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Rheology changes in oil-in-water emulsions stabilized by a complex system of animal and vegetable proteins induced by thermal processing

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ABSTRACT

Mixtures of animal and vegetable proteins were used to stabilize oil-in-water emulsions of a meat rich filling. Collagen and pea protein, combined in different proportions for a total protein of 9 g/100 g were used to prepare oil-water emulsions, to develop a new meat product. Texture and rheological parameters were measured to evaluate the behavior of the emulsions. Temperature sweeps from 20 to 90 °C and back (0.5 K/min) were applied and the impact on emulsions structure was monitored, in the rheometer, through the changes on the viscoelastic properties. All the mixtures studied exhibited a shear-thinning flow behavior and showed different viscoelastic properties. The tested systems, exhibited an increase of both viscoelastic moduli on cooling from 90 to 20 °C, where the storage modulus is always higher than the loss modulus. This increase in viscoelastic functions should result from intermolecular hydrophobic driven cross-linking as well as some hydrogen bonds and physical entanglements between proteins molecules on gel formation induced by the heating/cooling cycles. The 3:1 mixture of collagen and pea protein showed to be a potential formulation for the new meat-product development, as it shows texture values and rheological features according to product specifications.

1. Introduction

In recent years, there has been a noticeable increase in the application of vegetable proteins in the food industry resulting from their low cost and reduced influence on the environment (Barac et al., 2010). The use of globular proteins from legumes can be an interesting alternative to enhance the nutritional and technological performance of animal based food (Bollinger, 2001). Consumers are increasingly interested in products with protein from a vegetable source and these are not only the vegetarians, but also those interested in a balanced ratio of vegetable/animal protein intake and consumers that associate specific health benefits with specific protein types (Russell, Drake, & Gerard, 2006).

Proteins from leguminous seeds have gained increasing importance since they are used to provide desired functional properties, including gelling, emulsifying, fat-absorbing and water binding properties (Nunes, Batista, Raymundo, Alves, & Sousa, 2003; Nunes, Raymundo, & Sousa, 2006a; Liang & Tang, 2014). The emulsifying properties of the proteins are an important functional feature for the food industry (Foegeding & Davis, 2011).

In addition to soybean seed, which has an overwhelming advantage in the market, but is shaded by the GMO label, pea seeds are one of the commercially available alternative plant protein sources (Tian, Kyle, & Small, 1999), and exhibit similar functional properties to soybean protein products (Boye, Zare, & Pletch, 2010).

Pea proteins, which are still evolving as new industrial proteins, show a well-balanced profile of amino acids, especially a high content in lysine (Schneider & Lacampagne, 2000), and contain two major globulin proteins: legumin (11S) and vicilin (7S) (Boye et al., 2010). Besides nutritional characteristics, their functional properties, such as gelling, emulsifying and foaming (Bacon, Noel, & Lambert, 1990; Bora, Brekke, & Powers, 1994; Raymundo, Empis, & Sousa, 1998; Tomoskozi, Lásztity, Haraszi, & Baticz, 2001), have led to a greater interest in this protein source as a promising food ingredient and as an alternative to the soybean protein. These proteins are also attractive to the food industry because of their low allergenicity, nutritional value and non-GMO status (Barac et al., 2010). The increasing knowledge about the emulsifying

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properties of these proteins can greatly extend their application in the food industry.

The interaction between animal and vegetable protein isolates is being now widely investigated to develop balanced animal and vegetable protein mixtures to produce derived meat products. However, the field of animal and vegetable protein interactions is one area that has to be deeply investigated.

Collagen fibrous protein is an animal protein responsible for structurally sustaining several animal tissues, being the main protein present in skin, bones, tendons, cartilages, and teeth. It is also the raw material for production of gelatin, cosmetics and foods, as well as an alternative for edible and/or biodegradable packaging film manufacture. Collagen and gelatin have been widely used in food industries as ingredients to improve the elasticity, consistency, and stability of foods, and can be used as emulsifiers in foods due to their ability to facilitate the formation of an emulsion, improve the stability, and produce desirable physicochemical properties in oil-in-water emulsions (Surh, Decker, & McClements, 2006). Since there is a lack of specific information on the rheological behavior of collagen in complex mixed systems (Olivo & Shimokomaki, 2002), the use of this protein was studied.

Over the past few years, there has been a growing interest in converting oil-in-water emulsions into gels, resulting from practical application in food formulations (Chen & Dickinson, 1999). Several workers have investigated the rheological properties of such emulsion-gels which can be used to create foods with improved sensorial, texture profile and interesting preservation properties. (Line, Remondetto, & Subirade, 2005; Manoi & Rizvi, 2009). Nonetheless, in many cases a thermal treatment is needed to produce these emulsion-gels, so their application in food formulations has to be more investigated.

The aim of this work is to study the interactions between pea protein (VP) and collagen (AP), on the development of stable oil-in-water emulsion-gels, with suitable texture profile and pleasant sensory properties, to be used as the basis of new meat products.

2. Materials and methods

2.1. Materials

The materials used to develop this work are commercial grade and were provided by the company Nobre, Alimentação, Lda.; pea protein isolate – PEA PROTEIN 85 by Agridient (Amsterdam), collagen protein isolate COLPROPUR D (Protein Ingredientes naturales, S.A., Spain). The olive oil as in dispersed phases, a blend of refined olive oil and extra virgin olive oil, was provided by JCCoimbra; Sodium ascorbate (E-301) by Helm Iberica SA, and glucose syrup SFINC GLUCO 21 by Pelicula Food Ingredients.

2.2. Methods

2.2.1. Preparation of mixtures

Based on preliminary testing, targeting the characteristics of a filling product designed by the industry (marketing department), oil-in-water emulsions were prepared with 9.0 g/100 g of total protein, from a mixture of collagen protein isolate (AP) and pea protein isolate (VP). Five mixtures were prepared at varying AP-to-VP ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 (w/w), which are denoted as 100AP:0VP, 75AP:25VP, 50AP:50VP, 25AP:75VP and 0AP:100VP, respectively. All samples included fixed amounts of olive oil (39 g/100 g) (dispersed or lipid phase), deionized water (24.5 g/100 g) (continuous or aqueous phase), sodium ascorbate (2.0 g/100 g) and glucose syrup (3.0 g/100 g). The sodium ascorbate and glucose syrup were added to control oxidation and reduce water activity, respectively.

For each sample the proteins mixtures were hydrated in the deionized water for approximately 12 h at room temperature. The emulsion preparation was performed in a thermo-processor (Bimby-Worwerk) at room temperature for about 10 min at positions 6, the lipid phase was added gradually into aqueous phase for all mixtures tested. After the emulsification, all samples were placed into a glass flask and kept at 4 °C during 24 h to achieve equilibrium, and only after that time the stability determinations, as rheological behavior and texture profile, were done.

2.2.2. Rheological measurements

All rheological measurements were carried out in a controlled stress rheometer (Haake Mars III - Thermo Scientific), with an UTC - Peltier system to control temperature, using a serrated parallel-plate sensor system (PP20 and 1 mm gap), to overcome the slip effect (Franco, Raymundo, Sousa, & Gallegos, 1998).

The shear steady flow curves were obtained ranging the shear rate from 1.00×10^{-5} to 5.00×10^{2} 1/s and applying different temperature conditions - 20 and 40 °C. The characterization of the emulsions flow at 40 °C was investigated to predict the behavior of the emulsions at a crucial processing step of pumping, at this temperature, in the manufacturing sequence at industry. Therefore, it was important to understand the impact of this middle range temperature on the flow parameters during pumping, as these are complex materials with proteins of different sources.

The comparison of the flow curves was performed from the adjustment of the Carreau model:

$$\eta = \eta_0 \Big/ \left[1 + (\gamma \cdot / \gamma \cdot_c)^2\right]^s \quad (1)$$

where η_0 is the zero-shear rate limiting-viscosity (Pa.s), $\boldsymbol{\gamma \cdot_c}$ is the critical shear rate for the onset of the shear-thinning behavior (1/s) and **s** is a parameter related to the slope of this region.

Heating/cooling curves were applied to all samples to study the structural changes resulting from the variation of temperature, at the rheometer plate, heating from 20 °C to 90 °C, at 0.5 K/min heating rate, kept for 5 min at 90 °C, and cooling down from of 90 °C back to 20 °C, at the same heating rate, and kept at 20 °C for 5 min. This temperature profile was designed to reproduce the temperatures applied, after pumping, during this specific industrial processing sequence for these systems.

During the heating profile mentioned, carried out at 1 Hz of frequency and constant stress, within the linear viscoelastic region, previously determined by a stress sweep test, the viscoelastic functions (G'- storage modulus and G" – loss modulus) were registered.

To evaluate the impact of the thermal treatment on the emulsions structure a frequency sweep test was carried out, i.e. the mechanical spectrum before and after the thermal treatment at 20 °C was determined.

Mechanical spectra at 20 °C were obtained varying the frequency between 0.001 Hz and 100.0 Hz, at a constant shear stress within the linear viscoelastic region of the samples. Each test was repeated at least two times and reproducible results were obtained.

2.2.3. Texture and colour characterization

Emulsions macrostructure was evaluated using the texture profile analysis (TPA), as previously described by Raymundo, Franco, Partal, Sousa, and Gallegos (1999). Texture parameters were determined with a TA-XTplus (Stable MicroSystems, UK) texturometer using a 5 kg load cell. Penetration tests were performed with a cylindrical probe (25 mm diameter, 15 mm of penetration, 5 s of waiting time and 1 mm/s of crosshead speed), and the samples were placed in cylindrical glass flask (45 mm of

height and 60 mm of diameter). The experiments were carried out 24 h after preparation, to allow the emulsions to equilibrate at 5 °C. Before performing any measurements, emulsions were allowed to equilibrate at 20 °C for approximately 1 h in a temperature-controlled room. Results for each emulsion were determined at least four times.

According to Raymundo et al. (1999), firmness and adhesiveness are the texture features that better characterize the emulsions texture. Firmness (N) was considered as the maximum resistance to the penetration of the probe and was calculated as the height of the force peak during the first penetration cycle. Adhesiveness (-N.m) represented the work necessary to pull the probe away from the sample and was recorded as the negative force area when the probe recedes back.

To evaluate the impact of the temperature on the visual appearance of the emulsions, the colour of these systems submitted to 100 °C for 1 h was measured. This treatment is in excess of the usual processing temperatures and exposure times, to evaluate the behavior of the systems in extreme conditions. The colour of the emulsions was measured using a colorimeter CR-300 (Minolta, Osaka, Japan) with standard illuminant D65 and a visual angle of 2°. Tri-stimulus colour coordinates (CIELAB system) were used to measure the degree of lightness (L^*), redness (a) and yellowness (b).

2.2.4. Water activity determination (aw)

The water activity (aw) was measured at least three times for each sample, using the method aw Quick at 20 ± 1 °C in a Hygrolab equipment (Rotronic, USA).

2.2.5. Statistical analysis

The experimental results were statistically analyzed by determining the mean, standard deviation, and the significance level was set at 95%, for each parameter evaluated.

Statistical analysis was made by applying variance analysis, the one factor (ANOVA), and multiple comparisons (Tukey test).

3. Results and discussion

3.1. Steady shear flow curves at 20 °C and 40 °C

Flow curves of the emulsions at 20 °C and 40 °C can be seen in Fig. 1. All samples showed a typical shear-thinning behavior: an initial Newtonian region with constant viscosity was observed at low shear rates, and at a particular shear rate value around 3.0×10^{-3} 1/s viscosity began to decrease following a straight-line decay. The Carreau model was fitted to the steady shear flow curves at 20 °C and 40 °C, as in previous similar works e.g. Raymundo, Franco, Empis, and Sousa (2002) and Pires et al. (2012). The r values of the fitted curves ranged from 0.995 to 0.9995. The values of the main parameters that characterize the flow behavior are presented in Table 1.

Differences were observed between samples with different protein blend ratios. For both temperatures, as seen in Fig. 1, the single pea protein system (100VP) presented the highest viscosity. From this figure it is clear that the flow was not substantially affected by temperature, showing a slight increase on the upper viscosity limit (η_0), due to protein denaturation. Considering the detailed figures presented on Table 1, it can be stated that the flow of the developed emulsion systems is slightly sensitive to temperature variations from 20 °C to 40 °C. On the mixed emulsion systems this slight increase in the η_0 with temperature is compensated by a small increase in slope (s).

In these products, high viscosities are not desirable in the processing lines at the factory floor. Similar results were observed in pea protein systems by Tarrega, Ramírez-Sucre, Vélez-Ruiz, and Costell (2012), and Tomé, Pires, Batista, Sousa, and Raymundo (2015), who also noticed an increase in the resistance to flow with pea protein content in the blend.

3.2. Heating/cooling curves

To characterize the structural changes involved in the specific industrial processing scheme, after the pumping step, the emulsions were subjected to a heat treatment and further cooled down, to reproduce the temperature profile used. In Fig. 2 the heating curves of five mixtures of proteins studied are represented and in Fig. 3 the mechanical spectra of the systems at 20 °C before and after the heating cycle can be seen.

From Fig. 2 it can be seen that changing the proportions of animal and vegetable proteins affected the viscoelastic behavior, showing different features within the heating/cooling cycle. These curves reveal a predominant elastic behavior over the applied temperature range, i.e., storage moduli values (G′) were higher than the loss moduli values (G″). The shape of the cooling curves obtained for single collagen (100AP – 0VP) and for single pea protein (0AP – 100VP), Fig. 2A and Fig. 2B, were similar to that obtained by Nicoleti and Telis (2009) for pure collagen protein and by Nunes, Raymundo, and Sousa (2006b) for pea protein gels, respectively. The presence of pea protein induces a slight decrease in G′ and G″ during cooling, as reported in the case of other globular proteins (Nunes et al., 2006b; Renkema & Van Vliet, 2002). Thermal gelation of globular proteins involves the partial unfolding of protein molecular strands at high temperatures, subsequent aggregation, and cross-linking in order to form a gel network during cooling (Clark, Kavanagh, & Ross-Murphy, 2001). The establishment of the gel network occurs as a result of hydrophobic and chemical interactions (particularly hydrogen bonding) and physical entanglements, between protein molecules. These linkings are formed mainly during cooling and are important in stabilizing protein systems (Tang & Liu, 2013).

As opposed to the single protein systems, the mixed systems in Fig. 2C and Fig. 2D, showed a tendency to overlap and reverse the viscoelastic functions (G′ e G″) during the heating cycle, from 20 °C to 90 °C. After the subsequent cooling cycle from 90 to 20 °C, the G′ and G″ values increased and remained practically constant, i.e., there was a strong buildup of the system structure with cooling. This might indicate the prevalence of a gel structure over the emulsions structure on cooling.

During the heating and cooling cycle, the 25AP-75VP sample in Fig. 2E kept the viscoelastic functions almost constant and parallel, showing a profile typical of a weak gel system, i.e., G′ closer to G", both below 10^3 Pa, which may suggest that the vegetable protein (VP) has an important role in system structuration, but their structural behavior is weakened when the collagen protein concentration increases.

This behavior is typical of the animal myofibrillar proteins (AP) and was also observed by other authors, such as Romero et al. (2009). Some antagonistic effect between AP and VP is evident on the emulsions structure, during the heating cycle, on the different mixtures of 75:25 and 50:50 AP:VP, Fig. 2C and Fig. 2D respectively. The emulsions stabilized by these mixtures start with G′ values close to G″ and around 40 °C, there is a reverse effect where G″ takes over G′ until 80 °C. This is the breaking of the emulsion system, but after 80 °C up to 90 °C there is the building up of another structure that is typical for a gel and is further developed, and consistently maintained, during the cooling cycle. The breaking of the emulsions was detected in 75:25 and 50:50 AP:VP samples. In systems where pea protein concentration is higher than collagen (100VP and 25AP:75VP), the breaking of the emulsion structure is not apparent during heating, however the values of the viscoelastic

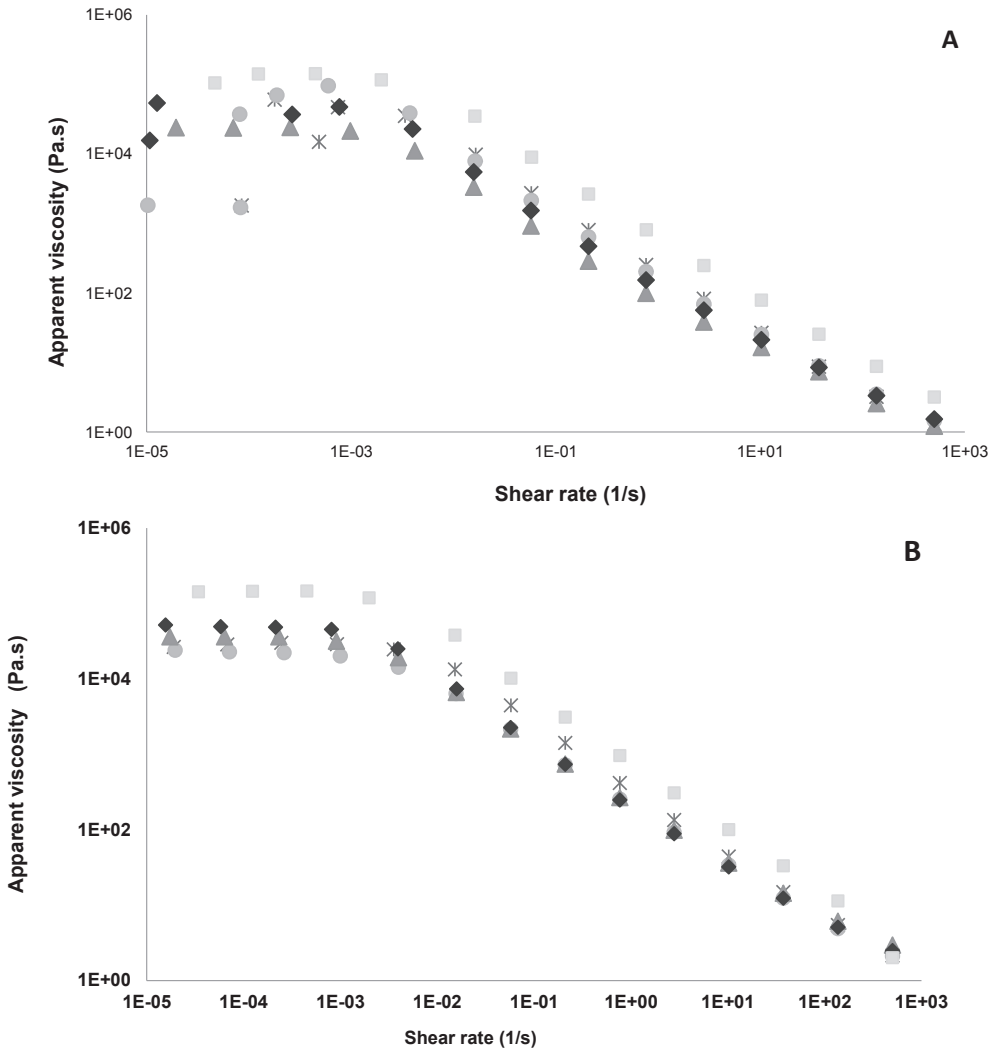

Fig. 1. Viscosity *versus* shear-rate curves, at 20 °C (**A**) and 40 °C (**B**), for samples for samples with 9.0 g/100 g of total protein and different proportions of collagen (AP) and pea protein (VP): ✳ 100AP:0VP; ● 75AP:25VP; ▲ 50AP:50VP; ◆ 25AP:75VP; ■ 0AP:100VP.

functions are reduced. Other authors as O'Kane, Vereijken, Gruppen, and Van Boekel (2005) and Shand, Ya, Pietrasik, and Wanasundara (2007) presented similar results, showing that pea proteins form gels of weak structure with high viscosity with a coarse texture, resulting from mainly hydrophobic protein–protein interactions (Pires et al., 2012; Tomé et al., 2015).

Fig. 3 shows the mechanical spectra obtained at 20 °C, before and after the heating/cooling cycles, for the five studied samples. These tests are useful to study the structural changes occurring on emulsions as a consequence of heating, simulating the industrial process that will be applied to these emulsions. These data allow the selection of the heat-resisting system leading to a more stable system. At first, a less structured system is required to facilitate the flow during the pumping phase, but after the subsequent industrial processing, subjected to high temperature conditions, a more structured system is formed, with lower aw values (Table 2), to increase the final product stability. This study is a simulation of the industrial process which leads to the manufacturing of the final product.

It was observed that more structured systems were formed after the application of the heating and cooling cycles (Fig. 3). This significant improvement of viscoelastic moduli is clearly induced by heat-treatment (Romero et al., 2009). The organization of protein molecules during the cooling stage generated more structured

Table 1
Parameters of viscosity curves obtained by Carreau model fitting to the steady shear flow measurements at 20 and 40 °C.

Samples	η_0 (20 °C) (k Pa s)	η_0 (40 °C) (k Pa s)	$\dot{\gamma}_c$ (20 °C) (s^{-1})	$\dot{\gamma}_c$ (40 °C) (s^{-1})	s (20 °C)	s (40 °C)
100AP:0VP	33.8 ± 2.84	47.9 ± 4,01	0.06 ± 0.001	0.05 ± 0.003	0.44 ± 0.003	0.47 ± 0.005
75AP:25VP	26.1 ± 1.56	37.8 ± 0,32	0.06 ± 0.002	0.02 ± 0.003	0.40 ± 0.007	0.50 ± 0.007
50AP:50VP	29.3 ± 3.06	33.5 ± 3.97	0.02 ± 0.001	0.02 ± 0.001	0.40 ± 0.003	0.45 ± 0.004
25AP:75VP	48.8 ± 2.29	42.5 ± 3.71	0.02 ± 0.001	0.02 ± 0.006	0.43 ± 0.021	0.44 ± 0.008
0AP:100VP	339.4 ± 4.79	441.6 ± 3.63	0.03 ± 0.005	0.03 ± 0.005	0.44 ± 0.021	0.46 ± 0.010

Abbreviations: AP – Animal Protein; VP – Vegetable protein.

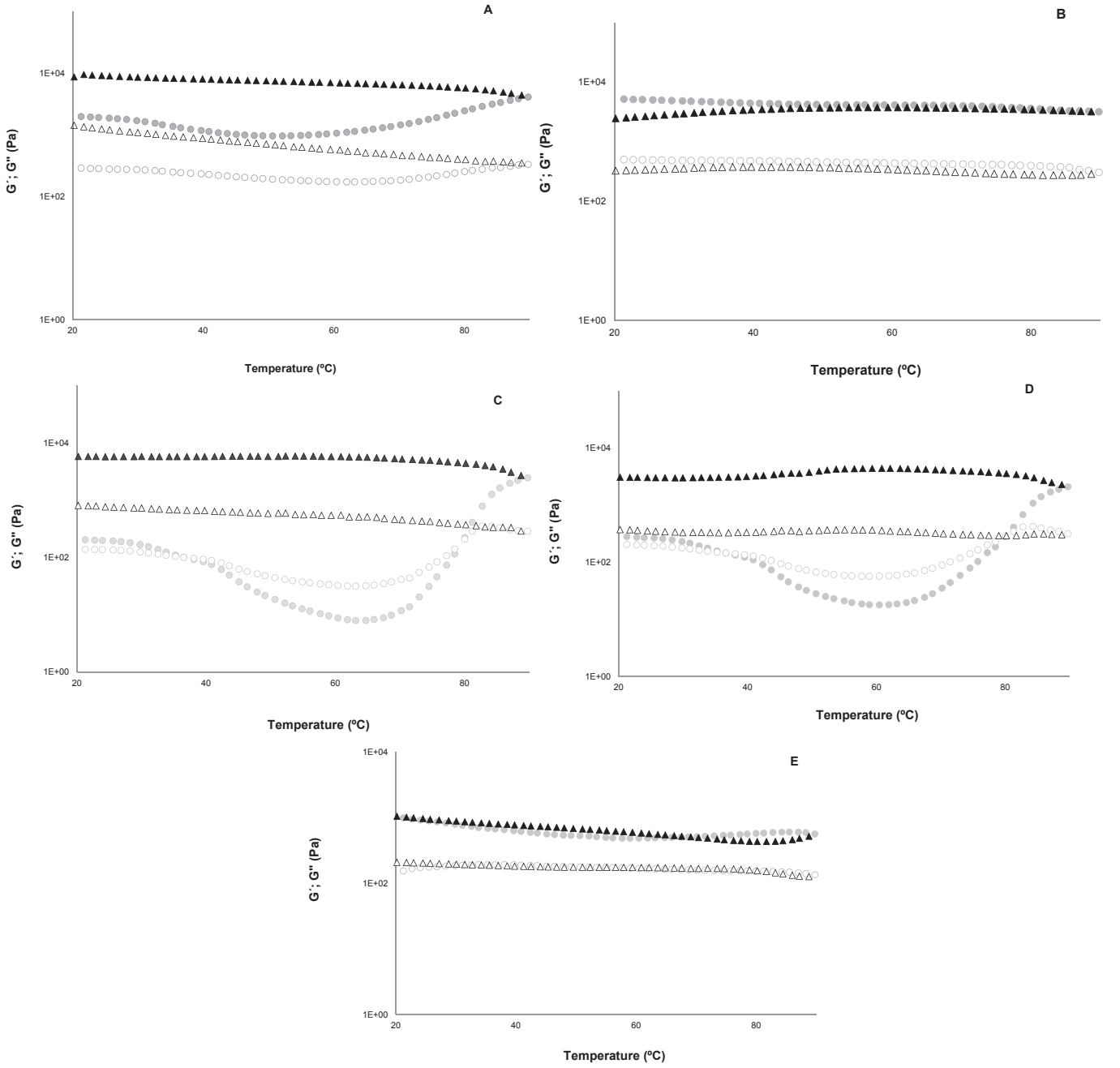

Fig. 2. Temperature sweeps from 20 to 90 °C and back to 20 °C at 0.5 °C/min. Heating (round symbols) and cooling (triangular symbols), full symbols (●, ▲) for G′ and open symbols (○, Δ) for G″, for samples with 9.0 g/100 g of total protein and different proportions of collagen (AP) and pea protein (VP): **A**- 100AP:0VP; **B**- 0AP:100VP; **C**- 75AP-25VP; **D**- 50AP-50VP and **E**- 25AP-75VP.

systems, called gels. It is also verified that when the amount of pea protein decreases in the formulations, G′ and G″ values increased, indicating that the higher proportion of collagen in the mixture induces a stronger gel structure at the end of the cooling cycle (Fig. 3A and Fig. 3C). The animal protein, collagen, had an important role in gel-structure, contributing for more stable systems.

From these set of tests, the protein mixture selected was the 75AP:25VP since it gathered all the important characteristics for the targeted meat product to be developed:

i) in the emulsion state it shows low viscosity (Fig. 1) and melts around 40.0 °C (Fig. 2C), important characteristics for this specific industrial processing;

ii) after the heating/cooling cycles it builds up structure into a gel-like system, more stable in terms of immobilizing water and fat.

3.3. Texture and colour characterization

Texture parameters (firmness and adhesiveness), obtained with a puncture test, for the emulsions studied are illustrated in Fig. 4.

This figure shows that the different protein proportions influences the results of firmness and adhesiveness of the emulsions and shows that emulsions with pea protein only (0AP:100VP) were firmer and more adhesive than emulsions prepared with collagen only (100AP:0VP). The existence of the antagonistic effect in the

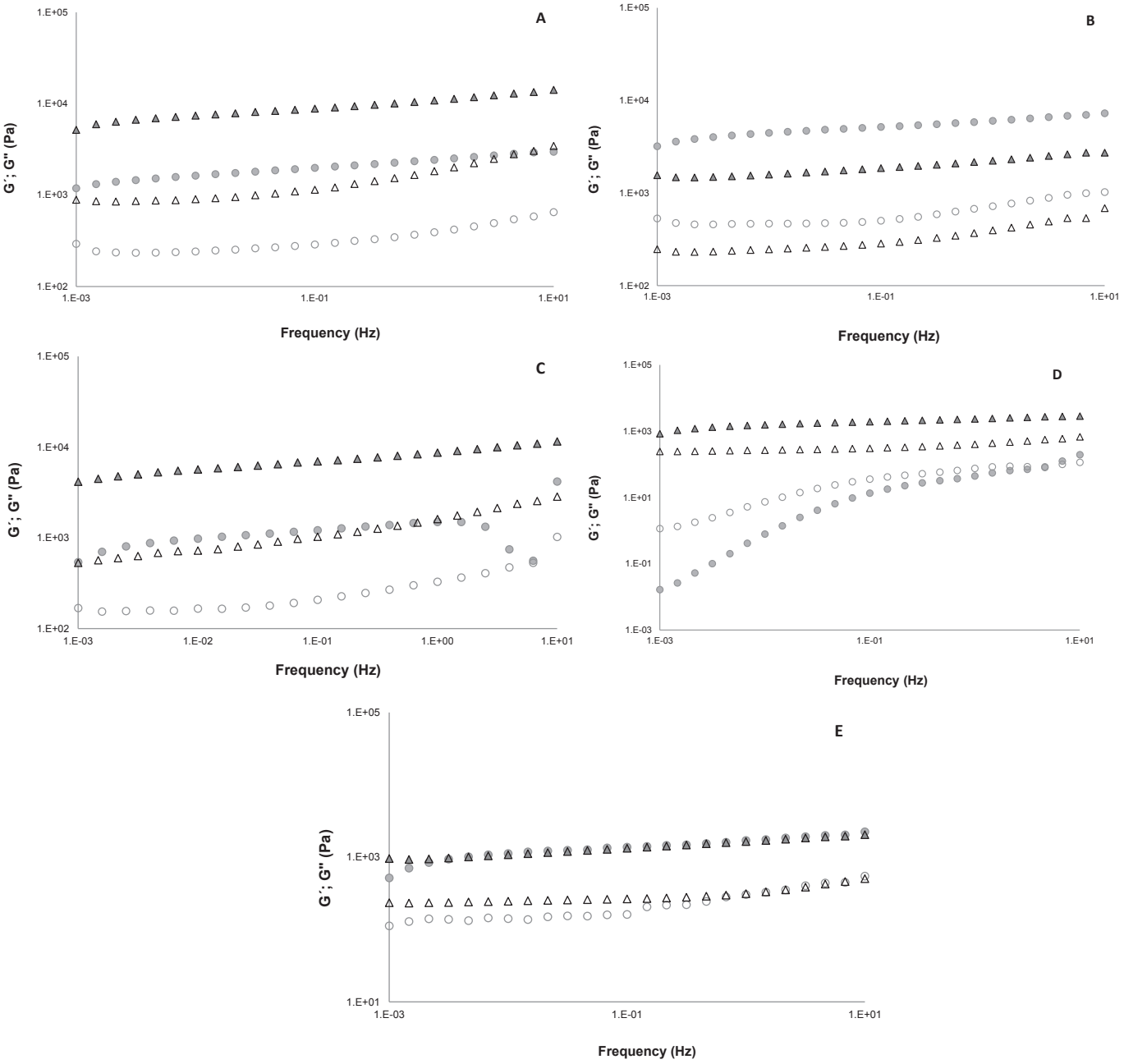

Fig. 3. Frequency sweep curves - change of the storage (G' - close symbols) and loss (G'' – open symbols) moduli with frequency, for emulsions (round symbols) and induced gel (triangular symbols) for samples with 9.0 g/100 g of total protein and different proportions of collagen (AP) and pea protein (VP), after heating cycle: **A**- 100AP:0VP; **B** - 0AP:100VP; **C**- 75AP-25VP; **D**- 50AP-50VP and **E**- 25AP-75VP.

Table 2
Emulsions and gels water activity (aw) comparison, obtained from different mixtures of collagen and pea protein at a total content of 9 g/100 g of protein, by heating at 100 °C for 1 h.

Samples	aw emulsions	aw induced gels
100AP:0VP	0.92 ± 0.006 [a]	0.75 ± 0.005 [f]
75AP:25VP	0.90 ± 0.001 [a]	0.84 ± 0.002 [b]
50AP:50VP	0.91 ± 0.002 [a]	0.86 ± 0.006 [c]
25AP:75VP	0.88 ± 0.003 [a]	0.81 ± 0.007 [d]
0AP:100VP	0.83 ± 0.005 [a]	0.79 ± 0.001 [e]

Note: In the same column, different letters mean statistically different values ($p < 0.05$).
Abbreviations: AP – Animal Protein; VP – Vegetable protein.

mixed protein systems was observed by the lower values of firmness and adhesiveness of the mixtures, mainly for 50AP:50VP and 25AP:75VP. The 75AP:25VP mixture presented a texture profile compatible with the target sensory characteristics, with values of firmness and adhesiveness of 1.5 N and - 6.0 × 10^{-3} N m, respectively. This behavior, featured for a better performance of the emulsions with protein mixtures than in the emulsions stabilized by one protein only, has also been verified by other authors such as Damodaran (2005) and Tomé et al. (2015).

To evaluate the impact of temperature on the colour of the product, the emulsions were heated up to 100 °C for 1 h. The gels lightness values (L*) were in the range of 21–36. All studied gels showed L* values below 50. With increasing proportion of pea

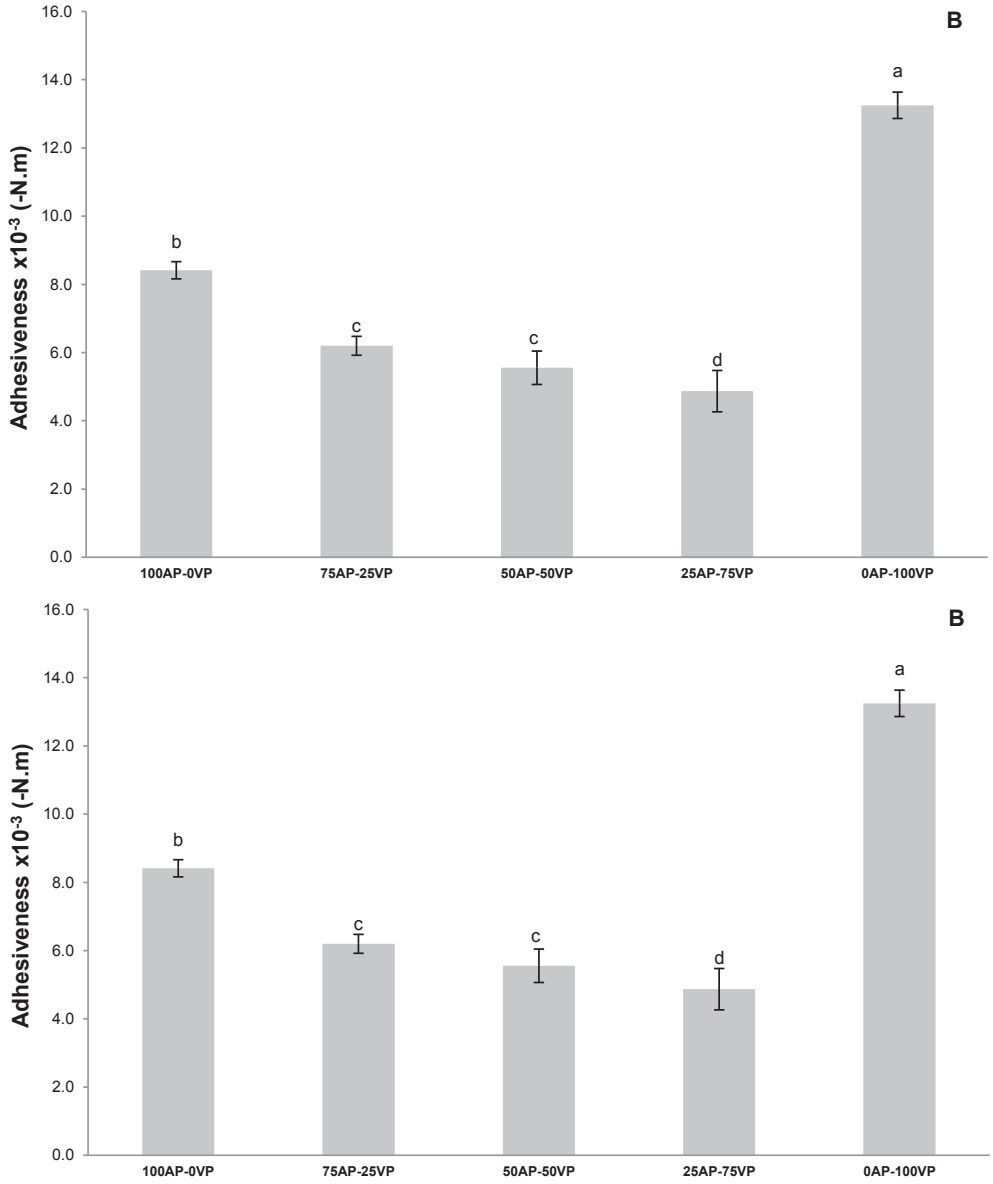

Fig. 4. Values of the texture profile parameters: **A** - Firmness (N); **B** - Adhesiveness (- N.mm), for emulsions with 9.0 g/100 g of total protein and different proportions of collagen (AP) and pea protein (VP): 100AP:0VP; 75AP:25VP; 50AP:50VP; 25AP:75VP and 0AP:100VP. Different letters mean statistically different values ($P < 0.05$).

protein the L* values of the emulsions significantly decreased ($p < 0.05$). For the redness values, a* ranged from 5 to 12, it was also found that with increasing amounts of pea protein (values above half of the concentration), gels tend to exhibit redness values significantly lower ($p < 0.05$). Regarding the yellowness values, b* ranged from 4 to 16 and a similar pattern was found: decreasing values of yellow component with increasing proportions of pea protein.

3.4. Emulsions and gels water activity (aw) comparison

The water activity parameters are crucial to the product stability, since they are directly related to shelf life. Therefore, the aw values were measured on the emulsions prepared with different proportions of collagen and pea proteins. The water activity values of the respective gels, i.e., after the heating/cooling treatment, were also measured (Table 2). No significant differences ($p < 0,05$) were found between aw values for the emulsions (full bars). Nevertheless, significant differences ($p > 0.05$) were found for the respective gels (empty bars). The induced gelation by heating/cooling, similarly to the industrial processing of this product, had an impact on water activity reduction, mainly on single protein systems, where the network formed is more structured. Gelation showed a better performance on immobilizing water and fat on the protein molecules network as observed by Lanier and Carvajal (2005). This is an interesting result, as immobilized water is not available for chemical and enzymatic degradation reactions as well as for the microorganism's development, improving the preservation and contributing to the extension of the final product shelf life.

4. Conclusion

The rheological properties and texture of the protein based systems with different animal/vegetable blends ratio were characterized to select the best proportion in order to develop a new meat product filling.

All the mixtures studied exhibited a shear-thinning flow behavior and showed different viscoelastic properties according to the amount of pea protein added to the formulations. Pea protein content above a quarter of the concentration value tend to originate weaker structures and may even cause instability.

According to the texture profile analysis, the mixture that showed suitable results was the 75AP-25VP, with values of firmness and adhesiveness between 1.5 N and − 6.0 N mm, respectively.

In terms of colour, the best colour coordinates were observed for the same mixture (75AP:25VP) contributing for the consumers acceptance of the product.

With respect to the water activity results, it was observed that the induced gelation by heating/cooling processing led to an aw reduction, more evident on the single protein systems.

In meat-based products, a certain combination of pea and collagen protein can be used to impart the desired texture and rheological features, contributing for a sensory pleasant product and nutritionally balanced between animal and vegetable proteins. From all the blends studied, the 75AP-25VP proportion showed to be the best combination to produce a filling with the required properties.

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