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REGULAR ARTICLE

Extraction and characterization of oil from Mudong bayberry (*Myrica rubra*) kernels

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ABSTRACT

The extraction of oil from Mudong bayberry (*Myrica rubra*) kernels was performed using the organic solvent leaching method. The extraction time, liquid/solid ratio, and temperature were optimized by the response surface methodology. The fatty acid content of kernel oil was also analyzed using a gas chromatography-mass spectrometry (GC-MS). The results showed that the optimal extraction time, liquid/solid ratio, and temperature for oil yield were 140 min, 7.5:1(v/w), and 48.5°C, respectively. Under optimum conditions, the oil yield was 62.52% (w/w). The GC-MS analysis showed that oleic acid accounted for 47.90% and linoleic acid accounted for 37.30% of total fatty acids in the Mudong bayberry kernels. Furthermore, a small amount of linolenic acid (0.12%), 11-eicosenoic acid (0.29%), and palmitic acid (0.88%) were also found in the extracted oil of Mudong bayberry kernels. This study revealed that Mudong bayberry kernels have a high lipid content, and the percentage of unsaturated fatty acids found is close to those found in other varieties of bayberry and olive oil. Mudong bayberry kernels have a great potential as alternative plant oil in the world.

Keywords: GC-MS; Kernel oil; Mudong bayberry; Organic solvent leaching extraction; Response surface methodology

INTRODUCTION

Bayberry (*Myrica rubra Sieb. et Zucc.*), which belongs to the family Myricaceae, is ubiquitous in China. About 400,000 tons is produced every year (Ministry of Agriculture of the People's Republic of China, 2013). According to previous reports, bayberry kernels contain high levels of oil (58.12-70.79%) (Cheng et al., 2009). Unsaturated fatty acids account for more than 85% of total fatty acids found in bayberry kernels (Zhang et al., 2012);, in which the contents of oleic acid and linoleic acid reach up to 40% and the content of linolenic acid is 0.1% (Xia et al., 2013, Cheng et al., 2008). Linoleic acid plays an important physiological role in reducing hypertension, hyperglycemia, and hyperlipidemia, as well as preventing fatty liver and atherosclerosis (Bhattacharya et al., 2006, Yurawecz et al., 1999). Linolenic acid is the dietary precursor for the long-chain omega-3 PUFA eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). In recent research, Zhang et al. (2012) have investigated the aqueous enzymatic extraction conditions of oil from bayberry kernels using the response surface methodology. Under optimal extraction conditions, the yield of bayberry kernel oil was 31.15%. According to the report from Chen et al. (2013), the oil from bayberry kernels was extracted via the aqueous enzymatic method with cellulase and protease, and the oil yield was 53.79%. The extraction conditions of supercritical carbon dioxide have been optimized for bayberry kernel oil through the response surface methodology by Dong et al. (2014). Through this method, the yield of bayberry kernel oil was approximately 61%. Although the extraction method provided a high extraction rate for bayberry kernel oil, it is costly. Organic solvent leaching is the commonly used method for extracting lipids from oil seeds due to its advantages, such as low costs, low labor intensity, relative high extraction rate, and easily achieved production automation (Zhang et al., 2012, Li et al., 2014).

Mudong bayberry (*Myrica rubra*) is the variety selected by the Agriculture Bureau of Jingzhou County of Hunan Province in 1983 from Mudong Village in Aoshang Town during the general investigation of the species resources of *Myrica rubra Sieb. et Zucc* found all over the county (Ma et al.,

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2006). In 2006, the plantation area of Mudong bayberry was approximately 2667 hectares, which is currently the largest plantation area in Hunan Province (Ministry of Agriculture of the People's Republic of China, 2013). To the best of our knowledge, there are no reports about the extraction of oil from Mudong bayberry kernels. Therefore, the aim of this work was to investigate the optimal extraction conditions for oil from Mudong bayberry kernels using the organic solvent leaching method based on the response surface methodology (RSM) (Wilkinson et al., 2014). The fatty acid compositions of Mudong bayberry kernel oil were also analyzed using the gas chromatography-mass spectrometry (GC-MS). The unsaturated fatty acid compositions of Mudong bayberry kernel oil were also compared with those of other varieties of bayberry kernel oil.

MATERIALS AND METHODS

Raw materials and reagents

Seeds of Mudong bayberries were collected from the Jingzhou County of Hunan Province, China. The n-hexane, aether and other reagents are of analytical reagent grade and purchased from Sinopharm Chemical Reagent Co., Ltd.

Preparation of raw material

Mudong bayberry kernels were obtained through the use of a crushing machine. The bayberry kernels were then dried at 100°C for 25 min in an oven until they reached a moisture content of 4.35%. Finally, the bayberry kernels were milled using a mortar and pestle, and passed through a 16-mesh sieve.

Soxhlet extraction

A conventional Soxhlet extraction (SE) was carried out to compare the extraction performance with the organic solvent leaching method under lab conditions. Briefly, the powder of bayberry kernels (5.00 g) was placed in a Soxhlet apparatus and then continuously extracted for 8 h using aether (34.6°C). After extraction, the aether was removed at 45.0°C under reduced pressure with a rotary vacuum evaporator (Mariod et al., 2015). The oil was then dried in an oven at 65.0°C to remove residual solvent and water. The obtained oil was weighed and the oil yield was calculated (Standardization Administration, 2008). The oil yield of the Mudong bayberry kernels was 67.0% using the SE.

Organic solvent leaching extraction

Bayberry kernel powder (10.00 g) was extracted with a certain proportion of n-hexane in the Erlenmeyer flask using a water bath. After filtering, the filtrate was transferred into an evaporation flask and the solvent was completely removed in a vacuum rotary evaporator. The residue was then dried and cooled. The total weight consisting of lipid and Erlenmeyer flask was measured, and the oil yield of bayberry kernels was calculated using the following formula:

$$P(\%) = \frac{m' - m}{M} \times 100 \quad (1)$$

Where,

P is the oil yield

m' = total weight of the lipid and the Erlenmeyer flask, g

m = the weight of the Erlenmeyer flask, g

M = the weight of the bayberry kernels, which, in this case, is 10 g.

The extraction time, liquid (n-hexane)/solid ratio, and extraction temperature were optimized. Each extraction was performed three times and the average values were calculated.

Experimental design and data analysis

The principle of the Box-Behnken design was followed, and a combination of the RSM and central composite design (CCD) was applied to survey the effects of extraction time, liquid/solid ratio, and temperature on the oil yield based on the mono-factor test, as shown in Table 1. All experiments, which include twelve factorial experiments and five zero-point experiments, were randomly performed. The zero-point experiments were conducted for evaluation of experimental error.

The design software Design-Expert V8.0.6 was used to determine the analysis of variance (ANOVA) and the coefficient of determination (R^2) to estimate the goodness of fit of the mode. The main effects were tested using *F*-tests and the means were compared using least significant difference ($P = 0.05$). ANOVA were used to estimate the correlation of the regression model with actual experimental data. The effects of the variables and their interactions were investigated using response surfaces and contour plots developed from the regression models.

Fatty acid analysis

The fatty acids of bayberry kernel oil were analyzed via a GCMS-QP2010 Ultra GC-MS (Shimadzu, Japan) in accordance with the ISO 5508:1990 method (ISO, 1990). Prior to injection, the extracted oil was converted to its fatty acid methyl esters (FAME) (ISO, 2000).

The flow rate of the carrier gas (He) was 1.0 ml/min^{-1} and the split ratio was 75:1. A 1 µl sample was injected into a

Table 1: The tested factors and levels for the design experiment

Factor	Levels		
	−1	0	1
A time (h)	1.5	2	2.5
B liquid/solid ratio (v/w)	6:1	7:1	8:1
C temperature (°C)	40	45	50

30 m x 0.25 mm x 0.25 μm J&W 122-3262 silica capillary column (Agilent, China). The injector temperature was 250°C. The initial column temperature was 160°C, and after 2 min increased by 6°C/min to 190°C. It was then maintained for 3 min, then increased by 5°C/min to 240°C and maintained for 15 min. The electron impact ionization (EI) mode of the mass spectrometer, set at 70 eV energy, was used. The ion source temperature was 250°C, and the scan range was 30~600 amu. The FAME peaks were identified by retention time compared to FAME standards. Each sample was analyzed in triplicate. The individual fatty acids were quantified from their peak areas and expressed as a percentage of the total fatty acid weight.

RESULTS AND DISCUSSION

Fitting the model

Based on the results from the mono-factor test, RSM was conducted to optimize the extraction conditions. Extraction time, liquid/solid ratio, and temperature at three levels were selected for response surface optimization experiments. The specific experimental design and results are shown in Table 2.

A quadratic regression model equation was established to estimate the relationship between the oil yield (*R*) and three factors, namely, extraction time (A), liquid/solid ratio (B), temperature (C) ($\alpha = 0.05$), and their interactions. The model can be expressed as:

$$R = 1.64 + 1.32A + 0.52B + 0.59C + 0.0025AB - 0.31AC - 0.035BC - 0.83A^2 - 0.26B^2 - 0.16\,C^2 \quad (2)$$

As observed in the regression model (Table 3), the *F* value of the quadratic regression model used in the experiments was 103.41. The *P*-value was less than 0.0001, indicating that the model was highly significant. The lack of fit (1.52) was larger than 0.05, suggesting that it did not reveal a significant impact. The determination coefficient (R^2) of the model was 0.9925, which suggests that the model has a high goodness of fit. The correlation coefficient, Pred R^2,was 0.9310, indicating that the calculated values were in agreement with the determined values.

The curve for actual values and predicted values in Fig. 1 revealed the consistency of the obtained model. The calibration coefficient of the model, Adj R^2, was 0.9829, suggesting that this model can explain the change of response. Based on the above factors, we deduce that the model was appropriate for the responses and had good precision and reliability.

From the above model and Table 3, it can be seen that the extraction time had the largest effect on the yield of oil, followed by the liquid/solid ratio ($p < 0.05$), and the interaction between time and temperature. Three second-order terms (the interactions between time and liquid/solid ratio, temperature and liquid/solid ratio, and quadratic term of temperature) of three processing parameters had negative effects on the oil yield.

Table 2: Experimental design and corresponding oil yield

Tests	Time (h)	Liquid/solid ratio (v/w)	Temperature (°C)	Oil yield (%)
	A	B	C	R
1	−1	−1	0	58.59
2	1	−1	0	61.26
3	−1	−1	0	59.83
4	1	1	0	62.51
5	−1	0	−1	58.48
6	1	0	−1	61.70
7	−1	0	1	60.22
8	1	0	1	62.21
9	0	−1	−1	60.14
10	0	1	−1	61.05
11	0	−1	1	61.45
12	0	1	1	62.22
13	0	0	0	61.46
14	0	0	0	61.62
15	0	0	0	61.83
16	0	0	0	61.71
17	0	0	0	61.56

Table 3: Results of the analysis of variance to the response surface model

Source	Sum of squares	df	Mean square	*F* value	*P*-value Prob >*F*	
Model	22.76	9	2.53	103.41	<0.0001	**
A-time	13.94	1	13.94	569.96	<0.0001	**
B-liquid/solid ratio	2.17	1	2.17	88.88	<0.0001	**
C-temperature	2.80	1	2.80	114.35	<0.0001	**
AB	0.00	1	0.00	0.00	0.9754	-
AC	0.38	1	0.38	15.47	0.0057	**
BC	4.9E-3	1	4.9E-3	0.20	0.6680	-
A^2	2.87	1	2.87	117.32	<0.0001	**
B^2	0.29	1	0.29	11.91	0.0107	*
C^2	0.11	1	0.11	4.30	0.0769	-
Residual	0.17	7	2.4E-2			
Lack of fit	9.1E-2	3	0.03	1.52	0.3396	-
Pure error	0.08	4	0.02			
Cor total	22.93	16				
R^2=0.9925 R^2 Adj=0.9829 R^2 Pred=0.9310						

*,**Indicates significance at *P*=0.05 and *P*=0.01, respectively

The cross-interaction between the extraction factors

Fig. 2 shows that the 3D plot of the response surface (a) and contour plots (b) for the extraction yield of oil is related to extraction time and liquid/solid ratio. With an increase in extraction time and liquid/solid ratio, the extraction yield rose at first, but then slowed down. A similar trend was also observed in other two interaction conditions

on the extraction yield, as shown Fig. 3 and Fig. 4. It demonstrated that a moderate extraction time, liquid/solid ratio, and temperature resulted in a high yield of oil. At a given liquid/solid ratio, oil yield increased obviously with an increase in extraction time, as is evident in Fig. 2b. The oil yield only increased slightly with an increase in liquid/solid ratio at any given time. This means that extraction time had a more significant effect on oil yield than the liquid/solid ratio.

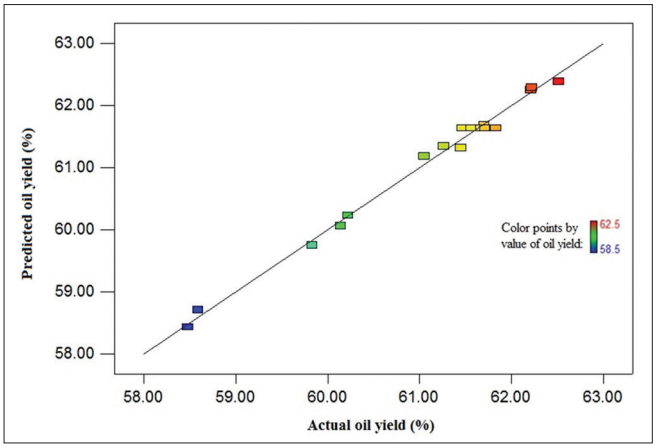

Fig 1. Experimental data vs. predicted data plot.

In Fig. 3, when the extraction time increased, the oil yield increased substantially first and after about 2.3 hours of extraction, remained at a constant amount. This was likely due to the fact that the oil had already been completely extracted, and there was very little oil left in the Mudong kernels. The extraction temperature had a significant effect on the oil yield, but less than the extraction time.

Verification of predictive model

In Fig. 4, we observed that the oil yield increased significantly with an increase in both the liquid/solid ratio and temperature at the given extraction time. The oil yield increased quickly from 40.0°C to 46.0°C. At high temperatures, there was a smaller increase in oil yield. This may be attributed to the fact that n-hexane is more volatile at high temperatures (n-hexane boiling point: 68.74°C).

The optimal parameters obtained using the proposed model are as follows: extraction time of 2.31 h, liquid/solid ratio of 7.62:1 (v/w), and extraction temperature of 48.46°C. Under these optimal conditions, the maximum predicted value for oil yield was 62.54%. In order to facilitate operations, the process parameters were changed slightly. Considering the factors involved, an extraction time of 140 min, liquid/solid ratio of 7.5:1 (v/w), and

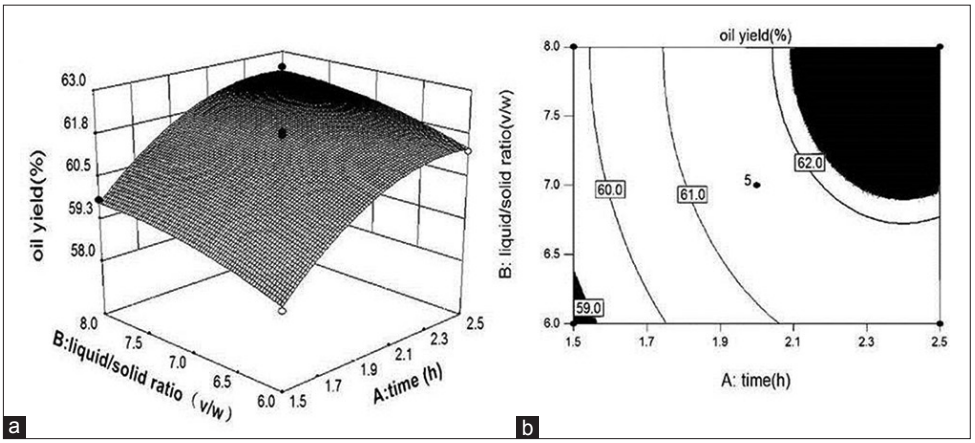

Fig 2. Response surface (a) and contour plots (b) for the effect of extraction time and liquid/solid ratio on the oil yield.

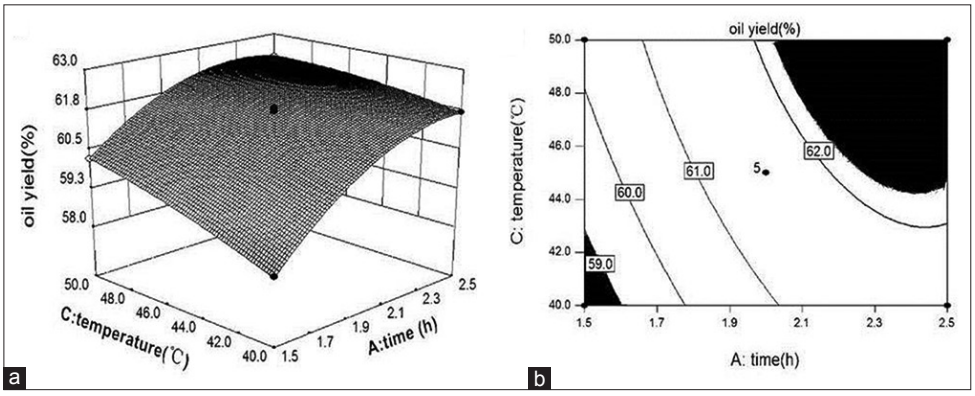

Fig 3. Response surface (a) and contour plots (b) for the effect of extraction time and temperature on the oil yield.

extraction temperature of 48.5°C are more suitable. The real laboratorial value of oil yield was 62.52± 0.22%.

These results indicate that the established model is reliable and the optimal extraction parameters can be applied in practical production.

Fatty acid composition of kernel oil

The fatty acid composition of Mudong bayberry oil are presented in Table 4 and Fig. 5. As shown in Table 4, Mudong bayberry oil is very abundant in unsaturated fatty acids, with a content of 86%, which is close to the unsaturated fatty acids content in Jiangxi wild bayberry oil (86%), Zhejiang Biqi bayberry oil (84%), Zhejiang Shui bayberry oil (85%), olive oil (80%), and camellia oil (90%) (Xia et al., 2013, Allalout et al., 2009). The proportion of oleic acid (47%) and linoleic acid (37%) in Mudong bayberry oil was close to that found in Zhejiang Biqi bayberry oil and Zhejiang Shui bayberry oil (Xia et al., 2013). In addition, a small amount of linolenic acid (0.12%) was detected. The total mass fraction of detected compounds is 99.98%. The difference of fatty acids composition in these bayberry kernel oils may be due to the cultivar, climatic conditions, genetics, and post-harvest treatment.

Table 4: Components of fatty acid in kernels oil and their relative content

Type	Fatty acid	Relative content (%)	Total content (%)
Unsaturates	Oleic acid (C18:1)	47.90±0.80	86.49±0.76
	Linoleic acid (C18:2)	37.30±0.18	
	Linolenic acid (C18:3)	0.12±0.07	
	Palmitoteic acid (C16:1)	0.88±0.04	
	11-Eicosenoic acid (C20:1)	0.29±0.01	
Saturates	Palmitic acid (C16:0)	10.40±0.17	13.49±0.10
	Stearic acid (C18:0)	3.00±0.15	
	Arachidic acid (C20:0)	0.09±0.01	

CONCLUSIONS

In the present study, Mudong bayberry kernel oil was first extracted and analyzed using an organic solvent. Extraction conditions were optimized through the response surface methodology. This study revealed that Mudong bayberry kernels have a high lipid content, and the percentage of unsaturated fatty acids found is close to those found in other varieties of bayberry and olive oil. The GC-MS

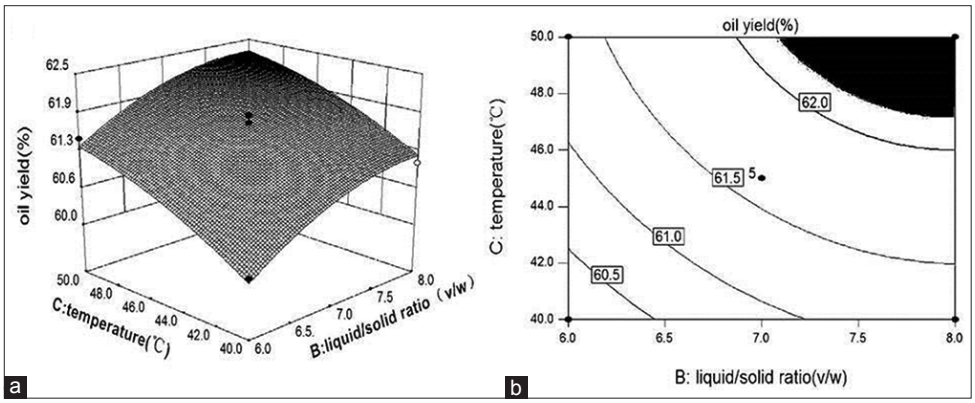

Fig 4. Response surface (a) and contour plots (b) for the effect of temperature and liquid/solid ratio on the oil yield.

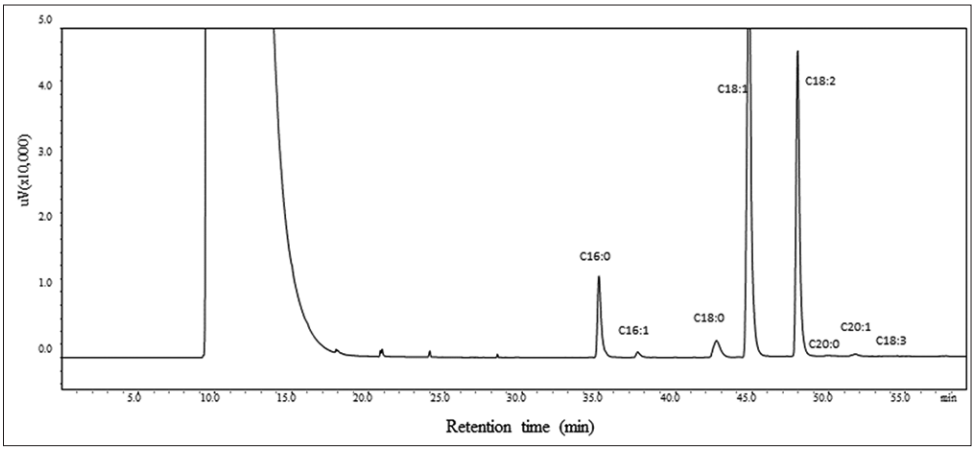

Fig 5. GC chromatogram of FAME analysis for oil extracted from Mudong bayberry kernel using organic solvent leaching method.

analysis showed that oleic acid accounted for 47.90% and linoleic acid accounted for 37.30% of total fatty acids in the Mudong bayberry kernels. Furthermore, a small amount of linolenic acid (0.12%), 11-eicosenoic acid (0.29%), and palmitic acid (0.88%) were detected in them. These results indicate that Mudong bayberry kernels have the potential to be exploited as a novel source for edible plant oil in the world.

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Authors' contributions

Ke Li designed and performed experiments and wrote the paper; Ya-Fang Huang andJin-Kui Ma analysed the data and wrote the paper; Luo-Ming Li and Zong-Jun Li developed analytical tools and supervised the project. All authors discussed the results and implications and commented on the manuscript at all stages.

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