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FUNCTIONAL CHARACTERIZATION OF LUPIN PROTEIN CONCENTRATE TREATED WITH BACTERIAL TRANSGLUTAMINASE

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The present paper describes the influence of bacterial transglutaminase, on the functional properties of the lupin proteins concentrate. Gelling and emulsifying properties of the lupin protein concentrate were studied as well as rheological behavior of the gel and emulsions. The degree of polymerization of the lupin proteins depended on the enzyme concentration, setting time and setting temperature; the best results in terms of functional properties were obtained with 0.25g transglutaminase/100g protein with 90 min setting at 50°C. Concerning rheological properties, both gels and emulsions based on lupin proteins, exhibited shear thinning behavior. These results confirm that transglutaminase may be used for producing lupin protein aggregates with enhanced functional properties.

Keywords: lupin proteins; bacterial transglutaminase; gels; emulsions; rheological behavior.

1. Introduction

Proteins of vegetal origin are used successfully in human diet due to their effect of lowering the cholesterol level, leading to reduced cardiovascular risks (Bakhit et al., 1994; Sirtori et al., 1998). Nowadays, there is an increased necessity to discover new sources of proteins to be used as functional ingredients in different food systems. Soybean proteins are often used as ingredients in different foods, to improve their processing and/or cooking behavior or mechanical and textural characteristics (Barbut, 1995; Rhee, 1994) and to reduce the cost price.

Lately, lupin proteins were increasingly used to replace soy proteins in different meat and low fat dairy products formulations. Lupin seeds are rich in proteins with an equilibrate balance in essential aminoacids, even better than of soybean proteins (Cerletti and Duranti, 1979). Moreover lupin proteins have a good solubility, emulsion formation capacity and gel-formation abilities (Pozani, et al., 2002; Kiosseoglou, et al., 1999; Mavrakis, et al., 2003).

In order to obtain convenient healthy food products, the methods of improving techno-functional properties of the proteins are of great interest. The treatment of proteins with bacterial transglutaminase (MTGase), leads to the modification of their hydration, gelation, rheological and emulsifying properties and of thermal stability (Dickinson, 1997; Lorenzen et al., 1998; Lorenzen and Schlimme, 1998; Motoki, et al. 1984, Motoki and Segura, 1998; Lorenzen, 2000), through the introduction of amines, cross-linking and deamination reactions (Ikura et al., 1992; Nielsen, 1995; Motoki and Segura, 1998).

Many research papers report specific applications of MTGase for processing: meat, fish, and fish/seafood products (Kuraishi, et al., 1997, 1998, Hammer, 1998, Jiang, et al., 2000; Soeda et al., 1996; Seguro, et al., 1995; Sakamoto, et al., 1995; Gómez-Guillén et al., 2001; Ionescu et al., 2008), bread and bakery products (Gerard, et al., 1998, 2000, 2001; Wang, et al., 2007), milk and dairy

products (Ikura et al., 1984; Nonaka et al., 1989; Han and Damodaran, 1996), eggs (Kato et al., 1991) and soybean (Nonaka, et. al., 1994; Soeda et al., 1995; Schafer, et.al., 2007). Nevertheless, there is not enough information about MTGase effect on mechanical properties of gels and emulsions based on lupin proteins.

The present work aimed towards the evaluation of the MTGase interaction with lupin proteins. In particular we studied the influence of the enzyme concentration, setting time and setting temperature on the functional and rheological properties of gels based on lupin proteins. Stability and thermal properties of emulsions were also analyzed.

2. Materials and methods

2.1. Materials

Lupin protein concentrate (Luppiro) was supplied by Enzymes and Derivates S.A. (Romania).

Microbial transglutaminase (Ajinomoto Inc. Teanec. N.J., USA) is a mixture containing 99% maltodextrin and 1% microbial transglutaminase (enzymatic activity of 100 UE/g). The enzyme is active over large ranges of temperature (2-60°C) and pH (5-8), and is inactivated at high temperatures, depending on the composition of the food.

2.2. Lupin protein concentrate characterization

Proximate composition and pH. The moisture, protein, fat and ash contents of the lupin protein concentrate were determined according to standard AOAC (1995) methods. All determinations were made in duplicate. pH measurements were made on the 10% protein solution (w/w) by means of a Hanna digital pH-meter.

Protein solubility. Protein solutions (2% w/v) were prepared by dispersing powdered protein concentrate in distilled water. The protein solution was homogenized with a magnetic stirrer for 20 min, stored overnight at 4^{o}C, centrifuged at 4500 rpm for 30 min, and then filtered through filter paper (Whatman No.1). The nitrogen content of the filtrate was determined by micro–Kjeldahl method (AOAC, 1995) and the soluble protein was obtained by multiplying the recorded nitrogen value by the nitrogen conversion factor (6.25).

Water absorption capacity. The method of Sathe et al., 1982 with slight modifications, was used to evaluate water absorption capacity. Approximately 0.5 g of dried lupin protein concentrate was placed into 15 ml centrifuge tube and mixed with 4.5 ml of distilled water. After incubating for 30 minutes at 20°C, the tubes were centrifuged for 30 minutes at 3500 rpm, decanted, and reweighed. Water absorption capacity was calculated by dividing the weight of absorbed water by the weight of the protein concentrate.

Emulsifying capacity. The method described by Beuchat et al. (1975), with slight modifications, was used to evaluate the emulsifying capacity. One g lupin protein concentrate was mixed with 50 ml of distilled water for 20 minutes. After complete dispersion, sunflower oil was added continuously during stirring with a Braun mixer at constant temperature (20^{o}C), until the mixture separated into two layers. The amount of oil added until the inversion point of the emulsion was used to calculate the emulsifying capacity reported as milliliters of oil per gram of lupin protein concentrate.

2.3. Preparation and characterization of lupin protein gels

The effect of the MTGase concentration, setting time and setting temperature on the functional properties of lupin protein gels, was tested by performing many tests. Lupin protein gels were prepared by first dispersing the protein concentrate in water to obtain a protein dispersion of 10% (w/v) with pH of 7.30-7.35. The protein dispersion was then mixed with MTGase (0.1; 0.15; 0.2 and 0.25 g MTGase/100 g proteins) for 2 minutes using a Braun mixer with temperature control. The samples were afterwards maintained at a constant temperature (4, 20, 30, 40 and 50^{o}C) for different setting times (30, 60, 90, 120 min). In order to inactivate the enzyme, the samples were subjected to thermal treatment (the temperature was increased at 1^{o}C/min) to achieve 85^{o}C in the thermal center of the

sample, temperature that was maintained for 5 min. The thermally treated samples were cooled in a mixture of water and ice to room temperature, and the obtained gels were stored overnight at 4°C.

Solubility of the proteins in sodium dodecyl sulphate (SDS) - The protein solubility in SDS was determined using the method indicated by Nishimoto et al., (1987) which was slightly modified. Gel (0.8 g) was homogenized with 15 ml of SDS solution (2% sodium dodecyl sulphate, 8M urea, 2% mercaptoethanol and 20 mM Tris-HCl; pH 8.0) using Ultraturax agitator at 10000 rpm for 3 min. The agitator was then washed with 10 ml distilled water, and the homogenized samples were stored at room temperature for 24 hours, with occasional stirring. Afterwards the samples were centrifuged at 4500 rpm for 20 min, and the proteins soluble in SDS were determined in the resulting supernatant by the Lowry colorimetric method (Lowry et al., 1951).

2.4. Preparation and characterization of lupin protein emulsions

The oil-in-water emulsions were obtained starting from the lupin gel, by homogenizing for 3 minutes the 2% protein dispersion (w/v) with sunflower oil (the ratio protein dispersion : oil = 1 : 2) using a Braun mixer. The temperature was maintained at 20°C by placing the emulsions on cold water bath.

Storage stability of emulsions

The emulsion (20 g) was placed into test tubes with inner diameter of 20 mm and height of 100 mm, and was stored at 20°C for seven days (Demetriades et al., 1997). The stability of the emulsions during storage (*ES*) was estimated as:

$$ES = (\text{final emulsion height/initial emulsion height}) \cdot 100 \quad (1)$$

Emulsion stability at centrifugation - 20 g of emulsion was placed in centrifuge tubes, and was centrifuged for 10 minutes at 3000 rpm, at 24°C. The emulsions stability at centrifugation (ES_C) was estimated as:

$$ES_C = (\text{emulsion height after centrifugation/initial emulsion height}) \cdot 100 \quad (2)$$

Thermal stability of emulsions

The emulsion was placed in test tubes and was heated at 1°C/min to achieve 80°C in the thermal center of the sample; this temperature was maintained for 10 min. Afterwards the sample was cooled to room temperature using an ice bath and stored overnight at 4°C. The water-like phase on top of the test tubes was removed carefully. The thermal stability of the emulsions was evaluated in terms of creaming and was reported as a creaming index at thermal treatment (Ic_t):

$$I_{Ct} = \frac{W_i - W_t}{W_i} \cdot 100 \quad (3)$$

where W_i and W_t are the weights of emulsion before and after thermal treatment, respectively.

2.5. Apparent viscosity measurements

The apparent viscosity of the enzyme treated lupin protein gels and of the emulsions based on lupin proteins was evaluated by means of a RHEOTEST-2 type rotating viscosimeter manufactured by VEB-MEDINGEN, Germany. Due to the medium viscosity of the samples the coaxial cylinder device S_3 was used and 50 g of sample was tested. The working frequency was 50 Hz and the shear rate (γ) was varied within the range 0.1667-145.8 s^{-1}. The shear stress (τ_r) was calculated starting from the value of α, which is the output value of the viscosimeter, using the relationship:

$$\tau_r = z \cdot \alpha \quad (4)$$

where: z is the device constant corresponding to the working domain I or II and to the cylinder type.

The apparent viscosity (η) was calculated as:

$$\eta = \frac{\tau_r}{\gamma} \quad (5)$$

2.6. Statistical analysis

Statistical analysis of the results was performed using Sigma Plot 2001/Statistics Date software. Three experimental batches were performed for each kind of test and the results are reported as mean values. Typical standard deviations are less than 5%.

Experimental data were fit using Table Curve 2D software and the regression equations were established based on statistical criteria (r^2, Fit Standard Error or F Statistics).

3. Results and discussions

3.1. Lupin proteins concentrate characterization

The results of chemical analysis showed that lupin concentrate is made up of 63.7±2.6% proteins, 15.2±0.66% carbohydrates, 10.8±0.46% fats, 5.9±0.24% water, and 3.89±1.80% ash. The pH value of the 10% protein solution was 7.30 at 20°C. Concerning the techno-functional properties, the lupin protein concentrate was characterized by a protein solubility of 66.22±2.14%, a water absorption capacity of 4.2±0.16 g H_2O/g protein concentrate and an emulsifying capacity of 100.8±2.6 ml oil/g protein.

3.2. Gels characterization

Apparent viscosity

The effect of the MTGase treatment on the gelling properties of lupin proteins was evaluated by measuring samples' viscosity.

The influence of the MTGase concentration on the properties of the lupin protein gels was estimated by setting the protein dispersions with the enzyme at different concentrations for 60 minutes at 40°C. Gelation properties of lupin proteins concentrate varied with the enzyme/substrate ratio. The rheological behavior of the gels prepared with different concentrations of MTGase is presented in Figure 1 as apparent viscosity vs. shear rate relations. The increase of the enzyme concentration up to 0.25 g MTGase/100 g proteins induced the improvement of the gels' viscosity (Figure 1), in agreement with the observations of Schafer, et al. (2007) who made a comparative study of gelation and cross-link formation during enzymatic texturisation of soybean and pea proteins.

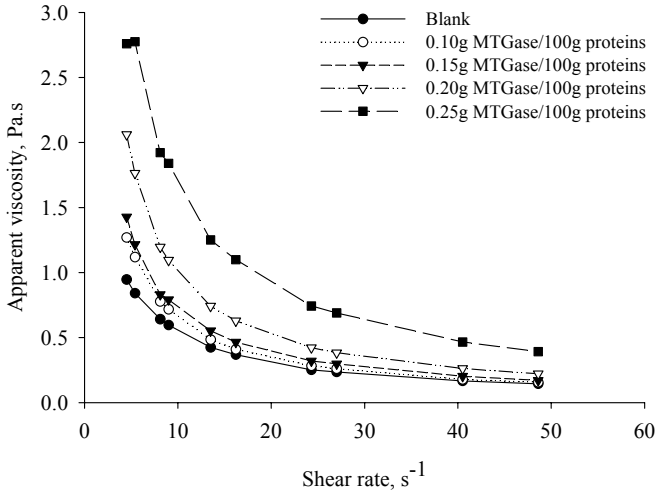

Figure 1. Influence of the MTGase concentration on apparent viscosity of lupin protein gels. Reaction conditions - setting temperature: 40°C, setting time: 60 min

Analyzing, for instance, the results obtained for the shear rate of 5.4 s^{-1} it can be seen that the apparent viscosity of the samples treated with 0.25 g MTGase and 0.1 g MTGase/100 g proteins increases by ~180% and ~20%, respectively, compared to the blank sample. We can explain the

increase of gels viscosity by the activity of MTGase of cross-linking the lupin protein molecules, which are rich in glutamic acid (19.2 g/100g protein) and lysine (5.6 g/100 g protein) (Sandre, et al., 2002).

In order to estimate the influence of the setting temperature on the intensity of the enzymatic reaction, the protein suspensions were incubated with MTGase (0.25 g MTGase/100 g proteins) for 60 minutes at different temperatures. The tested setting temperatures (4, 20, 30, 40 and 50^0C) are lower than the denaturation temperature of lupin proteins (76^OC) indicated by Kiosseoglou et al. (1999). The results (Figure 2) indicate that high temperatures induce high enzymatic reaction rates which reside in the large amount of the cross-links between polypeptide chains mediated by MTGase. The highest increase of the apparent viscosity (316% with respect to the sample set at 4°C) was recorded for the sample set at 50°C (Figure 2). In case of the samples set at 40 °C and 50°C we may also consider the contribution of the thermal treatment to the improvement of swelling and gelling capacity of lupin proteins concentrate.

The influence of the setting time on the properties of MTGase treated lupin proteins was estimated by setting the protein suspension with 0.25 g MTGase/100 g proteins at 40°C for different times. According to our results (Figure 3) the viscosity of the protein gel is directly correlated with the time of MTGase activity. The ε-(γ-Glu) lysine dipeptides cross-linking within the lupin proteins catalyzed by the MTGase at 40°C was more accentuated when increasing the setting time from 30 to 90 min. Longer setting times (120 min) at 40°C causes the viscosity decrease due to the isopeptides bonds disruptions.

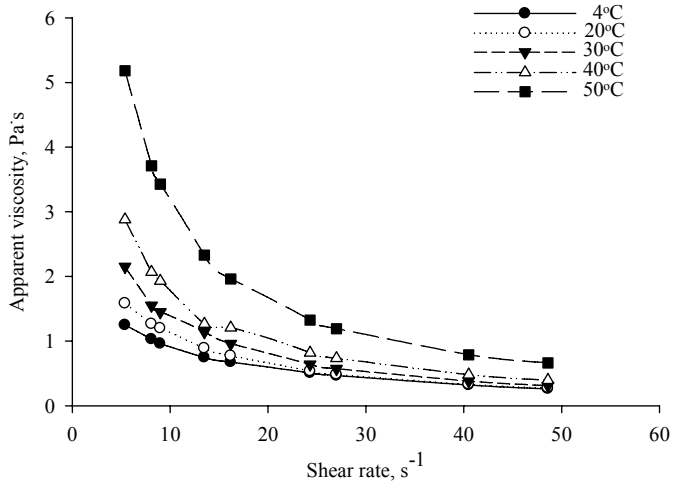

Figure 2. Influence of the setting temperature on the apparent viscosity of the lupin protein gels. Reaction conditions - MTGase concentration: 0.25 g/100 g proteins, setting time: 60 min

Shear thinning behavior

Shear thinning behavior was observed for all the studied gels in all enzyme concentration, time and temperature conditions. In all cases, the apparent viscosity of the gels decreased with the increase of the shear rate from 0.1667 to148.6 s^{-1} (Figure 1). This tendency gives indications about a high shear thinning as a result of mechanical destroying or unfolding of the protein aggregates and their orientation in the flowing direction.

Rheological behavior of the gels (Figure 1) was well described by the constitutive Herschel-Bulkely power low model:

$$\tau = a + b \cdot \gamma^c \tag{6}$$

where: *a* represents the yield stress, *b* is the consistency index and *c* is the power index.

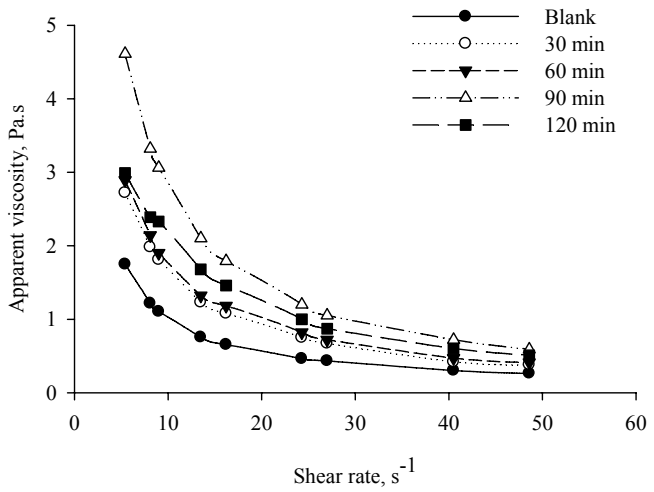

Figure 3. Influence of the setting time on the apparent viscosity of the lupin protein gels. Reaction conditions - MTGase concentration: 0.25 g/100 g proteins, setting temperature: 40°C

Solubility of the proteins in SDS

Formation of protein aggregates by inter- and intramolecular interactions mediated by MTGase, was estimated by determining the solubility of the gel proteins in SDS. The results indicated that by adding MTGase the solubility of the lupin proteins is reduced. In case of the sample with 0.25 g MTGase/100 g proteins set for 120 minutes at 40°C the proteins' solubility in SDS (1.54 g/g gel) was ~11.2% lower than the blank sample (1.73 g/g gel). In native state, globulins (vicilin, legumin and globulins) and albumins of the lupin seeds have good solubility (Duranti, 2006).

3.3. Emulsions characterization

The effect of MTGase treatment on emulsifying properties of lupin protein gel was determined by evaluating the emulsion stability and the apparent viscosity of the emulsions.

Emulsions stability

The stability of the emulsions based on MTGase modified lupin proteins was appreciated by evaluating the creaming index. The oil droplets, generally, have a lower density than surrounding aqueous phase and therefore move upwards during storage, leading to creaming. According to our result, the storage stability of the emulsions depends on the MTGase concentration, setting time and setting temperature.

Samples with MTGase concentrations ranging between 0.1 and 0.25 g MTGase/100 g proteins set at 40°C for 60 minutes, presented an *ES* of 100% after seven days. The creaming process occurred faster (five days) in case of the blank (enzymatically untreated) samples and the water-like phase remained turbid during storage. An *ES* of 100% was obtained also for the samples set with 0.25 g MTGase/100 g proteins for 60 minutes at temperatures between 4-30°C, and those ones set for different times between 30-90 minutes at 40°C. The variation of the creaming behavior of different samples may be explained by structural changes of the lupin proteins. The polypeptide cross-linking catalyzed by MTGase increases the molecular weight of the proteins and consequently limits their mobility to the water-oil interface. Therefore, the protein films surrounding the oil drops are not uniform enough and resistant, to prevent the tendency of emulsions' phases separation.

The results concerning the stability at centrifugation are presented in Figure 4. Emulsions based on lupin proteins modified with MTGase, were slightly less stable compared to the sample without enzyme (Figure 4).

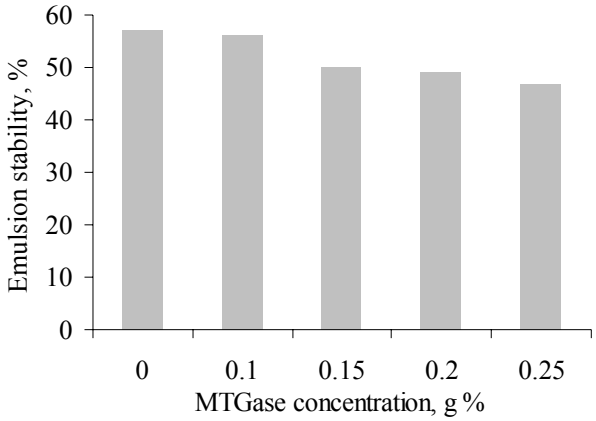

Figure 4. Influence of the MTGase concentration on emulsion stability after centrifugation. Reaction conditions - setting time: 60 min, setting temperature: 40°C

Concerning the stability at thermal treatment, the results indicate that the highest stability (85%) was obtained for the sample with 0.1 g MTgase/100 g proteins (Figure 5). The increase of cross-links may change the balance between hydrophile/hydrophobe ratio of emulsifier and protein stabilizer and reduces the number of exposed hydrophobic groups, leading to the release of the water-like phase from the emulsion system.

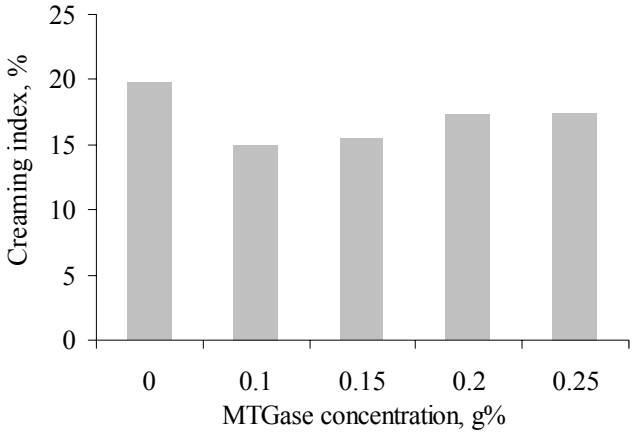

Figure 5. Influence of the MTGase concentration on the creaming index after thermal treatment of the emulsion. Reaction conditions - setting time: 60 min, setting temperature: 40°C

The oil-in-water emulsions are thermodynamically unstable systems and have the tendency to break down over time (McClements, 1999; Dickinson 1992). They are susceptible at destabilization due to some physical processes, such as flocculation, coalescence, physical ripening, gravitational separation and the phase inversion (Friberg and Larsson, 1997; McClements, 2000). The creaming is an example of physical instability of an emulsion. However, kinetically stable emulsion can be formed by adding emulsifiers and/or thickening agents to overcome the activation energy of the system. In our study, the lupin proteins were used as emulsifiers, diminishing creaming percentage, especially when protein was treated with the enzyme.

Shear thinning behaviour

Different enzyme concentrations were tested and the results are shown in Figure 6. The MTGase catalyzed cross-linking of the lupin polypeptides led to increased apparent viscosity of the emulsions

over the entire range of tested shear rates. The viscosity enhancement was directly correlated with the enzyme concentration.

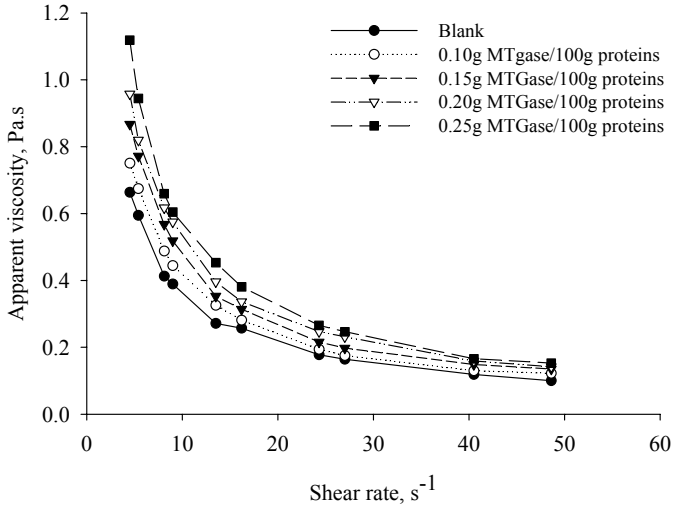

Figure 6. Influence of the MTGase concentration on the rheological behavior of the emulsions based on lupin proteins. Reaction conditions – temperature: 40°C, setting time: 60 min

The influence of the setting temperature and setting time on the rheological behavior of the lupin protein-based emulsions is presented in Figure 7 and Figure 8, respectively. For all shear rates, the apparent viscosity of the emulsions based on lupin proteins treated with 0.25 g MTGase/100 g proteins, increased on increasing the setting temperature from 4 to 50°C (Figure 7) and the setting time from 0 to 90 minutes (Figure 8). The apparent viscosity decreases for the whole domain of the tested shear rates, the decrease being more accentuated in the first domain. As the shear rate sufficiently increases to overcome the Brownian motion, the emulsion droplets become more ordered along the flowing field and offer less resistance to flow and hence the lower viscosity (MC Clements, 1999). The thinning behavior of the emulsions at shear flow appears as a consequence of structure degradation induced by the friction forces linked to the deflocculating mechanism of oil droplets.

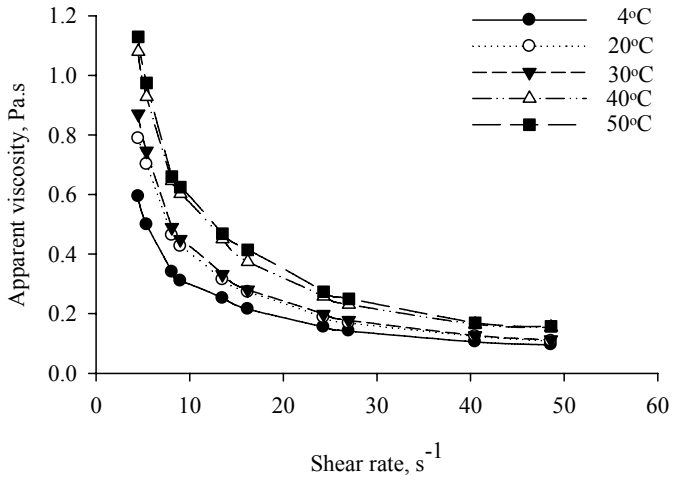

Figure 7. Influence of the setting temperature on the rheological behavior of the emulsions based on lupin proteins. Reaction conditions - MTGase concentration: 0.25 g/100 g proteins, setting temperature: 40°C

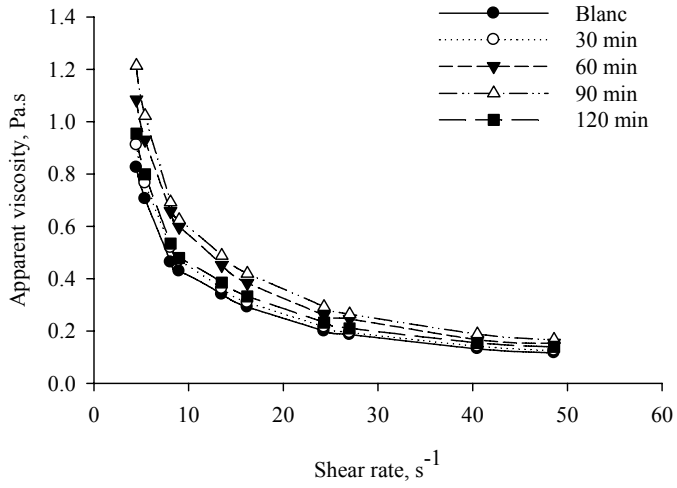

Figure 8. Influence of the setting time on the rheological behavior of the emulsions based on lupin proteins. Reaction conditions - MTGase concentration: 0.25 g/100 g proteins, setting temperature: 40°C

4. Conclusions

The MTGase treatment of lupin proteins led to the modification of their gelling and emulsifying properties. The intensity of these modifications was directly correlated with the enzyme concentration, the reaction temperature and the setting time during which the MTGase reacted with the lupin proteins.

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