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Research Article/Araştırma Makalesi

The influence of high pressure homogenization and high-intensity ultrasound on the functional properties of whey-protein/canola oil nanoemulsions during storage

Yüksek basınçlı homojenizasyon ve yüksek yoğunluklu ultrasonun depolama sırasında peynir altı suyu proteini/kanola yağı nanoemülsiyonlarının fonksiyonel özellikleri üzerindeki etkisi

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ABSTRACT

The current work was conducted in order to explore the influence of two non-thermal technologies (high pressure processing (HPH) and high-intensity ultrasound (HIU) on the droplet size, turbidity and lipid oxidation of whey protein / canola oil nanoemulsions (WPN) during a week. The outcomes exhibited that the HIU process have a significant impact on both droplet size and lipid oxidation ($p<0.05$) of WPNs. A smaller droplet size was obtained for HPH treated WPNs compared to the control (untreated) WPNs. On the other hand, the smallest droplet sizes were obtained for the samples exposed to 10 min HIU at 100% amplitude (614.7 nm). The same sample also showed the least lipid oxidation during 7 days of storage (124 mmol/kg). There is a positive relationship between the variables of droplet sizes and turbidities. All HIU-treated WPN samples exhibited less turbidity and smaller droplet size where the control WPN samples demonstrated most turbid structure (5.97) with the biggest droplet sizes (985.4 nm). Similar positive relationship was also figured out between the variables of droplet size and lipid oxidation. All HIU-treated WPN samples exhibited less lipid oxidation and smaller droplet size where the control WPN samples demonstrated most lipid oxidation with the biggest droplet sizes.

Key Words: High intensity ultrasound, High pressure homogenization, Droplet size, Lipid oxidation, Whey protein/canola oil nanoemulsion.

ÖZ

Mevcut çalışmada, iki termal olmayan teknolojinin (yüksek basınçlı işleme (HPH) ve yüksek yoğunluklu ultrasonun (HIU) peynir altı suyu proteini/kanola yağı nanoemülsiyonlarının bir hafta boyunca (WPN) damlacık boyutu, bulanıklığı ve lipid oksidasyonu üzerindeki etkisini araştırmak için yapılmıştır. Sonuçlar, HIU işleminin WPN'lerin hem damlacık boyutu hem de lipid oksidasyonu ($p<0.05$) üzerinde önemli bir etkiye sahip olduğunu göstermiştir. Kontrol (uygulama yapılmayan) WPN'lere kıyasla HPH uygulanan WPN'lerde daha küçük bir damlacık boyutu elde edilmiştir. En küçük damlacık boyutları ise %100 genlikte (614.7 nm) 10 dakika HIU uygulanan numunelerde elde edilmiştir. Aynı numune ayrıca 7 günlük depolama (124 mmol/kg) sırasında en az lipid oksidasyonunu göstermiştir. Damlacık boyutları ve bulanıklık değişkenleri arasında pozitif bir ilişki vardır. HIU uygulanan tüm WPN numunelerinde, daha az bulanıklık ve daha küçük damlacık boyutu görülmüştür; burada kontrol WPN numunelerinde, en büyük damlacık boyutlarıyla (985.4 nm) en bulanık yapıyı (5.97) göstermiştir. Damlacık boyutu ve lipid oksidasyonu değişkenleri arasında da benzer pozitif bir ilişki bulunmuştur. HIU uygulanan tüm WPN numunelerinde, daha az lipit oksidasyonu ve daha küçük damlacık boyutu görülmüş; kontrol WPN numunelerinde ise en büyük damlacık boyutu ve en fazla lipit oksidasyonu olduğu görülmüştür.

Anahtar Kelimeler: Yüksek yoğunluklu ultrason, Yüksek basınçlı homojenizasyon, Damlacık boyutu, Lipid oksidaasyonu, Peynir altı suyu proteini/kanola yağı nanoemülsiyonu

Introduction

Whey protein is a crucial material of functional protein components for several convential and novel food materials (Kumar et al., 2018). Whey proteins are recognized as complete proteins since they include all 9 essential amino acids. Lactose content is low in whey products. When the liquid whey is obtained as a by-product of cheese or yoghurt fabrication, it is subjected to different processes in order to make the protein content higher (Liu et al., 2014). After enough protein concentration is obtained, the liquid could be dried to develop whey protein concentrate (WPC) including nearly 80% protein. The major proteins found in whey can be listed as b-lactoglobuln, a-lactolbumin and bovin serum albümin (BSA), and these proteins are composed of almost seventy-percent of all whey proteins (Arzeni, 2012). These proteins are in charge of the functional features of WPC, such as solubility in water and propose various nutritional benefits to functionalized products (KrešicÅL et al., 2008).

Various methodologies have been promoted to alter the native protein structure for the purpose of improvement of the functionality. Modified whey proteins exhibit a very high level of functionality capacity. By molecular and physical alterations, it is achievable to reorganize protein compounds so that they develop into more practical and useful form. Both HIU and HPH applications are novel non-thermal technologies. Ultrasound (US) technology is a cost effective and fast application which has been employed to alter both the structure and functional properties of protein molecules (Mason et al., 1996; Jamrak et al., 2008; Yıldız, 2018). The impact of US treatment is accomplished by the chemical, molecular, and physical consequences of acoustic cavitation. The cavitation mostly defined as a creation, development, and powerful breakdown of tiny droplets in the solution. The cavitation could be the reason of protein structure modification thanks to hydrogen bonds and hydrophobic cooperations, and falling apart the protein molecules (Yildiz et al., 2017). By taking into account the benefits of HIU and HPH applications such as being a cost-effective, non-toxic, fast and efficient process, it is anticipated to reach a goal of advanced whey protein nanoemulsion functionality by using these applications (Yildiz, 2019). For this reason, the purpose of the present research is to analyze the impact of HIU and HPH applications on the droplet size, turbidity and lipid oxidation of WPNs during a week storage period.

Material and Method

Nanoemulsion preparation by HIU and HPH

Oil-in-water (O/W) WPN nanoemulsion was fabricated with canola oil and whey protein (WP). Canola oil (0.125 g) was mixed with 50 mL WP (10 mg/mL) and stirred powerfully for 5 min with a magnetic stirrer. HIU treatment was conducted using a VC-750 ultrasound generator at 20 kHz (Sonics & Materials, Inc., Newtown, CT, USA) for 5 and 10 minutes at 100% amplitude. In addition, The HPH application was employed via a high-pressure homogenizer (APV two-stage homogenizer; SPX Flow Technology, Denmark) at 800 bar for 5 and 10 minutes. The conditions for the HIU and HPH applications were listed in Table 1.

Table 1. The description of the WPNs and treatments

Sample names	Treatments
Control	Untreated WPN, no ultrasound
HIU5	Ultrasound treatment with 5 min at 100% amplitude
HIU10	Ultrasound treatment with 10 min at 100% amplitude
HPH5	HPH treatment with 5 min at 800 bar
HPH10	HPH treatment with 10 min at 800 bar

Droplet size and turbidity

The droplet sizes of WPN were measured following the methodology figured out by Yildiz et al. (2017) via dynamic light scattering (DLS) with the assist of NICOP 38 DSL instrument (Santa Barbar, CA, USA). WPNs were diluted 500-fold with deionized H_2O before achieving DSL analysis. All experiments were conducted at a stable scattering angle of 90^o along with the wavelengths of 658 nm at room environment. The

average of droplet sizes was achieved as the mean of 3 measurements where each measurement was performed for about a minute.

Turbidity of the WPN dispersions was figured out by a spectrophotometer according to the methodology proposed by Yildiz et al. (2017). DI water was used as the blank, and the absorbance at 600 nm was obtained.

Lipid oxidation

Lipid hydroperoxide values formed at storage of WPN were measured as stated in Min et al. (2003). WPN samples (around 5 mL) were added in a test tubes and let the oxidation under 25 ^{o}C in the dark. Lipid hydroperoxide value was determined after mixing 0.3 mL of whey emulsions with 1.5 mL of isooctane/2-propanol (3:1, v/v) via vortexing (around 10 s and 3 times) and isolation of organic solvent parts subsequent to centrifugation at 1000 g for about 2 min. The organic solvent part (200 μL) was mixed into 2.8 mL of methanol/1-butanol (2:1, v/v), and followed by 15 μL of 4 M amonium thiocynate and 15 μL of ferous iron solutions (created via blending 0.15 M $BaCl_2$ & 0.144 M FeSO4). Subsequent to 20 minutes time period, the absorbances of the whey protein solutions were determined at the wavelengths of 510 nm. Lipid hydroperoxide values of the WP nanoemulsion was determined at 1, 2, 3, 4, 5, 6, and 7th days.

Statistical analysis

For all treatment conditions, three independent experiments were conducted. The differences were achieved with the General Linear Model prosess in SAS (version 9.3, SAS Insttute, Inc., Cary, North Carolin, USA). A significant difference among the mean values was defined by Fisher's least signifcant difference (LSD) test at alpha = 0.05.

Results and Discussion

Droplet size and turbidity

Figure 1 displays the findings related to droplet sizes of the WPN samples exposed to HIU and HPH treatments. Both HIU- and HPH-treated WPN samples displayed significantly smaller droplet size in comporison with the control WPNs. Moreover, the smallest droplet size was obtained for the WPN samples exposed to 10 min US at 100% amplitude (HIU10: 614.7 nm). WPN samples treated with HIU for 10 min showed smaller particle size compared to HIU-treated samples for 5 min (Figure 1). An inverse relationship between the droplet size and ultrasound time was determined. The higher the time (5 to 10 min), the smaller the droplet sizes. It was obviously seen that enhancing US time advances the droplet size of whey protein nanoemulsions. On the other hand, HPH-treated WPN samples also displayed significantly smaller sizes in comparison with the control WPNs. However, they also showed significantly bigger droplet sizes compared to HIU-treated WPN samples. In contrary to ultrasound treatment, increasing HPH time did not cause a smaller size. While the droplet size was obtained as 774.1 nm for the HPH-treated WPN samples for 5 min (HPH5), bigger droplet sizes (805.2 nm) was determined for the HPH-treated WPN samples for 10 min (HPH10). Increasing HPH time from 5 to 10 min led to bigger droplet size. The unfolding process especially by ultrasound process may cause WPN samples to become more susceptible to break-down. The decline in the droplet sizes of plant protein sources (i.e., soy protein, and pea protein) were reported in previous works (Lee et al., 2016; Yildiz et al., 2017; Yildiz et al., 2018; Jiang et al., 2019). In the study of Jambrak et al. (2014) following application with an ultrasonic probe (20 kHz), high intensity ultrasound treatment led to a decrease in droplet size as well as narrowed their distribution, and significantly raise specific free surface ($p<0.05$) in whey protein specimens. When the use in protein suspensions, ultrasound treatment was expressed to significantly lower the droplet sizes of whey protein samples (Jambrak et al., 2008). Moreover, Karki et al. (2010) determined that the droplet sizes of defated soy flakes samples were decreased approximately 10-fold after ultraosund

application. It was figured out that the cavitation may be the explanation of the breakage of protein agregates, and decline in the droplet size (Azeni et al., 2012; Yildiz, 2019; Yildiz and Aadil, 2020). Gordan and Pilosopf (2010) accomplished to control particle size via high intensity ultraosund by merging several treatment periods, temperatures and ratios of whey protein dispersions. Ultrasound process develops a new surface and makes lower the sizes of the aggregates (Yildiz and Feng, 2019). In this case, the protein drople sizes are decreased due to the cavitation phenomena. This involves the degradation of protein aggregates and agglomerates. Ultrasonic cavitation is very efficient to break up protein substances and smaller particle aggregates the van der Wals's forces (Jambrak et al., 2014).

The turbidity findings of WPN specimens are demonstrated in Figure 2. Martini et al. (2010) handled with a power ultrasonic sound waves to lessen the turbidity of whey solutions. It was concluded that around 90% decrease was observed in the turbidity of samples treated with ultrasound processing. The highest decline in turbidity values was determined for the samples treated with HIU for 10 min at 100% amplitude (HIU10 samples). While the highest turbidity was obtained for the untreated WPN (5.97), the lowest turbidity was observed for the HIU10 samples (3.89). There is a positive relationship between the variables of droplet sizes and turbidities. All HIU- and HPH- treated WPN specimens showed less turbidity and smaller droplet size where the control WPN samples exhibited most turbid appearance and the biggest droplet size (Figure 2). In overall, HIU10 samples showed the smallest droplet size (614.7 nm) and least turbidity (3.89) compared to HPH-treated WPN samples. Both the number of soluble protein components in the dispersion figured out by solubility and the size of the soluble protein components determine the turbidities of a whey protein dispersion (Gregor,1998). Employing the US at 20 kHz raised the clearness and transparency of whey protein suspensions mostly because of the decrease in the size of the suspended insoluble protein components (Zisu et al., 2011).

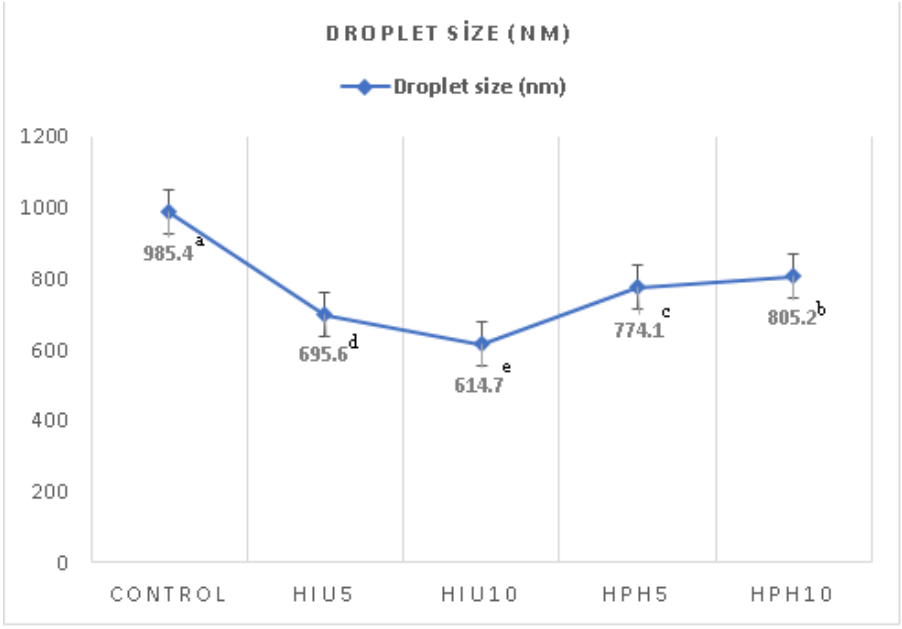

Figure. 1 Droplet size (nm) of WPN samples. ($^{a-e}$ Different superscript lowercase letters show differences between the droplet size ($P < 0.05$).

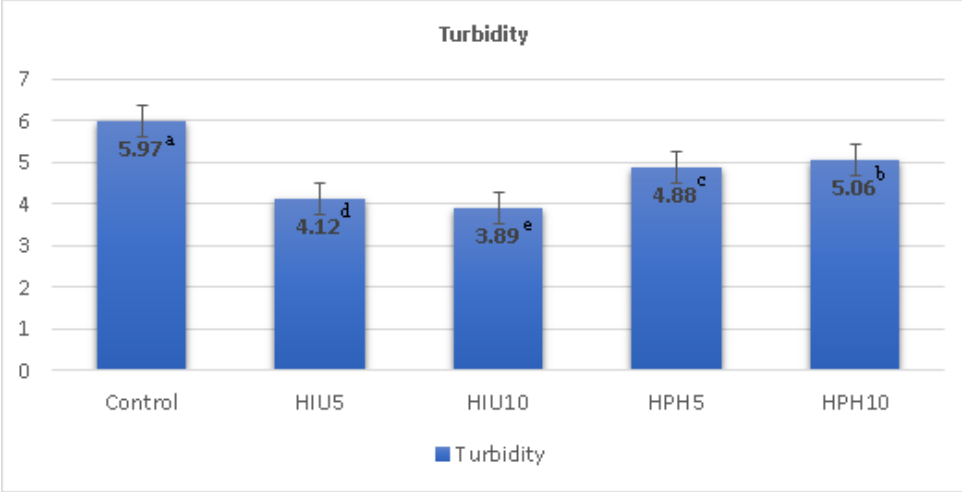

Figure. 2. Turbidity values of WPN samples ($^{a-e}$ Different superscript lowercase letters show differences between the turbidity ($P < 0.05$).

Lipid oxidation

Lipid hydroperoxide value of the whey protein nanoemulsions with oil concentration of 0.25% for 7-days (168 h in total) of storage period under 25 ^{o}C is demonstrated in Fig.3. No oxidized lipid particles were measured for the first and second days for all WPN samples including control samples. Starting from the second day, the lipid oxidation was started to defined. Significant increases in lipid oxidation beginning from second days to seventh days for control WPN specimens were determined. The tendency of whey protein's role as the chemical stopper in order to postpone lipid oxidation is proved at the first 6 days of storage period for HIU10 samples. For the WPN samples treated with 10 min HIU at 100% amplitude, no oxidized lipids were observed till the day of 6 (Fig.3). The lipid oxidation was detected in seventh days for the first time for HIU10 samples. On the other hand, lipid oxidation for HIU5 samples was observed in the sixth and seventh days. HPH-treated WPN samples compared to HIU-treated samples showed more and earlier lipid oxidation. The lipid oxidation was detected for HPH5 samples at starting from 5th days and for HPH10 samples starting from 4th days. It can be concluded that the encapsulation of secondary components with the whey protein nanoemulsions might be conducted within 144 hours (6 days) subsequent to preparation of WP nanoemulsions, right before oxidation stage of oil used in the nanoemulsion.

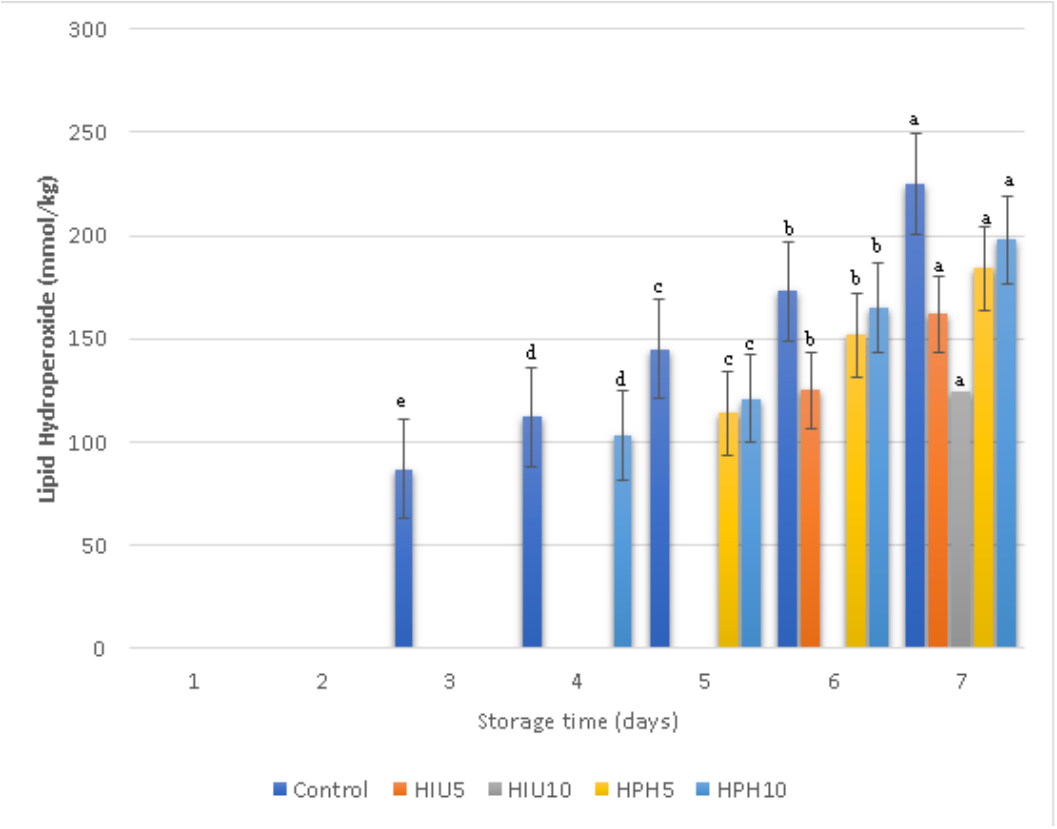

Figure. 3. Lipid hydroperoxide values of WPN during storage at 25 °C for 7 days ($^{a-e}$ Within the same sample different superscript lowercase letters show differences between the hydroperoxide values ($P < 0.05$).

Conclusion

HIU and HPH treatments were examined for the purpose of modification and enhancement of the WPN functionality. Compared with HPH treatment, a significant improvement in the droplet sizes and lipid oxidations of WPN samples was achieved with a HIU10 treatment. Overall, HIU10 is a promising treatment to strengthen the functional characteristics of WPNs as indicated within the present study by its ability to smaller droplet and less lipid oxidation right after ultrasonication. The findings of current research proved the potential of the HIU10 treatment as an effective method for protein modification. The functionalized WPN produced by HIU10 treatment can be used in a liquid food with less precipitation.

Conflict of Interest: The author declares that they have no conflict of interest.

Author Contributions: M. Murat CEYLAN conceived and designed the analysis, collected the data, performed the anlysis, wrote and submitted the manuscript.

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