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Effect of enzyme types on emulsifying properties of walnut emulsion

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Abstract

In order to explore the efficient investigate and utilization of emulsion produced by enzyme-assisted aqueous extraction of walnut oil, the effects of different enzyme treatment (including Alcalase 2.4L, Ban 480L α-amylase, Neutrase 0.8L, Pectinex SP-L, Celluclast 1.5L) on the characteristics of emulsion were studied to characterize its emulsifying characteristics The emulsion composition, particle size distribution, Zeta potential, rheological properties and protein surface hydrophobicity of emulsion were determined to characterize its emulsifying properties. The results showed that the particle size of the emulsion obtained from enzymatic hydrolysis showed a smaller trend and the absolute value of Zeta potential showed a larger trend in the presence of enzymes. On emulsion protein, hydrophobicity of emulsion protein was obtained by hydrolysis with different enzymes showed that the trend followed the order: Celluclast 1.5L> Ban 480L >Neutrase 0.8L >Pectinex SP-L >control> Alcalase 2.4L. The particle size, zeta potential, ability to increase emulsion viscosity and interfacial hydrophobicity of emulsion proteins affected the emulsion physical stability and the effect of demulsification. The results of rheological analysis show that emulsions from Ban 480L α-amylase, Neutrase 0.8L and Celluclast 1.5L hydrolysate still maintained their elastic structure damaged by shear rate and were more stable, and the emulsions obtained by Alcalase 2.4L and Pectinex SP-L were unstable which was easy to be broken.

Keywords: walnut oil; enzyme-assisted aqueous extraction; emulsion; protein; characteristic analysis.

Practical Application: On the one hand, the emulsion can be considered as a by-product of enzyme-assisted aqueous extraction, and can be processed and utilized by microencapsulation and freeze-drying techniques to produce powder oils that can be added to health foods. On the other hand, in recent years, it has been found that the emulsion produced by enzyme-assisted aqueous extraction still contains protein, fat, polysaccharide and other nutrients. Through intensive processing, the emulsion can be effectively used to prepare milk beverage and walnut powder, fully reflecting its economic value.

1 Introduction

Walnut is an important oil crop in China, which is rich in nutrients such as protein, fat, vitamin and mineral elements, and its oil content is up to 65% (Zhang et al., 2009). Walnut oil is a type of vegetable oil with a high content of unsaturated fatty acids. It is also rich in monounsaturated polyunsaturated fatty acids such as linoleic acid and α-linolenic acids. Moreover, Walnut oil contains many functional ingredients, such as vitamins, squalene, phenols, and trace elements (Yingjie et al., 2022). The walnut oil extraction processes used differed according to the research objective: (i) determination of walnut oil content, (ii) selection of certain compounds of interest, (iii) implementation of new systems in industrial installations to increase oil yield, to obtain oil free from oxidized products or to promote an eco-friendly technique (Catherine et al., 2022). At present, the traditional methods of extracting walnut oil include solvent extraction and squeezing method. The squeezing method is simple to operate, but easy to denature protein, and usually costs a lot. The quality of extracted oil which uses solvent extraction is good with the extraction principle, However, there is a drawback that can not be ignored is that residual organic solvents may harm human health (Luque-Rodríguez et al., 2005).Therefore, looking for a new efficient and safe way of oil extraction has become a research hotspot.

Biological dissociation technology, also known as enzyme-assisted aqueous extraction process(EAEP), is a biological technology that separates oil, protein and other components by taking advantage of the difference of affinity between protein and carbohydrate for oil and water on the basis of mechanical crushing (Niranjan & Hanmoungjai, 2004; Campbell & Glatz, 2009). This method has many advantages, such as simple equipment, so the operation is relatively safe, and no residual solvent. At present, it is mostly used in soybean, peanut, camellia, sunflower seeds, corn and other oil materials (Yang et al., 2009; Zongyuan et al., 2015; Ruijin et al., 2016). The problem of low extraction efficiency of this processes may be overcome by the use of hydrolytic enzymes which help release oil and increase the yield as some studies have shown (Ziting et al., 2020). However, the popularization and development of this technology has been limited due to the emulsification phenomenon produced in the biological dissociation system which affects the release

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of oil, leads to low oil extraction rate and poor utilization of by-products. Researchers found that enzyme treatment destabilized the emulsion and recovered oil from it, which in turn significantly ($P<0.05$) increased the free oil yield (Lamsal & Johnson, 2007). Therefore, the release and recovery of oils and fats from emulsions, also known as demulsification, has become research topic hotspot in this field.

In order to explore effective demulsification methods and seek high-value utilization approaches, researchers have made in-depth studies on enzyme-assisted aqueous extraction method. Surfactant and salt-aided aqueous extraction (SSAE) method was used to break the emulsion by Qiaona Geng et al. (2020). David González-Gómez et al investigated the effect of temperature and time on oil il extraction yield and emulsions in enzymatic extraction process (González-Gómez et al., 2019). However, the formation of emulsion and its emulsification characteristics directly affect the demulsification effect and oil extraction efficiency, therefore, efficient demulsification can be achieved through the adjustment of emulsion structure and stability. Wang Limin et al. (2018) studied the effects of enzyme addition amount and enzymatic hydrolysis time on the structure of soybean emulsion, and found that fewer short peptides and more macromolecular proteins in the system played a dominant role in emulsion stabilization. For extruded puffed soybean flour, the emulsification properties of soybean hydrolysates treated with alkaline protease and flavored protease differed significantly (Lianzhou et al., 2018).

At present, researches on enzymatic demulsification mostly focus on the influence of enzyme dosage, enzymatic hydrolysis time, temperature and pH on oil yield (Qingui et al., 2008; Shuangzhi et al., 2010; Xiaosheng et al., 2010; Lianzhou et al., 2017), but there are few researches on the formation and emulsification properties of the emulsion during enzyme-assisted aqueous extraction process by the type of enzyme preparation, which directly affect the stability and demulsification effect of the emulsion. Biodissociation technology mainly uses enzyme preparation to destroy the cell wall structure to release oil, and the cell wall of wallplants consists of five components, namely cellulose, hemicellulose, pectin, lignin and glycoprotein. In this study, different types (Alcalase 2.4 L, Ban 480 L α-amylase, Neutrase 0.8 L, Pectinex SP -L, Celluclase 1.5 L) of food-grade commercial enzyme preparations were used for the EAEP of walnuts, and the influence of the structure and emulsification properties of the emulsions obtained from the enzymatic digestion of different enzyme by characterizing the emulsion composition, hydrodynamic radius distribution, zeta potential, rheological characteristics and hydrophobicity of the emulsion protein surface. Based on the emulsification properties of the emulsions themselves, the stability of the emulsions will be further evaluated, indirectly laying the foundation for the exploration of efficient and green emulsion breaking methods.

2 Materials and methods

2.1 Materials and chemicals

Walnut kernel was provided by Guizhou Boxing Food Technology Co., Ltd (China). All enzyme preparation, including Alcalase 2.4 L, Ban 480 L α-amylase, Neutrase 0.8 L, Pectinex SP-L and Celluclase 1.5 L were purchased from Novozymes Biotechnology Co., Ltd (China). The properties of each enzyme preparation are shown in Table 1. All chemicals used in this study were of analytical grade.

2.2 Instruments and equipment

Mastersize2000 laser particle size analyzer was purchased from Malvin Instrument Co., Ltd., Worcestershire, UK, LTD which was used to determine the average particle size of emulsion droplets. Zetasizer Nanozs 90 potentiometer was from Brookhaven instruments and used for measuring the Zeta potential of emulsion. AR1000 rheometer was purchased from TA instruments (America) and used for determination of emulsion rheological properties. F2000 fluorescence spectrometer was from Japan's Hitachi; visible spectrophotometer was purchased from Shanghai Spectral Instrument Co., Ltd (China). LGR20-W table top high speed refrigerated centrifuge was purchased from Beijing Jingli Centrifuge Co., Ltd. (China), and LGJ-1 freeze-dryer was purchased from Shanghai Medical Centrifuge Factory.

2.3 Sample preparation method

Preparation test of emulsion sample

The biological enzymatic walnut emulsion was prepared according to the report by Jiang Lianzhou et al. (2018). Walnut kernel was crushed through a 60-mesh sieve to get walnut powder, which was mixed with distilled water at the rate of 1:6 (v/v), and the addition amount of enzyme was 1 mL/(100 g). The conditions of enzymatic hydrolysis were shown in Table 1. To further clarify the influence of enzymes, some sample was hydrolyzed at 50°C without adding any enzymes as a blank control. Remember hydrochloric acid (2 mol/L HCl) or sodium hydroxide (2 mol/L NaOH) should be used to adjust the optimal pH of the enzyme before enzymatic hydrolysis. The speed of enzymatic hydrolysis was 20 r/min, and the time of enzymatic hydrolysis was 3 h. After enzymatic hydrolysis, the enzyme was inactivated for 10 min in water bath at 100°C. Subsequently, the enzymatic hydrolysate was cooled to room temperature and then placed in a centrifuge under the centrifugation conditions of 5000 r/min and 5 °C for 20 minutes. After centrifugation, the emulsion was collected by standing separation in a separatory funnel and rinsed repeatedly with deionized water. The emulsion was recycled after centrifugation at room temperature for 30 min at a centrifugal force of 15,000 r/min and placed on a 200-mesh screen. Six emulsion samples were obtained and stored at 4 °C for further determination.

Table 1. Properties of enzymes for the experiment.

Name	Standard dynamic	Optimum temperature (°C)	optimum pH
Alcalase 2.4L	2.4AU-A/g	50	9
Ban 480L α-amylase	480KNU/g	70	6
Neutrase 0.8L	0.8AU-N/g	50	7
Pectinex SP-L	26000PG/mL	35	4.5
Celluclase 1.5 L	700EGU/g	50	5

Extraction of protein in emulsion

Protein was extracted from the emulsion by acetone precipitation (Chen et al., 2017).The resulting emulsion was mixed with acetone solution frozen for 2 h at the ratio of 1:20 (w/v) and fully stirred. The reaction was carried out at -18 °C for 2 h. After the reaction, the precipitate was obtained by centrifugation for 15 min at a centrifugal force of 12000 r/min. Then, the supernatant was washed and precipitated with acetone for 3~5 times until the supernatant changed from yellow to colorless after centrifugation. Finally, the protein powder was obtained by freeze-drying.

Determination of emulsion composition

The moisture content, crude protein content and total phospholipid content of the emulsion were determined according to GB 5009.236-2016, GB 5009.5-2016 and GB/T 5537-2008 (National Standard for Food Safety, 2016a, b, 2008), respectively, and the crude fat content was determined according to GB/T 24870-2010 (National Standard for Food Safety, 2010).

Determination of hydrodynamic mean radius and its distribution of emulsion

The measurement method of particle size distribution of emulsion according to reference (Shao & Tang, 2014), Mastersize2000 laser particle size analyzer was used to measure the average particle size of emulsion droplets. The emulsion (0.5 mL /g) was diluted 10 times with deionized water (50 mL) and the particle size was kept within the measurement range, the refraction index of the oil droplets and the dispersant (water) was set at 1.47 and 1.333 respectively. The emulsion was diluted immediately after preparation and measured at room temperature (20 °C) with an absorbance of 0.001. The particle size of the sample was characterized by the average volume diameter, and the experiment was repeated three times during measurement.

Zeta potential determination of emulsion

Zeta potential of emulsion was measured by Zetasizer nanozs 90 potentiometer (Niu et al., 2022). The emulsion sample was diluted to a mass fraction of 0.2% with phosphate buffer solution of 0.05 mol/L and pH 7.0, then the solution was measured with the sample volume of 1 mL and the temperature of 25 °C, each sample was repeated for 3 times.

Rheological properties of emulsion

AR1000 rheometer was used for measurement according to the method in literature (Yanna, 2014), parallel plates with an spacing of 1 mm and a diameter of 40 mm were selected in the vibration mode to conduct dynamic scanning rheological test. Dynamic frequency conversion rheological measurement: the temperature was set at 25 °C, the stress was 1%, the frequency was from 0.1Hz to 10 Hz, and the change of modulus (elastic modulus G ‘, G “) and compound viscosity (η*) with the shear rate was determined.

Determination of protein surface hydrophobicity in emulsion

The surface hydrophobicity of proteins in the emulsion was determined by ANS fluorescent probe (Xie et al., 2022). Firstly, the emulsion proteins under different enzymatic hydrolysis conditions were weighed and dissolved in 0.01 mol/L phosphate buffer with pH 7.0, then the resulting mixture was stirred at 25 °C for an hour and centrifuged at 10000 r/min at 4 °C for 30 min, later, the samples of each group were diluted with 0.01 mol/L phosphate buffer solution with pH 7.0 to obtain 5 protein solutions with concentration gradients of 0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL respectively. Finally, 0.8, 1.6, 2.4, 3.2, and 4 mL of the solution were taken and 0, 0.8, 1.6, 2.4, and 3.2 mL of 8 mmol/L ANS solution were added successively. After rapid and sufficient oscillation to ensure uniform composition, the solution was left standing at room temperature for 5 min to determine fluorescence intensity (FI). The conditions were set as follows: the excitation wavelength was set at 400 nm, the emission wavelength was set at 470 nm, the crack value was set at 5 nm, and the scanning speed was 10 nm/s. The surface hydrophobic value of protein is the slope of the initial segment of the trend line plotted between the measured FI value and the corresponding protein concentration.

2.4 Statistics and analysis

Statistical analyses were performed by ANOV A using the SPSS 20.0 software, The data were expressed as mean values ± standard deviations. Significant differences ($P < 0.05$) between treatments were determined using Tukey's multiple range test. Chart making and graph analysis were performed by Origin Pro 2021 software.

3 Results and discussion

3.1 Effects of different enzyme types on the composition of emulsion

The chemical compositions of walnut emulsion treated with five enzymes were determined and analyzed by biological dissociation process, and the results were shown in Table 2.

It can be seen from Table 2 that the main components of the emulsion formed in walnut enzymatic oil extraction process include moisture, protein, fat, ash. The moisture and protein content of the emulsion were increased with different enzyme treatment, compared with the blank group. We can see that the protein content in emulsion which was treated with BAN 480L and Celluclast 1.5 L was higher. Since the special parent structure of proteins plays an important role in the formation and stability of the emulsion, the protein concentration can also be used to characterize the stability of the emulsion, which indicated that the two kinds of emulsion have good stability. The fat content in emulsions treated with Celluclast 1.5 L was higher may be attributed to the enzyme can increase walnut oil extraction rate.

3.2 Influence of different enzyme types on hydrodynamic radius and distribution of emulsion

The hydrodynamic radius and distribution of walnut emulsion treated with different enzymes were analyzed. The results are shown in Figure 1.

Table 2. The chemical composition of emulsions.

The kinds of enzyme	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Blank control	60.30 ± 0.01^{c}	8.53 ± 0.15^{c}	26.13 ± 0.09^{b}	0.72 ± 0.02^{a}
Alcalase 2.4L	63.90 ± 0.06^{a}	8.68 ± 0.09^{bc}	25.77 ± 0.20^{b}	0.70 ± 0.00^{a}
Ban 480L	62.30 ± 0.03^{b}	10.04 ± 0.09^{a}	25.90 ± 0.06^{b}	0.5 ± 0.003^{d}
Neutrase 0.8L	64.50 ± 0.20^{a}	9.26 ± 0.02^{b}	25.07 ± 0.03^{b}	0.60 ± 0.00^{bc}
Pectinex SP-L	62.20 ± 0.40^{b}	9.02 ± 0.02^{bc}	25.80 ± 0.46^{b}	0.59 ± 0.003^{c}
Celluclast 1.5 L	61.60 ± 0.40^{b}	10.47 ± 0.30^{a}	28.43 ± 0.33^{a}	0.67 ± 0.03^{ab}

Note: Different lowercase letters in the same column represent significant differences between samples, $P < 0.05$.

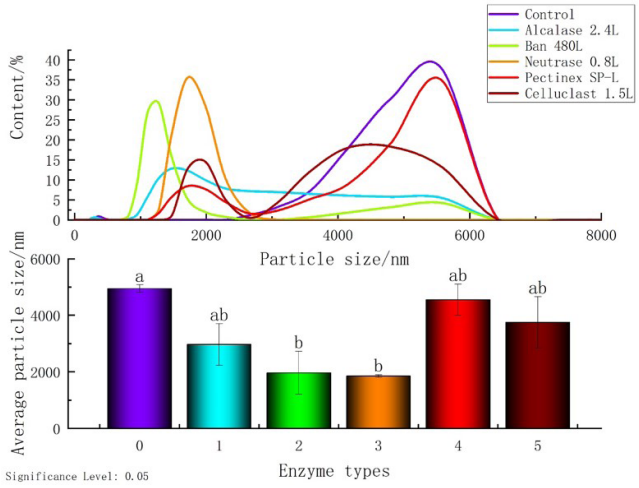

Figure 1. Effect of different kinds of enzyme on particle size distribution and the average particle size of emulsion (0-control,1- Alcalase 2.4L, 2-Ban 480L α-amylase, 3-Neutrase 0.8L, 4-Pectinex Ultra SP-L, 5-Celluclast1.5 L. (Different letters in the figure indicate significant difference between data, P<0.05).

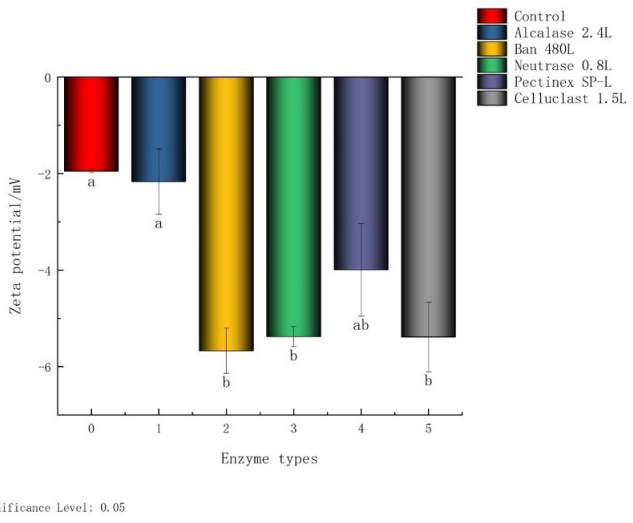

Figure 2. Effect of different kinds of enzyme on Zeta potential of emulsion (0-control, 1- Alcalase 2.4L, 2-Ban 480L α-amylase, 3-Neutrase 0.8L, 4-Pectinex Ultra SP-L, 5-Celluclast1.5L (Different letters in the figure indicate significant difference between data, *P*<0.05).

The dynamic radius and its distribution of the fluid are one of the important indicators for evaluating the stability of the emulsion, which can intuitively reflect the particle size distribution in the emulsion and at the same time characterize the aggregation and depolymerization behaviors between protein molecules and emulsion droplets in the emulsion (Liyuan, 2019). Generally speaking, the larger the particle size, the more likely the emulsion is to aggregate and fuse, and the more unstable the emulsion is. If the opposite is true, the more stable the emulsion is (Chen et al., 2020).As can be seen from Figure 1, The particle size distribution of blank group is relatively concentrated in the range of 3000~6000 nm and the average particle size is the largest. However, the particle size distribution of the emulsion obtained by adding enzyme hydrolysis showed a decreasing trend, which was conducive to the stability of the emulsion (Catherine et al., 2022). The average particle size plots showed that the average particle size of different emulsions was in the following order: Neutrase < α-amylase <Alcalase <Celluclase<Pectinex SP-L < The blank group, the average particle size of the five enzymatic hydrolysis decreased compared with the control group, which may be due to the thicker interface protein film outside the oil in the blank emulsion and the formation of larger pellet particles, while the enzyme preparation may hydrolyze the protein into small molecular peptides, which combine with the oil droplets to form fine, uniform and relatively stable emulsion (Liyuan, 2019).The average particle size of the emulsion obtained by BAN 480 L α-amylase and Neutrase 0.8 L was significantly lower than that of the blank control group ($P < 0.05$), the average particle size of the emulsion was smaller, suggesting that the emulsion obtained by these two enzymes was more stable.

3.3 Influence of different enzyme types on emulsion Zeta potential

Zeta potential is another indicator indicating the stability of emulsion system (Li et al., 2021). The greater the absolute value of Zeta potential, the stronger the interaction of charged particles, and the more stable the emulsion is (Chen et al., 2020). As can be seen from Figure 2, the Zeta potential of the emulsion samples obtained from biological dissociation in this experiment was all negative, which was consistent with the studies of Wang Limin et al. (2018) and ANN-DORIT (Let et al., 2007). Figure 2 showed that the absolute potential of the emulsion hydrolyzed by Ban 480L α-amylase was the highest, which was

-5.67mV, followed by Celluclast 1.5 L and Neutrase 0.8 L. Among all enzyme preparation, the absolute Zeta potential of the emulsion produced by Alcalase 2.4 L was the lowest, showing that the emulsion obtained by Ban 480L α-amylase, Celluclast 1.5 L and Neutrase 0.8L was relatively stable, which can be used to develop products with higher requirements on emulsification stability. Whereas, the stability of emulsion obtained by Alcalase 2.4 L was poor which was favorable for subsequent demulsification.

3.4 Influence of different enzyme types on rheological properties of emulsion

The emulsion system is a fluid containing water, protein and oil according to the composition analysis of the emulsion. The protein adsorption on the interface between oil droplets and water affects the viscosity, elasticity and other rheological properties of the emulsion system, which play a decisive role in the aggregation resistance of oil droplets (Yingyao & Zhang, 2009). Hence, the effect of different enzyme types on the rheological properties of the emulsion system was investigated.

Since the dynamic modulus is defined based on the linear viscoelastic region, the linear viscoelastic region is firstly determined according to the data. Within this region, the composite viscosity, storage modulus (G ‘) and loss modulus (G “) are plotted to obtain the frequency conversion rheological properties of the emulsion under different enzymatic hydrolysis conditions (Huang et al., 2022). Similar to the viscosity curve in the steady-state flow experiment that decreases with the increase of shear rate, the dynamic frequency scanning test results in the same variation trend of composite viscosity curve, as shown in Figure 3.

As can be seen from Figure 3, the viscosity of the emulsion varies with the frequency basically the same although the types of enzymes are different, that is, the viscosity of the emulsion decreases with the increase of frequency, indicating that the emulsion obtained after enzymatic hydrolysis has the characteristics of typical pseudoplastic fluid. Therefore, demulsification by high-speed shearing can be considered. At the same time, we also found that the initial viscosity and curve shape of different emulsion have some differences. The initial value of the emulsion hydrolyzed by Ban 480L α-amylase and Celluclast1.5 L was higher than that of the other three groups, which might be due to the fact that amylase and cellulase hydrolyzed macromolecules and were eventually adsorbed by the interface, resulting in higher initial compound viscosity than other emulsions. The addition of the two proteases not only hydrolyzed the interface proteins (Yingyao & Zhang, 2009), but also adsorbed the enzymatically hydrolyzed small molecular peptides, therefore, the viscosity was not different from the blank control group. And the viscosity of emulsion treated with pectinase is far less than that of other conditions, which is easier to flow and the stability is poor. It was worth noting that although the initial viscosity of the emulsion obtained by neutral protease hydrolysis is slightly lower, the effect of shear force on the emulsion is much less than that of other emulsions. Therefore, it can be inferred that the emulsion can inhibit the high-speed shear demulsification, which reflects another stability of the emulsion.

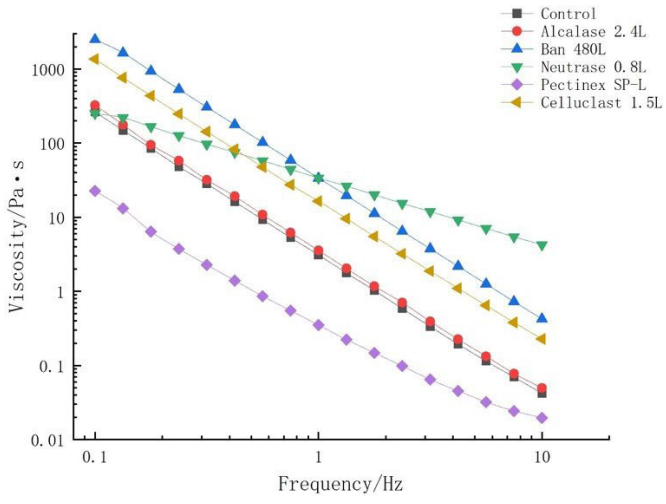

Figure 3. Effect of different kinds of enzyme on composite viscosity of emulsion.

The dynamic rheological properties of the emulsion were measured in the linear viscoelastic region. Figure 4 showed that the storage modulus (G ‘) and loss modulus (G “) of different emulsions as a function of frequency. As can be seen from Figure 4, the loss modulus of emulsion treated with pectinase gradually increases and surpasses the storage modulus with the increase of shear rate, forming a fluid system. However, the storage modulus and loss modulus of the emulsions treated by other enzymes increase slowly with the increase of frequency in the log-log coordinate, and the storage modulus (G ‘) is always greater than the loss modulus (G “), Therefore, it can be judged that the emulsion obtained under this condition is not destroyed by shear rate and still retains its elastic structure. Studies have shown that the physical stability of the emulsion increases with the increase of G ‘and G “ (Glusac et al., 2020). In conclusion, emulsion treated with Ban 480L α-amylase, Neutrase 0.8 L and celluclast1.5 L had a good stability.

Any substance is sticky and elastic at the microscopic level, which is reflected in the attraction and repulsion between molecules. If the attraction is greater than the repulsion, the object is sticky at the macroscopic level, otherwise, it is elastic. So the elasticity of the emulsion is determined not only by the elastic modulus, but also by the viscoelastic ratio, namely the tangent loss value. The tangent loss values of different emulsions were shown in Table 3. Except for Pectinex SP-L, the tangent

Table 3. The tangent loss value of different emulsions.

The kinds of enzyme	Tan(δ)
Blank	0.32 ± 0.04^{b}
Alcalase 2.4L	0.34 ± 0.05^{b}
Ban 480L α-amylase	0.25 ± 0.03^{b}
Neutrase 0.8L	0.27 ± 0.01^{b}
Pectinex SP-L	2.16 ± 1.25^{a}
Celluclast1.5L	0.43 ± 0.03^{b}

Note: Different lowercase letters in the same column represent significant differences between samples, $P < 0.05$.

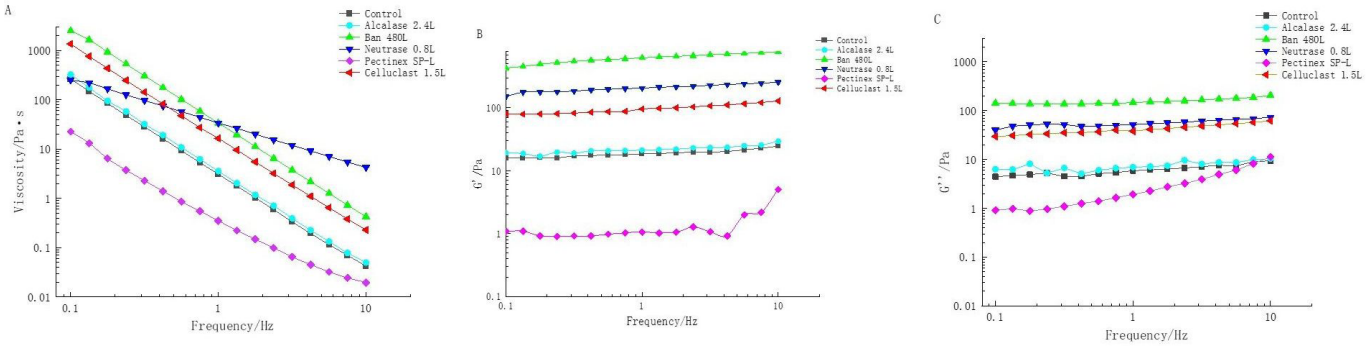

Figure 4. The modulus of the emulsion at different frequencies [Loss modulus: (A) Viscosity, (B) Storage modulus, (C) Loss modulus].

values of other emulsions are all small(less than 1), especially the emulsions hydrolyzed by α-amylase and neutral protease, which indicates that the emulsions have good elasticity. Elastic properties are mainly reflected in the elastic deformation of adsorbed proteins, starches and other macromolecules (Yingyao & Zhang, 2009). Thus, the more elastic the emulsion the more stable the corresponding emulsion structure is, which also indicates that the three-dimensional structure network on the surface of oil droplets is more complete and solid.

3.5 Effects of different enzyme types on the surface hydrophobicity of protein in emulsion

Surface hydrophobicity (S_0) is commonly used to characterize the number of hydrophobic groups on the surface of protein molecules, and it is also one of the important indicators reflecting whether the protein molecular structure has changed (Fischer et al., 2001). Therefore, in this experiment, the emulsion obtained from the biological separation of different enzymes was separately prepared and its surface hydrophobicity was measured, the results are shown in Figure 5. As can be seen from Figure 5, the effects of different enzyme types on the surface hydrophobicity of emulsion proteins were compared in order of size: Celluclast1.5 L >BAN 480 L alpha-amylase > Neutrase 0.8 L > Pectinex SP-L > Blank group > Alcalase 2.4 L.In other words, except alkaline protease, the surface hydrophobicity of the emulsion obtained by other enzymes is high. Literature (Zhang & Lu, 2015) 28 found that a large number of hydrophobic residues in protein molecules would be exposed under the action of enzymes, resulting in the increase of surface hydrophobicity of protein molecules. At the same time, the protein molecules exposed hydrophobic residues will happen between crosslinking gathered themselves together and formed a large number of aggregate in a long time and the high concentration of added amount of deep enzyme digestion reaction process. These soluble or insoluble aggregates will shield the hydrophobic region of the protein, and have a certain blocking effect on the effective binding of the hydrophobic group to the fluorescent probe ANS, thus causing the decrease of the hydrophobicity of the protein molecular surface (Tong et al., 2021). The protein molecular surface with low hydrophobicity has a reduced affinity to the surface of oil droplets, thus reducing the stability of the emulsion (Fischer et al., 2001). In conclusion, the emulsion obtained by Celluclast1.5 L, Ban 480 L α-amylase and Neutrase 0.8 L had good stability which is consistent with

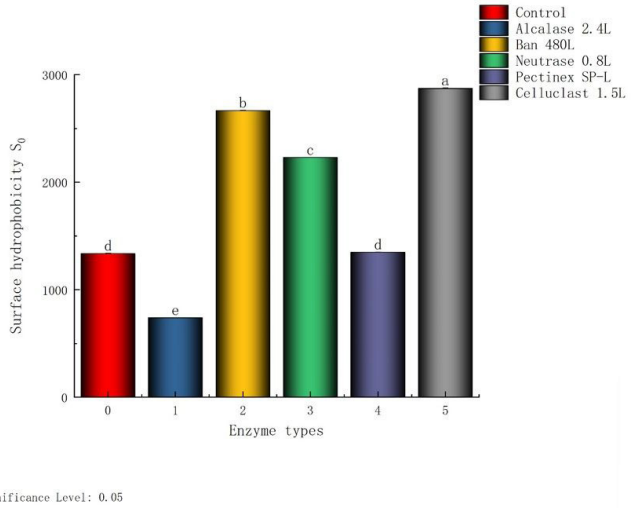

Figure 5. Effect of different kinds of enzyme on surface hydrophobicity of protein in emulsion (0-control, 1- Alcalase 2.4L, 2-Ban 480L α-amylase, 3-Neutrase 0.8L, 4-Pectinex Ultra SP-L, 5-Celluclast1.5L).

the above results, while the emulsion obtained by Alcalase 2.4 L had the worst stability and was easy to be broken.

4 Conclusion

The present study aimed to investigate the effect of different enzymes on the properties of walnut biodissociated emulsion by comparing and analyzing the composition of the emulsion, particle size distribution, potential, rheological properties and protein surface hydrophobicity. The results showed that there were significant differences in the composition of the emulsions hydrolysis by different enzyme preparation. Ban 480L α-amylase and Neutrase 0.8L produced the emulsions with smaller particle size, while Celluclast 1.5L produced the emulsions with relatively larger absolute potential. The rheological properties of the emulsion showed that the emulsion was shear thinning fluid, the initial viscosity of Celluclast 1.5L and Ban 480L α-amylase were higher, and the Neutrase 0.8L changed slowly with the shear rate increasing. Enzyme preparation can reduce the hydrophobicity of protein and thus reduce the stability of the emulsion. Notably, in the presence of specific types of enzymes, a large number of hydrophobic residues in

the protein molecule are exposed, which also causes an increase in the surface hydrophobicity of the protein molecule. Comprehensive comparison, the Ban 480 L a-amylase, the stability of emulsion obtained from Neutrase 0.8 L and Celluclast 1.5 L is good, which can be used to develop products with higher requirements on emulsion stability Such as powder oil products. and the stability of the emulsion with Alcalase 2.4 was the worst in the groups which was conducive to subsequent emulsion breaking work. In-depth analysis of the stabilization mechanism of emulsions produced by different enzymatic hydrolysis is conducive to the stability regulation of emulsions and provides a theoretical basis for demulsification in the EAEP process. Future research can focus on the application of EAEP emulsion systems, including emulsion delivery system, and the oil extraction by demulsification can make attempts to combined application of different types of enzymes.

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