STRUCTURAL CHANGES OF FILLING CREAMS AFTER *IN VITRO* DIGESTION. APPLICATION OF HYDROCOLLOID BASED EMULSIONS AS FAT SOURCE

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ABSTRACT

The objective of this study was to evaluate the application of hydrocolloid-based o/w emulsions as fat source in filling creams. Emulsions containing sunflower oil stabilized by different hydrocolloids (hydroxypropyl methylcellulose (HHPMC), methylcellulose (MC) and xanthan gum (XG)), were employed. The changes in the structure of the systems before and after *in vitro* digestion were studied by textural analysis and confocal and light microscopy, and they were related to lipid digestibility after intestinal digestion. The presence of XG led to a significant increase in consistency in the stomach digestion phase compared to stomach water dilution incubation (6.32 N.s and 4.72 N.s respectively). After the intestinal phase, an increase in globule size was observed in all creams (MC: from 8.4 (fresh cream) to 15.4 μm (after intestinal step); HHPMC: from 12.8 to 17.6 μm ; XG: from 27.5 to 32.0 μm), as well as the appearance of coalescence phenomena due to changes in fat stability, although fat globules are still visualized. Filling creams formulated with hydrocolloid-based o/w emulsion were more resistant to *in vitro* digestion than the control cream, exhibiting a significant decrease in fat digestibility (MC 240 g/Kg, HHPMC 230 g/Kg and XG 490 g/Kg reduction of fatty acids released).

Key words: cellulose ether; filling cream; lipid digestibility; structure; xanthan gum.

1. Introduction

The development of foods that reduce fat bioaccessibility or appetite represents a potential solution to mitigate obesity and related chronic diseases (Guo, Ye, Bellissimo, Singh, & Rousseau, 2017).

Fat exist in many different forms in food, including bulk lipids, water in oil emulsions (margarine, butter) and oil in water emulsions (cream, dressings) (McClements, 2018) and provides a wide variety of desirable properties affecting texture, mouthfeel and functionality of food (Co & Marangoni, 2012). The most commonly used fats in pastries and confectionery food are butter, margarine or hydrogenated oils (Talbot, 2009; Van den Brecht, Van hoed, Müllendorff, & Arnaut, 2012). The fat content of the creams can reach up to 60% and has a great effect on its sensory, rheological and textural properties (Miele, Di Monaco, Masi, & Cavella, 2015; Young & Wassell, 2008). As regards hydrogenated vegetable oils, they have been used in a wide variety of confectionery and pastry food in recent years. However, hydrogenated oils are the primary dietary source for *trans* fats and there is scientific evidence showing that the consumption of *trans* fatty acids is associated with coronary heart disease and diabetes (Mozaffarian, Katan, Ascherio, Stampfer, & Willett, 2006). An additional problem is that some alternatives to *trans* fats may lead to higher levels of saturated fatty acids.

The use of vegetable fat such as sunflower oil in reformulated food improves the lipid profile in the final product, but the low percentage of saturated fat in its composition cannot achieve the semi-solid structure associated with solid fat. One way to obtain the desirable characteristics is by incorporating hydrocolloids as stabilizers in an oil-water emulsion, which can act as saturated fat replacer (Tarancón, Salvador, & Sanz, 2013).

The presence of non-fat particles and the type and amount of fat in an emulsion play an important role in the physicochemical, sensory and nutritional properties, determining

the texture, mouthfeel, flavour stability, appearance and biological response in emulsion-based products (Chung, Smith, Degner, & McClements, 2016). However, besides using vegetable oils emulsified by hydrocolloids to improve the plasticity of fat (semi-solid character), it would also be interesting for the structure of the stabilized emulsion to be able to resist throughout the digestion process, thus influencing lipid digestion (Bonnaire et al., 2008; Qiu, Zhao, Decker, & McClements, 2015). Recently, cellulose ether or xanthan gum based oil in water emulsions have been recognized as a good strategy by which to reduce the fat digestibility (Espert et al., 2017; Espert, Salvador, & Sanz, 2019).

Many studies about fat bioaccessibility are based on model systems (such as an oil-water emulsion), but it would be interesting to obtain an approach on the digestibility mechanisms of fat in more real matrices such as a foodstuff.

Therefore, the objective of this study is to evaluate the physical properties and fat lipolysis of filling creams composed of o/w hydrocolloid-based emulsions as fat source. For this purpose, the structural changes of filling creams during *in vitro* digestion and their relationship with fat digestibility are evaluated.

2. Materials and Methods

2.1 Materials

Filling creams were composed of sugar (AB Azucarera Iberia, Madrid, Spain), skimmed powder milk (1 g/kg fat) (Central Lechera Asturiana, Granda, Spain), starch (CTex 06205, Cargill BV, Amsterdam, Netherlands), mineral water and o/w emulsion. For the control cream, the o/w emulsion was replaced by sunflower oil (Koipe Sol, Deoleo S.A., Madrid, Spain). Three different emulsions were prepared with sunflower oil, water and three different hydrocolloids (two types of cellulose ethers and xanthan gum (XG)).

Cellulose ethers with thermogelling ability were supplied by The Dow Chemical Co (Brussels, Belgium) (METHOCELTM K4M and A4M, from now on referred to as HHPMC (high hydroxypropyl methylcellulose) and MC (methylcellulose), respectively) with differing degrees of methoxyl content. Their percentage of chemical substitution is: HHPMC 225 g/Kg methoxyl, 77 g/Kg hydroxypropyl; MC 300 g /Kg methoxyl. Both celluloses have approximately the same molecular weight (MW) and a viscosity of 4000 mPa s (20 g/L aqueous solution at 20 °C measured by The Dow Chemical Company following reference methods ASTM D1347 and ASTM D2363). Xanthan gum (XG) (Satiaxane CX911), with a viscosity of 1500 mPa s in a 10 g/L aqueous solution (10 g/L KCl), measured by Cargill France SAS, was supplied by Cargill (Baupre, France).

Calcium chloride anhydrous, di-potassium hydrogen phosphate trihydrate and disodium hydrogen phosphate dihydrate were purchased from Scharlab S.L. (Barcelona, Spain) and sodium carbonate anhydrous, sodium chloride, sodium hydrogen carbonate, potassium chloride pure and calcium chloride 2-hydrate were provided by Panreac Química S.L.U. (Barcelona, Spain). Sodium phosphate monobasic dehydrate was provided by Acros Organics (Merelbeke, Belgium). α -amylase from porcine pancreas (A3176-1MU), mucin from porcine stomach (M2378), pepsin from porcine gastric mucosa (P7000), bile extract porcine (B8631), pancreatin from porcine pancreas (P1750) and Nile Red and Rhodamine B dyes were supplied by Sigma-Aldrich Chemical Company (St Louis, Missouri).

2.2. Emulsion preparation

200 g of emulsion was prepared using the following proportions: water 510 g/Kg, sunflower oil 470 g/Kg and hydrocolloid 20 g/Kg.

To prepare the cellulose o/w emulsion, cellulose ether was first dispersed in the oil using a Heidolph stirrer at the lowest speed (300 rpm) for five minutes. The mixture was then hydrated by gradually adding the water at 10 °C while continuing to stir for 30 s. Stirring continued using a homogenizer (Ultraturrax T18, IKA, Königswinter, Germany) with the S18N-19G dispersion tool at 6500 (1 min⁻¹) for 15 s, and subsequently at 17500 (1 min⁻¹) for 60 s until the emulsion was obtained.

In XG o/w emulsion, the XG was first dispersed in the water at room temperature using a Heidolph stirrer RZR 1 (Heidolph Instruments, Schwabach, Germany) at 300-500 rpm for 10-12 min. Then the oil was gradually added while continuing to stir, increasing the speed up to 1800 rpm. Stirring was maintained for an additional 15 min. The 200 g mixture was then homogenized using an IKA T18 Ultra-Turrax with the S18N-19G dispersion tool at 6500 (1 min⁻¹) for 60 s, 13500 (1 min⁻¹) for 60 s and subsequently at 17500 (1 min⁻¹) for 60 s.

The emulsions were stored at room temperature for 24 h before the cream preparation.

2.3. Cream preparation

The different cream formulations are shown in Table 1. The preparation of the cream was performed in a TM31 Thermomix (Thermomix, Vorwerk, Wuppertal, Germany) and was carried out in two stages. In the first step, sugar, milk powder, starch and water were mixed at speed 2 for 6 min at 90 °C and then the mixture was allowed to cool to room temperature. In the second stage, the fat (sunflower oil (in control cream) or o/w emulsion (in emulsion creams)) was gradually added by mixing at speed 2 for 5 min. Once the cream was made, it was stored at 5 °C for 24 h before the measurements.

2.4. In vitro digestion model

The digestion process was simulated using an *in vitro* digestion protocol previously reported by Borreani, Llorca, Quiles, & Hernando (2017) and Espert et al. (2017).

2.4.1 In vitro fluids

Simulated Saliva Fluid (SSF) was prepared according to the method described by Mishellany-Dutour et al. (2011), with some modifications. SSF was composed of 5.2 g of NaHCO_3 , 1.37 g $\text{K}_2\text{H}_2\text{P}_2\text{O}_7 \cdot 3 \text{H}_2\text{O}$, 0.88 g NaCl , 0.48 g KCl and 0.44 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, dissolved in 1 L of bi-distilled water. 0.70 g of α -amylase and 2.16 g of mucin were added to this solution.

Simulated Gastric Fluid (SGF) was prepared according to a previous study (Sanz, Handschin, Nuessli & Conde Petit, 2007) with some modifications. 3.10 g NaCl , 0.11 g CaCl_2 , 1.10 g KCl and 5.68 mL Na_2CO_3 (1 M) were dissolved in 1 L of bi-distilled water. The solution was adjusted to pH 2. 0.15 g of pepsin was dissolved in 1 L of SGF.

Simulated Intestinal Fluid (SIF) was composed of an electrolyte solution and bile and pancreatin solutions. The electrolyte solution was prepared by dissolving 1.25 g NaCl , 0.15 g KCl and 0.055 g CaCl_2 in 1 L of distilled water. Phosphate buffer solution was prepared (103.5 mg $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 44.5 mg $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 100 mL of distilled water) setting the pH to 7.0 if necessary to prepare bile and pancreatin freshly suspensions (Sanz et al., 2007).

2.4.2 In vitro digestion procedure

- To simulate *oral phase*, 50 g of fresh emulsion sample were gently mixed for 5 s with 1 mL of Simulated Saliva Fluid (SSF) in a water bath at 37 °C, to mimic the time the food spends within the mouth prior to swallowing.
- To simulate *gastric digestion*, the “bolus” sample from the mouth phase was mixed with 16 mL of Simulated Gastric Fluid (SGF) to obtain a final enzyme-sample ratio of 1:250 (v/v). The pH of the mixture was adjusted to 2.0 and incubated for 1 h under continuous agitation at 37 °C.
- To simulate *intestinal digestion*, 10.6 mL of bile extract (46.87 mg/mL) solution dissolved in phosphate buffer and 4 mL of electrolyte solution was added to the sample, and the pH was adjusted to 7.0 using NH₃ (25 g/100 g). Then, 5.34 mL of pancreatin dissolved in phosphate buffer was added to the mix (1:14 (v/v) ratio). The resulting mixture was incubated for two hours under continuous agitation at 37 °C.

To consider the effect of water dilution, samples were also incubated in mouth and stomach models, but only with the addition of distilled water. The incubation process (time, temperature and shaking conditions) was the same as that in the samples with enzymes.

2.5. Extrusion test

Texture analysis was evaluated using a TA-XT plus Texture Analyzer (Stable Microsystems, Godalming, UK). The samples (50 g) were filled in a bucket (5 cm in diameter and 7.5 cm in height) and a back extrusion test was conducted with the A/BE back extrusion rig cell (40 mm diameter), setting the distance force at 15 mm. A compression rate of 1 mm s⁻¹ and a trigger force of 10 g were selected. The area under the curve (AUC) (N s) was calculated from the force time profiles obtained.

2.6. Microstructure

The different creams were observed at 40 x magnification for confocal laser scanning microscopy (CLSM) according to Borreani et al. (2017) and at 10 x magnification for light microscopy (LM) (Borreani, Llorca, Larrea, & Hernando, 2016). Nile Red (0.1 g/L), Rhodamine B (2 g/L) and Toluidine blue (1 g/L) was used as staining agents.

The droplet size of the fresh creams and after the intestinal step was determined from several CLSM images. The diameter of at least 80 droplets from each sample was measured with the Fiji (ImageJ2) software.

2.7. Free fatty acid (FFA) measurement

The extent of lipid digestion was determined by measuring the free fatty acids (FFA) produced after small intestine digestion according to a method reported by Espert et al. (2017) adapted from a previously described titration method (Mun, Decker, & McClements, 2007).

2.8. Statistical analysis

The analyses were performed on triplicate samples prepared on different days, and results were expressed as the mean and standard deviations. Analysis of variance (ANOVA) was performed on the data using XLSTAT statistical software (version 2010.5.02, Addinsoft, Barcelona, Spain) and Tukey's test was used to assess the differences in mean values ($P < 0.05$).

3. Results and discussion

3.1. Textural properties

Figure 1 shows the extrusion profiles corresponding to the different creams before and after *in vitro* digestion steps and Table 2 shows the AUC values calculated from these curves. As can be seen, all the creams prepared with hydrocolloid-based emulsions showed a significantly greater consistency than the control cream, with the XG cream presenting significantly the highest force values (Table 2), which was associated with the greater thickening ability of XG in comparison to the cellulose ethers and starch. After the oral step, significantly lower consistency values were found in all the samples when they were incubated with Simulated Saliva Fluid (SSF) if compared to those incubated with water at the same dilution factor, this is because the α -amylase of saliva acts by hydrolyzing the starch causing the consequent loss of consistency, as previously observed by Sanz et al. (2007) in custards. In samples incubated with water (water saliva dilution), the control and XG creams were found to have no significant differences when compared with the fresh creams, and only a slight decrease was found in the cellulose creams when also compared with the fresh samples. The fresh XG cream exhibited significantly higher force values than the other fresh creams and after saliva addition, as well as after saliva water dilution incubation. These results may be in accordance with the results reported by Sasaki & Kohyama (2012), who concluded that the presence of XG in starch suspensions exert a suppressive effect on starch digestibility, what could explain the consistency of this cream.

MC cream was the second most consistent sample, followed by HHPMC and control creams, with there being no significant differences between the latter two. Gastric digestion originated a decrease in the consistency of all the samples if compared to fresh and oral digested ones. However, the reduction in AUC values in these samples was mainly due to the dilution effect and not due to enzyme action, as no significant differences were found between the incubated sample with Simulated Gastric Fluid

(SGF) and the incubated sample with stomach water dilution, except for the sample prepared with XG. Therefore, the presence of enzymes and gastric conditions had the same effect as that caused by water in control and cellulose samples. Nevertheless, the behaviour in the stomach phase was different in the cream with XG, where the stomach conditions exerted about a smaller decrease in consistency compared to the stomach water dilution sample. The cream prepared with XG showed significantly higher force values after gastric incubation than after the stomach water dilution, which implies an increase in consistency associated with the specific stomach conditions, which probably would be related to an increase in satiating capacity. Espert et al. (2019) also observed this increase in force values in XG-oil in water emulsion in the gastric phase, which was attributed to the sensitivity of the xanthan gum structure to the stomach conditions.

3.2. Microstructure of filling creams during in vitro digestion

The microstructure of the creams was observed using CLSM and LM as they passed through each phase of the *in vitro* digestion model (Figures 2 and 3). The creams exhibited a continuous red matrix consisting mainly of starch (arrows in Figures 2 A, E, I and 3 M) milk protein and carbohydrates, where the fat globules may be observed in green (Figure 2). Fresh control cream (Figures 2 A and 3 A) contained large (mean diameter = $30.3 \pm 10.0 \mu\text{m}$), rounded lipid globules of differing sizes. In creams made with celluloses (Figures 2 E, I, and 3 E, I), fat globules of differing sizes were rounded, but smaller (MC: $12.8 \pm 3.1 \mu\text{m}$; HHPMC: $8.4 \pm 2.8 \mu\text{m}$) than in the control cream. In all likelihood, this suggests that the fat globules in creams elaborated with cellulose emulsions were stabilized due to the formation of a hydrated cellulose network present in the aqueous phase. This network immobilizes the fat globules of the dispersed phase (Li, Wang, & Xu, 2003), preventing flocculation and coalescence phenomena due to the

decreased mobility of globules within the matrix (Aranberri, Binks, Clint, & Fletcher, 2006; Piorkowski, & McClements, 2014). Visually, it could be seen that fat globules in creams with HHPMC (Figures 2 E and 3 E) were smaller ($8.4 \pm 2.8 \mu\text{m}$) than those in creams with MC ($12.8 \pm 3.1 \mu\text{m}$) (Figures 2 I and 3 I), possibly because of the presence of hydrophobic substituent groups in the MC (Espert et al., 2017). In the case of XG cream (Figures 2 M and 3 M), although some fat globules appeared to be very big ($27.5 \pm 13.4 \mu\text{m}$), different sizes can be distinguished. In general, these globules were less rounded than in the other creams (control, HHPMC and MC creams). This is as expected, as xanthan gum is not an emulsifier, and only acts as an emulsion stabilizer by thickening the continuous phase.

After oral incubation, the continuous phase was observed to be more homogeneous in all the creams (Figures 2 and 3); starch granules (pointed to by arrows in Figures 2 A, E, I and 3 M) are not observed in the oral digested samples due to starch hydrolysis by the α -amylase, as previously observed in other studies (Robyt, 2008; Sanz & Luyten, 2006). In this phase, fat globule flocculation and coalescence were observed in the control cream (Figures 2 B and 3 B). This phenomenon is not observed in cellulose creams or xanthan gum creams (Figures 2 F, J, N and 3 F, J, N), because the hydrocolloid networks continue providing stabilizing capacity, as they are not affected by the enzymatic effect of saliva.

After the gastric digestion phase, the control cream (Figures 2 C and 3 C) exhibited smaller fat globules, while in cellulose creams (Figures 2 G, K and 3 G, K) and in xanthan creams (Figures 2 O and 3 O), the fat globules were similar in shape and size to the previous digestion steps. However, a lower density of fat globules can be observed, attributable to the diluting effect of the digestion fluids.

The intestine digestion step markedly affected the structure of all the systems, although it was more noticeable in the control cream (Figures 2 D and 3 D), in which the fat was mostly digested (mean diameter = $4.0 \pm 0.8 \mu\text{m}$) by the action of pancreatic lipase. In all the hydrocolloid creams, a significant increase in both the size of the fat globules (MC: $17.6 \pm 9.3 \mu\text{m}$; HHPMC: $15.4 \pm 8.7 \mu\text{m}$; XG: $32.0 \pm 17.0 \mu\text{m}$) and droplet coalescence was observed. At this level of digestion, some of the bile salts are able to displace the hydrocolloid molecules, causing a destabilizing effect (Li, Hu, & McClements, 2011). It has been reported that the hydrolysis of triglycerides in the small intestine is related to the available surface area, and an increase in droplet size is associated with a reduced surface area and a reduction in the rate of lipid hydrolysis (Lairon, 1997; Schneeman, & Gallaher, 2001). Even though the cellulose creams showed a clear change in their microstructure after *in vitro* digestion, the fat globules were not fully digested in either cream with cellulose. The creams elaborated with xanthan gum were not completely digested in the intestinal step, either. Similarly to both cellulose creams, a marked increase in both the size of the fat globules and droplet coalescence was observed in the xanthan cream. In all the hydrocolloid creams, the effect of intestine digestion is reflected by an increase in globule size and the appearance of coalescence due to changes in fat stability, implying an effect of the digestive fluids. However, fat globules are still visualized, implying that both celluloses and xanthan gum continue to exert a stabilizing effect.

3.3. FFA release after *in vitro* digestion

The extent of lipid digestion is dependent on the fat content, the physicochemical processes that occur during the digestion phases, the lipid droplet concentration and the droplet size, among other conditions (McClements, Decker, & Park, 2009; Li, &

McClements, 2010; Li et al., 2011). The FFA generated after *in vitro* digestion were used to quantify the extent of lipolysis. Table 3 shows the oleic acid values generated in the different filling creams during *in vitro* digestion. The extent of lipid digestion was found to decrease in creams with hydrocolloid-based emulsions compared to the control cream with non-emulsified oil. These results were similar to those found in the cellulose/xanthan gum oil in water emulsions (Espert et al., 2017; Espert, Salvador, & Sanz, 2019), where it is explained that the main mechanism of the hydrocolloid is acting as a physical barrier, preventing an effective action of the enzymes and conferring structural stability on digestion. MC and HHPMC creams exhibited the same fat release with small differences (240 g/Kg and 230 g/Kg fat reduction, respectively). Xanthan gum emulsion was the least-digested one, showing a decrease of fatty acids generated of 490 g/Kg. Probably, the greatest resistance of XG cream to digestion could be related to its greater values of force in texture, that is, its greater consistency. Lairon (1997), Schneeman and Gallaher (2001) and Lairon, Play, & Jourdhueil-Rahmani (2007) also investigate the use of gums and cellulose ethers for the purposes of reducing lipid digestibility. They attributed this behaviour to different mechanisms, such as direct interaction between dietary fiber-enzyme, the formation of a protective coating by the fiber around the lipid droplet, the binding of the bile salts or an increase in the viscosity.

4. Conclusions

In this study the application of non-digestible hydrocolloid-based emulsions in a filling cream matrix was investigated. The type of hydrocolloid influenced the consistency of the creams, as well as the structural changes during *in vitro* digestion, and consequently the lipid digestibility. The presence of hydrocolloid-based emulsion caused an increase in consistency. In particular, XG showed the highest force values, and stomach

conditions caused in this cream an increase in consistency compared to its water dilution. This fact could convert XG filling cream into a feasible option when an increase in the satiating capacity is desired. The creams formulated with MC, HHPMC or XG oil-in-water emulsion were more resistant to *in vitro* digestion than the control cream, exhibiting a significant decrease in fat digestibility. These results suggest that a vegetable oil-in-water emulsion stabilized by cellulose ethers or xanthan gum could be considered as a healthier alternative to conventional fat due to its lipid profile. Furthermore, by employing these low digestibility emulsions in a filling cream matrix, fat bioavailability would be lower, so problems related to the excessive consumption of fat could be reduced. The information acquired in this study may be useful for the design of emulsion-based functional products that provide healthier lipid profile while modulate fat digestibility and/or satiety.

Acknowledgements

The authors would like to acknowledge the financial support provided by the Spanish Ministry of Economy and Competitiveness (Project AGL2015-68923-C2-1-R).

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TABLES

Table 1. Ingredients of filling cream formulations.

Ingredients (g/400 g cream)	Control	MC/HHPMC/XG
Sugar	20	20
Milk powder	8	8
Starch	16	9
Water	262	163
Emulsion	-	200
Sunflower oil	94	-

MC: methylcellulose; HHPMC: high hydroxypropyl methylcellulose; XG: xanthan gum

Table 2. Area under the curve (AUC) (N.s) of the creams, before and after *in vitro* digestion (n=3).

Digestion step	CONTROL	MC	HHPMC	XG
Fresh	7.29 ^a (0.05)	10.57 ^a (0.08)	8.59 ^a (0.11)	13.25 ^a (0.17)
Simulated Saliva Fluid	3.47 ^b (0.57)	5.56 ^c (0.20)	3.17 ^c (0.38)	11.26 ^b (0.24)
Saliva Water Dilution	6.63 ^a (0.06)	8.96 ^b (0.63)	6.88 ^b (0.41)	13.02 ^a (0.28)
Simulated Gastric Fluid	1.84 ^c (0.12)	2.58 ^d (0.35)	2.20 ^d (0.14)	6.32 ^c (0.30)
Stomach Water Dilution	1.31 ^c (0.01)	2.66 ^d (0.21)	1.55 ^d (0.11)	4.72 ^d (0.26)

MC: methylcellulose; HHPMC: high hydroxypropyl methylcellulose; XG: xanthan gum.

^{abcd} Different letters in the same column and for the same cream type denote values with statistically significant differences ($p < 0.05$) according to the Tukey test.

Values in parentheses are standard deviations.

Table 3. Oleic acid values after intestine *in vitro* digestion of the filling creams (n=3).

Cream type	Oleic acid (g)	Oleic acid/initial fat
CONTROL	0.261 ^a (0.015)	0.168 ^a (0.009)
MC	0.198 ^b (0.007)	0.127 ^b (0.004)
HHPMC	0.201 ^b (0.008)	0.129 ^b (0.006)
XG	0.133 ^c (0.031)	0.085 ^c (0.031)

MC: methylcellulose; HHPMC: high hydroxypropyl methylcellulose; XG: xanthan gum

^{abc}Within the same column, values with the same letter are not statistically different according to the Tukey test ($p > 0.05$).

Values in parentheses are standard deviations.

FIGURE LEGENDS

Figure 1. Extrusion profiles of filling creams during *in vitro* digestion. A: control cream; B: MC (methylcellulose) cream; C: HHPMC (high hydroxypropyl methylcellulose) cream; D: XG (xanthan gum) cream.

Figure 2. Confocal micrographs of the different creams before and during *in vitro* digestion. White arrows point to starch granules. Nile Red and Rhodamine B staining. The scale bars measure 60 μm . MC: Methylcellulose; HHPMC: High hydroxypropyl methylcellulose; XG: xanthan gum.

Figure 3. Light microscopy micrographs of the different creams before and during *in vitro* digestion. Black arrows point to starch granules. Toluidine blue staining. MC: Methylcellulose; HHPMC: High hydroxypropyl methylcellulose; XG: xanthan gum.

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