Supercritical Fluid Extraction of Lemon Verbena (*Aloysia triphylla*): Kinetics, Scale-up and Chemical Composition

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Supercritical fluid extraction (SFE) and low-pressure solvent extraction (LPSE) of lemon verbena were conducted. Kinetic parameters of the overall extraction curve (OEC) and scale-up data are presented. The scale-up criterion used (maintaining solvent to feed ratio constant) was successfully used for a 14-fold scale-up from laboratory to pilot scale. Maximum yield obtained for SFE was 1.8 %, and for LPSE, 7.1 %. The chemical composition revealed different chemical profiles for SFE and LPSE extracts; the last ones presented more flavonoids and other heavy compounds, while SFE extracts were more concentrated on volatile compounds. By manipulating separation conditions, three different products, of different physical-chemical properties, were obtained in the three series separators of the pilot equipment. Therefore, the purpose of using the extract should influence the decisions of which are the best extraction method and the best operational conditions.

INTRODUCTION

Supercritical fluid extraction (SFE) is considered an emergent technology, since it has proven to be technically and economically feasible, presenting several advantages when compared to traditional extraction methods. However, after three decades of development, of the over 200 commercial plants in the world [1], none of them is located in Latin America. For developing countries, adding value to indigenous raw material using an environmentally friendly technology represents the possibility of increasing its competitivity in the global market of natural products.

The relations between processes conducted in bench, pilot and industrial scales cannot always be simply approached or predicted. The differences observed in procedures conducted in equipments of significantly different sizes must be carefully studied and evaluated to avoid rough mistakes when scaling-up a process. Therefore, studying the scale-up is important to establish a methodology that allows predicting the behavior of SFE process at industrial scale from laboratory scale data. LASEFI research group has been studying these issues for a few years [2-8], and the more recent works have concluded that the scale-up criterion keeping S/F (solvent to feed ratio) constant presents good agreement between bench-scale and pilot-scale data [7,8].

When validating scale-up criteria, it is necessary to assess their applicability to different types of raw materials, since the mass transfer mechanisms may differ among species and parts of the plants used for extraction [9]. Lemon verbena leaves present a completely different profile from the raw materials studied in our previous works, namely clove, sugar cane residue and grape seeds [7,8], which can be a good addition for scale-up data gathered so far.

Lemon verbena (*Aloysia triphylla* [L'Hérit.]), Verbenaceae family, is indigenous to South America, and it is also cultivated in Northern Africa and Southern Europe [10]. Its botanical synonyms include *Lippia citriodora* (Lam.) Kunth, *Aloysia citriodora* Palau and *Verbena triphylla* [11]. Lemon verbena leaves are widely used in folk medicine for their aromatic, digestive, anti-spasmodic, antibacterial [12-14], antioxidant [15,16] properties, etc.

There are some studies in literature for obtaining lemon verbena extract. Carnat et al. [10], Sartoratto et al. [14] and Silva [17], obtained yields of 0.82 %, 0.22 % and around 1 %, respectively, for hydrodistillation of the leaves. The harvesting season and time and the particle diameter presented influence on the yield [17]. Duarte et al. [18] obtained 0.50 % of yield for hydrodistillation and 20.80 % for maceration with ethanol:water 7:3. Pereira and Meireles [16] determined SFE global yield isotherms of lemon verbena leaves at 308-318 K/10-35 MPa, obtaining maximum yield of 1.49 % at 318 K/35MPa; for the same raw material, hydrodistillation yield was 1.15 %. The extracts obtained under milder extraction conditions presented chemical composition more similar to volatile oil, while more aggressive operational conditions leaded to extraction of heavier compounds.

Considering the rich biodiversity of South American flora, and the possibility of adding value to it without degrading the environment, technical and economical analysis inserted in South American reality is important to provide information for the installation of an industrial SFE unit in this region. Therefore, the objective of the present work was to study SFE kinetics and scale-up of lemon verbena leaves.

MATERIALS AND METHODS

Raw material characterization

Lemon verbena leaves were donated by Colflavor S.A. (Envigado, Colombia). They were comminuted in a knife mill (Marconi, model MA 340, Piracicaba, Brazil). The milled raw material was classified according to particle size using a vibratory system (Bertel, model 1868, Caieiras, SP) with 8-80 mesh sieves (Tyler series, Wheeling, USA), and then stored in a domestic freezer (Metalfrio, model HC-4, São Paulo, Brazil) at 255 K prior to extraction.

The mean particle diameter was determined according to ASAE Standards [19]; it was of 0.672 mm. The humidity of the raw material was determined by xylene distillation [20] in duplicate; the leaves presented mean moisture content of 5.3 %. The apparent bed density was calculated by dividing the feed mass by the vessel volume.

Low-pressure solvent extraction (LPSE)

In order to compare the extracts obtained by SFE and LPSE, the lemon verbena leaves were extracted in Soxhlet apparatus with ethanol during 240 min, using solvent:raw material of 10:1. After the extraction, the ethanol was removed using a rotary evaporator (Heidolph, model Laborota 4001 WB, Viertrieb, Germany) at 1 MPa and 323 K. The assays were conducted in duplicate.

Overall extraction curve (OEC) determination

A laboratory scale equipment (Applied Separations, model 7071, Allentown, USA) equipped with a 290 mL extraction vessel was used for determining the OEC of lemon verbena leaves.

The solvent used was carbon dioxide (99.9 % purity, Gama Gases, São Bernardo do Campo, SP). This OEC was determined in duplicate and used as reference for scaling-up the process to pilot scale.

The extraction bed was filled with 122 g of raw material, resulting in an apparent bed density of 421 kg/m^3. Operational conditions selected were 333 K/35 MPa. After pressurization of the vessel, the bed was submitted to 15 min of static period, and then the CO_2 was admitted in the system at a constant flow rate of 1.024×10^{-4} kg/s. The total extraction time was of 420 min, totalizing an S/F of 21. The extracts were periodically collected, at time intervals varying between 15 and 60 min. The separator consisted of a 50 mL glass vial immersed in ice bath at environment pressure.

The overall extraction curves obtained were adjusted to three straight lines, namely CER (constant extraction rate), FER (falling extraction rate) and DC (diffusion controlled) periods, according to the method described by Rodrigues et al. [21] and Meireles [22].

Scale-up study

The scale-up criterion adopted consisted in maintaining S/F constant. The solvent flow rate was calculated so that in pilot scale the kinetic behavior would reproduce laboratory scale data. The reproducibility of the kinetic behavior using this criterion was already determined in previous studies [7,8], so that only one total collection point was determined in the present work. The experiment was conducted in duplicate.

A pilot scale equipment (Thar Technologies, model SFE-2×5LF-2-FMC, Pittsburgh, USA) equipped with two 5.15 L extraction vessels and three 1 L separators displayed in series was used; only one extractor was used. The solvent used was carbon dioxide (99.0 % purity, Gama Gases, São Bernardo do Campo, Brazil).

For scale-up study, the extraction vessel was partially filled with 1760 g of raw material, and then completed with glass beads, resulting in apparent bed density of 410 kg/m^3. The OEC obtained in laboratory scale experiment was used as reference, so that operational conditions were the same, except for solvent flow rate, which was calculated using the scale-up criterion (S/F constant) as 1.47×10^{-3} kg/s. The separators were operated at 323 K/10 MPa (separator 1 – S_1), 303 K/7 MPa (separator 2 – S_2) and 313 K/3 MPa (separator 3 – S_3).

Chemical analysis of the extracts

The extracts were analyzed by TLC (thin layer chromatography), GC (gas chromatography) and GC-MS (gas chromatography coupled to mass spectrometry), in order to determine their chemical composition. The TLC analysis was used for determining classes of compounds present in the extracts, while the GC and GC-MS analyses were used for determining the composition of the extract's volatile fraction.

For the TLC analysis, the stationary phase consisted of an aluminum plate covered with silica gel (Merck, CCF-C/25, Silica gel 60, lot OB347654, Darmstadt, Germany) or UV-sensitive (Merck, CCF-C/25, Silica gel 60 F_{254}, lot OB347654, Darmstadt, Germany) plate. The extracts were diluted at 5 mg/mL in ethyl acetate, and then applied on the plates. The mobile phase used was composed by hexane and ethyl acetate at 8:2 proportion.

Two different spray reagents were used, one was specific for terpenoids, propilpropanoids, pungent principles and saponins (anisaldehyde) and the other one was specific for flavonoids (NP) [23]. The anisaldehyde reagent, sprayed on normal plates, was prepared with 0.5 mL of p- anisaldehyde (Sigma, lot 116K3531, St. Louis, USA), 10 mL of glacial acetic acid (Merck, 100 %, PA, lot K31358063 243, Darmstadt, Germany), 85 mL of methanol (Ecibra,PA – ACS, lot 17028, São Paulo, Brazil) and 5 mL of concentrate sulfuric acid (Vetec, 98 %, lot 993150, Rio de Janeiro, Brazil), added in this order; after spraying anisaldehyde reagent, the plates were submitted to heating at 373 K in an oven for the complete revelation of the compounds. The NP reagent, sprayed on UV-sensitive plates, was prepared with 0.5 g of 2-aminoethyl diphenylborinate (Sigma-Aldrich, lots C14H16BNO and 096K2612, Milwaukee, USA) in 50 mL of methanol (Ecibra,PA – ACS, lot 17028, São Paulo, Brazil); after spraying the NP reagent, the plates were observed under ultraviolet lamp (Mineralight ® Lamp, model UVGL-58, Multiband UV – 254-366nm, Upland, USA) at 366 nm in dark chamber (UVP-Chromato-VUE, model CC-10, Upland, USA).

For the GC analysis, it was used a gas chromatographer with FID detector (Shimadzu, model G 17A, Kyoto, Japan) equipped with a silica capillary column DB-5 (30 m × 0.25 mm × 0.25μm, J & W Scientific, Folsom, USA). The carrier gas was helium (White Martins, 99.9 %, Campinas, Brazil) at 1.1 mL/min. One microliter of the extract at a 5 mg/mL dilution in ethyl acetate (PA, Merck, lot K 39390823 847, Darmstadt, Germany) was injected. The sample split ratio was 1:20. The column was heated from 333 K up to 573 K, at a 5 K/min rate, and then kept under this temperature for 20 min. The injector and detector temperatures were 553 K and 573 K, respectively. The identification of the compounds present in the extracts was done by GC-MS analysis at the Central Analítica do Instituto de Química da Unicamp (Campinas, Brazil).

RESULTS

SFE kinetics and scale-up

Figure 1 presents the OEC obtained at laboratory scale and the total collection yield obtained in pilot scale for lemon verbena leaves SFE at 333K/35 MPa as a function of extraction time. In 420 min of extraction, 1.89 % (dry basis – d.b.) yield was obtained at laboratory scale. Pereira and Meireles [16] determined the global yield isotherms of lemon verbena leaves at 308-318 K/10-35 MPa, obtaining maximum yield of 1.5 % at 318 K/35 MPa. These authors observed that for pressures above 25 MPa, the vapor pressure of the solute presents significant effect over the yield, which increases with temperature at constant pressure. Therefore, the result found in the present study is in agreement with the expected behavior, since at 333 K/35 MPa, the yield found was higher (1.89 %) than the yield obtained by Pereira and Meireles [16] under this same pressure, but at 318 K (1.5 %).

Lemon verbena OEC presents the three characteristics extraction periods of SFE processes (CER, FER and DC steps). The parameters adjusted from the spline fitting were: t_{CER} (70 min), t_{FER} (195 min), M_{CER} (2.15×10^{-7} kg/s), Y_{CER} ($2{,}10 \times 10^{-3}$ kg extract/kg CO_2) and Y_{CER} (1.00 %). Usually, a viable SFE cycle is between t_{CER} and t_{FER} [22], so that the calculation of these periods allow a first estimation of the duration of batch cycle of an industrial process. The mass transfer rate (M_{CER}) and the concentration of the solute in the solvent stream at the extractor outlet (Y_{CER}) are also important kinetic parameters, which should be optimized in order to improve the SFE process.

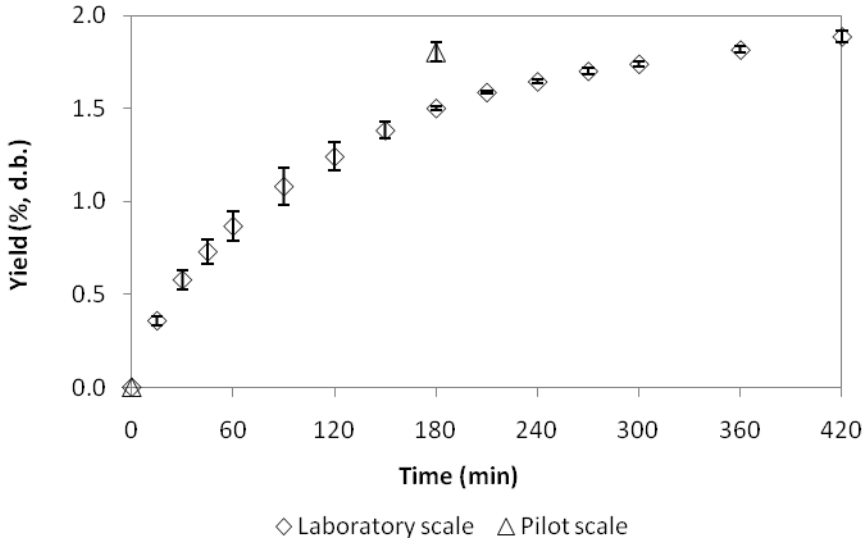

Figure 1. OEC of lemon verbena leaves at 333 K/35 MPa on laboratory and pilot scales for a 14-fold scale-up.

Since only 53 % of the extract was recovered during the CER period, the t_{CER2} (112 min) was determined. This period is the intersection of the first and third lines adjusted to the curve. This period can be considered as a more adequate initial estimation of a batch cycle, since around 63 % of the extract (1.20 % of yield) was recovered within this time. It is important to remember that these periods are only initial estimations, and that more accurate technical and economical evaluations should be carried out to determine the best cycle time of a SFE batch.

A 14-fold scale-up was applied from laboratory to pilot scale. For 180 min, the yield in pilot scale was 20 % higher than for the same time at laboratory scale. This results corroborates previous works [7,8], where the yield in pilot scale was higher than in laboratory scale when using constant S/F as scale-up criterion.

The co-extraction of water was observed in pilot scale; it was precipitated in all the separators. However, it was separated from the extracts by density difference. Around 30 % of the initial moisture content was removed in the process at pilot scale. On Figure 1, the results presented include only extract yield, without the water co-extracted.

The yields in S_1, S_2 and S_3 were 65 %, 8 % and 27 % of total extract, respectively. There is no information in literature on the solubility of lemon verbena extracts in supercritical CO_2. The only information available that could be used as an indication of solubility, despite not being measures, is the global yield [16]. Pereira and Meireles [16] observed that with pressure increase there is increase in the global yield, and that the highest the pressure, the highest the amount of heavy compounds recovered. When modifying operation conditions from 333 K/35 MPa (in the extractor) to 323 K/10 MPa (in S_1) and then 303 K/7 MPa (in S_2) and 313 K/3 MPa, the dark green and more viscous extract (S_1) was separated from the extracts presenting orange-brownish color and less viscosity (S_2 and S_3).

The ethanolic Soxhlet extraction yielded 7.1 %, a value almost four times higher than the one obtained by SFE. However, it is important to remember that the chemical composition of the

extracts must be determined prior to choosing an extraction method, since the method with higher yield may be less selective for target compounds.

Chemical composition of the extracts

Figure 2 presents the TLC plates of lemon verbena extracts obtained during the OEC and for Soxhlet extraction. It can be observed on Figure 2a that the extracts obtained by SFE presented higher concentration of terpenoids, while the extract obtained by Soxhlet presented higher concentration of heavy and/or polar compounds, which were retained in the basis of the plate. It can be noticed on Figures 2b and 2c the possible presence of flavonoids, in higher concentration in the Soxhlet extracts. According to Wagner and Bladt [23], red bands indicate the presence of flavonoid aglicons; these compounds were obtained for both extraction methods. On the other hand, the blue/green bands in the basis of the plate, also indicating flavonoids, were only obtained for Soxhlet extract. Therefore, the flavonoids composition may differ according to the extraction method used. It is also interesting to notice that in the SFE extracts, the concentration of flavonoids increases with extraction time.

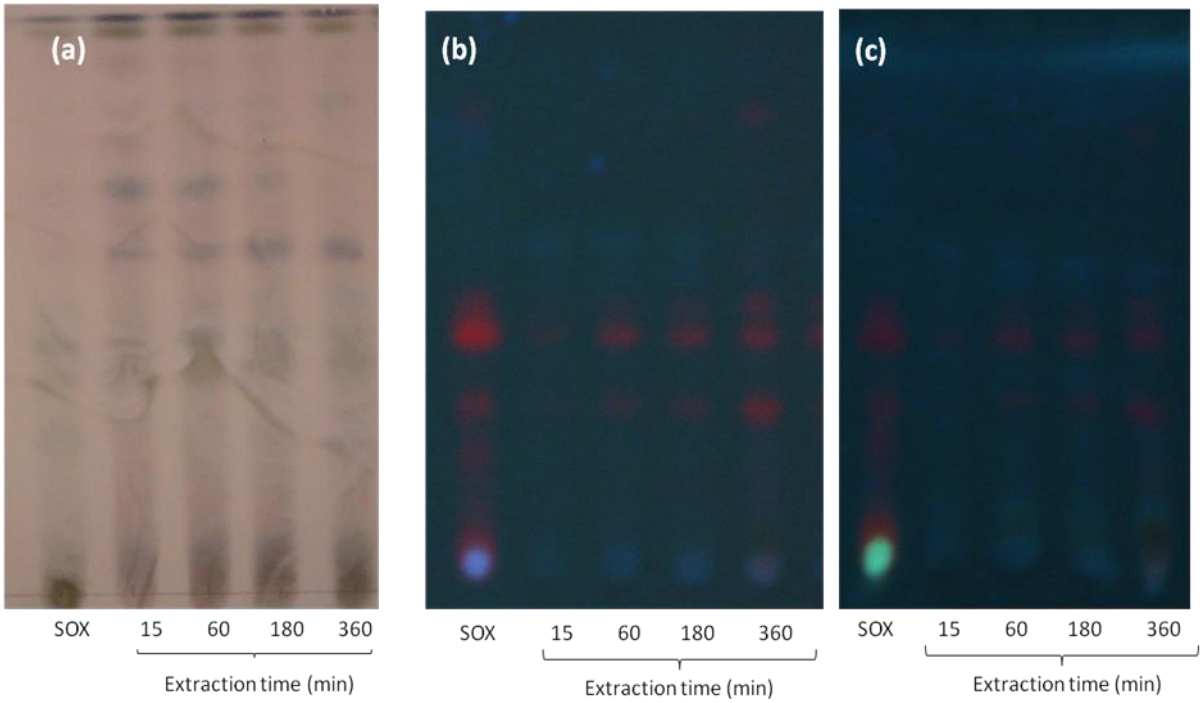

Figure 2. TLCs of lemon verbena extracts obtained by Soxhlet (SOX) and during the OEC, revealed with anisaldehyde (a), under UV light without revelator (b) and under UV light with NP (c).

Figure 3 presents the TLC plates of lemon verbena extracts obtained in the three separators of the pilot equipment. It can be noticed on Figure 3a the higher concentration of terpenoids in S_2 and S_3 when compared to S_1. As for Figures 3b and 3c, they show that the amount of flavonoids obtained was low, since few and weak bands could be observed under UV light, and that they were concentrated in S_1. When comparing Figures 2 and 3, it can be noticed that since the highest amount of flavonoids is recovered by the end of the SFE process, the concentration of flavonoids in the extract of pilot scale experiment was low because it was carried out during less than half of the time of the laboratory scale experiment.

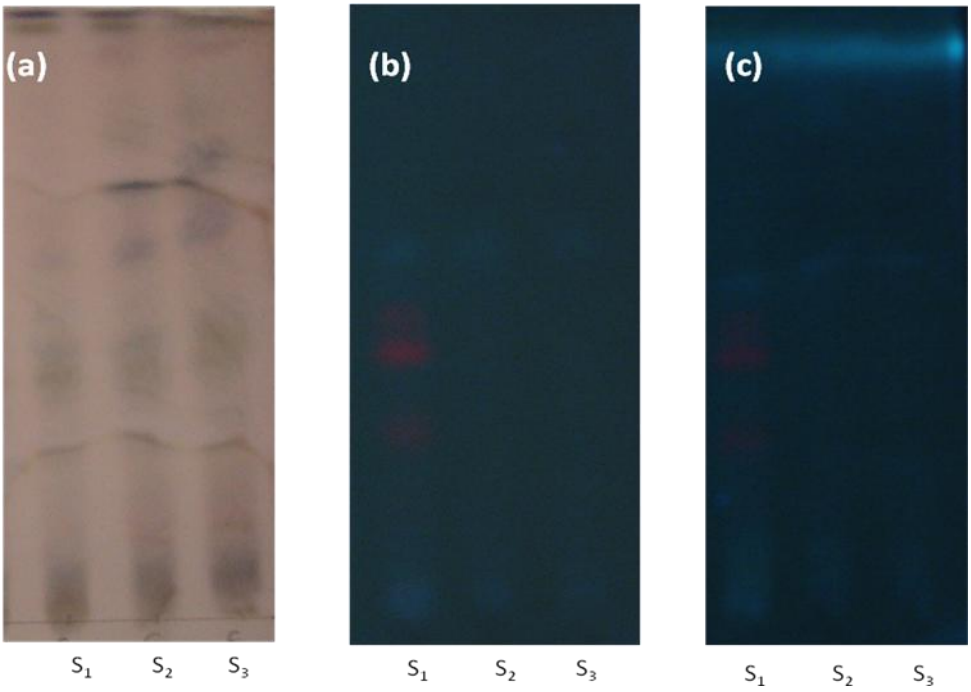

Figure 3. TLCs of lemon verbena extracts obtained by in the separators of the pilot equipment, revealed with anisaldehyde (a), under UV light without revelator (b) and under UV light with NP (c).

Table 1 presents the chemical composition determined by GC of the lemon verbena extracts obtained during the OEC, in the separators of the pilot equipment and in the Soxhlet extraction. Only 10 compounds were identified by GC-MS analysis, but over 70 different compounds were isolated in the GC analysis. Therefore, 25-55 % of the area composition could not be identified.

Observing the OEC data, it can be noticed that the light compounds are mostly extracted in the beginning of the process, while the heavy compounds' concentration increases with time. As for the extracts obtained in the separators, most of the light compounds were recovered in S_2 and S_3, while the heavy compounds presented higher concentration in S_1. Soxhlet extract presented similar composition to the extract obtained in S_1. These results corroborate the indication observed in TLC analyses. It is important to remember, however, that GC results in area percentage account only the volatile fraction of the extracts, which could be diluted in other heavy compounds. This can be clearly noticed on Figure 2a, where the Soxhlet extracts present little concentration of volatile compounds. Therefore, one should be cautioned when comparing extracts with different concentrations of heavy compounds non-detectable by GC.

The major compounds found in the extracts were spathulenol, phytol, octadecatrienal and a non-identified hydrocarbon. This composition is in partial agreement with literature data for lemon verbena volatile oil obtained by hydrodistillation, which includes mainly geranial, neral, limonene, caryophyllene oxide, t-caryophyllene and curcumene [14], and for lemon verbena SFE extract, with caryophyllene oxide, high molecular mass non-identified compound, nerolidol, α-curcumene and neral as major compounds [16]. The aggressive extraction conditions used in this work (333 K/35 MPa) were not used previously for lemon verbena extraction, which may explain the differences in composition, with higher concentration of heavier compounds. Moreover, the raw material used in this study was of Colombian origin, which may have influenced the chemical profile of the extracts.

Table 1. Chemical composition (GC, area %) of lemon verbena extracts obtained by SFE at laboratory and pilot scales and Soxhlet.

Peak	Compound	OEC - Time (min)														Separators			Soxhlet
		15	30	45	60	90	120	150	180	210	240	270	300	360	420	S_1	S_2	S_3	
1		0.5	0.4	0.4	0.5													0.6	
2		0.5	0.5	0.5	0.6	0.5											0.5	0.9	
3		1.8	1.4	1.2	1.5	1.4	1.3	1.1	0.9	0.7							0.9	2.3	
4		0.7	0.6	0.6	0.8	0.7	0.7	0.6									0.8	1.3	
5	E-citral	3.0	2.3	2.1	2.5	2.4	2.2	1.8	1.4	1.1	0.7	0.4					1.5	3.7	
6				0.3	0.4	0.4	0.5											0.5	
7			0.3	0.4	0.4	0.4											0.5	0.8	
8	Geranic acid	2.3	2.8	3.2	3.7	4.0	4.6	4.7	4.8	4.6	4.2	4.1	3.6	3.1	2.7	1.8	3.4	5.8	4.5
9		0.6	0.6	0.6	0.6	0.7	0.6	0.6									0.5	1.0	
10		0.6	0.4															0.5	
11																		0.5	
12		0.5	0.4	0.5	0.6	0.6	0.5										0.3	0.7	
13		1.2	0.9	0.7	0.7	0.6	0.3										0.4	0.9	
14		0.3			0.3													0.4	
15		0.4	0.5		0.5	0.5											0.5	0.7	
16	Curcumene	3.4	2.8	2.1	2.1	1.8	1.5	1.2	0.4	0.7							1.9	3.1	3.1
17		0.6	0.5	0.4	0.5				0.4								0.3	0.5	
18		0.8	0.7	0.6	0.6												0.7	0.8	
19		0.4	0.4	0.5	0.5	0.5	0.6	0.6	0.3	0.3							0.4	0.7	
20																	0.3	0.6	
21	Nerolidol	3.3	2.9	2.5	2.6	2.5	2.0	1.8	1.2	1.0	0.9	0.8	0.8	0.3	0.3	0.8	3.4	4.0	3.5
22																	0.3	0.4	
23	Spathulenol	9.5	8.6	7.4	7.7	7.7	6.4	6.0	4.3	3.7	3.3	3.1	3.0	2.8	2.6	3.5	9.1	10.9	9.9
24	Farnesene	2.8	2.4	2.0	2.1	2.0	1.6	1.5	1.0	0.9	0.8	0.7	0.7	0.7	0.3	0.1	2.5	3.3	2.7
25		0.3																0.5	
26		0.5	0.4	0.3													0.6	0.7	
27		0.4	0.5	0.5	0.6	0.5	0.6	0.7	0.8	0.8	0.9	0.8	1.2	0.6	1.4	0.8	0.4	0.3	1.9
28		1.2	1.2	1.1	1.2	1.2	1.0	1.0	0.7							0.1	1.1	1.5	1.8
29		0.9	0.8	0.7	0.7	0.7	0.4	0.3	0.2								0.9	1.0	
30		0.3	0.3	0.3	0.3												0.3	0.4	
31		0.8	0.7	0.7	0.7	0.7	0.6	0.3									0.8	1.1	

Peak	Compound	OEC - Time (min)														Separators			Soxhlet
		15	30	45	60	90	120	150	180	210	240	270	300	360	420	S_1	S_2	S_3	
32		0.6	0.5	0.5	0.5	0.5											0.6	0.7	
33		0.5	0.5	0.4	0.5	0.5											0.4	0.6	
34		0.3															0.3	0.5	
35	Spathulenol (isomer)	3.3	3.5	3.3	3.5	3.7	3.9	4.0	3.9	3.7	3.5	3.4	3.4	3.2	3.0	3.9	3.0	3.8	4.5
36		0.4	0.4	0.4	0.4												0.4	0.5	
37		1.1	1.1	1.1	1.1	1.2	1.2	1.2	1.1	1.1	1.0	1.0	0.9	0.9	0.4	0.9	1.1	1.3	0.6
38		0.8	0.9	0.9	0.9	1.0	0.9	1.0	1.0	1.0	0.9	0.9	0.8	0.8	0.4	0.9	0.8	0.9	
39				0.4	0.4	0.5	0.7	0.9	1.1	1.3	1.3	1.3	1.3	1.2	1.1			0.4	
40		0.5	0.5	0.5	0.6	0.6	0.6	0.7	0.6	0.6						0.5	0.5	0.7	
41		0.6	0.6	0.6	0.6	0.7	0.7	0.7	0.6							0.6	0.6	0.8	
42											0.6	0.6	0.8	0.8	1.0			0.5	4.2
43		0.6	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.3	0.3			0.6	0.6		
44		0.5	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.3						0.5	0.5	0.6	0.6
45																			0.7
46		4.0	4.8	5.3	5.5	5.7	6.2	6.4	6.9	7.2	7.5	7.8	7.6	7.6	8.0	5.8	3.7	3.7	6.9
47																			0.5
48																	0.3	0.4	
49		0.3																0.3	
50	Phytol	6.8	7.0	8.0	9.3	11.4	16.5	20.6	24.8	27.1	30.0	30.9	31.6	32.0	29.7	8.0	9.8	12.5	9.4
51		1.0	1.3	1.5	1.5	1.5	1.6	1.6	1.8	1.9	1.8	2.0	1.8	1.8	1.8	1.7	1.0	0.9	0.8
52	Octadecatrienal	5.3	7.4	8.8	8.9	9.1	9.9	10.2	11.5	12.1	12.0	13.1	12.3	12.4	12.7	10.9	6.3	4.8	1
53		0.3																0.6	
54																		0.6	
55										0.6	0.8	0.8	0.9	0.9	1.0				
56		0.9	0.8	0.7	0.6	0.5			0.5	0.7	0.8	1.1	1.1	1.1	1.6	1.0	0.9	0.7	0.8
57		0.4	0.3	0.3	0.3													0.3	
58		0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.6	0.6						0.9	0.6	0.8	0.5
59		10.7	10.1	8.3	6.9	6.2	4.4	3.8	2.3	1.8	1.6	1.4	1.3	1.3	1.7	10.9	10.9	3.9	8.5
60		0.7	0.7	0.7	0.6	0.6	0.6									0.7	0.5	0.5	
61		2.6	2.2	1.9	1.6	1.6	1.2	1.1	0.8	0.6	0.6					2.8	1.4	1.2	2.3
62		1.4	1.4	1.2	0.9	0.9	0.4	0.4								1.5	1.6	0.4	
63		0.4	0.4	0.4												0.6	0.4		
64		3.3	3.8	3.4	2.5	2.1	1.5	1.4	0.5	0.3					0.4	3.8	4.2	0.8	2.6

Peak	Compound	OEC - Time (min)														Separators			Soxhlet
		15	30	45	60	90	120	150	180	210	240	270	300	360	420	S_1	S_2	S_3	
65		0.7									0.5		0.5	0.5					
66		1.9	2.6	2.9	2.8	3.0	3.1	3.2	3.2	3.2	3.3	3.4	3.5	3.7	3.9	4.7	1.9	0.8	3.6
67		0.5															0.4		
68		1.2	1.1	0.9	0.8	0.7	0.4	0.3								1.5	1.0	0.4	0.5
69			0.4	0.5	0.4		0.5	0.5	0.5	0.6						0.7	0.4		
70		0.4	0.5	0.6	0.6	0.7	0.7	0.8	0.9	0.9	1.0	1.0	1.0	1.0	1.1	1.5	0.6		
71	Non-identified hydrocarbon	5.3	8.6	11.2	10.6	12.1	14.0	14.5	17.0	18.2	19.2	19.0	19.9	21.3	22.6	21.9	9.6	1.4	14.9
72		0.3	0.4	0.4	0.4	0.5	0.5	0.5								0.6	0.3		
73		0.3	0.5	0.5	0.5	0.5	0.5		0.3							0.9	0.4		
74			0.5	0.6	0.5	0.5	0.6	0.6	0.7	0.4	0.3	0.4				1.1	0.5		
75		0.8	1.5	1.8	1.6	1.7	1.8	1.7	1.9	1.9	1.8	1.7	1.7	1.7	1.7	3.1	1.3		0.8
76		0.7	0.2														0.5	0.4	
	Total non-identified	55.0	51.7	49.4	47.1	43.2	37.5	33.9	29.8	27.1	25.4	24.5	24.7	24.2	26.1	49.1	49.5	46.7	37.7

CONCLUSION

The scale-up criterion studied, maintaining solvent to feed ratio constant, was successfully used to extract lemon verbena leaves in laboratory and pilot scales with a 14-fold scale-up. Volatile oil and flavonoids were identified in the extracts; the major compounds present in the volatile fraction of the extracts were spathulenol, phytol, octadecatrienal and a non-identified hydrocarbon. Using three separators in series in the exit stream of SFE pilot equipment allowed obtaining three different products for one single run, with different physical aspects and chemical profiles.

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