

Assessing gelling properties of chia (*Salvia hispanica* L.) flour through rheological characterization

Susana Ramos,^a Patrícia Fradinho,^{b*} Paulina Mata^a and Anabela Raymundo^b

Abstract

BACKGROUND: Chia (*Salvia hispanica* L.) seeds are considered a promising ingredient for the development of functional products owing to their high nutritional value: 343 g kg⁻¹ lipids, 251 g kg⁻¹ protein and 226 g kg⁻¹ fibre. Considering chia's technological capacities, mainly the ability to swell when absorbing water and gel-forming properties, its addition to a food matrix can affect texture and rheological behaviour, acting as a texturing and stabilizing agent. The aim of the present work was to assess the gelling properties of chia flour through the rheological characterization of 100, 130 and 150 g kg⁻¹ chia flour gels.

RESULTS: According to the mechanical spectra, all gels presented weak gel-like structures, as G' was always less than a decade higher than G'' , but higher chia flour concentrations showed a considerable increase in viscoelastic moduli. The gels had relatively low maturation times, almost instantaneous for lower concentrations, but the cooling rate affected the dynamics of formation of the gel structure.

CONCLUSION: Based on texture and rheological properties, gels with 130 g kg⁻¹ of chia flour processed at 90 °C for 30 min showed the most suitable characteristics for use in the development of new food applications.

© 2016 Society of Chemical Industry

Keywords: chia seeds; chia gel; rheological properties; texture

INTRODUCTION

Salvia hispanica L., commonly known as chia, is a summer annual plant of the Lamiaceae family that grows in arid or semiarid climates and is native to southern Mexico and northern Guatemala. The plant produces small dark and beige oval seeds that were used by the Aztec tribes as a food, but also as a medicine and oil source.¹ Nowadays, chia consumption is considered safe by food authorities² and is a potential source of nutrients for the food and animal feed industries.^{1,3} Chia seeds have a high content of protein (150–250 g kg⁻¹), lipids (200–350 g kg⁻¹) and dietary fibre (180–380 g kg⁻¹).^{4–6} Chia also contains high amounts of natural antioxidants^{4,7} and is an important source of B vitamins and minerals.¹ Its composition, particularly the oil and fatty acid content, is affected by the ecosystem in which the chia plant is grown, rather than by the genotype⁸ or the seed coat colour.⁹

Chia is mainly valued for its oil, as it has one of the highest known contents of α -linolenic acid (ALA) (up to 680 g ALA kg⁻¹ chia oil).^{10,11} Chia in the form of seed and/or oil can be an efficient renewable resource to ensure supply of ω -3 fatty acids for enriching food formulations.^{11,12}

When chia seeds are soaked in water, a clear mucilaginous gel is released, forming a highly viscous solution.^{13,14} The exuded polysaccharide, located in the outer three layers of the seed coat, has a molecular weight from 0.8×10^6 to 2.0×10^6 Da. Lin *et al.*¹⁵ proposed a chemical composition for the polysaccharide structural unit based on a tetrasaccharide with 4-O-methyl- α -D-glucopyranosyluronic acid residues occurring as branches at O-2 of some β -D-xylopyranosyl residues

in the main chain consisting of (1 \rightarrow 4)- β -D-xylopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl-(1 \rightarrow 4)- β -D-xylopyranosyl. The potential of chia seeds and mucilage to be used as functional ingredients has been recognized by several authors.^{6,13}

All chia nutraceutical components support claims associated with health benefits: reduction of cardiovascular diseases and obesity, regulation of intestinal transit and cholesterol and triglyceride levels, as well as prevention of diseases such as type II diabetes and some types of cancer.^{11,12}

The objective of this study was to characterize the gelling properties of chia flour (from ground chia seeds) with the purpose of studying its potential for the development of functional foods. The rheological approach is determinant to understand the role of the polysaccharide's molecules in the development of the gel structure. The evolution of the gel viscoelastic functions during heating and cooling processes and the kinetics of gel maturation are key features to consider for the development of new food products such as gel desserts or salty gel preparations with addition

* Correspondence to: P Fradinho, LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, P-1349-017 Lisboa.
E-mail: pfradinho@isa.ulisboa.pt

^a LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, P-2829-516 Caparica, Portugal

^b LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, P-1349-017 Lisboa, Portugal

health benefits. Similar rheological studies have been done by the authors using other sources such as chestnut and rice flours¹⁶ and complex polysaccharide–protein systems.¹⁷

MATERIALS AND METHODS

Raw materials

Commercial chia seeds Midzu from Peru (provided by Efeito Verde, Lda) were ground in a domestic mill (Classic Moulinex 123-A320R1) at constant speed. Particle size separation was performed using a sieve system, and only the fraction smaller than 0.5 mm was used for gel preparation. This chia flour was the raw material for gel development.

Physicochemical characterization

Chia flour was characterized in terms of moisture and ash contents according to AOAC method 935.29¹⁸ and Portuguese standard NP 518¹⁹ respectively, based on gravimetric methods. Total lipid analysis was carried out according to Portuguese standard NP 4168.²⁰ Protein content was determined by the Kjeldahl method following ISO 20483,²¹ using a nitrogen conversion factor of 5.83.²² Soluble, insoluble and total dietary fibre contents of chia flour were evaluated according to the enzymatic/gravimetric method described by Prosky *et al.*²³ Total fibre content is included in carbohydrate content, which was calculated by difference.

The swelling power and solubility of chia flour were determined using the method developed by Leach *et al.*²⁴ for starches, with slight modifications. This method involves the suspension of chia flour (w_{sample}) in a known volume of water, with gentle stirring to keep it in suspension, followed by incubation at a selected temperature for 30 min and centrifugation at $3000 \times g$ for 10 min. Chia flour solubility was obtained by drying the supernatant at $105 \pm 1^\circ\text{C}$ (w_r) and expressed as

$$\text{solubility (\%)} = (w_r/w_{\text{sample}}) \times 100 \quad (1)$$

Swelling power was determined from the weight of the sediment (w_{sediment}) according to the equation

$$\text{swelling power (g g}^{-1}) = w_{\text{sediment}} / (w - w_r) \quad (2)$$

The temperatures selected for incubation, namely 25, 35, 50 and 90°C , were within the range of temperatures involved in the chia flour gelling process.

All chemical analyses were carried out at least in triplicate and the results expressed as mean \pm standard deviation.

Preparation of chia gels

The capacity of chia flour to form gels was evaluated for a range of concentrations from 10 to 150 g kg⁻¹. The effect of the binomial time/heating temperature on chia gel properties was also studied.

In preliminary studies, the critical chia flour concentration, i.e. the amount of chia flour need to produce a structured system with a rheological behaviour close to a gel dessert,²⁵ was established as 130 g kg⁻¹. For texture measurements, 130 g kg⁻¹ chia flour gels were prepared by dispersing the flour in water under mechanical stirring (Eurostar Digital, IKA-Werke Staufen, Germany) at $15 \times g$ for 30 min at $20 \pm 1^\circ\text{C}$. The time and temperature for chia hydration were optimized in preliminary studies performed according to Salgado-Cruz *et al.*,¹⁴ which showed that the chia mucilage expelling process required about 30 min. The suspensions were poured into glass containers (35 mm height, 32 mm

diameter) and heated in a water bath at gelation temperatures of 40, 50, 60, 70 and 90°C for 30 min. The effect of heating time was also studied by preparing gels at 90°C for 10, 30 and 60 min.

Gel rheological characterization was performed using different concentrations (100, 130 and 150 g kg⁻¹) of chia flour suspensions also hydrated at $20 \pm 1^\circ\text{C}$ for 30 min under magnetic stirring owing to the small quantity of each sample. Subsequently, the gelation occurred under controlled time and temperature conditions in the rheometer measurement device (gelation *in situ*).

Texture evaluation of chia gels

Texture analysis of chia flour gels was performed using a TA.XT-Plus texturometer (Stable Microsystems, Godalming, UK) in a temperature-controlled room at $20 \pm 1^\circ\text{C}$. Texture profile analysis (TPA) tests were performed on the gels contained in a cylindrical flask (35 mm height, 32 mm diameter), using a 10 mm cylindrical probe (10 mm penetration, 2 mm s⁻¹ crosshead speed, with a waiting time of 5 s between the two cycles). The tests were carried out 24 h after gel preparation, allowing the gels to achieve full maturation, at $4 \pm 1^\circ\text{C}$. Before performing the measurements, the samples were allowed to equilibrate at 20°C for 30 min. From the experimental force *versus* time data, the maximum force was calculated and expressed as gel firmness. From the TPA results, the cohesiveness and adhesiveness of gels were also obtained.

Rheological properties of chia gels

Dynamic oscillatory measurements were performed to characterize the mechanical properties of chia flour gels, using small-amplitude oscillatory shear (SAOS) measurements in a controlled stress rheometer (RS-300, Haake Karlsruhe, Germany) coupled to a UTC-Peltier system, with a serrated parallel plate with 35 mm diameter (PP35) and 1 mm gap.

The chia flour suspensions were transferred to the instrument plate, covered with a layer of paraffin oil to prevent moisture loss and stabilized for 5 min at 20°C . The subsequent gelation *in situ* was followed up.

The samples were heated from 20 to 90°C (heating rate 2°C min^{-1}), maintained at this temperature for 30 min, with no shear applied, and then cooled to 5°C at different rates (0.5, 2 and 5°C min^{-1}). These temperature sweep tests were carried out at $f = 1\text{ Hz}$ and $\tau = 1\text{ Pa}$ (within the linear viscoelastic region). Subsequently, a time sweep test at 5°C for 120 min ($f = 1\text{ Hz}$, $\tau = 5\text{ Pa}$) was performed to allow full chia gel maturation. To characterize the mechanical properties of the matured gels, frequency sweep tests ($\tau = 10\text{ Pa}$, $f = 0.01\text{--}100\text{ Hz}$) were conducted after the maturation process. Each test was performed in triplicate.

Colour measurements

The colour of chia gels prepared at 40, 60, 70 and 90°C for 30 min was measured instrumentally using a CR-300 colorimeter (Minolta, Osaka, Japan) with standard illuminant D65 and a visual angle of 2° . The colour parameters L^* , a^* and b^* (CIELAB system) were assessed, where L^* corresponds to the lightness, a^* to the degree of redness or greenness and b^* to the degree of yellowness or blueness. The total colour difference between the parameters obtained for all temperature/time conditions and $40^\circ\text{C}/30\text{ min}$ was calculated according to

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

Table 1. Proximate composition (g kg^{-1}) of chia flour

Moisture	Fat	Protein	Ash	Carbohydrate	Soluble fibre	Insoluble fibre
78.7 \pm 2.3	343.1 \pm 2.2	250.5 \pm 7.4	39.8 \pm 1.5	287.9 \pm 8.2 ^a	35.3 \pm 1.3	190.9 \pm 0.3

^a Carbohydrate standard deviation was obtained by combined uncertainty.

The colour difference is considered to be detectable by the human eye when $\Delta E^* > 5$.²⁶

The measurements were conducted 24 h after preparation at 20 \pm 1 °C and replicated at least eight times.

Statistical analysis

Experimental data were analysed by one-way analysis of variance (ANOVA) or the Kruskal–Wallis test. When the former analysis indicated differences among means, a Scheffé test or pairwise comparison was performed to differentiate means with 95% confidence ($P < 0.05$). All statistical treatments were done using SPSS Statistics Version 20 (IBM SPSS Statistics, New York, NY, USA).

RESULTS AND DISCUSSION

Physicochemical characterization of chia flour

Nutritional evaluation of chia flour

The proximate chemical composition of chia flour is summarized in Table 1. The values are in accordance with the characterization obtained by other authors.^{4–6,27,28} The slight differences should be primarily related to geographical origin and to different production factors such as temperature, light and soil type. The influence of these factors on the nutritional composition of chia flour is widely reported by Ayerza and Coates.⁸

Chia flour presented a 251 g kg⁻¹ protein, 288 g kg⁻¹ carbohydrate and 343 g kg⁻¹ fat content, accounting for a caloric value of 524.2 kcal per 100 g. According to Ayerza and Coates⁸ and Segura-Campos *et al.*,²⁹ about 70% of the fat fraction consists of ω -3 fatty acids.

The chia flour analysed had a higher content of insoluble (191 g kg⁻¹) than soluble (35 g kg⁻¹) fibre. The insoluble fraction results from the high proportion of insoluble pectins, cellulose, hemicellulose and lignin, which have already been described by other authors^{7,30} as the main constituents of the fibre fraction of chia.

Swelling power and solubility

The ability of chia flour to form gels is highly dependent on its swelling power and solubility. These parameters are closely related to the interaction of chia flour constituents with water molecules.¹³ Swelling power and solubility were evaluated at several different temperatures commonly associated with food storage and processing conditions, since water absorption is strongly dependent on the temperature conditions.³¹

The swelling power and solubility of chia flour stirred for 30 min at the studied temperatures are presented in Table 2. The values show that below 50 °C the swelling power and solubility are not significantly dependent on the temperature value. In a previous study, Raymundo *et al.*³² also reported that the swelling capacity and solubility of psyllium husk were influenced by the temperature.

For common flours containing starch, the solubility is a consequence of amylose leaching, which is dependent on the starch

Table 2. Swelling power and solubility of chia flour processed at different temperatures for 30 min

Temperature (°C)	Swelling power (g g ⁻¹)	Solubility (%)
25	19.74 \pm 0.95y	46.48 \pm 4.94a
35	18.76 \pm 1.03y	47.17 \pm 2.70a
50	18.80 \pm 0.53y	49.64 \pm 4.11ab
90	15.68 \pm 1.29x	59.90 \pm 3.55b

Different letters within the same parameter indicate significant differences at $P < 0.05$ level.

gelatinization temperature. However, since chia flour contains no starch,³³ the observed increase in solubility with temperature must result from other phenomena. When chia is soaked in water, the outer layer of the seeds swells into a firm gel layer which is tightly bound to the seeds,¹⁵ and a similar process is observed on chia flour particles. This mucilaginous gel is formed as a result of the chia soluble dietary fibre that is partially expelled from the seed as it comes into contact with water.

The observed increase in chia flour solubility with temperature has been noted by other authors such as Timilsena *et al.*,³⁴ who also mentioned the importance of high solubility of chia in water at ambient temperature to its promising application in food. In Table 2, one can also observe a parallel reduction in swelling power with increasing temperature. It is important to note that, according to the adapted Eqn (2) for non-starch systems, the swelling power is a measure of the water absorption by the insoluble fraction of sediment. This fraction decreases as the solubility increases, resulting from the migration of soluble compounds from the sediment to the outer layer.

In previous studies with psyllium,³² the opposite phenomenon was recorded, i.e. the solubility decreased with increasing temperature from 60 to 95 °C, although the fraction of soluble fibre in psyllium husk is higher than that of insoluble fibre. According to Lai *et al.*,³⁵ the presence of fibre may compete for water, modifying the solubility behaviour.

Effect of thermal treatment conditions on chia gel texture and colour

Preliminary studies (not presented) were performed to define the chia flour critical concentration to obtain a gelled structure. A range of chia flour concentrations from 10 to 150 g kg⁻¹ was tested and it was observed that 100 g kg⁻¹ is the minimum concentration required to obtain a structured material. However, the gel concentration of 130 g kg⁻¹ showed the greatest potential for future application. The impact of thermal treatment (time and temperature) on the texture and colour of this gel was studied (see Tables 3 and 4).

Several authors^{36–38} emphasize the importance of optimizing the time/temperature binomial in order to improve gel structure. The impact of heating temperature in the range 40–90 °C, applied

Table 3. Effect of heating time on textural properties of 130 g kg⁻¹ chia flour gels prepared at 90 °C

Time (min)	Firmness (N)	Adhesiveness (–N s)	Cohesiveness
10	0.053 ± 0.001a	0.040 ± 0.004x	0.489 ± 0.043i
30	0.181 ± 0.016b	0.096 ± 0.007y	0.306 ± 0.023h
60	0.147 ± 0.007c	0.092 ± 0.004y	0.383 ± 0.51hi

Different letters within the same parameter indicate significant differences at $P < 0.05$ level.

for 30 min on 130 g kg⁻¹ chia flour suspensions, on gel texture (firmness, adhesiveness and cohesiveness) is shown in Fig. 1. It can be seen that the gels prepared at 70 and 90 °C showed significantly higher firmness than those prepared at lower temperatures. The gel prepared at 90 °C exhibited the significantly ($P < 0.05$) highest value of adhesiveness and the lowest value of cohesiveness.

It is important to point out that there is a linear relationship of the three texture parameters studied with temperature ($r^2 > 0.9$).

Taking into account the high levels of protein and carbohydrate present in the chia flour studied (Table 1), it is expected that the gelation process is driven by the denaturation of proteins, which can be enhanced by their interaction with polysaccharides. These two phenomena are generally favoured by temperature increase, which can explain the gel formation at high temperature.

Ferry,³⁶ Tobitani and Ross-Murphy³⁹ and Nunes *et al.*⁴⁰ also reported an important dependence of the gel texture on the heating temperature. Increasing temperature induces a greater degree of unfolding of protein molecules and the formation of ordered structures or assembly zones by polysaccharides,⁴¹ leading to the consequent exposure of a greater number of hydrophobic groups, which is reflected in increasing gel firmness and adhesiveness. However, at lower temperatures (<70 °C), a gelled structure was also noticed. In this case, the driving force should be mainly related to mucilaginous polysaccharides that remain available for hydration in the chia flour after milling.³³

Considering the type of structure most suitable for future food development, the temperature of 90 °C seems to be the most appropriate for the chia flour gelation process. This temperature also has advantages in terms of microbiological conservation.

The effect of three heating times, 10, 30 and 60 min, at 90 °C on the textural properties of chia gels was evaluated (Table 3). It can be seen that 30 min processing time yielded a gel with significantly higher firmness ($P < 0.05$) than those prepared with 10 or 60 min processing time. Gels prepared at 90 °C for 30 and 60 min had significantly ($P < 0.05$) higher adhesiveness and lower cohesiveness than the gel processed for 10 min. The optimal thermal conditions for the preparation of a gel with a given texture are strongly dependent on the conformational state of the macromolecules. Excess thermal energy can lead to excessive protein denaturation, which may have a negative impact on gel texture development.^{16,17} To obtain chia gels with interesting characteristics for the development of new food applications, heat treatment at 90 °C for 30 min provides conditions that result in appropriate textural properties for the development of target gel desserts and salty preparations (developed in a parallel work).

In order to evaluate the effect of thermal treatment on the colour of chia gels (130 g kg⁻¹), instrumental colour measurements were performed on suspensions heated for 30 min at different temperatures. Colour parameters L^* , a^* and b^* obtained for different temperatures from 40 to 90 °C are presented in Table 4. In general, L^*

and b^* have values that do not change significantly ($P < 0.05$) with temperature. It can also be seen that the a^* value shows a tendency to increase with temperature. This reveals an increment in the red colour component and can be considered an indicator of a slight change in the colour of the gel matrix as a result of heat treatment.

The effect of heating time (10, 30 and 60 min) on colour parameters of gel prepared at 90 °C was also investigated (Table 4). For this temperature, the gel prepared with a heating time of 10 min exhibited significantly higher ($P < 0.05$) lightness (L^*) than gels prepared with longer processing times. It can be stated that an increase in heating time can induce a slight darkening of the gel. Regarding a^* and b^* , no direct relationship between the variation in these parameters with temperature can be seen.

In all heat treatment conditions studied, the colour changes observed, expressed in terms of ΔE^* (Eqn (3)), range between 3.1 and 7.4, but these variations are only detectable by the naked eye when $\Delta E^* > 5$. However, the range of colorations obtained is relatively neutral, which is desirable for food applications under development, i.e. gelled desserts and salty preparations, since they allow the expression of colour by adding other colouring agents (e.g. fruits or vegetables).

Considering the texture and colour results, the time/heating temperature binomial 30 min/90 °C was used for further work.

Rheological properties of chia gels

The effect of chia flour concentration on gel rheological properties was studied taking into account the different stages of the gelation process: heating, cooling and maturation. The type of structure formed in each case was also evaluated through a mechanical spectrum of the matured gel.

Heating phase

Figure 2A presents the heating curves (2 °C min⁻¹) for 100, 130 and 150 g kg⁻¹ chia flour suspensions. In all cases, the elastic modulus (G') is always higher than the viscous modulus (G'') in the temperature range studied. Even at the beginning of the heating process, at 20 °C, such behaviour is already evident. To explain this, it can be stated that the previous hydration of the suspension (30 min/20 °C) led to the formation of a gel-like structure. Thus, for the studied conditions, the gelling temperature cannot be evaluated, according to the procedure referred to by Winter and Chambon,⁴² based on the cross between G' and G'' .

It should also be noted that, for the highest chia flour concentrations (130 and 150 g kg⁻¹), a considerable increase in viscoelastic moduli is observed in comparison with the gel produced with 100 g kg⁻¹. This increase, which started to be noticeable at 50 °C, should result from a greater uptake of polysaccharide and protein molecules in the gel matrix, leading to the formation of more junction zones.^{43–45} At this temperature, reinforcement of the gel structure started to occur, corresponding to an appreciable increase in solubility and a decrease in swelling power (Table 2). This behaviour must be associated with the ability of soluble compounds of chia flour to contribute to reinforcement of the gel structure.

Cooling curve

After gradual heating (2 °C min⁻¹) of chia suspensions from 20 to 90 °C, samples remained at this temperature for 30 min. Then cooling from 90 to 5 °C at 2 °C min⁻¹ was performed for the three chia flour concentrations under study (Fig. 2B).

Table 4. Colour parameters of 130 g kg⁻¹ chia flour gels obtained by thermal processing at different temperatures for different times

Processing condition	<i>L</i> *	<i>a</i> *	<i>b</i> *	ΔE^*
40 °C/30 min	59.66 ± 1.03a	2.49 ± 0.55z	11.66 ± 1.38mn	–
60 °C/30 min	54.00 ± 0.59bd	3.93 ± 0.14xy	11.61 ± 0.61mn	5.8
70 °C/30 min	55.78 ± 1.80acd	3.43 ± 0.33y	12.95 ± 1.56m	4.2
90 °C/30 min	52.44 ± 0.81bc	3.50 ± 0.09yz	10.20 ± 0.80n	7.4
90 °C/10 min	57.46 ± 1.52ae	4.26 ± 0.13x	12.89 ± 1.11m	3.1
90 °C/60 min	54.23 ± 1.90bde	4.32 ± 0.84xy	13.25 ± 0.95m	5.9

Different letters within the same parameter indicate significant differences at $P < 0.05$ level. ΔE^* was calculated in relation to 40 °C/30 min condition.

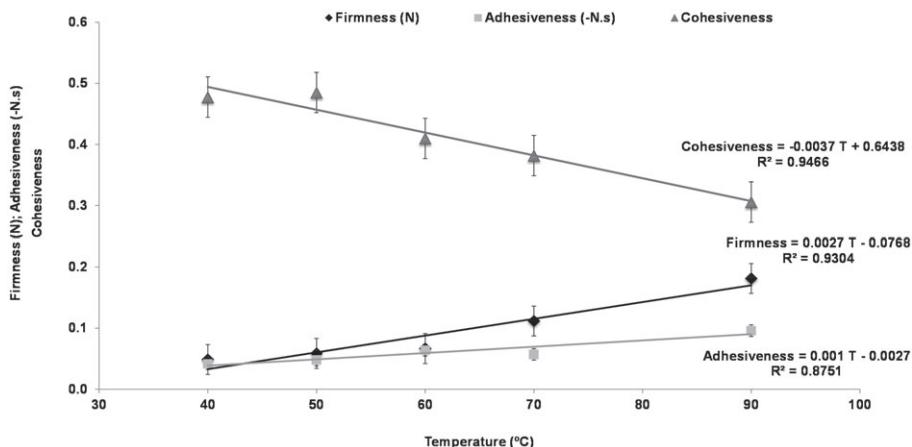


Figure 1. Influence of heating temperature on firmness, cohesiveness and adhesiveness of gels obtained from 130 g kg⁻¹ chia flour suspensions.

It can be seen that, for 130 and 150 g kg⁻¹ chia flour gels, G' and G'' increase sharply with temperature decrease, with no appreciable difference between the two concentrations. There are clearly two regions with different temperature dependence characteristics: (I) up to 50 °C, viscoelastic functions increase gradually with temperature decrease; (II) starting from 50 °C, G' and G'' increase sharply with temperature decrease. These behaviours may be associated with the gelling mechanism of chia flour, i.e. the process associated with the incorporation of molecules with different characteristics in the gel matrix. Thus the incorporation of proteins and polysaccharides in the gel matrix, whose three-dimensional dynamic organization is distinct,⁴⁶ should occur in phase I. After this stage, in phase II, the increase in G' reflects the increase in gel rigidity during cooling, resulting from the formation of intermolecular bonds, especially those from hydrogen bonds between polysaccharides and other components of chia flour (e.g. proteins), and among these and water molecules.¹³

For the 100 g kg⁻¹ sample, there was only a slight increase in viscoelastic functions with temperature decrease, showing a behaviour distinct from that of samples with higher concentration. In this case, the formation of a gelled structure is not evident.

Gel maturation

Considering the curves for the maturation kinetics of systems with 100, 130 and 150 g kg⁻¹ chia flour (Fig. 3), obtained after cooling to 5 °C, it turns out that in all cases the value of G' remains almost constant from 100 min maturation. The maturation of the three gels was studied during the same period in order to highlight major differences between them and to mimic real cooking conditions for preparing gelled desserts. It can also be seen that G'

and G'' increase with chia flour concentration, corresponding to more structured systems. Nevertheless, the time needed to attain the equilibrium state also increases with chia content: it is almost instantaneous for the gels prepared with 100 and 130 g kg⁻¹ chia flour but is longer for the highly concentrated gel (150 g kg⁻¹).

The presented gel maturation kinetics also highlight that from a practical viewpoint, depending on the chia concentration, the time needed for gel stabilization is different. Nevertheless, 2 h is enough to achieve structure stabilization even at the highest concentration. This is the time required to reach the equilibrium state of the gel structure.^{17,47,48} It is an important datum to take into account for planning food product development, as this will be the waiting time required between the preparation of the gel and the attainment of a stable structure. The time of 2 h is relatively short compared with other gelling systems; for example, the maturation time of vegetable protein gels is up to 20 h or more.⁴⁷

Gel structure – mechanical spectra

After the previously described gel maturation at 5 °C, the chia flour gels were subjected to sweep frequency tests within the linear viscoelastic region. Analysing the mechanical spectra obtained (Fig. 4), it is found that, for all chia flour concentrations, the elastic modulus (G') always show higher values than the viscous modulus (G'') over the frequency range studied. In all cases, a dependence of viscoelastic moduli on frequency is observed, showing a pattern generally associated with a weak gel-like structure.⁴⁹

It should also be noted that, for the gel with 100 g kg⁻¹ chia flour, there is a tendency for a crossing of G' and G'' at high oscillation

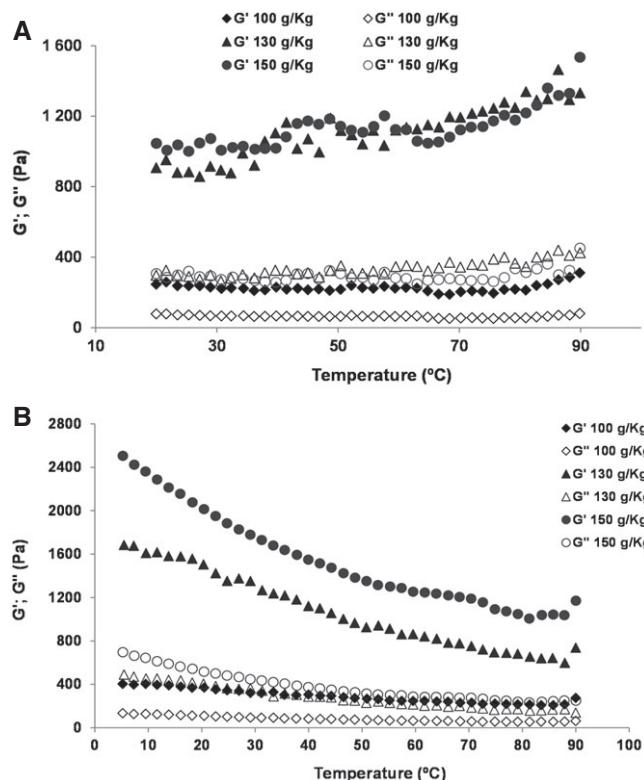


Figure 2. (A) Heating curves from 20 to 90 °C of 100, 130 and 150 g kg⁻¹ chia flour suspensions. (B) Cooling curves of 100, 130 and 150 g kg⁻¹ chia flour gels obtained after the heating process and a stage of 30 min at 90 °C. G' , storage modulus; G'' , loss modulus.

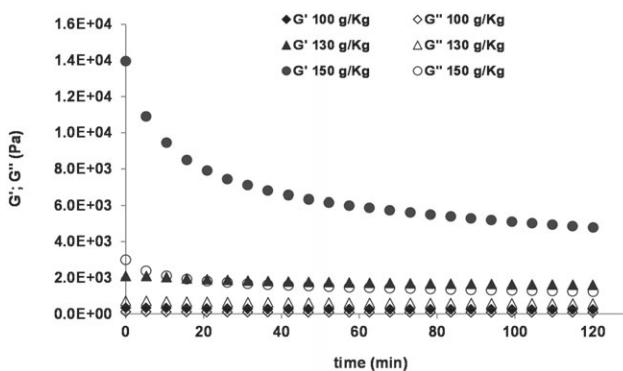


Figure 3. Maturation curves of gels prepared from 100, 130 and 150 g kg⁻¹ chia flour suspensions. G' , storage modulus; G'' , loss modulus.

frequency, which is associated with a certain weakness of the gel structure.

The increasing of G' and G'' with concentration results from a more complex structural organization of biopolymers and the intensification of their intermolecular bonds, associated with the production of firmer gels. Similar behaviour was obtained for the concentration effect of other biopolymers on gel structure.⁴⁹

Several authors (e.g. Batista *et al.*⁴⁸) have reported that the gel cooling rate has a direct influence on its level of structure, determining the way in which molecules become balanced in the gel matrix, which may have not only sensory and textural implications but also stability ones.⁵⁰

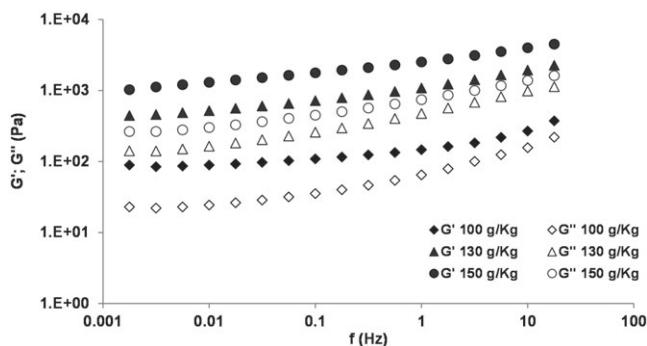


Figure 4. Frequency sweep tests of 100, 130 and 150 g kg⁻¹ chia flour gels obtained after maturation at 5 °C. G' , storage modulus; G'' , loss modulus.

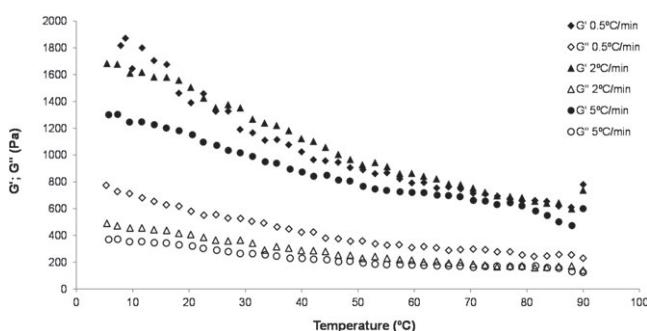


Figure 5. Cooling curves of 130 g kg⁻¹ chia flour gels cooled at different rates. G' , storage modulus; G'' , loss modulus.

The effect of cooling rate (0.5, 2 and 5 °C min⁻¹) was investigated for gels prepared with 130 g kg⁻¹ chia flour. These gels were subjected to the same treatment in terms of heating and maturation as described above. The resulting cooling curves are summarized in Fig. 5. It can be seen that, as the cooling rate increases, lower values of G' and G'' are obtained, corresponding to less structured systems. However, the shapes of the curves are very similar, which indicates that the gel matrix consolidation process is not substantially altered by the cooling rate. Similar results were described by Hesarinejad *et al.*,⁵¹ who studied the gelation process of clapping pepperweed seeds and observed that G' also decreased with increasing cooling rate, indicating that molecules did not have enough time to develop a firm network. The cooling time is directly related to the magnitude of the structural molecular bonds of the gelled system. Thereby slower cooling processes are generally associated with more organized molecular structures and more stable connections.¹⁷

CONCLUSIONS

Chia flour has an interesting nutritional composition related to its high protein, fat and fibre content, which confers on this ingredient good potential for use in several food applications involving gelation, with positive impact on health.

With chia flour concentrations of 130 g kg⁻¹ or higher, it is possible to obtain gels with structural features interesting for use in diverse food applications. Based on textural analysis and colour measurements, the optimal time/heating temperature binomial was found to be 30 min/90 °C.

The obtained gels have a short maturation time, which effectively allows optimization of the time between preparation and

consumption of the gelled food. The cooling rate influences the final system structure: more structured gels can be obtained by conducting a slower cooling process.

REFERENCES

- 1 Muñoz LA, Cobos A, Diaz O and Aguilera JM, Chia seed (*Salvia hispanica*): an ancient grain and a new functional food. *Food Rev Int* **29**:394–408 (2013).
- 2 EFSA, Opinion on the safety of 'Chia seeds (*Salvia hispanica* L.) and ground whole Chia seeds' as a food ingredient. *EFSA J* **996**:1–26 (2009).
- 3 Ixtaina VY, Nolasco SM and Tomás MC, Physical properties of chia (*Salvia hispanica* L.) seeds. *Ind Crops Prod* **28**:286–93 (2008).
- 4 Marineli RDS, Moraes EA, Lenquiste SA, Godoy AT, Eberlin MN and Maróstica Jr MR, Chemical characterization and antioxidant potential of Chilean chia seeds and oil (*Salvia hispanica* L.). *LWT – Food Sci Technol* **59**:1–7 (2014).
- 5 Segura-Campos MR, Ciau-Solís N, Rosado-Rubio G, Chel-Guerrero L and Betancur-Ancona D, Physicochemical characterization of chia (*Salvia hispanica*) seed oil from Yucatán, México. *Agric Sci* **5**:220–226 (2014).
- 6 Coelho MS and Salas-Mellado MM, Chemical characterization of chia (*Salvia hispanica* L.) for use in food products. *J Food Nutr Res* **2**:263–269 (2014).
- 7 Capitani MI, Spotorno V, Nolasco SM and Tomás MC, Physicochemical and functional characterization of by-products from chia (*Salvia hispanica* L.) seeds of Argentina. *LWT – Food Sci Technol* **45**:94–102 (2012).
- 8 Ayerza R and Coates W, Composition of chia (*Salvia hispanica*) grown in six tropical and sub-tropical ecosystems of South America. *Trop Sci* **44**:131–135 (2004).
- 9 Ayerza R, Effects of seed color and growing locations on fatty acid content and composition of two chia (*Salvia hispanica* L.) genotypes. *J Am Oil Chem Soc* **87**:1161–1165 (2010).
- 10 Ixtaina VY, Martínez ML, Spotorno V, Mateo CM, Maestri DM, Diehl BWK *et al.*, Characterization of chia seed oils obtained by pressing and solvent extraction. *J Food Compos Anal* **24**:166–174 (2011).
- 11 Sandoval-Olivero MR and Paredes-López O, Isolation and characterization of proteins from chia seeds (*Salvia hispanica* L.). *J Agric Food Chem* **61**:193–201 (2013).
- 12 Ali NM, Yeap SK, Ho WY, Beh BK, Tan SW and Tan SG, The promising future of chia, *Salvia hispanica* L. *J Biomed Biotechnol* **2012**:171956 (2012).
- 13 Muñoz LA, Cobos A, Diaz O and Aguilera JM, Chia seeds: microstructure, mucilage extraction and hydration. *J Food Eng* **108**:216–224 (2012).
- 14 Salgado-Cruz MP, Calderón-Domínguez G, Chanona-Pérez J, Farrera-Rebollo RR, Méndez-Méndez JV and Díaz-Ramírez M, Chia (*Salvia hispanica* L.) seed mucilage release characterisation. A microstructural and image analysis study. *Ind Crops Prod* **51**:453–462 (2013).
- 15 Lin K-Y, Daniel JR and Whistler RL, Structure of chia seed polysaccharide exudate. *Carbohydr Polym* **23**:13–18 (1994).
- 16 Torres MD, Fradinho P, Raymundo A and Sousa I, Thermorheological and textural behaviour of gluten-free gels obtained from chestnut and rice flours. *Food Bioprocess Technol* **7**:1171–1182 (2014).
- 17 Nunes MC, Raymundo A and Sousa I, Rheological behaviour and microstructure of pea protein/k-carrageenan/starch gels with different setting conditions. *Food Hydrocolloids* **20**:106–113 (2006).
- 18 AOAC, *Official Methods of Analysis*. AOAC International Method 935.29, 16th edition, Washington, DC (1998).
- 19 Instituto Português da Qualidade, *Cereais e Leguminosas. Determinação do Teor de Cinza*. Processo por Incineração a 550 °C. NP 518 (1986).
- 20 Instituto Português da Qualidade, *Cereais e Derivados. Determinação do Teor de Matéria Gorda Total*. NP 4168 (1991).
- 21 International Organization for Standardization, *Cereals and Pulses – Determination of the Nitrogen Content and Calculation of the Crude Protein Content – Kjeldahl Method*. ISO 20483 (2006).
- 22 Tkachuk R, Nitrogen to protein conversion factors for cereals and oilseed meals. *Cereal Chem* **46**:419–423 (1969).
- 23 Prosky L, Asp NG, Schweizer TF, DeVries JW and Furda I, Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. *J Assoc Off Anal Chem* **71**:1017–1023 (1988).
- 24 Leach HW, McCowen ID and Schoch TJ, Structure of the starch granule. I. Swelling and solubility patterns of various starches. *Cereal Chem* **36**:534–544 (1959).
- 25 Batista P, Nunes MC and Sousa I, Physical characterisation of commercial dairy desserts, in *Progress in Rheology: Theory and Applications*, ed. by Boza FM, Guerrero A, Partal P, Franco J and Munoz J. Publicaciones Digitales, Sevilla, Spain, pp. 449–452 (2002).
- 26 Castellar MR, Obón JM and Fernández-López JA, The isolation and properties of a concentrated red-purple betacyanin food colourant from *Opuntia stricta* fruits. *J Sci Food Agric* **86**:122–128 (2006).
- 27 Vázquez-Ovando JA, Rosado-Rubio JG, Chel-Guerrero LA and Betancur-Ancona DA, Dry processing of chía (*Salvia hispanica* L.) flour: chemical characterization of fiber and protein. *CyTA J Food* **8**:117–127 (2010).
- 28 Segura-Campos MR, Ciau-Solís N, Rosado-Rubio G, Chel-Guerrero L and Betancur-Ancona D, Chemical and functional properties of chia seed (*Salvia hispanica* L.) gum. *Int J Food Sci* **2014**:241053 (2014).
- 29 Segura-Campos MR, Salazar-Vega IM, Chel-Guerrero LA and Betancur-Ancona DA, Biological potential of chia (*Salvia hispanica* L.) protein hydrolysates and their incorporation into functional foods. *LWT – Food Sci Technol* **50**:723–731 (2013).
- 30 Vázquez-Ovando A, Rosado-Rubio G, Chel-Guerrero L and Betancur-Ancona DA, Physicochemical properties of a fibrous fraction from chia (*Salvia hispanica* L.). *LWT – Food Sci Technol* **42**:168–173 (2009).
- 31 Moreira R, Chenlo F, Prieto DM and Torres MD, Water adsorption isotherms of chia (*Salvia hispanica* L.) seeds. *Food Bioprocess Technol* **5**:1077–1082 (2012).
- 32 Raymundo A, Fradinho P and Nunes MC, Effect of Psyllium fibre content on the textural and rheological characteristics of biscuit and biscuit dough. *Bioact Carbohydr Diet Fibre* **3**:96–105 (2014).
- 33 Goh KKT, Matia-Merino L, Chiang JH, Quek R, Soh SBJ and Lentle RG, The physico-chemical properties of chia seed polysaccharide and its microgel dispersion rheology. *Carbohydr Polym* **149**:297–307 (2016).
- 34 Timilsena YP, Adhikari R, Kasapis S and Adhikari B, Molecular and functional characteristics of purified gum from Australian chia seeds. *Carbohydr Polym* **136**:128–136 (2016).
- 35 Lai P, Li KY, Lu S and Chen HH, Physicochemical characteristics of rice starch supplemented with dietary fibre. *Food Chem* **127**:153–158 (2011).
- 36 Ferry JD, Protein gels. *Adv Protein Chem* **4**:1–78 (1948).
- 37 Arntfield SD, Murray ED and Ismond MAH, Effect of salt on the thermal stability of storage proteins from fababean (*Vicia faba*). *J Food Sci* **51**:371–377 (1986).
- 38 Batista AP, Portugal CM, Sousa I, Crespo JG and Raymundo A, Assessing gelling ability of vegetable proteins using rheological and fluorescence techniques. *Int J Biol Macromol* **36**:135–143 (2005).
- 39 Tobitani A and Ross-Murphy SB, Heat-induced gelation of globular proteins. 1. Model for the effects of time and temperature on the gelation time of BSA gels. *Macromolecules* **30**:4845–4854 (1997).
- 40 Nunes MC, Raymundo A and Sousa I, Gelled vegetable desserts containing pea protein, *k*-carrageenan and starch. *Eur Food Res Technol* **222**:622–628 (2006).
- 41 Vries J, Hydrocolloid gelling agents and their applications, in *Gums and Stabilisers for the Food Industry*, ed. by Williams PA and Phillips GO. Royal Society of Chemistry, Cambridge, pp. 23–31 (2004).
- 42 Winter HH and Chambon F, Analysis of linear viscoelasticity of a crosslinking polymer at the gel point. *J Rheol* **30**:367–382 (1986).
- 43 Verheul M, Roefs SPFM, Mellema J and Kruif KG, Power law behavior of structural properties of protein gels. *Langmuir* **14**:2263–2268 (1998).
- 44 Mellema M, Walstra P, van Opheusden JJJ and van Vliet T, Effects of structural rearrangements on the rheology of rennet-induced casein particle gels. *Adv Colloid Interface Sci* **98**:25–50 (2002).
- 45 Renkema JMS and van Vliet T, Heat-induced gel formation by soy proteins at neutral pH. *J Agric Food Chem* **50**:1569–1573 (2002).
- 46 Van Vliet T, Martin AH and Bos MA, Gelation and interfacial behaviour of vegetable proteins. *Curr Opin Colloid Interface Sci* **7**:462–468 (2002).
- 47 Nunes MC, Batista P, Raymundo A, Alves MM and Sousa I, Vegetable proteins and milk puddings. *Colloids Surf B* **31**:21–29 (2003).

48 Batista AP, Nunes MC, Fradinho P, Gouveia L, Sousa I, Raymundo A *et al.*, Novel foods with microalgal ingredients – effect of gel setting conditions on the linear viscoelasticity of *Spirulina* and *Haematococcus* gels. *J Food Eng* **110**:182–189 (2012).

49 Farahnaky A, Askari H, Majzoobi M and Mesbahi G, The impact of concentration, temperature and pH on dynamic rheology of psyllium gels. *J Food Eng* **100**:294–301 (2010).

50 Nunes MC, *Interacções proteína/polissacárido em géis proteicos de origem vegetal*. Master's thesis, Instituto Superior de Agronomia, Universidade Técnica de Lisboa (2005).

51 Hesarinejad MA, Koocheki A and Razavi SMA, Dynamic rheological properties of *Lepidium perfoliatum* seed gum: effect of concentration, temperature and heating/cooling rate. *Food Hydrocolloids* **35**:583–589 (2014).