Study on Ultrasonic Extraction and Stability of Glucosinolate in Rapeseed Cake

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Abstract: This paper performs an ultrasonic-enhanced glucosinolate extraction test with the rapeseed cake as the object and ethanol as the solvent, and compares this method with the conventional one. Based on that, this paper studies the stability of glucosinolate. The result shows that under the optimal conditions (150W acoustic power, 70% ethanol, 4:1 (mL/g) liquid-to-material ratio, 60 °C, and the extraction frequency of 5 times/15 min), the glucosinolate yield can reach 62.1 mg/g. Compared with the conventional method, in order to reach the same yield, the method used in this paper can reduce the required temperature by 20 °C and shorten the extraction time by 40%. Stability test shows that the glucosinolate is sensitive to temperature and pH, more stable below 80 °C and in neutral environments; it degrades faster above 80 °C, and is easy to decompose in acidic and alkaline environments. Under the test conditions, its stability decreases with the increase of acoustical power.

1. Introduction
Every year, the world produces about 28 million tons of rapeseed cakes, which contain 8-10% fat and 33% ~ 37% crude proteins. The nutritional value of the protein is equivalent to that recommended by the Food and Agriculture Organization of the United Nations and the World Health Organization [1]. However, rapeseed cake contains the glucosinolate, an important secondary metabolite of cruciferous plants [2]. Glucosinolate is considered to be toxic for a long time, which restricted the application of rapeseed cakes. In order to remove this component, Wang [3] and Bu [4] tried to extract glucosinolate using methanol as the solvent, which is of high extraction rate and low cost. However, methanol is toxic and volatile, which may cause physical injury and environmental pollution. Ethanol is similar to methanol in terms of properties, but is less toxic and dissolves less proteins and sugars. Ren et al. [5] conducted a test using 45% ethanol, confirming that heat treatment can reduce the degradation rate of glucosinolate. Ishikawa S. [6] achieved a glucosinolate extraction rate of 3.0% by using 20% ethanol as the solvent. Bao [7] realized a removal rate of glucosinolate in rapeseed cake of 46% using 75% acetone-ethanol solution. It is found that the ethanol concentration, liquid-to-material ratio, and extraction temperature vary in different literatures. At the same time, current studies regard glucosinolate as a kind of poison, and none of them focuses on the ultrasonic extraction and stability analysis of glucosinolate in rapeseed (or rapeseed cake).

In recent years, glucosinolate and their degradation products in such plants as broccoli and arugula seeds have been proved to own cancer resistance and prevention and antioxidant effects[8-10].
Viewing from the structure, we can see that the glucosinolate in rapeseed and that in broccoli and arugula are composed of β-thioglucosyl, sulfonic oxime, and branch R [11], with the only difference lying in the branch R. Their degradation products are all composed of isothiocyanate, thiocyanate, and nitriles. Therefore, it is reasonable to believe that the glucosinolate and degradation products in rapeseed, which are very similar in structure with those in broccoli and arugula seeds, should have similar functions.

The glucosinolate content in rapeseed cake reaches 3-8% [12]. It is a valuable resource, with which we can make use of the high-quality protein. To this end, this paper conducts ultrasonic enhanced extraction test of glucosinolate with ethanol as the solvent and rapeseed cake as the raw material, and compares this method with the conventional one. Based on that, this author also conducts glucosinolate stability test. The purpose of this paper is to lay a foundation for the biological function research and further development of glucosinolate.

2. Material and method

2.1 Material, reagent and instrument

Materials and reagents: Rapeseed cake (provided by China Oil and Food Import and Export Corporation); glycerol; citric acid; ethanol; sodium hydroxide; concentrated hydrochloric acid; phenolphthalein; disodium hydrogen phosphate (the above drugs are analytically pure).

Instrument: Thermostatic waterbath pot with digital display (Jinjiang Guocheng Experimental Equipment Factory, Jintan City, Jiangsu Province); rotary evaporator (Shanghai Yarong Biochemical Instrument Factory); low speed centrifuge (SC-3614) (Anhui Zhongke Zhongjia Scientific Instrument Co., Ltd.); ultraviolet spectrophotometer (UV -1800) (Shimadzu, Japan); thermostatic waterbath (Tianjin Xianquan Industry and Trade Development Co., Ltd.)

The booster-type ultrasonic extraction device is assembled using ultrasonic cell pulverizer with replaceable time and power (HN-1000, Shanghai Hannuo Instrument Co., Ltd.). During the test, ultrasonic action (time) and gap (time) shall be constant. The sample and the ethanol solution were placed in a heat-insulated cup connecting to a super-thermostatic waterbath. The position and depth of the booster in the liquid shall be the same in each test.

2.2 Method

2.2.1 Measurement of glucosinolate yield

The method for measuring glucosinolate yield used in this paper is modified based on the method mentioned in the literature [13]. The glucosinolate is hydrolyzed to produce glucose, which is measured using 3, 5-dinitrosalicylic acid colorimetry. The set of hydrolysis conditions and the preparation of the participating solutions were based on the literature [13]. The measured volume concentration of the substance was used to calculate the mass of glucosinolate. Yield of glucosinolate (mg/g) =mass of glucosinolate in the extract (mg)/mass of glucosinolate in rapeseed cake (g). Each sample was measured three times in parallel, and the data was processed using Origin.

2.2.2 Operation method

(1) Ultrasonic extraction method

Weigh the rapeseed cake and put it in an insulated cup, and add the ethanol solution. Connect the cup to a thermostatic waterbath, turn on the ultrasonic equipment, and set the power and time. After extraction, remove the insulated cup, decant the supernatant, and add fresh solvent for multiple extractions. Mix the extract, measure the glucosinolate content, and calculate the yield. Conduct single factor experiments on the effect of temperature, acoustical power, time, and volume fraction of ethanol on the glucosinolate yield rate. As for the conventional comparative test, all operations are the same except that ultrasonic equipment is not used.

(2) Glucosinolate stability test
Add lead acetate to the glucosinolate extracting solution, shake the solution, and take the supernatant as a test raw material to study the impact of temperature, acoustical power, and solution pH on glucosinolate stability. The temperature was controlled using the waterbath and the time was fixed at 30 minutes. The pH was adjusted using citric acid-sodium citrate, and the depth and position of the booster in the solution were fixed each time.

3. Test result and analysis

3.1 Single factor experiment of the influence of various factors on ultrasonic extraction

Figure 1 shows the single factor experiment of ultrasonic extraction of glucosinolate. According to Figure 1 (a), the yield of glucosinolate reaches the highest at 60 °C, and decreases when the temperature is higher than 60 °C. This may be because that the combined effect of the temperature, ultrasonic heat and cavitation leads to partial glucosinolate degradation [14]. As shown in Figure 1 (b), when the acoustical power increases from 50W to 100W, the glucosinolate yield does not change significantly; when it rises to 150W, the yield rises rapidly to the peak; when it exceeds 150W, the yield declines continuously. This may be because that within a certain range, as the acoustical power increases, the cavitation intensity increases, and the extraction effect becomes better; however, overlarge acoustical power leads to the formation of a sound barrier at the transmitting end of the booster, which hinders the sound transmission and reduces the sound effect. At the same time, when the acoustic power is too large, the ultrasonic cavitation effect and thermal effect lead to the glucosinolate degradation. Therefore, as the acoustic power increases, the glucosinolate yield rises first and then decreases. Further study shall be conduct to explore whether the hindered sound transmission or the reduced glucosinolate stability in the ultrasonic field is the reason behind the phenomenon.

Figure 1 (c) shows that from 10 to 15min, the glucosinolate yield significantly increases, and reaches the maximum at 15min; after that, the yield gradually decreases. In theory, if the glucosinolate is of good stability, its yield will not decrease with time. Therefore, the decline is most likely due to the instability of the glucosinolate in the sound field. In the 15 minutes before the ultrasonic action, the
Fig.1. Effect of different factors on the yield of glucosinolate in ultrasonic field.

Enhanced effect of ultrasound caused a large amount of glucosinolate to dissolve. At this time, there was less glucosinolate in the solution, and degraded amount of glucosinolate was less than the dissolving-out amount; after 15 min, the dissolution of glucosinolate tended to achieve a balanced state, and the degradation effect of ultrasonic sulfur on the glucosinolate became more prominent due to the increasing glucosinolate concentration, leading to a rapidly yield decrease. Generally speaking, the polarity of the solvent is affected by the type and concentration. According to the theory that similarities can be solvable easily in each other, it can be judged that the 70% ethanol is similar to the glucosinolate in the system in terms of polarity [15], at which point the solubility can reach maximum. According to Figures 1 (e) and 1 (f), larger amount of solvent and number of extractions are beneficial to the extraction. However, when the liquid-to-material ratio is greater than 4:1 and the extraction has been conducted for over 5 times, the yield of glucosinolate increases slowly. With the comprehensive
cost taken into consideration, liquid-to-material ratio shall be taken as 4:1, and the extraction shall be conducted for 5 times.

Based on the above analysis, the optimal conditions for ultrasonic extraction are: 60 °C; 15min duration each time; 70% ethanol; liquid-to-material ratio=4: 1 (mL/g); 5 times of extraction. Under these conditions, the yield of glucosinolate can reach 62.1 mg/g.

3.2 Single factor experiments of conventional extraction method

Figure 2 (a) shows that temperature has a greater influence on the yield of glucosinolate. As the temperature rises, the yield of glucosinolate increases slowly below 70 °C, and reaches the highest at 80 °C; it then decreases rapidly above 80 °C. Theoretically speaking, extraction is a process of dissolution and diffusion. As the temperature rises, the system viscosity decreases and the molecular motion is intensified, which are the conditions beneficial to dissolution and diffusion. However, the boiling point of ethanol is 78 °C. So when the temperature is too high, especially higher than 80 °C, a large amount of ethanol will volatilize, thus reducing the effective ethanol concentration of the solution and also the amount of effective ethanol, which further leads to a smaller yield after the temperature exceeds the boiling point. At this time, we cannot rule out the possibility that glucosinolate is easily degraded above 80 °C [16-17], leading to a smaller yield. Compared with Figure 1 (a), the ultrasonic effect can help to accelerate extraction compared to the conventional method when the temperature is below 70 °C. But the yield delivered using the former method decreases when the temperature is lower than 60 °C, at which point the solvent is not likely to volatize in large quantities. So this phenomenon is mainly due to glucosinolate degradation. This shows that if the glucosinolate itself is not stable, then the ultrasound will further deteriorate its stability.

Figure 2 (b) shows that under the test conditions, the glucosinolate yield continues to increase with time, and the increase slows down after 25 min. This shows that the glucosinolate is of good stability. Figure 1 (c) shows that after 15min of ultrasonic effect, the yield of glucosinolate decreases rapidly, which again confirms that ultrasound can promote glucosinolate degradation. It can be seen from Figure 2-c that 70% ethanol is the best choice for conventional extraction, which is similar to the result of ultrasonic-enhanced extraction (Figure 1 (d)). But when the concentration of ethanol is low, ultrasound shows a significant strengthening effect. According to Figures 2 (d) and 2 (e), generally speaking, larger amount of solvent and larger number of extractions can deliver higher glucosinolate yield. However, when the liquid-to-material ratio is greater than 4:1 and the extraction is conducted for over 5 times, the glucosinolate yield changes very slightly. Therefore, the most appropriate liquid-to-material ratio should be 4:1, and the best number of extraction is 5 times. The influence of these two factors on glucosinolate yield is exactly the same as that of ultrasonic extraction.

Based on the above, the optimal conditions for conventional solvent extraction are: 80 °C; 25min duration each time; 70% ethanol; liquid-to-material ratio=4:1 (mL/g); 5 times of extraction. Under these conditions, the glucosinolate yield can reach 63.1 mg/g, which is similar to that obtained using the ultrasonic method. But the ultrasonic method requires a temperature lower by 20 °C and the time shorter by 40%.
3.3 Glucosinolate stability test

3.3.1 Impact of temperature

It can be seen from Figure 3 (a) that the glucosinolate remains stable when the temperature is lower than 80 °C, but its content decreases sharply when the temperature is higher than 80 °C. Therefore, it is suitable to store the glucosinolate in rapeseed cake under 80 °C.

In the infrared spectrum showed in Figure 3 (b), methylene groups vibrate at 2921 cm\(^{-1}\) and 2854 cm\(^{-1}\), and methylene groups exist on the R group and glycosyl groups of the glucosinolate; the peak at 1045 cm\(^{-1}\) is stretching vibration of C-O-C; the absorption peak at 3375 cm\(^{-1}\) is mainly hydroxyl; 1644 cm\(^{-1}\) shows the stretching vibration of glucosinolate molecule C=N. This figure shows no significant difference in the infrared spectrums of the samples treated at 50 °C and 100 °C, while Figure 3 (a) shows that the glucosinolate content is significantly reduced when the temperature is above 80 °C. This may be because that above 80 °C, the glucosinolate are hydrolyzed to generate free sugars, while the aglycone shows no significant change, thereby leading to lower glucosinolate content but no significant change in infrared spectrum has not changed significantly. Further research is needed in this regard, especially in the separation and identification of the post-treatment products.

![Fig.3. Effect of temperature on the stability of glucosinolate](image)

Fig.3. Effect of temperature on the stability of glucosinolate

![Fig.4. Effect of ultrasonic power on the stability of glucosinolate](image)

Fig.4. Effect of ultrasonic power on the stability of glucosinolate
3.3.2 Effect of Ultrasonic power

It can be seen from Figure 4 (a) that ultrasonic treatment has a significant effect on the stability of glucosinolate. As the acoustical power increases, the glucosinolate content continues to decrease. Figure 4 (b) shows that after ultrasonic treatment, the absorption peak at 3347 cm\(^{-1}\) becomes blunt and wider, indicating stronger association of hydrogen bonds; characteristic absorption peak of N=C=S (isothiocyanate) occurs at 2250 cm\(^{-1}\), indicating that glucosinolate is degraded to produce isothiocyanate; the peak at 1050cm\(^{-1}\), the stretching vibration of C-O-C, is shifted to 1043cm\(^{-1}\), and becomes broader and relatively weaker, which is mainly due to the change of glucosinolate glycosyl under the action of ultrasound.

3.3.3 Impact of pH

It can be seen from Figure 5 (a) that the pH has a great influence on the stability of glucosinolate. The stronger the acidity, the lower the glucosinolate content; when the pH=7, there is no significant change in the glucosinolate content; when the pH is greater than 7, glucosinolate content decreases. This shows that the glucosinolate is unstable in acidic or alkaline environments and is relatively stable in the neutral environment. Figure 5 (b) shows that when the pH=2, the peak at 1054cm\(^{-1}\), the stretching vibration of C-O, weakens significantly; the peak at 2930cm\(^{-1}\), the stretching vibration of -CH\(_2\)-, almost disappears; the peak at 1665cm\(^{-1}\), the stretching vibration of C≡N, disappears. All of them indicate that the strong acid can facilitate the degradation of glucosinolate. A new peak appears at 2460 cm\(^{-1}\), the stretching vibration of C≡N, indicating that the glucosinolate is degraded to form nitrile compounds after strong acid treatment.

The above tests show that temperature, acoustical power, and pH all affect the stability of glucosinolate. When the temperature is lower than 80 °C, the environment is neutral, and the acoustical power is small, glucosinolate tends to be more stable. In the aforementioned extraction, the temperature is lower than 80 °C, and the environment is also neutral. However, small acoustical power of ultrasound delivers no significant strengthening effect, while large acoustical power facilitates glucosinolate degradation. In combination with Figure 1 (c) and Figure 5 (a), the acoustical power should be strictly controlled at 100-150W, at which point the acoustical power has a significant strengthening effect and does not facilitate glucosinolate degradation.

![Fig.5. Effect of pH on the stability of glucosinolate](image)

4. Conclusion and prospects

(1) With the rapeseed cake as the raw material, the optimal conditions for ultrasonic extraction of glucosinolate are: ultrasonic power=150W; 70% ethanol; liquid-to-material ratio=4: 1 (mL/g); 60 °C; 15min duration each time; 5 times of extraction. Under these conditions, the yield of glucosinolate can reach 62.1 mg/g. The optimal conditions for solvent extraction of glucosinolate are: liquid-to-material ratio=4:1 (mL/g); 70% ethanol; 80 °C; 25min duration each time; 5 times of extraction. Under these conditions, the yield of glucosinolate can reach 63.1 mg/g. In comparison, to achieve the same glucosinolate yield, the time can be shortened by 40% and the temperature can be lowered by 20 °C.
after adding ultrasound.

(2) Glucosinolate is relatively stable when the temperature is below 80 °C, and it is easy to decompose when the temperature is above 80 °C. The larger the acoustical power, the more unstable the glucosinolate. Glucosinolate is more stable in the neutral environment, while acidic and alkaline environments will facilitate its degradation.

(3) This paper explores the ultrasonic-enhanced method for extracting glucosinolate from rapeseed cake using ethanol, and compares this method with the conventional one. In addition, this paper studies the stability of glucosinolate in different environments. Further studies are needed to explore the structural characteristics of degradation products of glucosinolate and the biological activities of glucosinolate and its degradation products.

Acknowledgments
This work was supported by the Science and Technology Program of Guangzhou, China (No.201604020074) and the Guangdong Provincial Science and Technology Project (No.2015B020215012).

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