Supercritical Carbon Dioxide Extraction of Mango Butter for Cocoa Butter Replacement

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The supercritical carbon dioxide extraction of Kaew variety mango seeds from Thailand was, for the first time to authors' knowledge, carried out in this research at various conditions, *i.e.* 300-500 bars and 40-60^{o}C. Actually, it is well-known that mango butter can be used as a cocoa butter substitute. Mango seeds were oven dried and ground to be in a powder form prior to the experiments. The 0.1 liter extractor was filled with about 10 grams of seed powder and 107 grams of glass beads. CO_2 of around 10 g/min was continuously passing through the reactor for the extraction time of 90 minutes. The findings suggested that the yield of mango butter was between 4-12% depending on the extraction conditions. The higher the pressure the higher the extraction yield as well as the extraction rate, whereas the effects of temperature seemed to be insignificant. In addition, the extracted oil samples were drawn out every 20 minutes and 4 different fractions were collected during the experiments. The GC analysis showed that there was no difference in fatty acid compositions among the different fractions and the mango butter mainly composed of approximately 45% C18:0, 40% C18:1, 7% C16:0, and 7% C18:3. The stearic and oleic acids percentages are close to those of cocoa butter, as it was expected.

Keywords: supercritical extraction, supercritical carbon dioxide, mango butter

INTRODUTION

Mangoes (*Mangifera indica L.*) are one of the most popular fruits in Southeast Asia and of Thailand's most economically important fruits. Each year the mango production in Thailand exceeds 20 million tons [1] and Kaew, one of the most common mango varieties in Thailand, is accounted for nearly 30% of the mango production [2]. Over the past decades, only mango flesh which accounts for 60% of total weight of the whole fruit has been consumed mainly in Thailand (90% of the production) whereas its seeds being discarded as agricultural waste. According to the survey study of Maisuthisakul and Pasook [2], it was found that currently mango seed kernel is not utilized for any commercial purposes. There were many studies reviewing the nutritional compositions of mango seeds that is it is rich in equal amounts of stearic and oleic acids (42%) [3], [4], [5]. Due to the fact that mango seed kernel contains fat around only 6-12% on the dry basis of the kernel, studies of the mango seed butter have not been going very fast as it should have been [4], [6]. Nevertheless, a high cost and shortage in cocoa butter in some areas recently especially in Asia attract several researchers to start focusing on extracting a type of fat called mango seed almond fat (MAF) containing a large amount of stearic acid similar to the cocoa butter (CB) and hence show a potential for CB

equivalent or replacement [7], [8]. It was found that MAF and CB have similar characteristics such as melting point, specific gravity, refractive index, and acid value, which are important for the textures of confectionary products [9]. In addition, according to the 2003 EU regulations MAF is one of the six vegetable oils that can be used in EU chocolate [10].

Similar to oil extraction of other fruits and plants, Soxhlet extraction with different solvents, typically hexane, has been a common extraction process for MAF; however, the process takes a long time as well as a severe condition (typically high temperatures with the use of organic solvents) is required [4], [6], [11]. Supercritical carbon dioxide (SC CO_2) extraction has gained an interest from food, pharmaceutical, and biotechnology industries for extracting edible oils from natural products due to several advantages. Not only it is an environmental friendly technique, but it also has favorable mass transfer characteristics and, moreover, the solubility of the extracted oil can be easily controlled by varying temperature or pressure. CO_2 is relatively low toxic, inexpensive, and has a considerably low critical temperature and pressure (31.1^{o}C and 7.39 MPa). Sahena *et al.* [12] studied different techniques for extracting fish oil and found that in terms of both extracted oil yields and fatty acid compositions of the extracted oils, there was no difference from different SC CO_2 techniques. In addition, the yields and compositions received from different fish parts (skin, flesh, head, and viscera) from SC CO_2 extraction are comparable to those received from Soxhlet extraction. However, SC conditions, i.e. pressure and temperature, can significantly affected the oil yield, the initial extraction rate, and the fatty acid compositions [13], [14], [15]. Similarity it was revealed from several studies that as the SC pressure increases, the extracted oil yields of many kinds of fruit seeds increase [13], [16]; however, the effects of SC temperature are not conclusive [14]. This research aims at investigating the effects of SC conditions on the MAF yields and their fatty acid compositions.

MATERIALS AND METHODS

Sample preparation

Kaew variety mango seeds were provided by a fruit processing factory in the western area of Thailand. The hard seed shells were removed and only the kernels were vacuum dried at 65^{o}C for 6 hours using a VOS-300SD Eyela Vacuum Oven prior of being milled into a powder form using a domestic processing machine. Moisture content of the powder was found to be lower than 10%.

Supercritical CO_2 extraction

Schematic diagram and details of the supercritical CO_2 extractor used in this research were previously described elsewhere [13]. In brief, high purity (> 99.99%) CO_2 from a high pressure cylinder was passed through an air driven piston pump to increase pressure till the set-point value. The high pressure liquid CO_2 was then passed through a heat exchanger where CO_2 become supercritical before passing through the extractor vessel inside which a tailor-made spacer (a stainless steel pipe) and a cylindrical basket of a volume of 0.1 L were placed, as shown in Figure 1. About 10 gram of the mango seed powder was uniformly

distributed in 107 gram of 1.5 mm diameter glass beads before loading into the cylindrical basket. The exit valve was initially closed to maintain the extractor in a static condition for 20 minutes before fluxing CO_2. Once the dynamic extraction proceeded, the CO_2 flux of about 16 g/min was continuously passed through the vessel for the extraction time of 90 minutes. The collected oil was weighted every 5 minutes and fractionated into 4 fractions by drawing out every 20 minutes. The extracted oil was stored in a freezer at a temperature of -20^{o}C prior to GC analysis. For the experiments focusing only on the extraction yield, the extraction periods were extended up to 200 minutes in some cases and the extracted oils were collected at the end of the experiments.

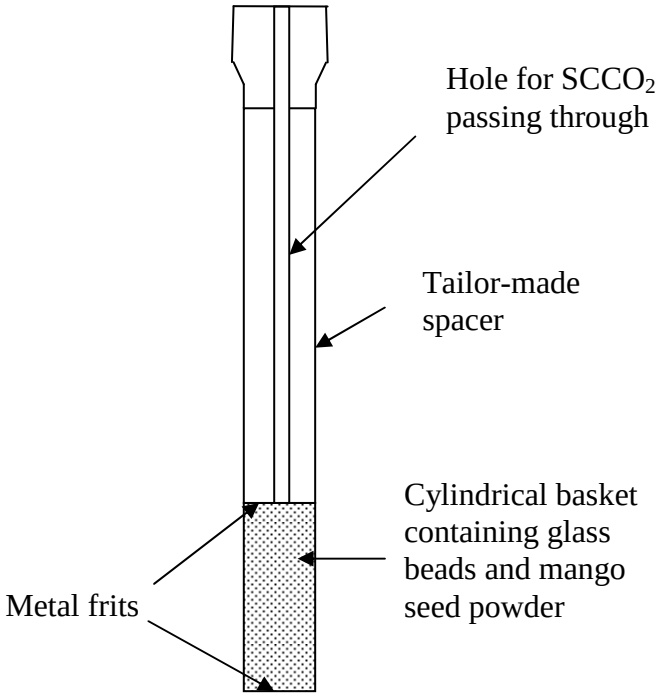

Figure 1. Schematic diagram of extraction vessel.

GC analysis

Prior to the fatty acids analysis of the extracted oil using a gas chromatography (GC), the transesterification was carried out. 5 mL of a 0.5M KOH solution in methanol was added into 200 μL of the oil. The reaction was performed at 60^{o}C for 3 hours and monitored using TLC (n-hexane/ethyl acetate 93:7). After the reaction was completed, neutralization process using sulphuric acid was followed and the organic solvent (yield 90%) was evaporated. Fatty acid methyl esters (FAMEs) were isolated by subjecting crude reaction material to a flash

chromatography on Silica gel with n-hexane/ethyl acetate gradient elution. FAMEs were collected in the first 1-3 fractions (overall yield 85%) whereas free fatty acids and sterols in the last ones.

A Carlo Erba mega series gas chromatograph, model 5300, equipped with a flame ionization detector was used to verify FAMEs. The carrier gas was helium. The chromatographic column used was a DB-WAX 30 m x 0.324 mm x 0.25 µm. The temperature of the column, injector and detector were maintained constant at 200, 250 and 300°C, respectively. Each analysis had a duration of 2 hours and was carried out in triplicate in order to quantify the precision of the results.

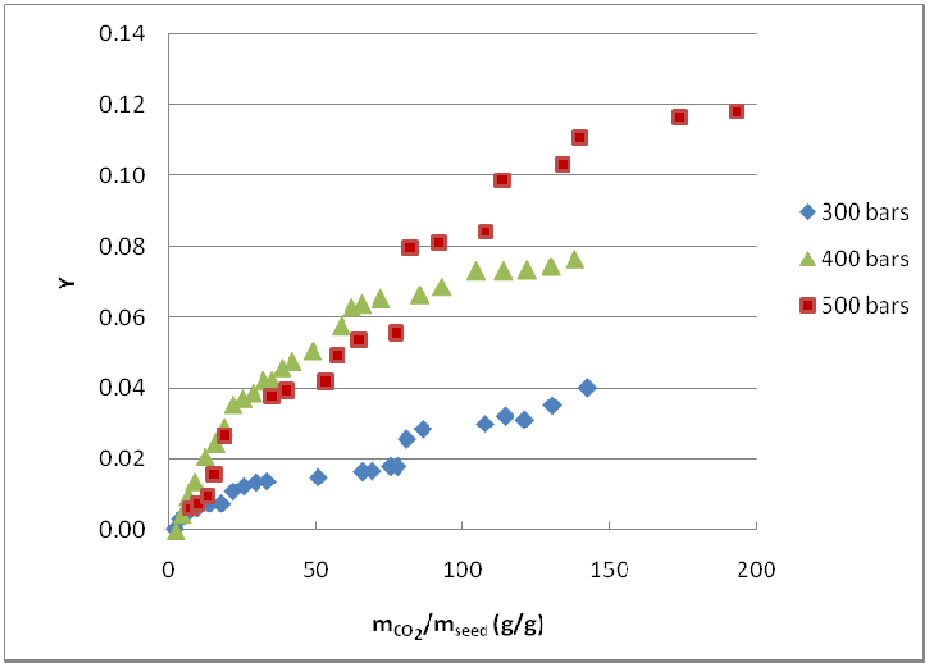

Figure 2. Extracted MAF yields at different SC CO_2 extraction pressures (50°C).

RESULTS

Qualitative Analysis

Figure 2 illustrates the extraction yield (Y), which is the ratio between the extracted oil and seed charge, as a function of the ratio of CO_2 consumption to seed charge (m_{CO2}/m_{seed}) at a constant extraction temperature of 50°C but at different extraction pressures. It can be seen that, except at 300 bars, the yields initially increased sharply as extraction time proceeded and then followed by a plateau which was the maximum yield of the extraction. However, in the case of 300 bars, the yield gradually increased at a lower extraction kinetics rate than those of 400 and 500 bars and did not reach the maximum yield in the period of testing. Similar findings were report for SC CO_2 extraction of other fruit seeds, *i.e.* neem, jojoba, and grape,

that at low pressures, the initial extraction rate and yield were significantly lower than those at high pressures [13], [14], [16]. As the SC CO_2 extraction pressure increased, the extraction rate increased due to the fact that the solubility of SC CO_2 increases with pressure. The MAF yields obtained from SC CO_2 extraction at the pressures of 300, 400, and 500 bars were 4, 8, and 12% respectively whereas the yield from Soxhlet extraction using hexane as a solvent was 7.28 ± 0.19% at the extraction temperature and time of 140^{o}C and 6 hours, respectively. It was reported that different mango varieties contain different amount of MAF, varying from 3.7-12.6% [4]. While the extraction pressure has showed a strong effect on the kinetics and yield of the process, extraction temperature revealed to be an insignificant factor as shown in Figure 3. The studies of Salgin [14], Zaidul *et al.* [15], and Tonthubthimthong *et al.* [16] agreed that at low pressures, the extraction yield decreased with temperature due to the low solvent density and hence solvating power. As the pressure increases, the higher solvent solubility plays an important role and therefore, the yield increases as the temperature increases.

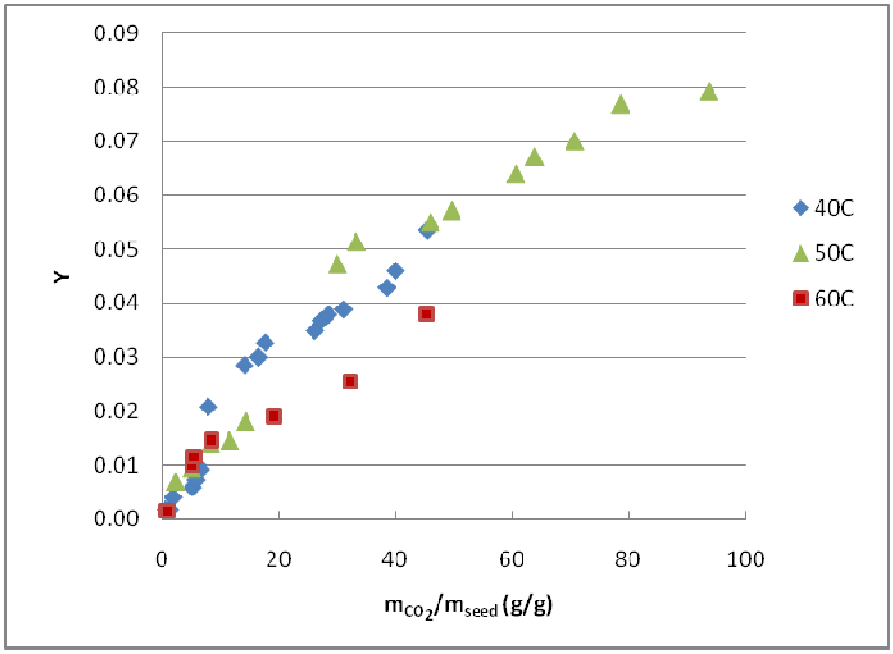

Figure 3. Extracted MAF yields at different SC CO_2 extraction temperatures (500 bars).

Quantitative Analysis

The fatty acid compositions of the MAF of each fraction SC CO_2 extracted at 60^{o}C and 400 and 500 bars are shown in Figures 4 and 5. As previously reported, MAF mainly contains of stearic (C18:0) and oleic (C18:1) acids of the amount of 45 and 40%, respectively [3], [4], [5]. Other components found were 7% C16:0, 7% C18:3, 1.5% C20:0, 0.2% C17:0, 0.1% C20:1, and 0.05% C14:0. Table 1 summarizes and compares the fatty acid compositions revealed in previous studies. A large variation can be seen from different mango varieties [4]; however, this research found a surprisingly higher C18:3 content compared to those reported elsewhere which is a good indication that MAF of Kaew variety mango is very high in omega 3, an essential unsaturated fatty acid that cannot be synthesized by the human body but is vital for normal metabolism. The high amounts in stearic and oleic acids make MAF attractive to confectionery and bakery industries due to the fact that its compositions is very closed to cocoa butter which contains around 25% C16:0, 35% C18:0, 35% C18:1, 3% C18:2, and 0.6% C14:0 [8], [15]. Even though MAF contains stearo-oleo-stearin (StOSt) trigycerols, it still lacks of the other two types of trigycerols that are the main compositions in CB, *i.e.* palmito-oleo-palmitin (POP) and palmito-oleo-stearin (POSt). Zaidul *et al.* [15], [17] attempted to mix palm oil and palm oil fraction to obtain CB replacers due to the fact that palm oil is rich in C16:0 and C18:1 whereas Solís-fuentes and Durán-de-bazúa [8] investigated the thermal properties of the blends of MAF and CB.

Even though the extraction pressure resulted in considerably different extracted oil yield, it did not seem to play any important role in the fatty acid compositions of the MAF. When considering the fatty acids of different MAF fractions, again the differences were unnoticeable. These findings were unlike those of Zaidul *et al.* [15], [17] where both yield and compositions of each of palm oil fraction were different.

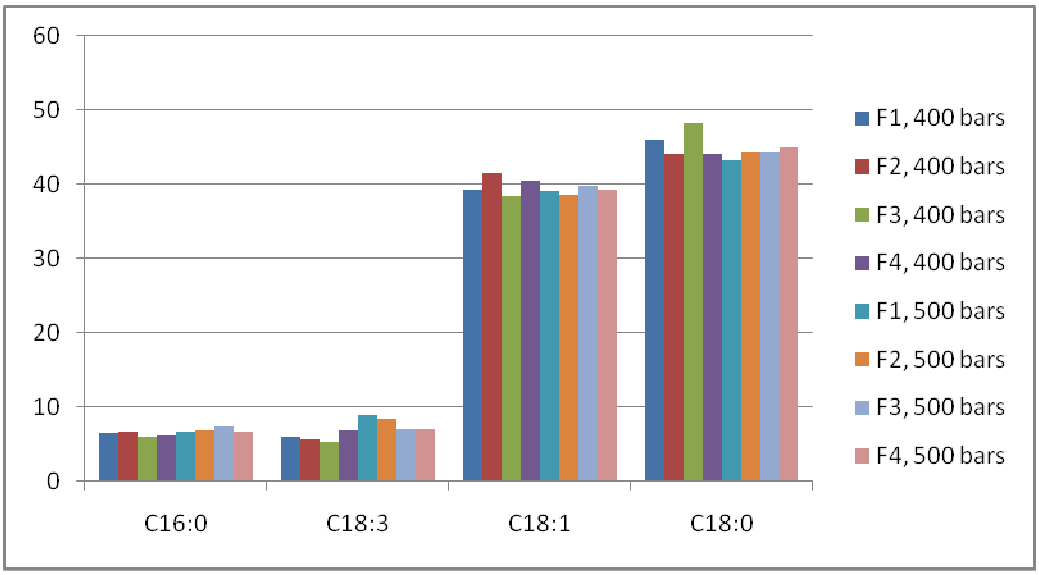

Figure 4. Major fatty acid compositions of each fraction.

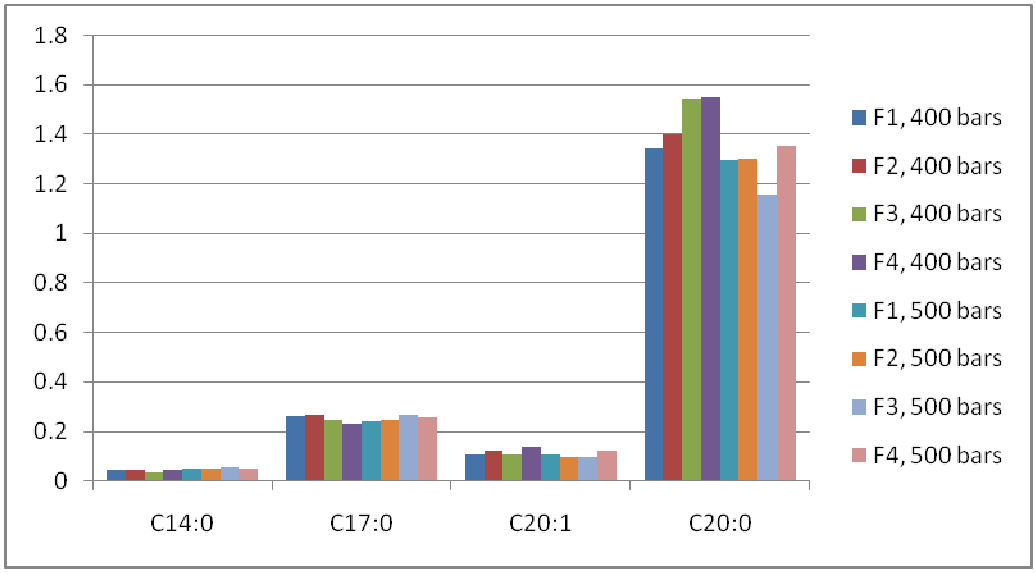

Figure 5. Minor fatty acid compositions of each fraction.

Table 1. Percentage fatty acid composition reported in literatures.

Fatty acids	This research	[3]	[4]	[5]	[7]	[8]	[11]
Lauric acid (C12:0)	-	-	-	-	0-0.3	-	-
Myristic acid (C14:0)	0.05	0.2	-	0.5±0.1	0.1-0.9	9.29	-
Palmitric acid (C16:0)	6-7	7.6	3-18	5.8±0.3	3.8-10.2	-	6.43
Palmitoleic acid (C16:1)	-	-	-	-	0.3-0.9	-	-
Margaric acid (C17:0)	0.2-0.3		trace–0.2	-	-	-	-
Stearic acid (C18:0)	42-48	41.1	24-57	38.3±1.2	26.6-49.2	39.07	37.73
Oleic acid (C18:1)	38-41	42.77	34-56	46.1±2.3	34.2-50.7	40.81	46.22
Linoleic acid (C18:2)	-	7.7	1-13	8.2±0.6	1.6-10.8	6.06	7.33
Linolenic acid (C18:3)	5-9	0.4	-	1.2±0.2	7.6-12.4	0.64	2.30
Arachidic acid (C20:0)	1.0-1.5	-	trace-4	-	0.6-3.4	2.48	-
Gadoleic acid (C20:1)	0.1	-	-	-	0.3-2.0	-	-
Behenic acid (C22:0)	-	-	-	-	-	0.64	-
Lignoceric acid (C24:0)	-	-	-	-	-	0.49	-

CONCLUSION

The SC CO_2 extraction of MAF has been successfully performed obtaining comparable or higher extracted oil yield (4-12%) compared to that obtained from the conventional Soxhlet extraction (7%). The higher the extraction pressure the higher the oil extracted as well as the initial extraction rate. This is due to the fact that a small increase in pressure can cause a large increase in density of SC CO_2 and hence increase the oil solubility. However, as the temperature increases, the density of the SC fluids decreases and the oil vapor pressure increases. The solubility of the oil therefore is needed to encounter between two effects, resulting in an insignificant effect of temperature on the extracted oil yield. The extracted oils from different extraction pressure nevertheless did not show any variations in fatty acid compositions. In addition, similar percentages of fatty acids were found at each MAF fraction. It is also demonstrated that MAF has a potential for CB alternative due to the equivalent amounts of stearic and oleic acids to those of CB. The shortage of palmitic acid in MAF can be regain by mixing it with palm oil or palm oil fraction and the research on these mixtures will draw more interest from several researchers. The physical and thermal characteristics of these MAFs will be carried out in the future.

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