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Effect of thermal treatment on microbiological, physicochemical and structural properties of high pressure homogenised hazelnut beverage

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RESEARCH ARTICLE

Abstract

The aim of this study was to investigate the effect of low (65, 72 and 85 °C) and high (105, 110 and 115 °C) temperature heat treatment on the microbiological, physical and chemical properties of high pressure homogenised hazelnut beverage. The total number of aerobic bacteria decreased with heat treatment and was not detected after 72 °C and higher heat treatments. The pH value of hazelnut beverage did not change significantly as a function of temperature (P>0.05). The total soluble content and soluble protein values of the low or high heat treated hazelnut beverage were significantly decreased after the heat treatments considered non-thermal treated ones, and also serum separation was adversely affected (P<0.05). Changes in colour components showed an increase in browning of hazelnut beverage by thermal treatment. The viscosity values of the samples significantly increased depending on the temperature except for the 65 °C treatment (P<0.05), and Herschel Bulkley's model was sufficient to describe the flow behaviour. Heat treatment at 85 °C for 5 min and higher temperatures led to an increase in particle size due to protein denaturation. Our results showed that the ideal temperature-time parameters were determined as 20 min at 72 °C and 1 min at 105 °C, based on microbiological results and better physicochemical properties.

Keywords: by-products, fruit crops, hazelnut beverage, thermal stability, microstructure

1. Introduction

Plant-based non-dairy beverages are good alternatives to animal based food products because animal milk has some health problems like lactose intolerance and allergy (Kurajdová *et al.*, 2015). So that, consumers avoid animal based dairy products due to medical reasons (lactose intolerance, allergy of cow milk, phenylketonuria), lifestyle reason (vegetarian-vegan diet) and concerns (antibiotic residues in milk, growth with hormone) (Makinen *et al.*, 2016). On the other hand, beverages other than dairy products are rich in polyphenols, vitamins, minerals and essential fatty acids and they have a low content of saturated fatty acids (Ahmadian-Kouchaksaraei *et al.*, 2014). The most known vegetable beverage is soy beverage and there are much more product varieties beside of soy beverage such as tiger nut, rice, coconut, oat, walnut, sesame, almond and hazelnut beverages (Bernat *et al.*, 2014a). Hazelnut beverage and hazelnut beverage derived products have been gaining popularity as a plant based beverage source because nuts provide good sources of phytochemicals, dietary fibres and carbohydrates with low glycaemic index (Bernat *et al.*, 2014b; Makinen *et al.*, 2016). Previous studies in our laboratory have shown that hazelnut beverage obtained from hazelnut cake as a by-product can be consumed favourably (Gul *et al.*, 2017, 2018a).

Thermal treatment is an important step in the production of food products which ensures microbiological safety and increases shelf-life of animal milk and vegetable beverages also. According to Sakkas *et al.* (2014), temperature time combinations, utilised heating methods and pretreatment

conditions determine the efficiency of thermal treatment on quality and technological and nutritional properties of products. On the other hand, thermal heat treatment had a negative effect on the sensory properties such as coked flavour and dark colour, and it may cause loss of nutritional value because of the Maillard reaction (Elliott *et al.*, 2003). Thermal treatments have been reported to have different influence on to obtain bacteriologically safe final products and to extend their shelf life for animal milks (Felfoul *et al.*, 2016; Giacometti *et al.*, 2016), soy beverage (Kwok *et al.*, 2002; Shimoyamada *et al.*, 2008), tiger nut beverage (Codina-Torrella *et al.*, 2018), almond beverage (Dhakal *et al.*, 2014) and other ones (Ahmadian-Kouchaksaraei *et al.*, 2014; Gan *et al.*, 2016). Nevertheless, information on effects of thermal treatment on hazelnut beverage is lacking in the literature. The aim of this study was to evaluate the influences of thermal treatment on microbiological, physicochemical and structural properties of hazelnut beverage. For this purpose, heat treatment was carried out at 65, 72 and 85 °C (low temperature) and at 105, 110 and 115 °C (high temperature) for high pressure homogenised hazelnut beverage.

2. Material and methods

Production of hazelnut beverage

Hazelnut beverage was produced from hazelnut cake as a by-product of oil industry according to method described by Gul *et al.* (2017). Unshelled vacuum-packaged hazelnut samples were obtained from Gursoy (Ordu, Turkey). After removing shells-brown skin, the hazelnut oil was removed from hazelnut by using a headed cold press machine (Ekotok 1, Izmir, Turkey).

The resulting cold press hazelnut cake (9.12% moisture) was ground for 10 min using the Waring blender (Conair Corporation, Stamford, CT, USA). The ground hazelnut cake was mixed with distilled water at a ratio 1:10 ratio and homogenised for 10 min at 10,000 rpm using an Ultra-Turrax (IKA-Werke GmbH & Co. KG, Staufen, Germany). Then, hazelnut beverage was homogenised using a high pressure homogeniser (Panda PLUS 2000, GEA Niro Soavi, Parma, Italy) at a pressure of 100 MPa. The physicochemical properties of homogenised hazelnut beverage were as follows: 6.58 pH, 9.81% total solid, 4.48% protein, 1.62% lipid, 2.44% carbohydrate and 0.68% ash determined by AOAC methods (AOAC, 1995).

Thermal treatments

Low temperature heat treatment (LTLT) and high temperature heat treatment (HTST) of homogenised hazelnut beverage were carried out by using a temperature controlled water bath (Nuve BM 402, Ankara, Turkey) and an autoclave (Nuve OT 100V), respectively. Selected heat treatment conditions were determined according to preliminary studies on the effect of different heat treatments on microorganism inactivation. The heating temperature/time combinations used in this study were 65 °C/30 min, 72 °C/20 min and 85 °C/5 min for LTLT, and 105 °C/1 min, 110 °C/1 min and 115 °C/1 min for HTST. Hazelnut beverage (200 ml) was transferred to sterilised glass bottles and semi-sealed. Heat treatments were applied after the temperature of beverage samples was reached to the specified temperature and then hazelnut beverage samples were immediately cooled to 4 °C in an iced water bath.

Microbiological analysis

Viable counts were assessed as follows: 10 ml of well stirred hazelnut beverages was mixed with 90 ml of sterile peptone-water (Merck, Darmstadt, Germany) and the resulting suspension was homogenised for 1 min using the Stomacher laboratory blender (Smasher; AES Chemunex, Bruz, France). After the serial decimally diluted with peptone-water, 1 ml of each dilution in duplicate was added into petri dishes and incubated under the following: total aerobic mesophilic bacteria (TAMB) on plate count agar (Merck, Darmstadt, Germany) at 30 °C for 72 h and yeasts and moulds on yeast glucose chloramphenicol agar (Merck) at 25 °C for 5 d. After incubation, the colonies were counted and the results were treated as log cfu/ml of hazelnut beverage.

Total soluble solids, protein solubility and pH

Total soluble solids (°Brix) of hazelnut beverage samples were carried out by using Hand refractometer at 20 °C. The soluble protein content of hazelnut beverage was determined using the Biuret method (Robinson and Hogden, 1940). Hazelnut beverage (0.1 ml) was mixed with 1 ml of Biuret reagent (pH 7.4) and homogenised by vortexing for 1 min. Samples were kept at room temperature for 20 min and absorbance at 550 nm was measured using a UV spectrometer (Helios Gama, Birmingham, UK). Protein solubility of the samples was calculated from a standard curve of bovine serum albumin. The pH measurement was performed with a calibrated pH meter at 25 °C (Eutech Cyberscan pH 2700, Ayer Rajah Crescent, Singapore).

Oxidative stability

Lipid hydroperoxides in hazelnut beverage were determined by the method based on the reaction Fe II/ thiocyanate, reported by Valencia-Flores *et al.* (2013) with some modifications. Two ml of homogenised hazelnut beverage was mixed with 2 ml of methanol and 4 ml of chloroform and stirred for 1 min. After centrifuging at 8,000×*g* for 20 min at 20 °C, the chloroform phase (1 ml) was transferred to a new test tube and mixed with 1 ml of Fe

(II)/thiocyanate in methanol/chloroform (1:1). The mixture was allowed to react for 10 min at room temperature and the absorbance was measured at a wavelength of 500 nm with a spectrophotometer (Helios Gama). Lipid peroxides were quantified from a calibration curve made according to the methodology described by the International Dairy Federation (IDF, 1991). The measurement was based on the spectrophotometric reading of a dilution series containing Fe^{3+} in chloroform/methanol (70:30) at 500 nm data were expressed as meq peroxide/l of the sample.

Particle size distribution and zeta potential

The particle size distribution of the samples was measured by light scattering (Malvern Mastersizer 2000 with Hydro 2000 G(A), Malvern Instruments Ltd, Malvern, UK) immediately after preparation using deionised water as dispersion medium. The hazelnut beverage sample was placed in a cuvette with a length of 1 cm and inserted into the cuvette holder. Before measuring the particle size at 25 °C, the sample was equilibrated at 25 °C for 120 s. The size distribution was characterised by $D_{0.5}$ indicating that 50% of the particles fell below the specified diameter.

The zeta potential of the samples was measured by using Malvern Zetasizer Nano-ZSP (Malvern Instruments Ltd). All experiments were carried out in distilled water as a dispersant using a disposable folded capillary cell with electrodes at 20 °C. The zeta potential values were reported as the average of five replicates.

Microstructural properties

The optical microstructure of hazelnut beverage samples was evaluated using an optical microscope (CX41; Olympus, Tokyo, Japan) with a 40× objective. For analyses, 25 µl of homogenised sample was placed on a glass slide and covered with a cover ship carefully rotated at 45° to guarantee the same orientation for the samples (Kubo *et al.*, 2013). The resulting photographic images were interpreted using imaging software (BP20; Olympus).

Solid particle sedimentation

Solid particle sedimentation was determined using phase separation method according to Kubo *et al.* (2013). Regarding the phase separation method, the hazelnut beverage samples were transferred to measurable centrifuge tubes and the height of the separate phase was quantified throughout the storage time (15 days) at 4 °C. Sodium azide at a concentration of 0.04 g/100 ml was added to the samples for preventing microbial growth during the storage period. The results were expressed as sedimentation index and calculated as follows:

$$Sedimentation\ index\ (\%) = \frac{V_t}{V_0} * 100 \quad (1)$$

where V_t is the sedimentation volume at storage time (ml) and V_0 is the initial volume (ml).

Rheological measurements

Rheological measurements were carried out with a rheometer (Haake Mars III rheometer, Thermo Scientific, Schwerte, Germany) equipped with cone-plate geometry (2° angle, 35 mm diameter, 0.104 mm gap size). The temperature was maintained constant at 25 °C by a Peltier system. Recording of shear stress values when shearing the samples at linearly increasing shear rates from 1 to 100 s^{-1} through 120 s can provide to identify flow behaviour of the samples. The flow behaviour of hazelnut beverage was modelled using the best fit with the Herschel Bulkley's model (Equation 2):

$$\tau = \tau_0 + K\,\dot{\gamma}^n \quad (2)$$

Where τ is shear stress (Pa), τ_0 is yield stress (Pa), K is consistency index (Pa.s^n), $\dot{\gamma}$ is shear rate (s^{-1}) and n is flow behaviour index (dimensionless).

Colour properties

A colorimeter (ColorFlex EZ colorimeter; Hunter Associates Laboratory, Inc., Reston, VA, USA) was used to measure colour values as L^* (Lightness/brightness), a^* (redness/greenness) and b^* (yellowness/blueness) of hazelnut beverage samples. The colorimeter was calibrated using white and black standard tiles. Illuminate D 65 and a 10° standard observer were used. Five readings were taken for each sample. After getting colour values of the samples, the colour difference (ΔE) was calculated as follows:

$$Colour\ difference = \Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a)^{*2} + (\Delta b)^{*2}} \quad (3)$$

The colour difference was calculated based on the non-heat-treated hazelnut beverage sample as a reference.

Statistical analysis

All experiments were performed three times and the data were expressed as mean ± standard deviation. SPSS statistics programme (SPSS version 21.0, Chicago, IL, USA) was used to determine the statistical performance of samples. Differences between the samples treated with different temperature-time conditions were determined using one-way analysis of variance (ANOVA) and Duncan's multiple range test was applied to compare the means with a confidence level of 95% (P<0.05).

3. Results and discussion

Microbiological analysis

The microbiological results of hazelnut beverage samples before and after low and high temperature treatment are given in Table 1. The TAMB of the control sample (after homogenisation at 100 MPa) was detected as 5.43 log cfu/ml and this result was in agreement with the results obtained by other authors in tiger nuts beverages (Codina-Torrella *et al.*, 2018). TAMB counts were significantly decreased after the thermal treatment at 65 °C with the reduction of the microbial count of 2.41 log cycles. Increasing heat treatment up to 72 °C and HTST caused completely inactivation of microorganism. This result was in agreement with the finding of Valencia-Flores *et al.* (2013) who reported that the microbial counts of almond beverage were below the detection level (<0.5 cfu/ml) after pasteurisation at 90 °C for 90 s and ultra-high temperature at 142 °C for 6 s. Yeasts and moulds were only counted as 3.51 log cfu/ml in the control sample, indicating that thermal treatment ensured the microbial safety of hazelnut beverage. Similar results were also obtained by Codina-Torrella *et al.* (2018) in tiger nuts' and Gul *et al.* (2018b) in hazelnut beverages.

Soluble solids, protein solubility and pH

Soluble solid values of thermal treated hazelnut beverage samples are shown in Table 2. Soluble solid contents of beverage samples significantly decreased due to temperature (P<0.05). The control sample had the highest soluble solids content (8.07 °Brix), while hazelnut beverage heated at 115 °C had the lowest (6.47 °Brix) value. The total soluble solid content decreasing with heat treatment may be related to decreasing protein solubility (Kasera *et al.*, 2012). Similarly, Dhakal *et al.* (2016) reported that the thermal treatment of almond beverage at 72, 85 and 99 °C for different times (0, 30, 180, 300 and 600 s) caused a 1.5 unit drop in the soluble solid contents of almond beverage.

Table 1. Microbiological properties (log cfu/ml) of hazelnut beverage samples after heat treatments for low temperature long time (LTLT) and high temperature short time (HTST).[1]

Samples		Total aerobic mesophilic bacteria	Yeasts and moulds
Control		5.43±0.2[a]	3.51±0.13
LTLT	65 °C/30 min	3.02±0.03[b]	ND
	72 °C/20 min	ND	ND
	85 °C/ 5 min	ND	ND
HTST	105 °C/1 min	ND	ND
	110 °C/1 min	ND	ND
	115 °C/1 min	ND	ND

[1] ND = not detected.

Protein solubility is a good indicator of protein denaturation which also affects the stability of food and some functional properties such as emulsification, foaming and gelation in food system (Qin *et al.*, 2013). The protein solubility value of the control sample was determined as 4.66 g/100 ml hazelnut beverage (as shown in Table 2). Soluble protein content was found to be significantly decreased after heat treatment of LTLT and HTST (P<0.05). However, the reduction in protein solubility was not significant at 65 °C. The highest reduction in protein solubility was found in hazelnut beverage samples processed at 85 and 115 °C temperatures for LTLT and HTST, respectively. Similarly, Dhakal *et al.* (2014) observed that a significant reduction in protein solubility at 85 and 99 °C (~50 and ~75%, respectively), probably due to protein unfolding and subsequent increase in surface hydrophobicity increasing protein-protein interactions leading to reduction of protein solubility. Reduction of protein solubility by heat treatment can also be explained by protein aggregation and/or precipitation (Shimoyamada *et al.*, 2008). In addition, heat treatment may cause deterioration in secondary, tertiary and quaternary structures of proteins that may adversely effect on protein solubility (Dhakal *et al.*, 2014). Protein solubility values of hazelnut beverage samples showed a good correlation with soluble solids with correlation coefficient of 0.857 and 0.94 for LTLT and HTST, respectively (data not shown).

The pH of the control sample was 6.58, slightly decreased by thermal treatments and reached to 6.44 by heat treatment at 105 °C. In contrast to our study, Gan *et al.* (2016) and Kuo *et al.* (2014) stated that the pH value of bean beverage samples increased slightly by thermal treatment. Bernat *et al.* (2015) also observed a slight increase in the pH values of hazelnut beverage after heat treatments and explained this change by conformational changes in the components during homogenisation which may inhibit the ionisation of some acidic groups. Overall, thermal treatment did not markedly change the pH and this result was consistent with Tsai *et al.* (2018), Codina-Torrella *et al.* (2018) and Dhakal *et al.* (2016) who reported that heat treatment did not statistically impact pH values of hazelnut, tiger nuts' and almond beverages, respectively.

Oxidative stability

The formation of hydroperoxides as primary products of autoxidation lead to increase in lipid oxidation which is associated with the presence of oxygen, light, enzymes as well as high temperature (Poliseli-Scopel *et al.*, 2012; Valencia-Flores *et al.*, 2013). In this study, the oxidation process was evaluated by the hydroperoxide concentration in hazelnut beverage samples (Table 2). The hydroperoxide

Table 2. Physicochemical properties of hazelnut beverage samples after heat treatments for low temperature long time (LTLT) and high temperature short time (HTST).[1]

Samples		Total soluble solids (°Brix)	Soluble protein (%)	pH	Hydroperoxide index (meq/l)	Sedimentation index (%)
Control		8.07±0.13^{a}	4.66±0.13^{a}	6.58±0.03^{a}	0.047±0.006^{d}	78.79±2.06^{a}
LTLT	65 °C/30 min	7.80±0.28^{ab}	4.57±0.21^{a}	6.47±0.06^{ab}	0.096±0.003^{b}	75.75±0.35^{ab}
	72 °C/20 min	7.45±0.21^{b}	3.89±0.11^{b}	6.46±0.08^{ab}	0.104±0.007^{ab}	75.50±0.71^{ab}
	85 °C/ 5 min	7.47±0.11^{b}	3.53±0.07^{c}	6.45±0.09^{ab}	0.101±0.002^{ab}	76.25±1.06^{a}
HTST	105 °C/1 min	6.75±0.07^{cd}	3.69±0.06^{bc}	6.44±0.03^{b}	0.079±0.003^{c}	73.55±0.69^{b}
	110 °C/1 min	7.00±0.07^{c}	3.78±0.08^{b}	6.49±0.01^{ab}	0.114±0.006^{a}	72.44±0.35^{b}
	115 °C/1 min	6.47±0.04^{d}	2.86±0.13^{d}	6.51±0.01^{ab}	0.116±0.011^{a}	71.00±0.10^{c}

[1] Values are means ± standard deviations. Means with different letters in the same column are significantly different (P<0.05).

value of the control sample was determined as 0.047 meq peroxide/l and hazelnut beverage samples showed significantly (P<0.05) higher hydroperoxide values after heat treatments than the control sample. On the other hand, the lipid peroxide concentration of the hazelnut beverage sample treated at 105 °C was significantly lower than other thermal treated samples (P<0.05). Similarly, Ferragut *et al.* (2015) stated that the hydroperoxide index increased significantly (P<0.05) with thermal treatment in almond and soy beverage samples compared to the base product. Poliseli-Scopel *et al.* (2012) reported that the hydroperoxide value of soy beverage after pasteurisation (95 °C for 30 s) and sterilisation (ultra-high temperature; 142 °C for 6 s) did not exhibit a trend in comparing the untreated samples, although the index changed in a narrow interval between 0.2 and 0.4 meq/l for all samples. Also an increase in peroxide index from 0.29 to 0.46 meq/ml was noticed between the thermal treated almond beverage and non-treated ones (Valencia-Flores *et al.*, 2013).

Particle size and zeta potential

The particle size distribution of hazelnut beverage samples is shown in Figure 1. The control sample showed a mono-modal particle size distribution due to homogenisation, causing particle size to shrink to 43.9 µm (Figure 1A). A similar particle size distribution was found in the heat treated samples at 65 and 72 °C, and particle size of the samples was not significantly different from the corresponding control sample (P>0.05), ranging from 41.3 to 42.6 µm. However, after heat treatment at 85 °C and also HTST, the samples exhibited bimodal particle size distribution. The particle size in the samples after heat treatment at 85 °C and above significantly increased probably due to the protein denaturation and aggregation. The number of larger particles was greater than that of the smaller ones in HTST samples, shown in Figure 1A. Our result is in agreement with Dhakal *et al.* (2016) who stated that particle size of heat treated almond beverage at 72 °C did not alter significantly when compared with untreated almond beverage, but the average particle size was markedly increased when the process temperature was increased to 85 °C. When the thermal treatment applied to sample, the disappearance of particles significantly changes due to the change in protein conformation and the promotion of particle aggregations, which reported earlier by Bernat *et al.* (2015).

The effect of the thermal treatment on the zeta potential value is shown in Figure 1B. Zeta potential values of control and heat treated hazelnut beverage samples are obtained at the neutral pH (as shown in Table 1) which is higher than the isoelectric point (between pH 4 and 5) of hazelnut proteins (Turan *et al.*, 2015). Zeta absolute potential results were found to be negative in the range of -8.6 to -21.3 mV. These results indicate that hazelnut beverage samples contained more negatively charged particles than positively charged ones. Heat treatment for LTLT and HTST was found to have a significant effect on the zeta potential values of hazelnut beverage samples (P<0.05). Zeta potential values increased with increasing temperature, but gradually decreased when the temperature increased from 110 to 115 °C. Turan *et al.* (2015) found that roasting process had a significant effect on the zeta potential values of hazelnut flour containing emulsions. In contrary to our results, Bernat *et al.* (2015) found that the thermal treatment led to a slight reduction in the charge of the particles and this reduction could be explained by lower particle charge due to protein denaturation and aggregation. On the other hand, zeta potential values of hazelnut beverage samples between -18.2 and -23.8 mV obtained by Bernat *et al.* (2015) are similar to our results.

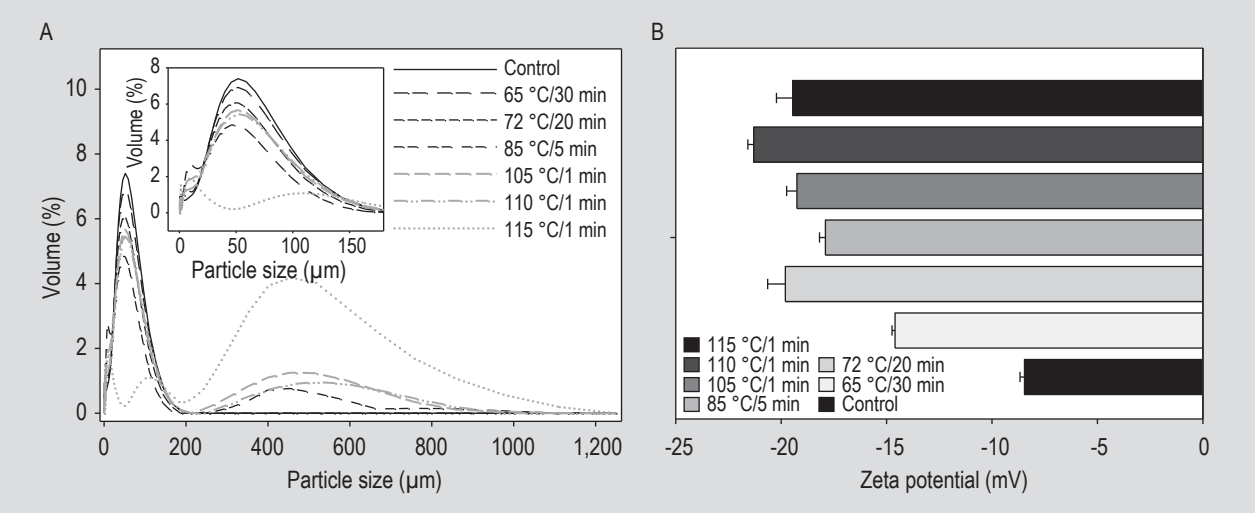

Figure 1. The influence of heat treatments for low temperature long time (LTLT) and high temperature short time (HTST) on (A) the particle size and (B) zeta potential in hazelnut beverage.

Microstructural properties

Microstructural properties of LTLT and HTST treated hazelnut beverage samples were examined with an optical microscope in order to better explain the experimental results, and micrographs were presented in Figure 2. It is clear from micrographs that the control and heat treated samples displayed significant differences. The control sample consisted mainly of deformable particles with irregular shapes and several small particles that were thought to be flocculated proteins due to the hydrophobic character (Bernat *et al.*, 2015; Gul *et al.*, 2017). As shown in Figure 2B and 2C, protein aggregation and/or flocculation were not observed for LTLT treated samples that could be due to the similar protein solubility of samples (Table 2), and protein particles in hazelnut beverages were well distributed by LTLT treatment and micrographs well supported to particle size distribution results. Heat treatment at 85 °C for 5 min led to the thermal aggregation of proteins (Figure 2D) which caused a significant increase in viscosity. HTST treatments of hazelnut beverages, except for 105 °C for 1 min, increased the formation of protein aggregates (Figure 2F and 2G) that significantly increased apparent viscosities, yield stress and particle sizes of samples. It was reported by Bernat *et al.* (2015) for almond beverages where high pressure homogenisation reduced particle size and increased to partial protein solubility, and high pressure and thermal treatment led to soluble protein denaturation and aggregation, as in a gel, thus the product microstructure was greatly modified. Denaturation and aggregation of soluble proteins with thermal process resulted in the formation of three-dimensional network structure, proven with increased yield stress. Therefore, the

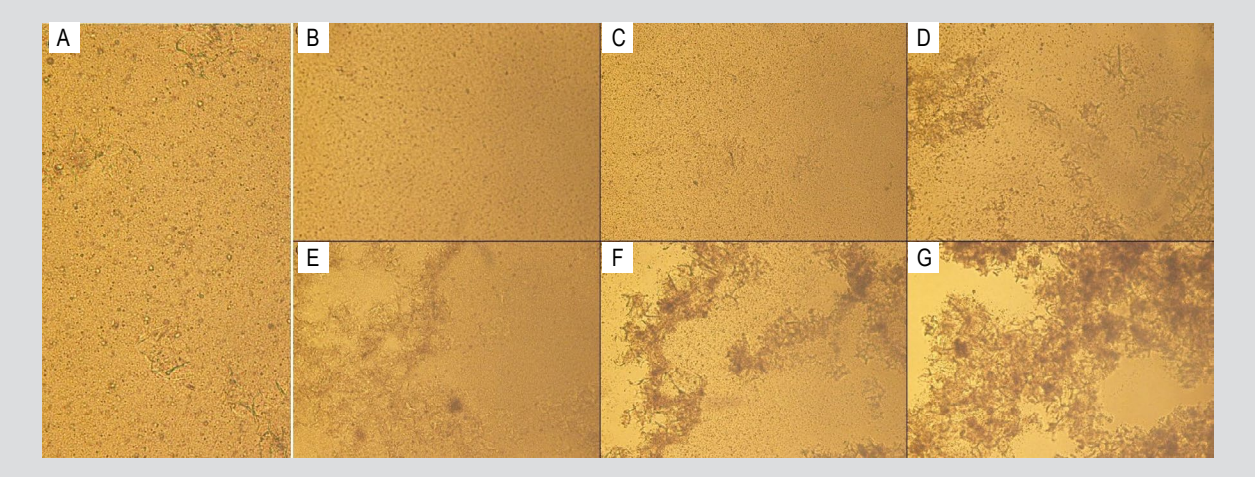

Figure 2. The influence of heat treatments for low temperature long time (LTLT) and high temperature short time (HTST) on the optical microstructure of hazelnut beverage samples: (A) control; (B) 65 °C for 30 min; (C) 72 °C for 20 min; (D) 85 °C for 5 min; (E) 105 °C for 1 min; (F) 110 °C for 1 min; (G): 115 °C for 1 min.

microstructural observations of hazelnut beverage samples revealed that the hazelnut proteins were thermally sensitive and denatured during thermal treatments that induced the formation of large aggregates.

Solid particle sedimentation

Solid sedimentation values of hazelnut beverage samples with phase separation method before and after heat treatment are given in Table 2. In both control and heat treated hazelnut beverage samples, the amount of sediment was increased over time and stabilised after almost 10 days (data not shown). The major changes were made in the first 3 and 7 days for HTST and LTLT treated hazelnut beverage samples. Particle sedimentation was occurred quickly in the HTST treated samples compared to the control and LTLT treated samples. As shown in Table 2, the heat treatment led to a reduction in colloidal stability values of the samples. This reduction was found significant for HTST treated samples (P<0.05). Similar particle sedimentation results, high sedimentation values compared to base product, were found by Valencia-Flores *et al.* (2013) for pasteurised and ultra-high temperature treated almond beverages. Similarly, Bernat *et al.* (2015) found that hazelnut beverage samples had a phase separation after storage due to a weak gel formation during thermal treatment. This could be related to the denaturation of the protein during the thermal treatment, leading to phase separation during storage of the product. Particle sedimentation was also reflected in the increase of the particle size in the samples after the heat treatment shown in Figure 1A. In contrary to our findings, Shimoyamada *et al.* (2008) found that heat treatment of soy beverage at temperatures above 90 °C caused to have more dispersion stability of the soy beverage protein or soy beverage emulsion consisting of proteins and lipids, because the precipitation of the soy beverage after heat treatment at 90, 100 and 115 °C was low correspond to the control sample.

Rheological properties

The flow curves (apparent viscosity vs shear rate) of LTLT and HTST treated hazelnut beverage samples are presented in Figure 3. As can be seen, the apparent viscosity of hazelnut beverages was higher at lower shear rates, but gradually decreased as the shear rate increased, which could be explained by structural breakdown and rearrangement induced by shear rates (Bortnowska *et al.*, 2013). The viscosity values of the control and heat treated hazelnut beverage samples decreased with increasing shear rates, indicating that the samples exhibited shear thinning behaviour. This behaviour was also reported by Gul *et al.* (2017) for high pressure homogenised hazelnut beverages.

Herschel Bulkley's model was found to be the best model in order to describe the flow behaviour of heat treated

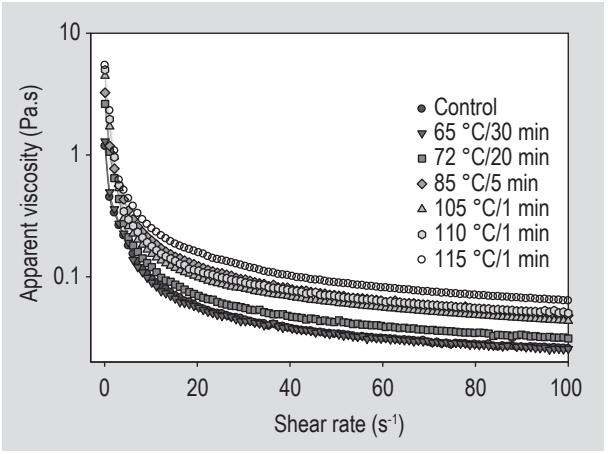

Figure 3. The flow behaviour of hazelnut beverage samples after heat treatments for low temperature long time (LTLT) and high temperature short time (HTST).

hazelnut beverage samples due to higher R^2 values of 0.988, and model parameters as well as apparent viscosity values of samples at 50 s^{-1} shear rate were given in Table 3. As can be seen, the heat treatment significantly affected to the viscosity values of the samples (P<0.05) and the highest viscosity values in LTLT and HTST processes were 0.069 and 0.093 Pa.s, respectively. Samples treated with LTLT did not significantly affect the apparent viscosity of samples, except for 85 °C. This may be related with similar particle size distributions (Figure 1) and microstructural images (Figure 3) of LTLT treated samples. HTST treatment significantly increased the apparent viscosity of samples except for 105 °C and led to a formation of weak gel like structure probably due to protein denaturation and subsequent cluster formation. The increased viscosity of the heat treated samples may also be related to the swelling of the starch granule and protein aggregation or the protein network strengthening due to thermal coagulation of proteins (Turan *et al.*, 2015). Moreover, the soluble fibre fractions in the hazelnut beverage can also contribute to the increase of apparent viscosity of hazelnut beverages by the extension and hydration of the biopolymer chains resulting from high temperature (Bernat *et al.*, 2015).

The yield stress obtained from the Herschel Bulkley's model is related to the minimum shear stress required to start the product flow and also provides information about the internal structure of the materials that must be broken for flow (Genovese and Rao, 2005; Tabilo-Munizaga and Barbosa-Cánovas, 2005). As shown in Table 3, the yield stress values of hazelnut beverages increased due to heat treatment, but LTLT and HTST treatments at 105 and 110 °C did not significantly affect the yield stress, while the HTST treatment at 115 °C caused a significant increase in yield stress. Yield stress is a typical characteristic feature of multiphase systems, such as vegetable based beverages, formed by dispersing insoluble sugars, minerals

Table 3. Rheological and colour properties of hazelnut beverage samples after heat treatments for low temperature long time (LTLT) and high temperature short time (HTST).[1,2]

Samples		Rheological properties					Colour properties			
		η_{app} (Pa.s)	τ_0 (Pa)	K (Pa.s^n)	n (-)	R^2	L^*	a^*	b^*	ΔE
Control		0.035±0.001^c	0.514±0.04^b	0.055±0.01^c	0.779±0.03^a	0.988	82.93±0.36^a	0.89±0.01^a	11.68±0.03^a	–
LTLT	65 °C/30 min	0.034±0.001^c	0.576±0.15^b	0.072±0.02^c	0.753±0.07^a	0.990	82.78±0.14^a	0.96±0.01^a	11.22±0.08^b	0.39±0.05^d
	72 °C/20 min	0.046±0.003^c	0.561±0.13^b	0.081±0.01^c	0.731±0.01^a	0.991	82.71±0.25^a	0.81±0.01^b	10.99±0.02^c	0.61±0.05^c
	85 °C/ 5 min	0.069±0.001^b	0.585±0.23^b	0.251±0.14^b	0.586±0.07^b	0.993	82.85±0.01^a	0.74±0.01^c	11.14±0.04^b	0.53±0.12^c
HTST	105 °C/1 min	0.040±0.001^c	0.555±0.07^b	0.087±0.01^c	0.728±0.02^a	0.989	80.80±0.18^b	0.97±0.07^a	11.78±0.04^a	1.14±0.16^b
	110 °C/1 min	0.077±0.021^{ab}	0.740±0.09^b	0.290±0.08^b	0.589±0.02^b	0.994	80.69±0.39^b	1.01±0.05^a	11.77±0.01^a	1.25±0.04^{ab}
	115 °C/1 min	0.093±0.009^a	1.054±0.11^a	0.479±0.17^a	0.561±0.02^b	0.995	80.54±0.08^b	0.95±0.13^a	11.87±0.09^a	1.39±0.14^a

[1] Values are means ± standard deviations. Means with different letters in the same column are significantly different (P<0.05).
[2] η_{app} = apparent viscosity at 50/s shear rate; τ_0 = yield stress; K = consistency index; n = flow behaviour index; R^2 = determination coefficient of Herschel Bulkley's model; L^* = lightness; a^* = redness; b^* = yellowness; ΔE = colour difference.

and proteins in a water solution (Sun and Gunasekaran, 2009). A similar tend with yield stress was observed from consistency coefficient (K) values of hazelnut beverages, significantly affected by temperature. The lowest K value was determined as 0.055 Pa.s^n for the control sample, while the highest value (0.479 Pa.s^n) was observed at 115 °C for 1 min treated sample. The flow behaviour index of the heat treated samples ranged from 0.561 to 0.779, and all samples showed shear thinning behaviour due to n<1. Samples treated with LTLT except for 85 °C displayed similar n values with HTST at 105 °C, however, n values of HTST treated samples were lower. Overall, the rheological results of heat treated hazelnut beverage showed that hazelnut proteins are sensitive to heat treatment. Thermal treatments of hazelnut beverages, it may be associated with protein aggregation, caused an increase in the sample viscosity and also as yield stress and consistency coefficient.

Colour properties

The L^*, a^* and b^* values obtained in the control and thermal treated hazelnut beverage are shown in Table 3 together with the colour difference between the control and heat treated samples. The L^* value for the control sample was 82.93, while the L^* value range for the heat treated samples was 80.85-82.80. LTLT treatment did not alter the whiteness properties of hazelnut beverage (P>0.05), but HTST samples were darker depending on the control sample (P<0.05). Similarly, b^* values of hazelnut beverages decreased compared to control sample. Whereas, the thermal treatment did not affect the a^* value of the samples. The darkening of the colour of samples at high temperature could be associated with the existence of Maillard reactions of free sugars activated by the temperature during thermal treatment (Codina-Torrella *et al.*, 2018) and also caramelisation reactions (Devi *et al.*, 2015).

It was observed that thermal treatment process had little influence on the total colour difference. Total colour changes are classified by Silva and Silva (1999) as 'imperceptible' (between 0 and 0.2), 'very small' (between 0.2 and 0.5), 'small' (between 0.5 and 1.5), 'distinct' (between 1.5 and 3.0), 'very distinct' (between 3.0 and 6.0), 'great' (between 6.0 and 12.0), and 'a very great' (>12.0) difference. Considering this classification, LTLT samples exhibited almost 'small' differences in colour, while a 'distinct' colour changes was observed in HTST samples. On the other hand, the colour differences occurring during the thermal treatment cannot be easily detected by the human due to the fact that they are lower than 3 units (Bernat *et al.*, 2015). Tsai *et al.* (2018) reported that the thermal treatment at 80 °C for 3 min had significant effect on hazelnut beverage colour (ΔE=17.05), leading to a darker colour of hazelnut beverage.

4. Conclusions

This study evaluated the effect of low and high heat treatment on microbial and physical stability of high pressure homogenised hazelnut beverage. Even though thermal treatment provided a better microbial inactivation in hazelnut beverage, significant differences were observed in physical and chemical properties of hazelnut beverage. The soluble protein content and also colloidal stability of the heat treated samples were negatively affected by the denaturation and/or aggregation of the hazelnut protein supported by microstructure photo graphs, particle size and rheological results. Thermal treatments at 72 °C for 20 min and 105 °C for 1 min for low and high heat treatment, respectively, were able to produce commercial hazelnut

beverage, which was considered to be bacterial safety as well as better chemical properties.

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