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RHEOLOGICAL CHARACTERIZATION OF GUMS-GEL OBTAINED FROM THE PROTEIC ISOLATE OF SESAME (*Sesamum indicum*)

CARACTERIZACIÓN REOLÓGICA DE GOMAS-GEL OBTENIDAS A PARTIR DE AISLADO PROTÉICO DE SÉSAMO (*Sesamum indicum*)

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Abstract
Sesame is produced in large amount around the world and could offer multiple alternatives for their use in industry. In this work were studied the obtaining and characterisation of protein isolate of sesame (*Sesamum indicum*) for the rheological studies of a blend product of protein/polysaccharide gel type with the incorporation of food hydrocolloids. Sesame seeds were ground to obtain oil and flour rich in proteins; this one was solubilized separating the flour paste by centrifugation, chemical composition was determined through a bromatological analysis and the rheological behaviour was studied by dynamic tests. Later, the concentration by isoelectric precipitation was made adjusting the pH (4-5), using a factorial design of 2^3 and three control samples for the elaboration of gums-gel, with xanthan gum and carrageenan. It was found an isolated with a concentration of 80.27% of proteins. Regarding formulations that had higher viscosity values were those that contained lower protein content and higher content of gums. Concerning dynamic viscoelastic tests, the different formulations of the product have an elastic behaviour rather than a viscous behaviour, being a gel-like product.
Keywords: sesame flour, protein isolate, gums-gel, bromatological analysis, rheological study.

Resumen
El sésamo se produce en gran cantidad en todo el mundo y podría ofrecer múltiples alternativas para su uso en la industria. En este trabajo se estudió la obtención y caracterización de proteína aislada de sésamo (*Sesamum indicum*) para el estudio reológica de un producto de mezcla de proteína / polisacárido tipo gel con la incorporación de hidrocoloides alimentarios. Las semillas de sésamo fueron molidas para obtener aceite y harina rica en proteínas; esta se solubilizó separando la pasta de harina por centrifugación, la composición química se determinó mediante un análisis bromatológico y se estudió el comportamiento reológico mediante pruebas dinámicas. Posteriormente, se realizó la concentración por precipitación isoeléctrica ajustando el pH (4-5), utilizando un diseño factorial de 2^3 y tres muestras de control para la elaboración de gomas-gel, con goma de xantana y carragenina. Se encontró un aislado con una concentración de 80.27% de proteínas. Con respecto a las formulaciones que tenían valores de viscosidad más altos, aquellos que contenían un menor contenido de proteínas y un mayor contenido de gomas. En cuanto a las pruebas viscoelásticas dinámicas, las diferentes formulaciones del producto tienen un comportamiento elástico en lugar de un comportamiento viscoso, siendo un producto similar a un gel.
Palabras clave: harina de sesamo, aislado de proteína, gomas-gel, análisis bromatológico, estudio reológica.

1 Introduction

Sesame (*Sesamum indicum*) is one of the most important oilseeds cultivated principally in Asia and Africa since centuries, because of its high content of edible oil and protein (Abou-Gharbia, 2000; Kapoor *et al*., 2015). Sesame seed is considered a vital source of protein and is one of the first crops processed for oil production. Within its non-food applications, the use in soaps, cosmetics, lubricants and medicines are remarkable. Sesame seeds oil possess sesamin and sesamolin which have a cholesterol-lowering effect in humans and to prevent high blood pressure. The oil has multiple medical and pharmaceutical applications. It is mildly laxative, emollient and demulcent (Anilakumar, 2010).

The sesame (*Sesamum indicum*) flour is a

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by-product obtained after oil extraction with a composition of 7.92% moisture, 27.83% fat, 30.56% protein (albumin 8.6%, globulin 67.3%, prolamin 1.4% and glutelin 6.9%), 6.22% fibre, 5.27% ash and 28.14% carbohydrates. The extraction of oil leads to an increase in the protein content of defatted sesame flour (41.15-49.58%) (Onsaard *et al*., 2010). Sesame proteins have been extracted by various methods, alkaline or salt extraction and isoelectric precipitation (pI) (Cano *et al*., 2011). Protein isolate has a low bulk density (0.71 g/mL) because it has a low amount of carbohydrates that increase the bulk density of the system (Krause *et al*., 2002).

Food hydrocolloids are known for their properties as thickening agents and gelling agents because they can form dispersions and/or viscous gels when dispersed in water, they can modify the rheology of a system including its viscosity and texture. The presence of a large number of hydroxyl (-OH) groups and other groups like sulphates (carrageenan gum) significantly increases their affinity for binding water and generate chemical interactions with other molecules (Dipjyoti and Suvendu, 2010; Li and Nie, 2016; Sánchez-Paz *et al*., 2018; Hernández-Téllez *et al*., 2018). These hydrocolloids tend to be polysaccharides or proteins which could improve stability in emulsions as was reported by Hernández-Rodríguez *et al*. (2017).

The interactions of protein-polysaccharide are of interest in a variety of scientific areas (López-Hernández *et al*., 2018). These interactions are found in many biological applications in foods, cosmetics, and pharmaceutical industries. The specific functionality of biopolymers can be enhanced through synergistic interactions between them, with implications for the stability, texture, and shelf life of food products (Pasquel, 2001). The use of these blends is gaining importance due to their synergistic interactions that offer the possibility of controlling or improving the texture of food, as well as modifying the organoleptic and rheological behaviour of the final product (Capitani, 2007; Sánchez-Paz *et al*., 2018). Rheology includes two basic properties flow behaviour (viscosity) and mechanical properties as a solid (texture), which invariably changes the sensory properties of the final product (Reyes *et al*., 2018; Sánchez-Paz *et al*., 2018). Rheology studies the relationship between stress and deformation in materials that are capable of flowing (Hoyo *et al*., 2012). The dynamic tests allow to obtain the mechanical spectrum of a gel through the variation of storage modulus (G') and loss modulus (G") depending on the frequency. Thus, from a rheological point of view, a gel is a viscoelastic material for which G' is greater than G" as a function of frequency (Romero, 2008). This work aimed to obtain and characterize a sesame isolated protein (*Sesamum indicum*) for the rheological study of a blend protein/polysaccharide gel product with the incorporation of food hydrocolloids.

2 Materials and methods

2.1 Obtaining sesame flour

Sesame seeds were purchased in the municipality of Carmen de Bolívar (Colombia). In the first place, they were cleaned by sieving removing stones and other debris. Then were selected the seeds with the best size and shape. After, seeds were milled using an electric mill (Cuisinart Prata Dbm-8). Subsequently, the oil extraction process was carried out in a hydraulic press. The separated oil was drained and collected in a container. In this stage of the process, partially defatted sesame flour rich in protein was obtained and separate for the subsequent utilization.

2.2 Obtaining sesame protein

The defatted sesame flour from mechanically pressed seeds was solubilized with water and 4.0 M NaCl in a ratio of 1:10 (w: v) to pH 7. The solubilization step was carried out under constant stirring for 90 min. After, the solubilized protein was separated from the flour paste by a centrifugation process at 3800 rpm for 15 min. Protein concentration was then carried out by isoelectric precipitation adjusting the pH of the solution with HCl to the isoelectric point (pH 4-5) using a Hanna HI 9124 pH-meter potentiometer. Finally, the suspensions were centrifuged at 3.800 rpm for 15 min. The precipitates were washed with an excess of water and dried at 60 °C.

2.3 Bromatological analysis

Water content, ethereal extract, ash and protein tests were determined on both the flour and the protein isolate using techniques described by the Association of Official Analytical Chemists.

2.4 *Ethereal extract*

Soxhlet equipment with petroleum ether was used for determination of ethereal extract. The sample was put into a filter paper inside the equipment, and the solvent was refluxed for 6 hours. Then the solvent was recovered by evaporation, and the ethereal extract was calculated by weight difference.

2.5 *Crude protein*

Crude protein was determined by the Kjeldahl method. Sample (10.00 g) was put in contact with sulfuric acid (H_2SO_4) in a Kjeldahl flask. The flask was heated until it became dark and neutralised with sodium hydroxide (NaOH). Subsequently, it was titrated with hydrochloric acid (HCl), and the crude protein was calculated by Equation 1.

$$\%P = \frac{(mLHCl \times NHCl \times 0.014 \times 6.25 \times 100)}{5} \quad (1)$$

2.6 *Moisture content*

Moisture content was carried out by drying in an oven at 60°C for 10h. Subsequently, the sample was placed in a desiccator, and its dry weight was taken.

2.7 *Ashes*

The ash content was determined by incinerating 5.00 g of sample in a muffle at 550ºC for 4 h.

2.8 *Design and formulation of sesame protein gums-gel*

Several formulations were studied to evaluate the influence of solids concentration on the rheological behaviour of sesame protein and its blend with two food gums. A factorial design of 2^3 with three factors and two levels was used and three control formulations were considered for a total of 11 formulations. The three factors chosen were xanthan gum, carrageenan and sesame protein with two levels for each one (low and high). Table 1 shows the experimental design, which was carried out using the Software Design Expert 9 for Windows.

2.9 *Elaboration of sesame protein gums-gel*

In order to evaluate the interaction of food gums and proteins in the elaboration of gels with application in the food industry, eight formulations and three control samples were elaborated from sesame protein, xanthan gums, carrageenan and distilled water as shown in Table 2.

The aqueous dispersions were prepared at 87°C for 45 min according to the technique described by Shimada and Cheftel (1998).

2.10 *Rheological behaviour*

Oscillatory shear tests of samples were carried out 48h after their elaboration using a Haake Mars 60 Advanced Modular Rheometer System. For this, shear stress was applied from 0.001 Pa to 1000 Pa at 1Hz, and the viscoelastic interval was determined. Subsequently, frequency sweeps were performed to obtain the mechanical spectrum applying a stress value within the linear viscoelastic interval in a frequency range between 10-2 and 102 rad/s at 25°C. The parameters obtained were the storage modulus (G'), loss modulus (G") and complex viscosity ($|\eta^*|$).

Table 1. Studied formulations.

Treatments	Xanthan gum	Carrageenan	Sesame Protein
1	1	0.5	10
2	0.5	1	5
3	0.5	0.5	5
4	1	0.5	5
5	0.5	0.5	10
6	1	1	5
7	1	1	10
8	0.5	1	10
Control A	1	—	10
Control B	—	1	10
Control C	1	1	—

Table 2. Experimental design for characterizations of gums-gel.

Formulations	Gum-gel (g)	Sesame protein (g)	Xanthan gum (g)	Carrageenan (g)	Water (g)
F1	100	10	1	0.5	88.5
F2	100	5	0.5	1	93.5
F3	100	5	0.5	0.5	94
F4	100	5	1	0.5	93.5
F5	100	10	0.5	0.5	89
F6	100	5	1	1	93
F7	100	10	1	1	88
F8	100	10	0.5	1	88.5
Control A	100	10	1	—	89
Control B	100	10	—	1	89
Control C	100	—	1	1	98

Table 3. Mean values and standard deviation of bromatological analysis of sesame flour (dry basis).

Sample	Moisture content (%)	Ash (%)	Ethereal extract (%)	Carbohydrates (%)
Defatted sesame flour	7.31±0.70	5.96±0.13	4.87±0.97	24.28±0.85
		Proteín		
Sesame flour		Defatted sesame flour		
35.65±1.23		51.31±0.87		

Dry basis

3 Results and discussion

3.1 Bromatological analysis of sesame flour

Table 3 shows the average values of moisture content, protein, ash, fat and carbohydrates obtained from both sesame flour without defatting and defatted sesame flour. The defatted sesame flour had a composition of 7.31% of moisture, 4.87 of fat, 51.31 of protein, 5.96% of ash and 24.28 of carbohydrates. The percentage of fat is higher than other results reported by Gandhi and Srivastava (2007), Essa *et al*., (2015) and Onsaard *et al*., (2010), who found that the fat content was 1.10%, 1.16% and 1.49%, respectively. It might be because of the pressing process used was artisanal, and its efficiency is limited. On the other hand, the flour contains a high concentration of proteins and carbohydrates according to Essa *et al*. (2015; Onsaard *et al*. (2010). The protein content in the defatted sesame flour was 15% higher than non-defatted flour. Similar results were reported by Inyang and Iduh (1996), who found that the protein content increased from 24.1% in sesame seeds to 59.7% in defatted sesame flour. Onsaard *et al*. (2010) reported that the protein content in the defatted and non-defatted flour was 41.15 and 30.56%, respectively. Similarly, Brewer *et al*. (2016) reported that the percentage of defatted sesame flour was 55 ± 0.05%.

Defatted flour contained 24.28% of carbohydrate. Similar values were reported by Gandhi and Srivastava (2017) with 26.84% and by Inyang and Iduh (1996) with 28.2%. In the same way, other authors found higher carbohydrate content as Onsaard *et al*., (2010) with 49.02% and Essa *et al*., (2015) with 41.74%. It must be considered that these results vary depending on the variety of sesame seed used. The high protein content of this by-product, higher than 50%, making it one as an attractive raw material for the preparation of products riches in proteins, such as concentrates, isolates and hydrolysates.

In general, the chemical composition agrees with the results presented by other works that used similar raw materials. Karr-lilienthal *et al*. (2006) evaluated the quality of soybean meal, and the percentage of protein was approximately 55.66%; González-Pérez (2002) carried out the isolation and characterisation of sunflower proteins (Helianthus annuus) reporting 26%; Vioque *et al*. (2001) also worked with oleaginous seeds in the obtaining and applications of protein isolates of chickpea (*Cicer*

Table 4. Mean values and standard deviation of bromatological analysis of isolated sesame protein (dry basis).

Sample	Moisture content (%)	Protein (%)	Ethereal extract (%)	Ash (%)	Carbohydrates (%)
Sesame isolated protein	49.82±0.11	80.27±0.12	2.53±0.22	2.81±0.31	5.58±0.28

Dry bases

arietinum) and sunflower (*Helianthus annuus*) finding protein percentages of 22.7 and 31.2%, respectively.

3.2 Bromatological analysis of isolated sesame protein

Table 4 shows the average and standard deviation values of moisture content, protein, ash, fat and carbohydrates obtained for the sesame protein isolate. The sesame protein isolate has a composition of 49.82% of moisture, 2.53% of fat, 80.27% of protein, 2.81% of ash and 5.58% of carbohydrates. The fat percentage of the protein isolate was higher than that found by Onsaard *et al*. (2010) with 0.51% and by Essa *et al*. 2015 with 0.26%. The percentages of carbohydrates and ash were similar than those found by Essa *et al*., (2015) with 4.50% and 2.8% respectively for isolated and flour of sesame. These results agree with those found by Vioque *et al*. (2001) for chickpea and sunflower protein. On the other hand, Onsaard *et al*. (2010); Essa *et al*. (2015) and Brewer *et al*. (2016) report a moisture content between 7.17% and 7.79%, presenting a significant difference with those reported in this work, it might due because of the lyophilisation process used (Ramírez, 2006).

A protein percentage higher than 80% was obtained. These results are similar to those reported by Brewer *et al*. (2016) with values of 80.43% and Onsaard *et al*. (2010) with 75.49%. The concentration of NaCl affects protein concentration (Brewer *et al*., 2016). This fact is due because of the salt ions interact with charged groups of proteins and decreases their electrostatic attraction among protein molecules promoting the flocculation (Hall, 1996). This effect results from the competition between the proteins and the salt ions for the water molecules necessary for their dissolution. At high salt concentrations, there are not enough water molecules available for the protein dissolution, since most of the water molecules are strongly bound to the salt ions. In this way, protein-protein interactions are stronger than protein-water interactions (AchourI *et al*., 2012).

As was expected, the other components of sesame protein isolate decreased respect to the sesame flour. This fact happens because the process of obtaining protein isolates involves steps aimed at eliminating or reducing the non-protein components like fibre, sugars, phenols and lipids. In the first stage, the proteins are solubilised for separating them from the rest of the insoluble compounds, mainly carbohydrates (Vioque *et al*., 2001). In the second stage occurs the concentration of proteins and especially their purification. To do this, isoelectric precipitation and subsequent separation by centrifugation are necessaries (Gonçalves *et al*., 1997). The isolate is recovered in the form of a concentrated insoluble gel. After, it is washing with water, centrifugate and dried.

The elimination of some non-protein compounds is essential because the sugars could produce a loss of protein quality through the Maillard reactions (Davies *et al*., 1998), producing a decrease in the contents of lysine, methionine and tryptophan. Also, the presence of lipids is very detrimental to the final product giving rise to problems of rancidity during the processing and storage (Millán *et al*., 1995).

3.3 Rheological study of gums-gel

The gels obtained were opaque, characteristic of aggregate or particulate gels. This type of gels is formed when the flocculates have a macroscopic size and disperse the light and some regions with high concentration are organized in networks. Similar results were found by Spahn *et al*. (2006), who evaluated the viscoelastic and textural properties of blend gels of whey protein and carrageenan. The carrageenan could be used as an effective crosslinking agent among proteins and other molecules (Elgadiry *et al*., 2015; Hernández-Téllez *et al*., 2018).

The formulations with higher consistency were CC and F6, and those with lower consistency were F5 and F1. The main difference among formulations was protein concentration. According to Lupano *et al*. (1992), the concentration of proteins is directly related to the firmness of the gels and could be influenced by the presence of gums or other components.

The formulation CB presented phase separation compared with the other formulations. Its rheological behaviour could not be determined since it presented a rupture of the structure. Several factors could determine this phenomenon, the first one is that the formulation did not have xanthan gum, indicating that this hydrocolloid can exert a strong synergic action with the other components. Similar results were reported by Suarez *et al*. (2012), who evaluated the influence of xanthan gum and guar gum on the rheological properties of cocoa-flavoured milk. This formation of separate phases is influenced by intrinsic and extrinsic factors, being the nature of the polysaccharide the factor that most strongly affects the separation of phases in polysaccharide/protein systems, as well as the molecular weight, load, the degree of branching, among others (Tolstoguzov, 1997).

3.4 Dynamic viscoelastic tests

The sweeps of shear stress obtained are shown in Fig. 1 representing the behaviour of the elastic and viscous modulus (G' and G" respectively) on the applied shear stress (τ) at 1 Hz. At the moment that the values of G' and G" decreased noticeably, it was considered that the gel has broken and does not support a higher shear stress.

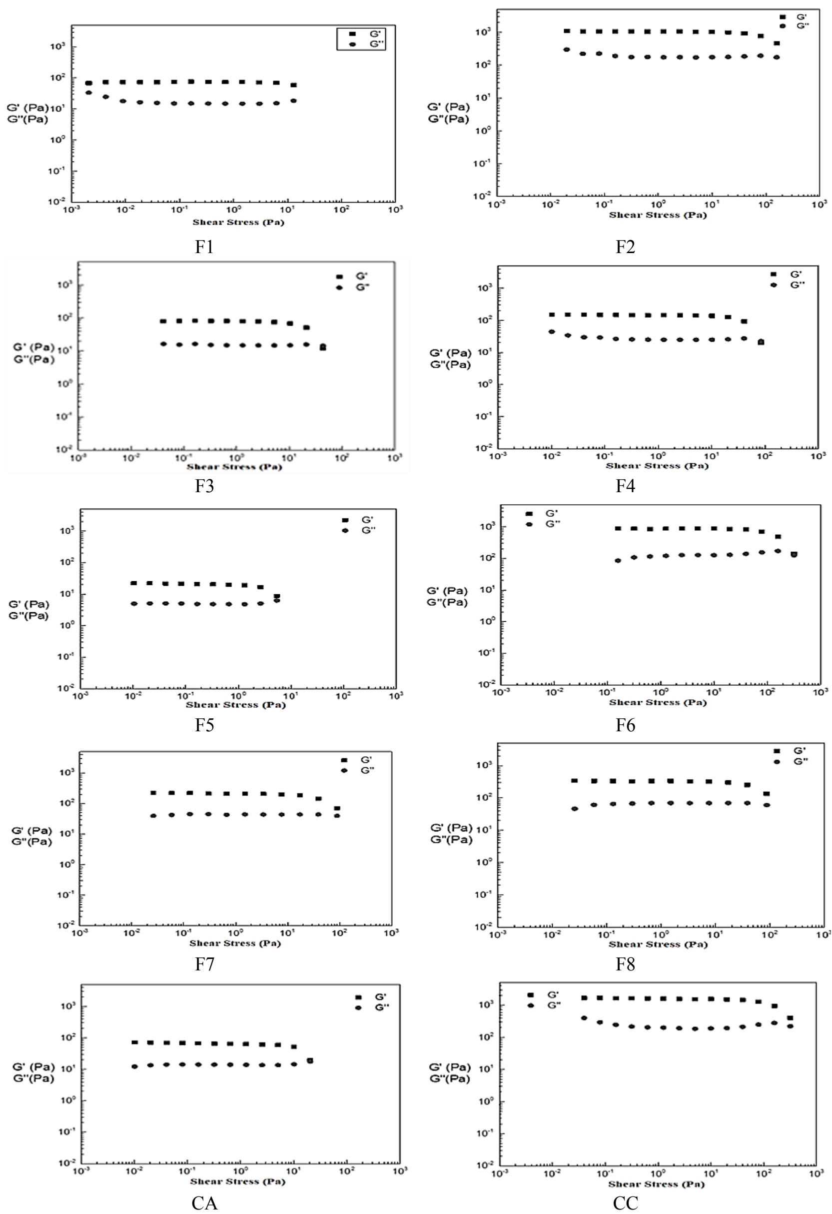

Fig. 1. Elastic modulus (G') and Viscous modulus (G") as a function of the Shear Stress at 25°C of the studied formulations

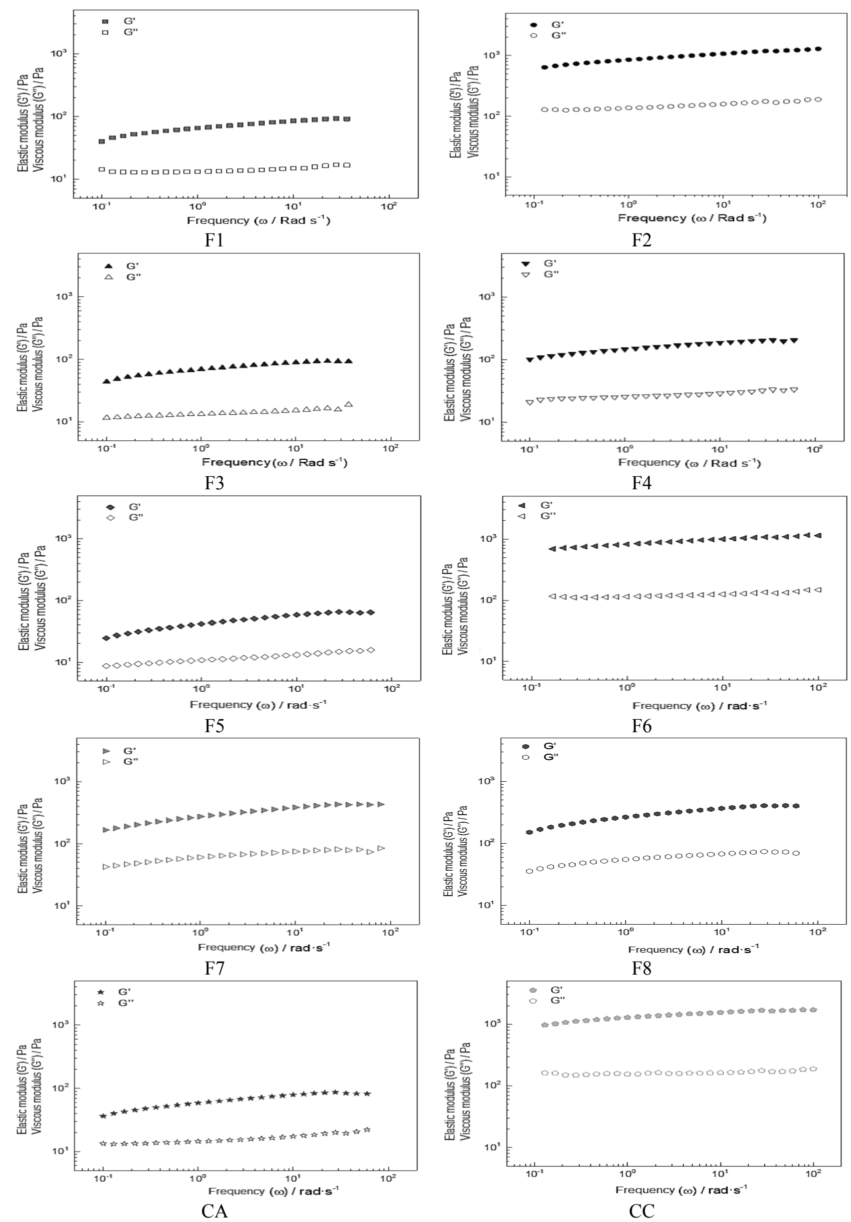

Fig. 2. Elastic modulus (G') and Viscous modulus (G") as a function of the frequency at 25°C of the studied formulations

The samples presented the linear viscoelastic region in a range of 0.001 to 30.0 Pa, then shear stress of 10 Pa was used in order to perform the frequency sweep tests from 0.1 to 100 Hz at 25°C (Fig. 2).

It is observed that the formulations F6 and CC achieve the highest shear stress before breaking. On the other hand, the F5 gum-gel was the weakest supporting an effort of 1 Pa; similar results were reported by Ibáñez (2013) in formulations with reduced capacity to form gel structure. Fig. 2 shows the mechanical spectra of the samples concerning G' and G" as a function of the angular frequency (rad/s) at 25°C for the studied formulations. In all cases, the storage modulus (G') and loss modulus (G") increased as the frequency increased, and it is also observed that the values of G' are higher than those of G", showing an elastic behaviour rather than viscous behaviour. Similar results have been reported for a blend of whey, flour and honey (Yamul, 2008) and a blend of whey protein and dextran (Spotti, 2013).

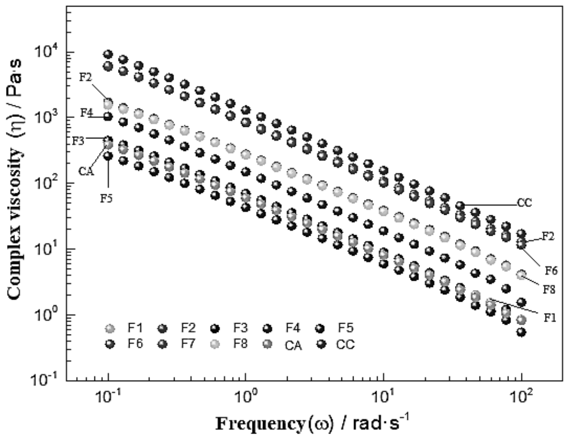

Fig. 3. Complex viscosity as a function of frequency at 25ºC of sesame protein formulations.

The elastic modulus G' in the studied region is always higher than the viscous modulus G", and both of them exhibit a slight increase with frequency. The gel structure is a complex matrix, and the linkage among molecules is a key factor for their mechanical properties. In this sense, the content of carrageenan is relevant for network firmness (López, 2007; Hernández-Téllez *et al.*, 2018). It can form a stable gel by combining into a double helix to form cross-linked bonds between molecules in a three-dimensional network (Ramírez and Vélez, 2009).

3.5 Analysis of complex viscosity ($|\eta^*|$)

Fig. 3 shows the complex viscosities for the studied gums-gel at 25°C as a function of frequency. It can be observed how this parameter decreases linearly with the increase of the frequency of oscillation and this parameter increases with the amount of gums added because a higher concentration implies a formation of a tighter structure with more active sites to interact with the other polymer chains and bound water. The formulation CC, F6 and F2 with lower protein content and higher gum content have the higher complex viscosity. On the other hand, for the formulations F5 and CA which contained a higher percentage of protein and a lower percentage of gums, the value of the complex viscosity was lower. This fact is due because proteins have not sufficient active sites to interact with water molecules compared with polysaccharides. In the case of high content of protein, the internal water of gel tends to release from the matrix, and the gel is destabilized.

Conclusions

The bromatological characterisation carried out on the defatted sesame flour shows that this by-product of the vegetable oil industry has a high protein content (51.3%), being an attractive raw material to produce protein-enriched products such as concentrates and isolates. From this, a protein isolate with a concentration of 80.27% proteins was obtained. Therefore, sesame flour can be used as a source of protein for the food industry. The gums-gel based on sesame protein obtained presented an elastic modulus higher than its viscous modulus for all the frequencies analysed. Mechanical properties of gels were improved with the increase of xanthan gum and carrageenan in formulations with low protein content.

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Nomenclature

G'	storage modulus
G"	loss modulus
% P	percentage of protein
pI	isoelectric precipitation
Greek symbols	
$\|\eta^*\|$	complex viscosity
τ	shear stress
ω	frequency

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