Extraction and surface activity of tea saponin from *Pu'er tea* seeds

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Abstract: Tea saponin was extracted from Pu’er Tea seeds using the ultrasound-ethanol method, the factors influencing the extraction rate were investigated, the extract was characterized and identified, and then the surface activity of the obtained tea saponin was explored from the aspects of surface tension, foamability and foam stability and detergency. The results show that the optimal extraction conditions include extraction time: 80 min, temperature: 70℃, liquid-solid ratio: 8:1, critical micelle concentration (CMC): 0.05%, and the surface tension is 30.01 mN/m under CMC concentration; the tea saponin has excellent foamability, high foam stability and strong detergency under CMC concentration.

1. Introduction

Surfactant has been extensively applied to various industrial fields such as detergents, cosmetics, etc. [1]. However, the conventional surfactant will unavoidably lead to major environmental pollution if widely used, as it belongs to a hardly biodegradable substance [2,3]. Therefore, pollution-free natural surfactants are urgently needed. To be specific, natural-occurring substances should be used to replace hazardous chemicals in order to develop fully degradable and multifunctional surfactants [1].

As a kind of natural glycoside, tea saponin is widely distributed in leaves, flowers and fruits of camellia plants. With both hydrophobic and hydrophilic groups, the molecular structure of tea saponin can change the physiochemical properties of the interface, thus endowing it favourable surface activity [4,5].

In recent years, increasing attention has been paid to the extraction, development and application of tea saponin. Nevertheless, the researches on the extraction of tea saponin from *Pu'er tea* seeds have not been reported yet. Yunnan Province in China abounds in *Pu'er tea*, which is considerably favoured by the market; however, a large number of *Pu'er tea* seeds have been wasted. The tea saponin was extracted from the *Pu'er tea* seeds through the ultrasound-ethanol method, the factors that influenced the extraction rate were investigated, the extract was characterized and identified, and the surface activity of the extracted tea saponin was explored from the aspects of surface tension, foamability and foam stability and detergency, etc., expecting to provide related references for the effective extraction, further development and utilization of tea saponin.
2. Materials and Methods

2.1. Experimental materials and equipment

*Pu’er* tea seeds were from Muga Tea Factory located in Pu’er City, Yunnan Province; absolute ethanol, cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS) and vegetable blend oil were all provided by Yunnan Scientific Apparatus and Chemical Glass Co., Ltd.

UA03MFD ultrasonic apparatus, Beijing Shinetek; 60MARM electronic balance, U.S. Ohaus; SHZ-DIII water circulating vacuum pump, Gongyi Yuhua; RE-2000A rotary evaporator, Shanghai Yarong; HJ-6A thermostatic magnetic stirrer, Jintan Jinfen; 2100Q turbidity meter, U.S. Hach.

2.2. Experimental methods

2.2.1. Research method of tea saponin extraction

The tea saponin was extracted from the *Pu’er* tea seeds using the ultrasound-ethanol method (ultrasonic frequency: 59 KHz, volume fraction of ethanol: 80%). On this basis, the influences of three factors—extraction temperature, liquid-solid ratio and extraction time—on the extraction rate of tea saponin were investigated through the control variate method. The optimal extraction conditions were obtained by taking the extraction rate as an evaluation index.

(1) The influence of extraction temperature on extraction rate: the extraction rates at 0℃, 50℃, 60℃, 70℃ and 80℃ were investigated under extraction time of 1 h and liquid-solid ratio of 8:1, and the optimal temperature was obtained.

(2) The influence of liquid-solid ratio on extraction rate: under the extraction time of 1 h and the optimal extraction temperature obtained in Step (1), the extraction rates at liquid-solid ratios of 6:1, 7:1, 8:1, 9:1 and 10:1 were studied, and the optimal liquid-solid ratio was obtained.

(3) The influence of extraction time on extraction rate: the extraction rates at 20, 40, 60, 80 and 100 min were explored under the above obtained optimal extraction temperature and optimal liquid-solid ratio, and thus the optimal extraction time was acquired.

2.2.2. Characterization and identification methods of tea saponin

IR characterization of the obtained tea saponin was performed, and whether the extract from *Pu’er* tea seeds was tea saponin was identified according to the peak position by comparing the IR spectrograms of the standard tea saponin and the extract.

2.2.3. Research method for surface activity of tea saponin

2.2.3.1. Determination of surface tension and critical micelle concentration (CMC)

The extracted tea saponin was prepared into to-be-determined solutions with mass concentrations of 0.001%, 0.004%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.10%, 0.15%, 0.20%, 0.40%, 1.00%, 2.00% and 4.00%, respectively. The surface tension was determined via an automatic surface tension meter at 25℃. Based on the obtained data, the negative logarithm of mass concentration was drawn on x-coordinate, and the measured surface tension was taken as y-coordinate to draw the change curve of \(-\log C\)-surface tension, and the catastrophe point on the curve was namely the CMC value of the extracted tea saponin.

2.2.3.2. Qualitative determination of foamability and foam stability

The foamability and foam stability were determined at 25℃ through the hand-cranking method. Tea saponin solutions (50 mL each) with mass concentrations of 0.1, 0.5, 1.0, 1.5 and 2.0 CMC were respectively prepared and poured into a 250 mL measuring cylinder with plug, after then, the plug was covered, the initial height of the solution was recorded, the cylinder was turned upside down once every second for 60 times with the middle of the measuring cylinder as the centre, the cylinder was placed on
an experiment platform, and the foaming height was recorded. 30 min later, the foaming height was recorded again as the determination result of foam stability.

2.2.3.3. Detergency determination

The gauze fully soaked in vegetable blend oil was taken as the deterging object to investigate the detergency performance of the tea saponin. Four clean gauzes which were totally the same in texture and size were used, where three were soaked in the same kind of vegetable blend oil of the same amount for 10 min, while the rest one was not soaked. The three soaked gauzes were taken out and placed into 100 mL of purified water (the content of tea saponin: 0, 0 CMC), tea saponin solution under 1 CMC and that under 2 CMC, respectively, and the non-soaked gauze was put into tea saponin under 2 CMC (control group, aimed to avoid the turbidity effect of tea saponin). Afterwards, each gauze was rinsed on a magnetic stirrer at 100 r/min for 20 min, the turbidity of cleaning fluid was determined using a turbidity meter in the end, the data were recorded, and the detergency performance of the extracted tea saponin was analysed by drawing the graph.

3. Results and Discussion

3.1. Influence factors for extraction rate of tea saponin

3.1.1. Influence of extraction temperature on extraction rate of tea saponin

As shown in Fig. 1, the extraction rate was gradually improved with the temperature rise, and reached a peak at 70℃. As the temperature continued to rise, the structure of tea saponin was damaged, the extraction rate was reduced on the contrary, so the optimal extraction temperature was 70℃.

3.1.2. Influence of liquid-solid ratio on extraction rate of tea saponin

As shown in Fig. 2, the extraction rate was gradually improved with the temperature rise, and reached a peak at 70℃. As the temperature continued to rise, the structure of tea saponin was damaged, the extraction rate was reduced on the contrary, so the optimal extraction temperature was 70℃.
From Fig. 2, as the extraction temperature was fixed at 70℃, the extraction rate from Pu’er tea seeds was gradually elevated with the continuous increase of liquid-solid ratio, it reached a peak at 8:1, and after then, it did not increase any longer with the increase of liquid-solid ratio even if the amount of seeds fed was increased. Therefore, the optimal liquid-solid ratio was 8:1 for the extraction.

3.1.3. Influence of extraction time on extraction rate of tea saponin

![Fig. 3 Influence of time on extraction rate](image)

It could be known from Fig. 3 that under fixed extraction temperature of 70℃ and liquid-solid ratio of 8:1, the extraction rate from Pu’er tea seeds was continuously elevated as the extraction time was continuously lengthened, it reached the peak at 80 min when the content reached a constant value, and since then, the extraction rate was not increased any longer with the increase of liquid-solid ratio. Hence, the optimal ultrasonic treatment time was 80 min.

3.2. Characterization of extract from Pu’er tea seeds

![Fig. 4 IR spectrogram of standard tea saponin](image)

![Fig. 5 IR spectrogram of extract from Pu’er tea seeds](image)
Fig. 4 and Fig. 5 show the IR spectral curves of standard tea saponin and extract from *Pu’er tea* seeds, respectively. Through a comparative analysis, within the characteristic spectral band, the characteristic peaks of hydroxyl and methylene appeared at about 3,650~3,200 cm⁻¹ and 2,935~2,925 cm⁻¹, respectively, while no characteristic peak existed at 2,500~1,900 cm⁻¹ or so, in other words, there were no triple bond or cumulative double bond. Nearby 1,850~1,600 cm⁻¹ was a characteristic peak of carbonyl, and at about 1,406 cm⁻¹ and 1,264 cm⁻¹ were characteristic peaks of -CH₂-. In the fingerprint region (1,350~650 cm⁻¹), the flexural vibration peaks of C-O-H appeared at about 1,266 cm⁻¹ and 1,264 cm⁻¹, and the stretching vibration peaks of C-O-C existed at about 1,047 cm⁻¹ and 1,049 cm⁻¹. Hence, the IR spectral curve of the extract from *Pu’er tea* seeds was basically identical with that of standard tea saponin, namely, the extract from *Pu’er tea* seeds was tea saponin.

### 3.3. Surface activity of tea saponin

#### 3.3.1. Surface tension and CMC of tea saponin

![Surface tension and -LgC](image)

As observed from Fig. 6, the surface tension gradually declined with the increasing mass concentration of tea saponin at 25°C. When the mass concentration increased to a certain degree, a catastrophe point appeared on the surface tension curve, the liquid surface reached the minimum energy state, namely, this point was CMC for the extract from *Pu’er tea* seeds, and it could be obtained from the figure that the CMC value was 0.05%.

The surface tensions of purified water, tea saponin under mass concentration of 0.05%, CTAB solution and SDS solution were determined as 69.02 mN/m, 30.01 mN/m, 31.21 mN/m and 36.64 mN/m, respectively. It could thus be seen that the surface tension of tea saponin was evidently lower than that of purified water, and its ability to reduce the solution surface tension was better than those of CTAB and SDS, indicating that the tea saponin was of good surface activity.

#### 3.3.2. Foamability and foam stability

![Foamability and foam stability](image)

As observed from Fig. 7, the foamability and foam stability of tea saponin showed good performance. The initial foam height increased with the increase in mass concentration, and after 30 min, the foam height remained relatively stable, indicating that the tea saponin had good foam stability.
As shown in Fig. 7, the foaming height of the tea saponin solution at 25℃ and that after 30 min were elevated with the increase of mass concentration from 0.1CMC (0.005%), 0.5CMC (0.025%), 1.0CMC (0.05%), 1.5CMC (0.075%) to 2.0CMC (0.1%). Before 1.0CMC (0.05%), the slope of the curve was large and the foaming height reached as high as 30 mL. Following the point of 1.0CMC (0.05%), the slope of the curve tended to be steady, and the height was stabilized at 10 mL after 30 min. This manifested that at 1.0CMC (0.05%), the tea saponin already possessed satisfactory foamability and foam stability. The stable foaming height was ascribed to the fact that the solution surface tension did not change any longer and the system surface tended to be unchanged; in addition, the foam was an unstable thermodynamics system, the foam breakdown run through the whole foaming process, a balance was reached between foaming and foam breakdown after the CMC was reached, and then the foamability of the solution would not be further enhanced.

3.3.3. Detergency determination

As shown in Fig. 8, the turbidity was only 3.01 NTU in the 2CMC control group not containing greasy dirt, and the solution was still transparent and clear after rinsing; the control experiment suggested that the influence of tea saponin on the turbidity of the cleaning system could be neglected. Meanwhile, after the oil fouled gauze was fully rinsed, the cleaning fluid of purified water (0 CMC) was still transparent and clear, and its turbidity was only 1.95 NTU; however, the tea saponin solutions under 1 CMC and 2 CMC both turned turbid with turbidities of 94 NTU and 106 NTU, respectively, and moreover, they presented uniform emulsion form, indicating a considerable part of greasy dirt was compatibilized and removed by the tea saponin. After reaching the CMC value, the tea saponin would have strong compatibilization capacity and superior detergency performance, but the detergency could not be significantly strengthened if the concentration of the tea saponin was further elevated.

4. Conclusions

The extraction of tea saponin from Pu’er tea seeds was realized through the ultrasound-ethanol method. The related factors that influenced the extraction rate were probed, the extract was characterized and identified, and the surface activity of the extracted tea saponin was analysed from the angles of surface tension, foamability and foam stability, and detergency, etc. According to the experimental results, the optimal extraction conditions consisted of extraction time: 80 min, temperature: 70℃, liquid-solid ratio: 8:1, and CMC: 0.05%, and the surface tension was 30.01 mN/m under these conditions. It could be observed that the surface tension of the system was obviously reduced, and the ability of the tea saponin to reduce the solution surface tension was better than those of CTAB and SDS; given this, the tea saponin shows excellent foamability, high foam stability and strong detergency performance at CMC.

In conclusion, the ultrasound-ethanol method can be used to effectively extract the tea saponin from Pu’er tea seeds, and the CMC value of the extracted tea saponin was low, along with low theoretical
use level and good surface activity. Furthermore, the tea saponin has broader application prospects in chemical engineering, environmental protection, etc. by virtue of pure natural, degradable, nontoxic and harmless advantages.

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