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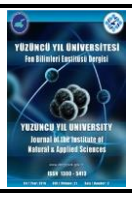

Research Article

# The Effects of Ultrasound Times and Amplitudes on the Particle Size and Emulsifying Properties of Whey Protein Concentrate

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**Abstract:** The current study was conducted to investigate the effects of ultrasound times and amplitudes on the particle sizes and emulsifying properties of samples of whey protein concentrate (WPC). The ultrasound (US) application was performed using VC-7500 ultrasonic power equipment at a 20 kHz frequency, at various times (10, 20, and 30 min at a 50% amplitude) and amplitudes (60%, 80%, and 100% for 5 min). The results showed that the US procedure had a significant effect ($p<0.05$) on both particle sizes and emulsifying properties ($p<0.05$). The smallest particle size was obtained for the WPC samples exposed to 30 min of US at a 100% amplitude (US310) (498.6 nm). The WPC samples treated at a 100% amplitude showed a smaller particle size compared to the other WPC samples at 60% and 80% amplitudes. While the WPC samples treated for 10 min had the biggest particle size (790.3 nm), those treated for 30 min had the smallest particle size (697.1 nm). On the other hand, among the treatments, US310 whey protein concentrate samples had the highest EAI (emulsifying activity index) (198 $m^2/g$) and ESI (emulsion stability index) (34.0 min), whereas the untreated WPC samples had the lowest EAI (56 $m^2/g$) and ESI (13.0 min). In general, 30-min US treatment at a 100% amplitude showed the lowest particle size (498.6 nm) and the highest emulsifying properties (EAI: 198 $m^2/g$ and ESI: 34.0) compared to the other sonication times and amplitudes.

# Peynir Altı Suyu Proteini Konsantresinin Partikül Boyutu ve Emülsifiye Edici Özellikleri Üzerine Ultrason Sürelerinin ve Genliklerinin Etkileri

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**Anahtar Kelimeler**
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**Öz:** Mevcut çalışma, peynir altı suyu proteini konsantresinin (PASPK) partikül boyutları ve emülsifiye edici özellikleri üzerindeki ultrason sürelerinin ve genliklerinin etkisini araştırmak için yapılmıştır. Ultrason (US) uygulaması, 20 kHz frekans ile farklı zamanlarda (10, 20 ve 30 dakika %50 genlikte) ve amplitüdlerde (5 dakika için %60, %80 ve %100) VC-7500 ultrasonik güç ekipmanı kullanılarak yapıldı. Sonuçlar, US işleminin hem partikül boyutları hem de emülsifiye edici özellikler üzerinde önemli ($p<0.05$) bir etkiye sahip olduğunu gösterdi. En küçük parçacık boyutu, %100 genlikte (US310) (498.6 nm) 30 dakika (dak.) US'ye maruz bırakılan PASPK numunelerinde belirlendi. %100 genlikte işlenen PASPK numuneleri, %60 ve %80 genliklerde diğer PASPK numunelerine kıyasla daha küçük parçacık boyutu gösterdi. US ile 10 dak. muamele edilen PASPK numunelerinin partikül boyutu en büyük (790.3 nm) düzeyde iken, 30 dak. süre ile muamele edilen PASPK numunelerinde en küçük

partikül boyutu (697.1 nm) tespit edilmiştir. Öte yandan, işlemler arasında US310 PASPK numuneleri en yüksek emülsiyon aktivitesi indeksine (EAİ) (198 $m^2/g$) ve emülsiyon stabilitesi indeksine ( ESİ) (34.0 dak.) sahipken, işlem görmemiş PASK numunelerinde en düşük EAİ (56 $m^2/g$) ve ESİ (13.0 dak.) saptandı. Genel olarak, %100 genlikte 30 dakikalık US uygulaması, diğer sonikasyon süreleri ve amplitüdleriyle karşılaştırıldığında en düşük parçacık boyutu (498.6 nm) ve en yüksek emülsiyon özellikleri (EAİ:198 $m^2/g$ ve ESİ: 34.0) gösterdi.

## 1. Introduction

Whey protein is a crucial material of functional protein components for many conventional and novel food materials (Kumar et al., 2018). Whey proteins are recognized as complete proteins since they include all nine essential amino acids. The lactose content is low in whey products. When the liquid whey is obtained as a by-product of cheese or yogurt fabrication, it is subjected to different processes to increase the protein content (Liu et al., 2014). After a sufficient protein concentration is obtained, the liquid can be dried to develop whey protein concentrate (WPC), including nearly 80% of protein. The major proteins in whey can be listed as b-lactoglobulin, a-lactalbumin, and bovine serum albumin (BSA), and these proteins constitute almost seventy percent of all whey proteins (Arzeni et al., 2012). These proteins are in charge of the physicochemical features of WPC, including soluble protein content in $H_2O$, and have various nutritional benefits to functionalized products (Krešic et al., 2008).

Various methodologies have been introduced to alter the native protein structure to improve its functionality. Modified whey proteins exhibit a very high level of functional capacity. Through molecular and physical alterations, it is possible to reorganize protein compounds so that they develop into a more practical and useful form. Ultrasound (US) technology is an inexpensive and fast application that has been employed to alter both the structure and functional properties of protein molecules (Mason et al., 1996; Jambrak et al., 2008; Yıldız, 2018). The impact of US treatment is realized by the chemical, molecular, and physicochemical consequences of acoustic cavitation. Cavitation is mostly defined as the creation, development, and powerful breakdown of tiny droplets in solution. Cavitation can be the reason for protein structure modification owing to hydrogen bonds and hydrophobic cooperation, and the breakdown of the protein molecules (Yıldız et al., 2017). Whey protein concentrates or powders are often used in emulsion products such as sausage, salami and mayonnaise to increase emulsion capacity and stability. Considering the benefits of US application, such as being an inexpensive, non-toxic, fast, and efficient process, it is anticipated to reach a goal of advanced WPC functionality using the US application. Therefore, the primary aim of this study is to explore the impacts of the US application on the particle sizes and emulsifying properties of whey protein.

## 2. Materials and Methods

### 2.1. Whey protein concentrate (WPC)

Whey protein concentrates (WPCs) were supplied from BulkSupplements (Henderson, NV, USA). The WPC consists of 80% protein on a dry base. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Fisher Scientific (Pittsburgh, PA, USA).

### 2.2. WPC samples and ultrasound application

The US application was performed using VC-7500 US power equipment at a 20 kHz frequency (Sonic & Material, Inc., USA), at various times (10, 20, and 30 min at a 50% amplitude) and amplitudes (60%, 80%, and 100% for about 5 min). Non-soluble WPC (3 g) samples were blended with 100 mL of distilled $H_2O$ and agitated for about 60 min at room temperature (RT) with a magnetic stirrer. The beaker was kept in a cup filled with ice cubes at the time of US treatment to prevent temperature rise. Following the US application, the protein solution was exposed to centrifugation (1200 g and 20 $^{\circ}$C) for around 15 min. Soluble WPC was collected immediately after the centrifugation stage. For the control WPC specimens, no US application was employed. Three grams of WPC in 100 mL water were agitated at 25

°C for about 30 min. While Table 1 contains the description of the WPC specimens and applications, Table 2 shows the processing steps applied for each treatment (Yıldız, 2018)

Table 1. The description of the WPC samples and treatments

| Sample names | Treatments |
|---|---|
| Control | Untreated WPC, no ultrasound |
| US1 | Ultrasound treatment for 10 min (50% amplitude) |
| US2 | Ultrasound treatment for 20 min (50% amplitude) |
| US3 | Ultrasound treatment for 30 min (50% amplitude) |
| US6 | Ultrasound treatment at a 60% amplitude (5 min) |
| US8 | Ultrasound treatment at an 80% amplitude (5 min) |
| US10 | Ultrasound treatment at a 100% amplitude (5 min) |
| US16 | Ultrasound treatment for 10 min at a 60% amplitude |
| US18 | Ultrasound treatment for 10 min at an 80% amplitude |
| US110 | Ultrasound treatment for 10 min at a 100% amplitude |
| US26 | Ultrasound treatment for 20 min at a 60% amplitude |
| US28 | Ultrasound treatment for 20 min at an 80% amplitude |
| US210 | Ultrasound treatment for 20 min at a 100% amplitude |
| US36 | Ultrasound treatment for 30 min at a 60% amplitude |
| US38 | Ultrasound treatment for 30 min at an 80% amplitude |
| US310 | Ultrasound treatment for 30 min at a 100% amplitude |

Table 2. The processing steps applied for each treatment

| Treatments | Stirring | US (10 min) | US (20 min) | US (30 min) | US (60% amplitude) | US (80% amplitude) | US (100% amplitude) | Centrifuge |
|---|---|---|---|---|---|---|---|---|
| Control | A | B | B | B | B | B | B | A |
| US1 | A | A | B | B | B | B | B | A |
| US2 | A | B | A | B | B | B | B | A |
| US3 | A | B | B | A | B | B | B | A |
| US6 | A | B | B | B | A | B | B | A |
| US8 | A | B | B | B | B | A | B | A |
| US10 | A | B | B | B | B | B | A | A |
| US16 | A | A | B | B | A | B | B | A |
| US18 | A | A | B | B | B | A | B | A |
| US110 | A | A | B | B | B | B | A | A |
| US26 | A | B | A | B | A | B | B | A |
| US28 | A | B | A | B | B | A | B | A |
| US210 | A | B | A | B | B | B | A | A |
| US36 | A | B | B | A | A | B | B | A |
| US38 | A | B | B | A | B | A | B | A |
| US310 | A | B | B | A | B | B | A | A |

(A: displays the stages applied; B: displays the stages not applied)

### 2.3. Particle size

The particle sizes of whey concentrates were measured following the methodology described by Yıldız et al. (2017) via dynamic light scattering (DLS) with a NICOP 38 DSL instrument (Santa Barbar, CA, USA). Whey concentrates were diluted 500-fold with deionized $H_2O$ prior to DSL analysis. All experiments were conducted at a stable scattering angle of $90^o$ along with 658 nm wavelengths under room conditions. The average of droplet sizes was achieved as the mean of 3 measurements where each measurement was performed for about a min.

### 2.4. Emulsifying properties

Emulsifying properties of whey concentrates were evaluated by employing the approach described by Yıldız (2018). Oil in $H_2O$ suspensions was made by adding a mL of canola oil in three mL of the whey concentrate specimens. The ratios of oil concentration were 0.25% (w/w) in this measurement. The combined oil and whey concentration solution was stirred harshly for approximately 5 min and then sonicated for about 5 minutes. After the suspension occurred, the absorbance of the whey concentrates was determined at 500 nm at 0 ($A_0$) and 10 min ($A_{10}$), subsequently. Both the emulsifying activity index (EAI) and the emulsion stability index (ESI) were calculated using the formulae below:

$$EAI\ (m^2/g)=2T\ A_0 \times \text{dilution factor}/c \times Q \times L \times 10{,}000$$

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

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).

### 2.5. Statistical analysis

The differences were achieved with the general linear model process in SAS (version 9.3, SAS Institute, Inc., Cary, North Carolin, USA). Significant variations between the average values were identified by Fisher's least significant difference (LSD) test at alpha = 0.05.

## 3. Results and Discussion

### 3.1. Particle size

Table 3 displays the findings regarding the particle sizes of the WPC samples exposed to different US treatments. All US-treated WPC samples had significantly smaller particle sizes in comparison with the control whey protein concentrates. In the study examining the effect of high-intensity ultrasound on whey protein isolate (Vargas et al., 2021), it was reported that a gradual decrease in WPI particle size (nm) was detected after ultrasound application. In addition, they found that ultrasonic treatments with particle size distribution applied in the study, similar to our study, had a significant effect on particle size reduction ($p < 0.05$) compared to untreated WPI (Vargas et al., 2021). Moreover, the smallest particle size was obtained in the WPC samples exposed to 30 min of US treatment at a 100% amplitude (498.6 nm). The WPC samples treated at a 100% amplitude had a smaller particle size than the other WPC samples at 60% and 80% amplitudes. In general, similar to our study, it was reported that the particle size decreased (from 50% to 100%) as the treatment amplitude increased in other studies (Jambrak et al., 2014; Lam, 2014; Vargas et al., 2021). An inverse relationship was detected between the particle size and ultrasound amplitude (Table 3). Particle sizes decreased with an increase in amplitude. While the amplitude was the lowest (60%), the particle size was the highest (798.6 nm). On the contrary, when the amplitude was the highest (100%), the particle size was the smallest (721.3 nm). It was clearly observed that increasing US amplitudes advanced the particle size of whey concentrate specimens. The WPC samples treated for 30 min had the smallest particle size (697.1 nm) compared to the WPC samples treated for 10 and 20 min (Table 3). Similar to the amplitude, ultrasound times also had an inverse relationship with solubility. Similar to our study, Vargas et al. (2021) in their

study on the application of whey protein isolate in high-density application; they used two different treatments (50,100%:%A; 2 and 4 minutes). According to the results of the study; In the same applications, it was determined that the 4 minute application had a lower particle size than the 2 minute application (Vargas et al., 2021). Increasing ultrasound time from 10 to 30 min led to smaller particle size. While the WPC samples treated for 10 min had the biggest particle size (790.3 nm), those treated for 30 min had the smallest particle size (697.1 nm).

The unfolding process, especially the ultrasound, may cause WPC samples to become more susceptible to breakdown. The decline in the particle sizes of plant proteins (i.e., soy protein and pea protein) has been reported in previous research (Lee et al., 2016; Yıldız et al., 2017; Yıldız, 2018; Jiang et al., 2019). In the study by Jambrak et al. (2014) performing the application with an ultrasonic probe (20 kHz), high-intensity ultrasound treatment led to a decrease in particle size as parallel to our research, narrowed their distribution, and significantly ($p<0.05$) increased specific free surface in whey protein specimens. When used in protein suspensions, ultrasound treatment was expressed to significantly reduce the droplet sizes of whey protein samples (Jambrak et al., 2008). Moreover, Karki et al. (2010) determined that the droplet sizes of defatted soy flake samples decreased approximately 10-fold after the ultrasound application. It was revealed that cavitation might explain the breakage of protein aggregates and decline in the droplet size (Arzeni et al., 2012; Yıldız, 2019; Yıldız & Aadil, 2020). Gordan & Pilosof (2010) managed to control particle size via high-intensity ultrasound by merging several treatment periods, temperatures, and ratios of whey protein dispersions. The ultrasound process develops a new surface and reduces the size of aggregates (Yıldız & Feng, 2019). In this case, protein particles decrease due to the cavitation phenomenon, which involves the degradation of protein aggregates and agglomerates. Ultrasonic cavitation is very efficient to break down protein aggregates and obtain smaller particle aggregates via the van der Waals forces (Jambrak et al., 2014).

Table 3. Particle size, EAI, and ESI of WPC samples

| Treatments | Particle size (nm) | EAI ($m^2/g$) | ESI (min) |
|---|---|---|---|
| Control | $897.2 \pm 0.3^{a}$ | $56 \pm 1.7^{l}$ | $13.0^{i}$ |
| US1 | $790.3 \pm 0.5^{c}$ | $69 \pm 0.2^{j}$ | $16.0^{h}$ |
| US2 | $726.6 \pm 0.3^{e}$ | $74 \pm 1.3^{i}$ | $18.0^{g}$ |
| US3 | $697.1 \pm 0.2^{f}$ | $96 \pm 2.1^{g}$ | $21.0^{f}$ |
| US6 | $798.6 \pm 0.1^{b}$ | $65 \pm 3.6^{k}$ | $15.0^{h}$ |
| US8 | $745.1 \pm 0.9^{d}$ | $75 \pm 1.5^{i}$ | $18.0^{g}$ |
| US10 | $721.3 \pm 0.1^{e}$ | $88 \pm 0.7^{h}$ | $19.0^{g}$ |
| US16 | $698.6 \pm 0.1^{f}$ | $95 \pm 0.4^{g}$ | $20.0^{f}$ |
| US18 | $644.4 \pm 0.8^{g}$ | $113 \pm 1.3^{f}$ | $23.0^{e}$ |
| US110 | $593.6 \pm 0.4^{j}$ | $144 \pm 1.9^{d}$ | $28.0^{c}$ |
| US26 | $628.7 \pm 0.6^{h}$ | $115 \pm 0.7^{f}$ | $25.0^{d}$ |
| US28 | $615.2 \pm 0.1^{i}$ | $121 \pm 2.2^{e}$ | $26.0^{d}$ |
| US210 | $588.8 \pm 0.5$ | $157 \pm 0.5^{c}$ | $31.0^{b}$ |
| US36 | $593.98 \pm 1.7^{j}$ | $145 \pm 1.1^{d}$ | $28.0^{c}$ |
| US38 | $552.3 \pm 0.8^{k}$ | $168 \pm 0.3^{b}$ | $32.0^{b}$ |
| US310 | $498.6 \pm 0.4^{l}$ | $198 \pm 0.8^{a}$ | $34.0^{a}$ |

[a-l] Mean ± standard deviation (n=3) of the feature with the same letter are not significantly different ($p < 0.05$)
*All the statistics were done separately for all and each variable (particle size, EAI, ESI)

### 3.2. Emulsifying properties

Table 3 shows the EAI and ESI of WPC samples treated with US. It was found that the WPC samples treated with US for 30 min at a 100% amplitude had the highest EAI (198 $m^2g^{-1}$) and ESI (34.0 minutes), while the whey concentrates without US treatment exhibited the lowest EAI (56 $m^2g^{-1}$) and ESI (13.0 min). Similar progression in the emulsifying properties of proteins with the US process was indicated in the study by Yıldız et al. (2017). It is possible to see an inverse relationship between particle

size and emulsifying properties (Table 3). Basically, the WPC samples with the smallest particle size, namely the WPC samples treated with US for 30 min at a 100% amplitude, also had high ESI and EAI. Some studies have reported that ultrasound treatment improves the foaming capacity, solubility and emulsifying properties of different proteins such as WPI, MPC, soy proteins (Jambrak et al., 2008; Abd El-Salam et al., 2009; Yanjun et al., 2014). In general, the use of ultrasound treatment, especially at higher amplitudes and times, resulted in an improvement of emulsifying properties (ESI and EAI).

## 4. Conclusion

In comparison with the other US treatments, a significant enhancement in the droplet sizes and emulsifying characteristics of WPC samples was achieved with US310 treatment. In general, US310 is alternative treatment to strengthen the physicochemical characteristics of WPCs, as indicated in the present study, due to its ability to obtain a smaller particle size (498.6 nm) and better emulsifying properties (EAI: 198 $m^2g^{-1}$ and ESI: 34.0) right after ultrasonication. The results of this specific research showed the potential of US treatment as an effective method for protein modification. In future studies, the interfacial properties of whey protein sources (isolate, concentrate, powder) can be explored and more detailed analyses can be conducted.

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