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Impact of chia seed mucilage on technological, sensory, and *in vitro* digestibility properties of a texture-modified puree

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ABSTRACT

The impact of adding chia seed mucilage (CSM) as alternative to common thickeners, such as modified starch (MS), in a texture-modified chicken and vegetables puree and their effect on technological, sensory, and *in vitro* digestibility properties were evaluated and compared. The thickened purees showed weak gel behaviour with good oral consistency, but MS puree was less accepted than CSM regarding the oral adhesiveness. CSM purees presented the highest consistency and viscosity during the oral processing, which would facilitate a safe swallowing process. Its flow behaviour was scarcely affected by the conditions of simulated oral and gastric phases. Protein digestibility was not compromised, whereas the digestion rate of starch in CSM was lower than that of MS. These results demonstrate the feasibility of tailoring the technological, sensory, and digestibility properties of purees by adding different thickeners, and the suitability of CSM to be used in dysphagia-oriented products.

1. Introduction

Dysphagia is characterised by impairments in foods or fluids transfer from the mouth to the stomach and is estimated to affect ~8% population around the world (~590 million people) (Cichero et al., 2017). Thickening dietary products is a practice commonly used to ensure safe consumption by people with swallowing problems, since it delays the fluid's flow rate and gives more time for safe swallowing (Herranz, Criado, Pozo-Bayón, & Álvarez, 2021).

Many of the currently available thickeners on the market are starch-based hydrocolloids (Moret-Tatay et al. 2015), which provide product stability and texture. Modified starch (MS) is a common and low-priced thickener that does not modify the flavour of the products at concentrations lower than 5% (Saha & Bhattacharya, 2010). Nevertheless, it is partially hydrolysed in the mouth by the salivary α-amylase, reducing samples' viscosity. The food industry is looking for alternative thickeners consisting of ingredients that increase the well-being of dysphagic patients regarding the nutritional content and safe swallowing. In this sense, Vieira, Oliveira, Salvaro, Maffezzolli, de Mello, and Cunha (2020) investigated the soluble polysaccharide part obtained from flaxseed (*Linum usitatissimum)* as a potential thickener for dysphagia-oriented products due to its excellent rheological and tribological characteristics coupled to nutritional properties. Chia (*Salvia hispanica* L.) is an herbaceous plant whose seeds, in contact with water, produce a transparent mucilaginous gel with a remarkable water-holding capability and good viscosities even at low concentrations (Segura-Campos, Acosta-Chi, Rosado-Rubio, Chel-Guerrero, & Betancur-Ancona, 2014). Chia seed mucilage (CSM) also provides nutritional and health benefits, resulting primarily from its dietary fibre content (Soukoulis, Gaiani, & Hoffmann, 2018). These properties make CSM an exceptional thickener for food applications, but its use in dysphagia management has been poorly evaluated so far. A recent study has shown its potential as texturing agent on texture-modified soups (Ribes, Grau, & Talens, 2022).

The study of textural and rheological properties of texture-modified foods is crucial to develop products with desired textural properties and appropriate flow characteristics for dysphagic patients. Moreover, oral processing including the role of saliva to lubricate the mouth and to produce a cohesive bolus must be also considered to ensure a safe swallow (Boehm, Yakubov, Stokes, & Baier, 2020). Some studies have carried out rheological and masticatory assays in thickened beverages (Moret-Tatay et al., 2015), purees (Sharma, Kristo, Corredig, & Duizer, 2017; Herranz et al., 2021), and creams (Talens, Castells, Verdú, Barat, & Grau, 2021). However, information on interactions between hydrocolloids and food matrix during gastrointestinal digestion and, thus, the effect on nutrient bioavailability is still scarce.

The main purpose of this work was to study the influence of adding chia seed mucilage, as alternative thickener to modified starch, on the

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technological (colour, texture, and rheology), sensory, and *in vitro* digestibility properties of a chicken and vegetables puree oriented for people with swallowing disorders.

2. Materials and methods

2.1. Materials

Ingredients to prepare chicken and vegetables puree were bought in a local supermarket. Thickeners used were Nutavant® - modified waxy maize starch (MS) -, bought in a local pharmacy, and CSM, which was obtained from chia seeds (Pedon S.P.A, Molvena, Italy) following the methodology proposed by Ribes et al. (2022).

Enzymes α-amylase and pancreatin from porcine pancreas, pepsin from porcine gastric mucosa, as well as porcine bile extract, mucin (type II) from porcine stomach, trinitrobenzenesulfonic acid (TNBS), and 3,5-dinitrosalicylic acid (DNS) were from Sigma-Aldrich, Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA) was purchased from Scharlau Chemie, S.A. (Sentmenat, Barcelona, Spain).

2.2. Chicken and vegetables puree preparation

The chicken and vegetables puree was prepared with 15% of chicken breast, 12% of carrots, 5% of onions, 3.5% of green beans, 6% of olive oil, 2% of salt, and 56.5% of tap water. The ingredients were mixed and cooked at 100 °C for 50 min in a kitchen robot (Thermomix^{TM} 31, Vorwerk M.S.L, Spain). After that, the broth was removed, and the remaining ingredients were pureed to obtain the control sample. Thickened purees were obtained by mixing the thickeners (MS and CSM) at 70 °C until being completely dispersed. The concentration of the thickeners utilised to formulate the purees was 1.5 % and 1 % (w/w) of MS and CSM, respectively, which was established to ensure an apparent viscosity (η) close to 2400 mPa·s (pudding-thick level) that was measured at 25 °C with a shear rate of 50 s^{-1} based on the National Dysphagia Diet Task Force (Vieira et al., 2020). The control sample had a η of 895 mPa·s (honey-thick level). Two independent batches of all puree samples were prepared.

2.3. Texture and colour characterisation

The purees were heated at 37 °C for 30 min in a water bath prior to run the texture and colour measurements, which were conducted in duplicate. Texture of samples was determined with a TA.XT2 Texture Analyser (Stable Micro Systems, Godalming, UK) by conducting a back-extrusion test according to Ribes, Estarriaga, Grau, and Talens (2021a). Colour parameters (L*, a*, and b*) were determined using a spectrocolorimeter (CM-3600d, Minolta Co., Tokyo, Japan) and the standard illuminant D65 and observer 10°. Chroma (C_{ab}*) and hue (h_{ab}*), as well as colour variations (ΔE*) of the thickened purees in comparison with the puree without addition of thickeners, were calculated by using Eq. (1), (2) and (3), respectively.

$$C_{ab}^{*} = \left((a^{*})^{2} + (b^{*})^{2}\right)^{0.5} \quad (1)$$

$$h_{ab}^{*} = arctg(b^{*}/a^{*}) \quad (2)$$

$$\Delta E^{*} = \left((\Delta L^{*})^{2} + (\Delta a^{*})^{2} + (\Delta b^{*})^{2}\right)^{0.5} \quad (3)$$

2.4. Flow rheological, viscoelastic, and combined squeezing flow and shear force test determinations

A rotational Kinexus Pro + Rheometer (Malvern Instruments Ltd., MA, USA), using a Peltier system for temperature regulation, was employed for these analyses. The determinations were run at 37 °C and in duplicate.

2.4.1. Flow rheological properties

The samples flow curves were obtained by recording for 300 s the shear stress values when the shear rate raised from 0.1 to 100 s^{-1}. Data from the curves of control and thickened purees were fitted to the power-law model (Eq. (4)) and Hershel-Bulkley model (Eq. (5)), respectively (Augusto, Cristianini, & Ibarz, 2012; Talens et al., 2021).

$$\sigma = K \cdot \dot{\gamma}^{n} \quad (4)$$

$$\sigma = \sigma_0 + K \cdot \dot{\gamma}^{n} \quad (5)$$

where σ is the shear stress (Pa), σ_0 is the yield stress (Pa), K is the consistency coefficient (Pa·s^n), $\dot{\gamma}$ is the shear rate (s^{-1}), and n is the flow behaviour index. The apparent viscosity values ($\eta = \sigma/\dot{\gamma}$) of all the samples measured at 10 s^{-1} and 50 s^{-1} were also reported. To corroborate the goodness-of-fit of the model, the correlation coefficient R^2 was used.

2.4.2. Viscoelastic properties

The viscoelastic characteristics of the purees were determined by conducting non-linear and linear viscoelastic assays.

The non-linear viscoelastic characteristics and the limit of the linear viscoelastic region (LVR) of the purees were evaluated by performing a stress sweep assay, with a stress ranging from 0.1 to 100 Pa at a frequency of 1 Hz. The changes in elastic and viscous modulus (G′ and G″, respectively) values, the stress values at the LVR ($Stress_{LVR}$), and the elastic modulus (G'_{LVR}) were reported (Talens et al., 2021).

The linear viscoelastic characteristics of the purees were determined by conducting a frequency sweep assay. The test was carried out from 0.1 to 10 Hz at 1 Pa. The G′, G″, complex viscosity (η*), complex modulus (G*), and loss tangent values (Tan δ), were reported by using rSpace for Kinexus software.

2.4.3. Combined squeezing flow and shear force test

The combined squeezing flow and shear force test was performed according to Chung, Olson, Degner, and McClements (2013). The purees were compressed, subjected to a constant shear rate (10 s^{-1}), and decompressed for ten cycles to mimic the motion of the tongue and palate during oral processing. This shear rate was established to simulate the in-mouth shear rate observed during eating (de Wijk, Prinz, & Janseen, 2006). The test was run at 37 °C, which could be the temperature of typical consumption and it is that of the human body (Herranz et al., 2021), as well as in absence and presence of artificial saliva that was mixed with the sample at a ratio 1:1 (v/w) to evaluate its impact during the oral processing. The standardised INFOGEST protocol (Minekus et al., 2014), with a slight modification, was used to prepare the artificial saliva that contained mucin (3 mg/mL), simulated salivary fluid, and α-amylase (75 U/mL).

2.5. Sensory analysis

The sensory analysis of the samples was made by 100 non-trained assessors (60 women and 40 men). They were selected according to their interest in the sensory analysis of thickened foods, availability, and absence of allergies, following the IFST Guidelines (IFST, 2020). Testing was carried out by employing a 9-point numeric scale (from 1 = very unpleasant to 9 = very pleasant. UNE-ISO 4121:2003; ISO, 2003) to determine the product acceptability. The assessors evaluated colour, flavour, oral consistency, oral adhesiveness, and general acceptance of three samples (control and both thickened purees) that were presented at 37 °C. Informed consent was obtained from all assessors.

2.6. In vitro gastrointestinal digestion

The simulated gastrointestinal digestion (GID) was conducted in 5 g of sample, in duplicate, based on the standardised INFOGEST method

(Minekus et al., 2014). In the oral phase (2 min, 37 °C, pH = 7), simulated salivary fluid with α-amylase (75 U/mL) were added to the sample (1:1, v/w). Then, simulated gastric fluid and pepsin (2000 U/mL) were added to the oral bolus (1:1, v/v) and mixed (2 h, 37 °C, pH = 3). Lastly, simulated intestinal fluid with bile salts (10 mM) and pancreatin (100 U/mL trypsin activity) were added to the gastric chyme (1:1, v/v), and incubated (2 h, 37 °C, pH = 7). Samples were promptly cooled in ice. Individual tubes were used per each digestion phase, and blanks (without sample) were run in parallel. Each puree sample was also mixed with water (1:1, w/v) to obtain samples before digestion. Aliquots of 3 mL were taken out, centrifuged (10,000 rpm, 10 min), and supernatants were stored for subsequent digestibility assays. The remaining sample was immediately analysed for rheological characterisation. In such a case, samples were also subjected to GID conditions but only adding water to consider the dilution effect of the samples by the addition of digestive fluids.

2.6.1. Determination of protein and starch digestion products

The proteolysis occurred in the samples during digestion was evaluated as proposed by Gallego et al. (2021) by determining the contents of total soluble proteins based on the Bradford method (Bradford, 1976), peptides soluble in 5% TCA, and free amino groups using the TNBS method (Adler-Nissen, 1979). The concentration of total reducing sugars released from starch digestion was also determined by the DNS method (Miller, 1959). All determinations were done in triplicate and data were expressed as mg/g sample.

2.6.2. Flow rheological properties of the digested samples

The flow rheological properties of the samples during *in vitro* GID were analysed as previously described in Section 2.4.1 but using the C25/PC25 sensory system of coaxial cylinders. The samples obtained during GID (after oral, gastric, and intestinal phases), as well as diluted samples (considering the dilution effect by the incorporation of digestive fluids at each stage) were evaluated. Results were fitted to the power-law model, and the η at 10 s^{-1}, n, K, and R^2 values were determined (Talens et al., 2021). A shear rate of 10 s^{-1} was selected as it was used to simulate the shear rate observed during digestion (Hardacre, Yap, Lentle, & Monro, 2015).

2.7. Statistical analysis

The data were analysed employing one-way analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) tests with Statgraphics Centurion XVII software (Statgraphics Technologies, Inc., The Plains, VA, USA). The data were expressed as the mean of replicates ± standard deviations (SD), and differences were considered statistically significant at $p < 0.05$. Pearson's Product Moment Correlation Coefficients were calculated to assess the relationships between instrumental and sensory data.

3. Results and discussion

3.1. Texture and colour texture characterisation

Table 1 presents the texture parameters (area and maximum force) and the colour analysis (L*, C_{ab}*, h_{ab}* and ΔE*) of the different purees at 37 °C. Concerning the texture parameters, significant differences ($p < 0.05$) were noted among the studied purees. MS and CSM purees exhibited higher area and maximum force values than the control, indicating greater consistency and firmness. According to Alpizar-Reyes, Román-Guerrero, Gallardo-Rivera, Varela-Guerrero, Cruz-Olivares, and Pérez-Alonso (2018), it could result from extended intermolecular interactions that lead to a greater water binding capability of hydrocolloids. The highest maximum force and area values were exhibited by the puree formulated with CSM as thickener. It could be attributed to the capability of swollen CSM particles to behave like filling agents, reducing the food matrix motion and rising the consistency and firmness of foods (Ribes, Peña, Fuentes, Talens & Barat, 2021b).

Table 1
Texture colour attributes, and flow rheological parameters from the steady flow behaviour test of the control and thickened chicken and vegetables purees measured at 37 °C.

Parameters		Control	MS	CSM
Texture	**Area (N·mm)**	11.67 ± 0.60^{c}	31.22 ± 0.10^{b}	36.30 ± 1.95^{a}
	Maximum force (N)	1.10 ± 0.07^{c}	3.14 ± 0.04^{b}	3.61 ± 0.18^{a}
Colour	**L***	70.71 ± 0.50^{a}	69.46 ± 0.12^{b}	65.69 ± 0.20^{c}
	C_{ab}*	39.89 ± 0.67^{a}	39.11 ± 0.36^{ab}	38.20 ± 0.02^{b}
	h_{ab}*	76.32 ± 0.04^{b}	76.31 ± 0.19^{b}	77.36 ± 0.03^{a}
	ΔE*	–	1.56 ± 0.82^{b}	5.35 ± 0.87^{a}
Flow rheology	**η at 10 s^{-1} (mPa·s)**	2155 ± 75^{c}	6849 ± 25^{a}	6427 ± 17^{b}
	η at 50 s^{-1} (mPa·s)	736 ± 30^{b}	2089 ± 26^{a}	2105 ± 26^{a}
	n	0.332 ± 0.004^{a}	0.262 ± 0.005^{c}	0.307 ± 0.006^{b}
	K (Pa·s^{n})	10.0 ± 0.3^{c}	37.4 ± 0.3^{a}	31.7 ± 0.4^{b}
	R^2	0.992	0.991	0.994

Control: puree without hydrocolloid; MS: puree thickened with modified starch; CSM: puree thickened with chia seed mucilage.
Texture parameters represent: Area under the curve, Maximum force. Colour attributes represent: L*, Luminosity; C_{ab}*, Chroma; h_{ab}*, Hue; and ΔE*, colour variation compared to control. Rheological parameters represent: η, apparent viscosity; n, flow behaviour index; K, consistency coefficient; R^2, correlation coefficient.
Values are the average of two independent experiments, and lowercase letters indicate significant differences among samples($p < 0.05$).

Regarding the colour analysis, MS and CSM purees showed slightly lower L* and C_{ab}* values than the control sample, being more evident when using CSM as thickening agent. Non-significant differences ($p > 0.05$) were observed between the control and MS puree regarding the h_{ab}* values, whereas slightly greater h_{ab}* values were reported by the CSM puree. Furthermore, the puree prepared with CSM exhibited the highest ΔE* value. The results observed in CSM sample could be explained by the existence of tannic substances or natural pigments coming from the chia seeds' tegument (Koocheki, Taherian, Razavi, & Bostan, 2009).

3.2. Flow rheological, viscoelastic, and combined squeezing flow and shear force test determinations

3.2.1. Flow rheological properties

The power-law and Herschel-Bulkley models were used to describe the viscous flow behaviour of the control and thickened purees, respectively. Table 1 summarises the data of the rheological parameters of the steady flow behaviour assay. The η values at 10 s^{-1} and 50 s^{-1} were reported due to the relevance of measuring the η at low shear rates, since a transitory increased η of the bolus and a decreased shear rate is connected to the swallowing procedure (Herranz et al., 2021). As expected, the viscosity (at 10 s^{-1} and 50 s^{-1}) increased in the thickened purees, which could be associated with the higher content of total solids resulted from the addition of the hydrocolloids (Alpizar-Reyes et al., 2018). All the purees presented an important reduction in the η values as the shear rate increased. It could be attributed to the structural breakage of molecules owing to the hydrodynamic forces created and the increased lining up of the molecules (Izidoro, Scheer, Sierakowski, & Haminiuk, 2008). This fact is indicative of the shear-tinning flow behaviour of the purees, and it was also reported by Herranz et al. (2021) in commercial dysphagia oriented-products.

The yield stress (σ_0) of a material is associated with the breakage of

its internal structure and corresponds to the least shear stress needed to start samples' flow (Augusto et al., 2012). The σ_0 of MS and CSM purees, obtained by the Hershel-Bulkley model, was close to 12 Pa. Thus, the addition of both thickening agents increased the least shear stress needed to cause samples' flow, which could be attributed to the capacity of thickeners to provide cohesion among food particles (Tashiro, Hasegawa, Kohyama, Kumagai, & Kumagai 2010). Moreover, significant differences ($p < 0.05$) were noted among purees regarding the flow behaviour index (n) and the consistency coefficient (K) values. Thickened purees showed greater K values than the control that could be associated with the greater water binding capability of thickeners by increasing intermolecular interactions (Alpizar-Reyes et al., 2018). This effect was more marked in CSM sample probably due to the capability of swollen CSM particles to behave like filling agents (Ribes et al., 2021b). These data fall in line with the results observed in the texture analysis. Finally, all the n values were below 1, confirming the shear-tinning flow behaviour. Based on that, both thickened samples could be considered appropriate for a swallowing procedure with a decreased aspiration risk by conferring the neuromuscular system more time to close properly the epiglottis (Nishinari, Turcanu, Nakauma, & Fang, 2019).

3.2.2. *Viscoelastic properties*

The changes in the elastic modulus (G′) and viscous modulus (G″) for all the purees as a function of the stress are presented in Fig. 1. For all the purees through the stress sweep applied, higher G′ than G″ values were observed, which is indicative of typical weak viscoelastic systems. After LVR, G′ values of all purees decreased as the stress increased. Nonetheless, all the purees presented a small increase in the G″ values that decreased when greater stress levels were applied to the samples. In relation to the categorisation of Hyun, Kim, Ahn, and Lee (2002) for the modulus behaviour of the samples, purees would present weak strain-overshoot behaviour beyond the LVR. The higher G″ values observed could denote the utilisation of greater quantities of energy through the deformation procedure. This outcome was also observed in carrot purees and pea creams prepared by using several gums and MS as thickeners (Sharma et al., 2017; Talens et al. 2021), and it could be attributed to microcracks formed in foods structure and layers' friction in the crack point leading to energy losses as heat (Sharma et al., 2017).

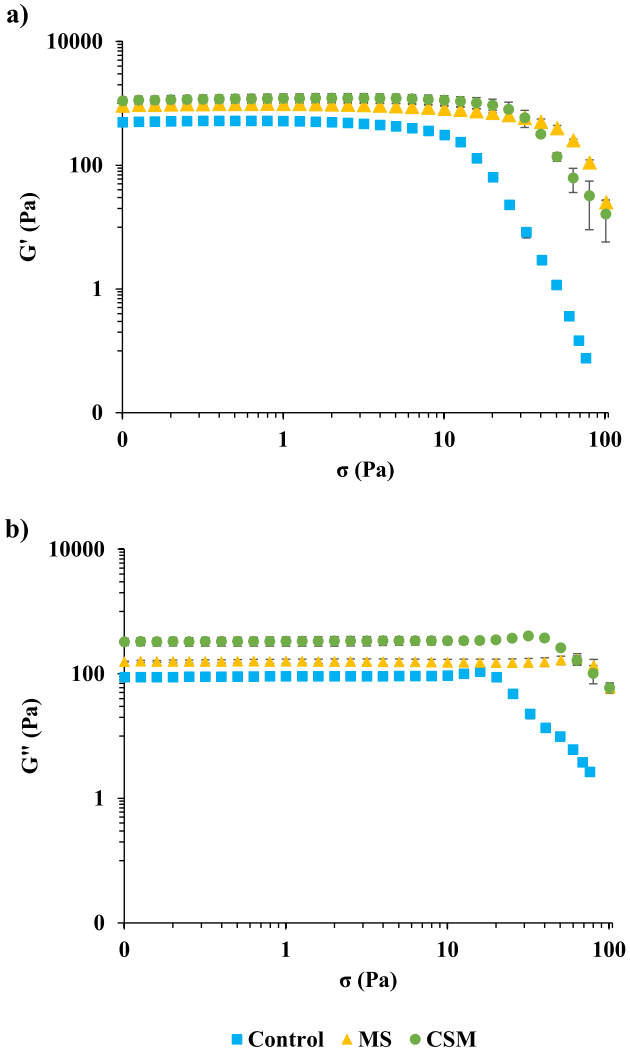

Fig. 1. Changes in a) elastic modulus, G′, and b) viscous modulus, G″, of the control and thickened purees at 37 °C with the shear stress. (MS: Modified starch and CSM: chia seed mucilage).

Table 2 shows the viscoelastic parameters (G'_{LVR} and $Stress_{LVR}$) of the different purees from the stress sweep test. G'_{LVR} is related to the product stiffness, meanwhile $Stress_{LVR}$ is referred to the stability of the product structure (Herranz et al., 2021; Talens et al., 2021). As expected, the control sample showed the lowest G'_{LVR} value, being consequently the lowest stiff puree. Contrarily, CSM showed the greatest stiff structure although non-significant differences ($p > 0.05$) were noticed between thickened purees. These findings are in accordance with those observed by Talens et al. (2021) when evaluating the stiffness of texture-modified pea creams. Concerning the $Stress_{LVR}$ parameter, CSM puree exhibited the highest value whereas non-significant differences ($p > 0.05$) were observed between the MS sample and control. This indicates that the CSM puree presented greater structural stability than MS and control purees. The differences observed between thickened purees could be ascribed to the existence of big polymeric molecules in CSM contrasted with MS.

To determine the linear viscoelastic characteristics of the purees, a frequency sweep test was carried out and the viscoelastic characteristics of the purees are summarised in Table 2. The viscoelastic parameters were reported at a frequency of 1 Hz for comparative purposes. For all purees, higher G′ than G″ values were observed, being commonly noticed in weak viscoelastic systems. Similar findings were presented by Sharma et al. (2017) and Herranz et al. (2021) in thickened carrot purees and commercial dysphagia-oriented products, respectively. Regarding the Tan δ, it gives information on the balance of the viscoelastic modulus of the food product by considering the contribution of both G′ and G″ modulus (Tan δ = G″/ G′) (Talens et al., 2021). All the samples presented Tan δ values lower than 1, also suggesting that the elastic properties predominate over the viscous ones. Tan δ can be used

Table 2
Viscoelastic parameters obtained from the stress sweep test and frequency sweep test at 37 °C.

Viscoelastic parameters		Control	MS	CSM
Stress sweep test	G'_{LVR} **(Pa)**	505 ± 13^{b}	975 ± 78^{a}	1219 ± 218^{a}
	$Stress_{LVR}$ **(Pa)**	1.591 ± 0.001^{b}	1.591 ± 0.004^{b}	3.171 ± 0.006^{a}
Frequency sweep test	G′ **(Pa)**	633 ± 132^{b}	1000 ± 25^{a}	1200 ± 13^{a}
	G″ **(Pa)**	108 ± 23^{c}	159 ± 4^{b}	323 ± 1^{a}
	G* (Pa)	643 ± 134^{b}	1013 ± 26^{a}	1242 ± 13^{a}
	η* (Pa·s)	102 ± 21^{b}	161 ± 4^{a}	198 ± 2^{a}
	Tan δ	0.170 ± 0.001^{b}	0.1588 ± 0.0004^{c}	0.269 ± 0.004^{a}

Control: puree without hydrocolloid; MS: puree thickened with modified starch; CSM: puree thickened with chia seed mucilage.
Stress sweep test: G'_{LVR}, elastic modulus value at LVR; and $Stress_{LVR}$, stress value at LVR. Frequency sweep test: G′, elastic modulus at 1 Hz; G″, viscous modulus at 1 Hz; G*, complex modulus at 1 Hz; η*, complex viscosity at 1 Hz; Tan δ, loss tangent at 1 Hz.
Values are the average of two independent experiments, and lowercase letters indicate significant differences among samples ($p < 0.05$).

as a rheological parameter of easy-swallowing boluses for dysphagic people when its values range between 0.1 and 1 (Ishihara, Nakauma, Funami, Odake, & Nishinari, 2011). The complex modulus (G*) is linked to the stiffness and rigidity of the product, and the complex viscosity (η*) denotes the total resistance of samples to flow with regard to the angular frequency (Talens et al., 2021). As expected, the thickened purees exhibited higher product stiffness and rigidity than the control. Despite non-significant differences ($p > 0.05$) were observed between the thickened samples, the puree formulated with CSM showed the greatest G* values (stiffness and rigidity) probably due to the network structure created by the molecules of the polysaccharide. The highest resistance to flow was also shown by the thickened samples, being more marked in CSM puree owing to increased internal cohesive forces (Ribes et al., 2021b). These results are in concordance with the G'_{LVR} values obtained in the stress sweep test.

3.2.3. Combined squeezing flow and shear force test

The assay was performed to mimic the movement of the tongue and palate during eating. In this assay, the maximum positive force achieved at the smallest gap between plates during the compression step was linked to the consistency of the puree, whereas the maximum negative force reached at the largest distance between plates during the decompression step was related to its adhesiveness. The η in the fixed gap stage was related to the sliding of the tongue against the palate (Chung et al., 2013). In addition, saliva plays a key role during oral processing. It contains a variety of substances including salts, mucin, and digestive enzymes such as α-amylase. Saliva has multifunctional roles in the oral cavity since it participates in food mixing, formation of a cohesive and lubricated bolus for a safe swallow, as well as in food disintegration and digestion. These processes may impact on the structural and rheological properties of the food in the mouth and are crucial for oral processing and sensory perception (Mosca & Chen, 2017).

Fig. 2 shows the changes in the maximum positive force, maximum negative force, and η during the compression/decompression cycles, both without and with saliva. In general, it was not observed a reduction in the values of the measured parameters as the number of cycles increased. Only CSM and MS samples presented a slight reduction in the maximum positive force and maximum negative force, respectively, from the first to the second cycle. The relatively constant values during the combined squeezing flow and shear force assay would be expected in pureed foods, in which the structure was broken during the grinding process rather than during the oral processing. In the absence of saliva, the consistency, adhesiveness, and η of the MS and CSM samples were significantly higher ($p < 0.05$) than the control, but no differences were found between the thickened samples (Fig. 2 a, c). Starch granules and fibres could form swollen hydrated networks that act as fillers in the food matrix and strengthen the network properties (Fabek, Messerschmidt, Brulport, & Goff, 2014; Morell, Hernando, Llorca, & Fiszman, 2015). In presence of saliva, a decrease in all the measured parameters for the control and thickened purees was observed, mainly due to the dilution effect (Fig. 2 b, d). But conversely to samples without saliva, MS puree presented similar values to the control that could be attributed to the hydrolysis of starch by the action of the α-amylase enzyme. CSM sample maintained the highest consistency, adhesiveness, and η when saliva was added. Viscous samples would be more miscible with saliva and result in a homogenous bolus (Talens et al., 2021), thus puree thickened with CSM could be more easily swallowed for dysphagic patients.

3.3. Sensory evaluation

A sensory evaluation was conducted to determine the acceptance of thickened purees prepared with MS and CSM as thickeners. Average scores of the different sensory attributes evaluated are shown in Fig. 3. Non-significant differences ($p > 0.05$) were observed in the acceptability of the different purees regarding colour, flavour, and general

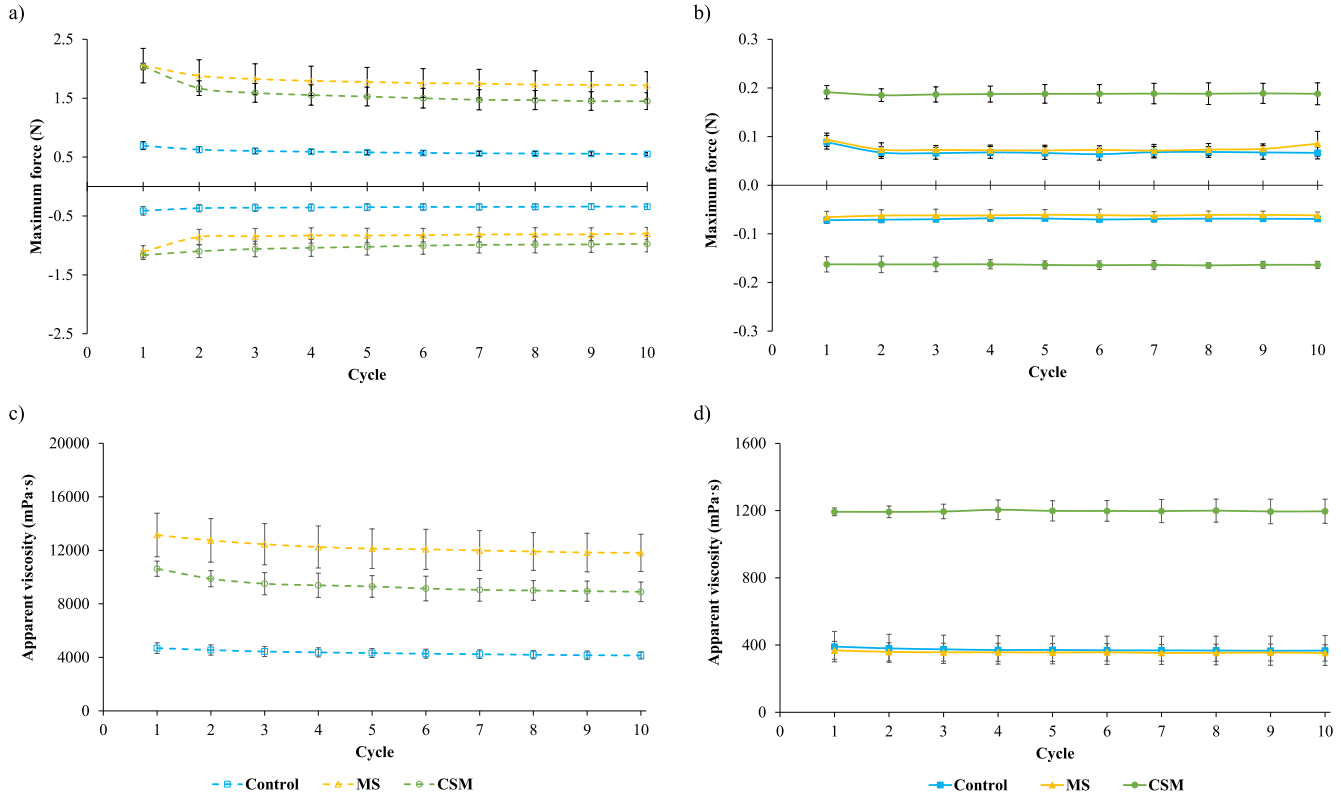

Fig. 2. Combined squeezing flow and shear force test of the control and thickened purees representing maximum force *vs* cycle in the a) absence and b) presence of saliva, and apparent viscosity (η) at 10 s^{-1} (mPa·s) *vs* cycle in the c) absence and d) presence of saliva. (MS: Modified starch and CSM: chia seed mucilage).

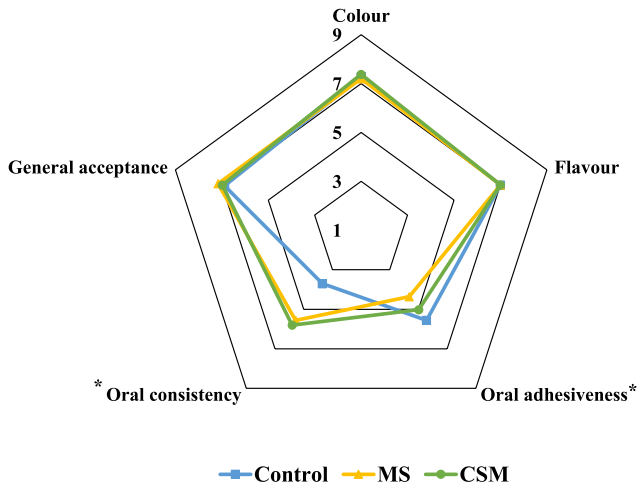

Fig. 3. Average score of the sensory attributes tested in control and thickened purees. Asterisk (*) denotes significant differences among purees ($p < 0.05$) (n = 100). (MS: Modified starch and CSM: chia seed mucilage).

acceptance. The addition of thickeners to achieve a pudding-thick level led to the highest scores for the oral consistency, but the MS sample was the least accepted in terms of oral adhesiveness.

3.4. Sensory and instrumental correlations

The relation between the sensory data and instrumental measurements could aid in developing thickened foods for dysphagic people. Table 3 shows the Pearson's correlations of the sensory attributes and instrumental measurements. A positive correlation was achieved between the sensory data of oral consistency and the instrumental measurements of area and K, which were obtained from the texture and flow rheological analysis, respectively. The CSM samples, with higher area and K values, would be more consistent at the beginning of the oral procedure and it is highly relevant when developing foods for people with swallowing problems. Moreover, the oral consistency attribute correlated positively with the instrumental measurement of η at 10 s^{-1} and 50 s^{-1}, meanwhile, the oral adhesiveness correlated negatively, indicating that high viscous purees would impart a lower adhesiveness and would be more easily swallowed by dysphagics. Regarding the viscoelastic analysis, the oral consistency attribute correlated positively with G'_{LVR} and G* values, indicating that samples with great stiffness and rigidity would need more energy to deform, particularly, at the beginning of the oral processing. It is important to highlight that Herranz et al. (2021) pointed out that foods with great resistance to deformation (high G* values) and great elasticity (low Tan δ values), like CSM, would be safe-swallowing.

3.5. Flow rheological characterisation of digested samples

Thickeners used in puree, such as MS and CSM that are mainly composed of soluble fibres and proteins, can modify the chyme viscosity and gastric emptying. During GID, the increased volume produced by adding digestive fluids, as well as the simulated conditions of the digestive tract due to enzymes action and changes in pH and ionic strength, would affect the behaviour and conformation of the hydrocolloid and the interaction between molecules, and thus the rheological properties of the samples (Chen et al., 2020). Changes in the rheological characteristics of the different puree samples during *in vitro* GID were evaluated. MS and CSM purees did not require a minimum shear stress to initiate samples' flow due to the dilution effect resulting from the digestion process. All the samples showed a non-Newtonian shear-tinning flow behaviour that can be described by the power-law model. The obtained parameters including the n, K, and R^2, as well as the η calculated at 10 s^{-1} are summarised in Table 4. The η of all the samples decreased as the *in vitro* GID advanced, which could be caused by both sample dilutions produced by the addition of digestive fluids and the action of digestive enzymes. To differentiate between both effects, the η values obtained for the digested samples were compared with the diluted samples (DE). Main differences in viscosity values were found for MS sample after the oral and gastric phases, as well as in CSM after the gastric phase, evidencing an additive effect of gastrointestinal conditions (enzymes, bile salts, and pH) to the reduction by dilution. As shown in Table 4, CSM presented higher η and K values than MS and control after the oral and gastric phases in both digested and diluted samples. These results indicate that CSM would be the sample less affected by the conditions of simulated digestion during the oral and gastric phases. This behaviour can be due to the crosslink formed through the fibre, water, and proteins to form a relatively compact network structure that in turn would determine the rheological properties of CSM sample (García-Salcedo, Torres-Vargas, del Real, Contreras-Jiménez, & Rodriguez-Garcia, 2018). Changes in physical characteristics and viscosity of soluble fibres during digestion would be determined by the fibre type, concentration, and their physicochemical properties, as well as by the digestion stage including dilution effect, enzymes, and changes in pH and ionic strength (Fabek et al., 2014; Vera, Laguna, Zura, Puente, & Muñoz, 2019). In fact, the capacity of fibres to retain or increase viscosity during GID would impart beneficial effects such as slowing digestion of nutrients, reducing serum cholesterol and blood glucose levels, and increasing satiety (Kendall, Esfahani, & Jenkins, 2010). Nevertheless, non-significant differences ($p > 0.05$) among samples were observed for η and K values neither in digested samples nor diluted samples after the intestinal phase, showing that the dilution effect was the most important factor in reducing the viscosity and consistency of all the samples at the end of the GID.

Table 3
Pearson's correlation coefficients of sensory attributes and instrumental measurements.

Instrumental parameters	*Sensory attributes*				
	Colour	**Flavour**	**Oral consistency**	**Oral adhesiveness**	**General acceptance**
Area (N·mm)	0.2568	−0.1135	**0.9927**	−0.7104	0.6325
ηat 10 s^{-1} (mPa·s)	−0.0076	0.1533	**0.9844**	**−0.8728**	0.8164
ηat 50 s^{-1} (mPa·s)	0.0756	0.0706	**0.9952**	**−0.8290**	0.7653
K (Pa·s^n)	0.1652	−0.0196	**0.9997**	−0.7753	0.7043
G'_{LVR} (Pa)	0.3771	−0.2456	**0.9253**	−0.5736	0.3546
G* (Pa)	0.4264	−0.2939	**0.9361**	−0.5515	0.4634
Maximum positive force (N)	**0.8578**	−0.7797	0.6099	0.0134	−0.1167
Maximum negative force (N)	**−0.9032**	0.8373	−0.5315	−0.1116	0.2140

Values in bold type correspond to $p < 0.05$.
η: apparent viscosity; K: consistency coefficient; G'_{LVR}, elastic modulus value at LVR; and G*: complex modulus at 1 Hz.
The values of the maximum positive and negative forces referred to the first cycle of the combined squeezing flow and shear force test with saliva.

Table 4
Flow rheological behaviour of the puree samples during *in vitro* gastrointestinal digestion (digested samples) and considering the dilution effect at each stage (diluted samples).

Digestion	Parameter	Digested samples			Diluted samples		
		Control	MS	CSM	Control	MS	CSM
Oral	**η (mPa·s)**	270 ± 16^{Ab}	244 ± 17^{Ab}	690 ± 102^{Aa}	288 ± 9^{Ac}	518 ± 17^{Ab}	895 ± 2^{Aa}
	n	0.34 ± 0.06^{Aa}	0.33 ± 0.02^{Aa}	0.28 ± 0.02^{Aa}	0.35 ± 0.01^{Aa}	0.33 ± 0.01^{Aa}	0.33 ± 0.01^{Aa}
	K (Pa·s^n)	1.25 ± 0.24^{Ab}	1.14 ± 0.02^{Ab}	3.65 ± 0.71^{Aa}	1.29 ± 0.05^{Ac}	2.40 ± 0.11^{Ab}	4.19 ± 0.13^{Aa}
	R^2	0.97	0.96	0.97	0.90	0.98	0.98
Gastric	**η (mPa·s)**	24 ± 5^{Bb}	19 ± 5^{Bb}	57 ± 5^{Ba}	27 ± 4^{Bc}	41 ± 4^{Bb}	87 ± 4^{Ba}
	n	0.36 ± 0.05^{Aa}	0.40 ± 0.07^{Aa}	0.36 ± 0.02^{Aa}	0.45 ± 0.06^{Aa}	0.41 ± 0.05^{Aa}	0.37 ± 0.03^{Aa}
	K (Pa·s^n)	0.10 ± 0.03^{Bb}	0.08 ± 0.03^{Bb}	0.25 ± 0.03^{Ba}	0.10 ± 0.03^{Bb}	0.16 ± 0.03^{Bb}	0.37 ± 0.05^{Ba}
	R^2	0.97	0.97	0.98	0.99	0.97	0.98
Intestinal	**η (mPa·s)**	5 ± 4^{Ba}	7 ± 5^{Ba}	13 ± 5^{Ba}	4 ± 4^{Ca}	4 ± 4^{Ca}	15 ± 6^{Ca}
	n	0.67 ± 0.34^{Aa}	0.60 ± 0.26^{Aa}	0.49 ± 0.12^{Aa}	0.80 ± 0.57^{Aa}	0.82 ± 0.59^{Aa}	0.34 ± 0.05^{Aa}
	K (Pa·s^n)	0.02 ± 0.02^{Ba}	0.02 ± 0.02^{Ba}	0.04 ± 0.03^{Ba}	0.01 ± 0.02^{Ba}	0.01 ± 0.02^{Ba}	0.07 ± 0.03^{Ca}
	R^2	0.96	0.97	0.96	0.96	0.96	0.88

Control: puree without hydrocolloid; MS: puree thickened with modified starch; CSM: puree thickened with chia seed mucilage.
Rheological parameters represent: η, apparent viscosity at 10 s^{-1}; n, flow behaviour index; K, consistency coefficient; R^2, correlation coefficient.
Values are the average of two independent experiments. Different superscript uppercase letters indicate significant differences ($p < 0.05$) among digestion phases within the same sample, whereas lowercase letters indicate significant differences ($p < 0.05$) among digested samples or diluted samples within the same digestion phase.

3.6. Digestibility of the samples

The addition of thickening agents has a great impact on food matrix and therefore on the structure of the chyme, which could modify or limit the action of digestive enzymes. As a result, the digestive process and rate of nutrient release can be affected (Turgeon & Rioux, 2011). In this regard, the digestibility of proteins and starch of the puree samples was evaluated throughout *in vitro* GID. During the oral phase, α-amylase enzyme initiates the hydrolysis of starch, but also food-saliva interactions can lead to the formation of new compounds, complexes, and microstructures. Even soft foods such as purees, which require minimal chewing and remain a short time in the mouth before swallowing, can undergo physical and biochemical changes during oral processing that determine further digestion (Mao & Miao, 2015; Mosca & Chen, 2017). Proteolysis starts in the stomach with the action of pepsin enzyme and it is completed in the intestinal phase by trypsin and chymotrypsin enzymes, releasing peptides of different sizes and free amino acids. In the small intestine, the hydrolysis of starch is also continued by the action of pancreatic amylase (Gropper & Smith, 2013).

The proteolysis profile of the puree samples was evaluated, before digestion and after the different digestion phases, by determining the contents of total soluble proteins, TCA-soluble peptides, and free amino groups (Table 5). Note that the fraction soluble in 5% TCA would contain small peptides (<10 residues) and amino acids (Chen, Shih, Chiou, & Yu, 2010). Before digestion, puree samples did not present significant differences ($p > 0.05$) in the content of soluble proteins. However, CSM showed the highest value of TCA-soluble peptides (1.94 mg/g), which might be derived from the proteins contained in the hydrocolloid. As the GID advanced, an increased solubility of proteins and a gradual release of peptides and amino acids was generally observed. It is noted that after the oral stage, where proteolysis is not expected to occur, the content of soluble proteins greatly decreased probably due to aggregation or complexation of proteins by interactions with the α-amylase (Crosara, Zuanazzi, Moffa, Xiao, Machado, & Siqueira, 2018). After the gastric phase, non-significant differences ($p > 0.05$) in the contents of soluble proteins and free amino groups were found among puree samples, whereas the content of TCA-soluble peptides was slightly higher in CSM. After the intestinal phase, MS presented the highest content of TCA-soluble peptides (3.21 mg/g compared to 2.7 mg/g in control and CSM), representing an increase by 2 times between the undigested and totally digested MS sample. A similar increment was observed for the control sample, whereas the content of soluble peptides for CSM sample increased <1.5 times when compared before and after GID. As previously commented, the dense and compact structure of CSM could hinder the access to digestive juices and enzymes, affecting protein digestibility (Lazaro, Puente, Zúñiga, & Muñoz, 2018). Nevertheless, the content of free amino groups obtained after the gastric and

Table 5
Changes in soluble proteins, TCA-soluble peptides, free amino groups, and reducing sugars in the different puree samples before and during *in vitro* gastrointestinal digestion.

Parameter	Digestion	Sample		
		Control	MS	CSM
Soluble proteins (mg/g)	Before	0.67 ± 0.10^{Ba}	0.69 ± 0.02^{Ba}	0.60 ± 0.00^{Ba}
	Oral	0.00 ± 0.00^{Ca}	0.02 ± 0.01^{Ca}	0.01 ± 0.08^{Ca}
	Gastric	0.72 ± 0.02^{Ba}	0.74 ± 0.04^{Ba}	0.68 ± 0.06^{Ba}
	Intestinal	2.46 ± 0.07^{Aa}	2.81 ± 0.27^{Aa}	2.59 ± 0.12^{Aa}
TCA-soluble peptides (mg/g)	Before	1.25 ± 0.14^{Dc}	1.59 ± 0.03^{Cb}	1.94 ± 0.01^{Ca}
	Oral	1.65 ± 0.03^{Cb}	1.63 ± 0.04^{Cb}	1.84 ± 0.06^{Da}
	Gastric	2.25 ± 0.04^{Ba}	2.07 ± 0.06^{Bb}	2.32 ± 0.02^{Ba}
	Intestinal	2.69 ± 0.10^{Ab}	3.21 ± 0.06^{Aa}	2.75 ± 0.01^{Ab}
Free amino groups (mg/g)	Before	1.12 ± 0.01^{Ca}	0.95 ± 0.01^{Cb}	1.06 ± 0.05^{Ca}
	Oral	1.12 ± 0.02^{Cb}	1.21 ± 0.03^{Cab}	1.26 ± 0.05^{Ca}
	Gastric	2.37 ± 0.06^{Ba}	2.52 ± 0.37^{Ba}	2.47 ± 0.00^{Ba}
	Intestinal	4.41 ± 0.12^{Aa}	4.35 ± 0.17^{Aa}	4.38 ± 0.26^{Aa}
Reducing sugars (mg/g)	Before	3.46 ± 0.25^{Dc}	4.91 ± 0.04^{Db}	5.48 ± 0.13^{Ca}
	Oral	16.48 ± 0.44^{Cc}	20.80 ± 0.25^{Ca}	17.68 ± 0.01^{Bb}
	Gastric	20.10 ± 0.25^{Bab}	23.54 ± 0.67^{Ba}	18.76 ± 1.89^{Bb}
	Intestinal	45.60 ± 0.08^{Ab}	58.67 ± 0.08^{Aa}	48.69 ± 1.76^{Ab}

*Control: puree without hydrocolloid; MS: puree thickened with modified starch; CSM: puree thickened with chia seed mucilage.
Values are the average of two independent experiments. Different superscript uppercase letters indicate significant differences ($p < 0.05$) among digestion phases within the same sample, whereas small letters indicate significant differences ($p < 0.05$) among samples within the same digestion phase.

intestinal phases did not present significant differences ($p > 0.05$) among samples, showing values close to 4.4 mg/g at the end of the GID. This fact suggests that the addition of hydrocolloids did not compromise the digestibility of the puree proteins.

The structure and ensuing viscosity of the hydrocolloids could also influence the hydrolysis of starch during GID, which was determined and expressed as the content of reducing sugars (Table 5). Before digestion, CSM sample showed the highest value of reducing sugars; however, MS showed the highest degree of starch hydrolysis after the oral and intestinal phases, which could be expected since MS is composed of modified starch that would be hydrolysed by amylases. Comparing the values between the undigested and totally digested samples, the control presented higher digestion rate (by 13 times) than MS and CSM samples, which presented increments about 12 and 8.8 times, respectively. Thus, the interaction of the hydrocolloids with the food matrix could slow carbohydrate digestion and affect glucose absorption.

4. Conclusions

MS and CSM showed similar technological properties when adding to chicken and vegetables purees to design dysphagia-oriented products, but the use of MS presented some limitations related to sensory adhesiveness and decreasing viscosity during the oral processing. However, CSM maintained the highest oral consistency and viscosity, so it could be more easily swallowed by dysphagic patients, and its flow behaviour was less affected at the beginning of the simulated digestion. Puree protein digestibility was not compromised by the addition of hydrocolloids, whereas the digestion rate of starch in CSM was lower than that of MS. The study of the interactions between hydrocolloids and food matrix, which determine the technological and sensory properties as well as the nutrient release during GID, can help to design products with specific characteristics to meet the necessities of specific populations. The results of the present work contribute to develop thickened foods with appropriate features for people with swallowing problems by employing alternative thickeners such as CSM. However, more works regarding the lubrication characteristics of products oriented to dysphagic people are needed to elucidate their behaviour during the oral processing.

Ethics Statement

The research did not include biological agents of risk, or clinical trials with humans or animal experiments.

CRediT authorship contribution statement

Susana Ribes: Conceptualisation, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualisation, Supervision. **Marta Gallego:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualisation, Supervision. **Jose M. Barat:** Writing – review & editing, Funding acquisition. **Raúl Grau:** Writing – review & editing, Project administration, Funding acquisition. **Pau Talens:** Conceptualisation, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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