



# Optimization of rice lipid production from ultrasound-assisted extraction by response surface methodology



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## ARTICLE INFO

### Article history:

Received 13 April 2015

Received in revised form

23 March 2016

Accepted 6 May 2016

Available online 7 May 2016

### Keywords:

Ultrasound-assisted extraction (UAE)

Convention extraction (CE)

Rice lipid

Response surface methodology (RSM)

### Abbreviations:

CCD

central composite design

CE

convention extraction

GC-MS

gas chromatography and mass spectrometry

RSM

response surface methodology

SFA

saturated fatty acid

UAE

ultrasound-assisted extraction

UFA

unsaturated fatty acid

## ABSTRACT

In this work, a new method of ultrasound-assisted extraction (UAE) of rice lipid was proposed. From the single-factor experimental design, extraction with hexane resulted in the highest yield compared to petroleum ether and isopropanol. The amount of extracted rice lipids yields enhanced with increasing sonication power, temperature and time, respectively. However, excessive sonication power, temperature and prolonged time would lead to the decrease of rice lipid. A response surface methodology (RSM) was used to optimize the parameters on UAE based on single factor experiment. Optimum combinations for lipid extraction yield (77.31%) were hexane, 123W, 42 °C and 37 min. The compositions of lipid were obtained by gas chromatography and mass spectrometry (GC-MS) and no significant differences were observed between UAE and conventional method.

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## 1. Introduction

Lipid concentration from either *japonica* or *indica* rice is about 2.2–2.6%, and there are almost no differences in the composition of lipid classes between the two subspecies (Mano et al., 1999). Though lipid has lower concentration than other compositions such

as carbohydrates and protein, it is regarded as one of the main factors that influence the cooking and eating qualities of rice varieties (Zhou et al., 2002). Lipid is reported to be positively correlated with rice palatability (Yoon et al., 2008). The function of lipid is mainly dependent on the fatty acid compositions such as linoleic acid which is reported to contribute to the high palatability of rice (Yoon et al., 2012). Among fatty acids, the predominant components are palmitic (21–26%), linoleic (31–33%) and oleic acids (37–42%). These three fatty acids are almost made up to 95% of all the fatty acid components in rice (Mano et al., 1999; Yoon et al., 2012).

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Conventionally, lipid is extracted by soxhlet extraction method, which has shortcomings such as time-consuming, low extraction efficiency, and consumption of chemicals and energy. Furthermore, the extraction solvent, petroleum ether is non-polar and unlikely to extract polar bran lipid from rice (Yoshida et al., 2011). In recent years, new separation techniques such as microwave extraction, supercritical fluid extraction, and ultrasound-assisted extraction emerged as alternative methods for lipid extraction. As an environmentally friendly new technique, the ultrasound-assisted extraction (UAE) offers a number of advantages in terms of productivity, yield, quality, and selectivity (Chemat et al., 2011; Chua et al., 2009). After interaction with subjected plant material, the UAE can accelerate its heat and mass transfer rate, and the ultrasound waves can alter plant physical and chemical properties. Besides, the gravitational effect of UAE facilitates the release of extractable compounds and enhances the mass transport by disrupting the plant cell walls (Chemat et al., 2011). As a high efficiency technique, the UAE is widely utilized to assist the extraction of lipid (Adam et al., 2012; Li et al., 2004; Zhang et al., 2008), phenolic compounds (Jermaun et al., 2010), protein (Zhu et al., 2009), enzyme (Yu et al., 2009) and other compounds.

The investigation of rice lipid is important because of its significant impact on seed qualities. Till now, there has been no report about ultrasound-assisted extraction of lipid from rice. The main objectives of this study were to investigate the influences of different solvents, sonication power, temperature and time on the extraction yield of lipid from rice. The results from UAE were compared with that obtained from the conventional solvent extraction. Besides, a three-factor central composite design (CCD) was employed to optimize the extraction parameters based on the results of single factor experiment. In addition, the fatty acid components of rice extracted by UAE and conventional method were compared using gas chromatography and mass spectrometry (GC-MS) analysis.

## 2. Materials and methods

### 2.1. Materials

The rice samples (cv. Koshihikari) were harvested in September 2012 at the Experimental Farm of Sichuan Agricultural University, Ya'an, Sichuan, China. The grains were dried in drying oven (DGG-9246A, Qixin experiment instrument Co., Shanghai, China) at 40 °C for 48 h, and then grounded with micromill. The sifted grain powder screened through 0.125 mm sieves (Daoux Experiment Instrument Co., Shangyu, Zhejiang, China) was collected, and then stored at -25 °C until use.

Extraction solvents (HPLC-grade), including petroleum ether and hexane were purchased from Kelong Chemical Regent Co. (Sichuan, China). Isopropanol was purchased from Xilong Chemical Industry Co., Ltd (Sichuan, China), and benzene and carbinol were purchased from Kermel Chemical Regent Co. (Tianjin, China).

### 2.2. Ultrasound-assisted extraction

Ultrasound-assisted extraction was carried out with an ultrasonic cleaner (KQ-400KDE, Kunshan Ultrasonic Instruments Co., Ltd, Kunshan, Jiangsu, China). Exactly 2.000 g samples were weighed and extracted with 20 ml of solvents in a glass tube, then mixed thoroughly using Vortex Genie 2 (Scientific Industries Inc. Bohemia, N.Y., USA). The tube was immersed into the ultrasonic cleaner bath and extracted with appropriate sonication power, time and temperature as described in the experiment design. The sample thimble was transferred to a centrifuge tube and centrifuged (Allegra 64R Centrifuge, Beckman Coulter, Inc. USA) for 10 min at

5000 rpm. The supernatant was then transferred to flask and evaporated using vacuum rotary evaporator (R-205, BÜCHI Labor-technik AG, Switzerland) at 60 °C, and the sample was finally dried and weighed.

### 2.3. Conventional extraction

Soxhlet extraction method was used as the conventional extraction (CE) according to China GB/T14772-93. The grain powder (2.000 g) was contained in the petroleum ether by using Soxhlet Solvent Extractor (Sox500, Hanon Group Inc., Jinan, China), and extracted for 5 h at 60 °C. The solvent was then evaporated and dried followed by weighing the sample as described above.

### 2.4. Yield determination

As described by Lou et al. (2010), the yield of rice grain lipid was calculated using the following formula:

$$\text{Yield}(\%) = (W_u/W_s) \times 100 \quad (1)$$

where  $W_u$  is the mass of lipid from UAE (g) and  $W_s$  is the mass of lipid from soxhlet extraction (g).

### 2.5. Experimental design

First, this study was to investigate the effects of different solvents (petroleum ether, hexane, and isopropanol), sonication powers (0, 50, 100, 150, 200 and 250 W), sonication temperatures (20, 30, 40, 50, 60 and 70 °C) and sonication time (10, 20, 30, 40, 50, 60, and 70 min), respectively, on the extraction yields of lipid from rice. All factors were tested by single-factor experimental design.

Then, the extraction parameters were optimized by response surface methodology (RSM), a central composite design (CCD) with three variables and five levels was applied to optimize the extraction conditions. The three factors were sonication power ( $x_1$ ), sonication temperature ( $x_2$ ) and sonication time ( $x_3$ ), respectively (Table 1). According to the first single-factor experimental design, the levels of each factor for CCD were identified. For this study, 20 treatments were prepared based on the CCD, and the experiment was carried out in a standard order. The behavior of independent variables for predicting the response variables was explained by the following equation:

$$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{33}x_3^2 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 \quad (2)$$

where  $Y$  is the predicted response,  $\beta_0$  is the intercept,  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  are the coefficients of the linear terms,  $\beta_{11}$ ,  $\beta_{22}$  and  $\beta_{33}$  are the coefficients of the quadratic terms, and  $\beta_{12}$ ,  $\beta_{13}$  and  $\beta_{23}$  are the coefficients of interactive terms. Accordingly, In this model,  $x_1$ ,  $x_2$  and  $x_3$  represent the independent variables. The confidence level was 0.05 and the  $P$ -Values of less than 0.05 by  $F$ -ratio were considered to be statistically significant. The regression model is determined

**Table 1**  
Levels of independent variables established according to the response surface methodology (RSM).

Independent variables	Independent levels				
	-1.682	-1	0	+1	+1.682
Sonication power, $x_1$ (W)	66	100	150	200	234
Temperature, $x_2$ (°C)	23	30	40	50	57
Sonication time, $x_3$ (min)	23	30	40	50	57

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