



# Extraction and Characterization of the Polar Lipid Fraction of Blackberry and Passion Fruit Seeds Oils Using Supercritical Fluid Extraction

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## Abstract

The study of the phytochemical composition of seed oils is of utmost importance for the food and cosmetic industries, mainly considering their associated biological properties. Extraction of seed oils using supercritical fluids (SFE) is an ecological and green alternative to conventional extraction processes since it is able to provide with potent bioactive extracts, avoiding degradation and transformation of the compounds present originally in the raw material. The objective of the present work was the extraction of pure fractions of polar lipids and their chemical characterization using chromatographic techniques such as GC-MS and LC-DAD-MS/MS of blackberry (*Rubus glaucus*) and passion fruit (*Passiflora edulis*) seed oils extracted by supercritical carbon dioxide. Oleamides derived from oleic acid were identified as the main compounds in both samples; in particular, 9-octadecenamide was the major identified oleamide. Besides, the extract obtained from passion fruit showed to be a source of linoleic acid, while the SFE extract from blackberry presented important concentrations of vanillin. The chemical composition of these seed oils can be of high interest for their further use in cosmetics and food industry.

**Keywords** Fruit by-product · SFE · Oleamides · *Passiflora edulis* · *Rubus glaucus* · GC-MS

## Introduction

Vegetable seed oils are mainly composed of triacylglycerides (TAGs), representing between 95 and 98% of their chemical composition. Other minor components such as sterols, tocopherols, mono- and diglycerides, carotenes, and polyphenols are also present and have been associated to an interesting biological activity of these oils. TAGs are composed by one molecule of glycerol esterified with three fatty acids (unsaturated or polyunsaturated). The unsaturated fatty acids are very

important compounds for nutritional and metabolic functions in the organism since they play an important role in the regulation of a variety of physiological and biological functions (Sanchez-Salcedo et al. 2016). Polyunsaturated fatty acids (PUFAs) are important for antioxidant, vascular, and anti-inflammatory properties (omega 3, 6, 9) (Opie et al. 2017). Besides, omega-3 fatty acids have shown anti-cancer prevention properties (Bodkowski et al. 2014; Sokoła-Wysoczańska et al. 2018). Likewise, its properties for the prevention of cardiovascular diseases are well recognized, mainly the omega-3 long-chain polyunsaturated fatty acids (18–22 carbon chain) family (Sokoła-Wysoczańska et al. 2018), that have been also associated to other health benefits related to skin diseases, arthritis, respiratory system diseases as asthma, lupus erythematosus, or multiple sclerosis (Abedi and Sahari 2014). Therefore, fruits seed are considered a valuable byproduct in the food industry that can be used for the development of new food products with improved nutritional characteristics (Villacís-Chiriboga et al. 2020).

Andean fruits have great potential value in terms of production and consumption for the agroindustrial field. The production of passion fruit in Colombia in 2019 was 137.456 t and represented 12.365 ha of the total cultivated area. At

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present, the fruit is commercialized as fresh fruit or preserved fruits. Concerning blackberry, the total production in 2019 reached 137.999 t occupying 15.902 ha of the cultivated area. The 55% of the blackberry production is destined to fresh fruit household consumption, while about 20% is sold to the agroindustry for the preparation of juices, pulps, preserved food fruits candies, and colorants (SIOC 2021). The oil from *Passiflora* seeds has considerable content of unsaturated fatty acids (Ballesteros-Vivas et al. 2019a; Ramaiya et al. 2019; Cuong et al. 2019; Pantoja-Chamorro et al. 2017a) being an important source of these compounds for the cosmetic and food industries (Malacrida and Jorge 2012; Pantoja-Chamorro et al. 2017b). The cosmetic industry appreciated the natural products with a high content of linoleic and linolenic acid for the high antioxidant and skincare properties (Urbanavičiūtė et al. 2019). Likewise, blackberry seed oils are an important source of unsaturated fatty acids as well (Pantoja-Chamorro et al. 2017b; Sanchez-Salcedo et al. 2016; Kitrytė et al. 2020); thus, the oils of blackberry fruit also have antioxidant, pharmaceutical, and food promising properties.

Fatty acid amides are compounds derived from fatty acids principally from oleic (oleamide), stearic (steramide), and linoleic (linoleamide) acids. Many properties have been described for these compounds, such as relevant effects on thermoregulation, analgesic activity in experimental models, and various biochemical activities (Cheng et al. 2010). Zeid et al. (2019) studied the use of 9-octadecenamide (oleamide) as a potential medicinal treatment for mood and sleep disorders. Also, different authors have reported that oleamide has cannabinoid-like activities (Oh et al. 2010; Cheng et al. 2010; Sudhakar et al. 2009). Other properties described in many works for oleamide are anti-inflammatory (Moon et al. 2018), influence in some mechanisms of the cardiovascular system (Hiley and Hoi 2007) as well as diuretic, carminative, and astringent properties (Nazeam et al. 2018; Tanvir et al. 2018).

Most of the effort carried out on the chemical composition of seed oils has been focused on the analysis of the non-polar fraction containing fatty acids, sterols, tocopherols, and diacylglycerols, while the study of more polar compounds such as fatty acid amides is really scarce (Amri et al. 2017). The main reason for this lack of information about the comprehensive characterization of the seed oils is the complex composition regarding the different families of compounds that present a wide range of polarities making both, the extraction and the chemical characterization a real challenge.

In the last years, supercritical fluid extraction (SFE) using carbon dioxide as extraction solvent has received special attention for the extraction of seed oils. SFE is an environmentally friendly technology that provides with important advantages compared to conventional extraction processes. The extraction yield and selectivity depend on different factors such

as the technique and the extraction parameters employed and the type of solvent or the geographical area where the fruits are grown (Pantoja-Chamorro et al. 2017a). SFE employs green solvents such as carbon dioxide and it commonly allows obtaining higher efficiency, selectivity, and recoveries working under mild conditions, thus favoring the extraction of bioactive and thermolabile compounds (Dorado et al. 2016; Herrero et al. 2006; Herrero et al. 2013).

Some manuscripts have been published focusing on the use of SFE for fruit's seeds oil extraction. Among them, the extraction conditions and chemical characterization of minor and major components of guava and passion fruits has been examined (Dorado et al. 2016; Castro-Vargas et al. 2010). Besides, the use of co-solvents for the extraction of antioxidant compounds and total phenols of guava seeds has been evaluated (Castro-Vargas et al. 2010) as well as the extraction of passion fruits seeds (*Passiflora edulis* sp.) using ultrasound-assisted SFE and the chemical analysis of major components such as fatty acids and tocopherols, tocotrienols, and antioxidant compounds (Manuel-Barrales et al. 2015). Moreover, fatty acids analysis and microencapsulation of blackberry seeds oil using supercritical CO<sub>2</sub> has been also reported (Figuroa et al. 2016).

Recently, the extraction and chemical characterization of bioactive metabolites from *Passiflora mollissima* seeds (Ballesteros-Vivas et al. 2019b) and mango seed kernel (Ballesteros-Vivas et al. 2019a) combining pressurized-liquid extraction and gas/liquid chromatography–high resolution mass spectrometry have been reported; however, to the best of our knowledge, no reports can be found in the literature involving the use of selective supercritical fluid extraction conditions for the fractionation and extraction of the polar lipid fraction and its chemical characterization from tropical fruits seeds or fruits. In this sense, considering both, the potential biological activity of the extracts and the advantages of SFE technology in terms of greenness of the processes, versatility and efficiency, the possibility of employing SFE as an environmentally clean and sustainable process to obtain oils enriched in bioactive minor components, mainly polar lipids, from blackberry (*Rubus glaucus*) and passion fruit (*Passiflora edulis*) seed oils from the Andes Natural Region of South America was studied. The chemical characterization of the minor polar lipid fraction of the seed oil extracts was carried out using chromatographic techniques such as GC-MS and LC-DAD-MS/MS.

## Material and Methods

### Samples and Reagents

Passion fruit and blackberry seeds were supplied by *Agroindustrial Products of Nariño* (INPADENA), Pasto,

Colombia. Supercritical seed oils extracts were obtained by the Laboratory of Supercritical Fluids and Natural Ingredients of the Emerging Technologies in Agroindustry research group of the Universidad de Nariño as described in “Extraction of the Seed Oils from Passion Fruit and Blackberry with Supercritical CO<sub>2</sub>.” Ultrapure water was obtained from a Millipore system (Billerica, MA, USA), whereas HPLC-grade methanol and acetonitrile were purchase from VWR Chemicals (Barcelona, Spain) and carbon dioxide from Cryogas-Indura (Cali, Colombia).

### Extraction of the Seed Oils from Passion Fruit and Blackberry with Supercritical CO<sub>2</sub>

SFE was carried out as described in a previous works by Pantoja-Chamorro et al. (2017a, 2017b). Briefly, samples were washed with water, then they were dried in a tray dryer (FIQ, Colombia) 8 h at 60 °C. The final moisture percentage was 7.60% and 7.8% for blackberry and passion fruit, respectively. The dried fruit seeds were ground with a grinder of hammers, blades, and discs, and they were stored at 4 °C until their use. The extractions were carried out using a pilot-scale SFE instrument (TharSFE-500 (Waters, USA)) equipped with a 0.5-L extraction cell and two 0.5-L separators with independent pressure and temperature controls. The determination of the optimal conditions was based on a kinetic study of the process. Two hundred and fifty grams of dried fruit seeds were used with a CO<sub>2</sub> flow rate of 30 g min<sup>-1</sup>. Extraction conditions were as follow: 350 bar and 60 °C for 150 min. Extraction was carried out in duplicate with neat supercritical CO<sub>2</sub>. After extraction, the oil extracts were collected in vials and stored at -20 °C and protected from light until analysis.

### Sample Preparation for the Analysis of seed Oil Polar Fraction

For the second step, the polar lipids were extracted from the SFE extract. One milliliter of 80% methanol was added to 200 µL of seed oils obtained under supercritical conditions. The sample was shaken at 6500 rpm in a shaker and centrifuged for 5 min at 10000 rpm, following the methodology described by Koubaa et al. (2015). The upper phase (polar fraction) was recovered and was kept under refrigeration at 4 °C until chromatographic analysis. The extraction was done by triplicate for each sample.

### Determination of the Polar Lipids Percentage in Seeds Oils

The percentage of polar lipids in the seed oils was determined by a gravimetric method previously described (Koubaa et al. 2015). The oil samples (200 µL) and the final extract (polar

fraction) were weighed in an analytical balance (Ohaus Pioneer).

### Analysis of the Seed Oils Polar Fraction by GC-MS

The polar fractions obtained as described in “Sample Preparation for the Analysis of seed Oil Polar Fraction” were dried under nitrogen stream and dissolved in methanol for their GC-MS analysis. A GCMS-QP2010 plus system (Shimadzu, Kyoto, Japan) was employed for the analysis, and the separation was carried out on a DB-5 ms (30 m × 0.25 mm × 0.25 µm, Agilent, Tech, Santa Clara, USA) using helium as carrier gas at 1.0 mL min<sup>-1</sup>. The separation was performed according to the following oven temperature program: initial temperature was 40 °C, then it was raised to 250 °C at 15° min<sup>-1</sup> maintaining this temperature for 5 min, followed by an increase to 300 °C at 10° min<sup>-1</sup>, finally this value was maintained for 20 min. The injection volume was 1.0 µL in splitless mode with the injector temperature set at 250 °C. The MS detection parameters were: interface temperature of 300 °C; source temperature of 230 °C; mass range *m/z* of 40–500; scan speed of 2500 amu s<sup>-1</sup>; and event time of 0.20 s. The analysis was carried out in triplicate. Data obtained in the analyses was handled using the GC-MS solution (ver. 2.71, Shimadzu) software. The identification of the compounds was carried out using commercial mass spectral database (Wiley and NIST) as well as their respective linear retention indices (LRIs) by the use of a hydrocarbon mixture ranging from C<sub>8</sub> to C<sub>30</sub> analyzed under the same conditions as the sample.

### Analysis of the Seed Oils Polar Fraction by LC-DAD-MS/MS

Analyses were carried out on a Agilent 1200 series liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump, an autosampler and a DAD, coupled to an ion trap mass spectrometer (Agilent ion trap 6320) with an electrospray interface. A Zorbax Eclipse XDB-C18 (4,6 × 150 mm, 5 µm, Agilent 248 Technologies, Santa Clara, CA, USA) column was employed for the separation using (A) water (0.1% formic acid) and (B) acetonitrile as mobile phases and a gradient elution of 0 min, 10% B; 20 min, 20% B, 35 min, 30% B; 45 min, 60% B; 55 min, 90% B; 60 min, 90% B; 61 min, 100% B; 70 min, 100%; 71 min, 10% B; 75 min, 10% B. The injection volume was 20 µL and flow rate was established at 0.5 mL min<sup>-1</sup>. Analyses were registered at 280 and 330 nm and the UV-Vis spectra were acquired from 190 to 550 nm. The MS was operated under ESI positive and negative ionization modes using the following setting: dry temperature 300 °C, mass range *m/z* 90–2200 Da, dry gas flow rate 12 L min<sup>-1</sup>, nebulization pressure 30 psi.

## Results and Discussion

### Determination of the Polar Lipid Percentage in Seeds Oils

SFE is known as a selective extraction technique for non-polar fractions due to the physicochemical characteristics of carbon dioxide at supercritical conditions. However, by the modification of some extraction conditions such as temperature, pressure, extraction time, or the use of co-solvents, the solubility of more polar compounds in the supercritical CO<sub>2</sub> can be improved, and therefore a more polar fraction can be extracted. In this work, 350 bar and 60 °C for 150 min were used as extraction conditions to obtain the potential bioactive compounds present in the seed oils from blackberry and passion fruit. The extraction conditions selected accordingly with a kinetic study for the fruit seeds provided the optimal conditions to maximize the oil extraction yield. The relation between temperature, pressure and time in SFE determines the quantity and quality of the extract composition, making possible the careful modification of these parameters to achieve selective lipid extractions (Sahena et al. 2009). In this work, a strong pressure-temperature interaction working at 350 bar was observed, being the optimal conditions of pressure and temperature 350 bar and 60 °C, respectively, which achieved the maximum extraction yield (Pantoja-Chamorro et al. 2017a, 2017b). These conditions allowed the extraction of major lipids characterized in the preliminary investigation (Pantoja-Chamorro et al. 2017a, 2017b) and minor lipids which were the target aim of this research. The optimization of the extraction conditions had the aim of extracting selectively the medium non-polar compounds; therefore, no co-solvent was employed to increase the polarity of the extraction solvent avoiding the extraction of polar compounds such as phenolic compounds.

The extraction yield for the lipid fraction in the SFE of the passion fruit and blackberry seeds were 15.7% and 14.5%, respectively. A liquid/liquid extraction of the polar lipids was carried out obtaining a recovery of 1.3% of the passion fruit SFE extract and 0.9% for the blackberry extract. These results are comparable to the results obtained with conventional solid/liquid extraction of polar lipids from different fruit seeds. For instance, Saïdani et al. (2004) reported the percentage of polar lipids extracted from other fruit seeds such as blood orange, sweet orange, lemon, and bergamot, obtaining 3.20, 1.20, 3.30, and 2.10%, respectively. In another study, the extraction yield of polar lipids was 7.8–9.8% in seeds from different citric fruits (Waheed et al. 2009). However, there is little information about the polar lipid extraction yield of the seed oils of blackberry and fruit passion.

The use of supercritical CO<sub>2</sub> for the extraction of seed oils has important advantages compared to conventional extraction methods, mainly solid-liquid extraction techniques,

which use organic solvents toxic to health and environment. Other characteristic disadvantages of these extraction techniques are the high flammability of the solvents for their use in the industry and the traces of solvents remaining in the oils after the extraction processes. Besides, SFE extraction allows a greater selectivity of compounds present in the oil fraction. In fact, slightly higher extraction yields have been obtained for the oil extraction of passion fruit seeds by compressed solvents using propane as extraction solvent at subcritical conditions (Pereira et al. 2017). In that work, and 23.68% extraction yield was achieved using propane in comparison to the extraction yield achieved in the present work using CO<sub>2</sub> (15.7%) due to the high affinity of propane to the very non-polar compounds present in the seeds. Comparing the extraction conditions of both methods, the same temperature resulted optimal for both extractions (60 °C), although softer pressure (20 bar) was used for the subcritical propane extraction; however, using propane, longer total extraction time (180 min) was needed for obtaining the maximum extraction yield. Moreover, the use of propane as extraction solvent has also some inconvenients such as its flammability and high cost in comparison to CO<sub>2</sub> (Akanda et al. 2012) and low solvent power at moderate temperatures (Del Valle & Aguilera. 1999). Therefore, the application of supercritical CO<sub>2</sub> for the extraction of the seed oil of the studied fruits represents a very novel and friendly technique.

### Characterization of the Seed Oils from Blackberry and Passion Fruit Obtained Under Supercritical CO<sub>2</sub> Conditions

As mentioned, a complete characterization of the blackberry and passion fruit seed oils was carried out in the present work using GC-MS. LC-DAD-MS/MS analysis were done to corroborate the purity of the extracted fraction confirming that no other kind of compounds were extracted under SFE conditions.

### Identification of Lipid Fraction by GC-MS in Seed Oils

The GC chromatograms of the blackberry (Fig. 1b) and passion fruit (Fig. 1b) seed oils profiles showed that the main compounds present in the seed oils were oleamides, fatty acids and fatty acid derivatives, low-polar phenolic compounds and derivatives, as well as, to a lesser extent, different tocopherols. As expected, TAGs were not detected; therefore, these extracts were considered rich in minority polar compounds. The tentatively identification of the separated compounds of both lipid extracts is summarized in Table 1, together with their relative percentage and LRI values.

Figure 2 depicts the graphical representation of the composition of the seed oils of each fruit (A: blackberry and B: passion fruit). As it can be seen, the most abundant compound



**Table 1** Analysis by GC-MS of the polar fraction of blackberry fruit seed oil and passion fruit seed oil including the tentative identification, the linear retention index (LRI) of the compounds (referred to a DB-5MS column) and the relative percentage of each compound in the sample

No	Retention time (min)	Tentative identification	Fragment ions (m/z)	Relative percentage (%) <sup>1</sup>	LRI
B1	6.56	Unidentified	43 (18), 73 (30), 104 (17), 117 (15), 223 (60), 253 (100)	Blackberry	985
BP1	8.65	N,N-bis (2-ethylbutyl) amine	41 (26), 54 (24), 56 (18), 85 (18), 114 (100), 185 (0.2)	Passion fruit	1210
P1	9.46	3-Ethyl-1,4-hexadiene	41 (42), 55 (23), 67 (28), 81 (100), 95 (12), 110 (10)		1292
P2	9.60	Thymol	77 (9), 91 (17), 107 (14), 115 (13), 135 (100), 150 (28)		1305
P3	9.72	(E,E)-2,4-decadienal	41 (20), 55 (13), 67 (14), 81 (100), 83 (14), 109 (6), 152 (6)		1319
B2	10.23	<i>o</i> -Vanillin	51 (24), 53 (26), 81 (48), 109 (27), 123 (26), 151 (96), 152 (100)		1380
B3	10.75	Vanillin	51 (25), 53 (24), 81 (37), 109 (25), 123 (22), 151 (100), 152 (81)		1430
P4	15.30	Palmitic acid	41 (83), 43 (100), 60 (80), 73 (92), 129 (36), 213 (12), 256 (12)		1970
P5	16.46	Methyl limoleate	41 (50), 55 (54), 67 (100), 81 (88), 109 (27), 263 (5), 294 (4)		2080
P6	17.10	Linoleic acid	41 (76), 55 (76), 67 (100), 81 (78), 95 (51), 109 (20), 280 (5)		2092
B4	17.14	$\Delta$ -lauro lactone	41 (78), 43 (86), 71 (55), 83 (40), 99 (100), 114 (18), 147 (7)		2154
BP2	17.54	Hexadecanamide	41 (16), 43 (20), 59 (100), 72 (41), 114 (5), 212 (2), 255 (0.6)		2190
P7	19.30	9-Octadecenamide isomer	41 (47), 59 (100), 72 (63), 128 (9), 281 (1)		2319
BP3	20.10	9-octadecenamide	41 (34), 55 (38), 59 (100), 72 (62), 126 (9), 281 (1.3)		2375
P8	20.42	Octadecanamide	41 (47), 59 (100), 72 (63), 128 (9), 283 (3)		2397
P9	21.45	Glycine-N-butoxycarbonyl-tetradecyl ester	43 (47), 55 (27), 57 (40), 101 (27), 117 (69), 130 (100), 239 (10), 371 (6)		2474
P10	21.70	Unidentified	43 (25), 55 (18), 75 (14), 117 (100), 129 (16)		2490
B5	23.60	Bornyl cinnamate	41 (20), 55 (20), 67 (26), 103 (28), 131 (100), 262 (3)		2650
P11	23.62	2,3-O-isopropyliden-1-O-(2-methoxy-4-hexadecenyl)-glycerol	41 (18), 55 (20), 67 (23), 81 (14), 131 (100), 262 (3)		2652
P12	24.30	$\beta$ -monolinolein	41 (73), 55 (98), 67 (100), 81 (76), 95 (51), 109 (23), 262 (20)		2709
P13	25.24	2-methyloctacosane	43 (61), 57 (100), 69 (15), 71 (73), 85 (60), 211 (2)		2793
P14	26.31	$\alpha$ -tocopherol	41 (13), 43 (30), 57 (20), 121 (9), 165 (100), 205 (10), 430 (54)		2890
B6	29.10	$\gamma$ -tocopherol	69 (13), 81 (34), 131 (14), 151 (100), 265 (10), 416 (30)		3010
P15	30.04	Hen triacontene	43 (66), 57 (100), 71 (70), 85 (50), 113 (11), 197 (1)		3127

B<sub>i</sub>, compound detected in blackberry; P<sub>i</sub>, compound detected in passion fruit; BP<sub>i</sub>, compound detected in blackberry and passion fruit

<sup>1</sup> Relative percentage average (%): numbers in parenthesis indicate SD (*n* = 3)

in both samples was 9-octadecenamide (peak BP3 of blackberry and passion fruit analysis (Fig. 1a and b)). The relative percentage of this compound was 23.46% and 39.8% in passion fruit and blackberry seed oils, respectively. Besides of 9-octadecenamide, other oleamides were identified in the samples: hexadecanamide and octadecanamide.

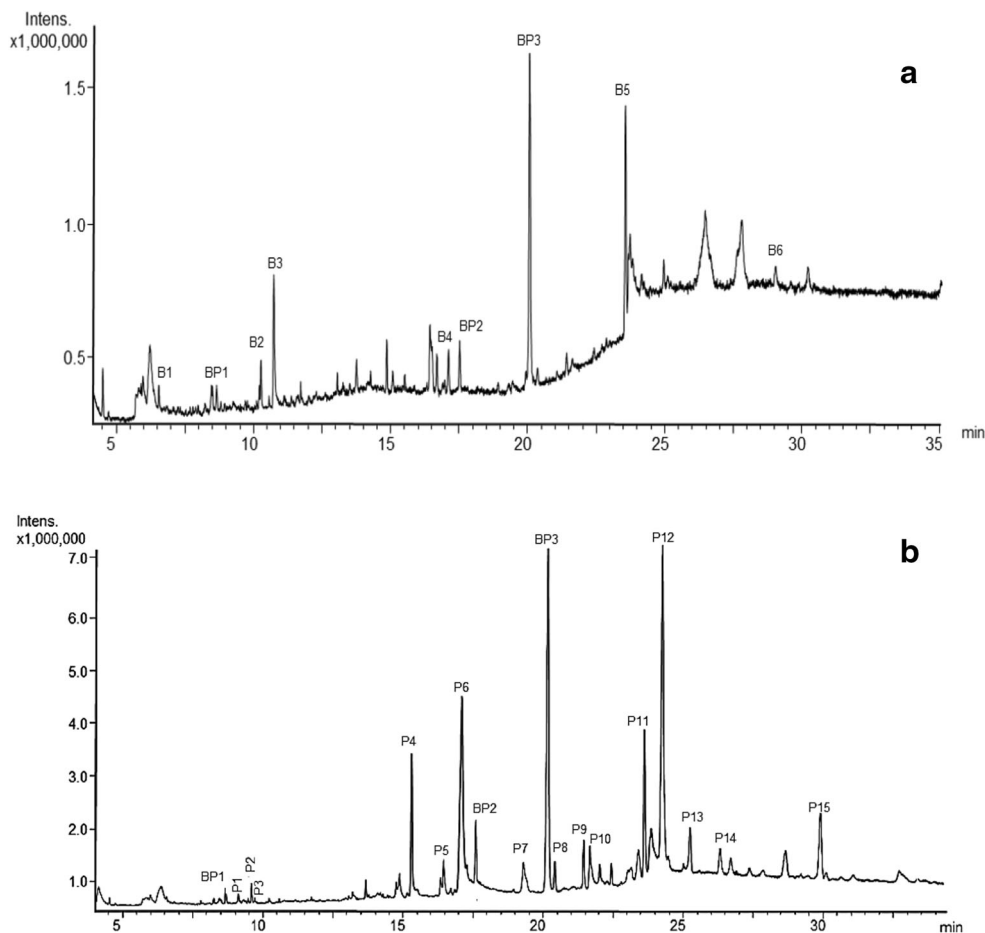
Comparing the presence of oleamides in the two studied fruits, hexadecanamide appears in both samples (peak BP2), whereas for octadecanamide, three different isomers were detected in passion fruit, including octadecanamide, 9-octadecenamide, and an octadecanamide isomer (peaks P7, BP3, and P8), while only 9-octadecenamide was observed in blackberry (peak BP3). The high percentage of 9-octadecenamide in the extracts could result of a great interest due to its health-promoting properties, since different biological activities have been reported for this oleamide, such as anti-inflammatory and antibacterial activities (Hadi et al. 2016). Moreover, this oleamide has been identified as an endogenous substance produced by animals to induce sleep and decrease the rate of blood circulation (Abu-Shandi et al. 2015) and has been associated with certain neurological activities, including the regulation of memory processes, the decrease of body temperature and the stimulation of mobility (Driscoll

et al. 2007). Some studies have demonstrated the relationship between oleamide and other endogenous lipids in the cannabinoids system (Wu et al. 2007). Besides, Cheng et al. (2010) determined that 9-octadecenamide extracted from *Cryptotaenia japonica* Hassk seeds had hypolipidemic activity, while Irshad et al. (2020) described that the extracts of Jujube (*Ziziphus jujube*) were effective against Alzheimer disease due to the presence of oleamide (Heo et al. 2003; Irshad et al. 2020).

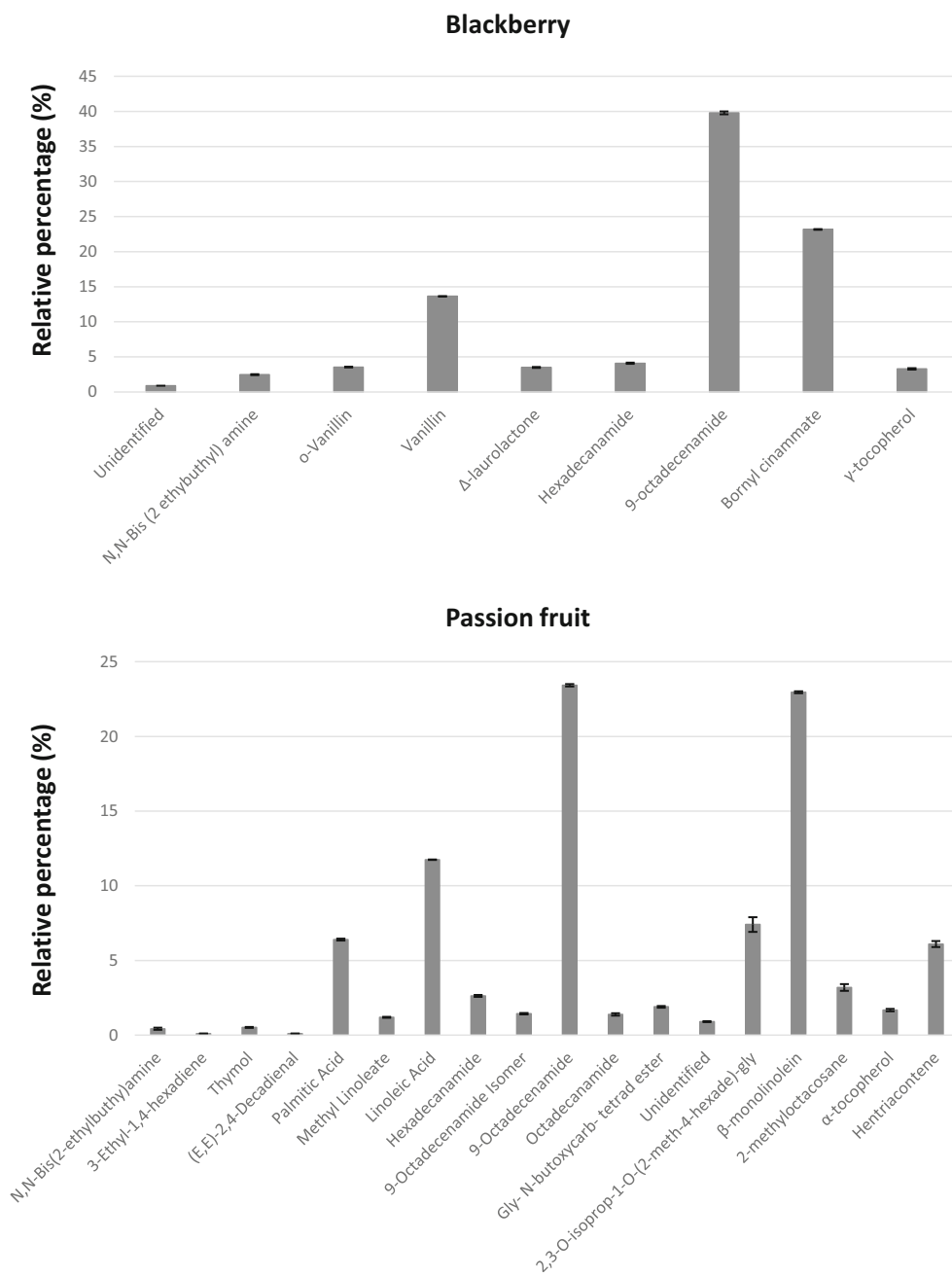
Regarding the presence of these compounds in oils, 9-octadecenamide, 9-octadecanamide, and tetradecanamide have been detected in the volatile composition of alhidran (*Boerhavia elegans Choisy*) seed oil, with percentages lower than 1.0% (Al-Farga et al. 2015). Sankar-Sethi et al. (2016) determined the presence of 9-octadecenamide and octadecanamide in *Jatropha* seeds, with percentages of 3.0 and 0.8% respectively. A higher content of oleamide (43.1%) was determined in *Lupinus termis* stems from Sudan (Hamed and Ayoub 2015).

Fatty acids also represented an important fraction of the passion fruit seed oil composition. Two main fatty acids were detected in this fruit, mainly palmitic acid and linoleic acid (peaks P4 and P6, respectively). In particular,

**Fig. 1** GC-MS of the polar fraction of blackberry (a) and passion fruit (b) seed oils. B, compound detected in blackberry; P, compound detected in passion fruit; BP, compound detected in blackberry and passion fruit. For compound identification see Table 1



**Fig. 2** Graphical representation of the GC-MS chemical characterization of blackberry (a) and passion fruit (b) SFE seed oils extracts. Error bars indicate the SD of the triplicate analyses



linoleic acid was one of the most abundant compounds in the passion fruit extract. This unsaturated fatty acid is an important compound in human diet since it has properties for prevention of certain cardiovascular diseases (Gupta et al. 2014). Unsaturated fatty acids such as linoleic ( $\omega$ -6) is considered essential components for the structure, development, and function of cells (Otero et al. 2020), and it is related to the reduction of diseases such as cardiovascular, inflammatory, cancer, and metabolic syndrome (González et al. 2016). Bardaa et al. (2016) reported that linoleic acid, as a precursor of arachidonic acid, is important in the inflammatory cascade (prostaglandins,

thromboxanes, and leukotrienes). Other beneficial properties for human health and care attributed to linoleic acid are the potential effects on the skin barrier, asthma, arthritis, and multiple sclerosis, and neurological diseases such as Alzheimer's disease or diabetes mellitus (Abedi and Sahari 2014; Tanvir et al. 2018; Vaughn et al. 2018; Su et al. 2015; Higashi et al. 2001; Su et al. 2013). Therefore, linoleic acid is one of the polyunsaturated fatty acids with more important applications for food, cosmetics, and pharmaceutical purposes (Ballesteros-Vivas et al. 2019a).

An interesting fatty acid derivate detected with high intensity in passion fruit seed oil extract was  $\beta$ -

monolinolein (peak P12, 22.96%). This compound (monoglyceride) derives from linoleic acid and presents a wide range of applications, including food additive, emulsifying, and surfactant (Sagalowicz et al. 2016). Regarding its biological activities, this compound has been related to potent cytotoxic activity on MDA-MB-231 breast cancer cells and in use together with stigmasterol it was efficient in inducing tumor regression (Sofi et al. 2018; Sofi 2019).

Interestingly, fatty acids were not detected in blackberry. However, the oil extracted from this fruit presented other important compounds such as low-polar phenolic compounds. In particular, two vanillin isomers (peaks B2 and B3) and a cinnamic acid ester derivative, bornyl cinnamate (peak B5). These compounds represented the second family of compounds more abundant in blackberry after the oleamines. Bornyl cinnamate was one of the most abundant compounds in blackberry seed oil, with a percentage of 23.18%. This compound has been previously identified in essential oils of tree leaves employed in the Chinese traditional medicine; for instance, Kumar et al. (2015) reported the anti-inflammatory effect of bornyl cinnamate obtained from *Liquidamber formosana* leaves. Also, the antileishmanial *in vivo* activities of this phenolic compound has been reported (Masic et al. 2015).

The presence of vanillin represented the 13.64% of the oil. In this sense, it is important to emphasize the interesting properties attributed to this compound such as antimutagenic, antineoplastic, anti-inflammatory, anti-apoptotic and antioxidant. Other studies have reported its anti-depressant effect (Ueno et al. 2019; Shoeb et al. 2013), comparable to the marketed drug fluoxetine (Anand et al. 2019; Shoeb et al. 2013). This compound has been reported in nopal seeds (Koubaa et al. 2015) as well as in olive oils from different origins (Peres et al. 2016; Brenes et al. 1999; Bianco et al. 2001). Moreover, it has been also identified in cumin seeds (*Cuminum cyminum*) (Al-Hashemi 2014) and soya seed oil (Schmidt and Pokorný 2005).

The chemical characterization of the two seed oils revealed that the SFE extraction conditions used in this work were adequate for the extraction of medium polar compounds present in the seed fruits. Although both fruits have oleamides as main compound, due to the different nature of the studied fruits, the extracted oils presented different chemical composition. Passion fruit was very rich in fatty acids, in particular, polyunsaturated fatty acids while in blackberry, the presence of low-polar phenolic compounds stood out. Therefore, relative pure extracts were obtained for each fruit with interesting bioactive perspectives for its use in the food, pharmacologic, or cosmetic industry. Moreover, to ensure the purity of these SFE fractions, LC-DAD-MS/MS analysis were carried out.

## LC-DAD-MS/MS Analysis of the Seed Oil Extracts

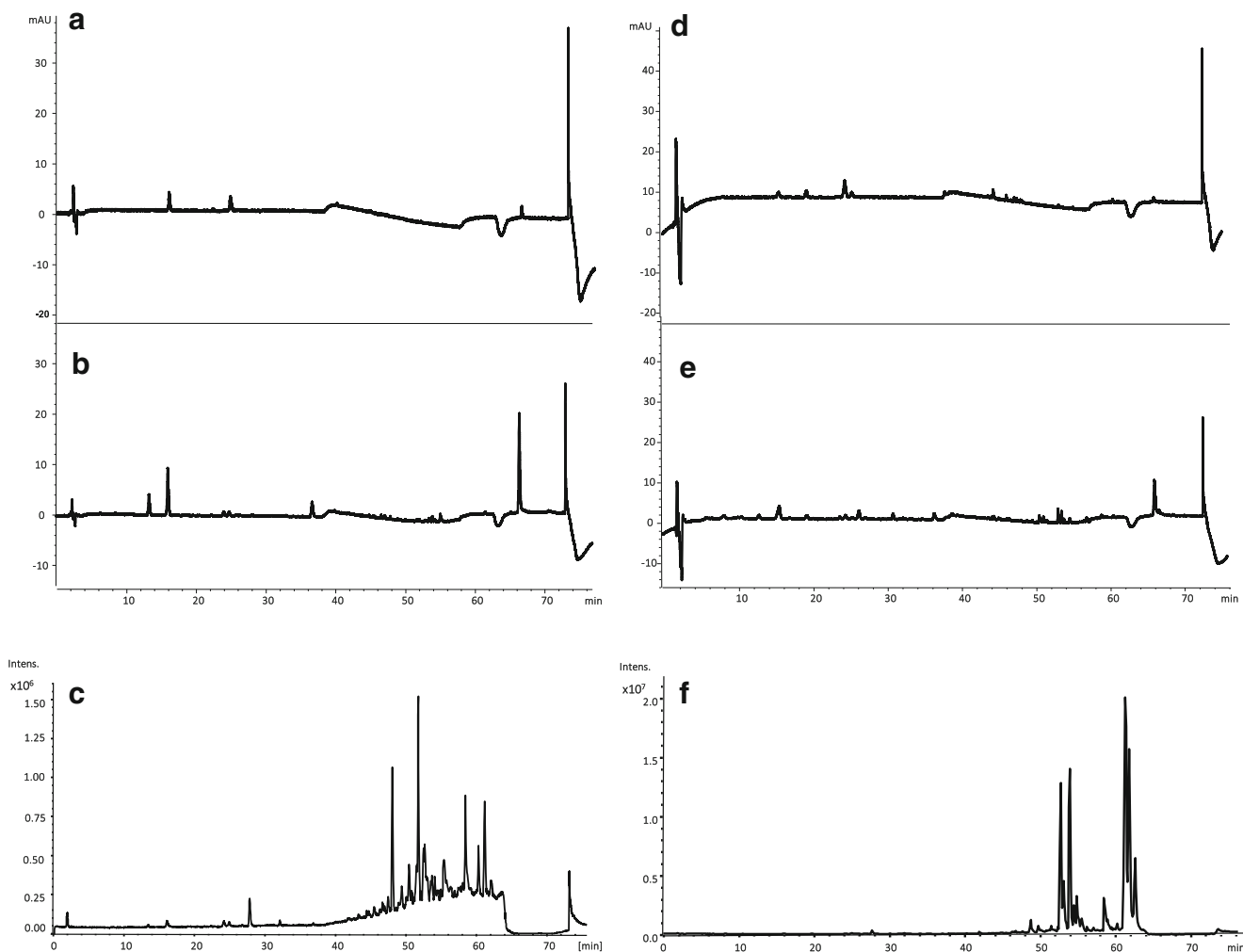
Several bioactive phenolic compounds have been widely described in the fruit seeds (Fazio et al. 2013; Caruso et al. 2015; De Santana et al. 2017; Castro-Vargas et al. 2019; Ballesteros-Vivas et al. 2019b; Narváez-Cuenca et al. 2020); however, the objective of this work was to apply selective SFE extraction conditions to obtain the polar lipid fraction of the seeds. Therefore, the SFE extracts of blackberry and passion fruit seeds were further investigated by LC-DAD-MS to evaluate the non-co-extraction of other kind of compounds like more polar phenolic compounds.

With this purpose, C18 RP column was chosen for the separation of the possible compounds present in the oil extracts. The information recorded by two different detectors (DAD and MS) was considered for the analysis of this extracts. Typical wavelengths for the detection of phenolic compounds were used, i.e., 280 and 330 nm as well as optimized MS parameters for phenolic compounds were applied. Figure 3 shows the DAD chromatogram at 280 and 330 nm and the TIC analysis of both extracts. The optimal conditions chosen for the analysis were long analysis (75 min) and slow gradient to cover all the polarities of possible compounds in a wide retention time. As can be seen in the DAD chromatograms of both extracts, very low abundant peaks were detected at 280 and 330 nm, being the intensity of the higher peak very low in both fruits. Considering the concentration of sample injected in the LC column (4.5 mg/mL) and the high injection volume (20  $\mu$ L) used, these peaks can be considered as a minimum trace of phenolic compounds.

Moreover, this affirmation was further corroborated with the MS and MS/MS analysis, where these compounds were not ionized, either in positive or negative mode, due to their very low intensity. Along the first 40 min, no peaks were detected and ionized. Only in the more non-polar part of the analysis (48–70 min) high signals were detected, corresponding to non-polar compounds different to phenolic compounds, since they were not detected at 280 and 330 nm by the DAD.

These results confirmed that the SFE extracts obtained from the studied fruits were rich and pure in potential non-polar bioactive compounds. Another conclusion of these results is that all the phenolic compounds present in the seeds would remain in the residue of the SFE extraction; therefore, a future work would be the study and optimization of the SFE conditions with the use of modifiers like ethanol, for the extraction of phenolic compounds from this residue, which can be considered lipid and non-polar compounds free. This study would match in a biorefinery approach for the complete recovery of important bioactive compounds in food waste like passion fruit and blackberry seeds.





**Fig. 3** LC-DAD-MS chromatograms of the polar fraction blackberry and passion fruit. **a** Chromatogram at 280 nm, **b** chromatogram at 330 nm, and **c** TIC of the blackberry sample. **d** Chromatogram at 280 nm, **e** chromatogram at 330 nm, and **f** TIC of the passion fruit sample

## Conclusions

In this work, a comprehensive extraction and analytical platform has been developed for obtaining a pure polar lipid valuable fraction from two food-by-products, namely passion fruit and blackberry seeds. This platform consisted on the use of the green extraction technique SFE together with two different analytical tools, i.e., GC-MS and LC-DAD-MS. To achieve this goal, the extraction method, previously optimized, was tested in terms of characterization of the final extracts to provide the accurate information about the chemical composition of the valued extracts. SFE was employed for the selective extraction of the lipid fraction from passion fruit and blackberry seed oils. After the extraction of the polar lipids, a pure fraction of minor polar compounds, free of TAGs, was obtained. GC-MS was applied for carrying out the chemical characterization of the polar fraction. These results revealed three main families of compounds: oleamines, fatty acids, and low-polar phenolic compounds. The main compound found

in both seed fruits was 9-octadecenamide. Passion fruit extract was rich in fatty acids, mainly linoleic acid, while blackberry seed oil presented more low-polar phenolic compounds. Besides, LC-DAD-MS/MS analysis showed the purity of the extracts. These extracts could present cosmetic and food interesting properties. Therefore, the use of a green extraction technique as SFE as well as analytical tools that determine the exact composition of the extract is considered useful for the extraction and characterization of, not only bioactive compounds from passion fruit and blackberry seed oils but also for other byproducts of the food industry that can be revaluated.

**Authors' Contribution** Conceptualization: David Arturo-Perdomo, Juan Pablo Jiménez Mora, Elena Ibáñez, Alejandro Cifuentes, Andrés Hurtado-Benavides, Lidia Montero; methodology: David Arturo-Perdomo, Juan Pablo Jiménez Mora, Lidia Montero; formal analysis and investigation: David Arturo-Perdomo, Juan Pablo Jiménez Mora, Elena Ibáñez, Alejandro Cifuentes, Andrés Hurtado-Benavides, Lidia

Montero; writing—original draft preparation: David Arturo-Perdomo, Lidia Montero; writing—review and editing: David Arturo-Perdomo, Juan Pablo Jiménez Mora, Elena Ibáñez, Alejandro Cifuentes, Andrés Hurtado-Benavides, Lidia Montero; funding acquisition: Elena Ibáñez, Alejandro Cifuentes, Andrés Hurtado-Benavides

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## Declarations

**Conflict of Interest** David Arturo-Perdomo declares that he has no conflict of interest. Juan Pablo Jiménez Mora declares that he has no conflict of interest. Elena Ibáñez declares that he has no conflict of interest. Alejandro Cifuentes declares that he has no conflict of interest. Andrés Hurtado-Benavides declares that he has no conflict of interest. Lidia Montero declares that she has no conflict of interest

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