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Research Paper / Araştırma Makalesi

Changes in Emulsifying Properties, Droplet Size, Turbidity and Lipid Oxidation of Pea Protein Nanoemulsions Exposed to High-Intensity Ultrasound and High-Pressure Homogenization during Storage

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ABSTRACT

The current work was conducted to explore the influence of two non-thermal technologies (high pressure homogenization (HPH) and high-intensity ultrasound (HIU)) on emulsifying properties, droplet size, turbidity and lipid oxidation of pea protein-stabilized nanoemulsions (PPNs) during seven days of storage. The smallest droplet size (198.3 nm) was obtained for the samples exposed to 10 min HIU at 100% amplitude among all treatments. The same sample also showed the least lipid oxidation (98 mmol/kg) during storage. There was a positive relationship between droplet size and turbidity values. All HIU-treated PPNs exhibited less turbidity and smaller droplet size where the control PPN samples demonstrated the most turbid structure (4.05) with the biggest droplet size (413.9 nm). Similar positive relationship was also found between the variables of droplet size and lipid oxidation. All HIU-treated PPNs exhibited less lipid oxidation and smaller droplet size where the control PPNs demonstrated the most lipid oxidation with the biggest droplet size. Last but not least, among the treatments, the PPNs exposed to 10 min HIU at 100% amplitude showed the highest emulsifying activity index (EAI, 212 m^2g^{-1}) and emulsifying stability index (ESI, 59 min), whereas the PPNs with no treatment showed the lowest EAI (69 m^2g^{-1}) and ESI (21 min).

Keywords: High intensity ultrasound, high pressure homogenization, emulsifying properties, lipid oxidation, pea protein nanoemulsion

Yüksek Yoğunluklu Ultrason ve Yüksek Basınçlı Homojenizasyona Maruz Bırakılan Bezelye Proteini Nanoemülsiyonlarının Depolanması Sırasında Emülsifiye Edici Özellik, Parçacık Boyutu, Bulanıklık ve Lipid Oksidasyonundaki Değişimler

ÖZ

Mevcut çalışmada, ısıl olmayan iki teknolojinin (yüksek basınçlı homojenizasyon (HPH) ve yüksek yoğunluklu ultrason (HIU)) bezelye proteini nanoemülsiyonunun (PPN) emülsiyonlaştırma özellikleri, parçacık boyutu, bulanıklık ve lipit oksidasyonu üzerindeki etkisi 7 günlük depolama süresince araştırılmıştır. Tüm muameleler arasında %100 genlikte 10 dakikalık HIU'ya maruz bırakılan numuneler en küçük parçacık boyutunu sergilemiştir (198.3 nm). Aynı örnek, 7 günlük depolama sırasında en az lipid oksidasyonunu göstermiştir (98 mmol/kg). Parçacık boyutları ile bulanıklık arasında pozitif bir ilişki tespit edilmiştir. HIU ile muamele edilmiş tüm PPN'ler daha az bulanıklık ve daha küçük parçacık boyutu sergilerken, kontrol PPN numuneleri en bulanık yapıyı (4.05) ve en büyük parçacık boyutunu (413,9 nm) sergilemiştir. Parçacık boyutu ve lipid oksidasyonu değişkenleri arasında da benzer pozitif bir ilişki bulunmuştur. HIU ile muamele edilmiş tüm PPN'ler, daha düşük lipid oksidasyonu ve daha küçük damlacık boyutu sergilerken, kontrol PPN'leri, en büyük damlacık boyutuyla en yüksek lipid oksidasyonunu göstermiştir. Son olarak, muameleler

arasında, %100 genlikte 10 dakika HIU'ya maruz kalan PPN'ler en yüksek EAI'yi (212 m^2g^{-1}) ve ESI'yi (59 min) gösterirken, kontrol PPN'ler en düşük EAI'yi (69 m^2g^{-1}) ve ESI'yi (21 min) göstermiştir.

Anahtar Kelimeler: Yüksek yoğunluklu ultrason, yüksek basınçlı homojenizasyon, emülsifiye edici özellikler, lipid oksidasyonu, bezelye proteini nanoemülsiyonu

INTRODUCTION

Legumes are not expensive and a high-quality source for foods compared to animal sources. They include high quantity of protein sources, dietary fiber, mineral substances and various vitamins. Today, soybean proteins are the most utilized and searched pulse proteins on the markets. The alternative potential legume protein has the identical and/or similar functional characteristics and nutritional properties as soy protein. Those alternative protein sources are needed to also have a price competitive to that of soybean proteins. An alternative pulse protein which is told to have a big potential for food processes are pea protein products. Pea proteins are also lesser in anti-nutritional compounds rather than soy proteins, and are not identified as an allergen (like soy and egg protein sources). Pea proteins are one of the few hypoallergenic plant protein sources with no genetic modification issues. Pea protein isolates (PPIs) are natural emulsifiers along with high nutritional quality. However, the application of PPI in food sources is limited because of its relatively poor solubility and functional properties [1].

Various methodologies have been promoted to alter the native protein structure for the purpose of improvement of the functionality. Modified pea 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 high-intensity ultrasound (HIU) and high pressure homogenization (HPH) applications are novel non-thermal technologies. HIU technology is a cost effective and fast application which has been employed to alter both the structure and functional properties of protein molecules [2, 3, 4]. The impact of HIU 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 cooperation, and falling apart the protein molecules [5]. 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 pea protein nanoemulsion functionality by using these applications. In this work, the purpose of the present research is to analyze the impact of HIU and HPH applications on the emulsifying properties, droplet size, turbidity and lipid oxidation of PPNs during a week storage period.

MATERIALS and METHODS

Chemicals

Pea protein isolate (PPI, NUTRALYS® S85F, 85% pea protein on dry basis) was provided by Roquette (Geneva, IL, USA). It was stored in a refrigerator at 4 °C before use. Reagents and chemicals were purchased from Bio-Rad (Hercules, CA, USA) and Sigma-Aldrich (St. Louis, MO, USA).

Nanoemulsion Preparation by HIU and HPH

Oil-in-water (O/W) nanoemulsions were prepared with canola oil and pea protein isolate (PPI). The oil concentration was 0.25 (w/w). Canola oil (5 mL) was mixed with 15 mL PPI and stirred powerfully for a 5 min with a magnetic stirrer. For the achievement of better homogenization, PPN specimens were sonicated at 100% amplitude for 5, 10 and 15 minutes using a VC-750 ultrasound unit (20 kHz, Sonic & Materials, Inc., Newton, CT, USA). In addition, The HPH application was employed via a high-pressure homogenizer (APV two stage homogenizer; SPX Flow Technology, Denmark) at 8000 psi for 5,10 and 15 minutes. The conditions for HIU and HPH applications (Table 1) and the processing steps of PPN production for each treatment were listed in Table 2.

Table 1. The description of the PPNs and treatments

Sample name	Treatment
Control	Untreated PPN, no ultrasound
PPN-HIU5	HIU treatment with 5 min at 100% amplitude
PPN-HIU10	HIU treatment with 10 min at 100% amplitude
PPN-HIU15	HIU treatment with 15 min at 100% amplitude
PPN-HPH5	HPH treatment with 5 min at 8000 psi
PPN-HPH10	HPH treatment with 10 min at 8000 psi
PPN-HPH15	HPH treatment with 15 min at 8000 psi

Table 2. The processing steps of PPN production for each treatment ("Q" displays the stages applied and "- "displays the stages that were not applied)

Treatment	Stirring (5 min)	HIU (5 min)	HIU (10 min)	HIU (15 min)	HPH (5 min)	HPH (10 min)	HPH (15 min)
Control	Q	-	-	-	-	-	-
PPN-HIU5	Q	Q	-	-	-	-	-
PPN-HIU10	Q	-	Q	-	-	-	-
PPN-HIU15	Q	-	-	Q	-	-	-
PPN-HPH5	Q	-	-	-	Q	-	-
PPN-HPH10	Q	-	-	-	-	Q	-
PPN-HPH15	Q	-	-	-	-	-	Q

(Control: Untreated PPN; PPN-HIU5: HIU treatment with 5 min at 100% amplitude; PPN-HIU10: HIU treatment with 10 min at 100% amplitude; PPN-HIU15: HIU treatment with 15 min at 100% amplitude; PPN-HPH5: HPH treatment with 5 min at 8000 psi; PPN-HPH10: HPH treatment with 10 min at 8000 psi; PPN-HPH15: HPH treatment with 15 min at 8000 psi)

Droplet Size and Turbidity

The droplet sizes of PPN were measured following the methodology figured out by Yildiz et al. [5] via dynamic light scattering (DLS) with the assist of NICOP 38 DLS instrument (Santa Barbara, CA, USA). PPNs were diluted 500-fold with deionized H_2O before achieving DLS 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 droplet size was achieved as the mean of 3 measurements where each measurement was performed for about a minute.

Turbidity of the PPN dispersions was figured out by a spectrophotometer according to the methodology proposed by Yildiz et al. [5]. DI water was used as the blank, and the absorbance at 600 nm was obtained.

Emulsifying Characteristics

Emulsifying properties of pea protein nanoemulsions was achieved by the approach of Jiang et al. [6]. Oil in H_2O suspensions were made with the addition of a mL of canola oil in three mL of the pea protein samples. The ratios of oil concentration were 0.25% (w/w) in this measurement. The combined oil and pea protein concentration solution were stirred harshly for around 5 min and then sonicated for about 5 minutes After suspension occurrence, the absorbances of the pea proteins were determined at 500 nm at 0 (A_0) and 10 min (A_{10}), subsequently. Both emulsifying activity index (EAI) and emulsion stability index (ESI) were determined by using the following formulas:

$$EAI\ (m^2/g) = 2T\ A_0 \times \text{dilution factor} / c \times Q \times L \times 10.000$$

$$ESI\ (\text{minute}) = A_0 / (A_0 - A_{10}) \times 10\ (\text{minute})$$

where T: 2.303, dilution element: 100, c: protein weight per unit volume (g/mL), L: width of the optical pathway (0.01 m), and Q: volumetric oil concentration (0.25).

Lipid Oxidation

Lipid hydroperoxide values formed at storage of PPN were measured as stated in Min et al. [7]. PPN samples (around 5 mL) were added in a test tubes and let the oxidation under 25°C in the dark. Lipid hydroperoxide value was determined after mixing 0.3 mL of pea 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 ammonium thiocyanate and 15 μL of ferrous iron solutions (created via blending 0.15 M $BaCl_2$ & 0.144 M FeSO4). Subsequent to 20 minutes time period, the absorbances of the pea protein solutions were determined at the wavelengths of 510 nm. Lipid hydroperoxide values of the PPN was determined at the days of 1, 2, 3, 4, 5, 6, and 7.

Statistical Analysis

The results were achieved using the General Linear Model process in SAS (version 9.3, SAS Institute, Inc., Cary, NC, USA). A significant difference among the mean values was defined by Fisher's least significant 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 PPN samples exposed to HIU and HPH treatments. Both HIU- and HPH-treated PPN samples displayed significantly smaller droplet size in comparison with the control PPNs. Moreover, the smallest droplet size was obtained for the PPN samples exposed to 10 min HIU at 100% amplitude (PPN-HIU10: 198.3 nm). PPN samples treated with HIU for 10 min showed smaller particle size compared to HIU-treated samples for 5 and 15 minutes (Figure 1). An inverse relationship between the droplet size and HIU times was determined for first 10 min. 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 pea protein nanoemulsions. On the other hand, HPH-treated PPN samples also displayed significantly smaller sizes in comparison with the control PPNs. However, they also showed significantly bigger droplet sizes compared to HIU-treated PPN samples. Similar to HIU treatment, increasing HPH time from 5 to 10 min caused a smaller size. While the droplet size was obtained as 306.5 nm for the HPH-treated PPN samples for 5 min (PPN-

HPH5), smaller droplet sizes (279.4 nm) was determined for the HPH-treated PPN samples for 15 min (PPN-HPH15). Increasing HPH time from 5 to 15 min led to smaller droplet size. The unfolding process especially by ultrasound process may cause PPN 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 [1, 5, 6, 8]. In the study of Jambrak et al. [9] 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 protein samples [3]. Moreover, Karki et al. [10] determined that the droplet sizes of defatted soy flakes samples were decreased approximately 10-fold after ultrasound application. It was figured out that the cavitation may be the explanation of the breakage of protein aggregates, and decline in the droplet size [11, 12]. Gordan and Pilosopf [13] accomplished to control particle size via high intensity ultrasound by merging several treatment periods, temperatures and ratios of protein dispersions. Ultrasound process develops a new surface and makes lower the sizes of the aggregates [14]. In this case, the protein droplet 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 Walls's forces [9].

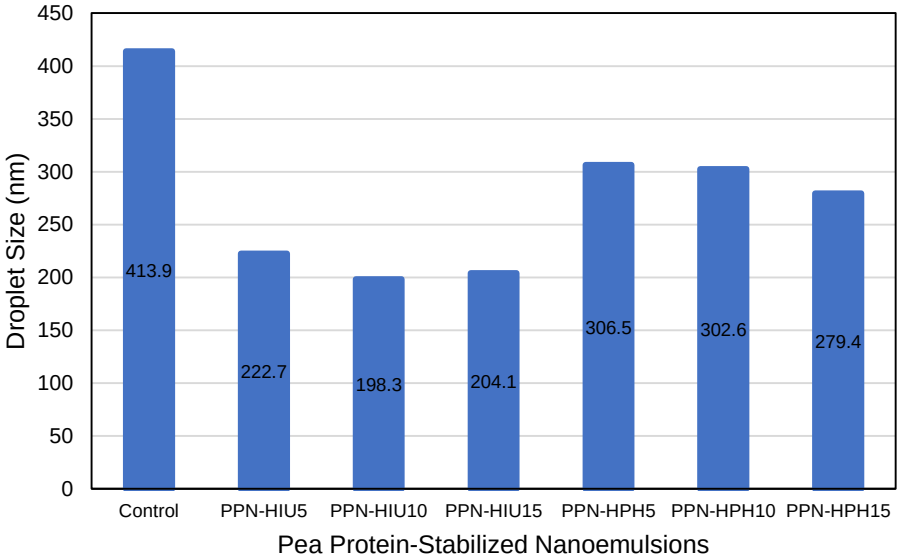

Figure 1. Droplet sizes (nm) of the pea protein-stabilized nanoemulsions (Control: Untreated PPN; PPN-HIU5: HIU treatment with 5 min at 100% amplitude; PPN-HIU10: HIU treatment with 10 min at 100% amplitude; PPN-HIU15: HIU treatment with 15 min at 100% amplitude; PPN-HPH5: HPH treatment with 5 min at 8000 psi; PPN-HPH10: HPH treatment with 10 min at 8000 psi; PPN-HPH15: HPH treatment with 15 min at 8000 psi)

The turbidity findings of PPN specimens are demonstrated in Figure 2. The highest decline in turbidity values was determined for the samples treated with HIU for 10 min at 100% amplitude (PPN-HIU10 samples). While the highest turbidity was obtained for the untreated PPN (4.05), the lowest turbidity was observed for the PPN-HIU10 samples (2.17). There is a positive relationship between the variables of droplet sizes and turbidities. All HIU- and HPH- treated PPN specimens showed less turbidity and smaller droplet size where the control PPN samples exhibited most turbid appearance and the biggest droplet size (Figure 2). In overall, PPN-HIU10 samples showed the smallest droplet size (198.3 nm) and least turbidity (2.17) compared to HPH-treated PPN 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 protein dispersions [15]. Employing the HIU at 20 kHz raised the clearness and transparency of protein suspensions mostly because of the decrease in the size of the suspended insoluble protein components [16].

Emulsifying Properties

Table 3 shows the EAI and ESI of PPN samples treated with HIU and HPH treatments. It was found that the PPN samples treated with 10 min HIU at 100% amplitude resulted with the highest EAI (212 m^2g^{-1}) and ESI (59.0 minutes), while the PPNs with no HIU and/or HPH treatments exhibited the lowest EAI (69 m^2g^{-1}) and ESI (21.0 min). Similar progression in the emulsion characteristics of proteins with HIU process was pointed out in the works of Yildiz et al. [5]. It is possible to see an inverse relationship between droplet size and emulsifying properties (Table 3). Basically, the PPN samples with the smallest droplet size, namely PPN-HIU10 samples, showed the highest ESI and EAI. On the other hand, HPH-treated PPN samples showed significantly higher EAI and ESI in comparison with control PPN samples (Table 3). PPN-HPH15 samples

where PPN exposed to HPH treatment with 15 min at 8000 psi showed the highest EAI and ESI among the HPH-treated PPNs. However, HIU-treated PPN samples showed better emulsifying characteristics compared to HIU-treated PPN samples. In overall, the use of HIU treatment, especially for 10 min, was resulted with an enhanced emulsifying property (ESI and EAI).

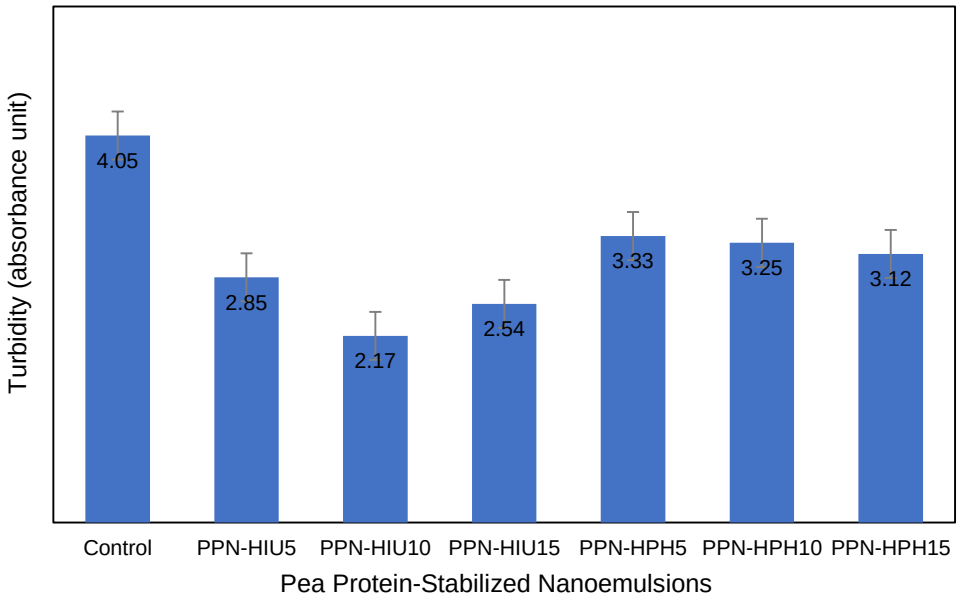

Figure 2. Turbidity values (absorbance at 600 nm) of the pea protein-stabilized nanoemulsions (Control: Untreated PPN; PPN-HIU5: HIU treatment with 5 min at 100% amplitude; PPN-HIU10: HIU treatment with 10 min at 100% amplitude; PPN-HIU15: HIU treatment with 15 min at 100% amplitude; PPN-HPH5: HPH treatment with 5 min at 8000 psi; PPN-HPH10: HPH treatment with 10 min at 8000 psi; PPN-HPH15: HPH treatment with 15 min at 8000 psi)

Table 3. EAI and ESI of PPN samples treated with HIU and HPH treatments

Treatment	EAI (m^2 /g)	ESI (min)
Control	69 ± 0.3^{f}	21.0^{f}
PPN-HIU5	198 ± 1.2^{c}	46.0^{c}
PPN-HIU10	212 ± 0.3^{a}	59.0^{a}
PPN-HIU15	205 ± 2.1^{b}	51.0^{b}
PPN-HPH5	144 ± 1.6^{e}	33.0^{e}
PPN-HPH10	175 ± 0.5^{d}	38.0^{d}
PPN-HPH15	178 ± 1.6^{d}	39.0^{d}

[a-f] Mean ± standard deviation (n=3) of feature with the same letter are not significantly different ($p < 0.05$); *All the statistics were done separately for all and each variable (Emulsifying activity index (EAI), Emulsifying stability index (ESI)); (Control: Untreated PPN; PPN-HIU5: HIU treatment with 5 min at 100% amplitude; PPN-HIU10: HIU treatment with 10 min at 100% amplitude; PPN-HIU15: HIU treatment with 15 min at 100% amplitude; PPN-HPH5: HPH treatment with 5 min at 8000 psi; PPN-HPH10: HPH treatment with 10 min at 8000 psi; PPN-HPH15: HPH treatment with 15 min at 8000 psi)

Lipid oxidation

Lipid hydroperoxide value of the pea protein nanoemulsions with oil concentration of 0.50% for 7-days (168 h in total) of storage period under 25°C is demonstrated in Figure 3. No oxidized lipid particles were measured for the first day for all PPN samples including untreated PPN samples. Starting from the second day, the lipid oxidation was started to defined. Significant increases in lipid oxidation beginning from the 2nd days to last days for control PPN samples were determined. The tendency of pea proteins' role as a chemical stopper for the purpose of delay lipid oxidation is proved at the first 6 days of storage period for PPN-HIU10 samples. For the PPN samples treated with 10 min HIU at 100% amplitude, no oxidized lipids were observed until the day of 6 (Figure 3). The lipid oxidation was detected in 7th days for the first time for PPN-HIU10 samples. On the other hand, while the lipid oxidation for PPN-HIU5 samples was observed in the sixth and seventh days, it was observed in the 5th, 6th, and 7th days for PPN-HIU15 samples. HPH-treated PPN samples compared to HIU-treated PPN samples showed higher and earlier lipid oxidation. The lipid oxidation was detected for PPN-HPH5, PPN-HPH10 and PPN-HPH15 samples starting from 4th days. It can be concluded that the encapsulation of secondary metabolites with the pea protein nanoemulsions might be conducted within 144 hours (6 days) subsequent to preparation of pea protein nanoemulsions, right before oxidation stage of oil used in the nanoemulsion.

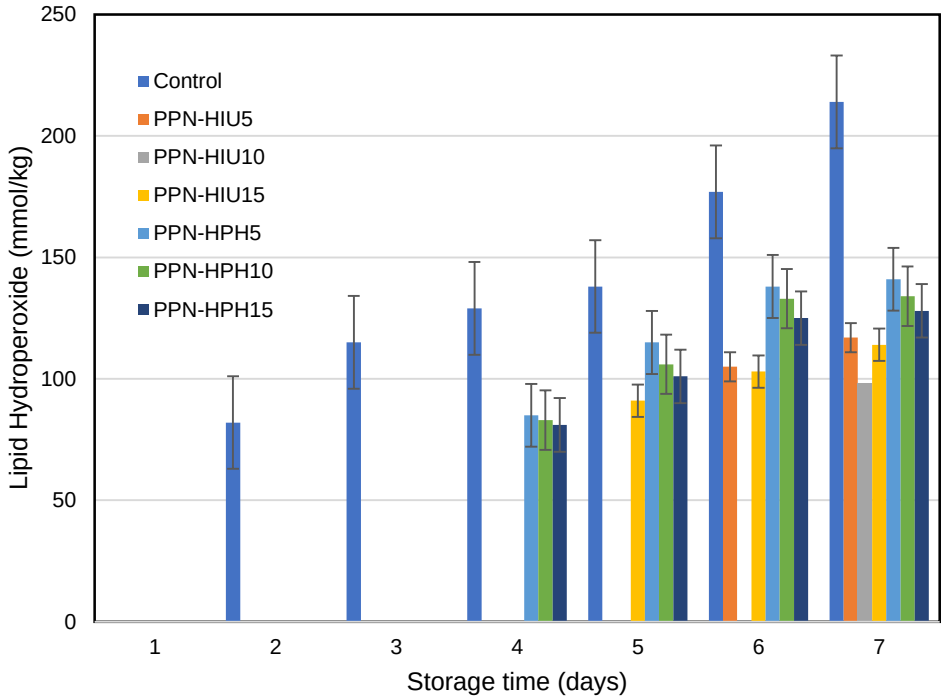

Figure 3. Lipid hydroperoxide values of pea protein-stabilized nanoemulsions during storage at 25°C for 7 days (Control: Untreated PPN; PPN-HIU5: HIU treatment with 5 min at 100% amplitude; PPN-HIU10: HIU treatment with 10 min at 100% amplitude; PPN-HIU15: HIU treatment with 15 min at 100% amplitude; PPN-HPH5: HPH treatment with 5 min at 8000 psi; PPN-HPH10: HPH treatment with 10 min at 8000 psi; PPN-HPH15: HPH treatment with 15 min at 8000 psi)

CONCLUSIONS

HIU and HPH treatments were examined for the purpose of modification and enhancement of the PPN functionality. Compared with HPH treatment, a significant improvement in the emulsifying properties, droplet size, turbidity and lipid oxidation of PPN samples was achieved with a PPN-HIU10 treatment. Overall, PPN-HIU10 (pea protein nanoemulsions treated with high-intensity ultrasound for 10 min at 100% amplitude) is a promising treatment to strengthen the functional characteristics of PPNs as indicated within the present study by its ability to smaller droplet size, less turbidity and lipid oxidation, enhanced emulsifying properties right after ultrasonication. The findings of current research proved the potential of the PPN-HIU10 treatment as an effective method for the protein modification. The PPNs produced by HIU treatment can be used in a liquid food with less precipitation. In addition, pea protein-stabilized nanoemulsions produced by this method are able to used as a great wall material alternative to animal-based proteins in order to encapsulate secondary metabolites.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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