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Ultrasonic Enhanced Walnut Protein Emulsifying Property

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Abstract

The effect of ultrasonic treatment on emulsifying property of walnut protein was investigated. The walnut protein concentration was adjusted to 1% and treated with ultrasonic at different temperature, power, frequency and time. The result showed that walnut protein emulsifying property firstly increased and then decreased with the temperature increase or time extension of ultrasonic, and it decreased with ultrasonic frequency enhancement. Below 300 W, the emulsifying property increased with the power enlargement. Treating walnut protein with ultrasonic at about 20 min, 45 kHz, 60°C and 180 W might enhance the emulsifying property of walnut protein.

Keywords: Ultrasonic; Walnut; Protein; Emulsifying

Introduction

Walnut is a nutritious nut, often pressed to acquire walnut oil at present. Walnut kernel residue is a by-product of extraction walnut oil, containing abundant protein [1]. The amino acids of walnut protein are complete compositions of 18 kinds of amino acid, and the contents of 8 kinds of necessary aminophenol are reasonable. Walnut protein is fitted with the model values of protein provided by FAO/WHO [2]. Furthermore, walnut protein has potential application value in food emulsification, which involves the stabilization of mixture consisting of hydrophilic and hydrophobic substances.

Ultrasonic is an electromagnetic wave with a frequency greater than 20 kHz and it has some effects such as cavitation, agitation and heating. At present, ultrasonic was applied in food sterilization, nutritional ingredient extraction, and so on [3]. To enhance the emulsification property of protein, the protein was often modified by physical or chemical methods [4,5]. Modifying protein with ultrasonic is a physical method and had relatively high food safety compared with food additive. Chalothorn K. and Warisnoicharoen W. found that ultrasonic treating could possibly affect the properties of whey protein isolate molecules at the oil-water interface as well as emulsifying efficiency [6].

This work aimed to enhance the emulsification property of walnut protein and expand the practical value of walnut protein in food industry. It may also provide reference for further developing walnut protein.

Materials and Methods

Materials

Walnut (*Juglans regia* L. cv. Soft walnut) was produced from Fenyang city, Shanxi province of China. Soybean oil (top-grade) was purchased from China National Cereals, Oils and Foodstuffs Corporation. Coomassie brilliant blue (G-250) and bovine serum albumin (standard reagent) were purchased from Sinopharm Chemical Reagent Co., Ltd.

Preparation of walnut protein solution

Walnut kernel was peeled by using 3% of lye, and the water in walnut kernel was drained away. Afterwards, the walnut kernel was dried at 60°C for 2 hours. And then the oil of walnut kernel was extracted at 60°C with 60 MPa using piston press machine (6YY-190, Jinxia Hydraulic Machine Co., Ltd, Luoyang, China). Thus, the walnut kernel residue was obtained. 100g of walnut kernel residue was firstly soaked with 1000 mL of deionized water for 30 min and then was smashed for 5min. The slurry of walnut kernel residue was filtrated at 160 mesh. The soluble protein concentration of slurry was adjusted to 1% through adding a little deionized water. The prepared protein solution was treated with ultrasonic using ultrasound clean equipment (KQ300VDE, Kun Shan Ultrasonic Instruments Co., Ltd, Kunshan, China) at different condition.

Determination of walnut soluble protein

The walnut protein was assayed using coomassie brilliant blue G-250 method [7]. An aliquot (1 mL) of a standard solution of bovine serum albumin with different concentrations (0, 20, 40, 80, and 100 μg/ml) was added to 5 mL of 0.01% coomassie brilliant blue G-250 solution. Each solution was thoroughly stirred. After 2 min, the absorbance of the mixture was determined at 595 nm versus the prepared blanks using a UV spectrophotometer (UV-1100, Shanghai Meipuda Instrument Co., Ltd., Shanghai, China). The abscissa denotes protein content, the ordinate reflects absence and the standard curve ($y=0.0045x+0.0096$, $R^2=0.9972$) was drew. The walnut protein content was measured according to narrative method.

Evaluation of walnut protein emulsifying property

The emulsifying property of walnut protein was determined according to the method of Dipak and Kumar with slight modification [8]. 20 mL of soy oil (V_{total}) was added into 20 mL of treated protein solution. And the mixture was homogenized under ambient temperature for 15 min at the highest speed (Agitator, HJ-4B, Guohua Electric Appliance Co., Ltd, Changzhou, China). The obtained emulsion was held with 50-mL centrifuge tube and centrifuged at 2800g for 10 min (Centrifuge, RJ-TDL-40C, Ruijiang Analysis Instrument Co., Ltd, Wuxi, China). The volume of suspension oil was measured ($V_{suspension}$).

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The emulsifying property was calculated as follows: Emulsifying property (%)=(1-$V_{suspension}$/V_{total}) × 100. Large numerical value stands for good emulsifying effect.

Statistical analysis

The data were processed by analysis of variance using DPS7.05 statistical software (Refine Information Tech. Co., Ltd., Hangzhou, China). The treatments were compared at P=0.05 using Tukey's test, which indicates the multi-comparison value in each case. The data were expressed as mean ± standard deviation.

Result and Analysis

The time of ultrasonic treatment

The walnut protein solution was treated with ultrasonic at 40°C through 150W of power and 45 kHz of frequency for 0, 10, 20, 30 and 40min. As shown in Figure 1, the emulsifying property of walnut protein first increased and then decreased with ultrasonic treating time extension. Before 20 min, it increased with time extension. And from 20 min to 40 min, it decreased with time extension. And at 20 min, the emulsifying property reached to the max value and was as about 2 times as control sample. The reason was probably that ultrasonic cavitation and mechanical action could destroy protein structural, and accordingly hydrophobic parts of walnut were exposed below 20 min. Thus, the affinity between protein and oil was enhanced and emulsifying property was increased as well. However, with treating time further extension, ultrasonic promoted part soluble protein to gather into a mass, which lead emulsifying property to decline [9].

The frequency of ultrasonic treatment

The walnut protein was treated with ultrasonic at 40°C through 150W of power and 45, 80, and 100 kHz of frequency for 20min. The effect of ultrasonic frequency on emulsifying property of walnut protein was described in Figure 2. The emulsifying property of walnut protein treated with ultrasonic at 45 kHz was the highest, the moderate at 80 kHz, and the lowest at 100 kHz. They were 100%, 70% and 48% higher than that of control sample. The intension of ultrasonic cavitation is related to frequency. In high frequency, bubble originated from ultrasonic cavitation is small and its intension is weak [10]. Thus, hydrophobic parts of walnut protein could not completely outspread under such small intension. Whereas, walnut protein was treated with lower frequency and bigger bubble occurred, which could modified protein and was beneficial to improve protein emulsifying property.

The temperature of ultrasonic treatment

The walnut protein was treated with ultrasonic at 20, 40, 60 and 80°C through 150W of power and 45 kHz of frequency for 20min. Below 60°C, the emulsifying property of walnut protein increased with temperature enhancement (Figure 3). Especially when the temperature was below 40°C, the emulsifying property increased fast and it is about 100% higher than that of control sample. However, it showed down trend from 60°C to 80°C, but there was no difference between them (P > 0.05). When walnut protein was properly heated, the molecules moved faster and hydrophobic parts was easily exposed under ultrasonic treating. Thus, the emulsifying property was improved. However, if protein was excessively modified under high temperature, many hydrophobic parts were exposed and the solubility of walnut protein decreased [11]. Thus, the emulsifying property of walnut decreased accordingly.

The power of ultrasonic treatment

The walnut protein was treated with ultrasonic at 40°C through 0, 60, 120, 180, 240 and 300 W of power and 45 kHz of frequency for 20min. As shown in Figure 4, the emulsifying property increased with ultrasonic power enhancement. It increased fast below 120 W and was 1-fold higher than that of control sample. From 120 W to 300 W,

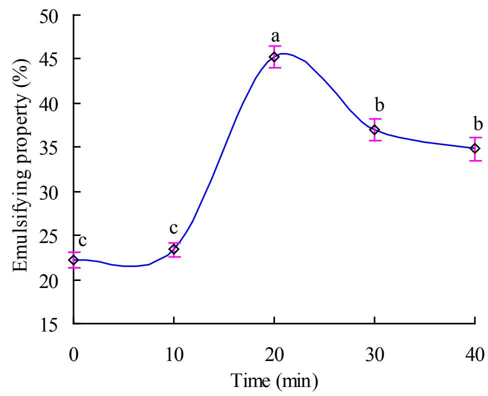

Figure 1: Effect of ultrasonic time on emulsifying property of walnut protein. Each point represents the mean value ± SD.

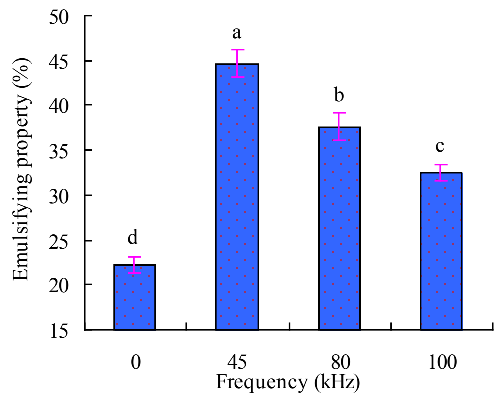

Figure 2: Effect of ultrasonic frequency on emulsifying property of walnut protein. Each point represents the mean value ± SD.

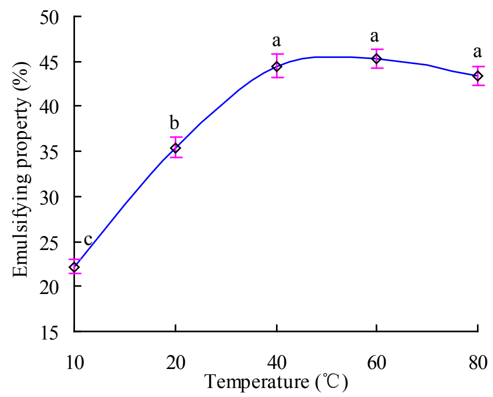

Figure 3: Effect of ultrasonic temperature on emulsifying property of walnut protein. Each point represents the mean value ± SD.

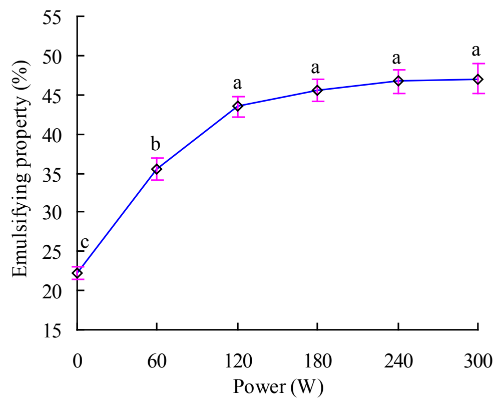

Figure 4: Effect of ultrasonic power on emulsifying property of walnut protein. Each point represents the mean value ± SD.

the emulsifying property did not significantly increase ($P > 0.05$). The reason was probably that ultrasonic cavitation intension strengthened with the power increase. Many hydrophobic parts were exposed and the emulsifying property was improved. When the ultrasonic power was 120W, the emulsifying property almost reached to the best effect. Though from 120 W to 300 W, the emulsifying property did not changed, we inferred it probably decreased if the ultrasonic power further increased. Whereas the maximal power of instrument used in our experiment is 300 W, the downtrend of emulsifying property was not observed [12].

Discussion

Walnut protein has two kinds of chemical groups. One is hydrophobic groups such as methyl, methylene, and so on. And the other is hydrophilic groups including hydroxyl, sulfhydryl and amino. The emulsifying property of protein is that oil phase was combined with aqueous phase through protein. Namely the oil was bound to hydrophobic groups of protein and the aqueous phase was theoretically bound to hydrophilic groups of the same protein molecule [13]. However, in natural protein structure, hydrophilic groups are located at outer layer and hydrophobic groups are prone to associate in inner region. This structure is beneficial to maintain protein conformation, but is disadvantage to emulsification. The emulsifying property of natural protein was worse usually owing to its compact and rigid structure stabilized by intramolecular bonds, namely hydro-phobic, hydrogen, electrostatic and disulphide bonds [14,15]. Ultrasonic treating could modify the protein conformation, and the hydrophobic groups were exposed. Thus the oil molecule was easily bound to protein and the emulsifying property was enhanced.

The emulsifying property firstly increased with ultrasonic power enlargement, time extension or temperature enhancement. However, treating walnut protein with ultrasonic should be moderate, and excessive ultrasonic treatment could reduce emulsifying property of walnut protein. One of reason is probably that excessive ultrasonic treatment destroyed the structure that was the best to emulsification owing to ultrasonic gathering effect [3]. The emulsifying property of walnut protein decreased with frequency enhancement, which is related to cavitation. In high frequency, the cavitation is weak, and the hydrophobic groups were not effectively exposed. Thus, the emulsifying property decreased accordingly [10].

Conclusion

Proper ultrasonic treatment could enhance emulsifying property of walnut protein. Emulsifying property firstly increased and then decreased with temperature enhancement, time extension or power enhancement (below 300 W). And it decreased with frequency enlargement. Treated with ultrasonic at about 20 min, 45 kHz, 60°C and 180 W, the walnut protein might have higher emulsifying property.

Acknowledgements

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