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Effect of thermosonication on functional and rheological properties of green lentil protein

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ABSTRACT

The objective of this study was to evaluate the effect of ultrasound in bath at 30, 50 and 70ºC for 5, 10, 20 and 30 min for improving functional and rheological properties of green lentil protein. The percentage of recovered lentil protein after alkaline extraction was calculated as 82.79%. The protein solubility of protein solutions significantly increased after sonication treatment. However, sonication treatment at 70ºC after 20 min caused a partial reduction of protein solubility. The thermosonication process markedly improved the emulsion activity and stability index up to 176.02 m^2/g and 40.68%, respectively, and also foaming capacity of sonicated protein suspension was 3.35-fold higher than that of the untreated sample. Protein suspensions displayed shear-thinning flow behavior, and results were satisfactorily fitted to Ostwald-de Waele model ($R^2 > 0.926$). As a result, the findings indicated that functional and rheological properties of proteins can be improved by ultrasound in combination with heating, and thus thermosonication process may be a valuable processing technology for modifying of lentil protein.

1. Introduction

Recently, there is a particular interest focused on plant-based proteins as alternative to animal based proteins in food industry due to consumer demands originating from health concerns, allergenicity, religious limitations and rising of vegetarian and also increasing global economic problems (Aydemir & Yemenicioğlu, 2013; Ladjal Ettoumi, Chibane, & Romero, 2016). Lentil, which belongs to the family Leguminosae, is considered particularly because of its high content of proteins (containing 20.6–31.4% protein on dry weight basis) and low content of fat (Joehnke et al., 2021). Lentil proteins are gaining importance by the food industry due to high nutritional quality and digestibility, their rich essential amino acid profiles such as lysine and leucine, and also excellent functional attributes include foaming, emulsifying and gelling (Boye et al., 2010; Liang & Tang, 2013). The major protein fractions of lentil proteins are globulin and albumin, which are represent 50–65% and 10–25% of the total protein, respectively (Boye et al., 2010; Yingxin Wang, Supratim Ghosh, & Nickerson, 2019). Also, glutelins and prolamins are present in minor levels, representing 11.2% and 3.5% of the total proteins, respectively (Aryee & Nickerson, 2012). Globulins primarily consist of two major protein fractions: legumin (11S) and vicilin (7S) with a molecular weight of 350-400 kDa and 150, respectively (Wang, Ghosh, & Nickerson, 2019). Albumin protein fractions with a molecular weight of 20 kDa consist of enzymatic proteins, protease inhibitors, amylase inhibitors, and lectins (Joehnke et al., 2021). In general, globulin and albumin protein fractions are substantially responsible for the functional properties of lentil proteins. Moreover, the functional properties of plant-based proteins are dependent on composition (e.g. vicilin/legume ratio), conformational stability, solubility and hydrophobicity (Ladjal Ettoumi et al., 2016). Additionally, environmental conditions, processing methods and storage conditions have an effect on functional properties of protein (Ladjal Ettoumi et al., 2016; Saricaoglu, 2020; Zhong & Xiong, 2020). Recently, non-thermal processing methods including high-pressure homogenization, high hydrostatic pressure and ultrasound have drawn considerable attention to modifying food ingredients (Liu et al., 2020; Saricaoglu, 2020; Sha, Koosis, Wang, True, & Xiong, 2021).

Ultrasound technology has been extensively used in the processing of food and application of sonication for modifying proteins and hence improving functional properties has been increasingly studied (Lafarga, Álvarez, Bobo, & Aguiló-Aguayo, 2018; Martinez-Velasco et al., 2018). Moreover, ultrasound treatment has been combined with heating that named thermosonication, which could be used to increase the effectiveness of modification for improving protein functionality due to synergistic effect of cavitation and thermal energy (Chen, Ettelaie, & Akhtar, 2019). Recently, thermosonication has been used for mung bean protein (Zhong

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& Xiong, 2020), white kidney bean protein (Ashraf et al., 2020) to improving functional properties. However, there is no information about the functional properties of green lentil protein with thermosonication technique. Therefore, the objectives of this study was to improve the functional properties of green lentil protein by thermosonication.

2. Materials and methods

2.1. Materials

The green lentils used for the preparation of protein isolate were obtained from a local market in Kastamonu, Turkey. Lentils were ground with a laboratory blender (Waring, USA) and sieved using a sieve to mesh size <250 µm. The flour was packed in polyethylene bag and stored at 4ºC until required. All reagents used for alkaline extraction and analysis were analytical grade. Sunflower oil was obtained from a local market.

2.2. Protein extraction and application of thermosonication

For alkaline extraction of lentil protein, the method described by Saricaoglu (2020) was performed. Lentil flour was mixed with distilled water at room temperature at flour : water ratio of 1/10 (w/v). The pH of the solution was adjusted to 8.0 with 2 M NaOH to solubilize the proteins and stirred using a magnetic stirrer for 2 h at room temperature. After the stirring, the resultant dispersion was centrifuged at 8.000 *x g* for 10 min at 4 ºC, using a centrifuge (NUVE NF-800R, Turkey). The collected supernatant was adjusted to pH 4.5 using 2 M HCl to precipitate the proteins, and then it was centrifuged at 8.000 *x g* for 10 min at 4 ºC. The precipitated protein was collected, re-dispersed with distilled water, dried in the oven at 45 ºC for 24 h. Lentil proteins were ground using a mill and stored in glass jar at refrigerating condition until further analysis.

For the preparation of lentil protein suspension, lentil protein (2 g) was dispersed in distilled water (100 mL). The pH of protein solution was adjusted to pH 9.0 using 2 M NaOH to obtain a good protein solubility, and stirred for 1 h at ambient temperature. The protein solution was thermosonicated using ultrasonic bath with varying temperatures (30, 50 and 70 °C) and times (5, 10, 20 and 30 min). After thermosonication process, the suspensions were immediately cooled using an ice water bath. The solution without thermosonication process was evaluated as the control sample.

2.3. Physicochemical analysis

The moisture, crude protein (N x 6.25), fat and ash of the lentil protein isolate were determined according to the AOAC (1990) standard methods. Physicochemical analysis in green lentil isolate was done by using proximate analysis that is explained as: protein and fat content were carried out by the Kjeldahl and Soxhlet methods, respectively. Meanwhile, the contents of moisture and ash were carried out by conventional methods. The percentage of total carbohydrates was calculated by subtracting the total percentage of other components from 100.

2.4. Protein solubility

Protein solubility in water was determined by the method of described by Robinson and Hogden (1940), with slight modifications. The treated and untreated lentil protein suspensions (200 mg) were added to 20 mL of distilled water, and stirred at 400 rpm for 30 min on a magnetic stir plate at room temperature. Then, the suspensions were centrifuged at 4100 rpm for 15 min to remove undissolved particles. One mL of supernatant was mixed with 1 mL of Biuret reagent and the absorbance of solution was measured at 550 nm with UV-Visible spectrophotometer (Shimadzu, 1800, Japan). Soluble protein content of sample was determined by using a standard curve of Bovine Serum Albumin (R^2 = 0.9997). The protein solubility was calculated as a percentage of total protein with the following equation:

$$Solubility\ (\%) = \frac{Protein\ content\ in\ supernatant}{Total\ protein\ content\ of\ sample} x100 \quad (1)$$

2.5. Emulsifying properties

Emulsifying activity (EAI) and emulsion stability indexes (ESI) were evaluated according to the method reported by Ogunwolu, Henshaw, Mock, Santros, and Awonorin (2009). Emulsions were prepared with the mixing of 300 mg lentil protein suspensions, 30 mL distilled water and 10 mL sunflower oil, and homogenized at 20,000 rpm for 2 min using a digital Ultra-Turrax homogenizer (Daihan Scientific, Seoul, South Korea). The formed emulsions (50 µL) were transferred to a tube promptly after the emulsification and after 10 min of emulsification, and mixed with 5 mL of 0.1 % sodium dodecyl sulfate solution. The absorbance of the diluted emulsions was recorded at 500 nm and the EAI and ESI were then calculated by following equations:

$$EAI = \frac{2\ x\ 2.303\ x\ A_0}{0.25\ x\ protein\ weight\ (g)} \quad (2)$$

$$ESI = \frac{A_{10}\ x\ \Delta t}{\Delta A} \quad (3)$$

Where; A_0 and A_{10} refer the absorbance after 0 and 10 min of homogenization, respectively; Δt=10 min; $\Delta A = A_0 - A_{10}$.

2.6. Foaming properties

Foaming properties (capacity and stability) were evaluated according to a method described previously (Saricaoglu, Gul, Besir, & Atalar, 2018), with some slight modifications. After thermosonication treatment, 300 mg lentil protein suspension was mixed with 30 mL distilled water, transferred into 100 mL of graduated cylinder, and homogenized at 15,000 rpm for 2 min. Foaming capacity (FC) was calculated after mixing as the percent increase in the volume of the suspension, while foaming stability (FS) was determined as the percentage of foam remaining after 30 min.

2.7. Rheological analysis

The flow properties of lentil protein suspensions before and after thermosonication treatment were determined by recording shear stress against shear rates between 1 and 100 s^{-1} by using a rheometer (Anton Paar, MCR 302, Austria) in parallel plate geometry, with a plate diameter of 35 mm. Ostwald-de Waele model (Eq. 4) was used for describing the relationship between shear stress and shear rate:

$$\eta_{app} = K \times \dot{\gamma}^{n-1} \quad (4)$$

where η_{app} refers the apparent viscosity (Pa.s); K refers the consistency index (Pa.s^n); $\dot{\gamma}$ refers the shear rate (s^{-1}) and n refers the flow behavior index (dimensionless).

2.8. Statistical analysis

The statistical analysis of the results was conducted with a SPSS Statistics 21.0 package program. ANOVA was performed and the Duncan multiple range test was applied for determining the difference between the mean values. All treatments were carried out in triplicate with two independent occasions, and the results were expressed as the mean ± standard deviation (SD).

3. Results and Discussion

3.1. Composition of protein isolate

The physicochemical composition of lentil protein isolate obtained after alkaline extraction was determined. The moisture, fat and ash contents of lentil protein isolate were measured as 5.68%, %0.89 and 2.05%, respectively. The content of carbohydrate was calculated as 7.59%. The protein content was determined as 82.79%, which was similar to the reports by Saricaoglu (2020) who found that the protein content of lentil protein isolate is 84.02% after alkaline extraction. Also, the result of protein content in our study was higher than the result (80.6%) obtained by Wang et al. (2019).

3.2. Protein solubility

Protein solubility is a thermodynamic index for the equilibrium between protein–protein and protein-solvent interactions, and it is a key index of functional properties of proteins, such as emulsification, surface-active, thickening, foaming, gelation, which are highly dependent on the solubility of proteins in water (Saricaoglu, 2020; Zhong & Xiong, 2020). Therefore, in the food industry, high water-solubility is an important attribute. The soluble protein contents of control sample and thermosonicated lentil protein solutions were shown in Figure 1. The protein solubility of solutions was significantly affected by both process time and temperature ($P<0.05$). The protein solubility increased significantly compared to the control sample when higher temperature levels and sonication times were used. However, only a partial reduction in sample treated at 70ºC was observed after 20 min, but it was not significant ($P>0.05$). The highest soluble protein content was obtained when a temperature was set at 50°C and a sonication time of 30 min (72.29%), which was larger than the untreated sample (41.64%). Our results are in accordance with the previous studies indicated that sonication treatment caused an increase in protein solubility for soy proteins (Hu et al., 2013; Jambrak, Lelas, Mason, Krešić, & Badanjak, 2009), meat proteins (Wang, Yang, Tang, Ni, & Zhou, 2017), mung bean proteins (Zhong & Xiong, 2020) and whey proteins (Jambrak, Mason, Lelas, Herceg, & Herceg, 2008). The increase in the protein solubility may be due to the conformational change during sonication process and formation of smaller soluble protein aggregates (Tang, Wang, Yang, & Li, 2009), which leads to increasing the interaction between the proteins and water molecules due to higher surface area, resulting in increased protein solubility (Hu et al., 2013). Furthermore, protein solubility could be improved during the formation water-protein hydrogen bonds, replacing former intramolecular hydrogen bonds (Zhong & Xiong, 2020).

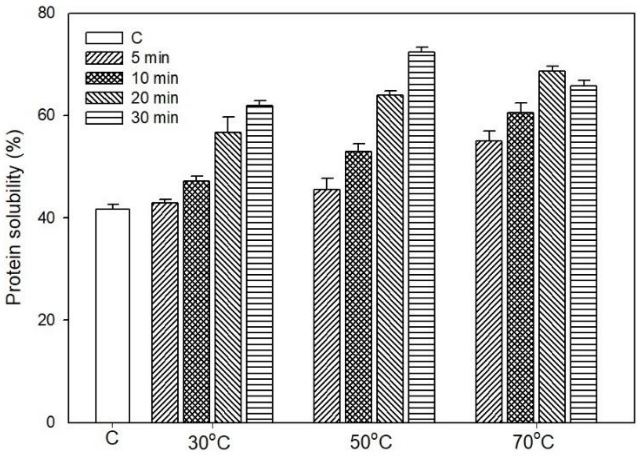

Figure 1. Protein solubility of green lentil protein untreated (control) and treated by ultrasound combined with heating at 30, 50 and 70 ºC for 5, 10, 20 and 30 min

3.3. Emulsifying properties

The effect of thermosonication treatment on emulsifying activity (EAI) and stability index (ESI) of lentil protein suspensions was measured because of the ability of proteins absorb to the oil-water interface that is important for many products due to formation and stabilization of emulsions (Zhu et al., 2018), and the results were given in Figure 2.

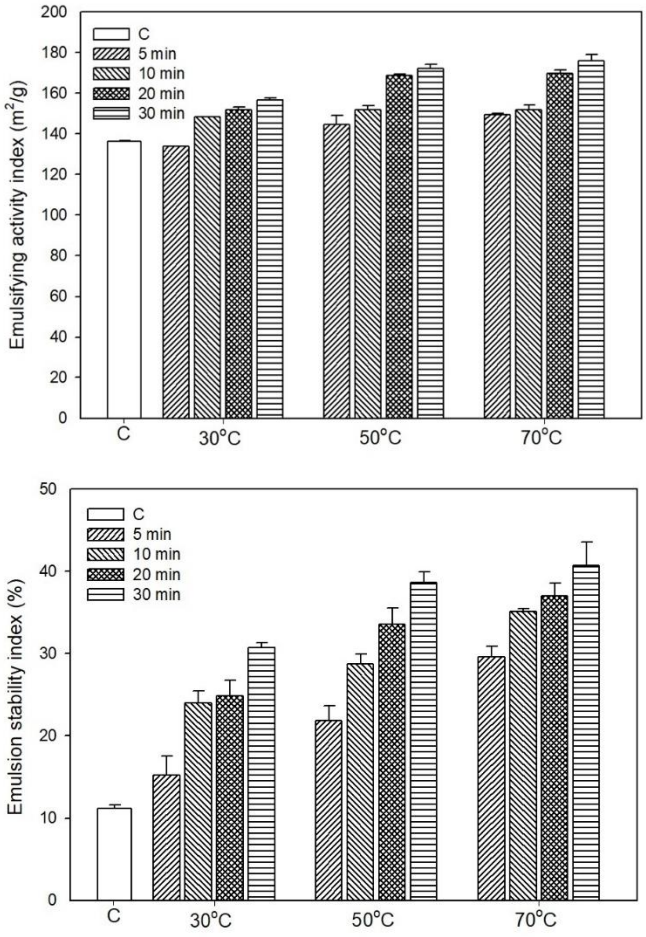

Figure 2. Emulsifying properties of green lentil protein untreated (control) and treated by ultrasound combined with heating at 30, 50 and 70 ºC for 5, 10, 20 and 30 min

The EAI and ESI values of control sample were determined as 136.34 m²/g and 11.15%, respectively, and

these values of lentil proteins significantly increased as the sonication temperature and time increased (P<0.05). A maximum value of EAI and ESI (176.02 m^2/g and 40.68%, respectively) was observed for the sample sonicated at 70°C for 30 min. This enhancement of emulsifying properties of lentil proteins after sonication process could be explained by a greater fraction of small soluble proteins available to adsorb to the oil-water interface or by some change in the surface chemistry of the proteins induced by sonication that increased their surface activity (Zhu et al., 2018). Similarly, Zhu et al. (2018) reported that the EAI and ESI values of the walnut proteins were higher in all the sonicated samples than in the untreated samples. In another study conducted by Jambrak et al. (2009), an increase in EAI values for soy protein isolate was observed after sonication process, which could be explained by the decrease in droplet size and increase in the percentage of adsorbed proteins as the ultrasound treatment is improved.

3.4. Foaming properties

Foaming capacity and stability are important functional properties of proteins used in food applications. The foaming capacity depends on the diffusion of the protein at the air-water interface by unfolding its structure, while the foaming stability is dependent on the formation of a thick cohesive layer around the air bubble (Flores-Jimenez et al., 2019; Khan et al., 2011). The effects of thermosonication treatment at different temperatures and times on the foaming properties of the lentil protein suspensions were shown in Figure 3.

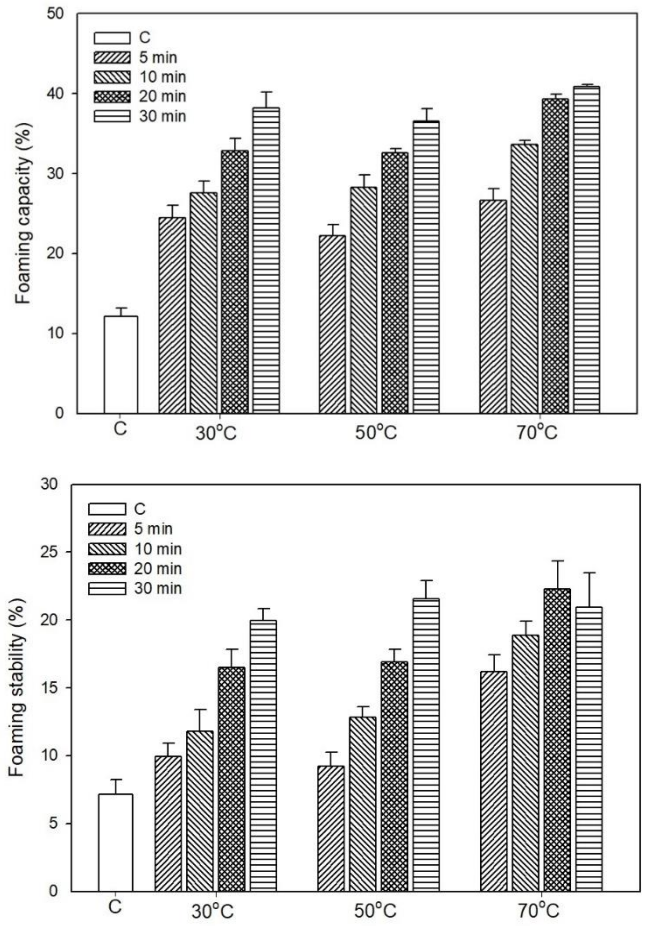

Figure 3. Foaming properties of green lentil protein untreated (control) and treated by ultrasound combined with heating at 30, 50 and 70 °C for 5, 10, 20 and 30 min

Both foaming capacity and stability were significantly affected by thermosonication treatment (P<0.05). The foaming capacity of sonicated lentil protein solution (at 50 °C for 20 min) was 3.35-fold higher than that of control sample. In addition, foaming stability of sonicated protein solutions was significantly higher than that of untreated sample (P<0.05). Similarly, other researchers have also reported an improvement in foaming capacity and stability for canola protein (Flores-Jimenez et al., 2019), pea protein (Xiong et al., 2018), faba bean protein (Martinez-Velasco et al., 2018) and whey protein (Jambrak et al., 2008) suspensions after sonication process. In general, foaming properties of proteins were related to the particle size, surface charge and hydrophobicity, and molecular flexibility (Zhang et al., 2014). However, the foaming stability of sonicated lentil protein suspension at 70 °C for 30 min was significantly decreased. This decrease might be explained by the partial denaturation during sonication treatment (Xiong et al., 2018), which are in good agreement with protein solubility results (Figure 1).

3.5. Rheological properties

The flow properties of thermosonication treated lentil protein suspensions were illustrated in Figure 4 as apparent viscosity vs shear rate.

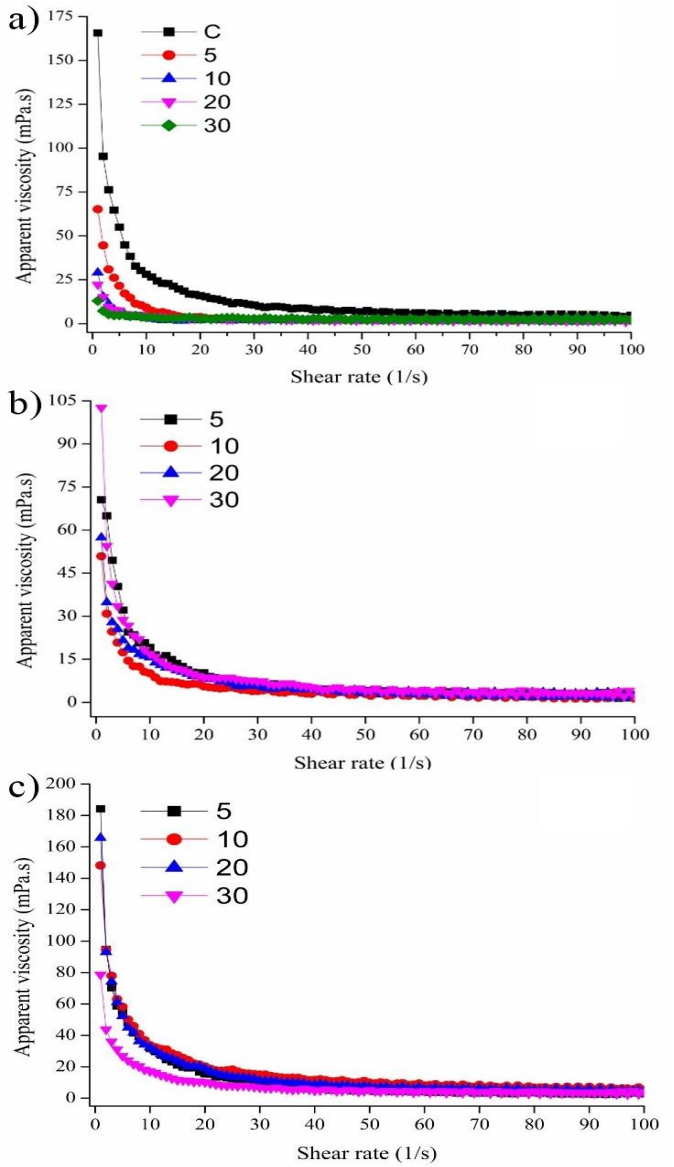

Figure 4. Flow properties of green lentil protein untreated (control) and treated by ultrasound combined with heating at 30, 50 and 70 °C for 5, 10, 20 and 30 min

Table 1. Effect of thermosonication treatment on consistency index (*K*) and flow behavior index (*n*) derived from Ostwald-de-Waele model of steady shear data.

Time	*K* (*Pa.s*^{n})			*n*			R^2		
(min)	30	50	70	30	50	70	30	50	70
C	169.30±2.72^{a}	169.30±2.72^{a}	169.30±2.72^{b}	0.214±0.01^{c}	0.214±0.01^{c}	0.214±0.01^{b}	0.994	0.994	0.994
5	70.13±3.16^{bC}	81.80±2.25^{cB}	184.10±3.59^{aA}	0.137±0.01^{dB}	0.290±0.01^{bA}	0.166±0.01^{cB}	0.974	0.950	0.988
10	27.36±2.67^{cC}	51.70±1.37^{eB}	153.70±2.62^{cA}	0.192±0.02^{cC}	0.275±0.02^{bB}	0.327±001^{aA}	0.926	0.992	0.993
20	26.95±2.01^{cC}	58.85±3.14^{dB}	166.90±2.04^{bA}	0.311±0.01^{bA}	0.334±0.02^{aA}	0.241±0.01^{bB}	0.937	0.982	0.995
30	8.92±1.60^{dC}	101.10±3.79^{bA}	78.13±3.31^{dB}	0.625±0.02^{aA}	0.211±0.01^{cC}	0.302±0.01^{aB}	0.955	0.996	0.993

C: Control sample without treatment; *K*: consistency index; *n*: flow behavior index; R^2: determination coefficient of Eq. (4).
a–d Values followed by different letters in one column show significant differences by Duncan's test at 95% significance (P<0.05)
A–C Values followed by different letters in one line show significant differences by Duncan's test at 95% significance (P<0.05)

It is clear that the apparent viscosity of all samples decreased with increasing shear rate showing shear thinning behavior, which is typical for protein suspensions (Fernandez-Avila, Escriu, & Trujillo, 2015; Saricaoglu, 2020; Saricaoglu et al., 2018). At high shear rates, the differences in apparent viscosity between samples treated with different thermosonication conditions were smaller than those at low shear rates. This could be explained by the structural breakdown and rearrangement induced by the shear rates. The apparent viscosity of control sample at the beginning of shear deformation had the highest value when compared with thermosonication treated samples at 30 and 50 °C, which had lower apparent viscosity values at the beginning of shear deformation. However, thermosonication treatment at 70 °C for 5 min caused a higher initial apparent viscosity value than control, and decreased with increasing application time. This could be related with the solubility of lentil proteins in hot water conditions.

The apparent viscosity vs shear rate data were well fitted to Ostwald de-Waele model (Eq. 4) and the related results (*K* and *n* values) were summarized in Table 1, as well as R^2 values between 0.926 and 0.996. The consistency index (*K*) of control sample was higher than thermosonication treated samples at 30 and 50 °C. However, the application of thermosonication at 70 °C significantly increased the *K* values, which was probably due to gelation of proteins causing viscoelastic structure with high consistency. The flow behavior index (*n*) of samples was lower than 1 supporting shear thinning behavior.

4. Conclusions

This study revealed that the functional and rheological properties of green lentil protein isolate treated with ultrasound in combination with heating were found to be markedly altered compared to untreated protein. The soluble protein content after thermosonication was increased up to 1.7 times as compared with the result of untreated sample. Additionally, thermosonication treatment significantly enhanced the emulsion and foaming properties of green lentil proteins. All protein suspensions displayed shear-thinning behavior with increasing shear rate and flow curves were satisfactorily fitted to Ostwald de-Waele model. It can be concluded from this study that thermosonication at 70°C up to 20 min could be a favorable technique for modifying protein functions, hence improving functional properties of green lentil proteins.

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