Enrichment of palmitoleic acid by a combination of crystallization and molecular distillation

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Abstract

Palmitoleic acid shows a variety of beneficial properties to human health. In this study, enrichment of palmitoleic acid from sea buckthorn pulp oil by crystallization and molecular distillation was investigated. Sea buckthorn pulp oil was first converted to its corresponding mixed fatty acids (SPOMFs) that contained 27.17% palmitoleic acid. Subsequently, the effect of various factors on crystallization (i.e., crystallization temperature, solvent, ratio of SPOMFs to solvent (w/v), crystallization time) and molecular distillation (distillation temperature) were assessed on a 5-g scale. It was found that optimal crystallization conditions were a 1:15 ratio of SPOMFs to methanol (w/v) at -20 °C for 12 h, while the optimal temperature for molecular distillation was 100 °C. These conditions were utilized to obtain a liquid oil comprising 54.18% palmitoleic acid with an overall yield of 56.31%. This method has great potential for adoption by the food and medical industries for the preparation of palmitoleic acid concentrate for nutritional studies.

Introduction

Palmitoleic acid, or (9Z)-hexadec-9-enoic acid, is a 16-carbon omega-7 monounsaturated fatty acid (Astudillo et al., 2018). This fatty acid has demonstrated a wide range of applications in nutrition, medicine and chemical industries (Luan et al., 2018). For example, in animal models of metabolic disease, adipose tissue has been shown to release palmitoleic acid, which suppresses hepatic steatosis and improves insulin sensitivity (Trico et al., 2019). Consequently, palmitoleic acid has been in the spotlight as a promising anti-inflammatory lipid that may help ameliorate metabolic disorders (Cao et al., 2008). In addition, palmitoleic acid is used in cosmetics to improve water retention and elasticity of the skin, delay the aging of skin, hair and nails, and improve eye health (Bal et al., 2011). At present many biopharmaceutical and nutrition companies are vigorously developing palmitoleic acid-based health products and pharmaceutical preparations; some of which have been successfully marketed.

Palmitoleic acid can be found in almost any oils of animal or plant origin, but usually in very low concentrations (Wu et al., 2012). At present, wild plants are the main sources of palmitoleic acid. The seed oil of cat's claw (*Doxantha unguis-cati* L.), a woody vine native to the Amazon rainforest, South America and Central America, comprises 64% palmitoleic acid in the oil (Wu et al., 2012). Macadamia nut oil contains 24%-36% palmitoleic acid (Aquino-Bolaños et al., 2016), and the pulp oil from sea buckthorn contains up to 30% palmitoleic acid (Smida et al., 2019). Of these three plants, only sea buckthorn is widely distributed and has good cultivation development potential in China. From other wild plants that contain high proportions of palmitoleic acid, only low extraction yields of palmitoleic acid are obtained, and the geographical distribution of these plants is narrow, making them less suitable for commercial cultivation compared to sea buckthorn. Therefore, sea buckthorn pulp oil (SPO) is considered to be the best raw material for palmitoleic acid for enrichment.

Fatty acids can be separated by crystallization, urea complexation, supercritical fluid extraction, molecular distillation, enzymatic transesterification and preparative liquid chromatography (Lei et al., 2016; Magallanes et al., 2019; Wang et al., 2020). To date, however, very little attention has been paid to the preparation of palmitoleic acid concentrate from natural sources. Chemical synthesis is commonly used to obtain highly pure palmitoleic acid, but this creates partial trans- palmitoleic acid (Guillocheau et al., 2020). Klaas and Meurer (2004) reported the enrichment of palmitoleic acid from natural sources, in which the palmitoleic acid concentration was increased by approximately 50% in a process of transesterification, distillation and urea crystallization. However, although this led to a product that was highly enriched in the ester of palmitoleic acid (81.9%), the overall yield of this method was very low (˜4%), and carcinogenic ethyl or methyl carbamate may be formed during urea inclusion (Solaesa et al., 2016), limiting the application of the extract in the food and pharmaceutical industries. In another study, Gutiérrez and Belkacemi (2008) crystallized SPO product (41.4% palmitoleic acid) at 15 °C in acetone, resulting in ˜53% enrichment and a 20% yield of palmitoleic acid. However, the proportion of palmitoleic acid in the liquid fraction was increased by only 27% compared with the initial proportion in crude SPO.

Various methods are available in the literature for the concentration or separation of unsaturated fatty acids, but only a few are feasible for scalable preparation (Patil and Nag, 2010). In this study, crystallization and molecular distillation were used to enrich palmitoleic acid from sea buckthorn pulp oil mixed fatty acids (SPOMFs). The operating conditions, namely the crystallization temperature, solvent, ratio of SPOMFs to solvent (w/v), crystallization time, and the distillation temperature, were optimized to achieve an acceptable concentration and yield of palmitoleic acid. Importantly, these methods are suitable for the scalable production of palmitoleic acid from an inexpensive and accessible natural source.

Materials and methods

Materials

SPO was purchased from Qinghai Kangpu Co., Ltd. (Xining, Qinghai). Standards of 37 fatty acid methyl esters were purchased from Sigma-Aldrich Chemical Co., Ltd. (Shanghai, China). Hexane, methanol, ethanol, acetone and isopropanol were provided by Sinopharm Chemical Regent (Shanghai, China). All of the other reagents were analytically pure and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Free fatty acid preparation by saponification of SPO

SPO (20 g) was combined with 2.2 g KOH and 40 mL 95% ethanol, and the saponification mixture was stirred at 75 °C in a water bath for 2 h. After the completion of the reaction, distilled water (100 mL) and hexane (200 mL) were added, and the mixture was transferred to a separating funnel to stand for separation. The unsaponifiable matters in the hexane phase were removed, and the collected lower layer containing soaps was acidified with 3 M hydrochloric acid to pH 2 to release free fatty acids. Subsequently, the solution was extracted three times with total 300 ml hexane. The upper layer containing free fatty acids was collected, and trace water was removed using anhydrous sodium sulfate. Then the extract was concentrated to dryness at 50 °C on a rotary evaporator to obtain SPOMFs. The final product was stored in a sealed aluminum pot at -20 °C before use.

Crystallization of SPOMFs

The crystallization of SPOMFs was performed at a controlled temperature in a 100 mL batch reactor. In general, SPOMFs of 5 g was mixed with a certain ratio of solvents, and then the mixture was allowed to crystallize at a selected temperature. At the end of the crystallization, the crystals were removed by low-temperature filtration to obtain the liquid fraction 1 that was rich in palmitoleic acid. Samples were obtained at regular time intervals, and solvent in the sample was evaporated under reduced pressure. The fatty acid composition was analyzed by GC after being diluted to 2 mg SPOMF/mL.

Several parameters were optimized, which were crystallization temperature, solvent, substrate ratio of SPOMFs to solvent (w/v) and crystallization time. Five solvents were used to prepare the free fatty acid

solutions, and they were hexane, methanol, ethanol, acetone and isopropanol. Crystallization temperature was in the range of 0 °C to -40 °C. Six different SPOMFs-to-solvent ratios in the range of 1:3 to 1:18 (w/v) were used, and it was found that the best range for investigation was 1:9 to 1:18. The crystallization time was increased from 4 h to 16 h. While one of these parameters was being optimized, the others were held at a fixed level. After one parameter optimization was completed, the optimal value of this parameter was used for the optimization of other parameters. The design for these optimization experiments is outlined in Table 1. The yield and enrichment of palmitoleic acid content were used as the main response variables for the optimization.

As a small amount of saturated fatty acids still remained in the liquid fraction 1, a secondary crystallization was conducted for further concentration of palmitoleic acid. Secondary crystallization was conducted at -40 °C by mixing 5 g liquid fraction 1 with 20 mL methanol for a period of time. The resulting crystals were separated from the liquid, giving liquid fraction 2. The fatty acid composition was analyzed (see below) and the yield of palmitoleic acid was calculated.

Molecular distillation of liquid fraction 2

The palmitoleic acid in liquid fraction 2 obtained from the two-step crystallization was further enriched using molecular distillation in a standard glass evaporator (KDL 1, UIC GmbH, Alzenau-Hoerstein, Germany). Molecular distillation was performed varying the evaporation temperature from 95 °C to 110 °C to obtain a liquid containing a high proportion of palmitoleic acid. The other operation parameters were fixed, and were as follows: a rotation speed of 400 rpm, a feed temperature of 70 °C, a feed rate of 1 mL/min, a vacuum level of 10^{-3} mbar and a condenser temperature of 55 degC. Finally, the composition of fatty acids was analyzed and the yield of palmitoleic acid was calculated.

Fatty acid composition analysis

Esterification of SPOMFs (50 mg) was achieved using 25% boron trifluoride (BF_3) in methanol (2 mL) at 70 degC for 3 min. The resulting fatty acid methyl esters (FAMEs) were extracted with hexane (2 mL). The upper layer was separated and dried over anhydrous sodium sulfate, centrifuged at 8000 rpm for 5 min, and then used for gas chromatograph (GC) analysis.

The FAMEs were separated on a DB-Fast FAME column (30 m x 0.25 mm x 0.25μm) and quantified by a flame-ionization detector (FID) mounted in a gas chromatograph (7820A, Agilent, USA). The injector and FID temperatures were 250 °C, the initial column temperature was maintained at 80 °C for 0.5 min and then increased to 165 °C (at a rate of 40 °C/min), following which it was maintained at 165 °C for 1 min. Finally, the temperature was increased to 230 °C (at a rate of 4 °C/min) and held at 230 for 4 min. The running time of the entire program was 23.875 min. The FAMEs were identified and quantified by comparison of the retention times of the sample peaks with those of a mixture of the FAME standards.

Statistical analysis

The yield of palmitoleic acid was calculated by the following equation:

$$\text{Yield of palmitoleic } (\%) = \frac{Weight\ of\ palmitoleic\ acid\ in\ a\ fraction\ (g)}{Weight\ of\ palmitoleic\ acid\ in\ the\ starting\ material\ (g)} \times 100$$

All analyses were carried out in duplicates, and the results were expressed as means ± standard deviations (SD). Statistical analyses were performed using the SPSS 24.0 (SPSS, Chicago, IL, USA), and one-way analysis of variance (ANOVA, Tukey's test) was performed to identify differences ($p < 5\%$, significant difference).

Results and discussion

The goal of this study was to enrich palmitoleic acid. As shown in Table 2, palmitic acid is the most abundant saturated fatty acid in the SPOMFs, accounting for 33.59% of total fatty acids, followed by oleic

acid (32.16%), and palmitoleic acid (27.17%).

First, the optimal temperature for low-temperature crystallization was evaluated, and then solvent type, substrate ratio of SPOMFs to solvent (w/v) and crystallization time during the first crystallization stage were optimized to improve the results of crystallization. Some residual saturated fatty acids still remained in the collected liquid fraction 1, thus a secondary crystallization was performed at -40 °C to remove these, and this resulted in the liquid fraction 2. Then, the liquid fraction 2 was subject to molecular distillation to yield a concentrated palmitoleic acid final product.

Effect of the crystallization temperature

First, the effect of crystallization temperature (0 °C, -20 °C, and -40 °C) on palmitoleic acid content and yield was investigated. When the free fatty acids were mixed with a selected solvent and then stored in a reduced temperature, saturated fatty acids, which have higher melting points and lower solubility in solvent particularly at a low temperature, will undergo crystallization and unsaturated fatty acids will remain in the liquid fraction (Vazquez and Akoh, 2012). Table 2 shows that the content of palmitic acid in SPOMFs was 33.59%, and the total contents of saturated fatty acids was 35.48%. Therefore, the main goal of the crystallization process was to remove saturated fatty acids. In this study, two solvents (methanol and acetone) were selected to examine the effect of crystallization temperature. It was observed that only few crystals were formed at 0 °C. Although the yields of palmitoleic acid were high, the content of palmitoleic acid was only increased slightly in two solvents compared to SPOMFs, as shown in Figure 1a and b. When the crystallization was conducted at -20 °C, the levels of palmitoleic acid using methanol and acetone as solvents increased to 38.55% and 33.82%, respectively, which were significantly higher than the initial content. In addition, the palmitoleic acid product obtained at -20 °C had a significantly higher content compared to 0 °C regardless of which solvent was used. However, when the crystallization was further decreased to -40 °C, the content of palmitoleic acid decreased slightly compared to that at -20 °C. The results might be due to the crystallization of palmitoleic acid from the solution at a lower temperature, causing the removal of palmitoleic acid from the liquid fraction (Mu et al., 2016).

The yield of palmitoleic acid, as shown in Figure 1b, was the lowest at -40 °C compared to yields at other temperatures (-20 and 0 °C). The yield of palmitoleic acid showed a significant reduction with decreased temperature because more fatty acid crystals formed at the very low temperature. The results are in agreement with a previous study (Zhang et al., 2017). Based on these results, the crystallization at -20 °C that had high palmitoleic acid contents and reasonable yields was determined as the optimum temperature, and this temperature was used in the following crystallization process.

Effect of various solvent types

In this study, hexane, methanol, ethanol, acetone, isopropanol were examined for the free fatty acid crystallization fractionation. These solvents are permitted at parts-per-million (ppm) residual concentrations in pharmaceuticals according to the United States Pharmacopoeia (USP). Methanol is a Class 2 solvent associated with an allowable concentration limit of 3000 ppm (Kameyama et al., 2019). Acetone, a polar solvent, is commonly selected as crystallization solvent for the separation of triglycerides and free fatty acids (Vazquez and Akoh, 2012). It is a permitted solvent in food industry. Hexane, a nonpolar solvent, is less toxic than methanol and widely used as the extraction solvent in oilseed crushing plants (Zhu et al., 2017).

Figure 2a shows the effect of solvent type on the content and yield of palmitoleic acid. Initially, crystallizations were performed with 5 g SPOMFs and 60 mL solvent at -20 °C for 12 h to investigate the effect of solvent type on palmitoleic acid enrichment. When methanol was used as the crystallization solvent, the palmitoleic acid content was up to 38.55%, which was significantly higher than those with other solvents. Therefore, methanol gave the highest content of palmitoleic acid in the liquid fraction 1, followed by hexane, acetone, isopropanol and ethanol. For acetone, isopropanol and ethanol, the increase of palmitoleic acid in the fraction was very limited and insignificant differences in palmitoleic acid level were found among these three solvents. Previous study is consistent with the results of this experiment. It is concluded that hexane was not the most suitable solvents for the concentration of free fatty acids by low-temperature crystallization (Vazquez

and Akoh, 2011).

Although the crystallization in methanol led to the highest proportion of palmitoleic acid in the liquid fraction, the yield needs to be improved. The yields of palmitoleic acid as affected by the treatments applied were compared and the results are shown in Figure 2a. There is no statistical difference in yield among solvents. Thus, taking both purity and yield into account, methanol was selected as an optimal solvent for further crystallization treatments.

Effect of the ratio of SPOMFs to solvent (w/v)

Subsequently, the effects of the ratio of SPOMFs to solvent (w/v) on purity (% palmitoleic acid relative to total fatty acid) and yield of palmitoleic acid were investigated, and the results are shown in Figure 2b. When crystallization was carried out at -20 °C, our preliminary study showed that SPOMFs: methanol ratios from 1:3 to 1:6 were inappropriate, because the solution froze too quickly to allow monitoring. Therefore, the SPOMF to methanol ratios were set at 1:9, 1:12, 1:15 and 1:18 (w/v), and the methanolic SPOMF solutions were stored at -20 °C for 12 h.

At shown in Figure 2b, when the ratio of SPOMFs to methanol (w/v) was changed from 1:9 to 1:15, the purity of the palmitoleic acid solution increased from 36.32% to 40.43%. This is due to the effective crystallization of the saturated fatty acid at high volume use of the methanol (Shahidi, 2016). However, as the SPOMFs to methanol ratio changed beyond 1:15, the proportion of palmitoleic acid in solution decreased, indicating solubilization of the saturated fatty acids due to the high solvent volume.

In terms of yield, no significant difference was found between SPOMFs to methanol ratios of 1:12 and 1:15. Considering both purity and yield of palmitoleic acid, an SPOMFs to methanol ratio of 1:15 (w/v) gave the best result. Hence, an SPOMF to methanol ratio of 1:15 (w/v) was used for further crystallization studies. Under these conditions, a solution with 40.43% palmitoleic acid was obtained, that translates to an 88.47% palmitoleic acid yield.

Effect of crystallization time

Time has an influence on the mass transfer between the solid and liquid phases in the crystallization process (Morales-Medina et al., 2016). Thus, four crystallization times (4 h, 8 h, 12 h and 16 h) were investigated with the SPOMFs to methanol ratio of 1:15 at -20 °C. It was observed that the change of crystallization time had a significant influence on the purity and yield of palmitoleic acid as shown in Figure 2c. Overall, the purity of palmitoleic acid increased with time, whereas the yield decreased with time. When the crystallization time ranged from 4 to 16 h, the purity of palmitoleic acid ranged from 27.17% to 41.55%. There were only slight increases in palmitoleic acid purity from 12 h to 16 h, suggesting that crystallization was almost complete at 16 h. Longer time will lead to the crystallization of the palmitoleic acid, reducing its yield significantly. Add what time is the best....

Secondary crystallization of liquid fraction 1

After completing the optimization of crystallization conditions at the first stage, the fatty acid composition of liquid fraction 1 was obtained under the optimal conditions. As shown in Table 2, the liquid fraction 1 contained low contents of saturated fatty acids, with palmitic acid being the most abundant, accounting for 9.37% of the total fatty acids. It is important that the fatty acids having [?]16 carbons are removed as much as possible from the liquid fraction because subsequent molecular distillation can only separate palmitoleic acid from the C18 fatty acids, but cannot separate palmitoleic acid from palmitic acid. However, it has been proven that multistage fractionation can further improve the purity (Jin et al., 2017). Based on these considerations, secondary crystallization of liquid fraction 1 was performed to remove palmitic acid.

A liquid fraction 2 was collected after re-crystallization at -40 degC. As shown in Table 2, the purity of palmitoleic acid in the liquid fraction 2 by secondary crystallization was 45.22%, with a yield of 95.49%. The content of palmitic acid was significantly reduced, from 9.37% to 2.13% in this fraction. Therefore, re-crystallization led to a significant increase of palmitoleic acid content in the liquid fraction, from 40.55%

to 45.22%. In addition, the oleic acid content in the liquid fraction 1 significantly differed from that in the liquid fraction 2, and no statistically significant differences were found in the proportions of linoleic acid and linolenic acid in solution after secondary crystallization. It should be noted that these contents are relative percentages. If one fatty acid is removed, others will tend to increase in relative percentage values.

Overall, secondary crystallization significantly decreased the proportion of saturated fatty acids in the liquid phase, and thus the unsaturated fatty acid content in liquid fraction 2 reached 97.52% after two steps of crystallization. Finally, we had obtained sufficiently enriched material for further purification by molecular distillation.

Effect of molecular distillation temperature on palmitoleic acid enrichment

Molecular distillation can be used for the separation of mixtures with different molecular weight and partial vapor pressure, and for the separation of homologs (Zhang et al., 2018). Compounds with different mean free paths are separated under vacuum condition, which decreases the evaporation temperature and the residence time (Solaesa et al., 2016; Zhang et al., 2018). Table 2 shows that the oleic acid in the liquid fraction 2 was 45.06% of the total fatty acids, which was approximately the same as the proportion of palmitoleic acid. Oleic acid is similar in structure to palmitoleic acid, with the former having two more carbons but with both containing one double bond. These two fatty acids are difficult to separate by crystallization. Thus, molecular distillation was applied to enrich palmitoleic acid in the liquid fraction 2.

The chain length and the number of double bonds of a fatty acid affect its boiling point, and the distillation order is palmitoleic acid > oleic acid [?] linoleic acid (Zhang et al., 2013). Thus, oleic acid is expected to remain in the residue while palmitoleic acid would be collected in the distillate under a proper temperature. In a study by (Solaesa et al., 2016), saturated fatty acids such as myristic acid and palmitic acid were evaporated in the distillate at 125 degC under a vacuum level of 10^{-3} mbar. Thus, considering the fatty acid composition difference in the feedstock in this study, evaporation temperature was first set at 120 degC to investigate the effect of separation. Unfortunately, this led to almost all products going in the distillate. At 95 degC, some separation was achieved, but the yield of the distillate was 17.79%.

Table 3 shows that as the temperature was increased, a greater yield (about 65%) of palmitoleic acid was obtained. At 100 degC to 110 degC, the purity of the distillate was ˜54%, while it was 54.18% at 100 degC with a yield of 63.64%. Accordingly, 100 degC was the best temperature for distillation. Thus, by combining the above steps of crystallization and distillation, a 54.18% pure liquid of palmitoleic acid was obtained, in an overall yield of 56.31%.

Conclusions

In this study, palmitoleic acid was enriched by combining crystallization and molecular distillation, leading to a high yield of a liquid containing a high proportion of palmitoleic acid. Methanol was found to be the optimum organic solvent to increase palmitoleic acid concentration in solution by crystallization. The optimal crystallization conditions were SPOMFs: methanol ratio of 1:15 (w/v), a crystallization temperature of -20 degC, and incubation time of 12 h. Then, molecular distillation performed at 100 degC can lead to a distillate that contains 54.18% palmitoleic acid in a yield of 56.31%, which was twice as enriched as the raw material. This demonstration of an effective method for obtaining a concentrated palmitoleic acid fraction from a natural source provides basis for industrial scale-up process of this unique fatty acid for the food and pharmaceutical industries.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure captions

Figure 1 The effect of crystallization temperature on content of palmitoleic acid (a) and the effect of crystallization temperature on yield of the palmitoleic acid at the first crystallization stage (b). Crystallization conditions: 5 g SPOMF, 60 mL solvent [SPOMF: solvent ratio 1:12 (w/v)], methanol and acetone as the solvents, crystallization time of 12 h.

Figure 2 Optimization of crystallization conditions at the first crystallization stage. Crystallization conditions (a): 5 g SPOMF, 60 mL solvent [SPOMF: solvent ratio 1:12 (w/v)], crystallization temperature of -20 degC, crystallization time of 12 h; (b): 5 g SPOMF, SPOMF to methanol ratio of 1:9 to 1:18 (w/v), crystallization temperature of -20 degC, crystallization time of 12 h; (c): 5 g SPOMF, 75 mL methanol [SPOMF to methanol ratio 1:15 (w/v)], crystallization temperature of -20 degC, crystallization time of 4 h to 12 h.

List of Tables

Table 1 Experimental design for optimization of palmitoleic acid purification by a combination of solvent crystallization and molecular distillation

Level	Solvent crystallization	Solvent crystallization	Solvent crystallization	Solvent crystallization	Secondary crysta
	X_1 (°C)	X_2	X_3 (w/v)	X_4 (h)	X_1 (°C)

Level	Solvent crystallization	Solvent crystallization	Solvent crystallization	Solvent crystallization	Secondary crysta
1	0	methanol	1: 9	4	-40
2	-20	ethanol	1: 12	8	
3	-40	acetone	1: 15	12	
4		Hexane	1: 18	16	
5		isopropanol			

X_1 = crystallization temperature, X_2 = solvent type, X_3 = ratio of SPOMFs to solvent, X_4 = time.

Table 2 Fatty acid composition of SPOMFs, liquid fraction 1, liquid fraction 2 and the distillate

Fatty acid	SPOMFs	Liquid fraction 1	Liquid fraction 2	The distillate
Myristic acid	0.37 ± 0.01^{a}	0.44 ± 0.02^{b}	ND	0.74 ± 0.01^{c}
Palmitic acid	33.59 ± 0.12^{c}	9.37 ± 0.06^{b}	2.13 ± 0.08^{a}	7.92 ± 0.22^{b}
Palmitoleic acid	27.17 ± 0.01^{a}	40.55 ± 0.16^{a}	45.22 ± 0.12^{b}	54.18 ± 0.27^{c}
Stearic acid	1.52 ± 0.05^{b}	0.40 ± 0.11^{a}	ND	ND
Oleic acid	32.16 ± 0.02^{b}	41.33 ± 0.48^{c}	45.06 ± 0.18^{d}	31.66 ± 0.38^{a}
Linoleic acid	3.50 ± 0.05^{a}	5.02 ± 0.28^{b}	5.11 ± 0.13^{b}	3.76 ± 0.06^{a}
Linolenic acid	1.70 ± 0.05^{a}	2.34 ± 0.04^{b}	2.50 ± 0.11^{b}	1.76 ± 0.05^{a}
SFA	35.48 ± 0.23^{c}	10.21 ± 0.03^{b}	2.13 ± 0.08^{a}	8.66 ± 0.23^{b}
UFA	64.53 ± 0.04^{a}	89.24 ± 0.32^{b}	97.52 ± 0.11^{c}	91.36 ± 0.22^{a}

SFA, saturated fatty acids, SFA= myristic acid + palmitic acid + stearic acid;

UFA, unsaturated fatty acids. UFA=palmitoleic acid + oleic acid + linoleic acid + linolenic acid;

ND, not detected;

Values in the same row with different letters are significant difference at $p < 0.05$

Table 3 Fatty acid composition of the distillate by molecular distillation

Fatty acid	Liquid fraction 2	95 °C	100 °C	105 °C	110 °C
Myristic acid	ND	0.75 ± 0.01^{a}	0.74 ± 0.01^{a}	0.71 ± 0.05^{a}	0.67 ± 0.00^{a}
Palmitic acid	2.13 ± 0.08^{a}	8.09 ± 0.06^{b}	7.92 ± 0.22^{b}	7.66 ± 0.14^{b}	7.57 ± 0.01^{b}
Palmitoleic acid	45.22 ± 0.12^{a}	58.4 ± 0.13^{c}	54.18 ± 0.27^{b}	53.56 ± 0.61^{b}	53.49 ± 0.51^{b}
Stearic acid	ND	ND	ND	ND	ND
Oleic acid	45.06 ± 0.18^{c}	28.47 ± 0.06^{a}	31.66 ± 0.38^{b}	32.44 ± 0.71^{b}	32.61 ± 0.47^{b}
Linoleic acid	5.11 ± 0.13^{c}	2.91 ± 0.03^{a}	3.76 ± 0.06^{b}	3.86 ± 0.08^{b}	3.87 ± 0.06^{b}
Linolenic acid	2.50 ± 0.11^{c}	1.46 ± 0.07^{a}	1.76 ± 0.05^{b}	1.79 ± 0.00^{b}	1.8 ± 0.01^{b}
Yield (%)	ND	17.79 ± 1.56^{a}	63.64 ± 1.43^{b}	65.65 ± 1.74^{b}	64.14 ± 1.13^{b}

ND, not detected;

Values in the same row with different letters are significant difference at $p < 0.05$

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`Figure.docx` available at https://authorea.com/users/355737/articles/478871-enrichment-of-palmitoleic-acid-by-a-combination-of-crystallization-and-molecular-distillation