Comparison of the composition and cost estimation of steam distillation and supercritical fluid extraction from leaves of *Lippia graveolens*

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ABSTRACT *Lippia graveolens*, mostly known as Mexican oregano, is an aromatic plant with great economic importance. However, very little is known about the reasons that could explain the variation observed in the yield and composition of the essential oil and extract obtained. The effect of steam distillation and supercritical fluid extraction processes on composition, yield, and economic, viability were evaluated. The essential oil and extract were obtained through steam distillation using analytical scale equipment and supercritical extraction equipment, respectively. The extracts were analyzed by GC-MS. Carvacrol was the majority compound in both processes. In the case of steam distillation, a maximum yield of 4.1 % on a dry weight basis was obtained, while the supercritical fluid extraction obtained provided a maximum yield of 2.8 % on a dry weight basis at the same bed porosity (0.91). The cost was obtained over a period of one year. The process of extraction by steam distillation showed good results to obtain essential oil from *L. graveolens*. It is observed that steam distillation remains the optimal choice.

KEYWORDS *Lippia graveolens*; Steam distillation; Supercritical fluid extraction; Cost estimation

1. Introduction

Lippia graveolens is a natural resource of economic importance of which Mexico is the main exporter. It has numerous uses in the pharmaceutical and food industries. It also represents a beneficial economic contribution to the people who cultivate and collect it. It is widely used in Mexico and North America, constituting an essential part of the regional food. The leaves are also used as remedies for anti-inflammatory, antipyretic, and analgesic purposes (Flores et al., 2016; González et al., 2019). Essential oils obtained from the genera Lippia are rich in carvacrol. One of these is a phenolic monoterpenoid that is found principally in the essential oil from oregano. A wide range of biological properties are attributed to it, such as acting as an antioxidant. Its specific antioxidant effect improves the activity of enzymatic antioxidants and the levels of non-enzymatic antioxidants. This is helpful in the conservation of seed oils and in the prolonged shelf-life of foods in active packaging, the antimicrobial-like bacteria and spores growth inhibition in vapor and liquid phases, inhibition of biofilms, and fungi growth inhibition. It participates in the activation of anti-cancer activities such as fighting against liver and breast cancer cell lines, liver cancer in vivo, and hepatic steatosis, as well as aiding in diminishing cancer-related pain, among others (Sharifi-Rad et al., 2018). Another research mentions that essential oil has antiviral activity, where carvacrol, from Mexican oregano, showed high antiviral activity against rotavirus (Ribas Pilau et al., 2011), it has also been reported antiviral properties of carvacrol and oregano oil against non-enveloped murine norovirus (MNV). The potential use of carvacrol as a surface sanitizer to control human norovirus and natural food has also been shown (Gilling et al., 2014).

Steam distillation is a method that has the advantage of not using organic solvents such as hexane, methane, and among others. In this case, water is used as a separator agent since it has a high processing capacity; that is, it can work with large quantities of plant material and with different physical characteristics which allows good profitability due to its economical and flexible construction (Meireles, 2009).

Meanwhile, one of the most recent extraction techniques that are of great interest in the scientific environment is the extraction with supercritical fluids, because they are more environmentally friendly, do not pose a health risk, as well as guarantee a better and superior quality of the products (Velásquez, 2008). Supercritical carbon dioxide (SC-CO_2) non-pollutant, is non-toxic and can be used at mild temperature conditions. It is recognized as a recommendable extraction fluid and is a highly selective extraction technique compared to other methods, as it allows for the adjustment of its properties through the manipulation of operating conditions such as pressure and temperature. By doing so, it exhibits a diffusivity resembling that of a gas and a density resembling that of a liquid. This enables the customization of CO_2 density

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(and consequently, its solvent capability) based on the specific compounds targeted for recovery (Zanotti et al., 2023).

On the other hand, manufacturing costs are an estimate of the cost used to plan a project according to the Association for the Advancement of International Cost Engineering (AACEI), since these costs are commonly used for strategic decisions, i.e. analyzing whether it is feasible to advance a project or failing to stop such a project. This cost estimate influences many factors that can be grouped into three cost categories: direct costs, fixed costs, and overhead. Direct costs consider costs that depend directly on production, including raw materials, utilities, and operating costs, among others. Fixed costs do not depend directly on production, even when production stopped, such as equipment costs, depreciation, taxes, and insurance. The overheads are composed of the amount needed to maintain the business and include administration costs, shipping costs, and research and development according to Meireles (2009).

It is calculated using the following equation:

$$C_{OM} = 0.304 \times F_{CI} + 2.73 \times C_{OL} + 1.23 \times (C_{RM} + C_{WT} + C_{UT}) \tag{1}$$

where, C_{OM} is cost of manufacturing which corresponds to the weighted sum of the five main costs, F_{CI} is capital investment cost, C_{OL} is cost of operational labor, C_{RM} is cost of raw material, C_{WT} cost of waste treatment and C_{UT} is cost of utilities.

2. Methods

2.1 Raw material

Dried *L. graveolens* leaves were obtained from the central market of the city of Merida. These leaves were cultivated in Tetiz, a town located in the northeast region of the state of Yucatan, Mexico, and were harvested in September, these leaves had a humidity of 11.2 % (±2.1), and the leaves were not ground for steam distillation. Subsequently, leaves were given a granulometric characterization for supercritical extraction, because the leaves were fragmented, where the particle size distribution was as follows: 7.5 % ±0.01 (4.7 mm), 5.5 % ±0.48 (4.0 mm), 31.4 % ±1.63 (2.4 mm), 6.8 % ±1.99 (2.0 mm), 23.1 % ±0.67 (1.4 mm), 3.7 ±0.12 (1.2 mm), 6.3 ±0.25 (1.0 mm), 4.4 ±0.37 (0.84 mm), as obtained, were chosen for both plant materials, those diameters where the highest percentage of particles retained in the meshes were, which correspond to a size between 1.4 mm and 2.4 mm.

2.2 Steam distillation

Analytical-scale experiments were carried out in 4.5 cm diameter and 0.334 L capacity analytical-scale glass equipment (Fig. 1) located in the Chemical Engineering Faculty of the Universidad Autónoma de Yucatán (FIQ-UADY). In these experiments, 20 g of dried *L. graveolens* leaves were packed and extracted with steam for 4 hours. Saturated steam at atmospheric pressure was used as the essential oil-extracting agent. After condensation of the vapor, essential oils were isolated by decanting (Soto-Armenta et al., 2017).

Figure 1: Steam distillation equipment.

Figure 2: Supercritical fluid extraction equipment.

2.3 Supercritical fluid extraction

A cell with a capacity of 1 L was used (Fig. 2) in which the amount of 88 g of leaves was introduced, where the bed density, porosity, particle size, temperature, and pressure, including static and dynamic extraction time, remained constant, these last ones are two stages of supercritical fluid extraction. A flow of 27.6 ±0.002 g/min was maintained throughout the dynamic extraction process.

The extraction was performed at 35 MPa and 60 °C, which were defined according to another study referring to the SFE of *Lippia graveolens* where different pressures and temperatures were evaluated, they reported that at 35 MPa and 60 °C obtained a maximum yield (Soto-Armenta et al., 2019).

2.3.1 Gases of operation

A flow of Industrial grade CO_2 with a purity of 99.9 % and extra-dry air with a purity of 99.99 %, were supplied by Praxair, Merida-Mexico.

2.4 Essential oil composition

All the experiments were analyzed by a chromatographer Agilent Technologies model 6890N coupled to an Agilent 5973N mass selective detector (MSD) with an electron impact ionization of 70 eV was used, with an initial temperature from 50 °C for 3 min, then rising from 50 °C to 250 °C at 20 °C/min, finally kept isothermal at 250 °C for 10 min, before post run from 280 °C for 5 min. Helium was used as carrier gas at 1 mL/min. An HP-5 MS (30 m×250 μm×0.25 μm) column was used. The injection volume was 2 μL (4 % essential oil/CH_2Cl_2 v/v) under a 100:1 split ratio. The injection and transfer line temperature were 150 °C and 280 °C, respectively (Ma et al., 2011).

2.5 Production costs

Cost of manufacture of essential oil obtained by steam distillation and supercritical fluid extraction. This preliminary cost estimate is commonly used for strategic decision-making, to advance or stop the project, being an important tool for evaluating the feasibility of a process.

The cost determination applied in this equation can be applied for both steam distillation and supercritical fluid extraction (Meireles, 2009; Navarro et al., 2009; Prado et al., 2017).

2.5.1 Capital investment cost (F_{CI})

The costs of steam distillation extraction equipment were \$9,150, 18,100, 24,900, and 50,000 USD, for equipment of 22, 50, 100, and 500 L respectively (company FIGMAY SRL 2017; Meireles, 2009), for the supercritical fluid extraction, equipment prices are around USD 280,786, 400,000, 1,253,000 and 1,659,000 USD (Meireles, 2009; Rocha-Uribe et al., 2014; Rosa and Meireles, 2005; de Melo et al., 2014), for equipment of 20, 50, 200 and 400 L. All this on an annual basis, where the investment fraction is calculated by multiplying the total investment by the depreciation rate, in this case, 10 % per year (Attard et al., 2015; de Melo et al., 2014; Prado et al., 2012).

2.5.2 Cost of operational labor (C_{OL})

The cost of labor was 3.00 USD per hour (Rosa and Meireles, 2005; Rocha-Uribe et al., 2014). The number of personnel required to operate an industrial plant depends on its capacity, and level of automation, in addition, to a lot of time. For supercritical extraction, not much energy is required to load and unload the extractors, in general, two operators are necessary for a large fully automated plant (Prado et al., 2017). It was considered a 24-hour day for 330 days per year, giving a total of 7200 hours.

2.5.3 Cost of raw material (C_{RM})

The cost of raw material for steam distillation extraction includes the costs for the plant material, and the amount of water used for the process, on the other hand, supercritical fluid extraction also includes the costs of plant material, which corresponds according to oregano producers in the region to an amount of USD 3644.28 per ton. It should be noted they were not given any initial pretreated. This section attaches the costs of the kilograms of CO_2 used, as well as the kilograms of CO_2 that are lost during the process (2 % according to Rocha et al., 2014).

2.5.4 Cost of waste treatment (C_{WT})

In both processes, the organic waste that is generated is not considered waste as it can be used as biomass. Wastewater resulting from the steam trawl extraction process is considered non-hazardous, which will result in a lower cost compared to the production of hazardous wastewater. In the case of supercritical fluid extraction, no water is required, therefore that cost is dismissed.

2.5.5 Cost of utilities (C_{UT})

For the steam distillation extraction process, the costs of the boiler were determined, while for supercritical fluid extraction, the costs generated by the pump, heater, and condenser were obtained, using the ASPEN PLUS program (Version 8.1, Aspen Tech, MA, USA).

3. Results and discussion

3.1 Yield obtained in both extraction processes

According to Martínez-Natarén et al. (2012), an average yield greater than 2 %, is considered high in the process of extraction of essential oils, demonstrating the potential to use the plant. The yields of essential oil obtained for *L. graveolens*, values like those achieved by Salgueiro et al. (2003) with a value of 3.5 % were found. Calvo-Irabién et al. (2014) indicate that they have achieved a percentage greater than 3 % by hydro-distillation with a Clevenger team for 1.5 hours, where they mention in their study, that the North region to the center of Yucatan are concentrated oregano plants with high yield values greater than 3 %, and with high content of carvacrol or thymol. Soto-Armenta et al. (2017) obtained a yield of 4.41 % on a dry-weight basis. In this investigation, an extraction yield of 4.10 % was obtained. Steam distillation is the most widely used extraction method due to its low investment requirements and operational costs in comparison to other methods such as subcritical carbon dioxide, ultrasound, microwaves, and nonpolar solvents. From an environmental perspective, steam distillation extraction is considered a clean method since water steam is used as the solvent. In contrast, organic solvents often necessitate post-separation processes, generate waste, require air treatment, exhaust systems, and sometimes even explosion-proof installations (Machado et al., 2022).

On the other hand, for supercritical fluid extraction, for a cell with a capacity of 0.1 L and specifically for *L. graveolens*, Soto-Armenta et al. (2019), mention having obtained a maximum yield of 2.6 % on a dry weight basis. Different research has been found where certain compounds of interest were obtained from *Origanum vulgare* by super-critical fluids extraction (Santoyo et al., 2006; Ocaña-Fuentes et al., 2010). In this study, under the same conditions used by Soto-Armenta et al. (2019) a yield of 2.8 % on a dry-weight basis was obtained in 1 L cell. One of the advantages of supercritical extraction compared to other more traditional is that it prevents the active compounds from degrading and decomposing, by applying reduced temperatures (Sánchez-Mesa et al., 2019). Other studies report that compared to conventional steam distillation and consecutive solvent extraction methods, supercritical fluid extraction was found to be more efficient and environmentally friendly for extracting aroma from *Salacca zalacca* (Gaertn.) Voss (Nadhirah et al., 2019). Ara et al. (2015) indicate that obtaining essential oil and fatty acids from *Descurainia Sophia* L. seeds through supercritical fluid extraction, using the appropriate conditions proved to be a more selective process, and yielded higher extraction efficiency compared to steam distillation.

Figure 3 shows the difference between both extraction processes in the yield extraction obtained. Steam distillation obtained a higher yield, while supercritical fluid extraction remained below, the extraction time for both processes was 240 min.

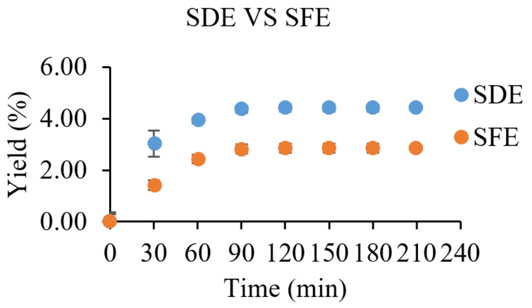

Figure 3: Kinetics of *L. graveolens* yield obtained concerning extraction time, obtained by steam distillation extraction (SDE) and the Supercritical Fluid Extraction (SFE).

3.2 Chemical composition

The chemical composition of essential oil and extract were identified by Gas Chromatography-Mass Spectrometry analysis, which involved steam distillation extraction. The analysis primarily identified three major compounds (Table 1): Carvacrol, followed by thymol, and cymene. While for supercritical fluid extraction carvacrol, hexadecanoic acid, and thymol as shown in Table 1.

The identification was made by comparison of their mass spectra with the mass spectra of the 2011 NIST library. The minimum accepted spectrum matching values for identification were 850, leaving a high confidence level, based on its probability percentage and spectrum matching rating.

The composition of *L. graveolens* essential oils coincide with those reported by Calvo-Irabién et al. (2014), for chemotype C individuals in the Yucatan region, Mexico, where carvacrol and thymol stand out in descending order as the majority compounds.

Carvacrol is a phenolic monoterpenoid found in essential oils of different plants, this compound possesses various types of bioactivities, which could be applied for medicinal purposes, among its activities, has been reported as an alternative to synthetic antioxidants for conserving the quality of roasted sunflower seeds (Quiroga et al., 2015). Also, possesses an antiviral bioactivity, specifically, shown a significant antiviral activity by inhibiting the herpes simplex virus proliferation process (Wang et al., 2020). Carvacrol is effective against *Escherichia coli*, *Bacillus cereus*, and *Salmonella* (Sharifi-Rad et al., 2018). The percentage of the two primary constituents found in Origanum vulgare ssp. hirtum essential oils, namely thymol, and carvacrol, can range from as low as 3 % to as high as 60 %, achieved through steam distillation. The major components consist of carvacrol and thymol, which collectively make up more than 80 % of the total oil. Carvacrol is typically the predominant component, although occasionally it may only be present in trace amounts. The concentration of active substances and the chemical composition of phytobiotics in the final products can vary significantly depending on the specific plant parts utilized, harvesting season, and geographical origins (Gholami-Ahangaran et al., 2022).

3.3 Determination of extraction costs

Figures 4 and 5 show the COM (manufacturing cost) values at different capacities of extractor cells, for the extraction processes by steam distillation and supercritical extraction. For the essential oil of oregano with steam distillation, it is shown that under the conditions proposed in this work, a COM of 70.28 USD/L was obtained in a 22 L equipment, compared to the value found in the market (198 USD/L) (Fig. 4), the difference is favorable.

When obtaining the COM for the products obtained by supercritical extraction (Fig. 5), the values turn out to be extremely high compared to the prices found in the market, due to the enormous investment cost that the use of super-critical extraction presupposes. In the case of oregano (*L. graveolens*), a period of 3 years is foreseen in super-critical equipment with a capacity of 400 L, thus reducing the manufacturing cost of the product by 15.1 % concerning the commercial value of the essential oil of oregano.

Table 1: Preliminary identification of the major compounds in the essential oil and extract using steam distillation and supercritical fluid extraction, respectively.

	% ÁREA (SD) Experiments *L. graveolens*	
COMPOUND	Equipment steam distillation	Equipment supercritical fluid extraction
Carvacrol	36.24 (3.96)	73.03 (1.72)
Thymol	17.19 (0.33)	6.43 (0.57)
Cymene isomers	5.96 (0.87)	ND
Phenol, 2-tert-butyl-4-methoxy	4.41 (0.12)	ND
Caryophyllene	3.76 (0.21)	ND
α-Caryophyllene	2.80 (0.42)	ND
Hexadecanoic acid	ND	7.36 (0.64)
Linolenic acid	ND	4.18 (0.31)

SD: Standard deviation; ND: Not detected.

Table 2: Preliminary identification of the major compounds in the essential oil and extract using steam distillation and supercritical fluid extraction, respectively.

Extraction	Equipment (L)	0.304* F_{CI} %	2.73* C_{OL} %	1.23* C_{RM} %	1.23* C_{UT} %	*COM* specific (USD)
SD	22	0.01	2.40	2.26	95.33	70.28
	50	0.005	1.07	2.29	96.63	69.34
	100	0.003	0.54	2.30	97.15	68.96
	500	0.002	0.13	2.87	96.99	55.26
SFE	12	5.06	79.72	15.16	0.06	1054.58
	50	4.96	52.94	41.94	0.16	381.16
	200	6.56	22.35	70.83	0.26	225.73
	400	5.98	12.77	80.94	0.30	197.51

F_{CI}: Capital investment cost; C_{OL}: Cost of operational labor; C_{RM}: Cost of raw material; C_{UT}: Cost of utilities; *COM*: Cost of manufacturing; SD: Steam distillation; SFE: Supercritical Fluid Extraction.

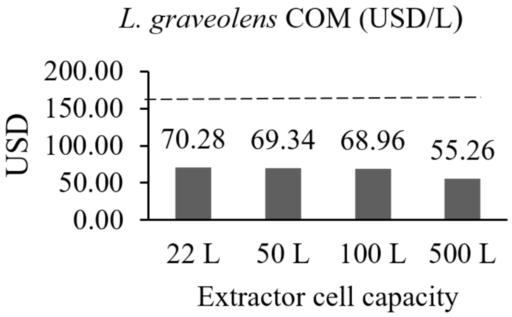

Figure 4: *COM* obtained by steam distillation at different scales, the broken line represents the price of essential oil in the national market.

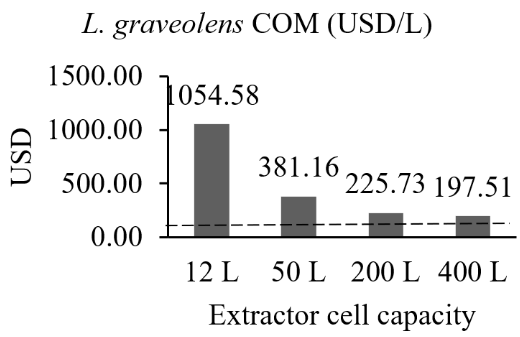

Figure 5: *COM* obtained by supercritical fluid extraction at different scales, the broken line represents the price of essential oil in the national market.

Table 2 shows the main costs for *L. graveolens* obtained during the extraction process by steam distillation and supercritical extraction corresponding to different equipment capacities. In these Table the corresponding cost was multiplied by its coefficient, according to Eq. 1, this was done to obtain the total costs in percentage. The raw material was what generated the most costs for all equipment. In the 12 and 50 L equipment used for supercritical extraction, operational labor was the one that generated the highest expenses.

4. Conclusions

Of the two extraction processes, the extraction process by steam distillation was the one that obtained the highest yield of essential oil. However, in the identification of compounds, in the supercritical extraction process, it is observed that it has a higher percentage of the area of the majority compound. Although different extraction processes and conditions were applied to *L. graveolens* leaves, carvacrol was the majority compound. The carvacrol compound has a higher percentage of area in the essential oil extracted by supercritical extraction compared to extraction by steam distillation. It is suggested to focus more research on the analysis of the essential oil composition. The manufacturing costs that were obtained in a period of one year for *L. graveolens* using the extraction process by steam distillation showed favorable results since under the most optimal conditions there was a difference of 72.2 % for *L. graveolens* concerning the oregano essential oil found in the market. The results obtained show that steam distillation continues to be the best extraction method to obtain essential oils. Therefore, it is considered feasible to undertake a more in-depth market study, and thus be able to take advantage of this valuable natural resource.

Nomenclature

C_{OL}: Cost of operational labor [USD].
C_{OM}: Cost of manufacturing [USD].
C_{RM}: Cost of raw material [USD].
C_{UT}: Cost of utilities [USD].
C_{WT}: Cost of waste treatment [USD].
F_{CI}: Capital investment cost [USD].
Y: Yield of essential oil [kg/kg].
Greek letters
ρ_f: Density of solvent [kg/m^3].
ρ_l: Apparent density of bed [kg/m^3].
ρ_s: Density of solid [kg/m^3].
ε: Porosity of bed [Adimensional].

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