Fractions of laurel essential oil obtained by molecular distillation with greater antioxidant and antimicrobial activities

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SUMMARY

This study aimed to analyze the chemical composition and the antioxidant and antimicrobial activities of *Laurus nobilis* L. essential oil (LEO) and its fractions obtained by short-path molecular distillation. According to the chemical composition, it can be said that LEO and its fractions proved to have antioxidant activity since both have a high content of total phenolic content (TPC). Short-path molecular distillation was used to separate essential oil fractions with superior antioxidant activity. Laurel residue (LR) exhibited the greatest antioxidant activity, with higher values of trolox equivalent antioxidant capacity with ABTS radical cation (TEAC-ABTS) assay and TPC. In addition, LR had the lowest value of IC50-DPPH. For antimicrobial activity, all natural products tested had an effect on all foodborne pathogenic microorganisms. LEO, as well as its fractions, showed antimicrobial, bacteriostatic, or bactericidal activity against Gram-positive and Gram-negative bacteria. The LEO and its fractions obtained by molecular distillation can be used as antimicrobials and as food preservatives to prevent oxidation. Also, consumers considered the addition of LEO or its fractions in food products as positive.

Keywords: *Laurus nobilis*, hydrodistillation, natural products, food preservatives.

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RESUMEN

Este estudio tuvo como objetivo analizar la composición química, la actividad antioxidante y antimicrobiana del aceite esencial de *Laurus nobilis* L. (AEL), y sus fracciones obtenidas por destilación molecular de camino corto. De acuerdo con la composición química, puede decirse que el AEL y sus fracciones tienen actividad antioxidante, ya que poseen un alto contenido de fenoles total (FT). La destilación molecular de camino corto se utiliza para separar las fracciones de aceite esencial con mayor actividad antioxidante que el original. El residuo de laurel (RL) exhibió la mayor actividad antioxidante, con

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valores más altos para los ensayos de la capacidad antioxidante equivalente a trolox con el radical catión ABTS (TEAC-ABTS) y FT. Además, RL tuvo el valor más bajo de IC50-DPPH. Para la actividad antimicrobiana, todos los productos naturales probados ejercieron una acción sobre todos los microorganismos patógenos utilizados. El AEL, así como sus fracciones, mostraron actividad antimicrobiana, bacteriostática o bactericida frente a bacterias Gram positivas y Gram negativas. El AEL y sus fracciones obtenidas por destilación molecular se pueden utilizar como conservantes de alimentos con funciones antimicrobianas y para prevenir oxidaciones. Asimismo, los consumidores consideraron positiva la adición de AEL y sus fracciones en productos alimenticios.

Palabras clave: *Laurus nobilis*, hidrodestilación, productos naturales, conservantes alimentarios.

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INTRODUCTION

The use of natural products as functional ingredients in foods and beverages is increasing as consumers look for these kinds of products in order to avoid synthetic additives (Soubra et al. 2007). There is a growing interest in antioxidants and antimicrobials, particularly in those expected to prevent the alleged harmful effects of free radicals in the human body and the deterioration of fats and other food components due to spoilage microorganisms. In all cases, there is a preference for those from natural sources rather than synthetic ones (Hamdo et al. 2014). Many natural antioxidants have been proven to be effective in lipid-rich foods. Essential oils (EOs) are possible alternatives to use in foods because they can inhibit or decrease microbial contamination and oxidation processes. Attention in EOs and their components is increasing since it is believed that natural products are not dangerous to human health and have functional properties (Cohen et al., 2021). Some researchers have reported the antioxidant activity of oregano (Quiroga et al., 2013; Asensio et al., 2015) and poleo EOs (Quiroga et al., 2013) and the antimicrobial activity of thyme and suico EOs (Prieto et al., 2020).

There are few cases of direct application of EOs in food (Quiroga et al., 2011, 2013; Asensio et al., 2014; Olmedo and Grosso, 2019) showing both antioxidant and antimicrobial activities. In these reports, EOs showed a lower activity than synthetic preservatives (Quiroga et al., 2013, 2015; Asensio et al., 2015; Riveros et al., 2016). In some cases, a strong preservative activity of EOs is achieved when they are used in high concentrations compared to synthetic ones, which implies a sensory effect that is often not desired in certain products. For this reason, an EO fraction enriched in certain molecules could have greater antimicrobial activity with respect to pure EO (Rocha-Guzmán et al., 2007; Borgarello et al., 2015; Mezza et al., 2018). The use of short-path molecular distillation technology is an alternative to separate fractions and concentrate different chemical compounds of EO (Borgarello et al., 2015, Mezza et al., 2018). There is evidence that oregano EO fractions separated by short-path molecular distillation increased their antioxidant activity (Olmedo et al., 2014). A fraction enriched with molecules with greater antioxidant power can

provide a greater effect, using lower doses than the EO from which it is derived. The *Laurus nobilis* (laurel) (LEO) has been demonstrated to have antioxidant activity (Mello da Silveira et al., 2012; Olmedo et al., 2015). The objective of this study was to separate fractions from LEO by short-path molecular distillation and to analyze their chemical composition and antioxidant and antimicrobial activities.

MATERIALS AND METHODS

Materials

Laurel essential oil (LEO) was purchased in a local market (Mendoza, Argentina). Bacterial strains *Salmonella* sp. (ATCC 700623), *Escherichia coli O157H7* (ATCC 43895), *Micrococcus luteus* (ATCC 9341), *Staphylococcus aureus* (ATCC 25212), *Enterococcus faecalis* (ATCC 29212) and *Bacillus cereus* (ATCC 11778) ceded by the Food Microbiology and General Microbiology Laboratories, Faculty of Exact, Physicochemical and Natural Sciences, National University of Río Cuarto.

Short path molecular distillation

The LEO was distilled with a short path molecular distillation (SPMD) apparatus following the method described by Olmedo et al. (2014), then it was placed in the reception chamber (capacity, 400 mL). The distillation conditions were at 20 mbar, 26 °C for evaporation, 2.5 °C for condensation and 1.18 mL/min for flow. After this distillation, two fractions called Distillate (LD) and Residue (LR) were obtained.

Chemical analysis of the essential oil and its fractions

Gas Chromatography

The chemical composition of the LEO and its fractions were determined by CG-MS using a Perkin Elmer gas chromatograph (Clarus 600, Waltham, USA) coupled with a mass spectrometry detector (MSD) with a capillary column DB-5 (30 m, 0.25 mm i.d., 0.25 mm coating thickness). The temperature programme was the 60 °C for 0 min at 5 °C/min rate and 200 °C for final temperature. The injector was held at 250 °C. Helium was used as the carrier gas with a flow rate of 0.9 mL/min. Ionisation was induced by electron impact at 70 eV. The compounds from LEO were identified by comparing their retention index, retention time and mass spectra with published data (Olmedo et al., 2015), NIST library (Shen et al., 2017) and Adams (1989). The Co-injection of Authentic standards (Sigma, St. Louis, USA) was also used for identification of the main components. The quantitative composition was obtained by peak area normalization, and the response factor for each component was considered to equal 1.

Antioxidant activity

Free-radical scavenging activity on DPPH

The free-radical scavenging activity of the LEO was determined using DPPH (Aldrich, Milwaukee, USA) according to Quiroga et al. (2011). Tested natural products solutions (100 µl) were added in 900 µl of DPPH (0.05 mM) and kept in dark for 30 min. The absorbance of the samples was measured on a spectrophotometer (Perkin Elmer, Lambda 1A, UV/VIS spectrophotometer, PerkinElmer Inc., Waltham, USA) at 517 nm. The inhibition percentage of the DPPH radical was calculated according to the following formula: % DPPH inhibition = (1-(*A*-*Ab*)/*Ao*)x100.

Where *A* is the absorbance of DPPH solution with essential oils, *Ab* is the absorbance of 60 % methanol with the natural products, and *Ao* is the absorbance of DPPH solution.

The inhibitory concentration 50 % (IC50) was calculated from the curve obtained by plotting the percentage of inhibition versus the final natural products concentrations (Quiroga et al., 2011).

Trolox equivalent antioxidant capacity (TEAC-ABTS assay)

For this assay, 10 µL LEO or their fractions were added to 990 µL of ABTS radical cation. The absorbance at 744 nm at room temperature (22 °C) was recorded after 25 min. Trolox (SIGMA ® St. Louis, USA) was used as an external standard. The antioxidant capacity of natural products was expressed as Trolox equivalent antioxidant capacity (TEAC) and TEAC was expressed as mg Trolox/mg sample (Asensio et al., 2015).

Total phenolic content (TPC)

TPC was determined by the Folin-Ciocalteu method measured at 760 nm in a spectrophotometer (Grosso et al., 2018). The concentration was calculated using gallic acid as standard. Phenolic content was expressed as milligrams of gallic acid

equivalents per g sample (GAE/g sample).

Accelerated oxidation test

Canola oil (CA) (Krol, Amerika 2001 SA, Entre Ríos, Argentina) was used for an oven test experiment. The following samples were prepared: CO added with 0.02% LEO (CO-LEO), 0.02% LD (CO-LD) and 0.02% LR (CO -LR) and 0.02% Butylhydroxytoluene (CO-BHT) as the reference antioxidant. CO without antioxidants was used as control sample (CO-C). The samples were stored during 50 days at 45°C in an oven and removed from storage at days 0, 14, 21 and 50. Two chemical indicators of lipid oxidation were evaluated in the samples: peroxide value (PV) and conjugated dienes (CD). The PV was expressed as miliequivalents of active oxygen per kilogram of oil (mEq O_2 / Kg oil) (Horwitz, 2010). Conjugated dienes were determined according to the COI method by reading the absorbance at 232 nm and the results were reported as the sample extinction coefficient E ($\%^{-1}$ cm^{-1}) (COI, 2001).

Antimicrobial activity of the essential oil and its fractions

Disk diffusion technique

Bacterial inoculums were placed in petri plates containing Müeller-Hinton Agar (MHA) and incubated for 18 h. Each sample (10µL) was impregnated in a filter paper disc placed in petri plates previously sown with microorganism. The plates were incubated at 37°C for 24h. Then, the inhibition halos were measured (Demo et al., 2005).

Determination of the Minimum Inhibitory Concentration (MIC)

The antimicrobial activity of LEO and its fraction was determined using the broth microdilution method described in Mann and Markham (1998) with modifications. The EO samples were diluted in factor two with a dimethyl sulphoxide (DMSO). The inoculum used was a microbial culture in Müeller-Hinton Broth (MHB) with 0.15% agar with a cell density required to reduce the redox resazurin indicator (Sigma-Aldrich, St. Louis, USA). The assay was performed in a sterile 96-well microtiter plate. Serial 10-fold dilutions of the overnight culture were prepared in MHB, and 170 µL of each dilution were dispensed into microtiter plates. The microtiter plates were incubated for 24h at 37°C and the appropriate dilution unable to reduce resazurin (blue) was chosen for the antimicrobial assays. To determine minimum inhibitory concentration (MIC), two trays were prepared for each strain and incubated at 37°C for 3.5h (Carezzano et al., 2017). After incubation, a 10 µL resazurin solution was added to all wells and incubated again for 2h at 37°C (Mann and Markham, 1998).

Minimum Bactericidal Concentration (MBC)

To perform the MBC, petri plates with MHA were used, which were inoculated with 100 µL of those dilutions that presented blue color. They were incubated at 37°C for 24h. The Minimum bactericidal concentration (MBC) was considered as the last dilution that did not show cellular growth (Asensio et al., 2014).

Sensory analysis

For the sensory analysis, an affective test was run. Consumer panelists residing in Córdoba (Argentina) participated for acceptance analysis. The trial was conducted with 92 panelists between 20 and 60 years old. For the evaluation, 3mL fresh canola oil samples (CO-C; CO-LEO; CO-LD and CO-LR) were placed on a 3 x 3 cm white bread piece. The samples were coded with 3-digit numbers. A glass of water and a paper napkin in a plastic tray were also presented with the samples. Panelists were instructed to consume the entire sample and rinse their mouths with water between samples to minimize any residual effects. A nine-point hedonic scale was used where 1 = Dislike extremely, up to 9 = like extremely (Peryam and Pilgrim, 1957).

Statistical analysis

All analyses were carried out in three repetitions. Data were analyzed using the InfoStat software, version 2018 (Di Rienzo et al., 2018). Means and standard deviations were calculated. ANOVA and FisherLSD test ($\alpha = 0.05$) were used to find significant differences among means.

RESULTS AND DISCUSSION

Chemical analysis of the essential oil and its fractions

Gas Chromatography

The chemical compositions of the studied

LEO and its fractions obtained by short-path molecular distillation are shown in the Table 1. Only components with concentrations >0.5% are reported.

The major components for LEO were 1,8 cineole (37.5%), α-terpinyl acetate (12.37%), linalool (8.71%) and sabinene (7.07%); for the LD fraction,1,8 cineole (47.56%), linalool 9.85%), α-terpinyl acetate (12.37%) and sabinene (7.45%); for the LR fraction, α-terpinyl acetate (23.2%), 1,8 cineole 19.92%), linalool (13.15%) and eugenol methyl ether (8.45%). Other authors reported 1,8 cineole as the main component of LEO. They also found α-terpinyl acetate among the first four main components. Flamini et al. (2007) observed high percentages of 1,8 cineole (37.5%) followed by trans-sabinene hydrate (9.7%) and α-terpinyl acetate (9.3%). Mello da Silveira et al. (2014) found 1,8 cineole (35.50 %) and linalool (14.10%) as major components of LEO, followed by lower proportions of α-terpinyl acetate (9.65%) and sabinene (9.45%). Taban et al. (2018) informed that the major components of the LEO obtained from the different extraction methods were eucalyptol (1,8 cineole) (34.37 – 50.07%), α-terpinyl acetate (14.93 – 18.78%), terpineol (4.72 – 6.02%) and sabinene (4.95 – 5.93%). Olmedo et al. (2014) reported 1,8 cineole (42.1%) as mayor component in LEO and α-terpinyl acetate (9.3%), they also found linalool (11.9%) between the main components in this study. Agreeing with Taban et al. (2018), LEO and its fractions are rich in oxygenated monoterpenes (such as 1,8 cineole and α-terpinyl acetate).

The differences found between the LEO and its fractions were mainly due to the difference in the molecular weight. In the LR there was an increase in terpenes with an alcohol function, such as linalool, terpinen-4-ol, α-terpineol, eugenol methyl ether, eugenol and α-terpineol acetate. These components were present in a minor proportion in LD. There were increased concentrations of terpenes, such as α-pinene, β-pinene, eucalyptol and sabinene in LD. Differences in the compound proportions were observed in the fractions due to the molecular distillation process that increased the concentrations of compounds with low boiling points (e.g. monoterpenes) in LD. On the other hand, compounds with a high boiling point (such as molecules with functional groups and sesquiterpenes) were mainly found in LR (Olmedo et al., 2014).

Table 1. Terpenoid concentrations (relative percentage) analyzed by GC–MS analysis from laurel essential oils and its distilled and residue fractions according to their elution order

		Relative percentage (%)		
RI	**Compounds**	**Laurel**	**Distillate**	**Residue**
938	α-tujene	0.87 ± 0.06	n.d.	n.d.
939	α-pinene	5.37 ± 0.29c	3.48 ± 0.07 b	1.3 ± 0.13 a
953	Camphene	0.97 ± 0.19	n.d.	n.d.
964	Sabinene	7.07 ± 0.99 b	7.45 ± 0.17 b	2.71 ± 0.40 a
981	β-pinene	4.18 ± 0.36 b	3.74 ± 0.34 b	1.37 ± 0.22 a
991	β-myrcene	0.84 ± 0.09 a	1.05 ± 0.10 b	n.d.
1012	α-terpinene	0.98 ± 0.24 a	1.15 ± 0.26 b	n.d.
1033	1,8 cineole	37.5 ± 2.77 b	47.56 ± 4.33 c	19.92 ± 3.10 a
1040	β-ocimene	2.05 ± 0.21 a	3.00 ± 0.35 b	n.d.
1074	ϒ-terpinene	1.20 ± 0.16 a	1.78 ± 0.27 b	n.d.
1098	Linalool	8.71 ± 0.26 a	9.85 ± 0.18 b	13.15 ± 0.93 c
1177	Terpinen- 4- ol	3.03 ± 0.36 a	3.15 ± 0.57 a	4.53 ± 0.10 b
1189	α-terpineol	3.58 ± 0.22 a	3.15 ± 0.39 a	6.27 ± 0.38 b
1257	Linalool acetate	n.d.	n.d.	1.17 ± 0.09
1285	Bornyl acetate	0.77 ± 0.04 b	n.d.	1.42 ± 0.03 a
1334	α-terpineol acetate	12.37 ± 1.16 b	8.36 ± 0.17 a	23.2 ± 2.84 c
1351	Eugenol	0.96 ± 0.12 a	n.d.	2.44 ± 0.17 b
1375	β-elemene	1.03 ± 0.09 a	n.d.	1.97 ± 0.33 b
1401	Eugenol methyl ether	3.90 ± 0.28 a	n.d.	8.45 ± 1.08 b
1418	Caryophyllene	1.46 ± 0.45 a	1.30 ± 0.05 a	3.01 ± 0.84 b
1440	Humulen	0.82 ± 0.15 a	n.d.	1.87 ± 0.25 b
1454	α-caryophillene	0.82 ± 0.11 a	n.d.	1.81 ± 0.13 b

Values with different letters in the same row are significantly different (LSD- Fisher; α = 0.05; n = 3). Abbreviation: n.d. = not detected; RI = retention index.

Antioxidant activity

Free-radical scavenging activity on DPPH

Essential oils from laurel have shown free-radical scavenging properties (Sacchetti et al., 2005; Olmedo et al., 2014, 2015; Olmedo and Grosso, 2019).

The DPPH-IC50 values of LEO and its fractions are shown in Table 2. LD and LR presented greater DPPH radical scavenging activity than the LEO. LR exhibited the best antioxidant activity with the lowest value of DPPH-IC50.

The differences in antioxidant activity and/or free-radical scavenging properties in LEO from different studies could be associated with the extraction method and chemotypes (Chizzola et al., 2008). The phenolic chemotypes express a higher antioxidant capacity than the non-phenolic chemotypes (Goudjil et al., 2015). Kaurinovic et al. (2010) found an IC50 value of 161.83 µg/mL for LEO and Fernández et al. (2019) reported a DPPH-IC50 of 257 ± 12 µg/mL for this EO.

Trolox equivalent antioxidant capacity (TEAC assay)

The mg Trolox/g EO value is shown in Table 2. LR exhibited the greatest antioxidant activity with higher values inTEAC assay. El et al. (2014) reported TEAC values of 2.4 ± 0.09 LEO obtained for hydrodistillation.

Total phenolic content (TPC)

The TPC values are shown in Table 2. LR had almost double phenolic content than LEO and LD have about half the TPC than LEO. Olmedo et al. (2015) reported a lower TPC value for LEO (10.48 mg GAE/g), while Ouchikh et al. (2011) reported a higher value of TPC in leaf laurel acetone extract (20.94 ± 0.97 mg GAE/g).

The TPC is not explainable simply based on phenol molecules as several studies have shown that other reducing compounds can also reduce Folin reagents and thus may be incorrectly considered phenolic compounds in assays of this type (Lester et al., 2012; Nielsen, 2017). TPC has been reported to be directly associated with antioxidant activity (Quiroga et al., 2011, 2013). The phenolics from vegetables present molecules with reducing capacity that are probably responsible for the antioxidant capacity of this EO.

Accelerated oxidation test

The chemical indicators (PV and CD) increased during storage in all canola oil samples. These indicators are shown in Figure1. As a consequence of the accelerated oxidation condition, high peroxide values were detected in the canola oil samples. The control samples (CO-C) and CO-LEO

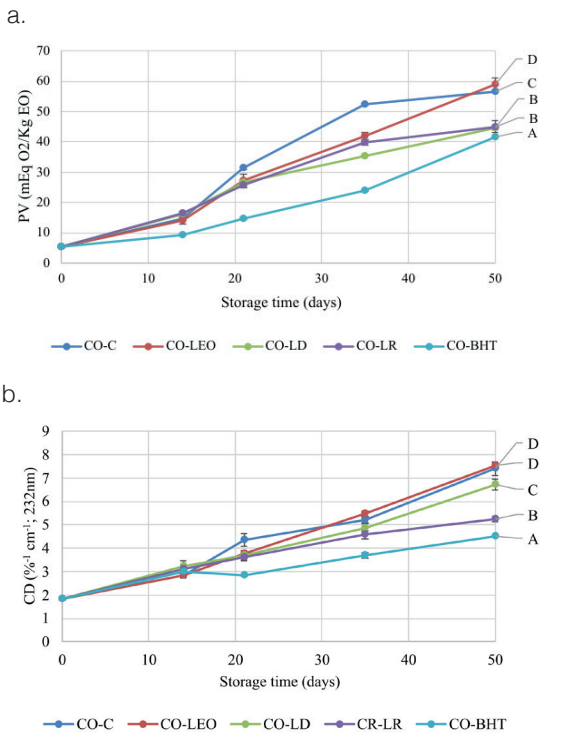

Figure 1. (a) Peroxide value (PV) and (b) conjugates dienes (CD) for canola oil control sample (CO-C) and canola oil samples added with laurel essential oil (CO-LEO) and distilled (CO-LD) and residue fractions (CO-LR) stored at 45 °C for 50 days. Different letters show statistically significant differences for the 50th day of storage (LSD- Fisher; α = 0.05; n = 3).

Table 2. DPPH-IC50, TEAC and TPC values for laurel essential oil (LEO) and distilled (LD) and residue fractions (LR)

	DPPH-IC50 (mg/mL)	TEAC (mg Trolox/g EO)	TPC
LEO	0.43 ± 0.01 c	2.61 ± 0.12 b	7.46 ± 0.25 b
LD	0.33 ± 0.02 b	1.28 ± 0.12 a	4.10 ± 0.10 a
LR	0.14 ± 0.01 a	4.46 ± 0.36 c	15.05 ± 0.82 c

Values with different letters in the same column are significantly different (LSD- Fisher; α = 0.05; n = 3).

exhibited the highest peroxide value with respect to samples with the addition of LEO fractions (LD and LR) after 50 days of storage (Figure 1a). At the end of storage, significant differences were observed between the samples. The CO-BHT showed the lowest values for PV, followed by LR and LD. The CD ($\%^{-1}$ cm^{-1}) values on storage day 50 were 7.40, 4.51, 7.55, 6.73, and 5.25 in CO-C, CO-BHT, CO-LEO, CO-LD, and CO-LR, respectively (Figure 1b). The CO-LD and CO-LR samples presented greater antioxidant activity than CO-LEO. In general, the CO-BHT sample exhibited lower CD values on storage day 14 than the samples with LEO or its fractions. Olmedo et al. (2015) showed that the volatile composition of the EO changed during the thermal stability study. The results of this study could be due to this and to the relationships among the different components in LEO and their fractions (Olmedo et al., 2015).

The antioxidant properties of EOs depend on several factors, such as the hydrophilic–lipophilic blend in a food product, the storage condition (temperature) and the chemical composition of the product. Before adding EO to a food product, it is essential to assay it to determine its real antioxidant capacity in that particular food.

Based on these results, it is observed that BHT, LEO and LEO fractions addition provides protection against the oxidation of canola oil. LR behaved better as antioxidant than LD, followed by LEO.

Antimicrobial activity of the essential oil and its fractions

Disk diffusion technique

All natural products presented activity against all microorganisms assayed. LR had in general the highest values of inhibition halo, showing the best antimicrobial activity. This sample was followed by LD fraction and, finally LEO, which showed the lowest activity for this technique (Table 3). Some authors have shown the ability of laurel to inhibit the growth of microorganisms using the disk diffusion assay (Djenane et al., 2012; Zazharskyi et al., 2019).

Determination of the minimum inhibitory concentration (MIC)

The MIC results are summarized in Table 4. LEO and these fractions showed activity not only against Gram-positive bacteria, but they also exhibited excellent activity against Gram-negative bacteria. Chmit et al. (2014) and Mello da Silveira et

Table 3. Inhibition halos for laurel essential oil (LEO) and distilled (LD) and residue fractions (LR)

	Inhibition halo (mm)		
Microorganism	**LEO**	**LD**	**LR**
Salmonella sp.	0.59 a	0.61 a	0.74 b
Escherichia coli O157 H7	0.90 a	0.88 b	0.69 ab
Pseudomona aeruginosa	NG	NG	NG
Micrococcus luteus	0.83 a	0.60 a	1.18 b
Baillus cereus	0.86 a	0.91 ab	1.05 b
Streptococcus aureus	0.75 a	0.78 a	1.01 b
Enterococcus faecalis	1.16 b	1.19 a	1.35 a

Values with different letters in the same row are significantly different (LSD- Fisher; $\alpha = 0.05$; n = 3). NG: did not grow.

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for laurel essential oil (LEO) and distilled (LD) and residue fractions (LR)

	MIC (mg/mL)			MBC (mg/mL)		
	LEO	**LD**	**LR**	**LEO**	**LD**	**LR**
Salmonella sp.	3.09 a	1.53 b	3.24 a	12.36 a	3.07 b	3.24 b
Escherichia coli O157 H7	12.36 a	3.07 b	3.24 b	12.36 a	3.07 b	3.24 b
Bacillus cereus	3.09 a	1.53 b	0.81 c	12.36 a	3.07 b	12.98 a
Micrococcus luteus	WM	12.27 a	3.24 b	WM	12.27 a	3.24 b
Streptococcus aureus	12.36 a	6.14 b	3.24 c	12.36 a	12.27 a	3.24 b
Enterococcus faecalis	12.36 a	6.14 b	1.62 c	12.36 a	12.27 a	1.62 b

Values with different letters in the same row are significantly different (LSD- Fisher; $\alpha = 0.05$; n = 3). WM = without MIC.

al. (2014) reported that LEO had inhibitory activity against these two types of bacteria.

LR presented the best inhibitory activity with the lowest MIC values against *B. cereus*, *M. luteus*, *S. aureus* and *E. faecalis* and LD showed the best antimicrobial activity against *Salmonella sp*. and *E. coli O157 H7*. As it was stated above, LEO presented antimicrobial activity against all tested strains, but it was the lowest one.

Different results were reported by Chmit et al. (2014), who found that LEO has good antimicrobial activity against *E. faecalis* and *P. aeruginosa* but not against *S. aureus* and *E. coli*. According to the antimicrobial classification by Holetz et al. (2002), the results of the current study show LEO as a weak antimicrobial, LD as a moderate/high activity antimicrobial, and LR as a high activity antimicrobial.

Minimum bactericidal concentration (MBC)

MBC results are summarized in Table 4. All natural products tested showed bactericidal activity against the assayed bacterial strains. LEO exhibited the lowest bactericide power, showing the higher MBC values for all strains tested. The LR fraction displayed the best antimicrobial activity against *M. luteus, S. aureus* and *E. faecalis*, and LD had the lowest MBC values for *Salmonella sp.*, *E. coli O157 H7* and *B. cereus*.

The antimicrobial or other biological activities of EOs have been thoroughly studied and there is a direct correlation between chemical composition and biological properties (Asensio et al., 2014, 2015, 2019). De Sousa et al. (2012) reported that 1,8-cinelole was effective in inhibiting Gram-positive and Gram-negative bacteria supporting the observations of other researchers about the efficacy of 1,8-cineole- in inhibiting the growth of gram-negative and gram-positive bacteria. Badr et al. (2021) found that α-terpinyl acetate and lavender EO containing α-terpinyl acetate in concentrations of 13.99 % had better antimicrobial activity against gram-positive than gram-negative bacteria; and α-terpinyl acetate had superior activity than lavender EO. Those results are in agreement with the results obtained for LR fraction, which has more quantity of α-terpinyl acetate than LEO and LD.

Sensory analyses

Acceptance and preference tests are subjective analysis concerning the degree of like or dislike of products by consumers. A consumer analysis was made in order to evaluate how the addition of LEO and its fractions on canola oil affects acceptability. For odor, flavor and overall acceptances, all canola oil samples showed means of consumer responses between 5 (neither like nor dislike) and 6.5 (like slightly) on a 9-point hedonic scale (Figure 2). Overall acceptance was high for LEO and all its fractions (hedonic scale point > 6).

The averages for flavor acceptance varied from 6.0 to 6.48 on a 9-point hedonic scale. Only between CO-LEO and CO-C there were significant statistical differences in flavor acceptances, which were higher in CO-LEO. Hence, consumers considered positive the addition of LEO. Samples added with natural products, did not exhibit significant differences in this attribute.

The means for odor acceptance varied from 5.50 to 6.22 on a 9-point hedonic scale and presented significant differences between samples. As in flavor attribute acceptance, odor acceptance of CO-LEO and CO-LR had no significant differences but, in this attribute, significant differences were found between CO-LEO and CO-LD samples. The differences in volatile composition of LEO and its fractions, when added to canola oil, resulted in variation in consumer odor acceptability. A volatile compound is seldom unique, and the common view is that characteristic odor will be represented by a blend of components, each having its own detection threshold and each present at different concentrations (Shahidi, 1998). The odor threshold is defined as the concentration where the odorant starts being perceived or as the lowest intensity at which a stimulus can be identified (Jele , 2012) and increases with increment of the volatility (Nagata, 2003; Bordiga and Nollet, 2019). In general, CO-LD had the lowest acceptance, which could be due to the fact that LD volatile composition has

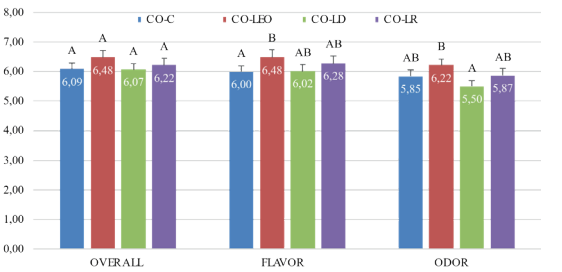

Figure 2. Odor, flavor and overall consumer's acceptance (9-point hedonic scale) for canola oil control sample (CO-C) and canola oil samples added with laurel essential oil (CO-LEO) and distilled (CO-LD) and residue fractions (CO-LR). Values with different letters are significantly different (LSD- Fisher; α = 0.05; n = 92).

more volatiles compounds than LEO and LR, thus, LD odor threshold could be lower than LEO and consumers perceived LD odor stronger than LEO or LR.

Food sample may be considered unacceptable to the consumer when the acceptability has a value minor to 5 on a 9-point hedonic scale. Acceptability values higher than 5 mean positive acceptance for a food product. Values higher than 5 (5 = neither like nor dislike) were observed in all consumer acceptance attributes and in all samples; therefore, all samples with or without natural products addition were positively accepted by consumers.

CONCLUSION

According to the results observed in the present research, the antimicrobial and antioxidant activities and sensory acceptance of LEO, LD and LR are directly correlated with chemical composition.

LR fraction behaved as better antioxidant than the LD and LEO, with LEO having the lowest antioxidant activity. All natural products showed antimicrobial, inhibitory, or bactericidal activity against Gram-positive and Gram-negative bacteria. The bacteriostatic capacity is of great importance in the food industry since it inhibits the development of food contaminating microorganisms.

Canola oil added with LEO, LD and LR are accepted by consumers with LEO being the most accepted. The addition of these natural products to canola oil help to slow the lipid oxidation process during storage. The use of natural additives instead of synthetic ones is convergent with the present trend in food technology; for this reason, LEO, LD and LR constitute natural sources of antioxidant additives useful in the food industry for preserving quality properties in products with high lipid contents.

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