Determination of free fatty acids in crude vegetable oil samples obtained by high-pressure processes

Carolina Medeiros Vicentini-Polette [a], Paulo Rodolfo Ramos [a], Cintia Bernardo Gonçalves [b], Alessandra Lopes De Oliveira [a,*]

[a] LTAPPN, Department of Food Engineering, Faculty of Animals Sciences and Food Engineering (FZEA), University of São Paulo (USP), Av. Duque de Caxias Norte, 225, Pirassununga, SP 13635-900, Brazil
[b] (in memoriam) LES, Department of Food Engineering, Faculty of Animals Sciences and Food Engineering (FZEA), University of São Paulo (USP), Av. Duque de Caxias Norte, 225, Pirassununga, SP 13635-900, Brazil

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ABSTRACT

This study determined the total acidity, fatty acids profile (TFAs), and free fatty acids (FFAs) present in sunflower and soybean oils obtained by green processes (supercritical carbon dioxide-scCO$_2$ and pressurized liquid extraction-PLE). The determination of the primary fatty acids responsible for product acidity can provide a higher quality product. Sunflower (scCO$_2$/PLE-ethanol) and soybean (PLE-ethanol/PLE-hexane) samples were evaluated. The TFAs profile was determined by gas chromatography - mass spectrometry. The total FFAs content was determined by titrimetric method. For the qualitative determination of the FFAs present in the oils, a new technique capable of repeatably identifying the main FFAs was applied, using GC/MS. The primary fatty acids (palmitic, stearic, oleic, linoleic, eicosenoic, and linolenic) were present in all samples, both as TFAs and FFAs. However, fatty acids of lesser intensity showed variations. The applied methodology provided relevant data on the FAs that cause acidity in vegetable oils obtained by green processes.

Introduction

Hexane has been used for decades in the conventional extraction of vegetable oils and fats. This solvent consists of a mixture of hydrocarbons that generally boils between 65 and 69 °C. Its commercial version contains approximately 65% of normal hexane, with the remaining 35% comprising cyclopentane and hexane isomers (Bailey & Shahidi, 2005).

However, new extraction alternatives are currently being investigated, such as processes that use renewable or "green" solvents to obtain vegetable oils. A conventional, more rustic extraction technique (mechanical pressing) has also been used, providing vegetable oils without petroleum-derived organic solvent residues but lower yields. Green and health-safe solvents have been employed in high-pressure systems, such as extractions using solvents in supercritical states (Supercritical fluid extraction, SFE) and pressurized solvents (Pressurized liquid extraction, PLE). SFE and PLE are considered clean extraction techniques since they also do not leave toxic solvent residues in the extracted vegetable oils and because they require smaller amounts of solvent (in some processes) and shorter periods of time to perform.

Supercritical fluid extraction is based on the interaction between a matrix (solid or liquid) and a supercritical fluid. The fluid becomes supercritical under conditions of pressure (P) and temperature (T) above its critical point, defined as the T and P in which the gaseous and liquid phases become indistinguishable. When a substance is heated and compressed above its critical point, its "supercritical phase" is formed. Carbon dioxide (CO$_2$) is the most used solvent in supercritical extraction processes of vegetable oils since it presents the advantage of having low values of relative critical temperature (31.1 °C) and pressure (7.4 MPa) (Dunford et al., 2003). Extraction with scCO$_2$ is indicated for lipophilic compounds and non-polar substances. The combination of relative low temperature and high pressure allows good separation of phytosterols and vitamin E, more efficiently, purely, and with lower waste compared to the conventional process, since tocopherol (vitamin E) is fat-soluble (Asl et al., 2020; Maul et al., 1996).

Recently, studies based on extraction using supercritical CO$_2$ have been directed towards some unconventional vegetable oils, such as wheat germ (Bojanić et al., 2019), palm (Promraksa et al., 2020), and "bacaba-de-leque" – a Brazilian palm tree (Cunha et al., 2019). Benito-

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Román et al. (2018) evaluated the quinoa oil extraction using CO_2, proving that the oil obtained by $scCO_2$ presented higher antioxidant capacity and tocopherol content than the oil extracted with hexane, and also that the extraction rate may be strongly controlled by the pressure rate (20–40 MPa).

Obtaining conventional oils using this technology has also been evaluated, such as canola oil (Sun et al., 2021) and coconut oil (Torres-Ramón et al., 2021). In addition, the technique also has excellent potential for extracting oil contained in byproducts, including olive oil cake (Durante et al., 2020), raspberry seeds (Pavlić et al., 2020), and coffee grounds (Muangrat & Pongsirikul, 2019).

PLE with intermittent extract purge is an efficient extraction method that was developed in the 1980 s for analytical purposes to extract compounds at low concentrations from samples (Richter et al., 1996). The technique started to be used to obtain extracts with active principles, including "araçá" (Bittencourt et al., 2019) and "candeia" (Santos et al., 2019) extract, and to optimize the conditions for the extraction of matrices, such as coffee grains (N. A. de Oliveira et al., 2018). This process enables extractions in shorter times and the use of renewable solvents, such as ethanol, for example. High temperatures (T) decrease solvent viscosity, facilitating its penetration and diffusion without solute degradation, while high pressure (P) maintains the solvent in the liquid state under T conditions, in which it would otherwise be in the gaseous state at atmospheric pressure (Wang & Weller, 2006). The optimization of the PLE of vegetable oils using pressurized alcohols, such as ethanol and isopropanol, has been the subject of research by the current research group (Bittencourt et al., 2019; Okiyama et al., 2018; N. A. de Oliveira et al., 2018).

The determination of free fatty acid (FFA) content is an important analysis for evaluating the quality of raw material and its degradation during storage, and throughout the shelf life of several vegetable oils, such as soybean, sunflower, rice, and canola. Also, the oxidation process may confer different quality and sensorial attributes to the oils (Coppin & Pike, 2001; Franco et al., 2018). The influence of temperature on oil degradation was assessed by Frega et al. (1999) in their study on oxidative stability.

The analysis of free fatty acids (FFAs) present in crude oils obtained by different extraction processes (conventional, PLE, and SFE) was standardized in the present study.

Two widespread analyses performed on oils are the determination of the total fatty acid (TFA) profile, which identifies the individual fatty acids that make up the triacylglycerols present in the oil, and the acidity index (AI), used to determine the FFA content. Thus, high acidity levels indicate high concentrations of FFAs in the oil (Santos et al., 2021).

Gonçalves & Meirelles (2004) determined the FFAs and TFAs in palm oil. According to the authors, although lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and arachidic (eicosanoic) acid esters were found in the TFA profile, only lauric, palmitic, stearic, oleic, linoleic, and arachidic acid were observed in the FFA.

According to the studies by Nimet et al. (2011) and Rai et al. (2016), who described the use of gas chromatography to determine the TFA profile in sunflower oil extracted using supercritical carbon dioxide ($scCO_2$), the extraction method did not influence the TFA profile, as was observed in coffee oil extraction (Cornelio-Santiago et al., 2017; De Oliveira et al., 2014). However, in Brazil nuts, Cornelio-Santiago et al. (2021) found differences in fatty acid content depending on how the oil was extracted.

Acidity can be determined by volumetric titration and can be expressed as a percentage by mass of lauric, palmitic, erucic, or oleic acid, depending on the oil's origin. This method is widely used for refined oils and presents good repeatability (Varona et al., 2021).

Although the identification of the TFA profile and the determination of titrimetric acidity are highly relevant for the characterization of oils, there is still no standardized methodology for the determination of FFAs in vegetable oils without the occurrence of triacylglycerol breakdown. Determining the acidity index and acid content does not allow for the individual identification of the free fatty acids. From a practical standpoint, the results of this study might provide insights leading to better understanding of the free fatty acids in crude vegetable oils extracted using green technologies.

Therefore, this study aims mainly at the development and detailing of a methodology for identifying FFAs in sunflower and soybean crude oils obtained by green processes, and stating the TFAs profile and total acidity levels for such oils as well as their qualitative FFAs profiles using the new procedure, determining eventual differences in FFA composition regarding the extraction method.

Material and methods

Reagents

In order to obtain the crude oils, 99.5% absolute ethanol (Dinâmica, Indaiatuba, Brazil), 99% carbon dioxide [CO_2] (Linde, Sertãozinho, Brazil), and *n*-hexane P.A. ACS (Êxodo Científica, Hortolândia, Brazil) were used.

Sample acidity was determined using ethyl ether P.A. (Dinâmica, Indaiatuba, Brazil), phenolphthalein P.A. (Synth, Diadema, Brazil), and 99% sodium hydroxide (Êxodo Científica, Hortolândia, Brazil).

For the determination of the free fatty acid content, the following reagents were used: 99% sodium hydroxide [NaOH] (Êxodo Científica, Hortolândia, Brazil), *n*-hexane P.A. ACS (Êxodo Científica, Hortolândia, Brazil), chromatographic grade *n*-hexane (VWR, Fontenay-sous-Bois, France), 14% boron trifluoride [BF_3] in methanol (Aldrich, St. Louis, USA), 98.5% hexane (Êxodo Científica, Sumaré, Brazil), and 99% anhydrous sodium sulfate P.A. [Na_2SO_4] (Synth, Diadema, Brazil); 99.9% compressed nitrogen (Messer, Barueri, Brazil) was used for drying the samples

Methods

Four vegetable oil samples obtained by alternative processes were analyzed, two sunflower oils and two soybean oils.

To determine the free fatty acids, the AOAC method 969.33 (AOAC, 2005) has been modified as an analytical basis so, instead of the total content, only the free fatty acids present in the crude oils would be determined.

Obtaining the crude oils

The sunflower oils were obtained by pressurized liquid extraction (PLE), with intermittent solvent purge, using 99.5 % ethanol as a solvent at a temperature (T) of 84 °C, and a rinse volume (RV) in each cycle corresponding to 110 % of the 100 mL extraction cell volume (110 mL). The rinse volume was divided into four cycles (N), each lasting 5 min, constituting the static time (St) of contact between the matrix and the solvent in each cycle. The extraction was conducted under optimized conditions, in which it was possible to recover 93.93 % of oil. The optimized conditions were determined in previous studies of the extraction process using rotated central compound delineation (CCRD) design. In the supercritical extraction using carbon dioxide ($scCO_2$), also performed under optimized conditions (60 °C; 32.1 MPa; flow rate of 10 g/s for 4 h), it was possible to recover 87.58 % of oil.

Meanwhile, the crude soybean oils were obtained by PLE, with ethanol and hexane as pressurized solvents. Upon extraction using 99.5 % ethanol, the optimized conditions (80 °C; RV corresponding to 60 % of the extraction cell volume of 100 mL, or 60 mL) were applied. The RV was divided into three cycles (N), with a St of 12 min; soybean oil recovery under this condition was 94.40 %. As for the PLE-hexane, also performed under optimized conditions (85 °C; RV of 60 % of the extraction cell volume, divided into 3 cycles (N); St of 13 min), the recovery rate was 86.16 % of oil.
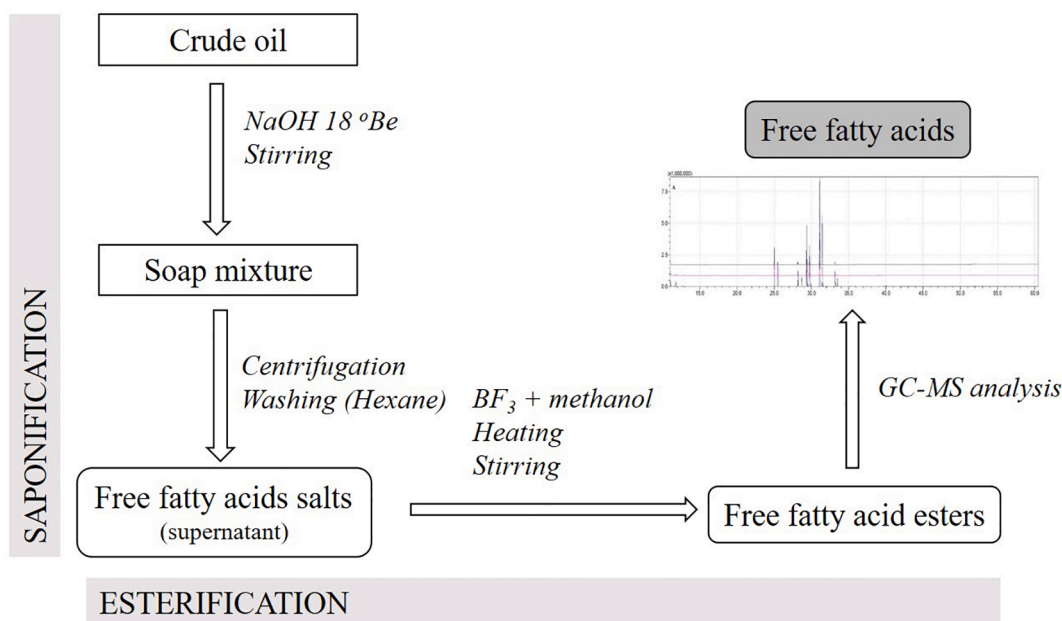
Fig. 1. Diagram of the processes used to determine the free fatty acid.

Determination of the total titratable acidity. The total titratable acidity was determined for all samples according to the Ca 5a-40 method (AOCS, 2004). Approximately 2.0 g of oil were used, dissolved in 25 mL of ether-alcohol solution (2:1), as well as two drops of 1 % phenolphthalein indicator in ethanol. Titration was performed using 0.1 M sodium hydroxide solution in deionized water until the appearance of pink color, with a minimum persistence of 30 s (Moretto & Fett, 1986). The FFA content was calculated according to Equation (1).

$$\% \ FFA = \frac{(V.M.28.2)}{m} \tag{1}$$

where % FFA represents the percentage of free fatty acids, V is the volume of solvent used, M corresponds to the Molarity of the NaOH solution, and m is the mass of the crude oil sample.

The results were expressed in oleic acid content (% m/m), and all analyses were conducted in triplicate.

Identification of total fatty acids (TFA). To the total fatty acid profile determination, the crude oil samples were saponified and esterified using hexane (method 969.33, AOAC, 2005), then diluted to 10 % (in hexane) and injected into the chromatograph.

The profile was obtained using a capillary column used was the SP 2560 (100 m × 0.25 mm × 0.2 μm), with helium as the carrier gas, at a flow rate of 1.59 mL.min^{-1}, by Gas Chromatography coupled to the Mass Spectrometer (GC–MS), equipped with a Split injector (1:40) (Shimadzu, GCMS-2010), with an automatic injector (model AOC-5000) at 250 °C and P = 300 kPa. The oven temperature was programmed to go from 100 to 250 °C, initially remaining at 100 °C for 1 min and gradually increasing by 5 °C.min^{-1} until reaching 195 °C. Next, the ramp was adjusted to gradually increase by 2 °C until it reached 250 °C, where it remained for 13 min for completion. Readings were taken between minutes 11 and 60.5, with a mass range of 40 to 350 *m/z*.

The obtained data were evaluated using the GC–MS Solutions software (v. 2.5, USA), coupled to the gas chromatograph and the mass spectrometer. The detector provided the ion chromatograms and molecular mass spectra for each sample. Identification was carried out by comparing the mass spectra of the compounds with those found in the NIST Library (National Institute of Standards and Technology, v. 11, USA), integrated in the detector. Peaks with an area ≥ 0.5% were considered.

Saponification. The saponification and esterification processes are outlined in Fig. 1.

A total of 3.0 g of crude vegetable oil were used. This mass was established considering the acidity of the crude sunflower and soybean vegetable oils used in the analysis (from 2.1 to 3.6 g/100 g oleic acid), estimating the amount required for detection by gas chromatography-mass spectrometry (GC–MS).

In order to determine the FFAs, it was necessary to ensure that all the FFAs present in the oil were completely neutralized. The present analysis was based on the study by Gonçalves & Meirelles (2004), who highlighted the use of an excess of 10 % of NaOH solution for complete acid neutralization. Since there was no prior knowledge of the acids contained in the oils, it was not possible to determine the molar mass of the compounds. Therefore, the used volume was estimated based on the aforementioned study.

Five milliliters of NaOH solution (12.69 %, m/m) in deionized water were added to each sample at room temperature, followed by vigorous stirring in a Vortex shaker (model Genius 3, IKA, Staufen, Germany) for 15 min. The mixture of NaOH with the oil results in the saponification of free fatty acids, *i.e.*, the formation of fatty acid salts. This reaction was performed at room temperature (Choe & Min, 2006) and using low concentration NaOH solution to avoid the tracylglycerol breakdown / hydrolysis.

Subsequently, the mixtures were centrifuged (Excelsea II 206-BL centrifuge, Fanem, Guarulhos, Brazil) at 5000 rpm for 10 min to separate the soap. Following centrifugation, three phases were observed. By tilting the tube and with the aid of a glass Pasteur pipette, the aqueous and oil phases were drained. Then, the white solid (soap) phase was carefully transferred to a new tube. In this step, it is crucial to avoid the transfer of neutral oil together with the soap. Therefore, the solid phase was carefully dried with absorbent paper.

Separation of the fatty acid salts. The soap was then washed with P.A. hexane. To this end, 3 mL of the solvent was added, vortexed for 15 s, and then centrifuged for 10 min. This process was repeated five times, carefully collecting the upper phase (hexane), where the salts are found.

The collected hexane was accumulated in a 5 mL vial. Subsequently, the solution containing the fatty acid salts was dried under N$_2$ flow in a sample concentrator (Marconi, Piracicaba, Brazil).

Esterification. Upon obtaining the fatty acid salts, esterification was conducted to volatilize their compounds. After drying, 3 mL of boron trifluoride (BF_3) in methanol were added to the samples at a concentration of 14 %. In a water bath, the vials were heated to 100 °C for 2 min, followed by rapid cooling in running water. For convenience and safety, the glass vials were placed in 50 mL screw-capped plastic tubes for heating.

Next, 6 mL of chromatographic grade hexane were added to the vials, which were then vortexed. After phase separation, the supernatant phase (hexane + fatty acid esters) was collected into a new glass flask, while the lower phase (BF_3 + methanol) was discarded.

Approximately 0.2 g of Na_2SO_4 was added to the collected phase, aiming at capturing possible water molecules that could be present. The vials were then shaken, after which phase separation was awaited.

Using a glass Pasteur pipette, approximately 1.5 mL of the supernatant phase were carefully transferred to the GC–MS injection vials.

Determination of free fatty acids. The free fatty acids in the oils were analyzed by gas chromatography-mass spectrometry (QP 2010 Plus, Shimadzu, Tokyo, Japan) in a device equipped with a Split/Splitless injector (AOC-5000, Shimadzu, Tokyo, Japan) with an automatic injector (model AOC-5000). A capillary column (100 m × 0.25 mm id × 0.20 μm df, SP-2560 Supelco, Bellefonte, USA) was used as a stationary phase, with helium as the carrier gas, at a flow rate of 1.59 mL/min. The samples were injected into an injector with a Split ratio of 1:40 and a temperature of 250 °C; the pressure in the column was 300 kPa.

The oven temperature and data treatment were applied similarly to the TFA analysis, however, this analysis considered peaks with an area ≥ 0.4 % in at least two of the three replicates of each sample.

The values were shown as the percentage of the total area detected in the analysis. Since this study proposed a qualitative analysis of the free fatty acids, the values were not normalized. Therefore, the actual mean values detected during the analysis were shown.

Results and discussion

The extracted crude oils were stored in a freezer at −20 °C, in the absence of light, until the moment of analysis.

Titratable acidity

The crude sunflower oils extracted by the green processes exhibited an acidity of 3.59 ± 0.010 and 3.17 ± 0.025 g oleic acid/100 g oil when obtained by $scCO_2$ extraction and PLE using ethanol as a solvent, respectively. In turn, the soybean oils presented an acidity of 2.10 ± 0.001 and 2.11 ± 0.008 g/100 g oleic acid in the PLE using ethanol and hexane as solvents, respectively.

An increase in the acidity index of an oil is an indication of the breakdown of triacylglycerols and the degradation of the product, given that the greater an oil's acidity, the greater its concentration of free fatty acids (Santos et al., 2021).

Some physical parameters, such as temperature, humidity, exposure to light, or the action of lipolytic enzymes, can accelerate the process of breaking down triacylglycerols into FFAs. Frega et al. (1999) used an accelerated oxidative test to analyze the influence of temperature (110 °C) on the oxidation of various oils and concluded that there is an increase in oil acidity with increasing duration of exposure to high temperatures.

The presence of light also accelerates the oxidation process and is one of the most common problems in the stocking and storage of oils and lipid-rich foods. Thode Filho et al. (2014) analyzed the deterioration rates of soybean, sunflower, canola, and corn oil in the presence and absence of light. After measuring oil acidity for 70 days, the authors noted that the acidity levels were higher in oils exposed to light as compared to those that remained in the absence of light.
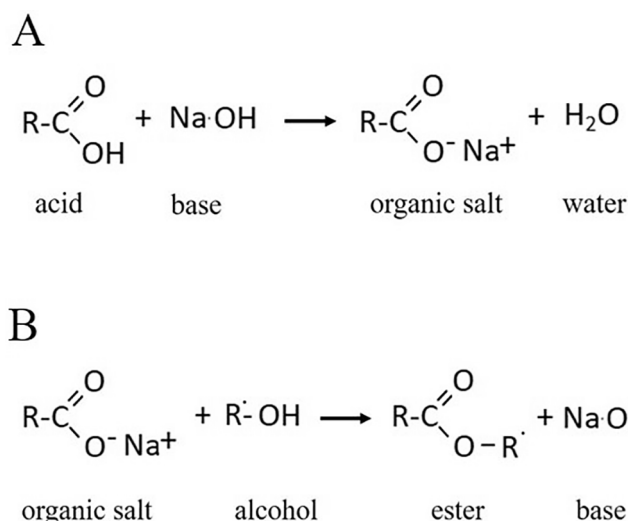

Fig. 2. Schematic of the neutralization reaction for the formation of fatty acid salts (A) and their esterification (B).

Refined sunflower oil has 0.13% equivalent in oleic acid, while soybean oil, 0.09% (Jorge et al., 2005). This result indicates that sunflower oil has a higher content of free fatty acids than soybean oil, even after refinement.

According to Correia et al. (2014), the authors found, in crude sunflower oil obtained by pressing and filtering, an acidity of 3.09 % in oleic acid. This value has the same order of magnitude as that observed herein, indicating that the crude sunflower oil has an acidity greater than 3 % in oleic acid; minor differences may be related to both the extraction process and seed variety.

Avramiuc (2013) evaluated the variation of the acidity index in sunflower oil extracted using a conventional method during storage under different conditions. The oil was stored in the presence of light and in the absence of light, at 4 °C and 20 °C. The author reported that the fresh oil had 4.5 mg KOH/g (2.26 g oleic acid/100 g oil). Based on the author's findings, in the presence of light at 4 °C, the acidity remained at 4.5 mg KOH/g (2.26 g oleic acid/100 g oil) on the 5th day of storage, increasing until 12.8 mg KOH/g (6.44 g oleic acid/100 g oil) on the 60th day; in the absence of light at 4 °C, the acidity ranged from 4.5 to 13.8 mg KOH/g (2.26 to 6.94 g oleic acid/100 g oil) in the same period. Meanwhile, at 20 °C, the authors observed a variation in acidity from 9.7 to 31.8 mg KOH/g (4.88 to 16.01 g oleic acid/100 g oil) in the presence of light and 10.5 to 30.3 mg KOH/g (5.29 to 15.25 g oleic acid/100 g oil) in the absence of light, on the 5th and 60th day, respectively. Therefore, it is evident that the correct storage of extracted oils is essential since the increase in acidity results from the degradation of triacylglycerols present in these oils, *i.e.*, loss of neutral oil. In the present study, the seeds were stored at −20 °C, in the absence of light, avoiding the acidification increase in oils, and the acidity observed in both treatments is close to the acidity reported by Avramiuc (2013) in the first 5 days.

In turn, Thode Filho et al. (2014) evaluated the variation in the acidity index of commercial soybean, sunflower, canola, and corn oil obtained by conventional extraction in the presence and absence of light for 70 days, kept in their plastic cartons and at a temperature of 30 °C. According to the authors, an increase in the acidity of the analyzed oils was observed both in the absence and presence of light in all the studied oils. Also, Nodar et al. (2002) claimed that soybean oil obtained by $scCO_2$ has higher total acidity when compared to oil obtained by conventional extraction (hexane).

Nodar et al. (2002) reported acidity of 0.8 % (oleic acid) in crude soybean oil extracted by $scCO_2$ (300 bar, 40 °C).
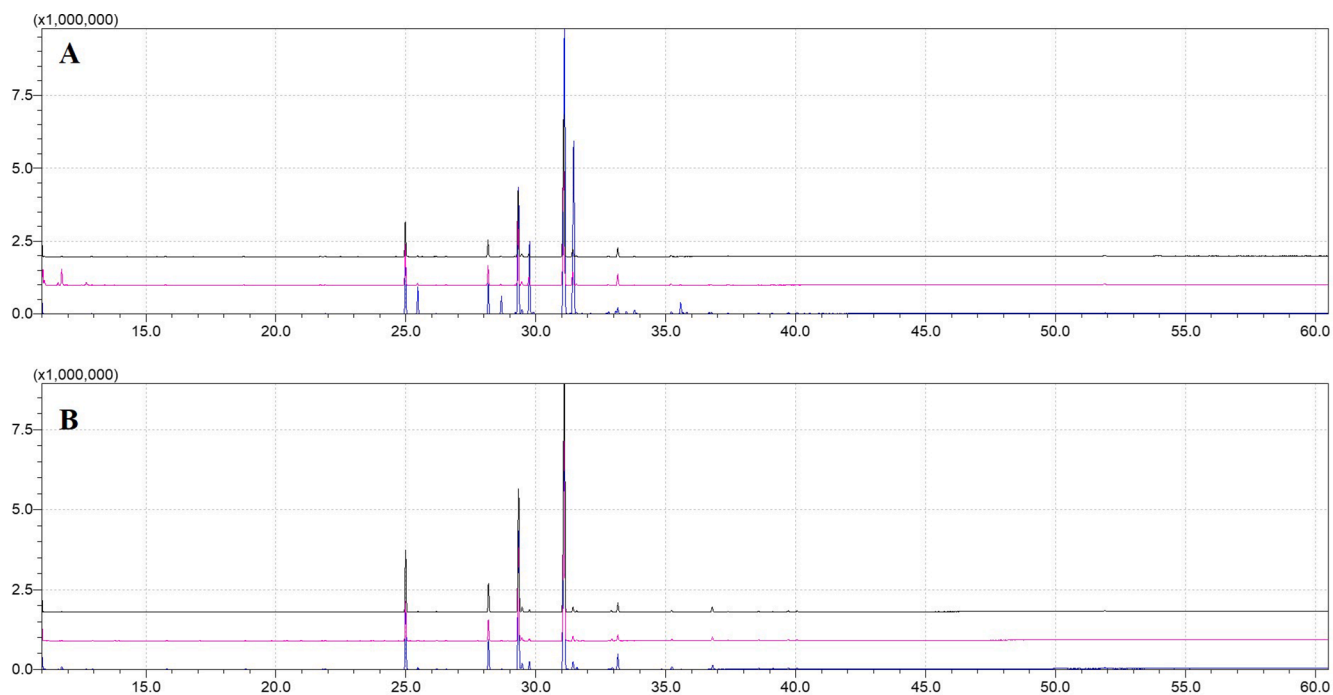
Fig. 3. Chromatograms for sunflower oil extracted by supercritical CO_2 (2) and pressurized ethanol (B).

FFAs

In order to determine the FFA, we ensured that all necessary steps were carried out so that the fatty acids could be saponified, separated, and esterified, enabling their detection via GC–MS.

The performed saponification procedure was equivalent to an acid-base neutralization reaction, in which an acid is neutralized by a base, forming salt and water. In oils and fats, the FFAs are neutralized by NaOH, forming water and organic salts, the latter being used in the next steps of the analysis. Fig. 2 shows the chemical equation that represents the neutralization reaction.

Triacylglycerols present in these oils can be degraded into FFAs by hydrolysis, also called hydrolytic or oxidative rancidity, a phenomenon that confers unpleasant odor and taste to the oils. The oxidation process is initiated by the breakage of the bond between hydrogen and the α-carbon, forming the first free radical. This newly formed radical reacts with oxygen, forming peroxides, which, in turn, steal hydrogen from another lipid molecule, restarting the cycle (Coppin & Pike, 2001).

The neutralization reaction was conducted at room temperature to avoid the breakdown of triacylglycerol molecules, since the increase in
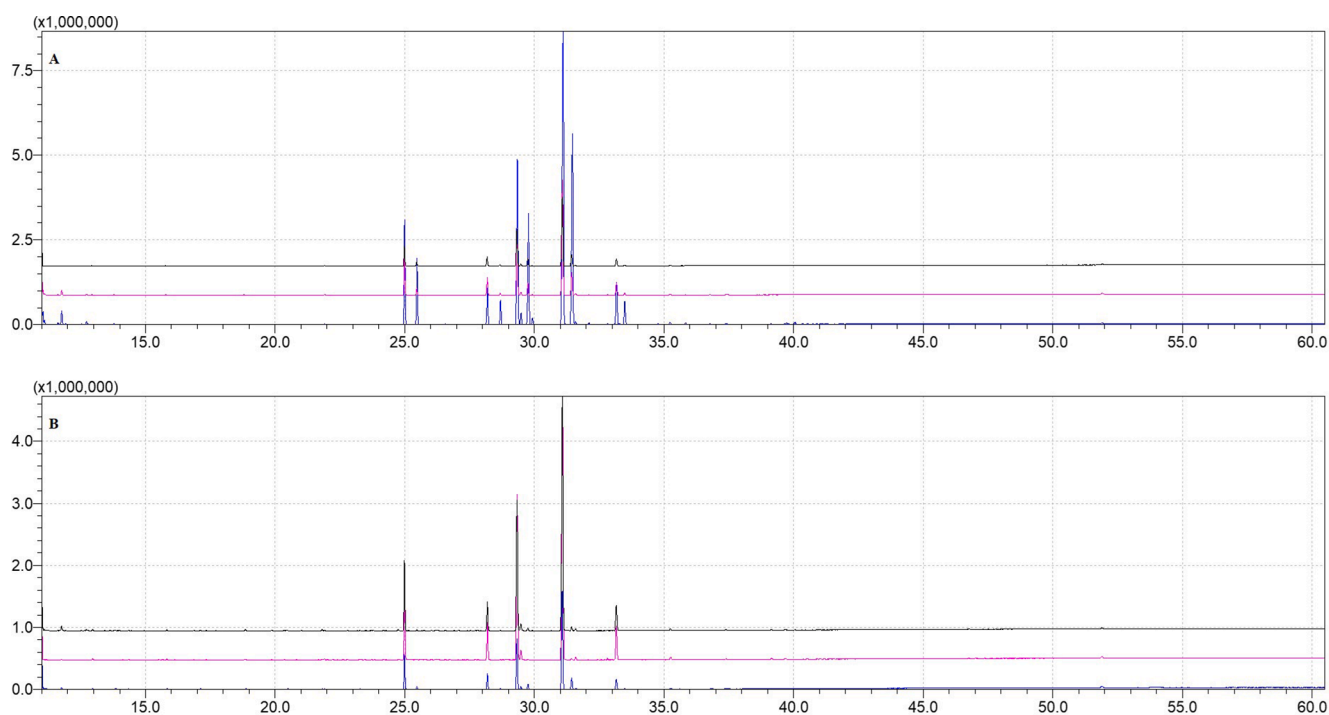

Fig. 4. Chromatograms for soybean oil extracted by pressurized ethanol (A) and hexane (B).
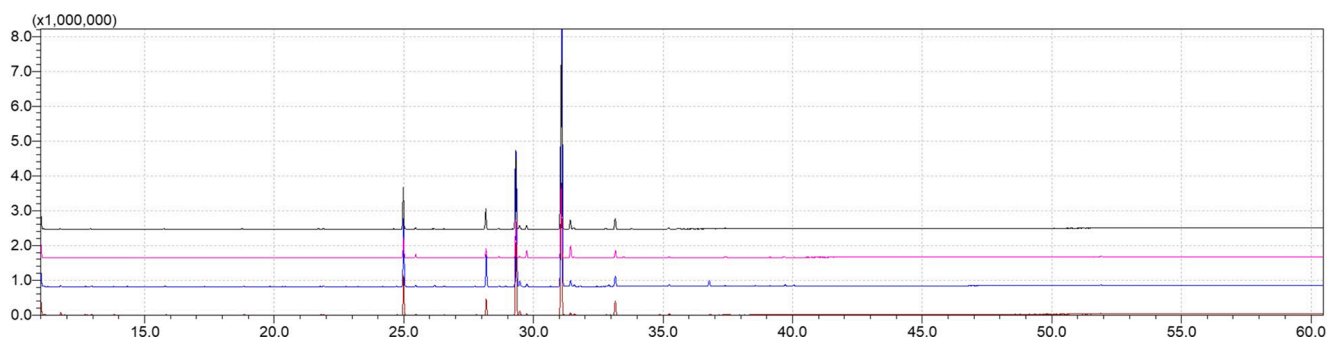
Fig. 5. Superimposition of the fatty acid esters in sunflower and soybean oil extracted using green technologies.

temperature favors the thermal degradation of triacylglycerol molecules into free fatty acids (Choe & Min, 2006). In addition, the temperature rise increases the agitation of the molecules and atoms forming the chemical bonds (Sobrinho & Paula e Souza, 2006).

The greater agitation of the molecules would favor the occurrence of shock between the triacylglycerols molecules and the hydroxyl of the base used for the neutralization reaction. The greater agitation/vibration of the atoms makes the bond more unstable and favors its breaking (Sanibal & Filho, 2002). Therefore, a lower reaction temperature disfavors the degradation of the triacylglycerol as well as the neutralization reaction.

In addition, free fatty acids have O—H bonds (acid group). Oxygen has a higher electronegativity than hydrogen, so the bond atoms between these two compounds are closer to oxygen than to hydrogen. This lower electron density around hydrogen causes the free hydroxyl molecules (OH–) to be more attracted to the hydrogen of the free acid molecules than to the O—C bonds of the triacylglycerol, since oxygen and carbon have similar electronegativities.

For these reasons, associated with the small amount of base used (in low concentration) there is the favorable neutralization of only the free fatty acids in the reaction medium and not the breakdown of triacylglycerols.

Therefore, the breakdown of triacylglycerol molecules was avoided. Thus, the salts were formed with the free fatty acids present in the crude oil. These hexane-soluble FFAs were collected by sequential washing, followed by drying in nitrogen. In this step, it is crucial that the supernatant phase be carefully collected in order to avoid carrying the neutral oil onto the following stages.

In GC–MS analyses, it is essential that the compounds of interest be volatile. Therefore, after the final drying step, the collected salts underwent esterification, aiming to obtain free fatty acid esters. To this end, BF_3 in methanol (alcohol) was added to the samples, followed by heating, the addition of hexane, and stirring. These steps ensure phase separation, allowing the collection of esters without the presence of secondary compounds. The esterification reaction is outlined in Fig. 2.

The previously described steps enabled the qualitative analysis of the free fatty acids present in the crude oils. However, further studies addressing their quantitative analyses are required.

Figs. 4 and 5 show the repeatability of the method for obtaining the different oils by different extraction techniques. The repetition of peaks was observed in all three replicates, indicating that the retention time (r't) (x-axis) is a reliable identification factor when applying the exact methodology described in this study.

Although this technique is not suitable for quantifying free fatty acids, it is possible to observe that oleic and linoleic acid stood out proportionally regarding the other acids, as was expected. This result was observed for both sunflower oil (Fig. 3) and soybean oil (Fig. 4), regardless of the extraction method used.

In the two Figures, it can also be noted that, in addition to the major compounds, the minor ones were also reproduced, indicating method reliability.

Table 1
Free fatty acid composition in sunflower and soybean oil extracted using green processes.

Matrix	Sunflower oil		Soybean oil	
	scCO$_2$	PLE-ethanol	PLE-ethanol	PLE-hexane
FA ester (group)	Mean peak area (%) ± SD			
Palmitic (C16:0)	10.21 ± 0.31	9.06 ± 0.74	11.38 ± 1.03	12.27 ± 0.49
Eicosanoic (C20:0)	0.40 ± 0.06		1.93 ± 0.02	
Stearic (C18:0)	5.18 ± 0.14	4.65 ± 0.09	5.13 ± 0.30	5.22 ± 0.14
Arachidonic (C20:4)			0.65 ± 0.03	
Oleic (C18:1)	21.93 ± 0.53	22.80 ± 0.47	20.48 ± 0.85	25.05 ± 0.17
Elaidic (*trans*C18:1)	1.51 ± 0.65		3.95 ± 0.17	
Linoleic (C18:2)	48.75 ± 3.20	56.25 ± 1.81	41.08 ± 1.06	47.75 ± 0.22
Eicosenoic (C20:1)	3.10 ± 0.60	1.05 ± 0.13	7.09 ± 0.20	2.59 ± 1.93
Linolenic (C18:3)	3.09 ± 0.04	1.89 ± 0.47	4.15 ± 0.01	5.17 ± 0.14

*FA = Fatty Acid; SFE = Supercritical Fluid Extraction; PLE = Pressurized Liquid Extraction;

Although slight differences were observed between the minor compounds of the different crude oils analyzed, Fig. 5 shows the superimposition of the four samples, indicating similarities between the free fatty acids of the vegetable oils obtained using green technologies.

The extraction method influenced the free fatty acid composition of the vegetable oils (Table 1), although only regarding minor fatty acids. The most present FFAs were equivalent among the samples, corroborating reports in the literature concerning TFAs.

Although this technique does not allow quantification, when using the criterion for selecting peaks with at least 0.4 % of total area in at least two of the three replicates per sample, it was noted that in sunflower oil, eicosanoic and elaidic acid were found when extraction with scCO$_2$ was applied, but not by PLE-ethanol (Table 1). This result is consistent with the observed acidity index, in which the acidity of the sunflower oil obtained by SFE-CO$_2$ was more pronounced. Thus, it is likely that the proportion of these fatty acids in the oil obtained by PLE-ethanol became undetectable or that their values were null or irrelevant in the whole.

When extracted by PLE-ethanol, the presence of arachidonic and elaidic acid was also observed, although no differences were found in the acid content of these oils extracted using different solvents.

The proportions shown in Table 1 were not normalized since there was a possibility that other fatty acids of lesser intensity, undetectable by this methodology, could be present.

In bleached and refined palm fat, Gonçalves & Meirelles (2004) observed the following fatty acids in the TFA profile: lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, and arachidic acid. Linolenic acid was found only in the bleached oil. However, in the FFA content of both treatments, only lauric, palmitic, stearic, oleic, linoleic,

Table 2

Total fatty acid profile (TFA) of sunflower (SF) and soybean (SB) oils obtained by optimized green processes (SFE and PLE), compared to relevant literature.

Matrix	Solvent Extraction	C16:0	C18:0	C18:1	C18:2	C18:3	C22:0
		Palmitic	Stearic	Oleic	Linoleic	Linolenic	Behenic
SF (this study)	CO₂	6.91	5.03	22.6	64.77	*nd*	0.69
SF (this study)	Ethanol	7.34	5.66	24.88	61.2	*nd*	0.92
SB (this study)	Hexane	11.62	4.71	24.83	49.45	6.29	*nd*
SB (this study)	Ethanol	12.84	7.49	24.49	39.06	8.15	*nd*
SF (Rai et al., 2016b)	CO₂	4.91–6.97	3.13–4.54	51.83–56.43	33.31–36.18	*nd*	0.36–1.04
SF (Correia et al., 2014)	Cold pressing	4.00	1.47	49.02	45.36	*nd*	*nd*
SB (Jokić et al., 2013)	CO₂	9.02–11.67	4.14–6.80	21.24–25.39	50.98–55.76	5.21–6.31	0.18–0.73
SB (Ivanov et al., 2011)	CO₂	16.95	5.15	16.02	47.57	12.11	0.39
SF* (Cuevas et al., 2009)	Ethanol	6.80	4.16	22.75	63.83	0.82	0.71
SF (Chowdhury et al., 1970)	ng	6.52	1.98	45.39	46.02	0.12	*nd*
SB (Chowdhury et al., 1970)	ng	14.04	4.07	26.27	52.18	5.63	*nd*

* degummed; ng = not given; nd = not detected.

and arachidic acid were observed. Those found in the FFAs had the highest mass percentage, except for lauric acid, which presented a mass of 0.67 % and 0.49 % in the bleached and refined oils, respectively.

TFAs

In general, the results observed in this study are close to the ones available in relevant literature (Table 2).

Information on the total fatty acid profile of sunflower oil extracted by PLE or SFE can be found in the literature (Nimet et al., 2011; Rai et al., 2016a), but not on the FFAs.

Velez et al. (2012) determined the composition of the total fatty acids (TFAs) found in commercial sunflower oil, which comprised 85 % in weight of oleic acid, 14 % in weight of palmitic acid, and 1 % in weight of stearic acid. Also regarding TFAs, higher concentrations of linoleic acid were observed, ranging from 33.3 to 68.7 %, and oleic acid, between 17.3 and 56.4 % (Cuevas et al., 2009; Nimet et al., 2011; Rai et al., 2016a). Correia et al. (2014) reported concentrations of 49.02 and 45.35 % of oleic and linoleic acids, respectively, in sunflower oil obtained by pressing. However, no information on the FFAs was provided.

The composition of the TFAs in the soybean oil determined herein corroborates the data obtained by several authors (Chowdhury et al., 1970; Ivanov et al., 2011; Jokić et al., 2013), who found higher concentrations of linoleic and oleic acid (52.18 and 23.27 %, respectively), in addition to similar concentrations of palmitic acid (11.74 %), stearic acid (5.15 %), and linolenic acid (6.24 %). Information on the FFAs of the soybean oil was also unavailable.

These results suggest that the profiles of TFA and FFAs are similar for major compounds, although minor compounds may vary. In the present study, the acids that generated the smallest peaks (area, Tables 1 and 2) varied among the samples.

Modern deacidification techniques based on the removal of oleic acid are under development, such as the method described by Ilgen & Dulger (2016). The authors used zeolite 13X as an adsorbent to remove the acid in sunflower oil, managing to desorb up to 91.6 % of the acidity present in the samples. The present study could validate the development of specific deacidification methodologies, as the one described by Ilgen & Dulger (2016), increasing its efficiency.

However, the various studies discussed herein reveal that, sometimes, the predominant oil found in TFAs is linoleic, suggesting the need for specific applications for each matrix, depending on the FFAs observed. Also, the present study could corroborate the development of specific deacidification methodologies, thus increasing the efficiency of currently used techniques.

Conclusion

It can be concluded that the used methodology rendered satisfactory results for the determination of free fatty acids in crude sunflower and soybean oil extracted using green technologies.

The primary free fatty acids observed in both oils were linoleic, oleic, palmitic, and stearic acid, listed in ascending order for proportion regarding the FFAs found.

Furthermore, it can be suggested that there are similarities between the major total fatty acids and free fatty acids among the different treatments, although the minor FFAs may vary. Thus, the present study provides relevant information on the determination of the FFAs in crude vegetable oils.

Authors' note

This study is dedicated to the memory of late Cintia Bernardo Gonçalves.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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