Food matrix impact on oral structure breakdown and sandiness perception of semisolid systems including resistant starch.

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Abstract

In this study, the influence of matrix structure and viscosity was investigated to gain insight into the mechanisms of sandiness perception. Granules of two types of resistant starch (RS types II and III) were used as edible particles of different size (RS type II, $D_{4,3} = 21.25 \mu\text{m}$; RS type III $D_{4,3} = 44.03 \mu\text{m}$). First the impact of adding the two RS types at two levels (1.5 and 3%) on rheology (flow behaviour) and sensory properties (ranking test) of custards was determined. Second, the impact of adding an extra thickener (starch, xanthan gum, carboxymethylcellulose, λ -carrageenan, or a mixture of xanthan and carrageenan) in the formulation on the viscosity decay at constant shear of 10 s^{-1} with saliva and the effect in sandiness perception was studied. The presence of RS granules that remained intact after oral digestion caused a sandiness sensation which increased with increasing levels of RS. Modifying the matrix (by adding an extra thickener) affected the sandiness perception, which depended more on the nature of the thickener than on the viscosity of the system. Viscosity decay curves show the extension and rate of structural breakdown also depended on the type of thickeners. In custards with xanthan gum, the part of the structure remaining after saliva hydrolysis may explain the less intense sandiness observed in this sample.

Keywords: sandiness perception, particle size, thickeners, saliva

1. Introduction

Grittiness, grainy texture, and sandiness are all mechanical qualities of foods that refer to the perception of particles in-mouth. This has been described in studies focused on the understanding of their origins and limits of their perception regarding food quality in a range of food products like chocolate (Liang & Hartel, 2004), chocolate brownies (Richardson et al., 2018), cream cheeses (Ningtyas, Bhandari, Bansal, & Prakash, 2017, 2019); Polowsky, Kindstedt, & Hughes, 2018; Sainani, Vyas, & Tong, 2004), ice creams (Ruger, Baer, & Kasperson, 2002), milk candy (“dulce de leche”) (Giménez, Ares, & Gámbaro, 2008), custards (De Wijk, Prinz, & Janssen, 2006; Godoi, Bhandari, & Prakash, 2017; González-Tomás, Bayarri, & Costell, 2009), fibre-enriched milk puddings (Ares, Baixauli, Sanz, Varela, & Salvador, 2009), yoghurts (Mantilla, Shewan, Shingleton, Stokes, & Smyth, 2020), gels (Laguna & Sarkar, 2016; Santagiuliana, Christaki, Piqueras-Fiszman, Scholten, & Stieger, 2018; Stribițcaia, Krop, Lewin, Holmes, & Sarkar, 2020), and food with encapsulated active ingredients (Patrignani et al., 2017; Ye, Georges, & Selomulya, 2018).

Sandiness or grittiness sensations seem to be especially relevant in semisolid foods because its presence is usually related to a decrease in the creaminess, one of main drivers of liking of these type of products (dairy desserts, sauces, and creams) (Frøst & Janhøj, 2007). However, it could be also due to the oral movements when eating a semisolid product facilitating the perception of this tactile sensation. Semisolid products are usually sheared against the oral surfaces while mixed with saliva (de Wijk, Janssen, & Prinz, 2011) and the continuous phase is squeezed between the oral surfaces, consequently any particles will be easily noticed.

Physiologically, particles are perceived by the filiform papillae in the oral cavity. These papillae are highly innervated by tactile sensors or mechanoreceptors that send the sensory input through the trigeminal ganglion to the brainstem receptive areas (Jacobs et al., 2002), where the sensation of sandiness is created. Oral tactile acuity varies between individuals (Laguna, Aktar, Ettelaie, Holmes, & Chen, 2016) and that might bring differences in food perception. However, these differences in an individual threshold are not important when the difference among food products is high enough, as all individuals (or a majority) can feel the differences.

Regarding the food factors influencing sandiness perception, particle properties such as hardness and geometry are considered key elements. Hard and irregular particles are perceived as more than round and soft particles of equivalent size (Engelen, Van Der Bilt, Schipper, & Bosman, 2005). In addition, the

59 proportion of people who perceived particles increased with particle size (Chojnicka-Paszun,
60 Doussinault, & De Jongh, 2014; Imai, Hatae, & Shimada, 1995).

61 Besides particle characteristics, studies have shown the possible influence of food matrix structures in
62 the particle's perception. In model systems with different microcrystalline cellulose concentrations used
63 as particles, and xanthan gum or agar as thickeners, less viscous solutions led to smaller size particle
64 identification (Imai, Hatae, & Shimada, 1995). Likewise, results were obtained from other research field,
65 in multiparticulate drugs, sandiness sensation was increasingly perceived with increasing amount and
66 size of particles, but this sensation was also counterbalanced by increasing viscosity (Lopez et al., 2016).

67 Different hydrocolloids can be used to increase viscosity and modify the food texture (Milani & Maleki,
68 2012). Starch is one of the most used in industry, and it is commonly combined with other thickeners
69 (carrageenan, xanthan gum, locus bean gum, guar gum, and cellulose derivatives) to provide other
70 properties like emulsification, and/or stabilisation (Li & Nie, 2016; Saha & Bhattacharya, 2010).

71 Although all hydrocolloids impart viscosity in semisolid systems (Saha & Bhattacharya, 2010), they are
72 different (branching structure, flexibility, molecular weight, and/or ionic charges) and create different
73 structure types in the food matrix that exhibit different rheological behaviour (BeMiller, 2018).

74 Changing the hydrocolloid used as a thickener affects the sensations perceived during product
75 consumption, affecting not only texture sensations but also flavour intensity and release (Yanes, Durán,
76 & Costell, 2002, Ferry et al., 2006, Arancibia, Jublot, Costell, & Bayarri, 2011; Dickinson, 2018). These
77 effects were attributed to the different in-mouth behaviour of the structure formed by different
78 hydrocolloids that, as showed in other studies, break differently depending on its resistance to saliva and
79 shearing. A study comparing custards thickened with four hydrocolloids (two modified starches: waxy
80 maize and tapioca; and two derivatives of cellulose: carboxymethylcellulose (CMC) and
81 hydroxypropylmethylcellulose (HPMC)) showed that after *in vitro* oral incubation, samples with starch
82 had a greater release of tyrosol because of the breakdown of the structure by salivary enzymes (Sanz &
83 Luyten, 2006). In another study, native and modified corn starch, λ -carrageenan, and guar gum were used
84 for creating milkshakes. The authors observed that in *in vitro* oral conditions, the structure of the
85 milkshakes prepared with λ -carrageenan and guar gum was better preserved (Morell, Fiszman, Varela,
86 & Hernando, 2014). Recently, present authors showed that when starch is combined with a second
87 hydrocolloid to thicken carrot puree, its rheological behaviour under *in vitro* oral conditions is modified.

88 In other words, viscosity profiles differing in the rate and extent of structure breakdown were observed
89 depending on the second thickener used (Laguna, Manickam, Arancibia, & Tarrega, 2020). Xanthan and

carrageenan were more resistant to *in vitro* oral digestion (slow and smaller viscosity reduction) whereas purees with starch and CMC showed a rapid reduction in viscosity (Laguna et al., 2020).

The hypothesis of this work is that not only the viscosity of the food matrix but also its in-mouth behaviour affects the oral perception of sandiness.

To evaluate this, two types of resistant starch (II and III) with different size were added to custards to create a sandiness sensation. Then, different hydrocolloids (starch, λ -carrageenan, xanthan gum, CMC, or a mix of xanthan gum with λ -carrageenan) were used to study how changing the matrix affected the *in vitro* viscosity decay induced by saliva and the sandiness perception.

2. Materials and methods

2.1. Materials

2.1.1. Artificial saliva components

Artificial saliva was freshly prepared daily, its composition was adapted from Minekus et al., (2014) containing sodium chloride (1.126 g L⁻¹), potassium phosphate (0.504 g L⁻¹), sodium bicarbonate (1.142 g L⁻¹), magnesium chloride hexahydrate (0.03 g L⁻¹), ammonium carbonate (0.006 g L⁻¹), calcium chloride dihydrate (0.221 g L⁻¹), 3 g L⁻¹ of mucin (porcine gastric mucin type II, Sigma Aldrich), and α -amylase (8.7 g L⁻¹) (A317, type VI-B from porcine pancreas, Sigma Aldrich) was added 10 minutes prior using the artificial saliva.

2.1.2. Custards

Custards ingredients

Commercial powder skim milk (Central Lechera Asturiana, Granada, Asturias) was purchased in a local store. Acetylated waxy maize adipate starch (S) (C*Tex 06205 Cargill Texturizing Solutions SL, Barcelona, Spain) from now on termed as starch, vanilla aroma (37548A; Lucta S.A., Spain), aspartame (Nutrasweet, Zug, Switzerland), and colourant (702679 beta carotene, CHR Hansen, Horshalm, Denmark) were provided by producers. The two resistant starches were from corn: resistant starch type II (RS2) (Hi maize 260, National Starch Food Innovation, Manchester, UK), and resistant starch type III (RS3) (Novelose 330, National starch, Manchester, UK); and the other thickeners used λ -carrageenan (Satiagum^{TM}, Cargill Texturizing Solutions SL, Barcelona, Spain), xanthan gum (Satiagum^{TM}, Cargill Texturizing Solutions SL, Barcelona, Spain), and sodium carboxymethylcellulose (CMC, Akzo Nobel Chemicals, Amersfoort, The Netherlands) were also provided by producers.

121 *Custard samples formulations*

122 Custard samples included 80% of powder skim milk, 0.1% vanilla aroma, 0.054% aspartame, and
123 0.052% colourant. Three sets of samples were prepared, varying the amount of RS2, RS3, and thickeners.

124 *Set 1.* To study the impact of RS type and concentration on custards; samples containing different
125 amounts (0, 1.5, and 3%) of RS2 or RS3 were prepared only using starch (4%) as the thickener.

126 *Set 2.* To study the impact of the matrix on custards with RS2; six samples with 3% RS2 were prepared,
127 using 3% starch as a thickener combined with an extra thickener (none, 1% starch, 0.06% λ -carrageenan,
128 0.3% xanthan gum, 0.4% CMC, or 0.15% mix of xanthan gum/ λ -carrageenan).

129 *Set 3.* To study the impact of the matrix on custards with RS3, six samples with 3% RS3 were prepared,
130 using 3% starch as a thickener combined with an extra thickener (none, 1% starch, 0.06% λ -carrageenan,
131 0.3% xanthan gum, 0.35% CMC, and 0.3% mix xanthan gum/ λ -carrageenan).

132 For sets 2 and 3, thickeners were first dissolved into water and stirred for 30 minutes. The amounts of
133 the second thickener were established according to a preliminary characterisation and comparison of
134 flow behaviour, to achieve custards with similar viscosity (equi-viscous) (Table 1). The objective of this
135 was to compare matrices of different hydrocolloid, having the same viscosity level.

136

137 *Custard preparation*

138 First, skim milk was prepared 24 hours in advance by dissolving milk powder in still water with low
139 mineral content (Cortes, Spain) and stored under refrigeration (4 °C). Then, the colourant, a sweetener
140 (aspartame), and the correspondent thickener were mixed in the prepared milk with stirring over 10
141 minutes at 250 rpm. The mixed solution was transferred to a kitchen robot (Thermomix, Vorwerk,
142 M.S.L., Madrid, Spain) and the temperature was set at 100 °C for 20 minutes while stirring at low speed
143 using a butterfly whisk. Time and temperature were set according to previous pasting experiments, to
144 ensure the starch was completely cook in the custards. After, the vanilla flavour was added to the robot,
145 as well as the water lost because of evaporation during cooking, calculated by weight difference, and
146 stirred at low speed for 1 min without applying temperature.

147 To ensure the cooling process was homogeneous, the custard was poured into a glass flask (1 L) in a
148 water bath (at 25 °C) with gentle stirring applied for 10 minutes with a square blade impeller (BR13,
149 Heidolph Instruments GmbH & CO. KG, Schwabach, Germany). Finally, the flask was sealed and kept
150 under refrigeration at 4 °C for 24 hours.

151

2.2 Particle size characterisation

Particle size distribution of the starch in dilution with and without heating (85 °C, 10 minutes) and of the custards formulations were determined using a laser diffraction particle size analyser (MasterSizer 2000, Malvern Instruments Ltd., Worcestershire, UK). A refractive index value of 1.5 for the dispersed phase and 1.33 for the continuous phase (water) were selected. Particle size calculations were based on Mie's scattering theory. Volume mean diameter values $D_{4,3}$, representing the diameter of bulk particle size (largest particles), and the percentage of volume corresponding to each observed population were obtained.

2.3. Flow behaviour characterisation

All rheological measurements were conducted in a controlled stress rheometer (AR-G2, TA Instruments, England). Flow properties were measured using cone-plates geometry (40 mm, 1 mm gap, 2° angle). Flow curves were obtained by recording shear stress values when shearing the samples at linearly increasing shear rates from 1 to 100 s⁻¹ over 60 s. From this curve, the values of apparent viscosity at 10 s⁻¹ were obtained for comparing custard viscosities, as this value (10 s⁻¹) is a good index of sensory viscosity in this type of systems (Laguna et al., 2020). Data from the ascending flow curve were adjusted to the Ostwald de Waele fit ($\sigma = K\dot{\gamma}^n$) where K (Pa sⁿ) is the consistency index and n is the flow index.

2.4. Viscosity decay induced by saliva measurement

Viscosity decay induced by saliva was measured using a starch pasting cell (impeller in a jacketed cell) adapted in a controlled stress rheometer (AR-G2, TA Instruments, Crawley, England). Twenty-five grams of custard were added to the probe cup and left covered to achieve 37 °C. Right before starting the test, 2 mL of artificial saliva were added to the cup using a pipette. Viscosity values when shearing at 10 s⁻¹ over 120 s were recorded using TA analysis software.

From the viscosity decay curves, the initial viscosity (η_0) corresponding to the viscosity value at time zero, and the final viscosity (η_f) were obtained. The final viscosity (η_f) was taken at 50 s for all samples, because the plateau region was reached and there was no further significant decrease in viscosity (< 1%). To quantify the rate of the structure breakdown, the Initial Breakdown Index (IBR) was calculated as the ratio between the viscosity at 10 s and the initial viscosity (η_0). To evaluate the extent of total structure breakage, the Total Breakdown Index (TBR) was calculated as the ratio between the final viscosity (η_f) and the initial viscosity (η_0).

2.5. Sensory evaluation

Sixty assessors ($n = 60$) evaluated sample sandiness using ranking tests. Assessors were instructed to not smoke, drink other than water, or eat the hour before the sensory evaluation.

Three sets of samples were evaluated over three days.

1) To evaluate the effects of the type and level of RS on sandiness, the base custard sample (without RS) and the four samples including RS2 or RS3) at different levels (1.5 and 3%) were first evaluated.

2) To evaluate the matrix impact on custard sandiness perception containing 3% RS2, six samples containing 3% starch and a different extra thickener (none, starch, xanthan gum, xanthan gum/ λ -carrageenan, λ -carrageenan, and CMC) were evaluated. 3) To evaluate the matrix impact on custard sandiness perception containing 3% RS3, six samples containing 3% starch and a different extra thickener (none, starch, λ -carrageenan, xanthan gum, CMC, or xanthan gum/ λ -carrageenan) were evaluated.

For each set, samples were served to the assessors together, and they were asked to rank the samples according to sandiness intensity (low to high) with ties allowed. Samples were served at 4 °C in 100 mL disposable cups with three-digit randomised codes. Sensory data were collected using Compusense Cloud software (Compusense Inc., Guelph, Canada).

This experimental procedure was approved by the Ethical Committee of CSIC (ethics reference 018/2019)

2.6. Data analysis

To study the effect of RS and thickener addition on the custard rheological properties (η_0 , η_f , IBR, and TBR) one-way ANOVA was used, and significance of differences among mean values of samples were determined by Tukey's multiple comparisons ($\alpha = 0.05$).

Friedman Analysis of Variance was applied to study the data obtained in the rank test, significant differences between samples were determined by a Nemenyi post-hoc-test ($\alpha = 0.05$). All analyses were performed with XLSTAT statistical software (version 2019, Microsoft Excel, Adinsoft, Paris, France).

3. Results

3.1. Particle size distribution of starch granules

3.1.1. Effect of heating on starch granules.

The particle size distribution, reported as the particle diameter is shown in Fig.1, for the non-resistant starch (S) and for each of the two resistant starches (RS2 and RS3) before (Fig. 1a) and after (Fig. 1b) heating in water at 85 °C for 10 minutes. Non-resistant starch (S) showed a population of particles whose diameter ranged from 6.6 to 92.1 μm with a volume mean diameter ($D_{4,3}$) of 19.50 μm . After the starch was heated, the curves shifted to the right showing an increase in size of starch granules (diameters ranged from 17.4 to 120.23 μm with a $D_{4,3}$ of 44.46 μm) corresponding to the swelling of the starch granules.

For RS2, the particle diameter distribution before heating ranged from 3.8 to 138 μm with a $D_{4,3}$ of 21.25 μm . After heating, the $D_{4,3}$ increased to 27.34 μm and the distribution curve showed two peaks. The first showed a population of particles with a size like observed before heating, and a second peak showed a population of bigger particles indicating gelatinisation had occurred for a part of the RS2 granules.

RS3 had the largest volume mean diameter ($D_{4,3} = 44.03 \mu\text{m}$) among the starches, and it showed a slight increase after heating ($D_{4,3} = 46.36 \mu\text{m}$).

3.1.2. Effect of saliva on starch granules size of custards

The custard's particle size distribution with and without resistant starch (RS2 or RS3) are shown in Fig. 2a. Without saliva, the size distribution was similar for the three custard samples, probably because of swollen starch granules (S) in the three custard samples. Custards without RS and with added RS2 presented similar $D_{4,3}$, 40.93 and 41.94 μm , respectively, whereas custard with added RS3 showed a higher $D_{4,3}$ (59.01 μm) because RS3 particles have a greater size.

After adding saliva (Fig 2b), the three custards presented different particles size distribution. For the custard without RS, volume mean diameter drastically decreased ($D_{4,3}$ custard S with saliva = 2.63 μm) indicating the starch granules were hydrolysed. However, for custards containing RS2 or RS3, a population of particles with a diameter distribution close to the observed granules before heating remained, indicating these granules were not hydrolysed by saliva.

3.2. Influence of type and concentration of RS on the physical and sensory properties of custards

3.2.1. RS effect in flow behaviour of custards

Fig. 3 shows all systems exhibited a shear-thinning flow behaviour (pseudoplastic), yet different curves were observed depending on the RS type and concentration.

For each curve, data were fitted to the Oswald de Waele model and the values of the consistency index (K) and flow index (n) and apparent viscosity at 10 s^{-1} (η_{10}) were obtained (Table 2). In comparison with the base system, adding any RS increased the consistency index and the viscosity of the system, and the increase was higher with concentration increment. Furthermore, RS3 had more consistency than RS2.

3.2.2. RS effect on the sandiness intensity perception

Ranking test data showed that the intensity of sandiness significantly varied among samples ($p = 0.001$). Custard samples without RS showed the least intense sandiness sensation. Adding 1.5% RS increased the sandiness sensation, but with no significant differences between RS2 and RS3. The increase of concentration (3%) increased the sandiness sensation similarly for RS2 and RS3 (Fig. 4).

3.3. Matrix effect on oral breakdown and sandiness perception of custards with RS

3.3.1. Viscosity of the custards with RS and extra thickeners

The flow behaviour of custards containing 3% RS2 or RS3, 3% starch, and different concentrations of an extra thickener (none, starch, λ -carrageenan, xanthan gum, CMC, or xanthan gum/ λ -carrageenan) were measured and the values of apparent viscosity at 10 s^{-1} (η_{10}) were obtained (Table 1).

For both sample sets (RS2 and RS3), an analysis of variance showed significant differences in the η_{10} values (F value = 16.64, $p = 0.04$). The value of the sample without extra thickener was significantly lower than the values of custards with extra thickener, as it can be seen in Table 1. As intended; the rest of the five samples containing an extra thickener did not show differences in the viscosity among them.

3.3.2. Structural breakage under shear and with artificial saliva

Fig. 5 shows the decrease in apparent viscosity with artificial saliva and constant shearing (at 10 s^{-1}) for custards with RS2 (Fig. 5a) and custards with RS3 (Fig. 5b). All custards showed an initial decrease in viscosity (structural breakdown) followed by a constant viscosity period. However, curves showed that the extent and the rate of viscosity decay were different for samples containing different thickener systems.

To characterise the viscosity breakdown of samples, the parameters η_0 and η_f were obtained (Table 3) and the IBR and TBR were calculated. For each set (custards with RS2 or custards with RS3) of custard, all parameters significantly varied ($p < 0.05$).

With custards with RS2 (Fig. 5a), the initial viscosity value was highest for samples with CMC, xanthan, and xanthan/ λ -carrageenan, whereas the lowest initial value was for the sample with no extra thickener.

As seen in Table 3 and Fig. 5a, the IBR (η_{10}/η_0) showed that during the first 10 seconds RS2 custards with xanthan had the biggest portion of structure breakdown (0.69), followed by the mixture of xanthan/ λ -carrageenan (0.67). After this initial breakdown, xanthan and xanthan/ λ -carrageenan custard viscosity remained constant, having the lowest total breakdown ratio (TBR). In contrast, RS2 custards with carrageenan or starch as an extra thickener had the lowest IBR (0.29–0.39 respectively), but the higher TBR (0.95–0.94 respectively), thus the structure was destroyed to a higher degree than xanthan containing samples, but this destruction was progressive.

For the custards set with RS3, the initial viscosity (η_0) values were lower for custards with no extra thickener (Fig. 5a). The custard without a second thickener (none), starch or carrageenan had higher IBR and TBR values. Similar to custards with RS2, RS3 custards containing xanthan (xanthan and xanthan/ λ -carrageenan mixture custard) had the lowest TBR values, thus the structure was more preserved than in the other custards.

3.3.3. Matrix effect on sandiness of custard samples enriched with RS

Results of the ranking tests evaluating sandiness of custards with RS and different thickeners systems are shown in Fig. 6a and 6b for the sets RS2 and RS3, respectively. In both cases Friedman tests shows that the intensity of sandiness significantly ($p = 0.001$) varied among samples.

In RS2 custards, sandiness sensation was significantly higher for samples containing CMC or xanthan/ λ -carrageenan as extra thickener than the sample containing xanthan.

In RS3, sandiness sensation was significantly higher for samples containing CMC, λ -carrageenan, or the xanthan/ λ -carrageenan than the samples containing only starch as extra thickener.

4. Discussion

4.1. Sandiness sensation elicited by resistant starch (RS) particles

In this study, the edible particles added (resistant starches) were first investigated alone in the custard under shear with artificial saliva.

The starch (S), used in all formulations, is modified and has undergone acetylation to avoid retrogradation, and has certain physical attributes that can resist extreme food processing (Berski et al., 2011). When heating, it undergoes a gelatinisation process when the granules amorphous regions expand, leading to a crystalline disruption, therefore absorbing water, which results in swelling (Donovan, 1979; Marques, Pérégó, Le Meins, Borsali, & Soldi, 2006) and an increase in particle size.

Granules of the RS3 were bigger than RS2. This difference is because of RS2 have non-gelatinised granules whereas RS3 are retrograded starch (Massarolo, Ferreira, de Borba, Kupski, & Furlong, 2019), this means that the RS3 starch granules have already gone through a phase swelling (increasing the size), and gelatinisation. Because RS3 granules are already retrograded also explains why they change in size much when heating. However, when heating RS2, a part of the granules increased in size. Previous studies report that RS do not change size during heating in water, as they have a really compact structure and low water absorption (Nugent, 2005). Therefore, the change observed in the RS2 was attributed to the richness of the RS2 used, as it was not pure (60/40), containing non-resistant starch that gelatinises, observed as particle enlargement.

After saliva was added, the starch matrix in which RS particles were imbibed disappeared, as the starch S (non-resistant) was hydrolysed by α -amylase; observed in Fig. 2 as the curve shifted to the left. This means that the peaks observed in the other custards correspond to RS2 or RS3 and were not attacked by α -amylase, thus will be intact during the oral process of the sample.

Ranking tests showed that the RS particles increased sandiness perception, especially at high concentration (3%). Previous authors, also using custards and including silicon beads with different sizes, described that roughness increased with particle size up to 80 μm (Engelen, Van Der Bilt, Schipper, & Bosman, 2005); thus, with increasing particle size, roughness perception increased, to an extent. When ranking the samples of this study, the increased in sandiness perception was observed when adding more RS (from 1.5 to 3.0%).

4.2. Effect of matrix on the viscosity decay induced by saliva and sandiness perception

First, to understand oral changes with custards, the viscosity decay was measured under constant shearing, artificial saliva, and a temperature of 37 °C was used. Table 3 and Fig. 5 show all custards decreased in viscosity. This viscosity decay can be attributed to the diluting effect of saliva addition to the action of the salivary α - amylase in breaking down the starch, and to the action of shear (de Wijk, Prinz, Engelen, & Weenen, 2004; Laguna et al., 2020).

Although all the custards experienced a viscosity decay, each hydrocolloid used showed a different breakage pattern. Custards with only S showed a near complete breakage of the structure after oral digestion. This was observed in previous research with starch base systems (custards and purees) in which once saliva was mixed with the product, the viscosity also decreased almost ten-fold (Janssen, Terpstra, De Wijk, & Prinz, 2007; Prinz, Janssen, & De Wijk, 2007; Laguna et al., 2020).

Likewise, systems with λ -carrageenan showed a high breakdown under oral conditions (95% at the end), that occurred progressively. This indicates that λ -carrageenan forms a network in presence of milk because of its interaction with casein micelles (Langendorff, Cuvelier, Michon, Launay, & Parker, 2000) that is fragile under shear and depends on the presence of starch, as the structure completely disappeared after oral processing. Custards with xanthan gum as a second thickener in both systems (RS2 or RS3) showed a rapid breakage of the structure, but the viscosity was maintained along the oral processing, indicating part of the structure remained. The ability of gums to partially preserve the structure with saliva has been reported by previous authors when researching thickeners for a dysphagia diet (Lee, Yoon, Yoo, & Yoo, 2016). This is probably because the xanthan, a dimeric helical polysaccharide, adopts an extended conformation, a rod like structure, that has both high rigidity and flexibility (Berth et al., 1996; Gunning, McMaster, & Morris, 1993) contributing to its film forming ability. The mixture of xanthan/ λ -carrageenan showed an intermediate behaviour with little breakage at the beginning and maintaining a certain degree of structure at the end.

Custards with CMC showed a slow breakage with part of the structure remaining after oral processing. Both CMC and xanthan have similar backbone structures (Viebke, 2005), however with hydration, the conformation of both hydrocolloids differs, as CMC chains interact within each other creating multiple connections (Kulicke et al., 1996).

Sandiness varied among products with different matrices. A previous study postulated that at the same particle concentration, an increase in viscosity helped mask the sandiness (Imai et al., 1995; Lopez et al., 2016). If this applied to our study, the sandiness in samples with extra thickener and higher viscosity would be lower than samples with extra thickener, but this effect was not observed. However, this difference between this study and the past is because the increase in the previous study's viscosity were ten-fold, as more hydrocolloid concentration was incorporated, whereas here it was lower (an increase of 2- to 4-fold) as we tried to keep a reasonable variation in the custard, in the range of the usual product consumption acceptability.

In addition, for some extra thickened samples (those containing CMC or carrageenan) sandiness was more intense than the base sample, which indicates a possible contribution of these hydrocolloids to a sandy mouthfeel sensation.

Comparing the samples with the same viscosity, differences in the sandiness' intensity are observed, depending on the extra thickener. Xanthan containing custards with the RS of smaller size (RS2) decreased the sandiness perception, but not for the bigger size (RS3). This was previously explained

because xanthan gum is a film forming hydrocolloid, which can decrease the friction coefficient and to create a slippery sensation in a particulate system. Still, this depends on the particle size as bigger particles create more friction coefficient and roughness sensation (Chojnicka-Paszun, Doussinault, & De Jongh, 2014).

In RS2 custards, a part of the CMC structure remained along the whole oral processing. Despite this, it did not remove the sandiness sensation, and it is unknown if this is because of the custard matrix or CMC. CMC is a negatively charged polymer that may repulse milk soluble proteins, that are also negatively charged, at the pH of the custard (≈ 6), thus this separated matrix might provide the sandiness perception. It can be hypothesised that as CMC does not form a rod like structure, its ability to create a film in the oral mucosa might be lower than xanthan, and cannot counteract sandiness perception.

Finally, it must be considered that this study has focused on the properties of food and not on the different perceptions of the participants. This study showed that despite the possible differences between participants, there were significant differences among matrices and particles. Still, future lines of research that consider inter-individual variability are still necessary for a complete understanding of sandiness perception. Further, as RS is considered as a source of fibre, and it could be added to fibre-enriched food products, it will be interesting to study how RS from other sources affect the sandiness perception.

5. Conclusions

In this study, two resistant starches were added as edible particles to a semisolid system. It was first observed that RS2 and RS3 particles were present after incubating the custards with saliva, causing sandiness sensation. Modifying the matrix using different hydrocolloids showed to affect sandiness perception. In contrast with previous studies, the increase in viscosity did not result in masking sandiness. However, the intensity of this sensation varied depending on the thickener used. Xanthan showed to decrease sandiness perception of smaller size particles (RS2), whereas λ -carrageenan and CMC increased the sandiness sensation.

Viscosity decay induced by saliva showed different rates of structural breakdown depending on the second thickener. Systems containing xanthan showed that part of the structure remained after oral processing and may explain the reduction in sandiness. Future studies to observe the friction coefficient with tribological studies, and the hydrocolloids structures by transmission electron microscopy, might help the further understanding of the sandiness perception in food matrices.

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Fig 1. Particle diameter distribution measured as the volume mean diameter of the starches, starch (S) (black line), resistant starch type II (RS2) (grey line), and resistant starch type III (RS3) (red line) before (a) and after (b) heating at a concentration in water of 3%.

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Fig 2. Particle diameter distribution measured as the volume mean diameter of a) custards or b) custards with artificial saliva. Colors of the curves represent the custards contained starch (black line), resistant starch type II (RS2) (grey line), and resistant starch type III (RS3) (red line).

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Fig 3. Flow behaviour of custards containing the two different resistant starches: RS2 (grey symbols) and RS3 (red symbols); with 1.5% (triangle) and 3% (square) of RS compared with the custard with no RS added (black symbols).

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Fig. 4. Rank mean values of sandiness evaluated by ranking tests for comparing custards containing two types of resistant starch: type II (RS2) and type III (RS3) at two concentrations (1.5 and 3%) and the custard without resistant starch (S). Different letters on top of bars mean significant differences according to the Nemenyi post-hoc test ($\alpha = 0.05$)

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Fig. 5. Viscosity decay induced by saliva for custards with 3% of resistant starch type II (RS2) (top) (a), or 3% of resistant starch type III (RS3) (bottom) (b). Samples were elaborated with different extra thickeners: none (—), starch (X), carrageenan ($\square$), xanthan ($\diamond$), CMC ($\circ$), and xanthan-carrageenan (Δ).

Fig. 6. Rank mean values of sandiness evaluation by ranking tests for comparing custards with none or different extra thickener. Two separate ranking tests were carried out for custards with resistant starch type II (RS2) (top), and for custards with resistant starch type III (RS3) (bottom).

Different letters on top of bars mean significant differences according to the Nemenyi post-hoc test ($\alpha = 0.05$)

629 Table 1. Mean and standard deviation values of apparent viscosity at 10 s⁻¹ for custards with both resistant
630 starches (RS2 and RS3) at 3% and the added second thickener
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Extra thickener added	RS2 η_{10} (Pa.s)	RS3 η_{10} (Pa.s)
(none)	0.8 ^a (0.07)	2.7 ^a (0.65)
Starch	3.6 ^b (0.33)	4.9 ^b (0.03)
Xanthan	3.8 ^b (0.17)	4.3 ^b (0.26)
Carrageenan	3.6 ^b (0.24)	4.3 ^b (0.17)
Xanthan/Carrageenan	3.9 ^b (0.14)	4.4 ^b (0.01)
CMC	3.8 ^b (0.27)	5.4 ^b (0.06)

632 Different letters in the same column indicate significant differences at $p < 0.05$ according to the Tukey's Test
633 CMC: sodium carboxymethyl cellulose
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657 Table 2. Mean and standard deviation of the flow behaviour parameters (K, consistency index; *n* flow
658 index) for custards with different percentages (1.5 and 3%) of the two resistant starches (RS2 and RS3)
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RS type and concentration	K (Pa s ⁿ)	<i>n</i>	η _{ap} (Pa.s) at 10 s ⁻¹
No RS added	4.79 ^e (0.43)	0.44 ^a (0.01)	1.33 ^e (0.01)
RS2- 1.5%	8.82 ^d (0.06)	0.42 ^a (0.01)	2.35 ^d (0.04)
RS3- 1.5%	15.62 ^b (1.03)	0.35 ^b (0.01)	3.53 ^c (0.06)
RS2- 3.0 %	14.09 ^c (0.05)	0.43 ^a (0.05)	3.78 ^b (0.13)
RS3- 3.0 %	21.55 ^a (0.14)	0.34 ^b (0.01)	4.70 ^a (0.04)

660 Different letters in the same column indicate significant differences (Tukey test, α = 0.05).

685 Table 3. Viscosity decay induced by saliva of custards with different thickeners. Mean values of the
 686 initial viscosity (η_0), initial breakdown ratio (IBR, η_{10}/η_0), and total breakdown ratio (TBR, η_f/η_0) for
 687 custards formulated with RS2 or RS3 and a second thickener or carrageenan/xanthan mixture
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Second thickener added	RS2			RS3		
	η_0	IBR	TBR	η_0	IBR	TBR
	(Pa.s)			(Pa.s)		
(none)	0.19 ^c	0.54 ^{ab}	0.93 ^a	0.30 ^c	0.89 ^a	0.93 ^a
Starch	0.30 ^c	0.39 ^c	0.94 ^a	0.60 ^b	0.84 ^a	0.96 ^a
Xanthan	0.89 ^a	0.69 ^a	0.73 ^c	0.94 ^a	0.69 ^b	0.75 ^c
Carrageenan	0.48 ^b	0.29 ^c	0.95 ^a	0.58 ^b	0.81 ^a	0.97 ^a
Xanthan/Carrageenan	0.83 ^a	0.67 ^a	0.80 ^b	0.70 ^b	0.61 ^b	0.74 ^b
CMC	0.89 ^a	0.43 ^{bc}	0.70 ^c	1.05 ^a	0.50 ^c	0.93 ^a

689 Different letters in the same column indicate significant differences at $p < 0.05$ according to the Tukey's Test
 690 CMC: sodium carboxymethyl cellulose
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