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Additional Information

Interpretive summary

Consumers demand for healthy and nutritious foods has force the scientific and industrial field to search for novel dairy products with lower fat content and free of chemical additives. However, reducing the fat content of yoghurts can lead to undesirable changes in their technological and sensory features. Plant-based mucilage can be used as natural fat substitute in fermented skimmed milk products, mimicking the technological properties of full-fat yoghurts. The current work opens doors to the use of chia seed mucilage as a fat replacer in the development of novel fat-free yoghurts with great technological properties and nutritional benefits for consumers.

Chia mucilage as fat replacer in yoghurts

Chia (*Salvia hispanica* L.) seed mucilage as a fat replacer in yoghurts: impact on their nutritional, technological and sensory properties

Susana Ribes, [1*]Nataly Peña,[1] Ana Fuentes,[1] Pau Talens,[1] Jose M. Barat[1]

[1]Departamento Tecnología de Alimentos, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

***Corresponding author:** *Departamento de Tecnología de Alimentos, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain. Tel.: +34 963877000; Ext.: 83612.*

E-mail address: surillo@upv.es

Highlights

- The use of chia seed mucilage as a fat substitute in skimmed yoghurts was studied
- The chia mucilage decreased syneresis degree of full-fat and skimmed yoghurts
- Fortification of yoghurts with the chia seed mucilage improved their nutritional value
- Enriched yoghurts showed equal or greater technological properties than controls
- The chia seed mucilage can be used as a fat replacer in non-fat yoghurts

ABSTRACT

Nowadays, the growing consumer demand for healthy and nutritious products has driven scientist and food manufacturers to design novel dairy products with higher fibre levels, lower fat content and free of chemical additives. Chia seed mucilage (CSM) is a healthy natural gel extensively employed as a dietary soluble fibre source, bulking agent and fat replacer in a huge variety of foods. In this study, the effect of CSM on the nutritional, technological and sensory properties of skimmed yoghurts was evaluated. The addition of 7.5 % CSM to yoghurt formula lowered samples' degree of syneresis during storage times compared to full-fat yoghurts. The nutritive value of enriched yoghurts improved due to higher levels of dietary fibre compared to full-fat and skimmed yoghurts. Moreover, rheological measurements revealed greater consistency, firmness and viscosity, as well as the formation of a highly structured network and better resistance to stress in those yoghurts containing 7.5 % CSM. The sensory acceptance of the yoghurts enriched with 7.5 % CSM was similar to the reference samples in acidity, creaminess and viscosity terms. These results confirm the feasibility of using CSM as a fat replacer to design novel skimmed yoghurts.

Keywords: chia seed mucilage; non-fat yoghurts; rheology; sensory analysis

INTRODUCTION

Yoghurt is an extensively consumed daily product obtained by the fermentation of milk by using a combination of *Lactobacillus* spp. and *Streptococcus* spp. bacteria. In recent years, the demand for low-fat yoghurts has risen as fat is associated with a high risk of obesity, arteriosclerosis, coronary heart illness, high blood pressure and some cancer types (Kaminarides et al., 2007). Nevertheless, reducing the fat content of yoghurts can lead to unpleasant changes in their physical and sensory properties (Zhao et al., 2018). To overcome these problems, hydrocolloids are widely employed in the dairy food industry as fat replacers thanks to their gelling and stabilising characteristics, and because they improve different yoghurt properties, such as texture, mouth feel, appearance, viscosity and consistency (Sanchez et al., 2000).

Chia (*Salvia hispanica* L.) is an herbaceous plant from southern Mexico and northern Guatemala (Capitani et al., 2012). Its seeds produce a transparent mucilaginous gel in contact with water, composed essentially of soluble fibre. This gel possesses an excellent water-holding capacity and good viscosities, even at low concentrations (Segura-Campos et al., 2014). These nutritional and technological properties make chia seed mucilage (**CSM**) an excellent candidate for food applications, such as thickener, gel former, fat replacer, and chelator (Capitani et al., 2012). Recently, CSM and other plant-based gels have been employed to fortify yoghurts by acting as fat substitutes given their ability to improve the rheological properties and stability of dairy products, while reducing calories (Darwish et al., 2018; Kim et al., 2020). However, it is important to highlight that yoghurts enriched with CSM present a fat content between 1 % and 3 % (Darwish et al., 2018), which are considered low-fat yoghurts because they contain no more than 3 g of fat per 100 g for solids, or 1.5 g of fat per 100 mL for liquids (Regulation (EC) 1924/2006, 20 December 2006). As far as we know, CSM has not been employed in fermented

skimmed milk products as a fat substitute by using full-fat yoghurts as reference products to achieve equal or better technological qualities.

Hence, this work aimed to evaluate the possibility of adding CSM as a fat replacer to skimmed yoghurts, and to investigate the effect of its incorporation on the nutritional, technological and organoleptic properties of skimmed yoghurts during their storage times.

MATERIALS AND METHODS

Yoghurt ingredients, media and chemicals

In the yoghurt formulation, full-fat and skimmed milk powder (Central Lechera Asturiana, Siero, Spain), and commercial chia seeds (Pedon S.P.A, Molvena, Italy) were purchased from a local Spanish market. Lyophilised *Streptococcus salivarius* subsp. *thermophilus* (CECT 801) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (CECT 4005), obtained from Colección Española de Cultivos Tipo (CECT, University of Valencia, Burjassot, Spain), were employed to prepare starter cultures.

For culture media, M17 and MRS (broth and agar) and D-Lactose monohydrate were employed, all of which were supplied by Scharlab (Barcelona, Spain). All the other chemicals were purchased from Sigma-Aldrich (Madrid, Spain).

Preparation of the starter cultures

Starter cultures were prepared by adding 1 mL of M17 broth, enriched with D-Lactose monohydrate (1 %, wt./v; **M17-lactose**), to the phial containing the lyophilised *S. salivarius* subsp. *thermophiles,* and 1 mL of MRS broth to the freeze-dried *L. delbrueckii* subsp. *bulgaricus* phial. They were mixed to obtain homogenous suspensions, which were transferred to test tubes containing 10 mL of M17-lactose for *S. salivarius* subsp. *thermophiles*, and 10 mL of MRS broth for *L. delbrueckii* subsp. *bulgaricus*. The M17-lactose tube was incubated aerobically at 37 ºC for 24 h, and the MRS tube was incubated anaerobically at 37 ºC for 72 h

using anaerobic jars. Test tubes were centrifuged (4,000 rpm for 5 min at 20 ºC), supernatants were discarded and 10 mL of each broth were added to the pellet. Serial dilutions were done and colonies were enumerated by plating 1 mL in M17-lactose agar and 1 mL in MRS agar. Finally, plates were incubated aerobically at 37 ºC for 24 h (*S. salivarius* subsp. *thermophiles*) and anaerobically at 37 ºC for 72 h (*L. delbrueckii* subsp. *bulgaricus*) using anaerobic jars.

Mucilage extraction from chia seeds

To extract CSM, whole chia seeds were submerged in distilled water (water:seed ratio of 30:3), and the mixture was gently stirred at 500 rpm for 3 h at 80 ºC. After cooling to 20 ºC, it was centrifuged at 10,000 rpm for 15 min at 20 ºC (Centrifuge 5804 R, Eppendorf AG, Germany). The obtained CSM was freeze-dried (LyoQuest-55, Telstar, España) for 48 h before being stored in sterile containers at room temperature prior to use.

Yoghurt manufacture

Five yoghurt types were prepared in this study: i) one full-fat yoghurt (**FFY**); ii) one skimmed yoghurt (**SY**); iii) three formulations of SY enriched with different CSM concentrations (2.5 %, 5.0 % and 7.5 %, wt./v).

Milk was prepared by adding 100 g of commercial full-fat or skimmed milk powder to 1 L of distilled water. The mixture was heated in a bath (JP Selecta S.A., Abrera, Spain) at 85 °C for 30 min. Afterward, milk was cooled to 45 ºC and CSM was added to the mixture at the previously indicated concentrations. No CSM was added to the FFY and SY samples. Each yoghurt culture was added at a concentration of 10^7 Colony Forming Units (**CFU**)/mL. Next 125 mL of the final mixture were poured into sterilised glass containers, which were placed in an incubator at 40 ºC and fermented until pH values of 4.5-4.6 were achieved. The glass containers were sterilised at 121 ºC for 15 minutes by using an autoclave. Finally, yoghurts

were stored at 4 ºC until analyses of 1, 7, 14 and 21 days. Each formulation was manufactured twice.

Physico-chemical characterisation of yoghurts

A Crison Basic 20+ pH meter (Crison S.A. Barcelona, Spain) was used to measure the pH of yoghurts. For the total titratable acidity (**TTA**) determinations, 10 g of each sample were mixed with 40 mL of distilled water and titrated with a 0.1 N NaOH solution until pH 8.30±0.01 (Vital et al., 2015). Titratable acidity was expressed as g lactic acid/100 g of yoghurt. Syneresis was determined by centrifuging 5 mL of yoghurts at 5,000 rpm for 20 min at 4 ºC (Centrifuge 5804 R, Eppendorf AG, Germany). The results were indicated as the volume of separated whey per 100 g of yoghurt.

The colour parameters (L*, a*, and b*) of yoghurts were measured by a spectrocolorimeter (CM-3600d, Minolta Co., Tokyo, Japan) with an observer 10º and illuminant D65. Whiteness index (**WI**) and colour variations (**ΔE***) were respectively calculated by Equation (1) and Equation (2):

$$WI = 100 - ((100 - L^*)^2 + a^{*2} + b^{*2})^{0.5} \quad (1)$$

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

Nutritional composition of yoghurts

The nutritional value of yoghurts (moisture, protein, fat, ash and total dietary fibre) was determined following AOAC procedures (AOAC, 2016). Protein, fat, fibre and ash contents were analysed by employing lyophilised yoghurt samples. Finally, carbohydrates were estimated by difference.

All the tests were run in triplicate.

Rheological and viscoelastic measurements

The rheological and viscoelastic measurements were taken by a stress controlled rheometer RS1 (ThermoHaake, Karslruhe, Germany). Assays were performed at 4 °C using a C60/2°Ti cone-plate geometry with a 2 mm gap. Samples were placed in the measuring system and allowed to stand for 300 s for structure recovery and temperature equilibration before being tested.

All the rheological measurements were taken in triplicate.

Steady shear rheological tests

The steady shear rheological tests were performed within the 0.1–200 s^{-1} range for 120 s. To rule out any possible dependence on flow time, a 4-step operation (two upward and two downward curves) was applied to samples and flow curve was fitted to the Hershel-Bulkley model (Sah et al., 2016). Finally, the yield stress ($\boldsymbol{\tau_0}$), consistency (**K**) and flow behaviour indices (**n**) were calculated, as was apparent viscosity ($\boldsymbol{\eta_{app50}}$) (Equation 3) (Morell et al., 2015).

$$\eta_{app50} = (K \times 50^{n-1}) \quad (3)$$

Dynamic rheological tests

To determine the linear viscoelastic region (**LVR**), stress sweeps were performed within a stress range from 0.01 to 10 Pa at 1 Hz. Frequency sweep tests were conducted at 1 Pa (in the LVR) to cover a 0.1-10 Hz frequency range at 4 °C.

The viscoelastic parameters, namely elastic or storage modulus ($\boldsymbol{G'}$), viscous or loss modulus ($\boldsymbol{G''}$), complex modulus ($|\boldsymbol{G^*}|$), loss tangent ($\boldsymbol{tan\ \delta}$) and complex viscosity ($|\boldsymbol{\eta^*}|$), were obtained from the rheometer software (RheoWin 3 Data Manager).

Creep and recovery tests

Creep and recovery tests were carried out by applying constant stress (1 Pa within the LVR), which was maintained for 300 s. Afterwards, stress was stopped and samples were released for recovery for 300 s.

The Burger model parameters (G_0= instantaneous elastic modulus of the Maxwell unit; η_0 residual viscosity) were taken from the rheometer software (RheoWin 3 Data Manager). The system's final percentage of recovery (**R**) was calculated by employing Equation (4):

$$R\ (\%) = (J_{max} - J_{\infty} / J_{max})\ x\ 100 \quad (4)$$

where J_{max} is the maximum deformation corresponding to the compliance value for the longest time (300 s) in the creep rest; J_{∞} is the residual deformation (Kurt et al., 2016).

Sensory evaluation

The sensory evaluation of yoghurts was made by a semi-trained panel. The participants were between 24 and 50 years old and consisted of 20 women and 10 men. Panel selection was emphasised on interest in the sensory evaluation of yoghurts, availability, non-smoker and non-allergic, following the general guidelines UNE-ISO 8586:2012. Training sessions were performed to introduce the panellists to the sensory analysis and to identify and score the quality attributes that defines the samples. A structured 9-point hedonic scale (1: very unpleasant; 9: very pleasant) (UNE-ISO 4121:2003) was used to evaluate the appearance, colour, viscosity, granularity, creaminess, acidity and global acceptance attributes. The panellists tested three different samples: i) FFY (prepared with whole milk powder); ii) SY (manufactured with skimmed milk powder); iii) SY + 7.5 % CSM (produced with skimmed milk powder enriched with 7.5% CSM). The evaluated samples were selected according to the data observed in the nutritional and rheological tests. The sensory analysis was carried out 24 h after preparing yoghurts, which were stored at 4 ºC. Samples were presented to the panellists in

a plastic cup, coded with three random digits. Sensory evaluations were made by considering the IFST Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods (IFST, 2015). Moreover, every panellist gave written consent before conducting the sensory analysis.

Statistical analysis

The results obtained from the physico-chemical characterisation, the nutritional composition, and the rheological assays of yoghurts were analysed by a multifactor analysis of variance (multifactor ANOVA) to evaluate differences among the samples and storage time. The results of the influence of using CSM on the sensory characteristics of yoghurts were analysed by a one-way ANOVA. The least significance procedure (LSD) was employed to test for any differences between averages at a 5 % level of significance. Data were statistically processed by Statgraphics Centurion XVI.

RESULTS AND DISCUSSION

Physico-chemical characterisation of yoghurts

The changes in pH, TTA and syneresis of the different manufactured yoghurts are shown in Figure 1. The pH values of all the samples progressively lowered while stored at 4 ºC, with a significant ($p<0.05$) decrease throughout the storage period. The differences observed among samples were significant ($p<0.05$) after 14 storage days.

A slight rise in TTA was perceived over the storage time as a result of greater lactic acid production, which could be provoked by constant fermentation and lactic bacteria stimulated growth. This leads to greater casein micelle matrix contraction, which means more whey is released (Fox et al., 2000). For instance, the initial lactic acid concentration in the stored yoghurts ranged from 0.617±0.002 g/100 g to 0.767±0.023 g/100 g, whereas this concentration ranged from 0.672±0.023 g/100 g to 0.815±0.009 g/100 at the end of the study (Figure 1B).

Furthermore, slightly significant differences ($p<0.05$) were observed in sample types. The TTA values of the skimmed yoghurts, with or without fortifying them with the extracted mucilage, were higher than those exhibited by the FFY sample. In line with this, Yazici and Akgun (2004) reported that higher TTA values could be attributed to the moisture content of low-fat and non-fat yoghurts.

Regarding samples' degree of syneresis, more whey was separated throughout the storage time for FFY and SY samples, which can be explained by increased acidity during storage, which would bring about casein micelle matrix contraction and, subsequently, released whey (Canbulat and Ozcan, 2015). Conversely, the sample prepared with the highest CSM concentration (7.5 %) had a lower degree of syneresis during the study, and this depletion came close to 2 % after 21 analysis days. The latter finding can be explained by interactions between casein micelles (positively-charged) and CSM (negatively-charged) which, in turn, enhance matrix stabilisation and reduce separated whey (Figure 1C).

Regarding the colour parameters, the L* values ranged from 86.9±0.5 to 89.8±0.1, and the WI values were between 83.4±1.2 and 86.4±0.6. Moreover, the ΔE* values ranged from 0.5±0.1 to 1.8±0.5 throughout the evaluation study (Figure 2). These results suggest that the ΔE* of yoghurts stored at 4 ºC for 21 days did not exceed the just noticeable difference (Baldevbhai and Anand, 2012).

Nutritional composition of yoghurts

Figure 3 presents the results of the nutritional composition of yoghurts. As expected, storage time did not significantly affect ($p>0.05$) moisture, protein, fat and ash contents, which remained practically constant over the study period for each sample type. Significant differences ($p<0.05$) in protein content were noted among formulations, being SY the product with the least protein. In addition, the yoghurts prepared with CSM exhibited slightly higher

protein values than SY (Figure 3B), may be due to the protein content of CSM, as indicated by Felisberto et al. (2015) while characterising lyophilised CSM.

FFY had a fat content of 3.9±0.2 g/100 g throughout storage. The fat content of the modified yoghurts increased with the CSM content (Figure 3C). This can be explained through data stated by several authors on proximate lyophilised CSM composition (Capitani et al., 2013; Felisberto et al., 2015). A similar trend was noted for ash content. The yoghurts made with CSM presented a slightly higher ash content than the control and SY samples (Figure 3D).

Figure 3E shows that the higher the mucilage concentration, the larger the fibre content. This fact can be accounted for the high dietary fibre levels present in the extracted mucilage (Felisberto et al., 2015), which allowed the nutritional characteristics of the novel yoghurts to improve. Storage time did not significantly affect the fibre content of yoghurts.

Rheological measurements

Steady shear rheological tests

Table 1 shows the results of the rheological parameters from the steady shear test of yoghurts. Yield stress (τ_0) is a typical characteristic of these products and is well correlated with their early firmness during sensory tests (Costa et al., 2019; Harte et al., 2007). As observed throughout the evaluation period, the formulations with higher yield stress (τ_0) were FFY and the yoghurt enriched with 7.5 % CSM. This outcome could be associated with the presence of an interactive or cross-linked structure (Yu et al., 2016), which was more pronounced in those samples with the highest amount of the CSM. A slightly reduction in this parameter was perceived during the storage time, which could be related to the preservation of the cross-linked structure with time.

The consistency index (K) of yoghurts is linked with different intermolecular interactions and attraction forces between proteins (Costa et al., 2019). Higher K values were observed for

the reference sample (FFY) during the study. Moreover, marked concentration dependency was noticed among the products containing the extracted mucilage. Addition of CSM to yoghurts significantly ($p<0.05$) increased the K values, being higher when employing larger amounts of the obtained mucilage. This could be explained by the capacity of swollen CSM particles to act as fillers, which reduces the protein matrix mobility and increases enriched yoghurts' consistency (Morell et al., 2015). Furthermore, significant differences ($p<0.05$) were perceived among samples during storage. As seen in Table 1, higher K values were detected in the samples stored for 21 days at 4 ºC. Similarly, Cardines et al. (2018) observed higher K values for all their samples throughout storage due to whey spontaneously migrating to the surface of the product.

The flow behaviour index (n) indicates the rheological nature of the fluid. As the fluid approaches Newtonian behaviour, the n value approaches one (Kaur and Kaler, 2008). The n values went below 1 for all the tested yoghurts, which indicates shear-thinning behaviour once yield stress was overtaken. Generally, n values lowered when using higher CSM concentrations. Similar results have been described by Paseephol et al. (2008) while studying the addition of oligofructose and medium-chain inulin to yoghurts. Conversely, a rise in those values was observed over storage time for each sample, which falls in line with the results stated by Paseephol et al. (2008).

A marked increase in the manufactured samples' viscosity (η_{app50}) was perceived in the SY products containing more CSM (Table 1). As the mucilage concentration rose, viscosity increased owing to the interactions between polysaccharides and diary proteins (Sodini et al., 2004). It is worth mentioning that by the end of storage, higher η_{app50} values were observed in the samples formulated with 7.5% CSM compared to FFY (Table 1).

Lastly, noticeable differences were found as a result of storage. The longer storage was, the lower the manufactured yoghurts' viscosity values (Aryana et al., 2006), which could be

284 explained by the depleted water-binding capacity of the proteins present in each formulation
285 (Jaster et al., 2018).

286 *Dynamic rheological tests*

287 Table 2 shows the viscoelastic properties of the different manufactured yoghurts. For
288 comparative purposes, the storage modulus (*G*') and loss modulus (*G''*) values were considered
289 at a frequency of 1 Hz. A predominant elastic behaviour (*G' > G''*) was observed during the
290 whole study, which is a common fact in weak viscoelastic systems (Morell et al., 2015). The
291 FFY sample presented higher *G'* and *G"* values than SY sample. Indeed, increasing *G'* and *G''*
292 values were noticed when using higher CSM concentrations. This can be explained by the
293 interactions taking place between negatively charged mucilage and the positively charged
294 surface of casein micelles, which leads to a highly structured network that provides samples
295 with good gel strength and makes them more viscous (Norton et al., 2011). Furthermore, for the
296 enriched yoghurts, and despite storage time, the highest *G'* and *G''* values were exhibited by the
297 yoghurts formulated with 7.5 % CSM. Thus, large amounts of CSM could increase skimmed
298 yoghurts' firmness due to the strong developed network structure, which would diminish the
299 mobility of particles and the syneresis rate, as above-mentioned.
300 Prolonged cold storage significantly affected ($p<0.05$) the *G'* and *G''* values of all the yoghurts
301 formulated in this study. Higher values were noticed after 21 storage days at 4 ºC, regardless of
302 yoghurt type (Sah et al., 2016). Cooling processes can bring about conformational changes in
303 products, such as xanthan gum and gelatine, reflected by increasing *G'* values of acid milk gels
304 (Pang et al., 2017). Hence conformational changes in CSM molecules during cooling storage
305 could explain the higher values found for the 7.5 % CSM yoghurts.

306 Complex moduli (|*G**|) is a measure of the product stiffness (Mezger, 2006). The higher
307 complex moduli |*G**|, the greater yoghurt firmness (Costa et al., 2019). Thus, FFY and 7.5 %

CSM were the formulations with the best firmness. This indicates that using larger amounts of CSM could increase skimmed yoghurts' firmness because of the created robust network structure.

Indeed, the firmness of all the products significantly ($p<0.05$) increased during storage. Walstra et al. (2006) pointed out that yoghurt gel firmness increased when pH values lowered. In this study, lower pH values were obtained at the end of storage, increasing $|G^*|$ values and improving gel firmness.

Tan δ values can be used to indicate the system's physical behaviour. *Tan δ* values above 1 are indicative of dilute solutions, while values between 0.1 and 1 denote weak gels (Irani et al., 2019). All the formulations had *tan δ* values below 1, which reinforces that elastic properties prevail over viscous ones. The *tan δ* values for all the formulations remained practically constant throughout storage. Moreover, the addition of CSM significantly ($p<0.05$) increased the complex viscosity ($|\eta^*|$) of the different samples suggesting that the obtained mucilage had a thickening effect on yoghurts and increased internal cohesive forces (Giri et al., 2018). Similarly, the increase in $|\eta^*|$ that took place during storage could be explained by post-acidification processes (Saint-Eve et al., 2008).

Creep and recovery tests

Figure 4 shows the creep and recovery curves of the different yoghurts. High compliance values *(J(t))* indicate a weaker product structure (Sozer, 2009). According to this view, the SY sample presented the weakest gel structure, whereas samples formulated with increasing CSM concentrations had lower *J(t)* values, which reflects more elastic behaviour. It is noteworthy that the FFY and 7.5 % CSM samples displayed similar behaviour for the first 7 analysis days. Afterwards, the *J(t)* values significantly ($p<0.05$) lowered for the sample enriched with 7.5 %

CSM. For this reason, it can be stated that employing CSM reinforced the gel structure of the developed yoghurts.

Regarding the Burger model parameters (Table 3), the instantaneous shear modulus (G_0) was higher when larger amounts of extracted mucilage were employed. This rise could indicate incremented elastic strength of the bonds forming the interfacial network structure of yoghurts (Karaman et al., 2012). Significant differences ($p<0.05$) were noticed in the G_0 values throughout the study as yoghurts presented higher G_0 values at the end of the study, suggesting the reinforcement of the gel matrix. These results agree with those previously observed.

On the contrary, the J_{max} and J_∞ values lowered when higher CSM concentrations were employed (Table 3). This fact could be explained by the final product's enhanced elastic bonds, which diminished the number of irreversible broken bonds (Kurt et al., 2016). Lastly, the herein obtained recovery rates (R, %) exhibited better elastic behaviour for 7.5 % CSM yoghurts than FFY and SY samples. This phenomenon was clearly observed when storage time ended. Thus, as suggested by Kurt et al. (2016), adding anionic polysaccharides to milk bases could increase resistance to stress due to stronger interactions among the constituents of foods.

Sensory analysis

The sensory analysis is an essential tool for determining the acceptance of new foods. For this reason, a sensory evaluation of FFY (control), SY and the yoghurt prepared with 7.5 % CSM was carried out. The incorporation of extracted CSM into yoghurts did not modify acidity, creaminess and viscosity compared to the FFY sample. Nevertheless, the appearance, colour and granularity of the manufactured yoghurts were affected when adding CSM (Table 4).

The colour attribute findings observed by the panellists were in accordance with those noticed while characterising the different yoghurt formulations. It is noteworthy that the global

355 acceptance of the sample enriched with 7.5 % CSM obtained a lower score probably due to the
356 effect of mucilage on the sample's granularity.

357 **CONCLUSIONS**

358 The use of chia (*Salvia hispanica* L.) seed mucilage in skimmed yoghurts displays relevant
359 changes in their nutritional, technological and sensory characteristics compared to full-fat and
360 skimmed controls. Enriching yoghurts with mucilage reduce the degree of syneresis during
361 storage compared to the full-fat and skimmed samples. Moreover, its incorporation improves
362 the nutritional value of yoghurts by increasing their fibre content. The rheological studies
363 suggest that the technological characteristics of the yoghurts formulated with chia seed
364 mucilage enhance in terms of greater consistency, high structured-network, firmness and
365 viscosity and resistance to stress *versus* skimmed and/or full-fat yoghurts. Storage time
366 positively affects the technological properties of the manufactured yoghurts. The sensory
367 evaluation shows that using chia seed mucilage as a fat replacer in yoghurts does not modify
368 the acidity, creaminess and viscosity of samples, whereas their appearance, colour and
369 granularity play an important role in products' global acceptance compared to full-fat yoghurt.
370 This work provides an interesting alternative for the food industry to reduce fat contents in
371 dairy products, increase their nutritional value and improve the technological features of
372 skimmed yoghurts. This fact could represent an advance in developing novel non-fat yoghurts
373 with equal or better characteristics than full-fat yoghurts, and with several functional benefits
374 for consumers. However, further studies need to be conducted to reduce its impact on products'
375 colour and sensory acceptance.

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Conflict of interest

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ORCID

Nataly Peña: 0000-0001-8779-3661

Ana Fuentes: 0000-0002-4144-2396

Pau Talens: 0000-0001-7318-3336

Jose M. Barat: 0000-0001-8487-7114

Table 1. Rheological parameters from the steady shear tests of the different yoghurt formulations during 21 storage days at 4 ºC: yield stress (τ_0), consistency coefficient (K), flow behaviour index (n) and apparent viscosity (η_{app50}). Mean values (n=3) ± standard deviation.

		Yoghurt type				
	Day of analysis	**FFY**	**SY**	**SY + CSM 2.5 %**	**SY + CSM 5.0 %**	**SY + CSM 7.5 %**
τ_0 (Pa)	1	3.192 ± 0.047^{bD}	1.982 ± 0.036^{bA}	2.469 ± 0.030^{bB}	2.853 ± 0.091^{bC}	3.157 ± 0.029^{cD}
	7	3.107 ± 0.065^{abD}	1.970 ± 0.058^{bA}	2.449 ± 0.048^{bB}	2.812 ± 0.043^{bC}	3.089 ± 0.042^{bD}
	14	3.069 ± 0.031^{aD}	1.778 ± 0.020^{aA}	2.204 ± 0.052^{aB}	2.767 ± 0.038^{abC}	3.044 ± 0.017^{bD}
	21	2.951 ± 0.113^{aCD}	1.731 ± 0.059^{aA}	2.163 ± 0.077^{aB}	2.722 ± 0.052^{aC}	2.968 ± 0.028^{aD}
K (Pa·s)	1	4.667 ± 0.138^{aC}	3.256 ± 0.092^{aA}	3.272 ± 0.036^{aA}	4.388 ± 0.064^{aB}	4.479 ± 0.105^{aBC}
	7	4.921 ± 0.106^{bD}	3.222 ± 0.014^{aA}	3.559 ± 0.042^{bB}	4.626 ± 0.050^{bC}	4.885 ± 0.092^{bD}
	14	5.216 ± 0.022^{cD}	4.160 ± 0.073^{bA}	4.303 ± 0.049^{cB}	5.070 ± 0.087^{cC}	5.088 ± 0.100^{cC}
	21	5.434 ± 0.063^{dE}	4.364 ± 0.040^{cA}	4.589 ± 0.047^{dB}	5.169 ± 0.029^{dC}	5.333 ± 0.041^{dD}
n	1	0.624 ± 0.009^{aA}	0.644 ± 0.005^{aB}	0.624 ± 0.007^{aA}	0.612 ± 0.019^{aA}	0.613 ± 0.026^{aA}
	7	0.631 ± 0.019^{abAB}	0.640 ± 0.011^{aB}	0.624 ± 0.010^{aA}	0.648 ± 0.010^{bB}	0.624 ± 0.005^{aA}
	14	0.656 ± 0.028^{bB}	0.686 ± 0.038^{abC}	0.673 ± 0.006^{bC}	0.619 ± 0.028^{aA}	0.644 ± 0.023^{abAB}
	21	0.671 ± 0.013^{bB}	0.696 ± 0.026^{bBC}	0.755 ± 0.073^{cC}	0.663 ± 0.004^{cB}	0.641 ± 0.014^{bA}
η_{app50}(Pa·s)	1	0.160 ± 0.011^{cC}	0.064 ± 0.008^{cA}	0.071 ± 0.007^{bA}	0.147 ± 0.008^{cB}	0.195 ± 0.023^{dD}
	7	0.135 ± 0.006^{bC}	0.054 ± 0.001^{bA}	0.059 ± 0.005^{abA}	0.128 ± 0.002^{bB}	0.165 ± 0.006^{cD}
	14	0.104 ± 0.009^{aB}	0.045 ± 0.011^{bA}	0.047 ± 0.014^{abA}	0.102 ± 0.016^{aB}	0.147 ± 0.012^{bC}
	21	0.090 ± 0.010^{aB}	0.028 ± 0.009^{aA}	0.030 ± 0.008^{aA}	0.085 ± 0.007^{aB}	0.106 ± 0.009^{aC}

Lowercase letters (a, b, c, d) indicate significant differences among the storage time expressed in days ($p<0.05$). Capital letters (A, B, C, D, E) indicate significant differences among the samples ($p<0.05$). (FFY: full- fat yoghurt; SY: skimmed yoghurt; CSM: chia seed mucilage).

Table 2. Viscoelastic properties, at a frequency of 1 Hz, of the different yoghurt formulations during 21 storage days at 4 ºC: elastic or storage modulus (G'), viscous or loss modulus (G''), complex modulus (|G*|), complex viscosity (|η*|), and loss tangent (tan δ). Mean values (n=3) ± standard deviation.

		Yoghurt type				
	Day of analysis	**FFY**	**SY**	**SY + CSM 2.5 %**	**SY + CSM 5.0 %**	**SY + CSM 7.5 %**
				f (Hz) = 1		
G' (Pa)	1	297 ± 91^{aC}	81 ± 42^{aA}	121 ± 89^{aAB}	169 ± 25^{aB}	281 ± 104^{aC}
	7	453 ± 62^{bC}	165 ± 49^{bA}	279 ± 38^{bB}	296 ± 90^{bBC}	445 ± 40^{bC}
	14	671 ± 39^{cD}	230 ± 91^{cA}	354 ± 20^{cB}	482 ± 22^{cC}	636 ± 91^{cD}
	21	721 ± 15^{dC}	399 ± 15^{dA}	413 ± 29^{dA}	626 ± 53^{dB}	770 ± 22^{dD}
G'' (Pa)	1	183 ± 43^{aD}	64 ± 24^{aA}	90 ± 11^{aB}	127 ± 36^{aC}	199 ± 31^{aD}
	7	236 ± 21^{bAB}	138 ± 23^{bA}	161 ± 50^{abA}	232 ± 32^{bAB}	250 ± 47^{abB}
	14	271 ± 36^{bcBC}	178 ± 21^{bcA}	240 ± 26^{bB}	275 ± 45^{bcBC}	287 ± 28^{bC}
	21	337 ± 19^{cD}	267 ± 14^{cA}	283 ± 12^{cB}	302 ± 18^{cC}	362 ± 19^{cE}
|*G**| (Pa)	1	287 ± 14^{aD}	109 ± 46^{aA}	183 ± 26^{aB}	235 ± 25^{aC}	293 ± 48^{aD}
	7	336 ± 45^{bB}	227 ± 31^{bA}	324 ± 51^{bB}	381 ± 23^{bC}	361 ± 37^{bBC}
	14	503 ± 51^{cB}	364 ± 42^{cA}	446 ± 40^{cAB}	496 ± 32^{cB}	598 ± 31^{cC}
	21	760 ± 15^{dD}	413 ± 10^{dA}	542 ± 31^{dB}	671 ± 83^{dC}	816 ± 18^{dE}
tan δ	1	0.596 ± 0.008^{bA}	0.709 ± 0.002^{aB}	0.746 ± 0.005^{cC}	0.756 ± 0.007^{cC}	0.710 ± 0.006^{cB}
	7	0.501 ± 0.009^{cA}	0.792 ± 0.002^{cD}	0.567 ± 0.004^{aB}	0.774 ± 0.001^{dC}	0.563 ± 0.008^{bB}
	14	0.434 ± 0.004^{aA}	0.756 ± 0.002^{bE}	0.680 ± 0.008^{bD}	0.560 ± 0.004^{bC}	0.461 ± 0.010^{aB}
	21	0.466 ± 0.008^{bA}	0.708 ± 0.006^{aD}	0.681 ± 0.007^{bC}	0.510 ± 0.004^{aB}	0.473 ± 0.004^{aA}
|*η**| (Pa·s)	1	61 ± 11^{aAB}	50 ± 1^{aA}	55 ± 17^{aAB}	66 ± 16^{aAB}	75 ± 24^{aB}
	7	94 ± 33^{abAB}	85 ± 15^{bA}	89 ± 16^{bA}	94 ± 31^{abAB}	100 ± 9^{aB}
	14	129 ± 45^{bA}	124 ± 26^{cA}	128 ± 44^{cA}	132 ± 35^{bAB}	170 ± 28^{bB}
	21	252 ± 38^{cB}	159 ± 23^{dA}	177 ± 33^{dAB}	187 ± 19^{cAB}	250 ± 39^{cB}

Lowercase letters (a, b, c, d) indicate significant differences among the storage time expressed in days (p<0.05). Capital letters (A, B, C, D, E) indicate significant differences among the samples (p<0.05). (FFY: full- fat yoghurt; SY: skimmed yoghurt; CSM: chia seed mucilage).

Table 3. Burger model parameters ((G_0 (Pa); η_0 (Pa·s)), maximum compliance (J_{max}) and final percentage of recovery (R) of the different yoghurts stored at 4 ºC for 21 days. Mean values (n=3) ± standard deviation.

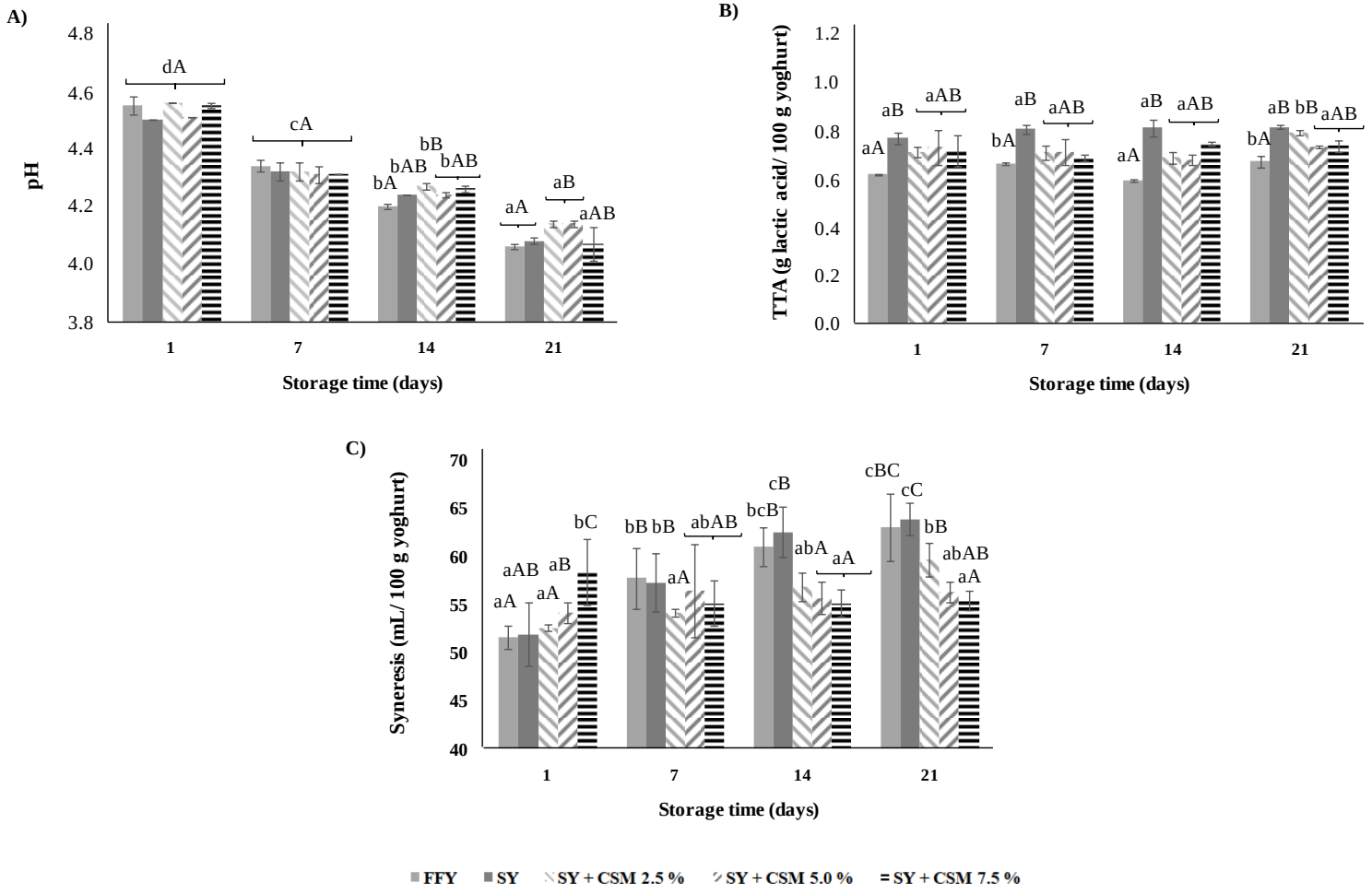
		Yoghurt type				
	Day of analysis	**FFY**	**SY**	**SY + CSM 2.5 %**	**SY + CSM 5.0 %**	**SY + CSM 7.5 %**
G_0 (Pa)	1	114.0 ± 10.0^{aB}	87.6 ± 5.3^{aA}	262.6 ± 33.3^{abC}	172.9 ± 71.2^{aBC}	282.6 ± 39.4^{aC}
	7	257.1 ± 47.7^{bAB}	187.6 ± 23.8^{bA}	264.7 ± 20.6^{abB}	213.9 ± 22.1^{abA}	481.4 ± 78.5^{bC}
	14	291.8 ± 22.3^{bB}	155.5 ± 48.6^{bA}	227.5 ± 71.3^{aAB}	260.6 ± 54.1^{bB}	671.3 ± 92.6^{cC}
	21	395.3 ± 15.9^{cC}	269.0 ± 38.6^{cA}	348.2 ± 23.2^{bB}	316.7 ± 66.2^{cAB}	1329.0 ± 36.5^{dD}
η_0x 10^5(Pa·s)	1	2.992 ± 0.199^{aC}	1.024 ± 0.161^{aA}	2.266 ± 0.295^{aB}	3.687 ± 0.502^{bD}	3.851 ± 1.376^{abCD}
	7	4.565 ± 0.994^{bC}	2.722 ± 0.358^{cA}	3.222 ± 0.189^{bAB}	2.935 ± 0.275^{abA}	3.912 ± 0.602^{aB}
	14	3.543 ± 0.502^{abB}	1.928 ± 0.604^{bcA}	2.685 ± 0.791^{abAB}	2.416 ± 0.622^{aAB}	6.735 ± 0.751^{cC}
	21	7.206 ± 1.094^{cC}	1.925 ± 0.365^{bA}	5.014 ± 0.908^{cB}	4.379 ± 2.326^{abAB}	5.014 ± 0.908^{bB}
J_{max} (Pa^{-1})	1	0.0031 ± 0.0001^{aA}	0.0144 ± 0.0002^{cC}	0.0047 ± 0.0002^{aB}	0.0041 ± 0.0008^{aAB}	0.0030 ± 0.0006^{abA}
	7	0.0040 ± 0.0008^{abAB}	0.0065 ± 0.0007^{bC}	0.0042 ± 0.0007^{aAB}	0.0057 ± 0.0005^{bB}	0.0031 ± 0.0002^{bA}
	14	0.0041 ± 0.0004^{bB}	0.0065 ± 0.0022^{abBC}	0.0059 ± 0.0009^{bC}	0.0047 ± 0.0025^{abAB}	0.0025 ± 0.0010^{abA}
	21	0.0029 ± 0.0006^{aB}	0.0047 ± 0.0004^{aC}	0.0040 ± 0.0011^{abBC}	0.0042 ± 0.0011^{abBC}	0.0019 ± 0.0007^{aA}
J_∞ (Pa^{-1})	1	0.0013 ± 0.0004^{aA}	0.0073 ± 0.0005^{cC}	0.0022 ± 0.0005^{aB}	0.0018 ± 0.0004^{aAB}	0.0015 ± 0.0005^{abA}
	7	0.0020 ± 0.0008^{abB}	0.0036 ± 0.0006^{abC}	0.0029 ± 0.0007^{abBC}	0.0030 ± 0.0003^{bBC}	0.0011 ± 0.0001^{bA}
	14	0.0027 ± 0.0010^{bB}	0.0042 ± 0.0002^{bC}	0.0030 ± 0.0009^{bB}	0.0028 ± 0.0013^{abAB}	0.0010 ± 0.0005^{abA}
	21	0.0018 ± 0.0006^{abB}	0.0029 ± 0.0004^{aC}	0.0027 ± 0.0011^{abBC}	0.0026 ± 0.0011^{abBC}	0.0007 ± 0.0003^{aA}
R (%)	1	58.07 ± 2.31^{cB}	49.31 ± 2.29^{bA}	52.75 ± 1.40^{cA}	56.10 ± 5.43^{cAB}	51.21 ± 5.04^{aAB}
	7	50.31 ± 5.02^{bB}	45.37 ± 5.26^{abAB}	33.78 ± 6.99^{aA}	48.37 ± 1.39^{bB}	63.99 ± 2.35^{bC}
	14	39.20 ± 6.12^{aA}	40.81 ± 6.45^{aA}	49.00 ± 2.30^{bB}	40.63 ± 0.83^{aA}	58.73 ± 4.63^{abC}
	21	38.68 ± 3.10^{aA}	42.44 ± 8.11^{abAB}	35.36 ± 6.64^{aA}	40.08 ± 3.45^{aAB}	64.57 ± 4.64^{bB}

Lowercase letters (a, b, c, d) indicate significant differences among the storage time expressed in days ($p<0.05$). Capital letters (A, B, C, D) indicate significant differences among the samples ($p<0.05$). (FFY: full-fat yoghurt; SY: skimmed yoghurt; CSM: chia seed mucilage).

Table 4. Score of the attributes tested in FFY and the yoghurts formulated with increasing CSM concentrations (2.5 %, 5.0 %, 7.5 %). 0: very unpleasant; 9: very pleasant. Capital letters (A, B) indicate significant differences among samples ($p<0.05$) (n=30).

Yoghurt type	Attributes						
	Appearance	**Colour**	**Viscosity**	**Granularity**	**Creaminess**	**Acidity**	**Global acceptance**
FFY	7.5 ± 0.4^{B}	7.5 ± 0.2^{B}	7.0 ± 0.3^{A}	7.5 ± 0.4^{B}	7.6 ± 0.5^{A}	6.2 ± 1.0^{A}	7.3 ± 0.2^{B}
SY	7.5 ± 0.4^{B}	7.5 ± 0.3^{B}	6.8 ± 0.6^{A}	7.6 ± 0.3^{B}	7.2 ± 0.5^{A}	6.3 ± 1.0^{A}	7.2 ± 0.2^{B}
SY + 7.5% CSM	6.4 ± 0.5^{A}	6.8 ± 0.3^{A}	6.8 ± 0.6^{A}	5.1 ± 0.9^{A}	7.2 ± 0.5^{A}	6.6 ± 1.3^{A}	6.3 ± 0.6^{A}

Capital letters (A, B, C, D, E) indicate significant differences among the samples ($p<0.05$).
(FFY: full-fat yoghurt; SY: skimmed yoghurt; CSM: chia seed mucilage).

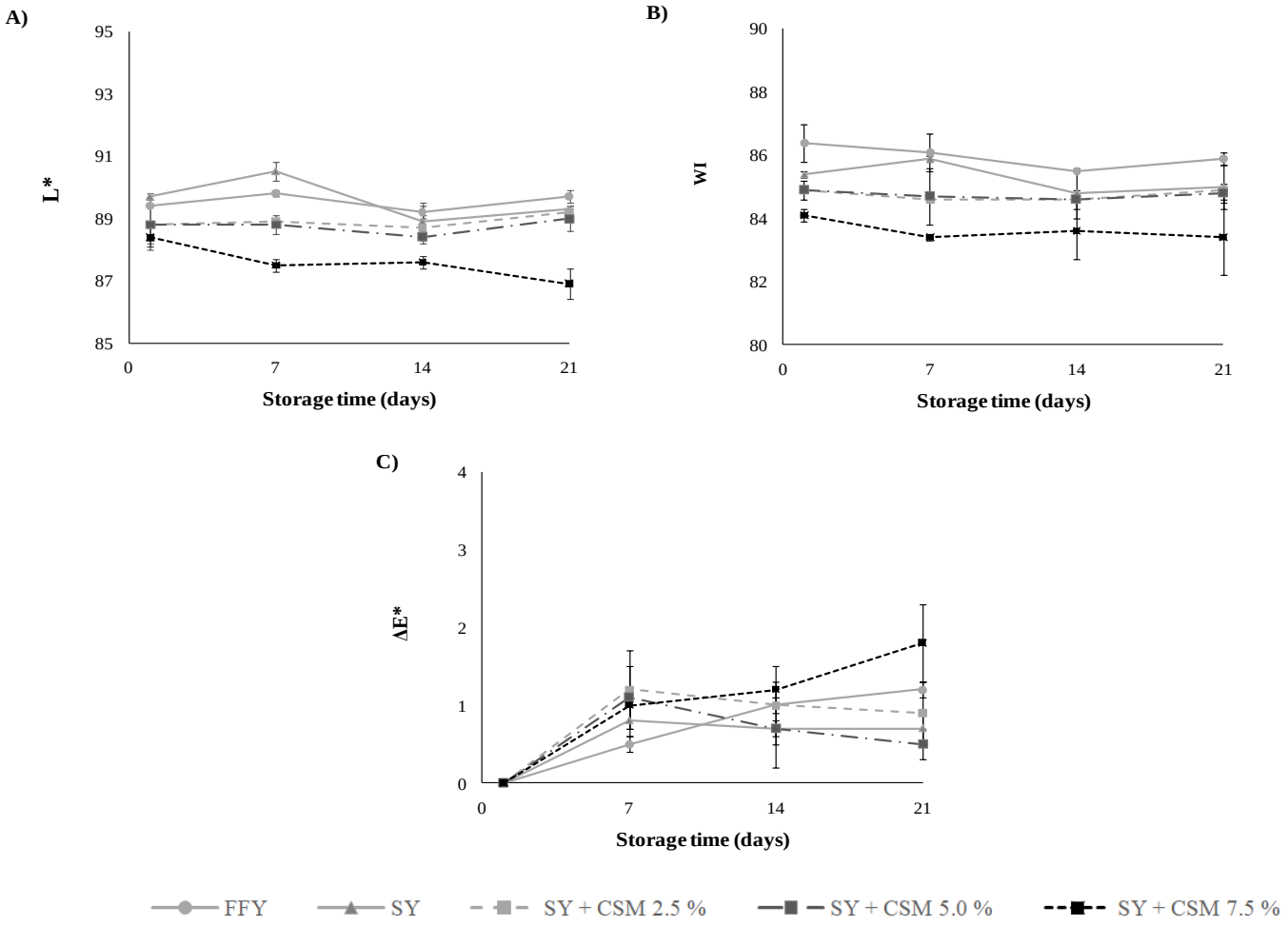


549

550 **Figure 1.** pH (A), total titratable acidity (B) and syneresis (C) of the different yoghurts manufactured during 21 storage days at 4 ºC. Mean

551 values (n=3) ± standard deviation. Lowercase letters (a, b, c, d) indicate significant differences among storage times ($p<0.05$). Capital letters (A,

552 B, C) indicate significant differences among samples ($p<0.05$). (FFY: full-fat yoghurt; SY: skimmed yoghurt; CSM: chia seed mucilage).



553

554 **Figure 2.** Colour parameters: A) luminosity (L*), B) whiteness index (WI) and, D) colour variations (ΔE*) of the different yoghurts stored

555 during 21 days at 4 ºC. Mean values (n=3) ± standard deviation. (FFY: full-fat yoghurt; SY: skimmed yoghurt; CSM: chia seed mucilage).

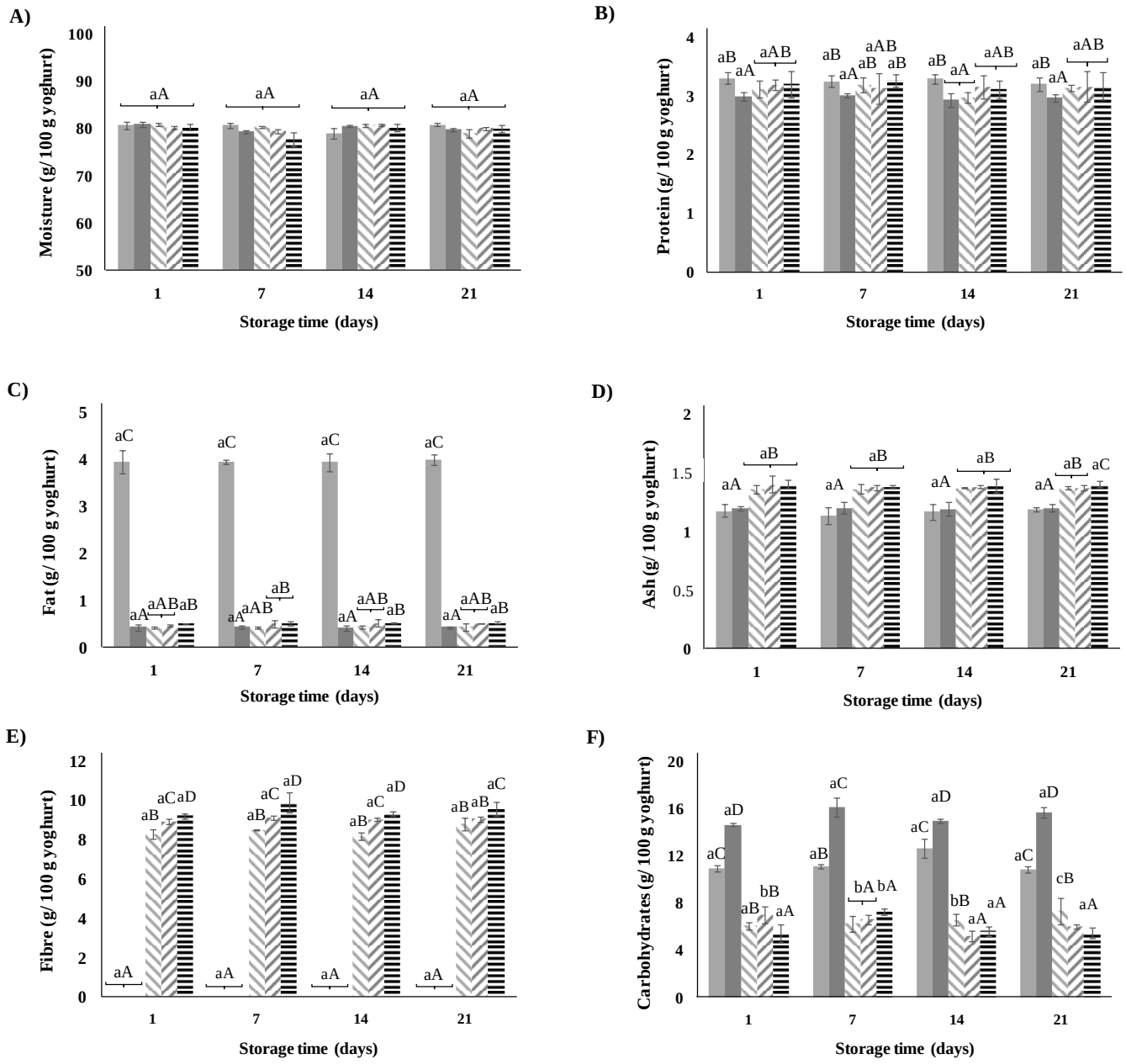
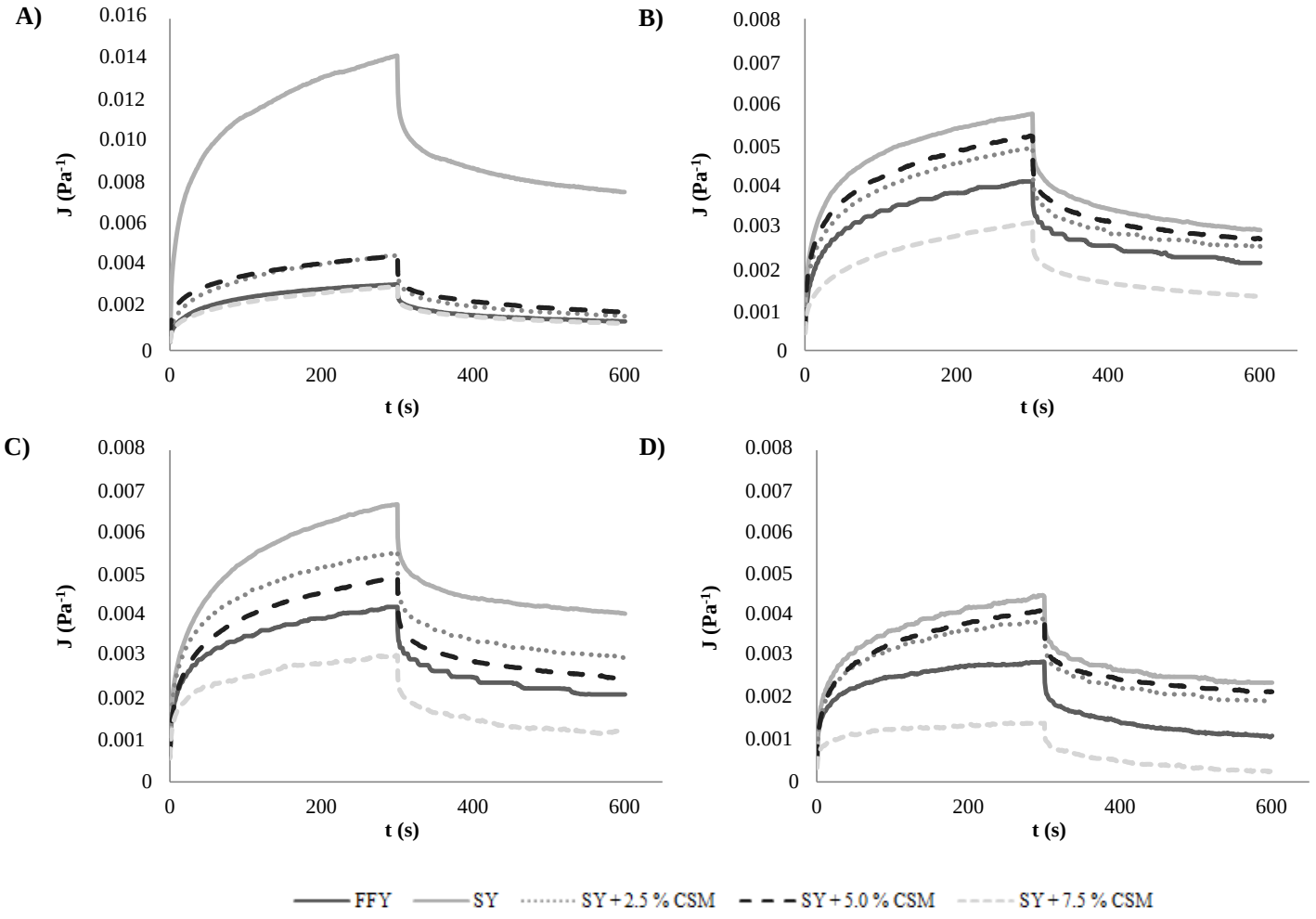


Figure 3. Nutritional composition (g/100 g of fresh yoghurt) for diverse yoghurt samples stored during 21 days at 4 ºC. Mean values (n=3) ± standard deviation. Lowercase letters (a, b, c) indicate significant differences among the storage time ($p<0.05$). Capital letters (A, B, C, D) indicate significant differences among the samples ($p<0.05$). (FFY: full-fat yoghurt; SY: skimmed yoghurt; CSM: chia seed mucilage).



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564 **Figure 4.** Creep and recovery curves of the different yoghurts stored at 4 ºC: A) 1 analysis day; B) 7 analysis days; C) 14 analysis days; and D)

565 21 analysis days. Mean values (n=3). (FFY: full-fat yoghurt; SY: skimmed yoghurt; CSM: chia seed mucilage).