Effect of ultrasonic and microwave processing on physicochemical property of silkworm pupae protein and mulberry seed meal protein to improve protein availability

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Abstract: Silkworm pupae and mulberry seed are good sources of protein, but low protein extraction rate and solubility result in proteins that are not efficiently and widely utilized. In the present study, ultrasound and microwave were used to treat degreased silkworm pupae and degreased mulberry seed meal, the solubility and other physicochemical properties of the treated protein were tested. The experimental results showed that the solubility, emulsifying properties and stability of the protein after ultrasonic and microwave treatment were increased. The effect of ultrasonic treatment was better than that of microwave treatment, and the response surface was used to optimize the treatment conditions, and the extraction rate of protein was up to 77%. Silkworm pupae and mulberry seed are widely used. On this basis, the modified protein was no longer limited by conditions such as low solubility and can be applied to more fields.
Keywords: Silkworm pupae protein, Mulberry seed meal protein, Ultrasound, Microwave, Physicochemical properties, Protein availability.

Introduction

Treatment and management of processing waste is a major challenge for industry [1]. Through the reuse of by-products or wastes, environmental pollution can be reduced, resource utilization can be improved, and high value-added products can be produced.

China is the origin center of mulberry, and mulberry, also known as mulberry, is one of the fruits with a long history in China. When mulberries are processed into fruit juice or wine making and vinegar making, they will produce a large number of coproducts-mulberry residues (containing mulberry seeds and other residues). In mulberry seed, there are 33.4% of oil, 25% of crude protein, 19.9% of total amino acids and 5.71% of essential amino acids. After deoiling, the protein content of mulberry seed meal was up to 40% [2]. However, this protein has not been properly used and resulting in huge waste. Silkworm pupae are a traditional characteristic resource in China, with an annual output of more than 600,000 tons. But the utilization of silkworm pupae after silk reeling was low. The protein content of silkworm pupae is up to 75% [3], however, the sensitization and the poor solubility of silkworm pupae protein and the special construction have led to the low degree of hydrolysis due to the difficulty of enzymes to attack the cleavage of the protein and limit its applications in industry.

Many treatments have been investigated to improve the solubility of protein. Various physical approaches, such as micro-fluidization, hydrothermal cooking, and high-pressure treatment have been reported [4, 5]. Ultrasound and microwave has also been tested for improving solubility and modifying the functional properties of proteins, which are considered an environmentally friendly and innovative technology [6, 7]. Ultrasound and microwave can change the secondary structure of the protein, thereby increasing the solubility of the protein and reducing its sensitization [8, 9].

Proteins perform a vast array of functions in both the food and pharmaceutical industries, such as emulsification, foaming, encapsulation, viscosity enhancement and gelation [10]. Therefore, in order to make full use of protein, we should not only consider the solubility of protein, but also consider its physical and chemical properties such as foaming, stability and holding oil capacity. Studies have shown that the particle size obtained from potato peel and sweet lime pulp are good enough for film formation treated by ultrasound for particle size reduction and ultrasound treatment improved the emulsion stability of animal and vegetable proteins [10, 11], Therefore, the processing of silkworm pupae protein and mulberry seed meal protein changed their physical and chemical properties, so that they can be applied to a wider range of fields.

This study using ultrasonic and microwave treatment silkworm pupae protein and mulberry seed meal protein respectively, based on the material ratio, processing time, processing temperature and the condition output power, optimization of process improve protein extraction rate, at the same time, after treatment were detected protein solubility, emulsibility, and other physical and chemical properties and comparison, in order to improve protein utilization degree provides reference data.

Materials and methods

Materials and chemicals

Mulberry seed was provided by Sericultural Research Institute, Chinese Academy of Agricultural Sciences. Silkworm pupae were acquired from Xinyuan Silk Group Co., Ltd, Nantong, Jiangsu province, China. The conventional reagents were purchased from the national pharmaceutical group.

Degreased treatment

Crush the raw materials with a crusher and pass through a 60-mesh sieve. Take the appropriate amount of materials in the beaker, add plenty of petroleum ether, after sealing with fresh-keeping film, stirring for 1 hour in a water bath pot at 50 °C, after draining and add proper amount of petroleum ether into beaker again, repeated 7-9 times until the filtrate was basically colorless. The filter residue was laid on the sample tray and a fresh-keeping film was laid on it. The residual solvents were removed overnight to obtain degreased silkworm pupae powder and degreased mulberry seed meal powder.

Extraction of silkworm pupae protein and mulberry seed meal protein

The degreased raw material and deionized water were prepared according to a certain ratio of solid to liquid and stirred. The pH was adjusted with 1 mol/L NaOH; the suspension was extracted for a period of time, and centrifuged for 15 min in a 10000 rpm/min refrigerated centrifuge. The supernatant was collected and adjusted to a constant point of 4.5 with 1 mol/L HCl, and the protein was precipitated and centrifuged again for 15 min. The precipitate was collected and washed with deionized water for 3 to 4 times, then adjusted to pH with 1 mol/L NaOH, and finally freeze-dried to obtain protein.

Ultrasound and microwave treatment of protein

In the process of ultrasonic processing, the substrate ratio of degreased raw material power and deionized water was set from 1:10 to 1:50, A 20 mL aliquot of solution was transferred to a jacket beaker (50 mL) and treated by ultrasound. The output power was set at 100, 200, 300, 400 and 500 W, treated time was 1, 3, 6, 9 and 12 min, temperature was set at 30-70 °C, respectively. After the treatment, the samples were readjusted to pH 4.5, and centrifuged at 10000 rpm for 15 min. Supernatants were collected and freeze-dried for 12 h.

Microwave processing was carried out using Microwave irradiation systems (MARSX, CEM, USA). The treatment process and parameter range were consistent with the ultrasonic processing.

Calculate the extraction rate of seed protein by the following formula:

$$\textit{Protein extraction rate}\ (\%) = \frac{\textit{Crude protein content in extract}\ (\mathrm{g})}{\text{Total protein content in raw materials (g)}} \times 100\% \quad (1)$$

Determination of protein solubility

Weigh 1.5g of the sample in a 250 mL beaker, add 75 mL of 0.2% KOH solution, and stir it on a magnetic stirrer for 20 min. Then transfer 50 mL of the solution to a centrifuge tube and centrifuge it for 10 min at 2700 rpm/min. The protein content of 15 mL supernatant was determined by Kjeldahl determination. Calculate the protein solubility by the formula below:

$$\textit{Protein solubility}\ (\%) = \frac{\textit{Crude protein content in supernatant solution}\ (\mathrm{g/mL})}{\text{Crude protein content in the sample (g/mL)}} \times 100\% \quad (2)$$

Detection of foaming capacity and foam stability of proteins

The protein was mixed with a 0.05 mol/L Tris-HCl buffer having a pH of 8.05 to prepare a solution having a solid-liquid ratio of 1%, and a certain volume of the protein solution was heating in a 25 °C water bathing for 10 min, and then dispersed twice at 13500 r/min using a high-speed disperser at room temperature, each time for 1 min. The solution was quickly transferred to a graduated cylinder and the foam volume (V_0) was recorded. After the solution in the cylinder was allowed to stand for 30 min, the remaining foam volume (V_r) was measured again. The foaming capacity (*FC*) and foam stability (*FS*) of the protein solution were calculated according to the following formula [6]:

$$FC(\%) = \frac{V_0}{\mathrm{V}} \times 100\% \tag{3}$$

$$FS(\%) = \frac{V_r}{V_0} \times 100\% \tag{4}$$

Detection of emulsibility and emulsion stability of proteins

The protein was mixed with 0.2 mol/L phosphate buffer to make a certain concentration (*C*) of protein solution. Corn oil was added and stirred thoroughly, and 50 μL of emulsion was taken and diluted 100 times with 1 g/L SDS buffer, then the sample solution was placed in an enzyme labeler for colorimetry with a wavelength of 500nm, and the absorbance of A_0 was determined. After standing for 10 minutes, the absorbance value detected again was V_{10}. The formula for calculating the emulsification activity index (*EAI*) and the emulsion stability index (*ESI*) of the protein was as follows [12], φ represents the optical path and θ represents the proportion of the oil phase.

$$EAI(\frac{m^2}{\mathrm{g}}) = \frac{2 \times 2.303 \times A_0}{C \times \varphi(1-\theta) \times 10^3} \tag{5}$$

$$ESI = \frac{A_0}{A_{10}} \times 100 \tag{6}$$

Detection of holding oil capacity and water retention of proteins

A certain amount of protein (m_1) was added with soybean oil or water and placed in a centrifuge tube and the total mass was m_2, the sample was shaken for 20 min, heated in a hot water bathing for 50 min, cooled to room temperature, and centrifuged at 3000 r/min for 5 min, the upper phase was removed, the mass of the precipitate (m_3) was weighed, and the formula for the holding oil capacity and water retention of the protein as follows:

$$OC = \frac{m_2 - m_3}{m_1} \times 100\% \tag{7}$$

$$WC = \frac{m_2 - m_3}{m_1} \times 100\% \tag{8}$$

Statistical analysis

Each preparation was investigated in triplicate. The reliability of the results can be verified by the standard deviation of the data and the analysis method was variance (ANOVA) method. The Pearson correlation coefficient was determined the significance of these data.

Results and discussion

Effects of different factors on protein extraction rate by ultrasonic treated

In this study, the degreased silkworm pupae and degreased mulberry seed meal were treated with ultrasound respectively, and the protein extraction rate after treatment was detected. Fig.1 shows the two protein extraction rates under different substrate ratios, different processing times, different temperatures, and different output powers, respectively. As shown in Fig.1a, the protein extraction rates of degreased silkworm pupae and degreased mulberry seed meal reached the highest values at substrate ratios of 1:30 and 1:40, and which were 64.90% and 68.28%, respectively. Similarly, the optimal treatment time for both proteins was 9 min (Fig.1b). Treatment temperature is an important factor affecting the rate of protein extraction, as can be seen from Fig.1c, the optimum treatment temperature for treat degreased silkworm pupae was 40 °C, the protein extraction rate of defatted mulberry seed meal was 76.73% at 60 °C and the protein extraction rate was 80.00% at 70 °C, Therefore, based on the maximum extraction rate, 70 °C can be selected, and 60 °C can be selected based on green environmental protection. The high temperature of 80 °C will affect the characteristics of the protein itself, so it is not used as an experimental object. Fig.1d shows the best output power was 300W for degreased mulberry seed peal and 600W for degreased silkworm pupae, respectively. The increase of protein yield with increasing output power intensity is attributed to a stronger cavitation effect due to a slight increase in collapse pressure, and more cavitation active volume gains over time. But as power increases, the yield of protein increases to a threshold and then decreases [13].

Studies have shown that compared with traditional protein extraction methods, ultrasonic treatment is conducive to disintegration of protein and the formation of protein solution, while ultrasonic treatment is relatively inexpensive and efficient [14].

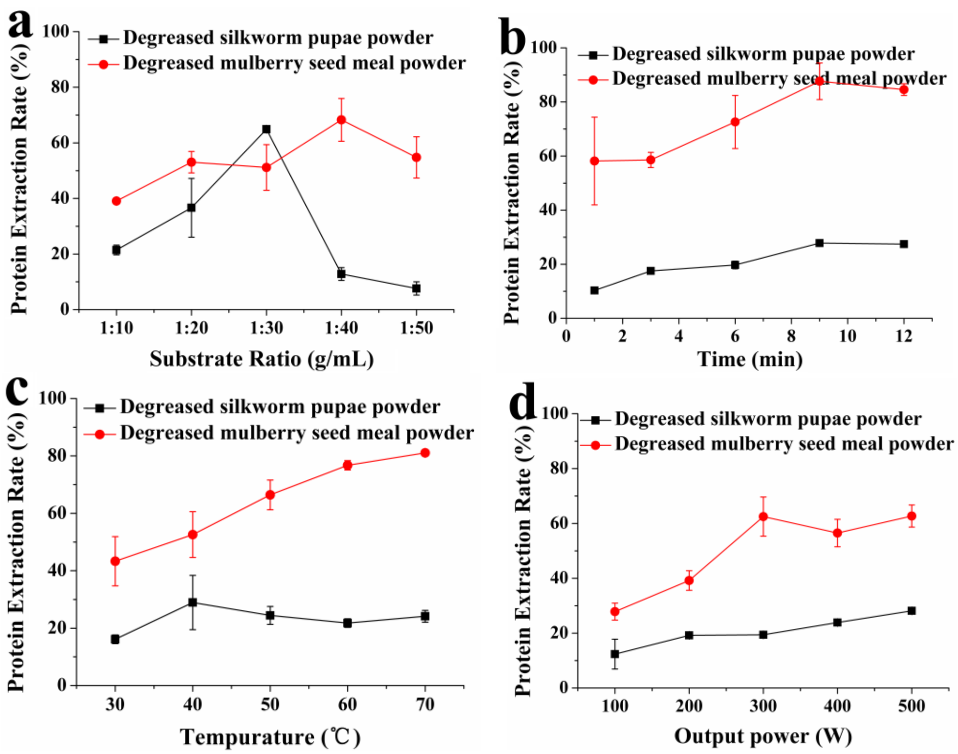

Fig. 1. Effects of different factors on extraction rate of silkworm pupae protein and mulberry seed meal protein by ultrasonic treated. (a different concentrations of substrate; b different treated time; c different treated temperature; d different output power). All data are means of three replicates; vertical bars represent error bars with the value equal to the standard error of the mean.

Effects of different factors on protein extraction rate by microwave treated

Microwave has the characteristics of uniform heating, quick heating speed and simple operation, and has been widely used in various fields in recent years. Protein can be traced to the mechanism of action of microwave processing of microwave dielectric heating effect and the electromagnetic polarization effect, on the one hand, in the microwave field protein polar groups through mutual friction, collisions produce large amounts of heat, make the protein temperature rise, resulting in protein structure and the physical and chemical properties change, on the other hand, the existence of microwave photonic energy will affect the chemical bonds in proteins and groups of electron cloud around configuration, so that the protein conformation change [8].

Fig.2 shows the two protein extraction rates under different substrate ratios (Fig.2a), different processing times (Fig.2b), different temperatures (Fig.2c), and different output powers (Fig.2d), respectively. From the microwave processing results, it was concluded that the optimal process conditions for degreased silkworm pupae were substrate ratio 1:10, treatment time 3 min, temperature 50 °C, output power 200 W; and the conditions for degreased mulberry seed peal were substrate ratio 1:30, treatment time 3 min, temperature 60 °C, output power 300 W.

By comparing the extraction rate of protein after ultrasonic treatment and microwave treatment, it can be seen that the extraction amount of raw protein after ultrasonic treatment is generally higher than that after microwave treatment. The amount of protein extracted from degreased silkworm pupae and degreased mulberry seed meal by ultrasound treatment was about 20% and 60%, respectively; but which were less than 5% and approximately 16% treated by microwave. The purpose of pretreatment was to improve the extraction rate of protein and improve the physicochemical properties of protein on a certain basis. Based on this, this study believes that ultrasound was more conducive to the extraction of proteins from the two raw materials. Therefore, this study validated the ultrasonic treatment conditions using the response surface method.

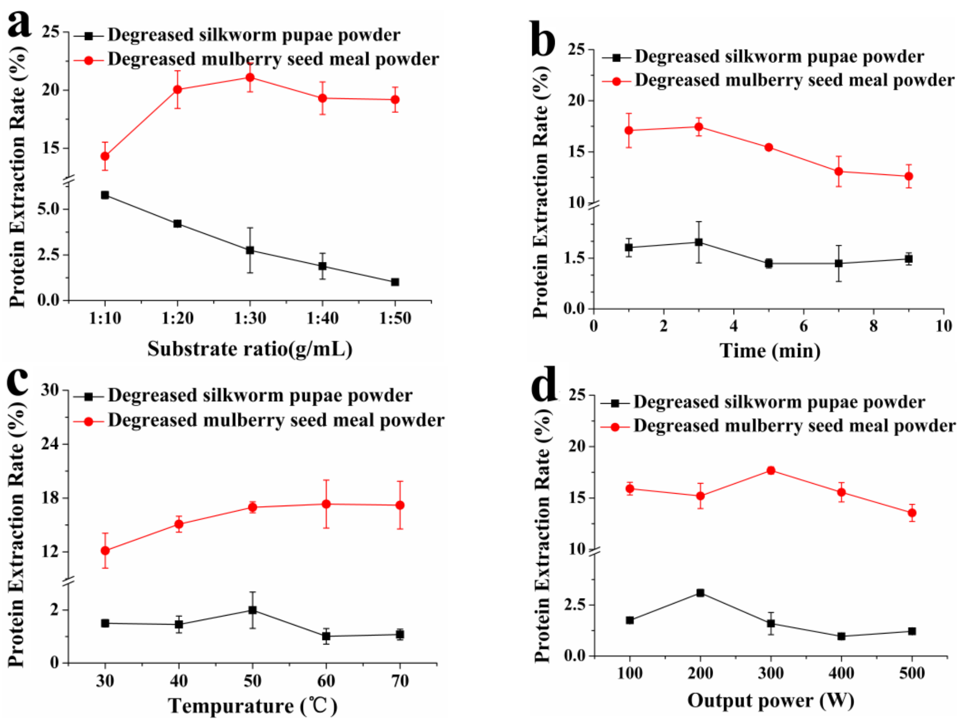

Fig. 2. Effects of different factors on extraction rate of silkworm pupae protein and mulberry seed meal protein by microwave treated. (a different concentrations of substrate; b different treated time; c different treated temperature; d different output power). All data are means of three replicates; vertical bars represent error bars with the value equal to the standard error of the mean.

Response surface optimization ultrasonic processing conditions

Response surface optimization was carried out according to the processing conditions obtained from the single-factor experiment to check whether the experimental conditions were appropriate as shown in Fig.3 and Fig.4, and the corresponding regression model equation (9) and (10) was obtained by the response surface fitting, Where A, B, C and D were the factors of each factor. It can be seen from Table 1 that the two response surface regression models are extremely significant ($p<0.01$), and the lack of fit were not significant ($p>0.05$), indicated that it was more appropriate to use model analysis and predict the effects of different processing conditions on protein extraction rate [15].

$$Y(\%) = 28.72 + 0.084\times A + 0.14\times B - 0.080\times C + 0.028\times D - 0.26\times A\times B + 0.29\times A\times C + 0.58\times A\times D - 0.25\times B\times C - 0.62\times B \times D + 0.34\times C\times D - 2.20\times A^2 - 2.08\times B^2 - 2.45\times C^2 - 1.91\times D^2 \quad (9)$$

$$Y(\%) = 77.06 + 0.50\times A + 0.56\times B + 0.34\times C - 0.39\times D + 0.37\times A\times B + 0.91\times A\times C - 0.043\times A\times D + 0.37\times B\times C - 1.24\times B \times D - 0.29\times C\times D - 4.18\times A^2 - 4.06\times B^2 - 4.43\times C^2 - 3.89\times D^2 \quad (10)$$

Table 1 Correlation parameters of ultrasonic processing regression model in response surface fitting.

Source	Sum of Squares	df	Mean Square	F Valve	p-value	
Model (Silkworm pupae)	380.68	14	27.19	15.84	<0.001	significant
Lack of fit	23.11	10	2.31	4.39	0.0581	Not significant
Model (Mulberry seed peal)	1384.16	14	98.87	11.29	<0.0001	significant
Lack of fit	51.07	10	5.11	0.32	0.9418	Not significant

As can be seen from the response surface diagram, with the constant increase of two factors, protein extraction firstly rose to a fixed value, and then presented a downward trend, according to the regression model of protein extraction rate and each factor, the quadratic term coefficient of each factor is negative, indicating that the parabolic opening is facing downward and the response value has an extreme value [16].

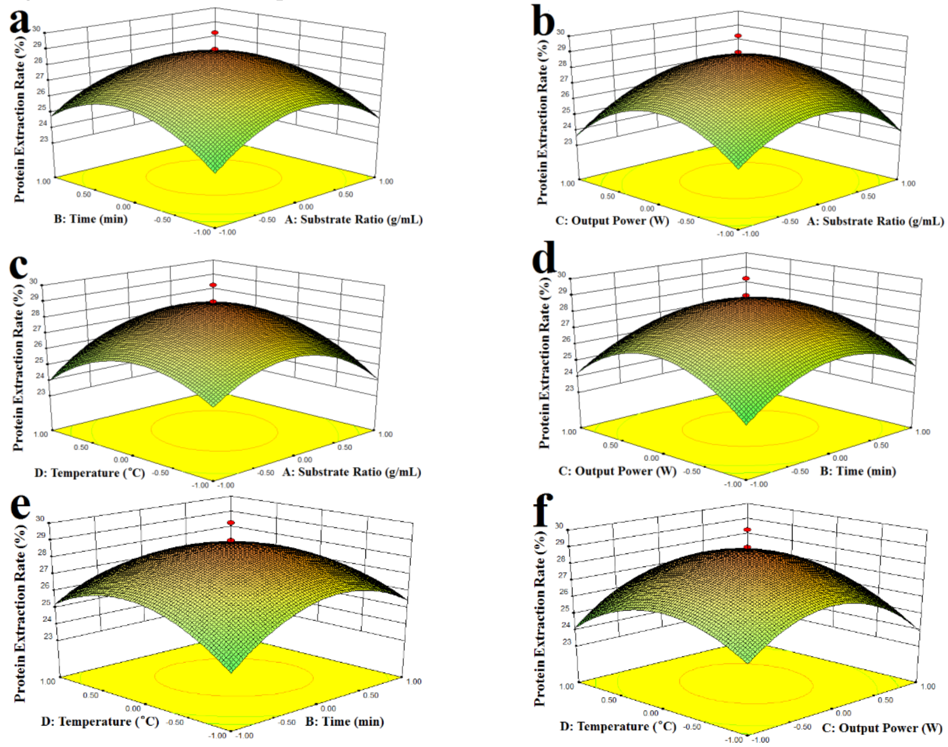

Fig. 3. Optimization of response surface for ultrasonic treatment of degreased silkworm pupae.

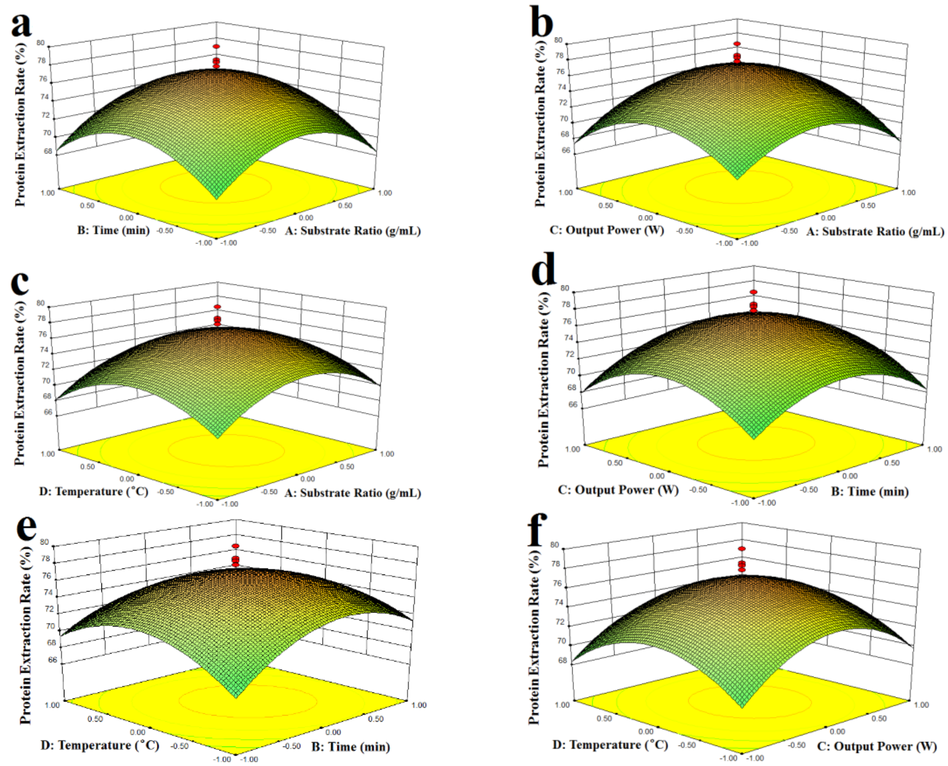

Fig. 4. Optimization of response surface for ultrasonic treatment of degreased mulberry seed peal.

The experimental results shown that the extraction rate of protein obtained under the above treatment conditions was the highest, the extraction rate of silkworm pupae protein was between 27-28%, and the extraction rate of mulberry seed protein was between 75-77%. Therefore, the results of single factor optimization can be used as a processing condition for efficient extraction of proteins

Effects of ultrasound and microwave on protein properties

The physical and chemical properties of the two proteins treated by ultrasound and microwave were tested. The results showed that the solubility, emulsifying properties and most of the properties of the protein after ultrasound and microwave treatment were improved, compared with the untreated protein (Table 2 and 3). These changes indicate that ultrasound and microwave treatment may induce molecular unfolding of protein by destroying hydrophobic interactions of the protein molecules, resulting in exposure of more hydrophobic groups [13].

Table 2 Effect of ultrasonic and microwave on physicochemical properties of mulberry seed meal protein.

Functional Properties	Mulberry seed meal protein	Protein treated by ultrasonic	Protein treated by microwave
Content of crude protein (%)	40.7±0.8	-	-
Solubility (%)	25.2±1.31	30.89±2.62	28.33±1.11
Foaming capacity (%)	20.00±4.3	27.63±2.31	22.2±4.0
Foam stability (%)	45.81±8.4	50.31±3.45	48.17±3.3
Emulsibility (m^2/g)	83.35±10.8	100.82±2.38	90.0±2.5
Emulsion stability (min)	18.46±1.05	20.03±4.12	18.59±1.2
Holding oil capacity (%)	220.67±13.1	280.67±8.77	234.27±1.0
Water retention (%)	123.12±0.89	151.35±1.23	130.45±2.2

Solubility depends on the level of protein denaturation and aggregation of the samples, the heat produced in the process of ultrasound and microwave treatment denatures the protein and increases its solubility; the foaming properties of protein were influenced by protein concentration, pH, high pressure, thermal treatment, foam procedure, by their nature and behavior at interfaces (denaturation, protein–protein interactions) and by their interactions with other ingredients [17], The improved stability of protein foam makes it a prerequisite for foaming agents.

Proteins in O/W emulsions form an interfacial protein film around oil droplets that influences the steric and electrostatic properties of the repulsive forces between lipid droplets, thus preventing flocculation and coalescence mechanisms that lead to creaming and syneresis, one factor that affect emulsification properties are protein solubility [18]. The physicochemical properties of the treated protein were improved, indicating that the treated protein possessed the functional properties of food protein.

Table 3 Effect of ultrasonic and microwave on physicochemical properties of silkworm pupae protein.

Functional Properties	Silkworm pupae protein	Protein treated by ultrasonic	Protein treated by microwave
Content of crude protein (%)	70.7±0.9	-	-
Solubility (%)	4.46±0.23	9.23±1.12	7.93±0.34
Foaming capacity (%)	21.50±1.12	47.38±1.21	42.15±1.12
Foam stability (%)	32.00±1.45	55.19±2.38	52.18±2.32
Emulsibility (m^2/g)	79.49±4.87	88.19±4.12	82.34±5.12
Emulsion stability (min)	33.21±1.77	57.11±2.25	41.20±2.23
Holding oil capacity (%)	130.67±13.1	250.67±8.77	234.27±1.01
Water retention (%)	138.12±0.89	180.35±2.12	140.45±3.20

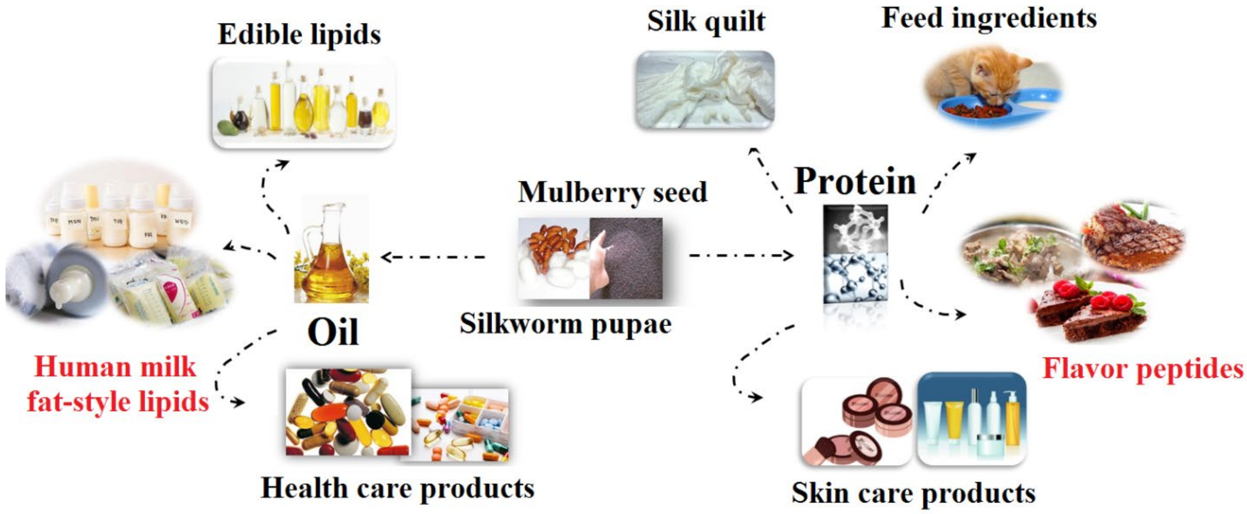

Fig. 5. Oils and proteins obtained from silkworm pupae and mulberry seed are used in many fields.

The oils and proteins obtained from the two substances are widely used in many fields. For example, the proteins can be used to prepare green films for packaged foods [14], silkworm pupae oil can be used to prepare breast milk-style fat substitutes and nutritionally balanced structural lipids [19], as well as produce health-care products, such as α-linolenic acid capsules already available on the market, produce protein skin care products, and novel taste peptides can be prepared or bioactive peptides for blood pressure [20], silkworm pupae protein also has anti-tumor effect and can prepare vaccine [21], high-grade feeds with extremely high protein content can be prepared [22].

Conclusions

Protein modification was achieved by ultrasound and microwave treatment, the solubility of silkworm pupae protein was more than doubled, and the solubility of mulberry seed protein was increased by 22.58%. At the same time, the protein extraction rate of degreased silkworm pupae and degreased mulberry seed peal also increased, up to 28% and 77%, respectively. The degree of proteolysis after modification will increase, making the protein easier to use, such as prepare high protein feed and high quality food additives.

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