Behavior of beeswax and edible oils mixtures in supercritical carbon dioxide at moderate temperature

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Abstract

This study investigated the behavior of lipid mixtures of beeswax with avocado oil or Brazil nut oil in supercritical CO2 processing. It entailed the measurements of melting temperature (Tm), solidification temperature (Ts), volumetric expansion (Ve), solubility of supercritical CO2, and solubility of the lipid mixtures in supercritical CO2 at fixed pressures. The Tm and Ts of lipid mixtures decreased progressively to a lower level (closer to 150 bar) that were then increased with increasing pressure. The volumetric expansion, solubility of supercritical CO2, and solubility of the lipid mixtures in supercritical CO2 increased as pressure increased. On the basis of the results, particle formation using these lipid mixtures as carrier materials could be feasible with supercritical CO2 at a temperature of 60 °C and pressures from 150 to 300 bar. The moderate temperature of the process could allow its application with thermally labile bioactive compounds.

1. Introduction

Solid lipid particles are promising carrier systems for lipophilic bioactive compounds because of their high compatibility with the solid lipids used as carrier material (Santos et al., 2019). These particles are required for the development of innovative cosmetic, food and pharmaceutical products (Panigrahi et al., 2019); therefore, it is necessary to overcome their potential disadvantages, such as insufficient load capacity and polymorphic transitions during storage that lead to the expulsion of the bioactive compound (Poovi et al., 2019).

One strategy to avoid the disadvantages of solid lipid particles is the use of solid-liquid lipid mixtures as carrier material in order to alter the crystalline lattice (Santos et al., 2019). For this purpose, beeswax (a solid lipid), which is a complex organic lipid-based material used as a carrier material in particle formation, could be mixed with edible oils (liquid lipids). Some of these edible oils are rich in bioactive compounds that could improve the bioactivity of the formed particles. For example, avocado oil is used in the cosmetic industry due to its anti-inflammatory and skin hydration power (Tan, 2019; Rydlewski et al., 2020), and Brazil nut oil could positively influence the modulation of the immune system and organic responses to inflammatory and hypolipidemic processes (Santos et al., 2012).

The formation of solid lipid particles can be achieved using different technologies, among which the supercritical CO_2 technology has advantages such as operation at moderate temperature and use of CO_2 as a green solvent. This technology involves some techniques like gas antisolvent precipitation (GAS), particles from gas saturated solution (PGSS), and rapid expansion of supercritical solution (RESS) (Spilimbergo et al., 2006). The GAS process is based on the supersaturation of a liquid solution by dissolving supercritical fluid in which initially the solute (immiscible in supercritical CO_2) is solubilized in an organic solvent that must be completely miscible with the supercritical fluid. This then proceeds to produce a volumetric expansion of the liquid solution together with the gradual addition of supercritical fluid to the solution that results in the

precipitation of the solute of interest. PGSS is used for materials with relatively low melting temperatures, such as polymers and lipids. In this technique the supercritical CO_2 dissolves in a melted lipid or plasticized polymer, and the rapid expansion of the solution through a nozzle results in precipitated particles (Akbari et al., 2020). In the RESS process, supercritical CO_2 is used as a means to extract the target material at high pressure; subsequently, the supercritical solution is depressurized to achieve high supersaturation for the generation of fine particles (Yang et al., 2020). Neither RESS nor PGSS uses organic solvents. Supercritical fluid technology has several advantages such as dry particles (water-free and solvent-free, except GAS that isn't solvent-free), process simplicity, low processing cost and a wide range of products due to the modulation of the supercritical fluid density by changing pressure, thus easy separation and recovery of solvent and antisolvent can be performed by a depressurization step (Akbari et al., 2020).

In this context, the design of the particle formation process requires knowledge of the behavior of the carrier material in supercritical CO_2 to define process parameters for verifying their economic and practical feasibility (Spilimbergo et al., 2006). Accordingly, measurements of melting temperature (T_m) and solidification temperature (T_s) of the lipid mixture at fixed pressures is necessary in defining the process temperature. Temperature is a key process parameter because it must be high enough to keep the lipid mixture in liquid state during processing, but low enough to allow the use of thermally labile bioactive compounds (Ciftci and Temelli, 2014). Furthermore, measurements of volumetric expansion, solubility of supercritical CO_2 on lipids and lipid mixtures and solubility of the lipid mixtures in supercritical CO_2 at the process temperature is important in explaining particle characteristics such as morphology and size when formed at different pressures. There are behavioral studies in supercritical CO_2 for many solid and liquid lipids. Nevertheless, there is no experimental data available in the literature about beeswax-edible oils mixtures.

Thus, in this investigation a behavioral study that covered the measurement of T_m and T_s, V_e, solubility of supercritical CO_2, and solubility of lipid mixtures of beeswax with avocado oil or Brazil nut oil in supercritical CO_2 at fixed pressures was carried out.

2. Materials and methods

2.1. Material

Beeswax from a farm located in Pirassununga, state of São Paulo, Brazil was used as solid lipid material. Edible oils of interest were commercial avocado oil (Hass, Bauru, Brazil) and Brazil nut oil extracted from commercial Brazil nuts (*Bertholletia excelsa* H.B.K.) from Pará (Brazil). The extraction of Brazil nut oil was performed with supercritical CO_2 (99%; Linde, Sertãozinho, Brazil) at 70 °C and 350 bar with solvent-to-feed ratio of 38.77 g CO_2/g sample. Lipid mixtures (1:1 w/w) consisting of beeswax with avocado oil or Brazil nut oil were prepared at 90 °C by manual mixing. These lipid mixtures were studied to discover their possible application as a new carrier material for particle formation.

2.2. Lipid behavior in supercritical CO_2 observed in phase monitor

A phase monitor (SFC/RESS, Thar Instruments Co./Waters, Pittsburgh, USA) (Figure 1A) was used for measurement of properties of lipids in supercritical CO_2 such as T_m, T_s, volumetric expansion (V_e) and solubility of supercritical CO_2. In these experiments, a height scale was placed inside the high-pressure vessel to measure the increase of the sample level due to the expansion with pressurized CO_2. The sample was melted at 90 °C to eliminate crystal memory, and about 0.2 g of each sample was placed in a clear glass tube (0.3 cm in diameter and 4.0 cm in height) fixed to the autoclave using adhesive tape and near the sapphire window. The tube was placed into the high pressure cell (5.5 cm in diameter and 5.8 cm in height) in a position that allowed its observation using a camera through the sapphire window.

2.2.1 Melting and solidification temperatures in supercritical CO_2

First, the cell was heated until the entire sample melted at atmospheric pressure. For T_s identification, the cell was cooled down slowly (about 0.5 °C/min) until sample solidification was visually observed. After T_s indentification, the sample was cooled down for extra 3 °C above T_s, awaited 10 minutes for equilibration and then the cell was heated slowly (about 0.5 °C/min) until the first brightness was visually observed,

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this temperature was identified as T_m. This process was repeated for all the pressures studied that ranged from 0 bar to 300 bar. The beeswax, beeswax-avocado oil mixture and beeswax-Brazil nut oil mixture were the studied lipids. The T_m and T_s were used to define and recommend a process temperature for particle formation. Thus, volumetric expansion, solubility of supercritical CO_2, and solubility of lipids in supercritical CO_2 were determined and carried out at this temperature.

2.2.2. Volumetric expansion in supercritical CO_2

The scale placed inside the high-pressure cell was calibrated by adding different volumes of distilled water into the glass tube at ambient conditions.

The sample volume of lipids in supercritical CO_2 was measured at pressures that ranged from 150 to 300 bar after 10 min at each pressure to clearly observe the lipid-CO_2 interface. Volumetric expansion was expressed as the percentage increase in volume relative to the initial volume at atmospheric pressure. In the case of solid samples at atmospheric pressure, the initial volume was recorded as the observed volume slightly above its T_m and at atmospheric pressure. Then, the cell was gradually pressurized to 150 bar and cooled to reach process temperature, maintaining the sample in liquid state to allow the measurement. The beeswax, avocado oil, Brazil nut oil, beeswax-avocado oil mixture and beeswax-Brazil nut oil mixture were the lipids studied. All measurements were done in duplicate.

2.2.3. Solubility of supercritical CO_2

The solubility of supercritical CO_2 in lipids (g of CO_2/kg of lipid) was calculated from volumetric expansion data. The variation of the volume of the lipid material at a fixed pressure was considered as the volume corresponding to the CO_2 mass dissolved in the known mass of lipid material. The CO_2 density in each pressure was used to calculate the CO_2 mass from the variation of the volume.

2.3. Solubility in supercritical CO_2

The static solubility of lipid mixtures in supercritical CO_2 (g/kg of CO_2) was determined based on the methodology described by Cornelio-Santiago et al. (2017) and carried out at the Laboratory of High Pressure Technology and Natural Products (LTAPPN) of the Faculty of Animal Science and Food Engineering of the University of São Paulo (FZEA / USP) (Pirassununga, Brazil). Figure 1B contains the schematic of the equipment used. The experiment initially consisted of the contact between the lipid mixture (5 g) dispersed in glass beads (sufficient amount to completely fill the equilibrium cell) and supercritical CO_2 under fixed conditions of pressure (150, 200 or 250 bar) and temperature (60 °C) in a balance cell (300 cm3). After a fixed contact time (1, 2 or 3 hours), a sample of the mixture, consisted by lipids solubilized in supercritical CO_2, was moved to a high-pressure collector of known volume (8.58 cm^3).

For this purpose, the CO_2 inlet valves for the balance cell and the solution outlet from the balance cell for the high-pressure manifold were opened simultaneously to maintain constant pressure inside the balance cell as a variation in pressure could destabilize the solution.

The solution sample contained in the high-pressure collector was depressurized into a collection bottle. After depressurization, the high-pressure collector and the depressurization line were washed with pressurized ethanol (about 75 mL) to remove possible remaining materials and then it was dried by CO_2 flow from another cylinder, all the ethanol used in this cleaning was also collected in the collector bottle. Finally, the mixture of lipid material and ethanol was placed in an oven at 70 °C for complete evaporation of ethanol until a constant weight was reached and the mass of lipid solubilized by CO_2 could be measured.

The temperature control of both the equilibrium cell and the high pressure collector of known volume was performed by immersion in a thermostatic bath (Suprilab, Campinas, Brazil) and the pressure control by a high pressure pump (Eldex AA100S, Napa, CA, USA). All experiments were done in duplicate. The values of the density of CO_2 under different conditions of temperature and pressure were calculated using the empirical equation of Huang et al. (1985). The means were compared using Tukey's test.

2.4. Fatty acid profile

Both edible oils and beeswax were evaluated in terms of their fatty acid (FA) profile. The oils were prepared in the form of fatty acid methyl esters according to the 969.33 AOAC method (AOAC, 2005) and then analyzed by gas chromatography (GC) to determine the FA composition. The beeswax was prepared by dilution in chloroform followed by an intense mechanical shake until total dissolution, following the methodology described by Svečnjak et al. (2019).

The GC analysis was carried out on a gas chromatograph coupled with a mass spectrometer (GC/MS) (QP 2010 Plus, Shimadzu, Japan) with automatic injector (AOC-5000, SWI). A non-bonded poly (biscyanopropyl siloxane) phase (100 m × 0.25 mm x 0.2 µm) capillary column (Supelco SP^{TM}2560, Bellefonte, USA) was used. The injection temperature was 250 °C, and the column temperature ranged from 100 °C to 195 °C with a temperature ramp of 5 °C/min and from 195 °C to 250 °C with a temperature ramp of 2 °C/min. The interface and ion source temperature was 250 °C, with a solvent cut time of 10 min. The injected volume was 1 µL with a split ratio of 12.5; helium was used as the carrier gas. The mass spectra were obtained by electron impact using 70 eV ionization energy as the quadrupole analyzer from 40 and 350 m/z. The fatty acid methyl esters were identified by comparison with mass spectra using GC/MS solutions v. 2.5 software, which has as database the NIST 11 and NIST 11 s libraries.

3. Results and discussion

3.1. Melting and solidification temperatures in supercritical CO_2

The T_m and T_s data for beeswax and lipid mixtures were divided into two regions referred to region 1 and region 2 (Figure 2). In region 1, both temperatures decreased with increasing pressure, while in region 2 the melting and solidification temperatures increased with the increase in pressure. In the transition between the two regions (at 150 bar) each sample presented a different behavior. For the beeswax, the T_s increased but the T_m continued to decrease. For the beeswax-avocado oil mixture, T_s remained constant and T_m increased, and for the beeswax-Brazil nut oil mixture, T_s decreased and T_m remained constant.

According to de Sousa et al. (2006), this behavior can be explained by two distinct phenomena: the increase in the melting temperature of the compounds due to the increase in pressure and the decrease in the melting temperature by CO_2 dissolution in the sample.

The plotted points of T_m and T_s (Figure 2) for each sample were used to determine the depression at the melting and solidification points, respectively, at a given pressure compared to atmospheric pressure. Figure 3A introduces T_m depression with increasing of pressure. The melting temperature depression for both mixtures of edible oil and beeswax were very similar, but the depression of melting temperature of pure beeswax was noticeably lower. This phenomenon may indicate that the fatty acid composition of the samples has a substantial effect on the T_m depression in pressurized CO_2 because beeswax has longer chain fatty acids than the other oils studied. Ciftci and Temelli (2014) observed a greater T_m depression for lipids containing shorter chain fatty acids. In Figure 3B, which represents the T_s depression for pressures up to 100 bar, all samples showed similar T_s depression; however, for higher pressures the T_s depression of the beeswax-avocado oil mixture was higher than that observed for beeswax-Brazil nut oil mixture; that of pure beeswax was the lowest, a fact that can be justified by the same argument used for the T_m depression, which is lipids or mixture of lipids that have heavy chain fatty acids have a lower T_s depression.

On the basis of the analysis of the T_m and T_s, the studied lipid mixtures could be used as carrier materials for particle formation with supercritical CO_2 at a temperature of 60 °C and pressures from 150 to 300 bar. This process temperature can ensure a safety margin for maintaining the lipid mixture in liquid state during the period of contact with supercritical CO_2.

3.2. Volumetric expansion in supercritical CO_2

The volumetric expansion of lipids in response to increased pressure varied for each sample, as shown in Figure 4. In an attempt to investigate the relationship between volumetric expansion and melting point depression, the V_e of lipids in pressurized CO_2 was studied at points referring to region 2 of the melting curve and at intermediate pressures to those points. However, the volumetric expansion of lipids increased

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with increasing pressure at 60 °C temperature (Figure 4). Typically, the solubility of CO_2 in the liquid lipid phase increases with pressure to a certain level and then reaches a plateau (Jenab and Temelli, 2012); however, in this study this phenomenon was only observed for the pure beeswax and beeswax-brazil nut oil mixtures in the pressure range used. The smallest V_e was observed for beeswax, and the largest V_e varied depending on the pressure range. For pressures below 225 bar, the beeswax-Brazil nut oil mixture showed the greater V_e. At 225 bar the volumetric expansions of this mixture and pure Brazil nut oil were similar, and for pressures above this the V_e of Brazil nut oil was high due to the fact that the binary mixture had reached the plateau. The behavior of the beeswax-avocado oil mixture and pure avocado oil were similar, with the V_e of the mixture slightly above 150 bar. For the other studied points, the V_e of pure avocado oil was slightly higher.

Comparing Figures 2 and 4, it can be seen that for the studied region there is a simultaneous increasing of volumetric expansion and melting temperature. According to Ciftci and Temelli (2014), there is a possibility of a slight increase in the T_m of fats and oils saturated with CO_2 for relatively high pressures, which is attributed to the concurrent effects of the increase in the T_m due to a higher hydrostatic pressure and lower T_m due to CO_2 dissolution.

3.3. Solubility of supercritical CO_2 in lipids

The increase in volume observed in the volumetric expansion tests is due to the dissolution of CO_2 in the studied lipids; therefore, determining the solubility of CO_2 in the lipids and in the liquid lipid mixture can be useful to interpret the behavior of the volumetric expansion of the oil in equilibrium with supercritical CO_2.

As shown in Figure 5, the solubility of CO_2 in the studied lipids increased with increasing pressure. This phenomenon probably occurs by two mechanisms. The first is that with the increase in pressure the density of CO_2 increases, becoming more similar with the density of the studied lipids; the second mechanism is that the increase in pressure facilitates the entry of CO_2 into the tube containing the lipids so that there is regulation of internal and external pressure to the tube.

A study carried out by de Sousa et al (2006) with Precirol® ATO5, Compritol® 888 ATO and Gelucire® 43-01 as lipid carriers, also verified the solubility of CO_2 in the lipids, varies by changing pressure, a behavior similar to that observed in this work.

3.4. Solubility of lipids in supercritical CO_2

The solubility of the studied lipid mixtures in supercritical CO_2 at 60 °C and different pressures (150, 200 and 250 bar) and contact time (1, 2 and 3 hours) ranged from 1.84 ± 0.27 g/kg of CO_2 to 6.51 ± 0.02 g/kg of CO_2 for the beeswax-avocado oil mixture and from 0.79 ± 0.10 g/kg of CO_2 to 3.87 ± 0.25 g/kg of CO_2 for the beeswax-Brazil nut oil mixture (Table 1). The solubility of lipid mixtures in supercritical CO_2 increased when pressure increased. Studies by McHugh and Krukonis (2013) show that the solvent power of a supercritical fluid is related to its density, which is reinforced by Rad, Sabet and Varaminian (2019) when they stated that an increase in pressure leads to an increase in density and solvation power of supercritical CO2. Based on this, we can infer that the solubility of lipid mixtures in supercritical CO2 probably increased when the pressure increased because the fluid density increased, becoming more similar to that of the lipid mixtures.

Solubility studies carried out by Rodrigues and collaborators (2005) with Brazil nut oil did not show a high correlation between the BNO solubility in supercritical CO2 and the increase in pressure, contrary to what was observed in our experiments; for pressure variation also between 150 and 250 bar the solubility measured by the authors ranged from 2.56 to 2.64 g/kg at 50 °C and from 2.14 to 2.79 g/kg at 70 °C. The authors did not perform measurements at 60 ° C.

These results revealed that particle formation with supercritical CO_2 at 60°C using the studied lipid mixtures can be conducted in a short contact time to minimize the degradation of heat sensitive bioactive compounds, which makes these mixtures good carriers for solid lipid particles. However, it must be considered that the

solubility of beeswax-avocado oil mixture in supercritical CO_2 at 60 °C and 200 bar was also affected by the static contact time.

3.5. Fatty acid profile

Total ion chromatogram of fatty acid found present in avocado oil, Brazil nut oil and beeswax are represented in Figure 6. It can be seen in the total ion chromatogram that several peaks presented by the samples have similar retention times, which indicates the presence of similar fatty acid or with similar functional groups. The qualitative fatty acid profiles of studied lipids are reported on Table 2.

Methyl elaidate, methyl 11-octadecenoate and linoleic acid ethyl ester were identified in both edible oils, and methyl margarate was identified in all studied lipids, including beeswax.

The differences on fatty acid profile probably has an influence on the other results obtained, such as the elevated solubility of the beeswax-avocado oil mixture in supercritical CO_2. The fatty acid profile affects the solubility of the lipids in supercritical CO2 due to the different polarity and density of each fatty acid directly affecting the characteristics of the whole lipid. In addition, beeswax, which is a wax and solid at room temperature, has a more complex fatty acid profile than that found for edible oils with only a similar fatty acid, which probably justifies its different behavior.

4. Conclusions

The T_m and T_s of the studied lipid mixtures were affected by the pressure; they decreased with increasing pressure up to a pressure of 150 bar and then began to increase linearly with the increase in pressure. Based on T_m behavior, it was observed that the temperature of 60 ° C ensures that both pure beeswax and mixtures of beeswax with edible oils are melted at pressures equal to or greater than 50 bar, however, to ensure that the CO2 present in the medium is in the supercritical state, pressures greater than 75 bar are recommended, which indicates that these materials are potential carriers for lipid particles loaded with bioactive compounds formed by supercritical precipitation using CO2 as a solvent.

The higher the pressure applied in the process, the higher the solubility of the CO_2 in lipid mixtures and the greater the volumetric expansion observed, in the studied pressure range.

These results could be used, as a preliminary study, for the design of lipid particles loaded with bioactive compounds by supercritical CO_2 technology employing at moderate temperature to be used in the formulation of innovative and functional food and pharmacological products.

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Table 1. Solubility of binary mixtures beeswax and avocado oil and beeswax and Brazil nut oil (g/Kg CO_2) in supercritical CO_2 (60 °C) at different pressures and contact times.

Pressure (bar)	CO_2 density (g/cm^3)	Time	Time	Time
		1 h	2 h	3 h
Beeswax and avocado oil	Beeswax and avocado oil	Beeswax and avocado oil	Beeswax and avocado oil	Beesw

Pressure (bar)	CO_2 density (g/cm^3)	Time	Time	Time
150	0.61	$1.95 \pm 0.19^{a,A}$	$2.45 \pm 0.26^{a,A}$	$1.84 \pm$
200	0.73	$2.83 \pm 0.12^{a,A}$	$4.84 \pm 0.23^{b,B}$	$6.51 \pm$
250	0.79	$4.98 \pm 0.60^{b,A}$	$5.78 \pm 0.37^{b,A}$	$5.79 \pm$
Beeswax and Brazil nut oil	Beeswax and Brazil nut oil	Beeswax and Brazil nut oil	Beeswax and Brazil nut oil	Beesw
150	0.61	$1.08 \pm 0.20^{a,A}$	$0.79 \pm 0.10^{a,A}$	$0.90 \pm$
200	0.73	$2.51 \pm 0.29^{b,A}$	$2.25 \pm 0.09^{b,A}$	$2.56 \pm$
250	0.79	$3.70 \pm 0.10^{c,A}$	$3.74 \pm 0.40^{c,A}$	$3.87 \pm$

Results expressed in mean ± standard deviation, based on triplicates.

Parameters followed by the same lowercase letters in the same column and uppercase letters in the same row, do not differ statistically from each other at the 95% confidence level by the Tukey's means test.

Table 2. Fatty acid present in avocado oil, Brazil nut oil and beeswax, determined by GC-MS analysis.

Fatty acid	Sample	Molecular formula	Molecular weight (g/mol)	CAS n
1-Pentacosanol	BW	C23H48O	368.7	26040-9
2-Octyldecanol	BW	C20H42O	298.5	5333-42
Cis-10-heptadecenoic acid, methyl ester	AO	C18H34O2	282.5	75190-8
Docosane	BW	C22H46	310.6	629-97-
Eicosyl pentafluoroptopionate	BW	C23H41F5O2	444.6	-
Heneicosyl trifluoroacetate	BW	C23H43F3O2	408.6	-
Heptacosanol	BW	C27H56O	396.7	2004-39
Heptadecane	BW	C17H36	240.5	629-78-
Linoleic acid ethyl ester	AO, BNO	C20H36O2	308.5	544-35-
Methyl 11,14,17-eicosatrienoate	AO	C21H36O2	320.5	55682-8
Methyl 11-octadecenoate	AO, BNO	C19H36O2	296.5	6198-58
Methyl elaidate	AO, BNO	C19H36O2	296.5	1937-62
Methyl hexacosanoate	AO, BW	C17H34O2	270.5	112-39-
Methyl margarate	AO, BNO, BW	C18H36O2	284.5	1731-92
Methyl nonadecanoate	BNO, BW	C20H40O2	312.5	1731-94
Methyl palmitate	BW	C17H34O2	270.5	112-39-
Methyl palmitoleate	BNO	C17H32O2	270.5	112-39-
Methyl pentadecanoate	BNO	C16H32O2	256.42	7132-64
Methyl tetracosanoate	BW	C25H50O2	382.7	2442-49
n-Eicosane	BW	C20H42	282.5	112-95-
n-Octacosane	BW	C28H58	394.8	630-02-
n-Tetracosane	BW	C24H50	338.7	646-31-
n-Triacontanol	BW	C30H62O	438.8	593-50-
Trans-Squalene	BNO	C30H50	410.7	111-02-

CAS: Chemical Abstract Service, AO: Avocado oil. BNO: Brazil nut oil, BW: Beeswax.

Figure Captions

Figure 1. Supercritical fluid extraction systems.

Subtitle:

Figure 1A : (1)CO_2 cylinder, (2)cooling bath, (3)high-pressure pump, (4)heat exchanger, (5) computer

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equipped with Thar SFC software, (6)automated back pressure regulator (ABPR), (7)autoclave, (8)thermometer, (9)depressurization cell.

Figure 1B: (1)CO_2 cylinder, (2)refrigerated bath, (3)CO_2 cooling tank, (4)high-pressure pump, (5) two bourdon-type manometers, (6)needle valves, (7)buffer tank, (8)equilibrium cell, (9)known volume collector, (10)glass bottle collector, (11)solvent bottle, (12)helical pump, (13)temperature controlled water bath.

Figure 2. Melting temperature (T_M) and solidification temperature (T_S) of beeswax (BW), binary mixture of beeswax and avocado oil (BW:AO) and binary mixture of beeswax and Brazil nut oil (BW:BNO) under different pressures.

Figure 3. Melting point depression (A) and solidification point depression (B) of beeswax (BW), binary mixture of beeswax and avocado oil (BW:AO) and binary mixture of beeswax and Brazil nut oil (BW:BNO) under different pressures.

Figure 4. Volumetric expansion at 60 °C of avocado oil (AO), Brazil nut oil (BNO), binary mixture of beeswax and avocado oil (BW+AO), binary mixture of beeswax and Brazil nut oil (BW+BNO) and beeswax (BW) under different pressures, expressed as a percentage of initial volume.

Figure 5. Supercritical carbon dioxide (CO_2) solubility in beeswax (BW), avocado oil (AO), Brazil nut oil (BNO), binary mixture of beeswax and avocado oil (BW:AO), binary mixture of beeswax and Brazil nut oil (BW:BNO) under different pressures, expressed in g CO_2/Kg lipid.

Figure 6. Total ion chromatogram for fatty acid in avocado oil, Brazil nut oil and Beeswax.

Subtitle:

Avocado oil : (1)Methyl hexadecanoate, (2)Cis-10-heptadecenoic acid, methyl ester, (3)Methyl margarate, (4)Methyl elaidate, (5)Methyl 11-octadecenoate, (6)Linoleic acid ethyl ester, (7)Methyl 11,14,17-eicosatrienoate. *Brazil nut oil* : (1)Methyl pentadecanoate, (2)Methyl palmitoleate, (3)Methyl margarate, (4)Methyl elaidate, (5)Methyl 11-octadecenoate, (6)Linoleic acid ethyl ester, (7)Methyl nonadecanoate, (8)Trans-Squalene. *Beeswax* : (1)Heptadecane, (2)Methyl palmitate, (3)n-Eicosane, (4)Methyl margarate, (5)2-Octyldecanol, (6)Docosane, (7)Methyl nonadecanoate, (8)n-Tetracosane, (9)1-Pentacosanol, (10)Methyl tetracosanoate, (11)n-Octacosane, (12)Heptacosanol, (13)Heneicosyl trifluoroacetate, (14)Methyl hexacosanoate.