FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 8(4), 284-289 (2022) • https://doi.org/10.3153/FH22026 *Research Article*

The effect of ultrasound times and amplitudes on the solubility and turbidity of whey protein concentrate

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Cite this article as:
Bulut, M. (2022). The effects of ultrasound times and amplitudes on the solubility and turbidity of whey protein concentrate. *Food and Health,* 8(4), 284-289. https://doi.org/10.3153/FH22026

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Submitted: 14.11.2021
Revision requested: 31.03.2022
Last revision received: 04.05.2022
Accepted: 05.05.2022
Published online: 19.08.2022

Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

The current work was conducted to explore the influence of ultrasound times and amplitudes on the solubility and turbidity of whey protein concentrate (WPC). Ultrasound (US) application was employed using VC-750 ultrasonic power equipment with the frequency of 20 kHz at various times (10, 20, and 30 minutes at 50% amplitude) and amplitudes (60%, 80%, and 100% for 5 min). The outcomes exhibited that the US process have a significant impact on both solubility and turbidity ($p<0.05$). The highest protein recovery was obtained for the samples exposed to 30 min the US at 100% amplitude (65.56%). WPC samples treated at 100% amplitude showed higher solubility compared to the other samples at 60% and 80% amplitudes. While the solubility of WPC samples treated with 10 min showed the lowest solubility (9.13%), samples treated with 30 min showed the highest solubility (38.14%). There is a negative relationship between solubility and turbidity. All US-treated samples showed less turbidity and higher solubility where the control WPC samples showed the most turbid structure (0.88 NTU) with the lowest solubility (4.15%). Overall, US treatment with 30-minutes at 100 % amplitude showed the highest solubility (65.56%) and least turbidity (0.26 NTU) compared to the other sonication times and amplitudes.

Keywords: Ultrasound, Amplitude, Protein solubility, Turbidity, Whey protein concentrate

Introduction

Whey protein is a crucial material of functional protein components for several conventional and novel food materials (Kumar et al., 2018). Whey proteins are recognized as complete proteins since they include all 9 essential amino acids. The 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-lactoglobulin, a-lactalbumin, and bovine 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 (Krešić et al., 2008).

Various methodologies have been promoted to alter the native protein structure for the purpose of improvement of functionality. Modified whey proteins exhibit a very high level of functional capacity. Through molecular and physical alterations, it is achievable to reorganize protein compounds so that they develop into more practical and useful forms. Ultrasound (US) technology is a cost-effective and fast application that has been employed to alter both the structure and functional properties of protein molecules (Masonn et al., 1996; Jambrak et al., 2008; Yıldız et al., 2018). The impact of US treatment is accomplished by the chemical, molecular, and physical consequences of acoustic cavitation. Cavitation is mostly defined as the creation, development, and powerful breakdown of tiny droplets in solution. The cavitation could be the reason for protein structure modification thanks to hydrogen bonds and hydrophobic cooperation, and the falling part of the protein molecules (Yildiz et al., 2017). By taking into account the benefits of the US application such as being a cost-effective, non-toxic, fast, and efficient process, it is anticipated to reach a goal of advanced WPC functionality by using the US application. For this reason, the purpose of the present work is to analyze the impact of US application on the protein recovery and turbidity features of whey protein.

Materials and Methods

Whey Protein Concentrate (WPC)

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

WPC Samples and Ultrasound Application

US application was progressed using a VC-7500 US power equipment along with the frequency of 20 kHz (Sonic & Materials, Inc., USA) at three different times (10, 20, and 30 minutes at 50% amplitude) and amplitudes (60%, 80, and 100% for 5 min). Insoluble WPC (3 g) was blended with a 100 mL distilled H_2O and stirred for about 60 min at room temperature (RT) conditions with the help of a magnetic stirrer. The beaker stayed in a cup filled with ice cubes at the time of US treatment for the prevention of temperature rise. The protein solution following the US application were centrifuged (1200 g and 20 ºC) for 15 min. Soluble WPC was collected right after centrifugation. For the control WPC samples, no US treatment was conducted; 3 g WPC in 100 mL water was agitated at 25 ºC for 30 min. While table 1 exhibits the description of the WPC samples and treatments, table 2 shows the processing steps applied for each treatment.

Table 1. The description of the WPC samples and treatments

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

Table 2. The processing steps applied for each treatment

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

(A: displays the stages applied; and NA: displays the stages that were not applied)

Protein Solubility

Solubility of the WPC samples was determined by a Bio-Rad Protein Assay based on the technique described by Bradford (1976). Bovine serum albumin (BSA) was utilized as the standard assay. Dye reagent was arranged by diluting 1 part of dye reagent concentrate into 4 parts of DI water, and filtered through a filter paper. The prepared solution was blended with soluble WPC. The protein concentration of soluble WPC was measured by spectrophotometer at the wavelength of 595 nm. Protein solubility was calculated as below and represented as "%":

$$Recovery\ of\ soluble\ protein = \frac{Protein\ concentration\ in\ soluble\ WPI}{Initial\ protein\ concentration} \times 100 \quad (1)$$

Turbidity (NTU)

The turbidity of the WPC 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 read.

Statistical Analysis

The differences were achieved with the General Linear Model process in SAS (version 9.3, SAS Insttute, Inc., Cary, North Carolin, USA). Significant differences between the mean values were identified by Fisher's least significant difference (LSD) test at alpha = 0.05.

Results and Discussion

Solubility

Table 3 displays the findings related to protein solubility values of the WPC samples exposed to different US treatments. All US-treated WPC samples displayed significantly higher solubility in comparison with the control whey protein concentrates. Moreover, the highest protein solubility was obtained for the WPC samples exposed to 30 min the US at 100% amplitude (65.56%). WPC samples treated at 100% amplitude showed higher solubility compared to the other samples at 60% and 80% amplitudes. A positive relationship between the solubility and ultrasound amplitude was determined. The higher the amplitude, the higher the solubility. While the amplitude was the lowest (60%), the solubility was the lowest (8.65%). On the other hand, while the amplitude was highest (100%), the solubility was highest (21.38%). It was clearly seen that increasing US advances the solubility of whey protein concentrates. WPC samples treated for 30 min showed the highest solubility (38.14%) compared to the WPC samples treated with 10 and 20 min (Table 3). Similar to the amplitude, ultrasound times have also positive relationships with solubility. Increasing ultrasound time from 10 to 30 min leads to higher solubility. While the solubility of WPC samples treated with 10 min showed the lowest solubility (9.13%), samples treated with 30 min showed the highest solubility (38.14%). Solubility is a main functional property for

whey protein (Hussain et al., 2012; Feng et al., 2022). Solubility is related to several functional features such as molecular weight, not the primary but the secondary and tertiary structure, hydrophobic, and electrostatic charges (Lee et al., 2016; Chang et al., 2021). Processing treatments used to manufacture whey protein may result in heat-induced protein denaturation, which then reduces whey protein solubility. Native whey proteins remain soluble at around pH 7; however, heat-induced denaturation renders whey proteins less soluble than native whey proteins (Jambrak et al., 2014). Thus, the protein solubility of whey protein is helpful to estimate protein denaturation (Morr and Ha, 1993). The development of protein solubility increase following a US application has been figured out in different works (Le et al., 2016; Jiang et al., 2017; Yildiz et al., 2017). The physical forces developed by US cavitation such as shear forces could alter the protein structure which comes out with developed protein solubility. Also, sonication can be the reason for the breakage of both non-covalent and covalent bonds which lead to protein solubility increase (Hue et al., 2003). Jambak et al. (2008) examined the influence of US (20 kHz probe & 40 kHz bath), on solubility, emulsifying and foaming attributes of different whey protein types consisting of whey protein isolate, whey protein concentrate, and whey protein hydrolysate. It was figured out whey protein solubility increased significantly for all whey samples for the treatment of 20 kHz probe and 40 kHz baths.

Table 3. Protein solubility (% recovery) & turbidity of WPC samples

Treatments	Solubility (%)	Turbidity (NTU)
Control	4.15 ±0.43^{j}	0.88 ±0.7^{a}
US1	9.13 ±0.58^{i}	0.79 ±0.2^{b}
US2	16.65 ±0.35^{h}	0.74 ±0.3^{bc}
US3	38.14 ±0.22^{f}	0.66 ±0.1^{c}
US6	8.65 ±0.11^{i}	0.79 ±0.6^{b}
US8	15.14 ±0.19^{h}	0.75 ±0.5^{bc}
US10	21.38 ±0.11^{g}	0.74 ±0.7^{bc}
US16	38.06 ±0.01^{f}	0.65 ±0.4^{c}
US18	44.14 ±0.81^{e}	0.63 ±0.3^{c}
US110	53.66 ±0.74^{c}	0.44 ±0.9^{e}
US26	48.73 ±0.62^{d}	0.55 ±0.7^{d}
US28	51.82 ±0.14^{cd}	0.51 ±0.2^{de}
US210	58.85 ±0.53^{b}	0.37 ±0.5^{f}
US36	53.19 ±1.17^{c}	0.45 ±0.1^{e}
US38	59.23 ±0.89^{b}	0.38 ±0.3^{f}
US310	65.56 ±0.46^{a}	0.26 ±0.8^{g}

$^{a-j}$ Mean ± standard deviation (n=3) of properties with the same letter are not significantly different ($p<0.05$)

* All the statistics were done separately for each parameter (solubility, turbidity)

Turbidity

The turbidity findings of WPC samples are demonstrated in Table 3. Both the solubility and particle size of soluble protein aggregates determines the turbidity of a protein dispersion (Lee et al., 2016). Martin et al. (2010) investigated the optimization of the use of power ultrasound to reduce the turbidity of whey solutions. It was concluded that around a 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 30 min at 100% amplitude (US310 samples). While the highest turbidity was obtained for the untreated WPC (0.88 NTU), the lowest turbidity was observed for the US310 samples (0.26 NTU). There is a negative relationship between the variables of solubility and turbidity. All US-treated samples showed less turbidity and higher solubility where the control WPC samples exhibited the most turbid appearance and lowest solubility (Table 3). Overall, US treatment with 30-minutes at 100 % amplitude showed the highest solubility (65.56%) and least turbidity (0.26 NTU) compared to the other sonication times and amplitudes. Both the number of soluble protein components in the dispersion figured out by solubility and the sizes of the soluble protein components determine the turbidity of a whey protein dispersion (Yildiz et al., 2017). 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; Ghasemi et al., 2018).

Conclusion

Ultrasound treatment was examined for the purpose of modification and enhancement of the whey protein functionality. Compared with other US treatments, a significant improvement in the solubility and turbidity properties of WPC samples was achieved with a US310 treatment. Overall, US310 is a promising treatment to strengthen the physicochemical characteristics of WPCs as indicated within the present study by its ability to higher solubility and less turbidity right after ultrasonication. The results of the current study showed the potential of the US310 treatment as an effective method for protein modification. The functionalized WPC produced by the US310 treatment can be used in a liquid food with high solubility and less precipitation.

Compliance with Ethical Standard

Conflict of interests: The author declares that for this article they have no actual, potential, or perceived conflict of interests.

Ethics committee approval: The author declares that this study does not include any experiments with human or animal subjects; therefore, no ethics committee approval is needed.

Funding disclosure: -

Acknowledgments: -

Disclosure: -

References

Arzeni, C., Martinez, K., Zema, P., Arias, A., Perez, O.E., Pilosof, A.M.R. (2012). Comparative study of high intensity ultrasound effects on food proteins functionality. *Journal of Food Engineering*, 108, 463-472. https://doi.org/10.1016/j.jfoodeng.2011.08.018

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* 72, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Chang, K., Liu, J., Jiang, W., Fan, Y., Nan, B., Ma, S., ..., Zhang, T. (2021). Structural characteristics and foaming properties of ovalbumin-Caffeic acid complex. *LWT*, *146*, 111383. https://doi.org/10.1016/j.lwt.2021.111383

Ghasemi, S., Jafari, S.M., Assadpour, E., Khomeiri, M. (2018). Nanoencapsulation of d-limonene within nanocarriers produced by pectin-whey protein complexes. *Food Hydrocolloids,* 77, 152-162. https://doi.org/10.1016/j.foodhyd.2017.09.030

Hu, H., Li-Chen, E.C.Y., Wan, L., Tian, M., Pan, S. (2003). The effect of high intensity ultrasonic pretreatment on the properties of soybean protein isolate gel induced by calcium sulfate. *Food Hydrocolloids,* 32(2), 303–311. https://doi.org/10.1016/j.foodhyd.2013.01.016

Hussain, R., Gaiani, C., Jeandel, C., Ghanbaja, J., Scher, J. (2012). Combined effect of heat treatment and ionic strength on the functionality of whey proteins. *Journal of Dairy Science,* 95, 6260-6273. https://doi.org/10.3168/jds.2012-5416

Feng, Y., Wang, J., Hu, H., Yang, C. (2022). Effect of oxidative modification by reactive oxygen species (ROS) on the aggregation of whey protein concentrate (WPC). *Food Hydrocolloids,*123,10718. https://doi.org/10.1016/j.foodhyd.2021.107189

Jambrak, A.R., Mason, T.M., Lelas, V., Herceg, Z., Herceg, I.L. (2008). Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. *Journal of Food Engineering*, 86, 281-287. https://doi.org/10.1016/j.jfoodeng.2007.10.004

Jambrak, A.R., Mason, T.J., Lelas, V., Paniwnyk, L., Herceg, Z. (2014). Effect of ultrasound treatment on particle size and molecular weight of whey proteins. *Journal of Food Engineering,* 121, 15-23. https://doi.org/10.1016/j.jfoodeng.2013.08.012

Jiang, S., Ding, J., Andrade, J., Rababah, T.M., Almajval, A., Abulmeaty, M.M., Feng, H. (2017). Modifying the physicochemical properties of pea protein by pH-shifting and ultrasound combined treatments. *Ultrasonics Sonochemistry.* 38, 835-842. https://doi.org/10.1016/j.ultsonch.2017.03.046

Krešić, G., Lelas, V., Jambrak, A. R., Herceg, Z., Brnčić, S. R. (2008). Influence of novel food processing technologies on the rheological and thermophysical properties of whey proteins. *Journal of Food Engineering,* 87(1), 64-73. https://doi.org/10.1016/j.jfoodeng.2007.10.024

Kumar, R., Chauhan, S.K., Shinde, G., Subramanian, V., Nadanasabapathi, S. (2018). Whey Proteins: A potential ingredient for food industry- A review. *Asian Journal of Dairy and Food Research*, 37, 283-290. https://doi.org/10.18805/ajdfr.DR-1389

Lee, H., Yildiz, G., Dos Santos, L.C., Jiang, S., Andrade, J., Engeseth, N.C., Feng, H. (2016). Soy protein nano-aggregates with improved functional properties prepared by sequential pH treatment and ultrasonication. *Food Hydrocolloids,* 55, 200-209. https://doi.org/10.1016/j.foodhyd.2015.11.022

Liu, Q., Li, J., Kong, B. H., Li, P.J., Xia, X.F. (2014). Physicochemical and antioxidant properties of Maillard reaction products formed by heating whey protein isolate and reducing sugars. *International Journal of Dairy Technology,* 67, 220-228. https://doi.org/10.1111/1471-0307.12110

Martini, S., Potter, R., Walsh, M.K. (2010). Optimizing the use of power ultrasound to decrease turbidity in whey protein suspensions. *Food Research International,* 43, 2444-2451. https://doi.org/10.1016/j.foodres.2010.09.018

Mason, T.J., Paniwnyk, L., Lorimer, J.P. (1996). The uses of ultrasound in food technology. *Ultrasonics Sonochemistry*, 3, 253–260. https://doi.org/10.1016/S1350-4177(96)00034-X

Morr, C.V., Ha, E.Y.W. (1993). Whey protein concentrates and isolates: processing and functional properties. *Critical Reviews in Food Science and Nutrition,* 33, 431-476. https://doi.org/10.1080/10408399309527643

Yildiz, G., Andrade, J., Engeseth, N.C., & Feng, H. (2017). Functionalizing soy protein Nan- aggregates with pH-shifting and mano-thermo-sonication. *Journal of Colloid and Interface Science,* 505, 836-846. https://doi.org/10.1016/j.jcis.2017.06.088

Yıldız, G. (2018). Physicochemical properties of soy protein concentrate treated with ultrasound at_various amplitudes. *Journal of the Institute of Science and Technology*, 8(4), 133-139. https://doi.org/10.21597/jist.436852

Zisu, B., Lee, J., Chandrapala, J., Bhaskaracharya, R., Palmer, M., Kentish, S., Ashokkumar, M. (2011). Effect of ultrasound on the physical and functional properties of reconstituted whey protein powders. *Journal of Dairy Research,* 78, 226-232. https://doi.org/10.1017/S0022029911000070