Vietnam Journal of Agricultural Sciences

ISSN 2588-1299

VJAS 2019; 2(4): 469-474
https://doi.org/10.31817/vjas.2019.2.4.03

Green Solvent Extraction and Quality Characteristics of Passion Fruit Seed Oil (*Passiflora edulis* Sims var. *edulis*)

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Abstract

The extraction of oil from passion fruit seeds with acetone, ethanol, ethyl acetate, isopropanol, and hexane was studied. The effects of the variables, namely type of solvent, material to solvent ratio, temperature, and extraction time, were investigated. The highest extraction yield was 78.52%, which was obtained using ethyl acetate with a material to solvent ratio of 1/10 at room temperature (28^{o}C) for 4h using a shaker. This yield was similar to that obtained when using hexane as a solvent. Our results indicate that ethyl acetate can replace the conventional hexane solvent in the extraction of oil from passion fruit seeds. The high content of polyunsaturated fatty acids in passion fruit seed oil suggests that this product has good potential for use in the human food, cosmetic, and pharmaceutical industries.

Keywords

Oil extraction, *Passiflora edulis* Sims var. *edulis*, passion fruit seed oil, polyunsaturated fatty acids

Introduction

The purple passion fruit (*Passiflora edulis* Sims var. *edulis*) and the yellow passion fruit (*Passiflora edulis* Sims var. *flavicarpa*) are two subvarieties of *P. eduliks*, which is the significant commercial value variety of the family Passifloraceae (Rehm & Espig, 1984). The purple passion fruit is mainly grown for fresh fruits and concentrated juice processing in Vietnam. The industrial processing of passion fruit juices produces large amounts of by-products, including seeds and peels, that account for up to 60% of the total fruit weight (Silva *et al.*, 2015). While discarding passion fruit seeds may lead to an increasing environmental problem, their high lipid content, around 30% (Oliveira *et al.*, 2014; Silva *et al.*, 2015), could be employed in the food and cosmetic industries (Oliveira *et al.*, 2013).

In general, the extraction of oil from the seeds can be done easily and efficiently using organic solvents, and the efficiency mainly depend

Received: June 9, 2019
Accepted: December 25, 2019

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on the choice of solvents (Cowan, 1999). Hexane is the most popular solvent for oil extraction due to its low cost and the high solubility of oils in hexane. However, hexane is not allowed to be used as a solvent "for all uses" in the food industry because of environmental and public health concerns. There are a number of solvents that are friendly to the environment, like acetone, ethanol, ethyl acetate, and isopropanol, because they generate less waste than other solvents (Oliveira *et al*., 2013) and can be used in the food industry (Directive 2009/32/EC of the European Parliament and of the Council). Besides solvents, the material to solvent ratio as well as the extraction temperature and time are generally known as factors affecting oil extraction from plant materials.

The objectives of this work were: (i) to investigate the effects of process variables on the oil extraction from passion fruit seeds using a shaker with green solvents (acetone, ethanol, ethyl acetate, and isopropanol) in comparison with using the conventional solvent (hexane); and (ii) to determine the chemical and quality characteristics of the oil obtained from the seeds of a purple passion fruit variety commonly grown in Vietnam. This study was the first step in investigating the exploitation of passion seeds as an additional dietary source of an essential polyunsaturated fatty acid (PUFA).

Materials and Methods

Sample preparation

Purple passion fruit seeds (*Passiflora edulis* Sims var. *edulis*) were obtained from the Nafoods Group (Nghe An, Vietnam) in November 2017. They were by-products of the production of passion fruit concentrated juice. Approximately 20kg of fresh seeds were collected and transported to the laboratory on the day of processing at the factory. The seeds were first washed with tap water to remove the aril around the seeds, rinsed in distilled water three times, and then dried under sunlight to 10% moisture content. Before extraction, the dried seed samples were ground into a fine powder form (from 0.5 to 1mm in diameter) with a laboratory grinder (HR2115, Philips) and kept in a sealed container (dark, dry, and at 4ºC).

Sample extraction

The dried passion fruit seed powder (5g) was mixed with different solvents (acetone, ethanol, ethyl acetate, isopropanol, or hexane) in Erlenmeyer flasks with rubber tops. The solvents were added at different sets of seed to solvent ratios of 1/4, 1/6, 1/8, 1/10, and 1/12. The Erlenmeyer flasks then were placed in an orbital incubator shaker Gyromax 727 (Amerex Instruments, Inc., USA) with a shaking speed of 150 rounds/min. The extraction times were 0.5, 1, 2, 3, 4, 5, and 6 hours at varying temperatures (room temperature, 40, 50, 60, and 70ºC). Afterwards, the crude extracts were transferred to 50mL falcon conical centrifuge tubes and centrifuged at 6,000rpm for 20 minutes with a Hermle Z400 (Hermle, Germany) tabletop centrifuge. The supernatants were concentrated using a rotary evaporator R-210 Rotavapor (Buchi, Switzerland) at the boiling point of the solvent under vacuum pressure. The seed powder was extracted in triplicate for each extraction treatment.

A single factor experiment was used to determine the suitable extraction conditions for passion fruit seeds. The influence of the extraction parameters, namely the type of solvent, material to solvent ratio, extraction temperature, and time, on oil extraction yield

Table 1. Independent parameters involved in the extraction of oil from passion fruit seeds

Factor names	Factor levels
Type of solvent	Acetone, ethanol, ethyl acetate, isopropanol, and hexane
Material to solvent ratio	1/4, 1/6, 1/8, 1/10, and 1/12 (*w/v*)
Extraction temperature	Room temperature, 40, 50, 60, and 70 (ºC)
Extraction time	0.5, 1, 2, 3, 4, 5, and 6 (hours)

Table 2. Experimental design studying the effects of different extraction parameters on the oil yield of passion fruit seeds

Experiment	Extraction parameters	Fixed parameter
Type of solvent	Acetone, ethanol, ethyl acetate, isopropanol, and n-hexane	1/8 g mL^{-1}, 50°C, 3h
Material to solvent ratio	1/4, 1/6, 1/8, 1/10, and 1/12 (*w/v*)	Selected solvent, 50°C, 3h
Extraction temperature	Room temperature (28°C), 40, 50, 60, and 70 (°C)	Selected solvent, material to solvent ratio, 3h
Extraction time	1, 2, 3, 4, and 5 (hours)	Selected solvent, material to solvent ratio, and extraction temperature

from passion fruit seeds were studied (Tables 1 and 2). The oil extraction yield was calculated using the equation:

$$\text{Oil extraction yield} = 100.O_e/O_s$$

where O_e is the oil content that was obtained in the extraction and O_s is the oil content in the seeds.

Methods

The oil content of passion fruit seeds (g/100g) was determined by measuring weight loss after a 6-cycle extraction with hexane in a Soxhlet apparatus. The acidity index of the extracted seed oil was determined by titration using a solution of KOH 0.1N according to EEC/TCVN 6127:2010. The peroxide value of the extracted seed oil was obtained by titration using a 0.01N solution of sodium thiosulfate according to AOCS Cd8b-90. The fatty acid profile of the extracted seed oil was determined by gas chromatography (GC-FID) at the National Institute for Food Control.

Statistical analysis

All experimental results in this study were expressed as mean values ± standard errors (SE) of nine measurements (n = 9). In the single factor experiments, the significant differences ($P < 0.05$) among means were subjected to one-way analysis of variance (ANOVA) with Duncan's test using the statistical software SAS 9.3.

Results and Discussion

Effects of the process variables on oil extraction from passion fruit seeds using a shaker

Table 3 shows the results obtained from the shaker extraction of oil from *Passiflora edulis* var. *edulis* seeds.

Solvent type had a strong effect on the oil extraction yield. The results showed that ethanol, hexane, and isopropanol gradually increased the oil extraction yield from 71.1 to 72.47% but they were not significantly different at a significance level of 5%. The remaining solvents, acetone and ethyl acetate, gave the highest oil extraction efficiencies and performed equally well as isopropanol and hexane. The only statistically significant difference in the extraction yield of passion fruit seeds was that ethanol was significantly lower than both ethyl acetate and acetone ($P < 0.05$).

With different polarities (hexane is a non-polar

Table 3. Effects of the process variables on oil extraction yield from passion fruit seeds

Solvent	Yield (%)	Material/ solvent ratio	Yield (%)	Extraction temperature	Yield (%)	Extraction time	Yield (%)
Acetone	72.98^{a}	1/4	63.10^{c}	Room temperature	75.66^{b}	1h	71.41^{c}
Ethanol	71.1^{b}	1/6	72.88^{b}	40°C	76.60^{ab}	2h	71.62^{c}
Ethyl acetate	73.67^{a}	1/8	74.51^{b}	50°C	77.00^{ab}	3h	75.26^{b}
Isopropanol	72.47^{ab}	1/10	78.01^{a}	60°C	77.46^{a}	4h	78.52^{a}
Hexane	72.07^{ab}	1/12	79.17^{a}	70°C	77.74^{a}	5h	79.49^{a}

Note: Values in the same column that do not share a common superscript are significantly different ($P < 0.05$).

solvent, ethanol and isopropanol are polar solvents, and ethyl acetate and acetone are aprotic polar solvents), different solvents resulted in different extraction efficiencies. The data indicated that the aprotic polar solvents were more suitable for the extraction of oil from purple passion fruit seeds since ethyl acetate and acetone had better oil extraction capacities than ethanol. Oliveira *et al.* (2014) also found the same result in that acetone was the best solvent compared to ethanol and isopropanol in extracting oil from passion fruit seeds.

In accordance with Directive 2009/32/EC of the European Union on April 23, 2009, both ethyl acetate and acetone are allowed to be used as solvents in the food industry because they generate less waste than other solvents. Because the boiling point of ethyl acetate is 77^{o}C, higher than that of acetone (56^{o}C), ethyl acetate was selected for the next experiment due to less solvent loss during oil recovery.

The oil content in the extracts strongly depended on the material to solvent ratio. There was an increase of the passion fruit seed oil yield with an increase of the material to solvent ratio (*w/v*). The material to solvent ratio of 1:12 (*w/v*) showed the highest amount of oil but was not significantly different from the ratio of 1:10 (*w/v*) (P >0.05). The opposite behavior is reported by Oliveira *et al.* (2014) who found that the ratio 1:4 of passion fruit seeds to solvent was the best ratio to extract oil from these seeds. Maybe differences between yellow and purple passion fruit seeds led to the differences in findings between the results of Oliveira *et al.* (2014) and our study.

A high material to solvent ratio could promote an increased concentration gradient, producing a higher chance of oil coming into contact with the extraction solvent, resulting in an increased diffusion rate that allows for greater extraction of materials by the solvent (Cacace & Mazza, 2003). These results were consistent with the mass transfer principle. However, the active component yields would not continue to increase once equilibrium had been reached (Herodež *et al.*, 2003). An equilibrium constant trend was observed at the material to solvent ratio of 1:10 (*w/v*) that indicated a sufficient amount of extracting solvent was used in the extraction of oil from passion fruit seeds and thus, this ratio was chosen for the determination of the extraction temperature and extraction time.

Regarding the extraction temperature, the results indicated a significant increase in the oil extraction yield when the temperature increased from room temperature (28^{o}C) to 60^{o}C (P <0.05) but the oil extraction efficiency did not significantly differ between the temperatures of 60 and 70^{o}C (P >0.05).

When performing extractions at low and mediate temperatures, from room temperature to 50^{o}C, the flexibility of the fatty constituents in the materials was not strongly promoted; hence, the extraction efficiency was around 76%. When the temperature was raised to 60^{o}C, the thermal conductivity increased, which made the diffusion coefficient of the solutes increase, consequently increasing their solubility (Al-Farsi & Chang, 2007) and thus, accelerating the whole extraction up to 78%. Although increasing the temperature to 60^{o}C resulted in a 2% increase in the oil extraction yield, 60^{o}C is not only closer to the boiling point of ethyl acetate (77^{o}C), which might cause significant losses in the solvent, energy consumption, and economic efficiency, but small constituents of materials are also easily dissolved at this temperature, leading to difficulties in oil recovery. Therefore, room temperature (28^{o}C) was selected for the extraction of oil from passion fruit seeds in the subsequent step.

The evaluation of the extraction time showed that the extraction capacity of passion fruit seeds increased sharply when the extraction time was increased to 4h, but at longer extraction times, the efficiency did not increase significantly (P >0.05). This finding is in agreement with the report of Oliveira *et al.* (2014) who reported that the highest oil extraction capacity value from passion fruit seeds was obtained after 4h of extraction. These phenomena could be explained by Fick's second law of diffusion, predicting that a final equilibrium between the solute concentration in the plant matrix and in the solvent might be reached after a certain time.

Chemical and quality characteristics of *Passiflora edulis* var. *edulis* seed oil

According to the chemical characteristics of passion fruit seed oil (Table 4), the oil content of *Passiflora edulis* var. *edulis* seeds was 27.28% on a dry basis indicating that these seeds were a good oil source when compared to soybean seeds, which contain about 25% on a dry basis (Hammad *et al.*, 2012). The oil content reported in this study was higher than the values of 18.5% for *P. edulis* var. *edulis* seeds and 20.6% for *P. edulis* var. *flavicarpa* seeds in Uganda found by Nyanzi *et al.* (2005), and lower than the figures of 30.39% and 30.22% for yellow passion fruit seeds in Brazil found by Malacrida & Jorge (2012) and Silva *et al.* (2015), respectively. Differences in the oil contents of *P. edulis* could be due to genetic, climate, and geographical attributes.

The acidity index of the extracted oil provides important information on the condition of oil conservation. A high acidity index indicates that the oil was partly oxidized or degraded. The value found in this study (1.61 ± 0.05 mg $KOHg^{-1}$) was equal with the value found by Silva *et al.* (2015) and 1.5 times lower than the quality parameter of 4.0 mg $KOHg^{-1}$ according to The Codex Alimentarius Commission (2008). This result indicates that the studied oil can be used for food purposes as it meets the required standard.

The determination of the peroxide value is used as an indicator of lipid oxidation. High peroxide values of extracted oil indicate that the oil was exposed to oxidative processes during the preparation of the raw material, extraction, or oil storage. In our study, the peroxide value of the seed oil from *P. edulis* var. *edulis* was 2.5 times lower than peroxide value of the seed oil from *P. edulis* var. *flavicarpa* found by Silva *et al.* (2015) of 1.54 ± 0.14 $meqO_2kg^{-1}$ and far lower than the value that the Codex Alimentarius Commission established for refined and crude oils in 2008 at 15 $meqO_2kg^{-1}$.

The GC analysis of the fatty acids of the *P. edulis* var. *edulis* seed oil showed a high content of unsaturated fatty acids such as linoleic and oleic (Table 5). Linoleic acid (C18:2n6), an essential polyunsaturated omega-6 fatty acid for humans, was the most dominant fatty acid making up 75.7% of the total fatty acids and was followed by oleic acid (C18:1n9) at 9%. These data were in agreement with the results reported by Nyanzi *et al.* (2005) in that linoleic and oleic acids made up the majority of the fatty acids found in the extracted oil of *P. edulis* var. *edulis* seeds with the values of 74.3% for linoleic acid and 13.6% for oleic acid.

In comparison to the fatty acid compositions of soybean oil and sunflower oil, two popular vegetable oils used in the food, cosmetic, and pharmaceutical industries (Rabasco & Gonzalez, 2000), the percentage of saturated fatty acids in

Table 4. Chemical characteristics of *P. edulis* var. *edulis* seeds oil

Component	Value*
Oil content (%)	27.28 ± 0.54
Acidity index (mg KOH g^{-1})	1.61 ± 0.05
Peroxide value ($meqO_2$ kg^{-1})	0.62 ± 0.03

*Note: * Each value is the mean ± SD of triplicate extractions and determinations.*

Table 5. Fatty acid composition of *P. edulis* var. *edulis* seed oil

Fatty acids	Composition (%)
Oleic acid (C18:1n9c)	9
Linoleic acid (C18:2n6)	75.7
Docosahexaenoic acid (C22:6n3)	0.94
Saturated fatty acid (SFA)	14
Unsaturated fatty acid (UFA)	86

passion fruit seed oil amounted to 14%, while the value obtained for soybean oil was slightly higher (15.1%) and the value for sunflower oil was a little lower (12.36%). Among the monounsaturated fatty acids, oleic acid was the main representative and its content was the lowest in passion fruit seed oil (9%) and highest in soybean oil (21.73%), while the level of oleic in sunflower oil was 15.93% (Zambiazi *et al*., 2007). Linoleic was the dominant fatty acid among the polyunsaturated fatty acids (PUFAs) and the linoleic acid amount in passion fruit seed oil was higher than the vales determined in sunflower oil and soybean oil, which were 71% and 56%, respectively. It can also be noted that sunflower oil's high PUFA content makes this oil a good salad oil source (Sullivan, 1980), and with the primary constituents of oleic, linoleic, and linolenic acids, soybean oil and sunflower oil can be employed in cosmetic products and pharmaceutical formulations (Rabasco & Gonzalez, 2000). Hence, the high content of PUFAs of the studied oil indicates this product is a good source of essential PUFAs for human use.

Conclusions

In this study, the effects of process variables on the solvent extraction of oil from passion fruit seeds were investigated using a shaker. The highest extraction yield was 78.52%, which was obtained using ethyl acetate at the ratio of 1:10 material to solvent at room temperature (28^{o}C) for 4h, and was similar to the results obtained with the conventional solvent hexane. Our results indicate that passion fruit seed oil extracted by ethyl acetate has a high content of PUFAs (around 77%) and thus, has good potential for use in the human food, cosmetic, and pharmaceutical industries.

Acknowledgements

The authors would like to acknowledge Vietnam National University of Agriculture for providing financial support under the Research Grant Scheme (Grant agreement T2017-08-55).

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