauquant, J., Tomé, D., & Gaudichon, C. (2006). Compared with casein or total milk protein, digestion of milk soluble proteins is too rapid to sustain the anabolic postprandial amino acid requirement. *American Journal of Clinical Nutrition*, *84*(5), 1070–1079.

Lobato-Calleros, C., Reyes-Hernández, J., Beristain, C. I., Hornelas-Uribe, Y., Sánchez-García, J. E., & Vernon-Carter, E. J. (2007). Microstructure and texture of white fresh cheese made with canola oil and whey protein concentrate in partial or total replacement of milk fat. *Food Research International*, *40*(4), 529–537.

Lundin, L., Golding, M., & Wooster, T. J. (2008). Understanding food structure and function in developing food for appetite control. *Nutrition & Dietetics*, *65*(SUPPL. 3), S79–S85.

Mahé, S., Roos, N., Benamouzig, R., Davin, L., Luengo, C., Gagnon, L., Gaussergès, N., Rautureau, J., & Tomé, D. (1996). Gastrojejunal kinetics and the digestion of [15N]β-lactoglobulin and casein in humans: The influence of the nature and quantity of the protein. *American Journal of Clinical Nutrition*, *63*(4), 546–552.

Mao, L., & Miao, S. (2015). Structuring Food Emulsions to Improve Nutrient Delivery During Digestion. *Food Engineering Reviews*, *7*(4), 439–451.

Marcano, J., Morales, D., Vélez-Ruiz, J. F., & Fiszman, S. (2015). Does food complexity have a role in eliciting expectations of satiating capacity? *Food Research International*, *75*, 225–232.

Matignon, A., Moulin, G., Barey, P., Desprairies, M., Mauduit, S., Sieffermann, J. M., & Michon, C. (2014). Starch/carrageenan/milk proteins interactions

studied using multiple staining and Confocal Laser Scanning Microscopy. *Carbohydrate Polymers*, *99*, 345–355.

Morell, P., Fiszman, S. M., Varela, P., & Hernando, I. (2014). Hydrocolloids for enhancing satiety: Relating oral digestion to rheology, structure and sensory perception. *Food Hydrocolloids*, *41*, 343–353.

Morell, P., Hernando, I., Llorca, E., & Fiszman, S. (2015). Yogurts with an increased protein content and physically modified starch: Rheological, structural, oral digestion and sensory properties related to enhanced satiating capacity. *Food Research International*, *70*, 64–73.

Morell, P., Piqueras-Fiszman, B., Hernando, I., & Fiszman, S. (2015). How is an ideal satiating yogurt described? A case study with added-protein yogurts. *Food Research International*, *78*, 141–147.

Munsters, M. J. M., & Saris, W. H. M. (2014). Body Weight Regulation and Obesity: Dietary Strategies to Improve the Metabolic Profile. *Annual Review of Food Science and Technology*, *5*(1), 39–51.

Nguyen, T. T. P., Bhandari, B., Cichero, J., & Prakash, S. (2015). Gastrointestinal digestion of dairy and soy proteins in infant formulas: An in vitro study. *Food Research International*, *76*(3), 348–358.

Remeuf, F., Mohammed, S., Sodini, I., & Tissier, J. P. (2003). Preliminary observations on the effects of milk fortification and heating on microstructure and physical properties of stirred yogurt. *International Dairy Journal*, *13*(9), 773–782.

Salvador, A., & Fiszman, S. M. (2004). Textural and sensory characteristics of whole and skimmed flavored set-type yogurt during long storage. *Journal of Dairy Science*, *87*(12), 4033–4041.

Solah, V. A., Kerr, D. A., Adikara, C. D., Meng, X., Binns, C. W., Zhu, K., Devine, A., & Prince, R. L. (2010). Differences in satiety effects of alginate- and whey protein-based foods. *Appetite*, *54*(3), 485–491.

Tunick, M. H., Ren, D. X., Van Hekken, D. L., Bonnaillie, L., Paul, M., Kwoczak, R., & Tomasula, P. M. (2016). Effect of heat and homogenization on in vitro digestion of milk. *Journal of Dairy Science*, *99*(6), 4124–4139.

Veldhorst, M., Smeets, A., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., Lejeune, M., Luscombe-Marsh, N., & Westerterp-Plantenga, M. (2008). Protein-induced satiety: Effects and mechanisms of different proteins. *Physiology and Behavior*, *94*(2), 300–307.

Wada, Y., & Lönnerdal, B. (2014). Effects of different industrial heating processes of milk on site-specific protein modifications and their relationship to in vitro and in vivo digestibility. *Journal of Agricultural and Food Chemistry*, *62*(18), 4175–4185.

Ye, A., Cui, J., & Singh, H. (2011). Proteolysis of milk fat globule membrane proteins during in vitro gastric digestion of milk. *Journal of Dairy Science*, *94*(6), 2762–2770.

Zhang, S., & Vardhanabhuti, B. (2014a). Effect of initial protein concentration and pH on in vitro gastric digestion of heated whey proteins. *Food Chemistry*, *145*, 473–480.

Zhang, S., & Vardhanabhuti, B. (2014b). Intragastric gelation of whey protein-pectin alters the digestibility of whey protein during in vitro pepsin digestion. *Food & Function*, *5*(1), 102–110.

Zhang, S., Zhang, Z., & Vardhanabhuti, B. (2014). Effect of charge density of polysaccharides on self-assembled intragastric gelation of whey

protein/polysaccharide under simulated gastric conditions. *Food & Function*, *5*(8), 1829–1838.

Table and figure captions

Table 1. Formulation of panna cotta samples with decreasing amounts of cream and the addition of different dairy proteins.

Table 2. Firmness values of the control sample and of the panna cottas formulated with extra protein and with different amounts of cream.

Figure 1. Microstructure of the panna cottas before *in vitro* oral plus gastric digestion. Nile Red stained fat *green-orange*, Rhodamine B stained protein *red*. Magnification 40x. The scale bars measure 40 µm. P: control; M: skim milk powder added; W: whey protein concentrate added; C: calcium caseinate added; Medium, Low, Zero: amount of cream.

Figure 2. Microstructure of four panna cottas during *in vitro* gastric digestion (0, 30, 60 and 120 min). Nile Red stained fat *green*, Rhodamine B stained protein *red.* Magnification 40x. The scale bars measure 40 µm. P: control; M_0: skim milk powder added, no cream; W_0: whey protein concentrate added, no cream; C_0: calcium caseinate added, no cream.

Figure 3. SDS-PAGE patterns under reducing conditions of panna cottas during *in vitro* gastric digestion (0, 30, 60 and 120 min). Std: Standard marker; P:

control; M_0: skim milk powder added, no cream; W_0: whey protein concentrate added, no cream; C_0: calcium caseinate added, no cream.

VITAE:

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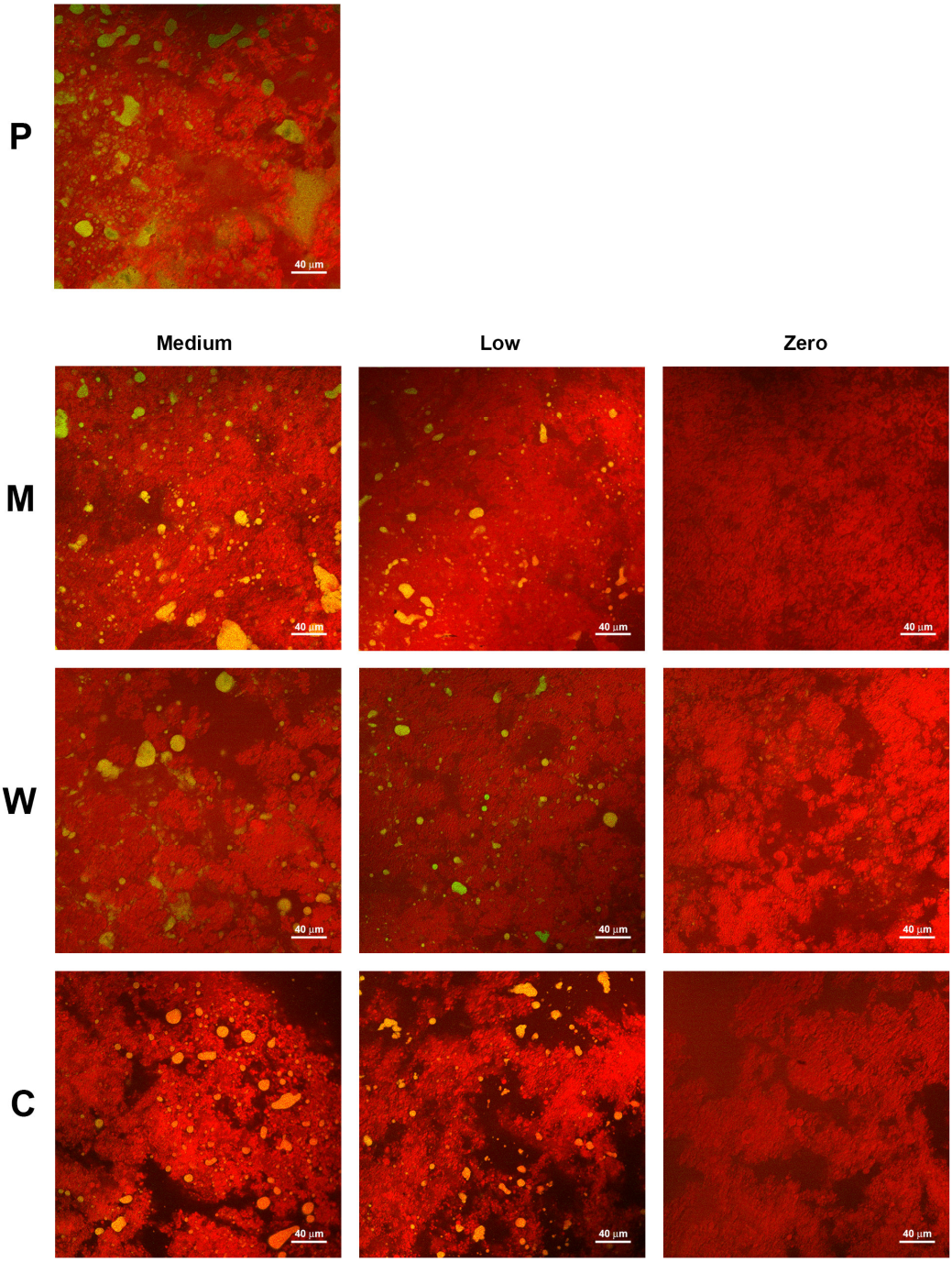
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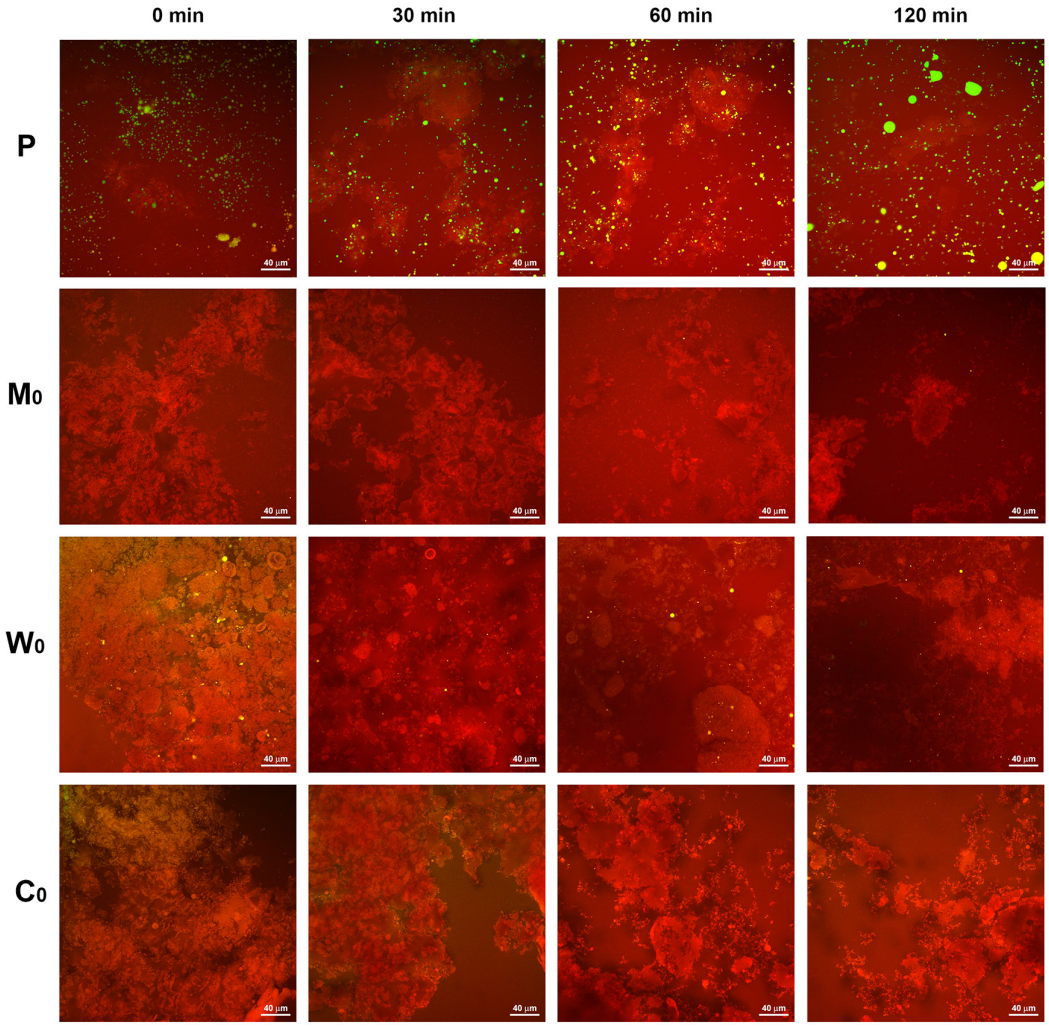

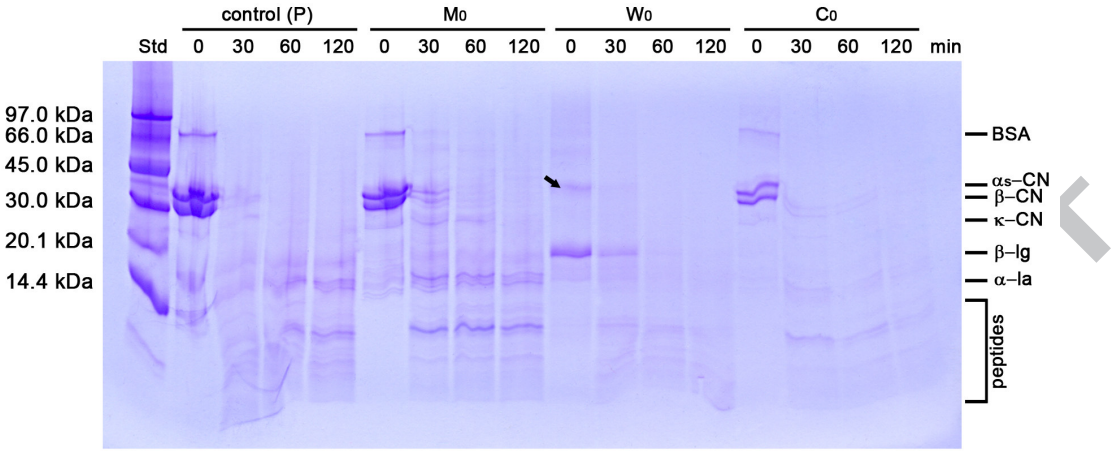

Ingredients	Samples									
	P	M_M	M_L	M_0	W_M	W_L	W_0	C_M	C_L	C_0
Skim milk (g)	50	100	100	100	50	50	50	50	50	50
Whey (g)	-	-	-	-	50	50	50	-	-	-
Caseinate (g)	-	-	-	-	-	-	-	50	50	50
Cream (mL)	200	50	25	0	50	25	0	50	25	0
κ-carrageenan (g)	3	3	3	3	3	3	3	3	3	3
Water (mL)	500	500	500	500	500	500	500	500	500	500

P: control; M_M, M_L, M_0: skim milk powder added and medium, low or zero amount of cream, respectively; W_M, W_L, W_0: whey protein concentrate added and medium, low or zero amount of cream, respectively; C_M, C_L, C_0: calcium caseinate added and medium, low or zero amount of cream, respectively.

Sample	Firmness (N)
P	1.10[a] (0.12)
M_M	1.12[a] (0.06)
M_L	1.29[b] (0.10)
M_0	1.32[b] (0.08)
W_M	3.95[c] (0.11)
W_L	4.59[d] (0.07)
W_0	4.24[e] (0.06)
C_M	2.92[f] (0.05)
C_L	3.30[g] (0.07)
C_0	3.21[g] (0.09)

Values in parentheses are the standard deviations. Different superscript letters in the same column denote values with statistically significant differences ($P < 0.05$) according to the LSD multiple range test.

P: control; M_M, M_L, M_0: skim milk powder added and medium, low or zero amount of cream, respectively; W_M, W_L, W_0: whey protein concentrate added and medium, low or zero amount of cream, respectively; C_M, C_L, C_0: calcium caseinate added and medium, low or zero amount of cream, respectively.

Highlights

- Low-cream extra-dairy protein panna cotta exhibited densest, firmest matrix
- Panna cottas with extra dairy proteins would have a high satiating capacity
- Whey proteins in panna cottas were more resistant to pepsin digestion than caseins
- Dairy proteins in panna cottas behaved in the same way as in model systems