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Cream replacement by hydrocolloid-stabilized emulsions to reduce fat digestion in panna cottas

Jennifer Borreani, Isabel Hernando*, Amparo Quiles

Food Microstructure and Chemistry Research Group, Department of Food Technology, Universitat Politècnica de València, Camino de Vera, s/n, 46022, Valencia, Spain

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ABSTRACT

The effect of emulsions based on different hydrocolloids (xanthan gum, hydroxypropyl methylcellulose and methylcellulose) on the structural, textural and sensory properties and lipid digestibility of panna cottas was investigated. The hydrocolloid-based emulsions presented similar microstructures. However, the panna cottas formulated with these emulsions presented large microstructural differences, which therefore also led to different textural and sensory properties. The differences in the initial microstructure of the panna cottas, the microstructural changes that they exhibited at the beginning of lipid digestion and the mechanisms of emulsification imparted by the hydrocolloids led to different extents of digestion. The panna cotta prepared with the hydroxypropyl methylcellulose-based emulsion was well accepted by the consumers and presented lower initial rate and extent of digestion (rate: 1,52% free fatty acid (FFA)/min; extent: 49.72% FFA) than the control panna cotta (3.69% FFA/min; 61.81% FFA). These results may contribute to the manufacture of reduced lipid digestion foods which could be used in weight management.

1. Introduction

Maintaining a proportionate intake of fat is nutritionally necessary for health and wellbeing. However, overconsumption of dietary lipids can lead to an excessive daily energy intake, which is seen as a contributing factor to the obesity epidemic (Norton, Fryer, & Norton, 2013). Reducing the fat content of foods has commonly been proposed as a method for reducing the consumers' energy intake (Lett, Norton, & Yeomans, 2016a) and still remains one of the most effective approaches that can be taken to minimize the impact of obesity and its related health issues (Norton et al., 2013).

However, reducing the fat content of foods usually decreases their desirable sensory qualities, because fat plays an important role in determining appearance, texture and taste. Foods with reduced fat levels must therefore be carefully formulated to ensure that they maintain their desirable physicochemical, sensory and nutritional properties, as otherwise they will not be acceptable to consumers (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2014).

An alternative approach that may maintain consumer satisfaction is to attempt to use other strategies associated with controlling fat digestion (Espinal-Ruiz et al., 2014). An understanding of the fate of lipids within the gastrointestinal human tract is important for designing functional foods to control the rate and extent of lipid digestion and absorption (Mun, Kim, McClements, Kim, & Choi, 2017). In general, the human body has excess capacity for fat digestion and the rate of fat digestion is controlled by the ability of lipase to bind to emulsion interfaces, which in turn is controlled by the emulsion size and interfacial composition. The initial composition and structure of the interfacial layer surrounding the lipid droplets in a food can be controlled by selecting specific emulsifier(s) and homogenization conditions to prepare an emulsion (McClements, Decker, Park, & Weiss, 2008).

Hydrocolloids are used in dairy products for two reasons: because they improve the texture and protect against creaming or flocculation and because of their function as thickeners, gelling agents, stabilizers, etc. In addition, hydrocolloids are known to have an impact on the behavior of lipids within the gastrointestinal tract, influencing lipid digestion through a variety of mechanisms: (i) binding to various intestinal components (e.g. bile salts, calcium ions, fatty acids, and lipase), (ii) altering the aggregation state of oil droplets, (iii) forming protective coatings around lipid droplets, or (iv) increasing the macroscopic viscosity of gastrointestinal fluids (Espinal-Ruiz et al., 2014; Qin, Yang, Gao, Yao, & McClements, 2016; Qiu, Zhao, Decker, & McClements, 2015).

For this study, three hydrocolloids (xanthan gum and two cellulose ethers) were selected because of their different characteristics. Xanthan

* Corresponding author.
E-mail addresses: jenbor@upvnet.upv.es (J. Borreani), mihernan@tal.upv.es (I. Hernando), mquichu@tal.upv.es (A. Quiles).

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gum is an anionic polysaccharide that thickens and stabilizes emulsions due to its ability to modify the rheology of the continuous phase (Dickinson, 2009). Hydroxypropyl methylcellulose and methylcellulose are non-ionic hydrocolloids which present good emulsifying properties due to their surface activity and can also be used as thickeners and gelling agents due to their thermal gelation ability. These two cellulose ethers differ principally in their degree and type of chemical substitution. In addition, panna cottas were chosen for being a dairy dessert with high calorie and fat contents that could be occasionally consumed as an indulgent choice.

The aim of this study was to obtain dairy desserts (panna cottas) with reduced lipid digestion whilst maintaining their sensory qualities. To this end, milk fat emulsions stabilized with different hydrocolloids were used to replace all the dairy cream content in traditional panna cottas. The emulsions were formulated with the same fat content and type (butter) as dairy cream in order to maintain the sensory properties of the dairy dessert. Stabilizing the emulsions with the hydrocolloids was expected to affect lipid digestion and so obtain an indulgent product (panna cottas) with reduced lipid digestion which could be used in weight management.

2. Materials and methods

2.1. Materials

Liquid cream (Pascual, Calidad Pascual SAU, Burgos, Spain) with a 35% fat content, butter (Consum, Iberleche SL, A Coruña, Spain), drinking water (Bezoya, Calidad Pascual SAU, Burgos, Spain), instant coffee (Carrefour, SEDA Outspan Iberia SL, Palencia, Spain) and liquid sweetener (12% sodium cyclamate and 1.2% saccharin) (Consum, Krüger GmbH & Co. KG., Bergisch Gladbach, Germany) were purchased from local supermarkets. Skim milk powder was kindly supplied by Central Lechera Asturiana (Siero, Spain). Xanthan gum (Satiaxane CX™ 911) and κ-carrageenan (Satiagel™ ME5) were obtained from Cargill France SAS (Saint-Germain-en-Laye, France). Sodium alginate (MANUCOL DMF) was purchased from FMC Biopolymer (Philadelphia, PA, USA). The cellulose ethers — hydroxypropyl methylcellulose (METHOCEL™ F4M; 6.8% hydroxypropyl, 29% methoxyl) and methylcellulose (METHOCEL™ A4M; 30% methoxyl) — were kindly supplied by The Dow Chemical Company (Bomlitz, Germany).

Lipase from porcine pancreas Type II (L3126), bile extract (B8631) and sodium phosphate (monobasic, monohydrate) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Calcium chloride ($CaCl_2$), sodium chloride (NaCl), sodium hydroxide (NaOH), sodium phosphate (dibasic, dodecahydrate) and toluidine blue O (C.I. 52040) were purchased from Panreac Química (Barcelona, Spain).

2.2. Emulsion preparation

The emulsions were prepared with butter (42.68 g/100 g emulsion), drinking water (57.07 g/100 g emulsion) and one of the hydrocolloids (0.25 g/100 g emulsion): (xanthan gum (XG), hydroxypropyl methylcellulose (HPMC) or methylcellulose (MC)).

The aqueous phase for the emulsion prepared with XG was prepared by dispersing XG in water (80 °C) using a stirrer (RZR 1, Heidolph, Schwabach, Germany) at 283 rpm for 1.5 min and subsequently at 464 rpm for 2.5 min.

The aqueous phase for the emulsions prepared with cellulose ethers was prepared with the "hot/cold" technique (Espert et al., 2017).

The butter was heated at 60 °C and added to the aqueous phase. The mixture was homogenized (Ultraturrax T18, IKA, Staufen, Germany) for 1 min at each speed (6400, 10000, 140000 and 18000 rpm), obtaining the following emulsions: EX (emulsion with XG), EH (emulsion with HPMC), or EM (emulsion with MC).

2.3. Panna cotta preparation

The reformulated panna cottas (PX, PH or PM) were prepared by replacing all the liquid cream content in the control panna cotta formulation with the relevant emulsion (EX, EH or EM, respectively). All the panna cottas had the same fat content (25.98 g/100 g panna cotta).

The panna cottas were prepared according to the method used by Borreani, Llorca, Quiles, and Hernando (2017c) and stored at 4–5 °C until they were analyzed.

2.4. Microstructure analysis

2.4.1. Light microscopy (LM)

For light microscopy (LM) observation, one drop of the emulsion (cream, EX, EH or EM) or a slim section from a frozen (−20 °C for 24 h) cube of the panna cotta was placed in the center of the microscope slide and observed according to Borreani et al. (2017c).

2.4.2. Cryo-scanning electron microscopy (Cryo-SEM)

The samples were prepared and observed according to Hernández-Carrión, Vázquez-Gutiérrez, Hernando, and Quiles (2014).

2.4.3. Particle size measurement

The particle size (mean area of fat droplets and aggregates) of emulsions and fresh, pre-digested and t = 0 min panna cottas was determined from LM and cryo-SEM images with the microscope software (NIS-Elements D, version 4.2, Nikon, Tokyo, Japan).

2.5. Texture analysis

A penetration test was performed in accordance with Borreani, Hernando, Salvador, and Quiles (2017b). The firmness of the panna cotta was defined as the maximum force (N) measured during sample penetration and the stiffness ($N\ s^{-1}$) as the slope of the curve before the rupture point. The texture analysis was performed in triplicate.

2.6. Sensory analysis

The sensory analysis was carried out with 70 consumers (43 men and 27 women) recruited among the employees and students of the University. The samples (control, PX, PH and PM) were analyzed in a sensory laboratory equipped with individual booths (ISO, 2007, pp. 9–11), according to the method described by Borreani et al. (2017b).

The consumer acceptance test was performed using a nine-point hedonic scale (from 1 = dislike extremely to 9 = like extremely). For each panna cotta, the consumers scored their degrees of liking in the following order: "appearance", "texture", "taste" and "overall acceptability".

2.7. In vitro intestinal model

An *in vitro* digestion model that simulated the small intestine was used, following a slightly modified version of Li, Hu, & McClements' (2011) method. Panna cotta samples (5 g) were mixed with phosphate buffer (pH 7.0) at a 1:3 ratio. The mixture was incubated at 37 °C for 10 min with continuous stirring at 760 rpm (pre-digestion step), then 5 mL of bile extract solution (275 mg of bile extract dissolved in phosphate buffer, pH 7.0) and 1 mL of salt solution (30.5 mg of $CaCl_2$ and 244.1 mg of NaCl dissolved in phosphate buffer, pH 7.0) were added to the samples and the mixture was adjusted to pH 7.0 (at this step the withdrawn aliquot corresponds to t = 0 min). Afterwards, 1.5 mL of freshly prepared lipase suspension (522 mg lipase powder dispersed in phosphate buffer; 1:2.5 enzyme/substrate ratio) were added to the mixture and titration started. The mixture was maintained at 37 °C and 760 rpm for 2 h to mimic conditions in the small intestine, withdrawing aliquots at intervals during the small intestine stage

(t = 30, 60 and 120 min). The final composition of the sample in the reaction cell was 1305 mg of lipid, 10 mg mL^{-1} of bile extract, 19 mg mL^{-1} of lipase, 10 mM $CaCl_2$ and 150 mM NaCl. A pH-stat automatic titration unit (Mettler-toledo DL 50, Greinfensee, Switzerland) was used to automatically monitor the pH and maintain it at pH 7.0 by titrating appropriate amounts (mL) of NaOH solution (0.5 M). The volume of NaOH added to the sample was recorded and used to calculate the concentration of free fatty acids (FFA) generated by lipolysis, in other words, the number of moles of NaOH required to neutralize the FFA (assuming 2 FFA produced per 1 triacylglycerol molecule). The measurement was carried out in triplicate.

2.8. Statistical analysis

One-way analysis of variance (ANOVA) was used to study the differences between formulations in all the experiments, employing XLSTAT statistical software (Addinsoft, NY, USA). The least significant differences (LSD) were calculated by Fisher's test ($P < 0.05$).

3. Results and discussion

3.1. Microstructure analysis

3.1.1. Emulsions

In all the emulsions (Fig. 1), the fat was observed as circular globules distributed homogeneously throughout the sample. The fat globules in the commercial cream (Fig. 1A) were smaller (area ≈13 μm^2) and more homogeneous in size than those of the other emulsions (EX: 148 μm^2, EH: 142 μm^2, EM: 158 μm^2). This was probably due to the different homogenization processes used in preparing the commercial cream (high-pressure homogenizer) and the EX, EH and EM emulsions (ultraturrax). Moreover, the higher protein content in the commercial cream than in milk fat emulsions (EX, EH and EM) could be an additional explanation to the smaller droplet size in the commercial cream. The microstructure of emulsions EX, EH and EM (Fig. 1B–D) was similar and few differences in globule size were observed between them. Similar globule sizes were observed in emulsions prepared with different cellulose ethers with thermo-gelling ability (Borreani et al., 2017a).

3.1.2. Panna cottas

The control panna cotta presented a homogeneous microstructure (Fig. 2A) with the fat (uncolored) embedded in the continuous network formed by proteins and polysaccharides (purple). In panna cottas PX and PH (Fig. 2B and C), well-defined round fat globules (black arrows) homogeneously distributed inside the continuous network were observed. However, these panna cottas (PX and PH) also presented some fat aggregates (red arrows in Fig. 2B and C), which seemed to be smaller in PX than in PH. In contrast, panna cotta PM (Fig. 2D) exhibited a heterogeneous microstructure in which most of the fat was observed to have flocculated and/or coalesced, resulting in clumps of fat.

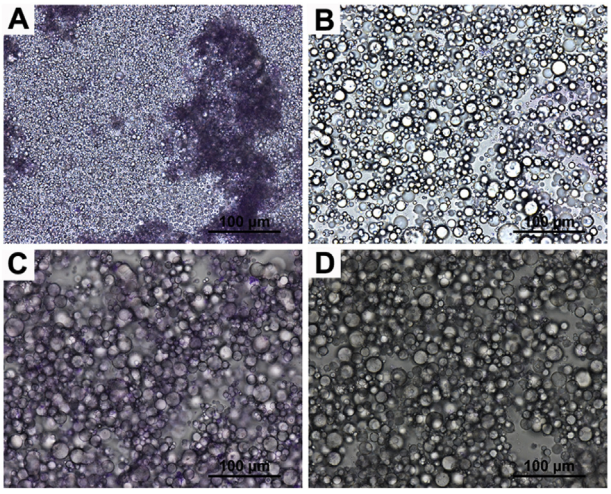

Fig. 1. Light microscopy images of the emulsions employed to prepare the panna cottas. A: commercial liquid cream; B: emulsion with XG (EX); C: emulsion with HPMC (EH); D: emulsion with MC (EM). The scale bars measure 100 µm.

With cryo-SEM, the fat in the control panna cotta was observed as round, well-defined fat globules, homogeneously distributed through the network (Fig. 2E) and smaller and more homogeneous in size than those in panna cottas PX and PH (Fig. 2F and G). This was due to the commercial cream having a smaller initial droplet size than in emulsions EX and EH, as previously observed in Fig. 1. The fat aggregates of panna cottas PX and PH (red arrows in Fig. 2B and C) seemed to be fragments of the emulsions (EX or EH, respectively) embedded in the continuous network formed by proteins and polysaccharides (red arrows in Fig. 2F and G). In these fragments of emulsions, fat globules (white arrows) remained trapped in the entangled hydrocolloid network. Because of xanthan gum possesses stable viscosity within a wide range of temperatures (Katzbauer, 1998) and HPMC possesses thermal gelation around 70 °C, emulsions EX and EH could have maintained their structure during the preparation of panna cottas PX and PH respectively and thus, some fragments of these emulsions formed by stirring were present. The heterogeneous aspect of panna cotta PM in Fig. 2D was due to the undefined shape of the fat and the disrupted network (Fig. 2H). Therefore, emulsion EM seemed to have been destabilized during the preparation of panna cotta PM and thus, some flocculation and coalescence occurred resulting in the presence of clumps of fat.

Although no major microstructural differences were observed between emulsions EX, EH and EM (Fig. 1B–D), great differences were observed between their panna cottas, respectively PX, PH and PM (Fig. 2B–D and F–H). This shows that each emulsion was affected differently by the panna cotta preparation conditions and/or that different interactions took place between the emulsions and the other ingredients. Therefore, the use of different hydrocolloids leads to different complex matrix structures in the panna cottas.

3.2. Texture analysis

The firmness values (Table 1) of the different panna cottas analyzed in this study ranged from 0.80 N (PM) to 0.85 N (control). Panna cottas PH and PM exhibited significantly ($P < 0.05$) lower firmness values than the control and PX panna cottas. As regards the stiffness values, the control was significantly ($P < 0.05$) stiffer than all the other panna cottas.

The differences in firmness and stiffness values could be attributed to the different hydrocolloids used and/or to the distribution of the fat in the complex matrix of the panna cottas. On the one hand, the xanthan gum improved the firmness in comparison with the cellulose ethers, due to their different chemical structures and thus to their different thickening properties. Xanthan molecules are not considered to be surface-active at the oil-water interface, but they can interact with pre-adsorbed protein molecules during the formation of panna cottas. Conversely, hydroxypropyl methylcellulose and methylcellulose molecules are surface-active compounds at the oil-water interface and they can act as both emulsifying and stabilizing agent. Xanthan gum forms a high-viscosity pseudoplastic material and possesses stable viscosity within a wide range of pH values, temperatures and salt contents (Katzbauer, 1998), whereas cellulose ether solutions undergo a sol – gel transition upon heating and return to a solution on cooling (Sanz, Falomir, & Salvador, 2015). The temperature at which the gelation process starts and the strength of the gel formed depend on the type and degree of cellulose substitution, the molecular weight and the presence

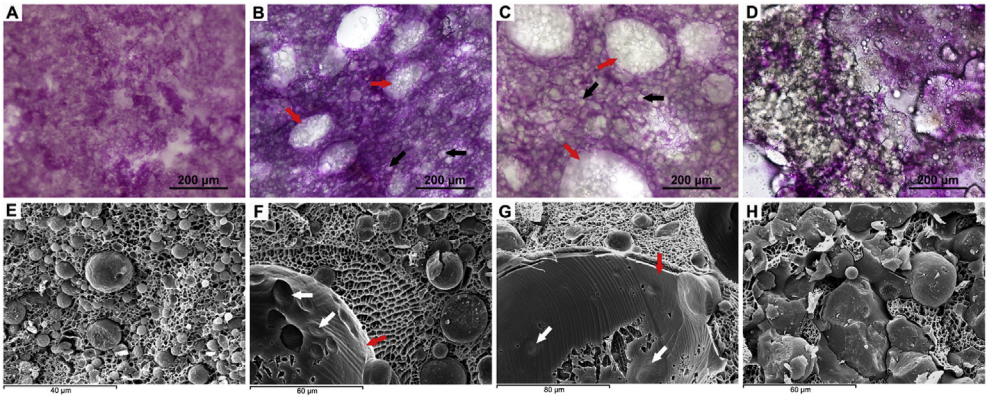

Fig. 2. Light microscopy (A–D) and cryo-SEM (E–H) images of the panna cottas. A and E: control; B and F: PX; C and G: PH; D and H: PM. The scale bars measure 200 μm (A–D), 40 μm (E), 60 μm (F, H) or 80 μm (G). Red arrows show emulsion fragments, black arrows show fat globules embedded in the protein and polysaccharide network and white arrows show fat globules in the emulsion fragments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Influence of cream replacement by milk fat emulsions on the texture of the panna cottas (n = 15).

Sample	Firmness (N)	Stiffness (N s^{-1})
Control	0.85 ± 0.02 ^{a}	2.05 ± 0.10 ^{a}
PX	0.84 ± 0.03 ^{a}	1.76 ± 0.10 ^{b}
PH	0.81 ± 0.05 ^{b}	1.82 ± 0.13 ^{b}
PM	0.80 ± 0.05 ^{b}	1.80 ± 0.09 ^{b}

Mean values ± standard deviations. Values with different letters within the same column are significantly different ($P < 0.05$) according to the LSD multiple range test.

Table 2
Liking scores for appearance, texture, taste and overall acceptability of the panna cottas (n = 70).

Sample	Appearance	Texture	Taste	Overall acceptability
Control	7.1 ± 1.2 ^{a}	7.3 ± 1.4 ^{a}	6.8 ± 1.8 ^{a}	6.9 ± 1.6 ^{a}
PX	7.1 ± 1.5 ^{a}	6.2 ± 1.7 ^{b}	4.8 ± 2.1 ^{c}	5.2 ± 1.9 ^{c}
PH	6.7 ± 1.3 ^{a}	6.4 ± 1.7 ^{b}	5.9 ± 2.0 ^{b}	6.0 ± 1.7 ^{b}
PM	6.1 ± 1.8 ^{b}	6.0 ± 1.7 ^{b}	5.6 ± 1.8 ^{b}	5.5 ± 1.6 ^{bc}

Mean values ± standard deviations. Values with different letters within the same column are significantly different ($P < 0.05$), according to the LSD multiple range test.

and concentration of electrolytes (Sanz et al., 2015). As a result, the low temperature of the panna cottas (stored at 4–5 °C until texture analysis) may have reduced gel network formation in the panna cottas prepared with the cellulose ethers, which could be the reason for their exhibiting lower firmness values than those prepared with xanthan gum. Moreover, these results were in accordance with the microstructure results (Fig. 2), where panna cotta PX presented a more compact network than the cellulose ether panna cottas. On the other hand, since the PX panna cotta contained 0.19% w/w of hydrocolloid (XG) due to the incorporation of emulsion EX, it was expected to exhibit higher firmness than the control panna cottas, however, no significant differences were observed ($P > 0.05$). Therefore, the microstructure of the panna cottas also contributed to their texture characteristics. The small, well-defined fat globules homogeneously distributed in the continuous network of the control panna cotta (Fig. 2A and E) could provide a higher degree of cross-linking of protein molecules than in panna cotta PX, which exhibited larger fat globules and fragments of emulsion, and, especially, than in panna cotta PM, where the fat formed clumps (Fig. 2D and H). The higher degree of protein molecule cross-linking could result in three-dimensional networks with a more compact structure that would exhibit higher resistance to deformation (Lobato-Calleros et al., 2007) and thus provide higher firmness and stiffness values. Moreover, the high protein content of the control panna cotta, due to using cream in the formulation, could lead to the formation of a harder network surrounding the fat globules.

3.3. Sensory analysis

The consumer acceptability results for the different panna cottas are shown in Table 2. The control and PX panna cottas obtained the same appearance score. This could be due to the smooth appearance of both (results not shown). Despite the slightly lumpy appearance of panna cotta PH and thus its lower appearance score, no significant differences ($P > 0.05$) were observed between the control and PH panna cottas. As expected, panna cotta PM obtained significantly ($P < 0.05$) the worst result due to having the lumpiest appearance.

As regards the texture acceptability results, the control panna cotta was rated significantly ($P < 0.05$) the best. Surprisingly, no significant differences were found between panna cottas PX, PH and PM.

Replacing the cream with milk fat emulsions (EX, EH or EM) significantly affected ($P < 0.05$) the taste of the panna cottas. The control obtained the best score and PX the worse score, while PH and PM, the panna cottas formulated with cellulose ethers, obtained intermediate scores. On the one hand, Lett, Yeomans, Norton, and Norton (2016b) found that flavor intensity (vanilla and cream) significantly increased with decreasing droplet size. Thus, in line with this finding, the control panna cotta with the smallest droplet size (Fig. 2A and E) could lead to increased flavor intensity in comparison with the other panna cottas. The increased contact between the sample and the surface of the mouth could have enhanced flavor intensity (Lett et al., 2016b). Increased flavor intensity was expected to positively influence liking scores. On the other hand, the results demonstrated that the taste of the panna cottas seems to be dependent on hydrocolloid type, as Arancibia, Castro, Jublot, Costell, and Bayarri (2015) observed in their study on dairy desserts. It is generally believed that texture influences flavor perception and that an increase of viscosity, for example due to addition of hydrocolloids, generally leads to a decrease in aroma and taste perception (He, Hort, & Wolf, 2016; Tournier, Sulmont-Rossé, & Guichard, 2007). This is attributed to lowering diffusion rate of tastant molecules from the interior of the sample to the taste receptors on the tongue due to the increased solution viscosity. Nevertheless, some studies indicated that the impact of hydrocolloids on flavor perception appeared to be related to the physicochemical properties of the taste and aroma compounds (Bylaite, Adler-Nissen, & Meyer, 2005; Tournier et al., 2007), as well as to the nature of hydrocolloids (He et al., 2016). Therefore, the decrease of aroma and taste perception induced by hydrocolloids may also be the result of specific molecular interactions between the flavor compounds and the hydrocolloids (Bylaite et al., 2005). It was reported that xanthan decreases sweetness perception (He et al., 2016; Tournier et al., 2007), as well as aroma intensity of butyric acid and dimethyl sulphide (Tournier et al., 2007). In the present study, texture acceptability scores did not show significant differences ($P > 0.05$) between panna cottas PX, PH and PM. Therefore, we hypothesized that the worse taste found in PX compared with panna cottas

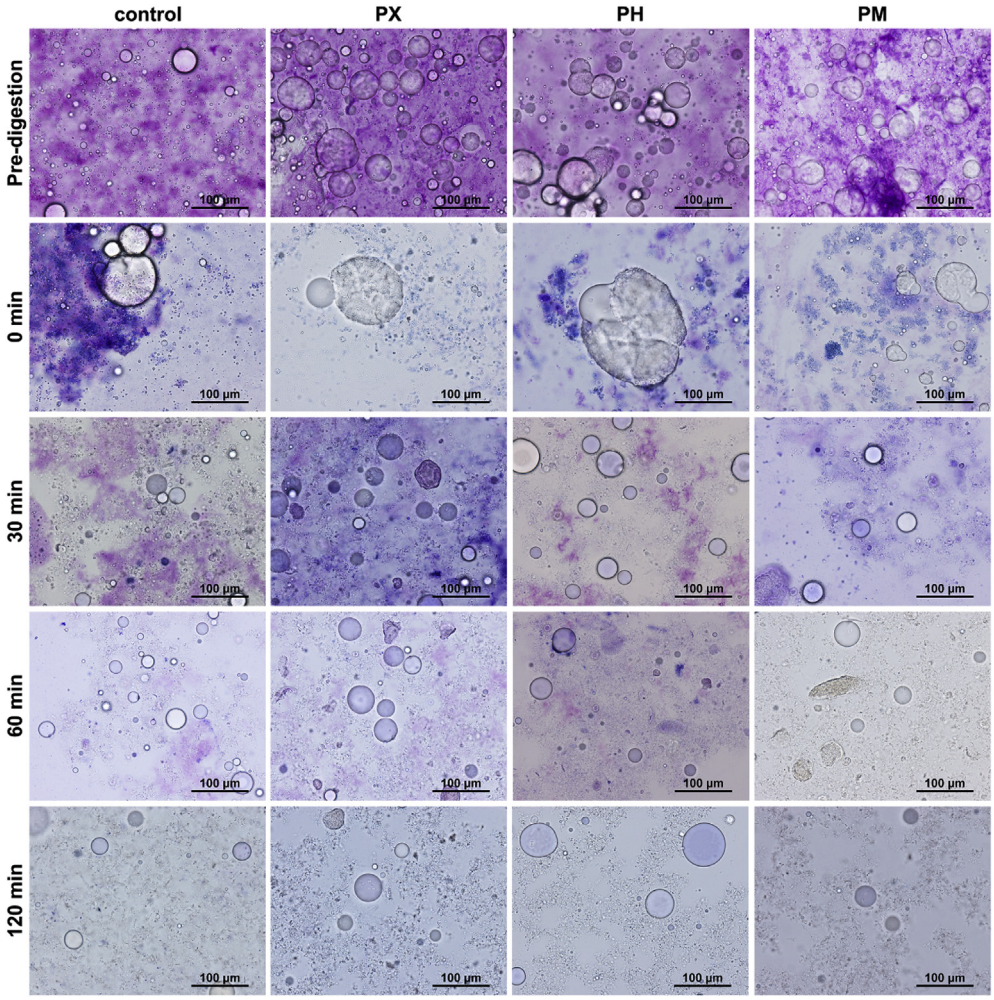

Fig. 3. Light microscopy images of the different panna cottas during the *in vitro* small intestine digestion. Pre-digestion: samples diluted with phosphate buffer during 10 min at 37 °C; 0 min: after the addition of bile salts and electrolytes solutions; 30–120 min: lipase digestion. The scale bars measure 100 µm.

PH and PM, could be due mainly to different interactions (hydrophobic binding, hydrogen bonding, and/or entrapment) between xanthan gum and aroma and tastant compounds more than to different textures.

The overall liking scores for the different samples showed that the consumers preferred the control panna cotta, followed by PH, PM and finally PX. However, no significant differences ($P > 0.05$) were found between panna cottas PX and PM, or between panna cottas PH and PM.

In conclusion, although the fat type and content was maintained in the reformulated panna cottas (PX, PH and PM), the incorporation of different hydrocolloids significantly affected the sensory properties of the panna cottas. However, panna cotta PH, the best-rated among the reformulated panna cottas, seemed to be well accepted by the consumers.

3.4. *In vitro intestinal digestion*

At the end of the pre-digestion step (samples diluted with phosphate buffer), the main change observed in the microstructure was the diluted appearance of all the panna cottas, as the fat was still embedded in the purple network (Fig. 3).

After the addition of bile salts and electrolyte solutions (t = 0 min), important changes were observed in all the samples. The protein-hydrocolloid network seemed disrupted and many fat globules had coalesced, resulting in fat aggregates, and only a few small fat globules remained visible at this magnification. The coalescence phenomena were mainly due to the presence of bile salts and mineral ions. The bile salts may have fully or partially displaced the original emulsifier molecules from the lipid droplet surface, then the cationic sodium and calcium ions may have promoted droplet flocculation (Li & McClements, 2010) and thus the subsequent coalescence.

After 30 min of lipase digestion, well-defined fat globules were observed in all the panna cottas. This would suggest that the fat aggregates formed at t = 0 min as a result of coalescence phenomena had been attacked by the lipase molecules. From 30 to 120 min, no great changes were observed, only some coalescence phenomena and a lower number of fat globules due to the progress of digestion. This indicates that the main structural changes took place during the first 30 min and that the panna cottas, whatever their initial structure, underwent major changes during *in vitro* small intestinal digestion.

The lipid digestion profiles of all the panna cottas followed a similar pattern (Fig. 4). A rapid increase in FFA release was observed in the initial period (the first 10–20 min), followed by a more gradual increase over longer times. Therefore, lipid digestion seemed to occur mainly during the first 20 min, as observed in the microstructure findings (Fig. 3). The slower rate of FFA release after 10–20 min could be associated with an accumulation of lipolysis products at the droplet surface, which could compete with the lipase molecules for adsorption at the interface, reducing the lipase activity (Bellesi, Martinez, Pizones Ruiz-Henestrosa, & Pilosof, 2016; Mun, Decker, & McClements, 2007).

To obtain a clearer comparison between the samples, the initial rate of FFA release was calculated from Fig. 4 by fitting a straight line to the first 10 min, according to the Chang and McClements (2016) method,

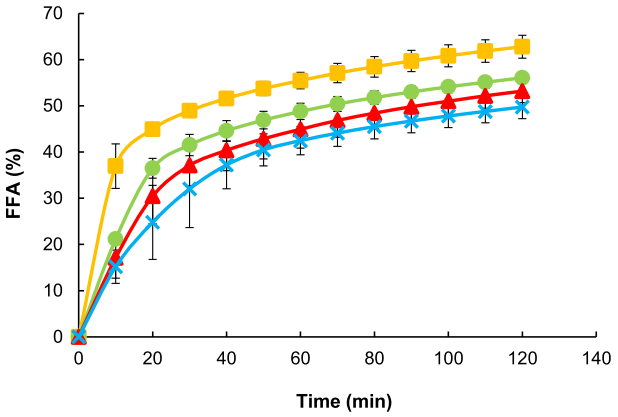

Fig. 4. Free fatty acid (FFA) released under *in vitro* small intestinal conditions from the different panna cottas (control ■, PM ●, PX ▲, PH X). The error bars represent standard deviations.

Table 3
Influence of the emulsion structure and composition on the initial digestion rate and extent of final digestion of the panna cottas (n = 3).

Sample	Initial rate (%FFA/min)	Extent (%FFA)
Control	3.69 ± 0.48 ^{a}	62.81 ± 2.47 ^{a}
PX	1.71 ± 0.44 ^{b}	53.19 ± 2.46 ^{bc}
PH	1.52 ± 0.36 ^{b}	49.72 ± 2.49 ^{c}
PM	2.12 ± 0.07 ^{b}	56.06 ± 1.25 ^{b}

Mean values ± standard deviations. Values with different letters within the same column are significantly different ($P < 0.05$), according to the LSD multiple range test.

and calculating the final extent of digestion at the end of 2 h incubation. These results are presented in Table 3.

The initial digestion rate was appreciably influenced by substituting milk fat emulsions for the cream. The control panna cotta was initially digested faster than the other panna cottas. This could be attributed to the smaller size of the lipid droplets in the control panna cotta (Fig. 2, ≈54 μm^2 and Fig. 3 at the pre-digestion step, ≈ 71 μm^2) and the slight coalescence phenomena observed before the addition of lipase (Fig. 3 at t = 0 min, ≈ 9 mm^2). As a result of its small droplet size, the control panna cotta presented an increase in surface area and therefore more sites for lipase molecules to bind to the lipid substrate, resulting in a relative increase in the rate of lipolysis. Although panna cotta PM at t = 0 min (Fig. 3) exhibited less extensive coalescence (≈10 mm^2) and therefore a higher initial digestion rate (Fig. 4) than panna cottas PX and PH (≈23 mm^2 and 26 mm^2, respectively), no significant ($P > 0.05$) differences between them were observed (Fig. 4 and Table 3). After the first 10 min, a clear differentiation between panna cottas PX, PH and PM was observed (Fig. 4), and the final extent of digestion of all the panna cottas showed the following order: control > PM ≥ PX ≥ PH (Fig. 4 and Table 3). The different digestion rate and extent results could be due mainly to the different initial structures of the panna cottas (observed in Fig. 2) and to the changes observed at the beginning of digestion (at the pre-digestion step and at t = 0 min), but also to the different mechanisms of emulsification imparted by the hydrocolloids. The control panna cotta presented the smallest fat globule size initially (Fig. 2A and E, ≈ 54 μm^2) and the least extent of coalescence at t = 0 min (Fig. 3, ≈9 mm^2), providing more accessible sites for lipase molecules, and thus was the most-digested sample. Panna cottas PX and PH initially presented some fragments of emulsion (red arrows in Fig. 2) and larger fat globules (Fig. 2B and F, ≈ 500 μm^2 and Fig. 2C and G, ≈ 771 μm^2) than the control panna cotta (Fig. 2A and E, ≈ 54 μm^2), as well as larger fat aggregates (Fig. 3, ≈23 and 26 mm^2) at t = 0 min (due to coalescence phenomena), so the fat was less accessible to the lipase molecules and these were the least-digested samples. Although the fragments of emulsion were larger in panna cotta PH (Fig. 2C, ≈ 31 mm^2) than in panna cotta PX (Fig. 2B, ≈ 11 mm^2), the fat aggregates at t = 0 min were fairly similar in size (Fig. 3, PH ≈ 26 mm^2 and PX ≈ 23 mm^2). Consequently panna cotta PH did not present significant differences ($P > 0.05$) with panna cotta PX in both digestion rate and extent. Despite xanthan gum does not have surface activity, it has the ability to thicken the system, which could imply a physical impediment for the lipase to reach the interface, as well as it could interact with pre-adsorbed protein at the oil-water interface and thus hinder lipase to attach to. This could also explain why panna cotta PX exhibited lower digestion extent than the control and PM panna cottas. Moreover, HPMC has been reported to form a physical barrier on the interface, which is resistant to displacement by bile salts. Consequently, it is difficult for lipase to access the interface required for lipid digestion (Torcello-Gómez & Foster, 2016) and thus, this could also explain why panna cotta PH was the least-digested sample. In the case of the PM panna cotta, the clumps of fat (Fig. 2D and H) could present a smaller surface area than the small droplets in the control panna cotta (Fig. 2A and E) and thus, the PM panna cotta obtained a lower result for the extent of digestion than the control panna cotta. Moreover, because emulsion EM was destabilized during the preparation of the relevant panna cotta (PM), methylcellulose molecules could have formed a weak protective layer around fat droplets; consequently, panna cotta PM was digested to a higher extent than panna cottas PX and PH.

4. Conclusions

On the one hand, this study shows that different hydrocolloids influence the microstructure of the panna cottas. Therefore, although a dairy fat was used in all the panna cottas and fat content was maintained, the reformulated panna cottas possessed different textural and sensory properties from the control panna cotta, being PH the most liked reformulated panna cotta. On the other hand, this study shows that both the superficial area of the fat and the different mechanisms of emulsification imparted by the hydrocolloids have an impact on lipid digestion. Thus, because of panna cotta PH presented the fewest bind sites for the lipase molecules (high superficial area of the fat and strong surface-activity of HPMC molecules), it presented lower initial rate and extent of digestion than the control panna cotta. These results may contribute to the manufacture of reduced lipid digestion foods which could be used in weight management.

Declaration of competing interest

The authors declare the absence of conflicts of interest

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