Applied Biological Research 25(3): 303-310; (2023)
DOI: 10.5958/0974-4517.2023.00035.6

QUALITY CHARACTERISTICS OF *Symplocos paniculata* (Thunb.) Miq. FRUIT OIL OBTAINED BY DIFFERENT EXTRACTION METHODS

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(Received 25 March, 2023; accepted 30 June, 2023)

ABSTRACT

The aim of present study was to develop an optimum extraction method for high quality edible oil from *Symplocos paniculata* fruit. *S. paniculata* fruit oils were extracted by three methods *viz.*, cold pressing (PCP), subcritical extraction (SBE) and Soxhlet extraction (SE). A comparative analysis was performed for their physiochemical properties, fatty acid composition and bioactive substances. The oil extraction rate from SE, SBE and PCP was 36.6, 35.7 and 8.4%, respectively. Total unsaturated fatty acids present were in the range of 79.04-79.35% with 48.13-48.52% oleic acid, and 30.08-30.18% linoleic acid. The ratio of saturated fatty acids to total unsaturated fatty acids was 0.26025~0.2652. PCP extraction oil had the highest content of polyunsaturated fatty acids, while SBE extracted oil had highest content of unsaturated fatty acids, along with rich bioactive substances. Based on the criterion of extraction rate, oil composition and bioactive substances, the SBE method appeared optimum method for extracting high quality edible oil from *S. paniculata* fruits.

Keywords: Bioactive substances; fatty acids; oil extraction method; *Symplocos paniculata*

INTRODUCTION

Symplocos paniculata (Thunb.) Miq., a member of family Symplocaceae, is widely distributed in tropical and subtropical areas of the world (Fu *et al.*, 2003) and has long been used as traditional herbal medicine of treating mastitis, lymphadenitis, enterodynia, gastric cancer, boils, skin itching, hernia and urticarial in China (Yang *et al.*, 2019). Its root and leaf contain bioactive compounds that control pathogen. Its fruit are rich in oil, characterized by unsaturated fatty acids, and have significant antimicrobial, analgesic and anti-inflammatory activities (Semwal *et al.*, 2011). The fruits of *S. paniculata* have been exploited for extraction of industrial and edible oil. Besides, *S. paniculata* fruit oil possess bioactive substances *viz.*, sterols, vitamin E (Ve) and squalene, which are beneficial for health (Li *et al.*, 2016). However, bioactive compounds are affected by the conditions under which the oil extraction is carried out. In other words, the oil extraction method directly affects rate of oil extraction and oil quality (Zhao *et al.,* 2019). Among the different extraction methods followed, physical cold pressing (PCP) method reportedly is operated easily and involves low process cost and is environment-friendly, but oil yield is low (Li *et al.,* 2016). Compared to the physical PCP, and Soxhlet extraction （SE) [refers to the method of extracting oil with organic solvents] gives higher oil yield with less oil in residue (Wang *et al*., 2015). However, there was an amount of residual solvent left in the crude oil. The subcritical extraction (SBE) method is an excellent method because of its advantages of recovering the residual solvent, which would lead to a better oil quality as compared to the traditional SE (Ma *et al.,* 2010). So the use of a suitable oil extraction method is key to get high quality oil (Wang *et al*., 2015). The oil yield of coconut obtained by SBE extraction method was high with rational fatty acid composition, and health bioactive substances (Zhou *et al*., 2017). In present study, the characteristics of fruit oil extracted by three methods (PCP, SE and SBE) were analysed for oil quality, and to find the optimum extraction method for *S. paniculata* fruit oil. The study, therefore, aimed to find the scientific basis and technical reference for the utilization of *S. paniculata* resources.

MATERIALS AND METHODS

Plant material, seed conditioning, and handling

Matured fruits of *Symplocos paniculata* were collected from the experimental station at Hunan Academy of Forestry (Changsha city, Hunan Province, China). *S. paniculata* fruits were dried in an oven at 60°C for 4 days until the weight remained constant, then the fruits were ground in a hammer mill (RT-02 miniature plant pulveriser) and sieved through serial stainless steel sieves to 0.15 ram.

Oil extraction methods

Physical cold pressing (PCP): The powdered fruit samples (500 g) were placed into PCP apparatus (Biodiesel Research Engineering Center, Hunan Province, China) and processed at 10 rpm screw rotation speed (Naebi *et al.,* 2022). During processing, the temperature was maintained at lower than 50°C.

Subcritical extraction (SBE): The powdered fruits (500 g) were placed into a sub-critical fluid extraction apparatus (Biodiesel Research Engineering Center, Hunan Province, China), and the oil extracted using butane (C_4H_{10}) at room temperature and 0.5 Mpa pressure in a sub-critical fluid extraction device for 7 h (Li *et al.*, 2016). The collected micelle was then vacuum-evaporated to obtain the extracted oil.

Soxhlet extraction (SE): The powdered fruits (10 g) and 60 mL hexane were placed to a SZE-101Fat Analyzer (Shanghai ShineJan Instruments Co., Shanghai, China). The contents were rapidly heated to reflux for 6 h with vigorous stirring After cooling, the contents were filtered. The hexane was evaporated to dryness in a rotary vacuum evaporator at 62.5°C, and the oil was recovered.

All the extracted oils were immediately centrifuged at 9000 rpm for 15 min, placed in amber-coloured glasses, and stored in a fridge till use. The final weight was recorded and used to calculate the oil content (w) using the following formula:

$$x = \frac{(\mathrm{Ma} - \mathrm{Mb})}{\mathrm{Ma}} \times 100\%$$

Where Ma represents the weight of sample prior to extraction and Mb denotes the weight of sample subsequent to the extraction (with a precision determined at 0.0001 g).

Physiochemical properties of fruit oil

Assessment of the characteristics of fruit oil: Refractive index was determined using refractive index tester (Shanghai INESA Optical Instrument Co., Shanghai, China). The colour of samples was determined by WSL-2 Lovibond colorimeter (Nanjing Jiaozi Teng Scientific Equipment Co.). Iodine value (IV) was measured as per to the Chinese national standard method GB/T 5532–2008. Acid value (AV) and saponification value (SV) of oil samples were determined using automatic titration analyser and hydroxide-ethanol titration (Sun *et al.,* 2020). The phospholipid content of oil samples was measured as per the Chinese national standard method GB /T 5531-2008.

Fatty acid composition: The fatty acid composition was determined by gas chromatography after transesterification (Liu *et al.,* 2015). The fatty acid methyl esters (FAMEs) were prepared by vigorously shaking the solution in oil (0.1 g), n-hexane (4 mL) with 0.5 mol mL^{-1} KOH-methanol (2 mL) at 40°C for 20 min. The upper phase was transferred to sample vials for analysis. The analysis was performed on an Nexis GC-2030 gas chromatograph equipped with a Shim-pack VP-ODS capillary column (100 m × 0.25 mm i.d., 0.25 µm film) and a flame ionization detector (FID). Nitrogen was used as carrier gas at a flow rate of 50 mL min^{-1}. The column temperature was initially set at 100°C for 13 min; subsequently increased to 180°C at a rate of 10°C min^{-1}, where it was held for 6 min; then increased to 200°C at a rate of 1°C min^{-1}, where it was held for 20 min; and finally increased to 230°C at a rate of 4°C min^{-1}, where it was held for 10.5 min. The mass spectrometer was operated in electron impact energy of 70 eV, and scanning quality ranged from 15 u to 500 u. The FAMEs were identified by the retention time compared to the corresponding standards. The individual FAMEs were expressed as weight percent (area normalization). The analysis by gas chromatography of methyl esters of fatty acids was accredited to GB/T 17377-2008. by China National Standardization Management Committee and the State Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China. The fatty acid components were identified using the Wiley mass spectral library. Relative percentage of fatty acid compositions was determined on the basis of the peak areas (Wang *et al.,* 2018).

Bioactive substances

Phytosterol content: Oil sample (~ 0.5 g) was mixed with 2 mol L^{-1} KOH-ethanol (2 mL) at 80°C for 3.5 h. The saponified oil was extracted using hexane. The collected oil was vacuum-evaporated (40°C, vacuum 0.6 bar and rpm 50) to obtain the extracted oil (Zhao *et al.,* 2018) and then sampled on dried silica gel plate, spread, scraped down the strip into glass tube. After that, 10 mL anhydrous ether was added to extract the unsaponifiable matter for three times. All the organic phases were combined and evaporated. Then 0.8 mL pyridine and 0.2 mL trimethylchlorosilane were added and silylated at 105°C for 15 min, and the resulting product redissolved in 0.5 mL hexane.

The phytosterols were analyzed using a GC-MS equipped with a HP-5 capillary column. The initial column temperature was set at 300°C. Helium was used as the carrier gas with a flow rate of 1 mL min^{-1}. The FID detector temperature was 360°C, and the injection volume 1 µL. The oven temperature was programmed to increase to 285°C and maintained at 285°C for 30 min, and then increased to 300°C at a rate of 10°C min^{-1}, and finally maintained at 300°C for 5 min. According to the corresponding phytosterols standard product, the standard curve was drawn to calculate the content of phytosterols in the sample.

Squalene content*:* The oil sample (2 g) was added to 50 mL KOH anhydrous ethanol solution (1 mol L^{-1}) in a 250 ml flask (Tong *et al.,* 2018). In a constant temperature (80°C) water-bath, water reflux saponification reaction was carried out at room temperature for 50 min (Wu *et al.*, 2021). The saponification material was transferred to a 250 mL split funnel; and then 50 mL n-hexane added to it and rested for 10 min (Tong *et al.,* 2018). After the solution was stratified, the upper organic phase was transferred to another 250 mL split funnel. The three hexane extracts were placed in the same funnel. The organic phase was washed to neutral with 10% ethanol solution. The remaining water was removed with anhydrous sodium sulphate. The solvent was rotated and evaporated in 40°C water bath. Oil (1 g) was dissolved in 10 mL n-hexane and then filtered through a 0.45 µm organic membrane filter. The 15 µL product was introduced into the HPLC system for analysis.

The squalene contents were measured using GC-mass spectrometry (GC-MS) equipped with a HP-5 capillary column (30 mm × 0.25 mm × 0.25 µm) (Wu *et al.*, 2021). The oven temperature was programmed to increase from 160 to 280°C at a rate of 15°C min-, and maintained at 280°C for 5 min; then increased to 300°C at a rate of 5°C min^{-1}, and maintained at 300°C for 5 min. The injector and detector temperatures were 300 and 330°C, respectively. Helium was used as carrier gas at an average velocity of 30 mL min^{-1} and air was injected with a flow rate of 400 mL min^{-1}. According to the corresponding squalene standard product, the standard curve was drawn to calculate the content of squalene in the sample.

Ve content: The Ve content in oil was determined by using HPLC-FLD as per the method described by Tuberoso *et al*. (Liu. *et al.*, 2020) with a slight modification. For this, 1 mL oil was filtered through a 0.22 µm organic membrane into a liquid flask. The conditions maintained were: mobile phase: 1, 4-

dioxane: heptane = 3:97; flow rate: 1 mL min^{-1}; silica gel column: 4.6 mm × 250 mm × 0.5 µm, column temperature 30℃; fluorescence detector wavelength: λex = 292 nm, λem = 325 nm. The standard curve was drawn to calculate the content of VE in the sample by using corresponding Ve standard product.

EXCEL2021 and Origin software are used for data collation, analysis and chart production.

RESULTS AND DISCUSSION

Oil extraction rate of different methods

The oil extraction rate from *S. paniculata* fruit during Soxhlet extraction (SE), subcritical extraction (SBE) and physical cold pressing (PCP) was 36.6, 35.7 and 8.4%, respectively. Obviously the PCP method showed the lowest extraction efficiency. PCP extracted 63.4% less oil than SE. There was no significant reduction in oil content between SE and SBE (Fig. 1). Of the three extraction methods tested, SE yielded the highest oil content. However, it has certain drawbacks such as requiring a high process time and temperature, which can have adverse effects on the quality of the oil (Khoddami *et al*., 2011), while the SBE produced oil with non-toxic and harmless properties, along with high oil extraction efficiency, which was suitable for large-scale oil production (Juhaimi *et al*., 2019).

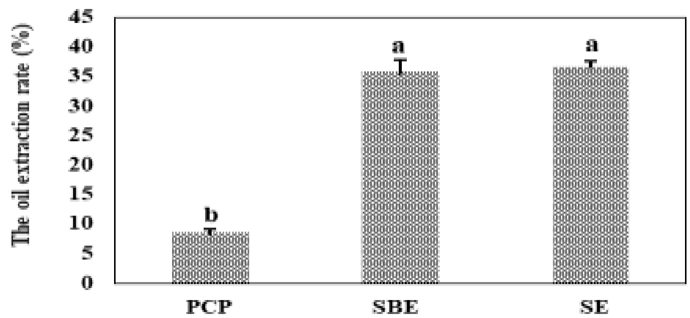

Fig. 1: Effect of different methods on *Symplocos paniculata* oil extraction rate; PCP: Physical cold pressing, SBE: Subcritical extraction, SE: Soxhlet extraction; Results were expressed as mean ± standard deviation (n = 3), and same lower case letters labelled indicate oil recovering rate were not significantly different among treatments by Tukey's honestly significant difference (P <0.05).

Fatty acid compositions of oil extracted by different methods

Although the oil content was affected by oil extraction methods, there were no remarkable difference in fatty acid components of the oils extracted by different methods (Fabrice *et al.*, 2018). The oils mainly composed of two saturated fatty acids (SFA) *viz*., palmitic acid (C16:0) and stearic acid (C18:0); and three unsaturated fatty acids (UFA) *viz*., oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) (Fig. 2). Similar phenomena were observed in the extraction of *Torreya grandis* kernel oil, with insignificant difference in the fatty acid components using various techniques (Shi *et al.*, 2018).

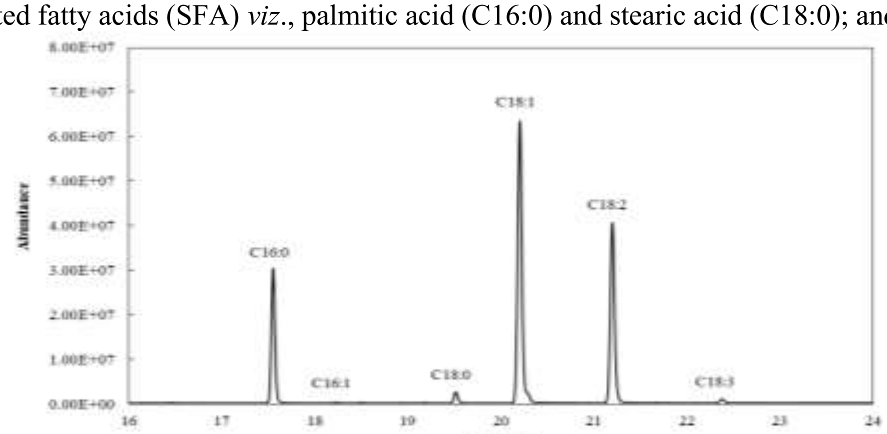

Fig. 2: GC chromatogram of *Symplocos paniculata* oils using sub-critical fluid extraction

There were no statistical differences (P>0.05) in fatty acids composition of the oils extracted by the three test methods (Table 1). The oleic acid content in all the oils was higher than linoleic acid, and the average relative content of oleic acid as high as 48%. The total content of polyunsaturated fatty acids extracted by PCP was 30.91%. The relative content of polyunsaturated fatty acids such as linoleic acid and linolenic acid in the oil obtained by SBE method was the lowest, but the oleic acid content was the highest with the value of 48.52 %, and the oil from SBE had more unsaturated fatty acid (79.35%) than the other two methods.

Table 1: Fatty acid composition of *Symplocos paniculata* fruit oils extracted by different methods

S, No.	Fatty acids	Retention time (min^{-1})	Relative fatty acid content (%)		
			PCP	SBE	SE
1.	Palmitic acid (C16:0)	17:55	19.16	18.98	19.10
2.	Stearic acid (C18:0)	19:53	1.80	1.67	1.80
3.	Oleic acid (C18:1)	20:20	48.13	48.52	48.23
4.	Linoleic acid (C18:2)	21:20	30.18	30.08	30.13
5.	Linolenic acid (C18:3)	22:37	0.73	0.75	0.74
	- Saturated fatty acids		20.96	20.65	20.90
	- Monounsaturated fatty acids		48.13	48.52	48.23
	- Polyunsaturated fatty acids		30.91	30.83	30.87
	- Unsaturated fatty acids		79.04	79.35	79.10

Physicochemical properties of oils extracted by different methods

Physicochemical properties of oil are the most important indices to evaluate oil quality. The physical properties of *S. paniculata* fruit oil extracted by PCP and SBE methods were orange viscous liquid with slightly flocculent precipitation. While the oil extracted by Soxhlet method was yellow and transparent without precipitation (Fig. 3; Table 2). The fruits oil obtained from SBE had lowest acid value (1.32 mg KOH g^{-1}), which expectedly would reduce the oil refining cost. While PCP produced the oil having highest saponification value of 181.4 mg KOH g^{-1} (Table 3). There were no significant differences in saponification value, iodine value and refractive index of fruit oil extracted by test methods. Cong *et al.* (2020) reported similar findings during walnut oil extraction.

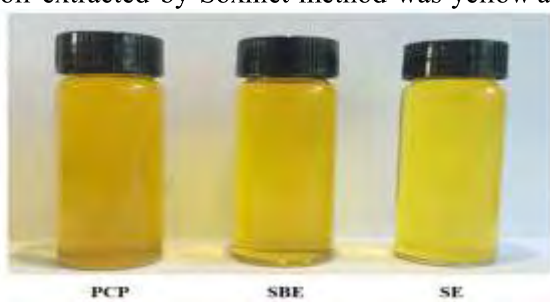

Fig. 3: The appearance of *S. paniculata* fruit oils obtained by different extraction methods

Table 2: Physical property of *Symplocos paniculata* oil extracted by different methods

Physical property	Oil extraction methods		
	PCP	SBE	SE
Colour (Lovibond)	Yellow 28, Red 9.7	Yellow 17, Red 2.5	Yellow 21, Red 1.6

Table 3: Chemical property of *Symplocos paniculata* oil extracted by different methods

Chemical properties	Oil extraction methods		
	PCP	SBE	SE
Acid value (mg KOH g^{-1})	0.91^{b}	1.32^{a}	0.54^{c}
Iodine value (g 100 g^{-1})	85^{a}	88^{a}	83^{a}
Saponification value (mg KOH g^{-1})	181.4^{a}	180.5^{a}	183.4^{a}
Refractive index (25°C)	1.4692^{a}	1.4676^{a}	1.4665^{a}

Means with same lowercase letters within a chemical property are not significantly different among oil extraction methods by Tukey's honestly significant difference multiple comparison at P < 0.05.

Bioactive substances in the oils extracted by different methods

The oil accompanying bioactive substances have many physiological functions like phytosterol control cancer cell and regulate endocrine (Zhang *et al.,* 2018). Vitamin E (Ve) is widely used as antioxidant in the oxidative inhibition of unsaturated fatty acids and the scavenging of free radicals generated by lipid peroxides (Wang *et al*., 2017). Squalene contains natural bioactive ingredients that can prevent some diseases and enhance immunity, so is widely used in health care and cosmetics (Peng *et al.,* 2018). The contents of phytosterol, vitamin E, and squalene in *S. paniculata* fruit oil extracted by three test methods were determined. Of the three methods, SBE extraction oil had highest content of 2.42 mg g^{-1}, 15.24 mg g^{-1} and 0.146 g kg^{-1} of phytosterol, vitamin E, and squalene content, respectively (Table 4). The oil extracted by PCP method had less contents of phytosterols, vitamin E and squalene than that of SBE, but higher than that of oil extracted by SE. PCP squeezed out all water and fat-soluble substances, which resulted in deep oil colour and lustre; while SE and SBE extraction solvents appeared to have different selectivity to water-soluble and fat-soluble bioactive substances in the fruit. The hexane in SE is a non-polar solvent so probably are mainly involved in the dissolution and/or solubilisation of oil in solvent. Whereas, the solvent butane in SBE is both water and fat soluble under pressure condition, so appears to have been used for oil solubilisation, but also allowed to keep the water-soluble in oil and fat-soluble bioactive substances better (Teixeira *et al.,* 2018).

Table 4: Bioactive substances of *Symplocos paniculata* oils extracted by different methods

Bioactive substances	PCP	SBE	SE
Phytosterol content (mg g^{-1})	1.12^{b}	2.42^{a}	0.353^{c}
Vitamin E content (mg 100 g^{-1})	13.59^{ab}	15.24^{a}	9.78^{b}
Squalene content (g kg^{-1})	0.0631^{b}	0.146^{a}	0.0215^{c}

[z]Means with same lowercase letters within bioactive substances' content are not significantly different among oil extraction methods by Tukey's honestly significant difference multiple comparison at $P < 0.05$.

Oxidative stability of oils extracted by different methods

The high phospholipid content in oil and grease can easily increase the water content in oil and promote hydrolysis and rancidity of oil. In addition, phospholipid has emulsification property so may produce a lot of foam on heating which may promote the oxidation of oil and make oil black and bitter after heat, thus affecting the use of oil. Therefore, in oil refining process it is necessary to remove the phospholipids by hydration and degumming. The high content of phospholipids increases the cost of oil refining. The different extraction methods exhibited great influence on phospholipid content of *S. paniculata* fruit oil. The phospholipid content was high (1.8251%) in the oil extracted by SE, followed by SBE method (1.6727%), and the phospholipid content in oil extracted by PCP was lowest (0.4293%). The phospholipid content in oil extracted by PCP was significantly lower than that obtained by SE and SBE methods, which may be due to phospholipids are insoluble in organic solvents. Therefore, the use of PCP can simplify the subsequent refining process and reduce the cost of degumming.

Conclusions: The highest extraction rate of oil was in SE method (36.6%), while it possessed lowest bioactive. Extraction methods showed no significant effect on refractive index, fatty acid composition of crude fruit oil, but had remarkable effects on saponification value and acid value of crude fruit oil. The polyunsaturated fatty acids content was highest in PCP extracted oil. Unsaturated fatty acids like monounsaturated fatty acids and polyunsaturated fatty acids are beneficial to human health. The essential fatty acids required by human body are polyunsaturated fatty acids which favourably influence in lowering blood lipid, improving blood circulation, inhibiting platelet agglutination, inhibiting atherosclerotic plaque and thrombosis, and have good prevention and treatment effects on cardiovascular and cerebrovascular diseases. The oil accompanying bioactive substances by using SBE method appears to be relatively better and more valuable. Phospholipids affect the oil colour and taste, etc. Therefore, it is important to consider phospholipids content while selecting the oil extraction method. If one wants to save the refining cost, then PCP is a better option. Our results will serve as a foundation to explore extraction methods especially oils of *S. paniculata* to achieve higher fruit oil quality. Also, the findings may provide reference for other researches on woody oil plants. There are still many questions need a further researching, whether extraction temperature and particle size the effects of on the quality oil?

Acknowledgments: The study was financially supported by Natural Science Foundation of Hunan Province (2022JJ31001), Science Foundation of Education Department of Hunan Province (21B0244), State Key Laboratory of Utilization of Woody Oil Resource (GZKF202102) and the talent acquisition project of Central South University of Forestry & Technology (2020YJ011).

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