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REVIEW

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The ultrasonic-assisted enzymatic extraction, components and activities of vegetable oils

Shiyu Huang and Gangliang Huang *

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As a green extraction technology of vegetable oil, aqueous enzymatic extraction has the characteristics of simple operation, mild extraction conditions and excellent oil quality. However, there are some problems in the aqueous enzymatic extraction process of oil, such as long enzymolysis time and a large amount of enzyme preparation, which restrict its industrial application, and the application of ultrasonic-assisted technology can effectively improve the efficiency of aqueous enzymatic extraction of oil. Herein, the mechanism of ultrasonic-assisted aqueous enzymatic extraction of vegetable oils was elaborated in detail and the application of ultrasonic-assisted technology in destroying the plant cell structure, improving the enzyme effect and promoting emulsion breaking by the aqueous enzymatic method was emphatically summarized. The bioactive components in vegetable oil and their health benefits were summarized and analyzed to provide a reference for human health.

Sustainability spotlight

What is the situation and why is it important to address/understand this? The ultrasonic-assisted aqueous enzymatic method is a green vegetable oil extraction method which combines the aqueous enzymatic method and ultrasonic technology. This technology has been widely used in scientific research and industrial application in recent years. It is green and safe, can retain nutrients and active ingredients, improve the utilization rate of resources, can meet the needs of food safety and health, and promote the industrialization of non-thermal processing technology. What is the sustainable advancement of the work? The sustainable development of ultrasonic-assisted aqueous enzymatic extraction of vegetable oil is mainly reflected in its core advantages such as greenness, high efficiency, low energy consumption and high-value utilization. How does the work align with the UN SDG(s) (https://sdgs.un.org/goals)? Ultrasonic-assisted aqueous enzymatic extraction is not only an advanced oil extraction technology, but also an important tool to realize green manufacturing and a circular economy, which fully echoes the core concept of the United Nations 2030 Agenda for Sustainable Development.

1. Introduction

Vegetable oil is an important energy-supplying substance to maintain the normal physiological activities of the body, and it is rich in fatty acids, sterols, tocopherols and other active substances needed by the human body. Vegetable oil is widely used in food, feed, cosmetics and other fields because of its positive effects in improving immunity and metabolism and anti-inflammatory and anti-oxidation properties. At present, the preparation methods of vegetable oil mainly adopt the pressing method, leaching method and pre-pressing and leaching method. Although there is no solvent residue in the product of the press method, the residual oil in the cake is high and the energy consumption is high, and the excessive friction produces a lot of heat, which easily denatures proteins. The residual oil in the oil extracted by the leaching method and pre-pressing-leaching method is low, but there are solvent residues in the oil, and the quality of crude oil is poor, which requires strict refining. At the same time, there are environmental pollution and production safety problems. With the increasing demand of consumers for high-quality edible oils, aqueous enzymatic extraction technology came into being.[1–3]

Herein, the mechanism of ultrasonic-assisted aqueous enzymatic extraction of vegetable oil was introduced in detail and the research progress of ultrasonic-assisted aqueous enzymatic extraction of vegetable oil and its influence on oil quality was summarized. The existing problems and development direction of ultrasonic-assisted aqueous enzymatic extraction of vegetable oil were summarized and discussed. At the same time, the components and biological activities of vegetable oil were summarized and analyzed.

2. Mechanism of ultrasonic-assisted aqueous enzymatic extraction of vegetable oils

The initial stage of the aqueous enzymatic method is called the aqueous method, which separates oil and hydrophilic components with the help of water. The aqueous method causes little damage to the quality of vegetable oil, but the extraction rate is

Key Laboratory of Carbohydrate Science and Engineering, Chongqing Normal University, Chongqing 401331, China. E-mail: huangdoctor226@163.com

low, which makes it difficult to adapt it to industrial production and market demand. Since the 1970s, some scholars have combined the aqueous method with the enzymatic method, which effectively improved the efficiency of oil extraction. Compared with traditional oil extraction methods, the aqueous enzymatic method has obvious advantages, simple operation and high safety, and the prepared vegetable oil has better quality.[4,5] However, there are some limitations of the aqueous enzymatic method, which restrict its popularization and development, such as long enzymolysis time (2–5.5 h) and large dosage of enzyme preparation (1.25–3.5%).[5–7] At present, researchers have combined the water enzymatic method with various physical and chemical auxiliary technologies such as heat treatment, microwave, extrusion and solvent extraction, and the synergistic effect is remarkable, but the application effect is different.[8] The comparative study shows that ultrasonic-assisted technology has the advantages of higher efficiency and environmental protection. The unique physical effect of ultrasonication can destroy the cell structure and oil–fat complex of plant oil and improve the enzyme activity, and at the same time, ultrasonic-assisted technology can be applied to the demulsification process of the aqueous enzymatic method, reducing the interfacial stability of emulsion.[9–11] Ultrasonic-assisted technology can not only reduce the enzyme dosage, shorten the enzymolysis time, and significantly improve the demulsification efficiency, but also reduce the oxidation loss of fatty acids and bioactive substances in oils and fats caused by high temperature, high pressure or solvent residues and better retain beneficial components.[8,9,12–14]

The aqueous enzymatic method is a green and efficient extraction method to release oil by adding pectinase, cellulase, hemicellulase, protease and other biological enzymes to destroy oil cell walls or hydrolyze macromolecular complexes[15] such as lipoproteins and lipopolysaccharides. It mainly uses the differences in water affinity and density of oil and non-oil components (proteins and carbohydrates) to achieve three-phase separation and synchronously separates oil from non-oil components.

Ultrasonic-assisted technology plays three main roles in the application of the aqueous enzymatic method. Ultrasound generates extreme high temperature, high pressure and high shear force by using mechanical effects such as micro-jets caused by periodic oscillation and collapse of cavitation bubbles. On the one hand, it acts on plant cell walls, which can enhance the heat and mass transfer effects in the process of plant oil separation and extraction, enhance the permeability of plant tissues, better destroy the structure of oilseed cells and cell walls, and promote the release of cell contents;[9,16] Secondly, physical effects such as the thermal effect and cavitation effect produced by ultrasound make the enzyme disperse more evenly in the reaction system, which increases the contact area between the enzyme and the substrate, and the collapse of cavitation bubbles can destroy the weak interaction within the enzyme molecule, change the structure and activity of the enzyme, and improve the efficiency of oil extraction by the aqueous enzymatic method;[10,11] In addition, ultrasound can be used in the demulsification of aqueous enzymatic emulsions. The ultrasonic mechanical action drives the collision of oil bodies in the emulsion, and the oil droplets aggregate and the average particle size increases, which is beneficial for breaking the interfacial film, reducing the stability of the emulsion and improving the demulsification efficiency.[17,18]

3. Ultrasonic-assisted aqueous enzymatic extraction of vegetable oil

3.1 Study on the effect of ultrasound on the cell wall of oilseeds

The structure of plant oilseed cells mainly includes a cell wall and an intracellular protoplast. The cell wall is composed of polysaccharides such as pectin, cellulose and hemicellulose, which can maintain cell stability and prevent intracellular components from spreading outward. The protoplasts of mature oilseed cells formed a complete lipid and protein structure. Taking peanut as an example,[19] the lipid bodies in peanut cells fill the whole cytoplasmic network and tightly wrap the protein bodies dispersed in it. These storage subcellular organs are constantly developed and formed, which promotes the accumulation of a large number of lipids and proteins in the cells. Therefore, destroying the cell structure of plant oil is helpful for the release and extraction of effective components such as oil.

Researchers have conducted extensive research on ultrasonic damage to the structure of oil-bearing cells in plants, mainly using the cavitation effect to cause structural damage to oil-bearing cells, improve matrix porosity and accelerate the decomposition of cell walls.[20,21] At the same time, the periodic oscillation of cavitation bubbles increases the intracellular space of oil-bearing cells, improves the permeability of cell membranes[22] and promotes the outflow of intracellular dissolved substances. Zhang *et al.*[23] studied the ultrasonic-assisted extraction of papaya seed oil, and the results showed that the extraction rate of papaya seed oil increased from 25.27% to 32.27% after ultrasonic treatment. By observing the microstructure of oil seeds, it was found that ultrasonic cavitation aggravated the collision between particles in the extraction process, which caused serious damage to the cell membrane and cell wall of papaya seed, leading to cell disintegration, thus making the oil easier to be released from cells.

The combination of ultrasonic technology and aqueous enzymatic oil extraction has a synergistic effect on enzymatic hydrolysis of the cell wall, which can significantly improve the efficiency of oil extraction. Wang *et al.*[24] compared the effect of ultrasonic treatment on aqueous enzymatic extraction of gardenia fruit oil and found that after ultrasonic treatment, the oil yield increased from 13.68% to 17.9%, the pores and gaps of gardenia fruit enzymatic residues became larger and the surface became rougher. These morphological changes also indicated that the cell structure was further destroyed which promoted the release of intracellular lipids. Our research group studied the changes in cell characteristics in the process of extracting peanut oil by ultrasonic coupling enzyme treatment and found that compared with enzymatic treatment and ultrasonic treatment, polysaccharides such as pectin, cellulose and hemicellulose in the cell wall were depolymerized at different degrees after ultrasonic coupling enzyme treatment, which led to the greater fragmentation of the cell wall and a decrease in the grease residue in the ultrasonic coupling enzymatic hydrolysis residue, indicating that the oil was fully released from the cells after ultrasonic treatment. To sum up, the application of ultrasound in the process of aqueous enzymatic extraction of plant oil can aggravate the destruction of the cell barrier, accelerate the degradation of cell wall polysaccharides, fully release oil and effectively improve the efficiency of oil extraction.

3.2 Study on the effect of ultrasound on enzyme characteristics

Oil plant cell walls are mainly composed of polysaccharides such as pectin, cellulose and hemicellulose. Under the action of cell wall-breaking enzymes such as cellulase, hemicellulase and pectinase, cell wall polysaccharides are gradually hydrolyzed, and the cell structure is destroyed. At the same time, protein hydrolases such as neutral protease and alkaline protease hydrolyze the oil complex, breaking its stability, thus promoting the release of oil.[12,24–26] Therefore, the effect of enzyme is highly related to the extraction efficiency of the aqueous enzymatic method. Studies have shown that in the process of ultrasonic-assisted aqueous enzymatic extraction of vegetable oils, the cavitation effect and mechanical effect of ultrasound can not only break the cell structure, but also improve the effect of enzymes and affect the extraction efficiency of aqueous enzymatic extraction.[27]

The mechanical effect of ultrasonic treatment can improve the dispersion of enzyme polymers, increase the contact area between the enzyme and substrate, improve the reaction rate and shorten the reaction time;[27,28] At the same time, the mechanical effect produced by ultrasound directly acts on the enzyme molecules, driving weak interactions such as hydrogen bonds and van der Waals forces to change their secondary and tertiary structures,[29] thus improving the activity and stability of the enzyme.[27] Li *et al.*[30] found in the research of ultrasonic-assisted cellulase hydrolysis of rice bran that at an ultrasonic power density of 1.67 W cm^{-3} for 30 min, the specificity of cellulase to cellulose was enhanced, the catalytic activity and efficiency were significantly improved, and ultrasound promoted the diffusion of cellulase in the hydrolysis system. Ma *et al.*[31] studied the mechanism of ultrasonic modification of pectinase, and the results showed that the ultrasonic mechanical effect was the most important factor for the increase of enzyme activity, and the pectinase activity was the highest when the ultrasonic power density was 4.50 W mL^{-1} and the time was 15 min. At the same time, the effects of ultrasonic treatment on pectinase with different concentrations were compared, and it was found that the pectinase activity at concentrations of 0.1, 1.0 and 10.0 mg mL^{-1} was increased by 68.24% and 20.98% respectively.

The application of ultrasonic-assisted technology in the process of aqueous enzymatic oil extraction can not only improve the efficiency of oil extraction, but also effectively reduce the enzymolysis time and enzyme dosage. Goula *et al.*[9] studied the ultrasonic-assisted enzymatic extraction of pomegranate seed oil and found that the enzyme–substrate interaction in the reaction system was effectively improved by adding ultrasonic treatment, which increased the extraction rate of pomegranate seed oil by 18.4% and shortened the extraction time by 91.7%. At the same time, due to the improvement of the action effect in the reaction system, the amount of enzyme is correspondingly reduced and the cost is reduced. Al Loman *et al.*[32] found that the yield of soybean oil could be increased from 70% to 81–87% by pulse ultrasonic treatment under the condition that the enzyme dosage was 2 mL g^{-1}, but the group without ultrasonic treatment needed to add about 2–4 times the enzyme to achieve the same level of extraction rate. However, the physical effect and thermal effect caused by excessive ultrasound may lead to denaturation of the enzyme[27] and reduce the enzymatic hydrolysis effect. Therefore, the optimization of ultrasonic technology is very important. The researchers optimized the ultrasonic-assisted aqueous enzymatic extraction process of the oil from *Erythrina cochinchinensis* and found that at the same reaction volume, when the ultrasonic power was increased within 30–60 W, the oil extraction rate increased significantly, but after 60 W, the oil extraction rate of *Erythrina cochinchinensis* would decrease if the ultrasonic power continued to increase.[33] Too high ultrasonic power will affect the expansion and collapse of bubbles and reduce the cavitation effect, and too high ultrasonic power will lead to the decrease or even inactivation of enzyme activity, thus affecting the efficiency of oil extraction. Therefore, analyzing the properties of the enzyme and optimizing the ultrasonic parameters can maximize the biocatalytic potential of the enzyme and improve the extraction rate of oil by aqueous enzymatic extraction.

When extracting vegetable oil, the effect of ultrasonic waves on enzyme is mainly reflected in enhanced enzymatic hydrolysis efficiency through physical action, but its effect is regulated by key factors such as particle size, enzyme ratio and enzyme selection.

3.2.1. The influence of particle size. The smaller the particle size, the higher the extraction rate, but there is a critical point. Ultrafine pulverization (such as with an 80 mesh sieve) can significantly increase the cell fragmentation rate, which is beneficial for the action of enzymes and ultrasound. Too fine (>80 mesh) may lead to an increase in viscosity and emulsification of feed liquid, but reduce the efficiency of oil separation. Ultrasound can further destroy the cell structure, especially in particles with small size, and the synergistic effect is more obvious, which promotes the release of oil.

3.2.2. Effect of enzyme ratio. The proportion of compound enzymes needs to be optimized, and different enzymes work synergistically. Cellulase destroys the cell wall and protease degrades the lipoprotein complex, and the two cooperate to improve the oil yield. The study of wheat germ showed that the oil yield was the highest when cellulase : protease = 1 : 5. In the extraction of rice germ oil, the addition of complex enzyme reached saturation at 1.2%, and the higher proportion did not increase significantly. Ultrasonic waves can enhance the contact between enzyme and the substrate and further improve the reaction rate at the optimal enzyme ratio.

3.2.3. Effect of enzyme selection. The types of enzymes directly affect the extraction efficiency. In the study of camellia seed oil, Alcalase 2.4L (alkaline protease) has the best effect, which is better than that of cellulase, Viscozyme L and so on. In the extraction of peanut oil, Alcalase 2.4L and cellulase + pectinase composite system all showed high efficiency. Ultrasound may change the conformation of the enzyme and enhance its activity under some conditions, but excessive ultrasound may also lead to enzyme inactivation.

Ultrasonic waves are mainly used as pretreatment or synergistic means, and their cavitation effect, shearing force and micro-stirring can accelerate enzymolysis and improve mass transfer efficiency, but it is necessary to avoid enzyme denaturation or the emulsification reaction caused by long time or high power.

When extracting vegetable oil, ultrasonic waves can improve the efficiency of enzyme, mainly through the following mechanisms. Destruction of the cell wall structure: the cavitation effect (tiny bubbles burst violently) and mechanical shear force produced by ultrasonic waves can effectively destroy the plant cell wall, making the oil in cells more accessible to enzymes, thus improving the efficiency of enzymatic hydrolysis. Enhancing mass transfer: ultrasound promotes the mixing between solvent and the substrate, reduces the diffusion boundary layer and accelerates the contact rate between enzyme and the substrate. Improving the combination of enzyme and substrate: moderate ultrasonic treatment can partially expose cell contents (such as oil), increase the action site of enzyme and improve hydrolysis efficiency. Ultrasonic power has a double effect on enzyme activity: low to medium power is usually beneficial, while too high power may damage the enzyme structure. 100–400 W: in this range, ultrasonic waves significantly improved the oil extraction rate, and the enzyme activity remained at a high level. For example, in the extraction of rice germ oil, the extraction rate reached the peak (88.3%) at 400 W and the enzymatic hydrolysis was still effective. Suggestion on power density: if laboratory ultrasonic equipment is used, 20–40% amplitude (corresponding to medium and low power) is often used to protect enzyme activity. For example, when peroxidase is extracted, 40% amplitude can retain more than 90% enzyme activity. >500 W: it may lead to excessive local temperature and severe cavitation, resulting in denaturation and inactivation of enzymes or destruction of the protein structure. In the study of rice germ oil, the extraction rate decreased significantly at 500 W, suggesting that the enzyme or substrate may be damaged. Long-term high-power continuous treatment: even if the power does not exceed the standard, the continuous action may inhibit the enzyme activity due to local high temperature or free radical generation. Ultrasonic waves are often combined with the water enzymatic method. In this kind of process, the concentration of enzyme is usually between 2% and 5%, depending on raw materials and enzyme types. In palm oil extraction, the extraction rate reached 34.62% when cellulase was used and the amount was 4.2% (relative to the material quality), combined with ultrasonic assistance. In the extraction of gourd seed oil, the amount of enzyme was 2.5% and the extraction rate was increased to 88.5% with ultrasonic pretreatment (55 °C, 500 W, 6 min). In the extraction of kiwifruit seed oil, the enzyme dosage was 2.50%, the ultrasonic power was 400 W, and the extraction rate was 92.57%.

3.3 Study on the effect of ultrasound on emulsion demulsification by the aqueous enzymatic method

In the process of extracting vegetable oil by the aqueous enzymatic method, the oil is mechanically crushed and phospholipids in the oil body combine with natural hydrophobic proteins such as oil body proteins, oil body troponin and oil body sterol proteins to form an interfacial film, and oil and protein form a stable water–oil system in emulsion, which affects the separation of oil. Solving the problem of difficult demulsification is an important link to promote the industrial application of the aqueous enzymatic method. Common demulsification methods include physical demulsification, chemical demulsification, enzymatic demulsification and composite demulsification.[13,17,34–37]

Ultrasonic demulsification mainly uses its mechanical action, cavitation effect and thermal effect to change the structural characteristics of proteins,[38] which leads to a change in interaction and binding ability between protein and oil, thus reducing the stability of emulsion[18] and promoting the aggregation of oil droplets. Lu *et al.*[17] found that ultrasonic pretreatment with isopropanol not only effectively reduced the formation of emulsion from 5.3% to 2.03%, but also significantly increased the oil recovery rate from 45.62% to 52.48%.

In the process of ultrasonic demulsification of aqueous enzymatic emulsions, ultrasonic power, ultrasonic temperature and ultrasonic time will all affect the yield of vegetable oil. Li *et al.*[37] used ethanol and ultrasound to demulsify the emulsion for the first time, and the recovery rate of soybean oil could reach 92.6% 3.4% after optimizing the ultrasonic process. Hu and Huang *et al.*[13] found that the demulsification rate increased from 83.93% 2.23% to 96.30% 1.20% when the emulsion volume fraction was 60%, the ultrasonic power was 400 W, the temperature was 40 °C and the time was 30 s. At the same time, the study also shows that the effect of ultrasonic-assisted microwave demulsification is significantly improved with the increase of emulsion volume fraction, and the volume fraction reaches the highest at 60%. If the volume fraction continues to increase, the demulsification effect will be reduced.

Ultrasonic technology has potential application value in aqueous enzymatic demulsification, but the demulsification effect depends on the precise control of the critical value of ultrasonic parameters. Below the critical value, the increase in ultrasonic intensity is beneficial for emulsion demulsification, while above the critical value, the emulsification degree of emulsion deepens,[39] which reduces the oil extraction rate. At present, most of the research focuses on the laboratory scale, and there are great differences in reaction volume, material flow and heat and mass transfer between the laboratory and large-scale production[40] (Fig. 1). Ultrasonic-assisted oil recovery

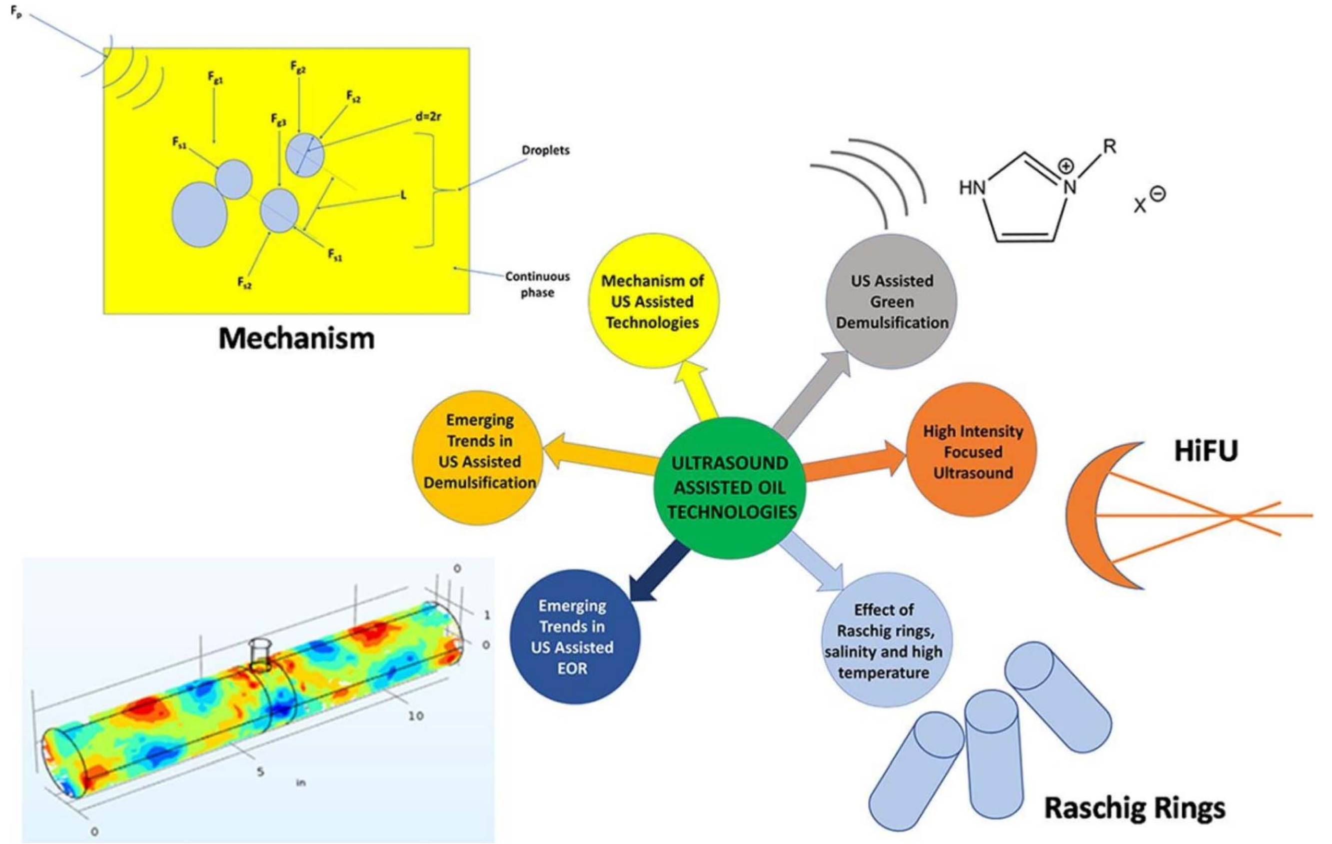

Fig. 1 The developments in the ultrasound (US)-assisted oil technologies.

technology is a physical method that uses high-frequency sound waves (usually above 20 kHz) to improve the seepage conditions of oil layers and improve the oil recovery and belongs to the category of tertiary oil recovery. This technology realizes the effects of plugging removal, viscosity reduction, wax prevention and scale prevention through mechanical vibration and the cavitation effect and thermal action and has the advantages of greenness, low energy consumption and no damage to the formation. Therefore, researchers use the response surface method, artificial neural networks and other mathematical models to optimize the ultrasonic-assisted extraction process, which can effectively improve the extraction efficiency of the target object and predict the ultrasonic parameters.[41] According to the characteristics of oilseeds and enzymes, a prediction model of ultrasonic parameter control was constructed by collecting ultrasonic demulsification parameters for future research, so as to accurately control ultrasonic parameters and ensure the best extraction effect and product quality in industrial application.

4. Ultrasonic-assisted aqueous enzymatic extraction of vegetable oil

4.1 Analysis of physical and chemical characteristics of vegetable oils

The quality of oils is usually evaluated using the acid value, peroxide value and saponification value. The smaller the acid value and peroxide value, the greater the saponification value and the higher the quality of oil. Ultrasonic-assisted treatment can not only significantly improve the efficiency of aqueous enzymatic extraction of vegetable oil, but also improve the physical and chemical properties of oil. The physicochemical properties of oil extracted by the ultrasonic-assisted aqueous enzymatic method were studied, and the results are shown in Table 1.

As a pretreatment method, ultrasound is used in the process of extracting oil by the aqueous enzymatic method, which has a positive effect on the physical and chemical properties of vegetable oil extracted by the aqueous enzymatic method. Kumar *et al.*[12] extracted freeze-dried seabuckthorn fruit oil by ultrasonic pretreatment assisted by the aqueous enzymatic method and determined the optimal ultrasonic parameters through response surface methodology. It was found that the physicochemical properties of seabuckthorn fruit oil prepared by this process were better than those of the oil obtained by Soxhlet extraction. Zhang Yana *et al.*[42] compared the quality of sesame oil extracted by ultrasonic pretreatment, freezing-microwave thawing pretreatment assisted by the aqueous enzymatic method and the pressing method. The results showed that the acid value and peroxide value of sesame oil extracted by the ultrasonic-assisted aqueous enzymatic method were lower than those of the oil extracted by the other two methods. Although the author thinks that this is related to the fact that the alkaline enzymatic hydrolysis environment of this

Table 1 Physicochemical properties of ultrasonic-assisted aqueous enzymatic extraction of vegetable oil

Plant raw materials	Extraction method	Physicochemical properties	References
Sesame	Ultrasonic-assisted aqueous enzymatic method	The acid value was 0.13 ± 0.12 mg g^{-1} and the peroxide value was 0.43 ± 0.14 mmol kg^{-1}	42
	Lixiviation process	The acid value was 1.18 ± 0.15 mg g^{-1} and the peroxide value was 1.86 ± 0.16 mmol kg^{-1}	
Walnut	Ultrasonic-assisted aqueous enzymatic method	Meq per kg, the saponification value is 194.82 ± 0.92 mg KOH per g and iodine value is 103.34 ± 0.46 g I_2 per 100 g The acid value was 3.47 ± 0.05 mg KOH per g and the peroxide value was 2.07 ± 0.03	43
	Squeezing method	Meq per kg, the saponification value is 194.75 ± 1.25 mg KOH per g and iodine value is 103.84 ± 0.27 g I_2 per 100 g	
Zhangshuzi	Ultrasonic-assisted aqueous enzymatic method	The acid value is 0.52 ± 0.01 mg KOH per g and the saponification value is 273.19 ± 6.12 mg g^{-1} and the iodine value 2.97 ± 0.06 g I_2 per 100 g	26
	Lixiviation process	The acid value is 0.60 ± 0.02 mg KOH per g and the saponification value is 270.65 ± 5.88 mg g^{-1} and the iodine value 2.86 ± 0.07 I_2 per 100 g	
Idesia	Ultrasonic-assisted aqueous enzymatic method	The acid value was 2.42 ± 0.03 mg g^{-1} and the peroxide value was 0.02 ± 0.00 g per 100 g, the iodine value is 135.13 ± 0.36 g per 100 g and saponification value is 194.19 ± 0.65 mg g^{-1}	33
	Squeezing method	The acid value was 1.72 ± 0.02 mg g^{-1} and the peroxide value was 0.07 ± 0.00 g per 100 g, the iodine value is 129.61 ± 0.46 g per 100 g and saponification value is 184.40	
Seabuckthorn fruit	Ultrasonic-assisted aqueous enzymatic method	The acid value was 6.77 ± 0.305 mg KOH per g and the peroxide value was 4.33 ± 0.252 Meq per kg, the iodine value is 138.67 ± 3.512 g I_2 per 100 g	12
	Squeezing method	The acid value is 9.2 ± 0.104 mg KOH per g and the peroxide value is 6.4 ± 0.100 Meq per kg, the iodine value is 123.5 ± 2.291 g I_2 per 100 g	
Peanut	Ultrasonic-assisted aqueous enzymatic method	The acid value is 0.38 ± 0.04 mg g^{-1} and the peroxide value is 0.15 ± 0.00 g per 100 g	44
	Aqueous enzymatic method	The acid value is 0.33 ± 0.04 mg g^{-1} and the peroxide value is 0.10 ± 0.01 g per 100 g	

process can neutralize free fatty acids to a certain extent, the acid value of oil is low, but subsequent studies have carried out enzymatic hydrolysis in acidic and neutral environments,[26,43] and the results also show that the oil prepared by ultrasound has good quality, which is consistent with the previous research results, verifying that ultrasonic pretreatment can improve the physical and chemical properties of oil extracted by the aqueous enzymatic method.

In addition, ultrasound is often used as a synchronous treatment method to assist aqueous enzymatic extraction of vegetable oils. Researchers[33] comparatively studied the acid value, peroxide value, iodine value and saponification value of tung oil extracted by ultrasonic synchronous enzymolysis and hot pressing and found that ultrasonic-assisted extraction avoided the high-temperature extraction environment and reduced the degree of oil oxidation and rancidity and the iodine value and saponification value of oil were higher. Dong Yifan *et al.*[44] found that the acid value and peroxide value of peanut oil extracted by the ultrasonic-assisted enzymatic method were higher than those of the oil extracted by the aqueous enzymatic method, presumably because the extraction conditions were milder than those employed in the aqueous enzymatic method, adding that ultrasonic action would generate instantaneous high temperature and promote oil oxidation.

4.2 Analysis of composition characteristics of vegetable oils

Fatty acid is an important component of oil and an important index to evaluate the quality of vegetable oil. Fatty acids include saturated fatty acids and unsaturated fatty acids, among which unsaturated fatty acids have physiological functions such as lowering blood pressure, reducing blood lipids, preventing cardiovascular diseases and preventing cancer.[45]

Compared with aqueous enzymatic extraction, squeezing extraction and solvent extraction, ultrasonic-assisted aqueous enzymatic extraction is more green and efficient, which helps retain sensitive components such as polyunsaturated fatty acids in oils. Wang Yuhan *et al.*[46] studied the quality of ultrasonic-assisted aqueous enzymatic extraction of blackcurrant seed oil. The results showed that the oil prepared by ultrasonic-assisted aqueous enzymatic extraction was low in trans fatty acids and saturated fats and rich in ω-3 linolenic acid (33.38%) which was beneficial for the human body. Wei *et al.*[26]

comparatively analyzed the fatty acid composition of camphor tree seed oil extracted by the ultrasonic-assisted aqueous enzymatic method and solvent extraction, and the results showed that the saturated fatty acid content of camphor tree seed oil extracted by the ultrasonic-assisted aqueous enzymatic method was low and the monounsaturated fatty acid content was high. Long *et al.*[47] also found that the content of unsaturated fatty acids in linseed oil extracted by the ultrasonic-assisted aqueous enzymatic method was 1.5% higher than that in the oil extracted using organic solvent. Wang Jin *et al.*[48] compared the extraction of walnut oil by the ultrasonic-assisted aqueous enzymatic method, mechanical pressing and supercritical fluid extraction and found that the oil prepared by the ultrasonic-assisted enzymatic method was unsaturated. The fatty acid content can reach more than 90%, which is significantly higher than that obtained by the other two processes. To sum up, ultrasonic-assisted aqueous enzymatic extraction is more conducive to the extraction of high-quality vegetable oils, and the composition and content of fatty acids in the extracted vegetable oils are more in line with human needs.

4.3 Analysis of bioactive components of vegetable oils

Plant oil is rich in many bioactive components, such as phytosterols, tocopherol, total phenols and squalene, which have antioxidant, anti-inflammatory and immune enhancement effects.[49]

It is considered that the ultrasonic-assisted aqueous enzymatic extraction conditions are mild, so the loss of bioactive components in oil is reduced.[48] At the same time, ultrasound can reduce the degree of complexation between bioactive substances and intracellular proteins and polysaccharides and promote the release of bioactive components.[43,44] Li *et al.*[50] used ultrasonic pretreatment combined with a step-by-step enzymatic method to extract gardenia fruit oil, which not only optimized the enzymatic hydrolysis system, but also yielded the oil with much higher contents of tocopherol and squalene than the oil extracted by traditional solvent extraction and pressing method. Liu *et al.*[43] evaluated the quality of walnut oil extracted by mechanical pressing and ultrasonic-assisted aqueous enzymatic extraction, and the results showed that the content of total phenols, squalene and phytosterols in the oil prepared by ultrasonic-assisted aqueous enzymatic extraction was high. Some researchers compared the bioactive components extracted by the ultrasonic-assisted aqueous enzymatic method with those extracted by the traditional aqueous enzymatic method and also found that the content of antioxidant active substances extracted by the ultrasonic-assisted aqueous enzymatic method was higher.[46] In the study of Chen *et al.*,[14] it was found that the total phenol content in coconut skin oil extracted by the ultrasonic-assisted aqueous enzymatic method was higher, which showed better antioxidant activity. It was proved that this was because ultrasound effectively destroyed the cell structure of coconut skin and promoted the release of cell contents during the extraction process. In addition, the thermal effect of ultrasound affects the quality of oil, and too strong ultrasound will cause overheating of the system, resulting in a loss of bioactive substances in oil.[8] Moradi and Rahimi *et al.*[51] comparatively analyzed the quality of sunflower seed oil extracted by the aqueous enzymatic method and ultrasonic-assisted enzymatic method and found that the degree of oxidation of oil prepared by ultrasonic-assisted extraction was high and the content of tocopherol decreased significantly, from 553.1 mg kg^{-1} to 543.5 mg kg^{-1}. To sum up, ultrasonic-assisted aqueous enzymatic extraction of vegetable oil can not only promote the release of bioactive components in oil cells, but also retain bioactive components to the greatest extent under mild extraction conditions. In practical application, the effects of the ultrasonic thermal effect and mechanical effect on the reaction system should be comprehensively considered to avoid the loss of bioactive components caused by excessive ultrasound.

5. Summary and prospects

The synergistic effect of ultrasonic-assisted technology in the process of extracting vegetable oil by the aqueous enzymatic method is remarkable. Ultrasonication can accelerate the decomposition of the plant cell wall structure and promote the release of intracellular dissolved substances. The intensity of ultrasonic action has a significant influence on the structure of enzyme. Proper intensity is beneficial to maintain or enhance the activity of enzyme, reduce the dosage of enzyme and shorten the enzymolysis time to improve the efficiency of aqueous enzymatic processing. At the same time, ultrasonic technology has potential application value in aqueous enzymatic demulsification. The cavitation effect can reduce the stability of emulsion, promote oil droplet aggregation, facilitate the extraction of vegetable oil, improve the efficiency of aqueous enzymatic extraction, and effectively maintain the good quality of oil.

At the same time, the ultrasonic-assisted aqueous enzymatic method has limitations that restrict its development and popularization. Due to the diversity of the cavitation effect, mechanical effect and thermal effect in the ultrasonic process, it is difficult to accurately control the ultrasonic parameters. If the parameters exceed the critical value, the oil will be degraded or isomerized, thus reducing the oil yield and quality, and its mechanical effect will aggravate the emulsification of feed liquid, leading to the difficulty of demulsification in the later stage. In addition, the cost of ultrasonic equipment used in the ultrasonic-assisted aqueous enzymatic method is high, and the processing capacity is small. When the ultrasonic equipment is scaled up from the laboratory scale to the industrial scale, there will be many technical problems, such as the ultrasonic process parameters determined at the laboratory scale, which may need to be re-optimized and adjusted in large-scale production; how to make ultrasonic energy evenly distributed in large-scale reaction systems to avoid local energy being too high or too low, which will affect the enzymatic hydrolysis effect and product quality; for the equipment needed for mass production, the investment cost is high, which increases the production cost. To realize the application of ultrasonic-assisted aqueous enzymatic extraction of vegetable oils in practical production, it is necessary to further explore the mechanism of ultrasonic degradation of cell walls, improvement of enzyme action and promotion of emulsion demulsification in future research. At the same time, a prediction model of ultrasonic parameters can be established with the help of machine learning methods, and the ultrasonic parameters can be accurately adjusted according to the characteristics of vegetable oils and enzymes to maximize the application effect of ultrasound in aqueous enzymatic extraction and facilitate large-scale production application.

Vegetable oil mainly contains minerals such as vitamin E, vitamin K, calcium, iron, phosphorus, potassium and fatty acids. Fatty acids in vegetable oil can make skin moist and shiny. Palm oil and coconut oil in vegetable oil are mainly composed of saturated fatty acids, which are the same as animal fats, so they are solid at room temperature. Most vegetable oils such as peanut oil, safflower oil, canola oil, corn oil, linseed oil, nut oil, sesame oil, soybean oil and sunflower oil are mainly composed of unsaturated fatty acids, so they are liquid at room temperature. It is recommended to eat vegetable oils mainly composed of monounsaturated fatty acids. Vegetable oils rich in monounsaturated fatty acids include canola oil, olive oil and peanut oil. Vegetable oils with high polyunsaturated fatty acid content include corn oil, safflower oil, soybean oil, sunflower oil and sesame oil, all of which have unique tastes.

Conflicts of interest

There are no conflicts to declare.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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