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5	Comparing $\lambda$ -carrageenan and an inulin blend as fat replacers in carboxymethyl
6	cellulose dairy desserts. Rheological and sensory aspects.
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## 11 Abstract

Carbohydrate-based fat replacers are of growing interest because, besides their 12 physicochemical properties, they also have health-friendly characteristics. The study 13 14 reported here aims to compare the effect of adding both  $\lambda$ -carrageenan and a blend (50:50) 15 of short and long-chain inulin on the rheological behaviour and sensory properties of low-fat 16 carboxymethyl cellulose (CMC) semi-solid dairy desserts. Low-fat samples with 0.03%  $\lambda$ -17 carrageenan or with 9% of the inulin blend displayed similar rheological behaviour to the full-fat control sample, i.e. there were no significant differences in either flow ( $\sigma_0$ , *K*,  $\eta_{10}$  and 18 19 *n*) or viscoelasticity (G', G'', tan  $\delta$  and  $\eta^*$  at 1 Hz and  $\eta^*_{8Hz}$  at 8 Hz). In general, samples 20 with the same rheological behaviour but different fat content were perceived as having 21 similar thickness, creaminess and smoothness. However, the substitution of fat by  $\lambda$ -22 carrageenan or inulin influenced both perceived sweetness and flavour.

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#### 26 Introduction

27 Sodium carboxymethyl cellulose (CMC) is being used as an alternative thickener to starch in 28 semisolid dairy products (Jellema, Janssen, Terpstra, de Wijk, & Smilde, 2005) due to its 29 technological and nutritional advantages. Studies have paid less attention to the sensory and 30 rheological properties of CMC-milk systems than to the starch-milk systems, although some 31 information has been published regarding the effect of CMC concentration on flavour and textural properties of custard desserts (Jellema et al., 2005; Van Ruth, de Witte and Uriarte, 32 33 2004). Less information exists regarding how the elimination or reduction of fat affects 34 rheological and sensory characteristics of CMC-milk systems. In previous works (Bayarri, 35 Dolz & Hernández, 2009a; Bayarri, González-Tomás, & Costell, 2009b), the viscoelastic 36 properties of aqueous and milk systems with CMC were studied by oscillatory and creep-37 recovery tests. Both the type of dispersing media (water, skimmed and whole milk) and the 38 CMC concentration clearly affected the viscoelastic behaviour of samples. In general, when 39 fat content decreased, elasticity did so too.

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41 The elimination or reduction of fat in dairy foods modifies its composition and structure as 42 well as the expected interactions among components, giving rise to, in most cases, 43 perceptible changes in colour, flavour and, especially in texture (Guinard, Zoumas-Morse, 44 Mori, Uatoni, Panyam & Kilara, 1997; González-Tomás, Bayarri, Taylor & Costell, 2007). 45 Certain ingredients are commonly used to compensate or reduce the problems associated 46 with the reduction or elimination of fat content in foods, such as effects on texture. There are 47 several types of fat replacers and in each case the selection depends on the composition and 48 characteristics of each food (Sandrou & Arvanitoyannis, 2000). Among fat replacers, carbohydrate-based substances such as starch, cellulose, pectin, inulin, xanthan gum or 49

carrageenan, are of growing interest because, besides their physicochemical properties, they
also have health-friendly characteristics (Warrand, 2006).

52 In general, the most suitable structure-forming hydrocolloids for dairy products are 53 considered to be carrageenans, mainly  $\kappa$  and  $\iota$ -carrageenan, due to their ability to combine 54 and form double helices and their interaction with casein (Michon, Chapuis, Langendorff, 55 Boulenguer, & Cuvelier, 2005; Spagnuolo, Dalgleish, Goff, & Morris, 2005). Lambda-56 carrageenan does not form gels in aqueous solutions; however,  $\lambda$ -carrageenan is able to form 57 gels in the presence of milk due to its interaction with casein micelles (Langendorff, 58 Cuvelier, Michon, Launay, Parker, & De Kruif, 2000; Shchipunov and Chesnokov, 2003). 59 To the best of the authors' knowledge, there is no information available about the influence 60 of  $\lambda$ -carrageenan as a fat replacer in low-fat CMC-milk semisolid desserts.

61 Inulin is a linear non-digestible polysaccharide of  $\beta$ -(2-1) linked fructose residues with a 62 terminal glucose residue unit. In addition to its beneficial effects on health as a dietetic fibre 63 and as a prebiotic ingredient (Flamm, Glinsmann, Kritchevsky, Prosky, & Roberfroid, 2001) 64 it can be used as a low-calorie sweetener, fat substitute or texture modifier (Tungland & 65 Meyer, 2002). These properties are linked to the polymerization degree of its chain. Long-66 chain inulin is more thermally stable, less soluble and more viscous than native and short-67 chain inulins (Wada, Sugatami, Terada, Ohguchi & Miwa, 2005) and it has been used as a 68 fat replacer in several dairy products. Villegas, Carbonell & Costell (2007) showed that to 69 obtain low-fat milk beverages with a similar thickness and creaminess to those perceived in 70 whole-milk beverages, long-chain inulin must be added at a concentration above 8%. 71 González- Tomás, Coll-Marqués & Costell (2008) observed that by adding 7.5% of long-72 chain inulin to low-fat starch-based custard desserts, they obtained similar viscoelastic 73 properties to the whole-milk sample with the same starch concentration. Meanwhile, the 74 addition of long-chain inulin improved creaminess and consistency of low-fat custards,

75 mimicking those of full-fat custard, but also reduced smoothness and increased the sensation 76 of roughness (González- Tomás, Bayarri & Costell, 2009). The latter effect is probably due to the presence of small crystals or crystal aggregates of long-chain inulin in the product. 77 78 Because the crystallization process of inulin depends on both the inulin chain size and the 79 initial inulin concentration (Bot, Erle, Vreeker, & Agterof, 2004) we hypothesised that 80 reducing long-chain inulin concentration would decrease the formation of inulin crystals, 81 even though by lowering long-chain inulin concentration, its fat-replacing ability can be 82 expected to decrease. One possibility is to use a blend of short and long-chain inulin as fat 83 replacer. In a previous work (Tárrega, Rocafull and Costell, 2010) addition of inulin blends 84 to low-fat samples was not enough to completely emulate the rheological behaviour of the 85 full fat-custard. However the low-fat sample with the inulin blend 50:50 was perceived to 86 have the same creaminess and thickness than the full-fat sample. From the nutritional point 87 of view, the mixture of short and long-chain inulin in the ratio 50:50 affords some extra 88 advantages in addition to improving prebiotic effectiveness. This blend ratio has been shown 89 to enhance calcium absorption and bone mineralization in pubertal adolescents (Abrams et 90 al., 2005) and to reduce the amount of gas produced while increasing or maintaining the 91 ability to support beneficial bacteria in the colon (Ghoddusi, Grandison, Grandison, & 92 Tuohy, 2007). In addition, the prebiotic effect of inulin could be boosted by supplementing it 93 with CMC in dietary treatments (Júskiewicz and Zdunczyk, 2004). These authors observed 94 that when a diet of inulin supplemented with CMC was given to rats, it led to an increase in 95 the size of the caecum, lower pH and ammonia concentration in the digestive tract as well as 96 a greater production of short-chain fatty acids.

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98 The study reported here aims to compare the effect of adding both  $\lambda$ -carrageenan and a 99 blend (50:50) of short and long-chain inulin on the rheological behaviour of low-fat

carboxymethyl cellulose semi-solid dairy desserts. Therefore, low-fat samples with similar
 rheological behaviour to full-fat samples were selected and their sensory properties were
 compared.

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#### 104 **2. Materials and Methods**

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## 106 **2.1. Sample preparation and composition**

107 Samples were prepared with carboxymethyl cellulose (CMC) (Akucell AF3265 Akzo 108 Nobel, Amersfoort, The Netherlands), sucrose, commercial whole (25% w/w protein, 39% 109 w/w carbohydrate, 26% w/w fat and 1.2% w/w calcium) and skimmed (34% w/w protein, 110 52% w/w carbohydrate, 1% w/w fat and 1.2%w/w calcium) milk powder (Central Lechera 111 Asturiana, Siero, Spain), vanilla aroma (375 48A Lucta S.A., Barcelona, Spain), vellow-112 orange colorant (Vegex NC 2c WS mct, CHR Hansen S.A., Barcelona, Spain), λ-113 carrageenan (Satiagum<sup>™</sup> ADC 25, Barcelona, Spain) and two types of inulin that differed in 114 the average chain length: long chain length ( $\geq 23$  monomers) (Frutafit® TEX!) and short 115 chain length inulin (7-9 monomers) (Frutafit® CLR), both of which were provided by 116 Sensus (Brenntag Química, Barcelona, Spain).

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Whole and skimmed milk were prepared in advance by dissolving 13.5 % (w/w) whole and skimmed milk powder, respectively, in mineral water to obtain a final fat content of 3.5%and 0.14%, respectively. Milk powder was dispersed in mineral water, at 250 rpm and 85 °C for 10 min, with the help of a magnetic stirrer and a hot plate (Ared, Velp Scientifica) and stored at  $4\pm1$ °C overnight to ensure complete hydration of the milk proteins. To prepare samples, a dry blend of sugar with CMC was added to the rehydrated milk, with the colorant, and stirred (Heidolph RZR 1, Germany) at room temperature for 35 min. Five min before the end vanilla aroma was added. Samples were transferred to a closed flask and stored (4±1°C; 24 h) prior to measurements. Low-fat samples containing  $\lambda$ -carrageenan were prepared in the same way except that  $\lambda$ -carrageenan powder was added to the skimmedrehydrated milk together with the dry blend of sugar and CMC. Long chain length inulin and short chain length inulin were mixed in the ratio of 50:50 and low-fat samples were prepared by dispersing skimmed-milk powder and the corresponding inulin concentration in mineral water just before the aforementioned heat treatment.

Low-fat samples were prepared at two CMC concentrations – 1.3 and 1.5% w/w - each with three  $\lambda$ -carrageenan contents – 0.01, 0.03 and 0.05% w/w - or three concentrations of the 50:50 blend of short and long chain inulin (SC/LC inulin blend) - 7, 9 and 11% w/w -. Two more samples without inulin or  $\lambda$ -carrageenan, one prepared with whole milk (full-fat) and the other prepared with skimmed milk (low fat), were used as control. The amounts of sugar (6% w/w), colorant (0.052%), vanilla aroma (0.016% w/w) and the weight of rehydrated milk (80% w/w) were fixed.

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# 140 **2.2. Rheological measurements**

141 Rheological measurements were carried out in a controlled stress rheometer RS1 142 (ThermoHaake, Karlsruhe, Germany), using parallel-plates geometry (60 mm diameter; 143 1mm gap). A sample temperature of  $10\pm1^{\circ}$ C, selected as representative of the usual 144 consumption temperature of dairy desserts, was kept during measurements by means of a 145 Haake circulating water bath. Two batches of each concentration combination were prepared 146 and each batch was measured twice, using a fresh sample for each measurement. After 147 loading the sample, a waiting period of 10 min was used to allow the sample to recover itself 148 and reach the desired temperature. After placing the sample between the plates carefully, the 149 excess material was wiped off with a spatula.

# 150 2.2.1. Flow behaviour.

Sample flow was measured by recording shear stress values when shearing the samples at an increasing shear rate from 1 to 200 s<sup>-1</sup> for a period of 60 s and in reverse sequence for the same time. Experimental data of descending flow curves were fitted to Herschel-Bulkley model (eq. 1) using Rheowin Pro software (v. 3.61, Haake),

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$$\sigma = \sigma_0 + K \dot{\gamma}^n \qquad (eq. 1)$$

156 where  $\sigma$  (Pa) is the shear stress,  $\sigma_0$  (Pa) is the yield stress, *K* (Pas<sup>n</sup>) is the consistency index, 157  $\dot{\gamma}$  (s<sup>-1</sup>) is the shear rate and *n* is the flow behaviour index. Shama & Sherman (1973) stated 158 that the stimulus associated with the perception of viscosity in semisolid products could be 159 the shear stress developed at a constant rate of 10 s<sup>-1</sup>. Thus, apparent viscosity values at this 160 shear rate ( $\eta_{10}$ ) were calculated (eq. 2) as an index of sensory viscosity.

161 
$$\eta_{10} = (\sigma_0 / \dot{\gamma}) + K \dot{\gamma}^{n-1}$$
 (eq. 2)

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# 163 2.2.2. Viscoelastic behaviour.

164 Stress sweeps were made between 0.02 and 300 Pa, at a frequency of 1Hz, in all the systems 165 studied to determine the linear viscoelasticity zone. Frequency sweeps tests at 0.05 Pa, 166 which is within the linear viscoelastic region, were then performed from 0.01 to 10 Hz. The 167 oscillatory rheological parameters used to compare the viscoelastic properties of the samples 168 were storage modulus (G'), loss modulus (G''), complex dynamic viscosity  $(\eta^*)$  and loss 169 angle  $(\tan \delta)$  at 1 Hz. In semisolid products with viscoelastic behaviour, several authors have obtained a good correlation between the perceived thickness and the complex dynamic 170 viscosity value at 50 rad s<sup>-1</sup> (Hill, Mitchell & Sherman, 1995). In this work, values of  $\eta^*$  at 8 171 Hz (equivalent to 50 rad s<sup>-1</sup>) ( $\eta^*_{8Hz}$ ) were determined as an index of oral thickness. 172

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# 174 **2.3. Sensory evaluation**

175 A group of 40 panellists, with previous experience (more than three years) in evaluating 176 sensory differences in dairy products, including dairy desserts, evaluated the intensity 177 differences in sweetness, vanilla flavour, thickness, creaminess and smoothness between 178 samples by paired comparison tests (ISO 5495-2005). Panellists were previously selected 179 according to their taste sensitivity and their capacity to detect differences in intensities of the 180 above mentioned attributes (ISO 8586-1:1993). A total of eight pairs of samples were 181 evaluated. Each pair was composed of two samples containing the same CMC concentration. 182 For each CMC concentration (1.3 and 1.5% CMC), four pairs of samples were evaluated. 183 The first pair consisted of control samples (full-fat and low-fat prepared with whole milk and 184 skimmed milk, respectively, and without fat replacer). The second and the third pairs were 185 composed of the control full-fat system and two low-fat samples containing  $\lambda$ -carrageenan 186 or SC/LC inulin blend at concentrations chosen on the strength of the rheological results. 187 Finally, the attribute intensities of low-fat samples with  $\lambda$ -carrageenan and with SC/LC 188 inulin blend were also compared.

189 Two pairs of samples were evaluated in each session and a total of four sessions were 190 performed. All sessions were carried out between 11:00-13:00 in a standardised test room 191 (ISO-8589, 2007) with separate booths. Samples (40 mL) were served at 10±1 °C in white 192 plastic vessels coded with three random digit numbers and the serving order was balanced in 193 such a way that each sample was evaluated first an equal number of times. Panellists tasted 194 approximately the same volume of each sample (half of a spoon) and were asked to indicate 195 which sample, within each pair had a higher intensity of sweetness, vanilla flavour, thickness, 196 creaminess or smoothness. Mineral water was provided to the assessors for mouth rinsing 197 between each pair of samples.

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# 199 2.4. Statistical analysis

200 *Rheological data analysis.* One-way ANOVA, considering composition as a factor, was 201 applied for each CMC concentration. Significant differences between individual samples 202 were determined by the Fisher's test ( $\alpha$ =0.05). Principal Component Analysis (PCA) was 203 applied to the average values of flow and viscoelastic parameters. All calculations were 204 carried out with XLSTAT-Pro software v.2007 (Adinsoft, Paris, France).

205 Sensory data analysis. Sensory data were processed using Compusense  $\mathbb{R}$  five release 4.6 206 (Compusense Inc., Guelph, ON, Canada). Tests were considered two-tailed and significant 207 differences were established for  $\alpha$ =0.05.

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# 209 **3. Results and discussion**

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# 211 **3.1.** λ-carrageenan as fat replacer. Influence of composition on rheological behaviour.

212 Figure 1 shows the flow curves for control samples and for low-fat samples with different  $\lambda$ -213 carrageenan concentrations. All samples showed observable hyteresis loops when they were 214 sheared during a complete cycle, indicating that sample flow was time-dependent (Figure 1). 215 This thixotropic behaviour increased with both  $\lambda$ -carrageenan and CMC concentration. Full-216 fat samples prepared with both CMC concentrations and low-fat samples with the higher 217 CMC and  $\lambda$ -carrageenan content showed an overshoot in the stress-rate curves. The maximum in the stress represents the yielding transition since the continuous network 218 219 changes to a discontinuous state at this point (Mujumdar, Beris, & Metzner, 2002). Similar 220 behaviour was observed on studying the flow properties of CMC dairy systems made with 221 whole milk at similar CMC concentrations (Bayarri and Costell, 2009a): particles seemed to 222 form many more connections, but the systems were more brittle as a consequence (i.e. a 223 clear overshoot in their stress-rate curves). Similarly, Lizarraga, Vicin, Gonzalez, Rubiolo, 224 & Santiago (2006) studied the flow curves of whey protein concentrate (WPC) and  $\lambda$ - 225 carrageenan aqueous mixtures and observed that increasing the WPC concentration 226 produced a more structured system, which partially broke down with increasing shear rates 227 and over time, as reflected by the overshoot in the shear-stress versus shear-rate profiles.

228 The flow curves of all the systems showed a shear thinning with yield stress behaviour. Therefore, the shear stress versus the shear-rate profiles of all the samples were consistent 229 230 with the Herschel-Bulkley model. Due to the presence of an overshoot in the ascending 231 rheograms of several samples, the experimental data of descending rheograms were fitted to the Herschel-Bulkley model. The fit was good in all cases ( $0.9997 < R^2 < 0.9999$ ). As expected 232 the 1.5% CMC systems gave higher values on the yield stress ( $\sigma_0$ ), consistency index (K) 233 and apparent viscosity at 10 s<sup>-1</sup> ( $\eta_{10}$ ) and lower ones on the flow index (*n*) than the 234 equivalent samples made with 1.3% CMC (Table 1). The flow index values varied between 235 236 0.4 and 0.6, and demonstrated clear pseudoplastic behaviour (Table 1). Low-fat samples 237 showed lower consistency and lower shear thinning than either full-fat or low-fat samples 238 with  $\lambda$ -carrageenan. Results from ANOVA showed that composition effect was significant 239 (P<0.05) on all flow parameter values except for flow index values in 1.5% CMC systems 240 (P=0.146). In both CMC systems, mean values of  $\sigma_0$ , K and  $\eta_{10}$  of low-fat samples increased 241 with  $\lambda$ -carrageenan concentration (Table 1). For both CMC systems, there was no significant 242 difference ( $\alpha$ =0.05) in  $\sigma_0$ , K,  $\eta_{10}$  and n between full-fat samples and low-fat samples with 243 0.03%  $\lambda$ -carrageenan.

With regard to the viscoelastic behaviour, all samples exhibited the viscoelastic properties usually observed for weak-gel systems, which is typical in this type of product: the elastic response predominated over the viscous one. Both dynamic moduli showed slight variation with oscillation frequency, being higher the frequency dependency of loss modulus (Figure 2). As expected, viscoelastic parameter values were higher, except tan  $\delta$ , in 1.5% CMC samples than in their counterparts formulated with 1.3% CMC, which confirms previous

250 results obtained by the authors Bayarri et al. (2009ab). For comparison purposes, G', G'', tan  $\delta$  and  $\eta^*$  at 1 Hz and  $\eta^*_{8Hz}$  at 8 Hz values were considered (Table 2). The elastic 251 252 contribution for full-fat control samples was higher than low-fat control samples, suggesting 253 a stronger gel structure. In general, all viscoelastic parameters were significantly affected by 254 the composition (P<0.05), but there were no differences in G'' and  $\eta^*_{8Hz}$  values (P>0.10) in 255 the case of 1.5% CMC systems due to fat or  $\lambda$ -carrageenan concentration. Within each CMC 256 concentration, mean values of the G', G'' and  $\eta^*$  (both at 1 and 8 Hz) increased and tan  $\delta$ 257 decreased when  $\lambda$ -carrageenan was added to low-fat samples. At 1.3% CMC concentration, 258 as in the case of flow behaviour, the full-fat sample did not differ statistically ( $\alpha$ =0.05) from 259 low-fat sample with 0.03%  $\lambda$ -carrageenan for any of the viscoelastic parameters studied, 260 while for the highest CMC concentration (1.5%), the full-fat sample did not differ ( $\alpha$ =0.05) 261 from low-fat samples with both 0.01 and 0.03%  $\lambda$ -carrageenan.

262 Rheological properties of low-fat samples showed an increase in the consistency and 263 mechanical strength with increasing  $\lambda$ -carrageenan concentration. Similar results have been 264 reported previously in other systems containing milk proteins (Lizarraga et al., 2006; 265 Lagendorff et al., 2000). The interactions between carrageenans and milk proteins and their 266 influence on rheological properties have been the subject of study (Depypere, Verbeken, 267 Torres, & Dewettinck, 2009). While kappa and iota carrageenans form gels in aqueous 268 solutions, lambda is unable to do so and is used as a pure thickener. However, lambda-269 carrageenan is able to form gels in the presence of milk. Most authors agree that carrageenan 270 associates with casein micelles through an electrostatic interaction between its negatively 271 charged sulphate groups and a positively charged region of  $\kappa$ -casein. In the case of  $\kappa$ -, and 1-272 carrageenan, the stabilization of gel network is achieved due to crosslinking of chain 273 fragments between the micelles by double helices. For  $\lambda$ -carrageenan, the gelation is ensured 274 only by binding with casein micelles (Shchipunov & Chesnokov, 2003).

275 To study the joint variability of rheological parameters Principal Component Analysis was 276 applied to their mean values (Figure 3). The first component accounted for 95.70% of total 277 variability and clearly separated the samples according to CMC concentration. Samples with 278 1.5% of CMC, in the positive part of the first dimension, showed higher values for most of the rheological parameters (G', G',  $\eta^*$ ,  $\eta^*_{8Hz}$ ,  $\sigma_0$ , K,  $\eta_{10}$ ) than samples with 1.3% of CMC, 279 280 which were in the negative part of the first dimension. The second component, which only accounted for a low percentage of variability (3.21%), correlated well with tan  $\delta$  and with 281 282 the flow index. For both CMC concentrations, low fat sample with 0.03%  $\lambda$ -carrageenan 283 exhibited the same rheological behaviour as the full-fat control sample in terms of both flow 284 and viscoelastic properties.

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# 3.2. Inulin blend as a fat replacer. Influence of composition on rheological behaviour Figure 4 shows the flow curves for control samples and for low-fat samples with different concentrations of SC/LC inulin blend. All samples exhibited thixotropic behaviour, which

290 increased with both CMC and inulin concentration. As in the case of full-fat samples, low-291 fat samples with 1.5% CMC and inulin displayed stress overshoot in the upward rheogram. 292 Similar behaviour was observed by Bot et al. (2004) on studying the influence of 293 crystallisation conditions on the large deformation rheology of inulin gels. The overshoot in 294 the stress-strain curve becomes more pronounced at higher inulin concentrations, therefore 295 an increase in fracture stress was observed on increasing inulin concentrations. Also in this 296 case, the fit to the Herschel-Bulkley model was good for all the samples  $(0.9997 < R^2 < 0.9999)$ . Flow index values ranged from 0.52 to 0.58 for samples prepared with 297 1.3% CMC and from 0.43 to 0.47 for samples with 1.5% CMC, which is indicative of shear 298 299 thinning behaviour (Table 3). The analysis of variance showed a significant effect (P<0.05) of composition on all flow parameters studied except for K and n in 1.5% CMC systems (P>0.05). In general, considering low-fat samples,  $\sigma_0$ , K and  $\eta_{10}$  increased, and flow behaviour index decreased with inulin concentration. Within each CMC concentration, fullfat samples did not differ statistically ( $\alpha$ =0.05) from low-fat samples with 9% inulin for any of the flow parameters studied.

305 Concerning the viscoelastic properties of the systems, low-fat samples with inulin showed a 306 similar mechanical spectra to that observed for low-fat samples with  $\lambda$ -carrageenan, *i.e.* all 307 samples showed weak-gel behaviour (Figure 5). Storage and loss modulus values of 1.5% 308 CMC systems were higher than in 1.3% CMC samples, which is indicative of a stronger 309 structured matrix. With the exception of tan  $\delta$  values in 1.5% CMC systems (P=0.142), all 310 viscoelastic parameters were significantly (P<0.05) affected by the composition of the 311 system. Within each CMC concentration, inulin addition to low-fat samples led to higher values of G', G'' and  $\eta^*$  and lower values of tan  $\delta$  (Table 4). The amount of SC/LC inulin 312 313 blend required to match the viscoelastic properties of full fat samples was dependent on 314 CMC concentration: 9% of inulin in 1.3% CMC systems and 7 or 9% in 1.5% CMC systems. 315 Principal Component Analysis was applied to mean values of rheological parameters 316 obtained for both flow and viscoelastic behaviour (Figure 6). The first component accounted for 87.86% of total variability and clearly separated the samples according to CMC 317 318 concentration. Samples with 1.5% of CMC, in the positive part of the first dimension, 319 showed higher values for most of the rheological parameters, except for tan  $\delta$  and n, than 320 samples with 1.3% of CMC, which were in the negative part of the first dimension. The 321 second component, which accounted for only a small percentage of variability (8.21%), was 322 related in its positive part with the variation in the flow index (n) and in tan  $\delta$  and in its negative part with the consistency index (K) and with apparent viscosity at  $10s^{-1}$  ( $\eta_{10}$ ). 323 324 Mapped sample distribution with respect to the first dimension (Figure 6) showed that for

both CMC concentrations, the low-fat sample with 9% inulin blend was the one
demonstrating rheological behaviour most similar to that of the whole-milk control sample.
Therefore, low-fat samples with inulin were successfully formulated to produce the same
rheological behaviour as full-fat samples.

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# 330 **3.3.** λ-carrageenan and inulin blend as fat replacers. Sensory properties

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332 According to the rheological results obtained, both fat replacers could be used in the 333 formulation of low-fat CMC-based dairy desserts to obtain products with similar rheological 334 behaviour to that obtained with full-fat milk. However, this does not guarantee it matches the 335 sensorially perceived texture. This is logical if one bears in mind that during ingestion and 336 swallowing, the thickness perceived depends not only on the shear forces in the mouth but 337 also on the effect of saliva, mouth temperature and also, on other textural characteristics of 338 the product. Although in many cases there is a close relationship between the values of one 339 or various rheological parameters and the perceived thickness in semisolids foods (van Vliet, 340 2002), rheological information alone may not be enough to explain all the textural 341 differences perceived particularly when products with different composition and structure 342 are being compared (González-Tomás & Costell, 2006). Some textural attributes, creaminess, 343 fattiness or smoothness, which strongly affect the final acceptance of semisolid dairy foods, 344 mainly depend on the food microstructure, on some surface properties and on certain crossed 345 interactions between texture attributes and some flavours. Moreover, besides the direct 346 influence that the elimination or reduction of fat has on texture, it also strongly influences 347 the mechanisms involved in flavour release and perception (Bayarri & Costell, 2009b).

In order to analyse the role fat plays in the sensory attributes of CMC-based dairy desserts,the sensory properties of full-fat and low-fat control samples were compared (Figure 7). For

350 both CMC concentrations, full-fat samples were perceived as thicker than low-fat samples, 351 which is in agreement with the rheological results obtained previously. Low-fat samples 352 were perceived as smoother while no significant differences were detected in creaminess 353 between each pair of samples with different fat content. In addition, low-fat control samples 354 were perceived as sweeter and as having greater vanilla flavour intensity than their full-fat 355 counterparts. These results are in accordance with those obtained in previous studies on 356 starch-based custards, showing that fat reduction leads to textural changes and modifies the 357 perception of their flavour (González-Tomás et al., 2007).

According to the rheological results obtained in the above sections, both in the samples containing 1.3% CMC and those with 1.5% of CMC, by adding 0.03% of  $\lambda$ -carrageenan or 9% of the inulin blend, low fat products were obtained with similar rheological behaviour to that of full-fat samples. A paired comparison test was carried out to assess the differences in vanilla flavour, sweetness, thickness, smoothness and creaminess between the low-fat custard dessert with 0.03% of  $\lambda$ -carrageenan or the low-fat custard dessert with 9% of the inulin blend and the control full-fat sample with the same amount of CMC.

365 When  $\lambda$ -carrageenan was used as a fat replacer (Figure 8a and 8b), no significant differences 366 were found in the intensity of the texture attributes between the samples with different fat 367 content. The addition of  $\lambda$ -carrageenan to low-fat samples increased thickness and decreased 368 smoothness, compensating the variation detected in the texture by reducing the fat content of 369 these products. However, the substitution of fat by  $\lambda$ -carrageenan also influenced the flavour 370 perceived. In general, both the sweetness and the vanilla flavour of low-fat samples were 371 significantly stronger ( $\alpha = 0.05$ ) than in the full-fat samples. This fact reflects the influence 372 of fat on flavour release and perception. For example, Lethuaut et al. (2005) studied the 373 aroma-sweetness interactions on dairy desserts with different textures and concluded that the 374 dessert with  $\lambda$ -carrageenan, which had the softest texture and was perceived as the sweetest,

375 was also perceived as the most highly flavoured. In this work, flavour differences may be explained by rheological and textural changes, but in the present study, samples showed 376 377 similar rheological properties and the variations in flavour intensity may have been due to 378 other factors. It is widely known that flavour release and perception decrease as lipid levels 379 increase in the food matrix, with the exception of hydrophilic compounds showing partition 380 coefficient values near or below zero (van Ruth, King, & Giannouli, 2002; Miettinen, 381 Hyvönen, Linforth, Taylor, & Tuorila, 2004). Bayarri, Smith, Hollowood, & Hort (2007), 382 working with model o/w emulsions with different oil contents and a composition adjusted to 383 deliver iso-release aroma in vivo and the same in-mouth viscosity, observed that samples 384 containing the highest oil content were perceived as significantly less sweet. This confirms 385 that fat content influences flavour in two ways: directly, it has a significant effect on the 386 release of chemical stimuli from the food matrix into the mouth and indirectly, due to its 387 influence on the product texture.

When the fat was replaced with the SC/LC inulin blend, its effect on texture was found to be 388 389 different depending on the CMC concentration. When CMC concentration was 1.3%, the 390 low-fat sample with the added inulin blend was perceived as less thick and significantly 391 smoother than the control sample while no significant difference were detected in 392 creaminess (Figure 8c). This confirmed that even when the rheological behaviour of 393 products with different fat content was alike, during consumption the orally perceived 394 texture is not always the same. A similar fact was found by Gallardo-Escamilla, Kelly, & 395 Delahunty (2007) when they observed that equiviscous fermented whey beverages, with 396 different added hydrocolloids, were perceived as having different thicknesses and by 397 Villegas, Carbonell, & Costell, (2008), who detected differences in perceived thickness 398 between equiviscous milk and soymilk vanilla beverages. In samples with 1.5% of CMC the 399 effect of adding inulin was different, being similar to that observed when  $\lambda$ -carrageenan was

400 added. In samples with this CMC concentration, there were no significant difference in 401 thickness, smoothness and creaminess between the full-fat sample and the low-fat sample 402 (Figure 8d). With regard to flavour, as happened when  $\lambda$ -carrageenan was added, low-fat 403 samples were perceived as being significantly sweeter and having a more intense vanilla 404 flavour.

405 Differences in sensory attribute intensities were also analysed between low-fat samples with 406 either of the fat replacers, *i.e* 0.03%  $\lambda$ -carrageenan and 9% inulin blend (Figure 9). For both 407 CMC concentrations, the samples with  $\lambda$ -carrageenan were perceived as significantly thicker 408 and there was a tendency to qualify their texture as less smooth and less creamy than the 409 samples with the inulin blend, although the differences were only significant for smoothness 410 in the samples with 1.3% CMC. Brennan & Tudorica (2008) observed that, the incorporation 411 of native inulin at high levels (6%) to low-fat products significantly improved the perceived 412 creaminess and mouthfeel of the product, and the resulting texture was perceived as 413 smoother. Regarding flavour differences, samples with the inulin blend were perceived as 414 significantly sweeter and as having greater vanilla flavour intensity than the samples with  $\lambda$ -415 carrageenan (Figure 9). Differences in sweetness and vanilla flavour can be explained by 416 both the higher consistency perceived in samples with  $\lambda$ -carrageenan and the higher 417 proportion of mono and disaccharides in short-chain inulin.

418

## 419 **Conclusion**

The results obtained showed that the two fat replacers,  $\lambda$ -carrageenan and SC/LC inulin blend, were successfully used to formulate low-fat products with similar texture properties to full-fat CMC semisolid dairy desserts. Nevertheless, to obtain products with different fat content but similar flavour perception, sweetener and aroma concentration should be adjusted.

425

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**Table 1.** Average values (n=2) of yield stress ( $\sigma_0$ ), consistency index (K), flow index (n) and apparent viscosity at 10 s<sup>-1</sup> ( $\eta_{10}$ ) of dairy desserts with different concentrations of CMC and  $\lambda$ -carrageenan.

556

	Low-fat	Low-fat -	Low-fat -	Low-fat -	Full-fat
	control	$0.01\% \lambda C^b$	$0.03\% \lambda C$	0.05% λC	control
		1.	3% CMC		
$\sigma_0$ (Pa)	7.3 <sup>a</sup>	8.1 <sup>a</sup>	8.9 <sup>a</sup>	11.8 <sup>b</sup>	8.2 <sup>a</sup>
K (Pa s <sup>n</sup> )	6.1 <sup>a</sup>	8.6 <sup>a</sup>	13.2 <sup>b</sup>	18.2 <sup>c</sup>	14.0 <sup>b</sup>
n	0.58 <sup>d</sup>	0.56 <sup>c</sup>	0.52 <sup>b</sup>	$0.49^{a}$	0.52 <sup>b</sup>
η <sub>10</sub> (Pa s)	3.1 <sup>a</sup>	3.9 <sup>b</sup>	5.2 <sup>c</sup>	6.8 <sup>d</sup>	5.4 <sup>c</sup>
		1.	5% CMC		
$\sigma_0$ (Pa)	13.0 <sup>a</sup>	15.6 <sup>ab</sup>	18.7 <sup>bc</sup>	19.7 <sup>c</sup>	19.4 <sup>bc</sup>
K (Pa s <sup>n</sup> )	22.5 <sup>a</sup>	29.3 <sup>ab</sup>	36.7 <sup>bc</sup>	43.7 <sup>c</sup>	35.1 <sup>bc</sup>
n	$0.47^{a}$	0.45 <sup>a</sup>	0.43 <sup>a</sup>	$0.42^{a}$	0.43 <sup>a</sup>
η <sub>10</sub> (Pa s)	7.9 <sup>a</sup>	9.7 <sup>b</sup>	11.7 <sup>cd</sup>	13.3 <sup>d</sup>	11.5 <sup>c</sup>

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558 <sup>a</sup> In each row, different superscript letters denote significant differences between samples ( $\alpha$ =0.05).

559  $b\lambda$ -Carrageenan

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	Low-fat	Low-fat -	Low-fat -	Low-fat -	Full-fat
	control	$0.01\% \lambda C^{b}$	0.03% λC	0.05% λC	control
		1.	3% CMC		
G' (Pa)	99.9 <sup>a</sup>	133.5 <sup>b</sup>	189.7 <sup>c</sup>	233.5 <sup>d</sup>	173.2 <sup>c</sup>
G'' (Pa)	$47.7^{\rm a}$	55.9 <sup>ab</sup>	60.8 <sup>bc</sup>	68.4 <sup>c</sup>	58.1 <sup>abc</sup>
Tan δ	0.48 <sup>d</sup>	$0.42^{\circ}$	0.32 <sup>b</sup>	$0.29^{a}$	0.33 <sup>b</sup>
η*(Pa s)	17.6 <sup>a</sup>	23.0 <sup>b</sup>	31.7 <sup>c</sup>	38.7 <sup>d</sup>	29.8 <sup>c</sup>
$\eta *_{8Hz}$ (Pa s)	4.3 <sup>a</sup>	5.2 <sup>b</sup>	6.4 <sup>cd</sup>	7.2 <sup>d</sup>	6.4 <sup>c</sup>
		1.	5% CMC		
G' (Pa)	300.2 <sup>a</sup>	353.2 <sup>ab</sup>	391.7 <sup>b</sup>	488.5 <sup>°</sup>	378.3 <sup>b</sup>
G" (Pa)	92.5 <sup>a</sup>	101.9 <sup>a</sup>	103.4 <sup>a</sup>	120.1 <sup>a</sup>	107.0 <sup>a</sup>
Tan δ	0.31 <sup>c</sup>	0.29 <sup>bc</sup>	$0.26^{ab}$	0.25 <sup>a</sup>	$0.28^{abc}$
η*(Pa s)	50.0 <sup>a</sup>	58.5 <sup>ab</sup>	64.5 <sup>b</sup>	80.1 <sup>c</sup>	62.6 <sup>b</sup>
$\eta *_{8Hz}$ (Pa s)	9.9 <sup>a</sup>	10.82 <sup>a</sup>	11.16 <sup>a</sup>	13.1 <sup>a</sup>	10.8 <sup>a</sup>

Table 2. Average values (n=2) of storage modulus (G'), loss modulus (G''), loss angle

tangent (tan\delta) and complex viscosity ( $\eta^*)$  at 1 Hz and complex viscosity at 8 Hz ( $\eta^*{}_{8\text{Hz}})$  of

564 dairy desserts with different concentrations of CMC and  $\lambda$ -carrageenan.

566 <sup>a</sup> In each row, different superscript letters denote significant differences between samples ( $\alpha$ =0.05).

567 <sup>b</sup>λ-Carrageenan

	Low-fat	Low-fat -	Low-fat -	Low-fat -	Full-fat
	control	7% Inulin	9% Inulin	11% Inulin	control
		1.	3% CMC		
$\sigma_0$ (Pa)	7.3 <sup>a</sup>	7.3 <sup>a</sup>	8.4 <sup>a</sup>	15.4 <sup>b</sup>	8.2 <sup>a</sup>
K (Pa s <sup>n</sup> )	6.1 <sup>a</sup>	10.8 <sup>b</sup>	12.2 <sup>bc</sup>	14.9 <sup>d</sup>	14.0 <sup>cd</sup>
n	0.58 <sup>c</sup>	0.53 <sup>ab</sup>	0.53 <sup>ab</sup>	0.55 <sup>b</sup>	0.52 <sup>a</sup>
η <sub>10</sub> (Pa s)	3.1 <sup>a</sup>	4.4 <sup>b</sup>	5.0 <sup>bc</sup>	6.8 <sup>d</sup>	5.4 <sup>c</sup>
		1.	5% CMC		
$\sigma_0$ (Pa)	13.0 <sup>a</sup>	14.7 <sup>ab</sup>	20.9 <sup>c</sup>	33.2 <sup>d</sup>	19.4 <sup>bc</sup>
K (Pa s <sup>n</sup> )	22.5 <sup>a</sup>	27.4 <sup>a</sup>	27.2 <sup>a</sup>	26.3 <sup>a</sup>	35.1 <sup>a</sup>
n	$0.47^{a}$	$0.45^{a}$	$0.47^{a}$	$0.47^{\mathrm{a}}$	0.43 <sup>a</sup>
η <sub>10</sub> (Pa s)	$7.9^{\mathrm{a}}$	9.2 <sup>ab</sup>	10.0 <sup>bc</sup>	11.8 <sup>d</sup>	11.5 <sup>cd</sup>

**Table 3.** Average values (n=2) of yield stress ( $\sigma_0$ ), consistency index (K), flow index (n) and

apparent viscosity at 10 s<sup>-1</sup> ( $\eta_{10}$ ) of dairy desserts with different concentrations of CMC and

574 SC/LC inulin blend.

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576 <sup>a</sup> In each row, different superscript letters denote significant differences between samples ( $\alpha$ =0.05).

577

	Low-fat	Low-fat -	Low-fat -	Low-fat -	Full-fat
	control	7% Inulin	9% Inulin	11% Inulin	control
		1.	3% CMC		
G' (Pa)	99.9 <sup>a</sup>	107.9 <sup>a</sup>	155.7 <sup>b</sup>	285.2 <sup>c</sup>	173.2 <sup>b</sup>
G'' (Pa)	47.7 <sup>a</sup>	55.0 <sup>a</sup>	54.3 <sup>a</sup>	92.7 <sup>b</sup>	58.1 <sup>a</sup>
Tan δ	$0.48^{b}$	0.51 <sup>b</sup>	0.35 <sup>a</sup>	$0.32^{a}$	0.33 <sup>a</sup>
η*(Pa s)	17.6 <sup>a</sup>	19.3 <sup>a</sup>	27.0 <sup>b</sup>	47.7 <sup>c</sup>	29.8 <sup>b</sup>
$\eta *_{8Hz}$ (Pa s)	4.3 <sup>a</sup>	4.7 <sup>a</sup>	6.0 <sup>b</sup>	8.8 <sup>c</sup>	6.4 <sup>b</sup>
		1.	5% CMC		
G' (Pa)	300.2 <sup>a</sup>	338.8 <sup>ab</sup>	381.8 <sup>b</sup>	555.3°	378.3 <sup>b</sup>
G'' (Pa)	92.5 <sup>a</sup>	95.9 <sup>a</sup>	105.1 <sup>a</sup>	143.5 <sup>b</sup>	107.0 <sup>a</sup>
Tan δ	0.31 <sup>a</sup>	$0.28^{a}$	$0.28^{a}$	$0.26^{a}$	$0.28^{a}$
η*(Pa s)	50.0 <sup>a</sup>	56.0 <sup>ab</sup>	63.0 <sup>b</sup>	91.3 <sup>c</sup>	62.6 <sup>b</sup>
$\eta *_{8Hz}$ (Pa s)	9.9 <sup>a</sup>	$10.0^{a}$	11.5 <sup>a</sup>	17.6 <sup>b</sup>	10.8 <sup>a</sup>

Table 4. Average values (n=2) of storage modulus (G'), loss modulus (G''), loss angle

tangent (tan\delta) and complex viscosity ( $\eta^*$ ) at 1 Hz and complex viscosity at 8Hz ( $\eta^*_{\text{8Hz}}$ ) of

581 dairy desserts with different concentrations of CMC and SC/LC inulin blend.

583 <sup>a</sup> In each row, different superscript letters denote significant differences between samples ( $\alpha$ =0.05).

588

# 589 Figure captions

**Figure 1.** Flow curves of control samples ( $\Delta$ = low-fat;  $\diamond$ = full-fat) and low-fat samples with different  $\lambda$ -carrageenan concentrations ( $\circ$ =0.01%;  $\Box$  = 0.03%;  $\nabla$ =0.05%) containing 1.3% (a) and 1.5% (b) of CMC.

**Figure 2.** Mechanical spectra of control samples ( $\blacktriangle$ =low-fat;  $\blacklozenge$ = full-fat) and low-fat samples with different  $\lambda$ -carrageenan concentrations ( $\bullet$ =0.01%;  $\blacksquare$ = 0.03%;  $\blacktriangledown$ =0.05%) containing 1.3% (a) and 1.5% (b) of CMC. G' (filled symbols) and G'' (empty symbols).

596 **Figure 3.** Principal component analysis bi-plot for rheological parameters of low-fat 597 custards with  $\lambda$ -carrageenan at different CMC (triangle=1.3%; square=1.5%) concentrations. 598 Full-fat (filled symbols) and low-fat (empty symbols) control samples.

**Figure 4.** Flow curves of control samples ( $\Delta$ = low-fat;  $\diamond$ = full-fat) and low-fat samples with different SC/LC inulin blend concentrations ( $\circ$ =7%;  $\Box$  = 9%;  $\nabla$ =11%) containing 1.3% (a) and 1.5% (b) of CMC.

Figure 5. Mechanical spectra of control samples ( $\blacktriangle$ =low-fat;  $\blacklozenge$ = full-fat) and low-fat samples with different SC/LC inulin blend concentrations ( $\bullet$ =7%;  $\blacksquare$ = 9%;  $\blacktriangledown$ =11%) containing 1.3% (a) and 1.5% (b) of CMC. G' (filled symbols) and G'' (empty symbols).

Figure 6. Principal component analysis bi-plot for rheological parameters of low-fat
custards with the blend of SC/LC inulin at different CMC (triangle=1.3%; square=1.5%)
concentrations. Full-fat (filled symbols) and low-fat (empty symbols) control samples.

608 Figure 7. Sensory evaluation of the differences between full-fat ( $\square$ ) and low-fat ( $\square$ )

609 control desserts at different CMC concentrations (a=1.3%; b=1.5%). The line indicates the

610 minimum value of response for which the difference is significant ( $\alpha$ =0.05).

Figure 8. Sensory evaluation of the differences between the low-fat custard dessert with 0.03% of  $\lambda$ -carrageenan ( $\square$ ) and the control full-fat sample ( $\blacksquare$ ) at different CMC concentrations (a=1.3%; b=1.5%) and between the low-fat custard dessert with the 9% of the blend of SC/LC inulin ( $\blacksquare$ ) and the control full-fat sample ( $\blacksquare$ ) at different CMC concentrations (c=1.3%; d=1.5%). The line indicates the minimum value of response for which the difference is significant ( $\alpha$ =0.05).

617 Figure 9. Sensory evaluation of the differences between the low-fat custard dessert with

618 0.03% of  $\lambda$ -carrageenan ( $\square$ ) and with the 9% of the blend of SC/LC inulin ( $\square$ ) at different

619 CMC concentrations (a=1.3%; b=1.5%). The line indicates the minimum value of response

- 620 for which the difference is significant ( $\alpha$ =0.05).
- 621

Fig 1





Fig. 2.



Fig. 3

Fig 4





Fig. 5.



Fig. 6.







Fig. 8



