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# Simultaneous extraction of oil and soy isoflavones from soy sauce residue using ultrasonic-assisted two-phase solvent extraction technology

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ABSTRACT

Simultaneous extraction of oil and soy isoflavones from soy sauce residue (SSR) was investigated by means of the two-phase solvent extraction intensified by ultrasonication. A single factor test was first carried out to study the effects of ultrasonic time, ethanol concentration, ratio of ethanol/water phase to raw material, ratio of hexane phase to raw material, and ultrasonic power on the extraction rates of oil and isoflavones, then response surface methodology was applied to further optimize and simulate four key factors. The results showed that the extraction rates of oil and isoflavones of 92.07% and 92.53%, respectively, could be obtained when ethanol concentration was 74.88%, ratio of ethanol/water phase to raw material 15:1, ratio of hexane phase to raw material 8.64:1, extraction time 20 min, and ultrasonic power 160 W. The predictive rates were well matched with the experimental ones. The quality analysis of oil and soy isoflavones from SSR showed that the extracted oil with high acid value could be a potential raw material for biodiesel production instead of cooking. The extracted soy isoflavones existed mainly in aglycones form, which might afford higher bioactivity than that from the other soybean products.

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## 1. Introduction

Soy sauce, brewed by soybean and wheat, is a conventional condiment in Asia, and more and more popular in the Western countries due to its special flavor. China is the birthland and the largest produce base of soy sauce. The annual production of soy sauce in China is approximately 6 million tons, accounting for over 65% of the total world production [1]. With the rapid development of soy sauce industry, however, the treatment and utilization of soy sauce residue (SSR) has been paid many attentions because of the environmental problems. Though SSR is regarded as a waste traditionally, it is actually rich in cellulose, oil, soy isoflavones, protein, as well as a number of other useful components [2]. Therefore, SSR is also considered as a potential cheap biomass resource.

Recently, there have been a number of investigations on extracting high value-added components from SSR, including soy isoflavones used as an antioxidant ingredient, and oil used as an alternative of edible oil for biodiesel. Among these, soy isoflavones were extracted usually by mixing/stirring the substrate in organic solvents, and various intensification processes such as microwave assisted heating [3], pressurized liquid extraction [4], superheated water extraction [5] and ultrasonic-assisted extraction [6–9] were employed to improve isoflavones yields from soy substrates.

Oil was abstracted commonly using solvent extraction or press method. Two-phase solvent extraction (TSE) has been shown great potential for oil extraction in recent decades due to its unique advantages [10,11]. In the process, oil is leached by nonpolar solvent phase, while some impurities or coloring matters are extracted by a water-miscible polar solvent phase. Thus the quality of oil is superior to the crude oil obtained from the traditional press process. Because the TSE technology can simplify the refining steps to obtain high quality vegetable oil [12,13], and is a wet extraction technique, which is free from drying process of wet materials before oil extraction, TSE is a time and energy conservation process [14]. To date, the method was applied for oil extraction from canola, rapeseed, Jatropha curcas seed, waste coffee grounds and so on [12–15].

According to the different solubility between soy isoflavones and oil, the simultaneous extraction of oil and soy isoflavones from SSR using TSE was explored and a satisfactory result was obtained [16]. However, the process still suffered from long extraction time and relatively large quantities of solvent. The ultrasonic assisted extraction is considered as an alternative procedure of the traditional reflux or stirring extraction and as a greener methodology that allows for a high reproducibility in shorter time, simplified manipulation, significant reduction in organic solvent consumption and temperature, and lower energy input [17]. Therefore, the

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objective of this study was to investigate the feasible and efficiency of ultrasonic-assisted two-phase solvent extraction technology (UTSE) in extraction of oil and soy isoflavones, in order to provide an economic and effective path for utilization of SSR. The effects of ultrasonic time, ethanol concentration, ratio of ethanol/water phase to raw material, ratio of hexane phase to raw material, and ultrasonic power on the extraction rates of oil and isoflavones were studied firstly to determine the level of the above factors, then response surface methodology (RSM) was applied to optimize the extraction process. Finally, the qualities of oil and soy isoflavones from SSR were characterized to evaluate their further application.

## 2. Experimental

### 2.1. Experimental material and apparatus

The SSR was provided by Guangdong Meiweixian Flavoring Food Co., Ltd., Guangdong, China. All organic solvents used for UTSE process were of analytical grade and purchased from Beijing Beihua Fine Chemical Co., Ltd., Beijing, China. Acetonitrile used for HPLC was of chromatographic grade (Fisher Scientific, USA), and water used was deionized water. Daidzein, glycitein, genistein and glycitin, used as the standard to determine the yield of total-isoflavones, were purchased from J&K Chemical Co., Ltd., Beijing, China.

The ultrasonicator (Branson, S450D, USA) was used in this study. A 1.27 cm diameter ultrasonic horn probe was fitted to the generator. The ultrasonic frequency of the system was set to 20 kHz, while the power was adjustable, ranging from 0 to 400 W. The system was operated in a pulse mode: 5 s for pulse on followed by 5 s for pulse off in order to avoid overheating.

The high performance liquid chromatography (HPLC, Shimadzu, LC-20AT, Japan) was equipped with two LC-20AT pumps, a SIL-20A autosampler, a CTO-20A column oven and a SPD-20A UV–Vis detector.

### 2.2. Pretreatment of SSR

In general, SSR contains about 10–20 wt% salt. Therefore, desalination of SSR is necessary before extraction. SSR was washed thoroughly with deionized water, then the solid residue was collected by filtration under vacuum with a 400 mesh filter cloth until the filtrate was free from chloride ion. The desalted residue was dried at 60 °C in an oven (DHG-9140, Shanghai Yiheng Technology Co., Ltd., Shanghai, China) until a constant weight was obtained, and the dried residue was ground in a knife mill. The powdered sample was sieved to select particles of 20–40 mesh and stored in dryer for subsequent experiments.

### 2.3. Determination of total oil content in the pretreated SSR

Soxhlet extraction was carried out to determine the total oil content of the pretreated SSR. 5.0 g pretreated SSR and 100 ml hexane were added for reflux in a water bath of 70 °C. After 8 h extraction, hexane was separated from the oil sample by rotary evaporator. The oil was left in the oven for 6 h at 104 °C to remove any hexane that might still be present in the extracted oil. All the experiments in this study were performed in triplicate and the average values were presented.

### 2.4. Determination of total soy isoflavones content in the pretreated SSR

2.0 g pretreated SSR was poured into a 250 ml centrifuged bottle, followed by 40 ml water:ethanol (4:1, v/v) and the mixture was shaken for 2 h at 65 °C. After Cooling to room temperature, the mixture was added into 3 ml 2 mol $L^{-1}$ NaOH and shaken for 10 min, subsequently mixed with 1 ml glacial acetic acid. The mixture was centrifuged at 4500 rpm for 5 min, and the supernatant was filtered through a 0.22 μm membrane filter and its volume was measured precisely. The resulting solution was injected into the HPLC for total isoflavones analysis. All the experiments in this study were performed in triplicate.

### 2.5. Simultaneous extraction of oil and soy isoflavones from SSR using UTSE

3.0 g pretreated SSR, ethanol/water solution with various compositions and hexane were added to a 500 mL three-necked flask, which was equipped with a reflux condenser and an ultrasonic horn probe. The extraction process was carried out at different extraction time and under different ultrasonic power according to the experimental design. After the extraction process, the extraction mixture was poured into a Buchner funnel for the separation of solid phase and liquid phase. The solid phase was dried at 60 °C in an oven until a constant weight was obtained for composition analysis. Then the liquid phase was transferred to a separatory funnel for the separation of ethanol/water phase and hexane phase. The hexane phase was treated by rotary evaporation in order to remove hexane, and the oil sample was left in the oven for 6 h at 104 °C and weighed accurately. The ethanol/water phase was filtered through a 0.22 μm membrane filter and precisely measured its volume. The resulting solution was injected into the HPLC for isoflavones analysis. The extraction rates of oil or isoflavones were calculated as follows:

Extraction rate (%) = extraction amount of oil or isoflavones (g/g)/total oil or isoflavones content in the pretreated SSR (g/g) × 100

### 2.6. Analytical methods

The isoflavones content in the extracts was analyzed using HPLC. A 10 μL sample was loaded onto a symmetry C18 column (TC-C18, 250 × 4.6 mm, 5 μm particle size, Agilent, USA) through an autosampler. The mobile phase was composed of acetonitrile (A) and phosphoric acid/water solution with pH value of 3 (B). The elution was performed with a nonlinear gradient: 0 min, A:B of 12:88; 10 min, A:B of 18:82; 23 min, A:B of 24:76; 30 min, A:B of 30:70; 50 min, A:B of 30:70; 55 min, A:B of 80:20; 56 min, A:B of 12:88, according to Chinese standard GB/T 23788-2009. The eluent flow rate was 1.0 ml/min and absorption was measured at 260 nm.

The fatty acid content of the extracted oil was measured by a gas chromatography (GC) equipped with a flame ionization detector and using nitrogen as carrier gas. The sample was methyl esterized firstly into fatty acid methyl ester (FAME) by $BF_3$/methanol reagent. 1.0 g FAME sample and 0.2 g internal standard were dissolved in 8 ml n-hexane and 1 μl of this solution was injected into the GC. The sample injected was separated in a stainless steel column (2 m × 4 mm). The oven temperature of the GC was programmed from 150 °C to 215 °C at an increasing rate of 5 $°C\cdot min^{-1}$ and was held at 215 °C for 20 min. The injector and detector temperatures were 260 °C and the flow rates of nitrogen, hydrogen and air were 19, 40 and 300 $mL\cdot min^{-1}$, respectively. The fatty acid content was calculated based on the area of FAME over the internal standard [12,13].

The acid value of the extracted oil was measured by the methods described in Chinese standard GB/T 5510-2011. The sample was mixed well with 50 vol% ethanol solution containing a phenolphthalein indicator. The sample was then titrated with a 0.05 N potassium hydroxide solution to the inflection point which was indicated by a color change of the phenolphthalein from yellow

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