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Abstract

Peels of citrus species from Argentina and United States were subjected to hydrodistillation to obtain their essential oils. Gas chromatography/Mass Spectrometry was carried to determine the chemical composition of all the essential oils. Limonene was found as the major compound with many minor components varying according to the different species. Antioxidant assays were conducted to determine the ability of essential oils as antioxidants. The antimicrobial activity was tested against Leuconostoc mesenteroides MS1, Escherichia coli and Lactobacillus plantarum ES147 and ATCC 8014. No marked trend about antioxidant profile of citrus essential oils. A broad variation in antimicrobial properties of the oils was observed. Grapefruit and lemon essential oils showed consistently strong antimicrobial activity against all tested bacteria, so they were selected for determining the minimum inhibitory concentration and minimum bactericidal concentration values against E. coli. Minimum Inhibition Concentration values ranged between 0.33 and 0.55 mg/mL and Minimum Bactericide Concentration values between 0.42 and 0.95 mg/mL.

Keywords	Essential oils, antioxidant activity, antimicrobial activity, United States, Argentina, GC-MS

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San Francisco, May 15th, 2020

Dear Editor-in-chief Joe Regenstein,

I am honored of submitting a revised version of the manuscript entitled "*Antioxidant and antimicrobial activity of citrus essential oils from Argentina and the United States*" in collaboration with my colleagues Raspo, Vignola and Andreatta. This manuscript describes original work and is not under consideration by any other journal.

> *The manuscript deals with Citrus species having an important contribution in the fruits and vegetables world market and are also important sources of industrial products such as essential oils. Essential oils are considered valuable industrial crops and have many non-foods uses such as cosmetics, fragrances and pharmaceutical preparations. In addition, essential oils can be used as bioactive compounds, since they are widely recognized for their use as antimicrobial agents and antioxidant capacity. Essential oils are obtained from the peels that were traditionally discarded. The identification of new uses and applications of this crop waste is an important strategy to find applications for industrial products. The aim of this work was to assess differences and similarities between the essential oils of different citrus species from Argentina and the U.S. but also compare different profiles such as their chemical compositions, antioxidant activities and activities as antimicrobial agents, in order to find new applications.*

We declare there are no conflicts of interest regarding the publication of this article.

We appreciate your time and look forward to your response.

Sincerely,

Rodolfo Juliani

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Response to the Co-Editor-in-Chief

We are thankful for the positive feedback and the valuable comments of the editor that contributed to the clarity and improvement of this work.

Please, see below our comments and answers to the editor observations (*in italics*).

Minimal changes were introduced to the manuscript in order to answer the reviewer request.

We appreciate your consideration for publication in **Food Bioscience**.

Comments:

In the manuscript:

All changes were done (yellow marked).

Highlights

Few chemical differences were observed between the U.S. and Argentinean citrus essential oils.

Potential selective antibacterial activity of citrus essential oils against pathogenic bacteria.

Mandarin oils showed the highest antioxidant capacity.

Citrus essential oils can provide useful bioactivities for different applications.

Full title: Antioxidant and antimicrobial activity of citrus essential oils from Argentina and the United States

Running title: Antioxidant and antimicrobial activity of citrus essential oils

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Abstract

Peels of citrus species from Argentina and the United States were subjected to hydrodistillation to obtain their essential oils. Gas chromatography/mass spectrometry was carried out to determine the chemical composition of all the essential oils. Limonene was found as the major compound with many minor components varying according to the different species. Antioxidant assays were done to determine the antioxidant activities of essential oils. The antimicrobial activity was tested against *Leuconostoc mesenteroides* MS1, *Escherichia coli* and *Lactobacillus plantarum* ES147 and ATCC 8014. Mandarin essential oil from the USA showed the strongest antioxidant capacity in different assays. Grapefruit and lemon essential oils showed consistently strong antimicrobial activity against all tested bacteria, so they were selected for determining the minimum inhibitory concentration and minimum bactericidal concentration values against *E. coli.* Minimum inhibition concentration values ranged between 0.33 and 0.55 mg/mL and minimum bactericide concentration values ranged between 0.42 and 0.95 mg/mL.

Keywords

Essential oils, lemon, grapefruit, orange, citrus, United States, Argentina

1. Introduction

With an average production of 10 million tonnes and 3 billion dollars between 2007 and 2017, citrus species are important global commodities. Argentina and the United States have large citrus plantations in their territories due to fertile soils and an appropriate climate.

The main products, such as orange, lemons and graperfruit, with further processing yield juices and food products that come from the pulp of the fruit (endocarp). The peel, and specifically the flavedo, are important sources of industrial products such as essential oils (EO) and other products (Iglesias et al., 2007). EO are a valuable natural products as they have many non-food uses including cosmetics, fragrances and pharmaceutical preparations (Finch et al., 2014). The discarded parts such as peels are still discarded and represent a potential source of natural additives, which are often preferred by consumers.

Similar research has been done on citrus EO (Jing et al., 2014; Viuda-Martos et al., 2008). However, most research has focused on antimicrobial activity without studying the composition of the EO, nor has work comparing citrus species from different geographic areas been done.

CEO have a volatile fraction usually >90%. Monoterpenes and sesquiterpenes are found mainly in the volatile volatile fraction, with limonene being the major compound. The USA Food and Drug Administration considered limonene as a GRAS (Generally Recognized as Safe) material. Aissou et al. (2017) have used limonene from agro-industrial waste streams as a primary chemical to obtain different oxidized and high added-value compounds, such as α-terpinolene, 3-methyl-cyclopentanone and cis-Linalool oxide. Several authors have used limonene as a polymer precursor using catalytic reactions (Gutiérrez et al., 2014). Linalool and *β*-pinene are other important compounds present in CEO, which have shown antidepressant and sedative activities when used in alternative medicines (Guzmán-Gutiérrez et al., 2012). Haselton et al. (2015) have shown that α-pinene had repellent properties against the house fly (*Musca domestica*) with laboratory conditions. Myrcene and linalool, have been shown to have anesthetic properties (Taheri Mirghaed et al., 2016).

Although the constituents of CEO are mostly monoterpenes, CEO has poor antioxidant capacity (Ghoorchibeigi et al., 2016). However CEO has biological activity against a range of bacterial species. For example, Randazzo et al. (2016) have shown that oxygenated monoterpenes of CEO inhibit *Listeria monocytogenes*. Other species studied included *Salmonella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Adukwu et al., 2012; Luciardi et al., 2016).

CEO show different bioactivities depending on their composition, species and origin (Celiktas et al., 2007). The present study looked at different species of citrus essential oils (grapefruit, lemon, mandarin and orange) from different origins (Argentina and the USA), in terms of their chemical composition, antioxidant capacity and antimicrobial activity.

2. Materials and methods

2.1. Plant material and CEO extraction

Grapefruit (*Citrus paridisi*), lemon (*Citrus lemon*), mandarin or tangerines (*Citrus reticulata*) and orange (*Citrus sinensis*) fruits were purchased during summer in local markets of San Francisco, Córdoba, Argentina and New Brunswick, NJ, USA. The sources of the fresh fruits were the litoral region of Argentina (AR) and California (USA).

The EO were extracted from the peels of fruits after manual peeling. To improve the extraction of EO, the citrus peels were ground (Allaf et al., 2013) with a food processor at the maximum setting for 60 sec (Oster, Boca Raton, FL, USA) . EO were extracted by hydrodistillation using a Clevenger-type apparatus (IVA S.A., Buenos Aires, Argentina) for two hr. The EO were stored at 4 °C for a maximum of 12 wk.

For comparison purposes, grapefruit, lemon, mandarin and orange commercial (CM) 100% pure EO obtained from citrus planted in the state of California (USA) were purchased (Plant Essential Oils, Los Angeles, CA, USA).

2.2. Gas chromatography with mass spectrometry

The chemical profile of each CEO was analyzed using a Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to a mass spectrometry detector (MSD) (Agilent Technologies) and a flame ionization detector (GC/MS-FID). Two capillary columns were used for each detector (HP-5 column, 30 m long, 0.25 mm internal diameter, and 0.25 mm coating thickness, Agilent Technologies). Helium was the carrier gas with a flow rate of 0.9 mL/min. Ionization was done by electron impact at 70 eV. Mass spectral data were acquired in the scan mode in the m/z range 35–450. The oven temperature was programmed at 60-200 °C (4 °C/min). For the MSD run, the injector and detector were maintained at 200 and 280 °C, respectively. The FID was at 220 °C. To calculate retention indeces (RI) injection of n-alkanes (8–20 carbon) (Sigma-Aldrich Co., St. Louis, MO, USA) was done in both columns connected to the MSD and FID. Then, the Kovats retention indices of the compounds were calculated:

$$RI = 100 \times [n + (N - n)(\log t_{unk} - \log t_n)/(\log t_N - \log t_n)$$

where n represents the number of carbon atoms in the smaller n-alkane, N is the number of carbon atoms in the larger n-alkane (N=n+1), and t represents the retention time of the related compounds between n and N. The oil components were identified by comparison of their RI and mass spectra with those from literature (Adams, 2007) and libraries (www.wiley.com).

2.3. Antioxidant capacity

Many antioxidant assays are based on the single electron transfer reaction that determines a change of color when the antioxidant is reduced. Assays based on the consumption of stable free radicals (ABTS and DPPH) and assays based in the capacity of antioxidants to reduce ions (FRAP and CUPRAC), were carried out to evaluate the antioxidant capacity of each CEO.

2.3.1. ABTS assay

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay was done using the method of Re et al. (1999) with slight modifications. Briefly, 1.3 mL of ABTS reagent (Sigma-Aldrich) was diluted in 100 mL of absolute ethanol (Sigma-Aldrich). Then, 10 µL of the EO were

mixed with 990 µL of the diluted reagent. The absorbance was measured at 734 nm using an HP 8453 model UV-Visible Spectrophotometer (Agilent Technologies). The ABTS antioxidant capacity of CEO was quantified as Trolox (TR) (Sigma-Aldrich) equivalent antioxidant capacity (TEAC) and expressed as mg of Trolox E/mL CEO.

2.3.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay measures the ability of antioxidants to reduce iron in acidic medium. The assay was carried out using the method of Benzie and Strain (1996). EO (10 µL) and 990 µL of FRAP reagent (ferric chloride and TPTZ (2,4,6-Tris-(2-pyridyl)-s-triazine), ratio 1:1) (Sigma-Aldrich) in acetate buffer (0.3 M, pH 3.6) were mixed and the FRAP values obtained at 593 using a calibration curve of ascorbic acid (AA) to get mM AA E/mL CEO.

2.3.3. DPPH assay

DPPH assay was determined using the method of Siripatrawan and Harte (2010), where the purple chromogen radical 2,2-diphenyl-1-picryhydrazyl (DPPH) (Sigma-Aldrich) is reduced by an antioxidant to the pale yellow hydrazine. Briefly, 60 µL of CEO were mixed with 240 µL of DPPH solution and incubated for 30 min. The scavenging capacity was measured at 517 nm. The DPPH antioxidant capacity of CEO was also expressed as TEAC.

2.3.4. Cupric Reducing Antioxidant Capacity (CUPRAC) assay

The CUPRAC was determined using the method of Apak et al. (2004), with slight modifications. Briefly, 70 µL of copper (II) chloride solution (0.01 M), 70 µL of neocuproine (0.0075 M), 70 µL of ammonium acetate buffer (1M) (Sigma-Aldrich) and sample dilutions to reach a final volume of 300 µL, were mixed. The test tubes were stoppered and incubated at room temperature (20 to 25° C) for 1 h. A change of color was obtained from pale blue to orange. The absorbance at 450 nm was measured against a reagent blank and the results were also quantified as TEAC.

2.4. Antimicrobial activity

2.4.1 Detection of antimicrobial activity of CEO

All CEO were initially screened to measure their antibacterial activity using a disk diffusion method (Clinical and Laboratory Standards Institute, 2013). This screening was carried out using a potential pathogenic bacterium, *Escherichia coli* ATCC 8739 (Gram-negative); a foodborne bacteria, *Leuconostoc mesenteroides* MS1 (Gram-positive); and two strains of beneficial bacteria *Lactobacillus plantarum* ES147 and ATCC 8014 (Gram-positive). *E. coli* ATCC 8739 and *L. plantarum* atcc 8014 belong to the culture collection of CEPROCOR (Centro de Excelencia en Procesos y Productos de Córdoba, Córdoba, Argentina), *L. plantarum* ES147 belongs to the culture collection of ICYTAC (Instituto de Ciencia y Tecnología de Alimentos Córdoba, CD, AR) and was isolated from raw cereal (Salvucci et al., 2016), and *L. mesenteroides* MS1 belongs to the culture collection of the laboratory in AR and was isolated from industrial sausages (Serra et al., 2018). *E. coli*, *L. mesenteroides* MS1 and *L. plantarum* ES147 and ATCC 8014 were grown on tryptic soy broth (Laboratorios Britania S.A., Buenos Aires, AR) for 24 h at 37 °C, de Man, Rogosa and Sharpe (MRS) broth (Laboratorios Britania) for 48 h at 30 °C, and MRS broth for 24 h at 37 °C, respectively. Then, plates were inoculated with the respective bacterial inoculum. The inoculation was prepared using the direct colony suspension method in a physiological saline solution to obtain, through a previously prepared calibration curve, a 0.5 density using the McFarland scale, which is equivalent to ~1.5 x 10^8 CFU/mL (McFarland, 1907). Ten µL of each CEO solution were placed on a 5 mm diameter sterile paper disc (125 mm, Munktell, Helsinki, Finland), which was transferred to the inoculated agar plate. Tests were done in triplicate. The agar plates were incubated at 37 °C for 24 h for *E. coli* and *L. plantarum* ES147 and ATCC 8014 and at 30 °C for 48 h for *L. mesenteroides*. Inhibition zone diameters were measured including paper disk (5 mm) with a digital caliper (accuracy: ±0.01 mm) (Model 500-196-30B, Mitutoyo Co., Mitutoyo, Japan). The positive controls were implemented with the commercial antibiotic gatifloxacin (0.5% w/w, Laboratorios Poen, Bermudez, Buenos Aires, AR) and its dilutions, which showed antibacterial action against a range of aerobic Gram-positive and Gram-negative bacteria; sodium hypochlorite (2.5% w/w, Clorox S.A., Aldo Bonzi, Buenos Aires, AR); and ethyl alcohol (96% w/w, Porta

160 Hermanos, Córdoba, AR). For the negative controls, sunflower oil (100%, AGD SA, General
161 Deheza, CD, AR) and granulated soy lecithin (70% w/w, Modelife, CD, AR) were used.
162 *2.4.2. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal*
163 *Concentration (MBC)*
164 MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of
165 a microorganism after overnight incubation. MBC is defined as the lowest concentration of an
166 antimicrobial that will prevent the growth of an organism after subculture on an antibiotic-free
167 media (Andrews, 2002). The MIC and MBC of the most active EO were determined using a serial
168 broth dilution method in tryptic soy broth for *E. coli*. A stock solution of each CEO containing 1
169 mL of grapefruit and lemon EO + 5 mL of soy lecithin aqueous solution (2 wt%) was prepared to
170 facilitate solubilization. The initial maximum concentration of each CEO was 0.125 g/mL
171 (grapefruit EO USA), 0.14 g/mL (lemon EO USA), and 0.12 g/mL (lemon EO AR) and were finally
172 diluted to a minimum concentration of 0.2, 0.3 and 0.3 mg/mL, respectively. Each tube was
173 inoculated with a loop of bacterial suspension, prepared as described before, to achieve a final
174 concentration of ~1.5 x 10^8 CFU/mL. Tubes were incubated at 37 °C for 24 h for *E. coli* along with
175 a control tube without CEO. Survival or not was determined by plating an aliquot from each tube
176 onto tryptic soy agar plates.
177 *2.5. Statistical analysis*
178 All statistical analysis was carried out using InfoStat Software (2016, CD, AR). Cluster analysis
179 was done based on Euclidean distances, using the average linkage method with a maximum cluster
180 number arbitrarily set to two. The cluster was made using the chemical composition of CEO as
181 variables. One-way analysis of variance (ANOVA, α=0.05) and the DGC test (Di Rienzo et al.,
182 2002) was done to determine significant differences between means in antioxidant assays. The
183 variability between the different CEO and the results of antioxidants assays and the antimicrobial
184 analysis was measured with a multivariate analysis of the principal components (PC)
185

3. Results and Discussions

3.1. Sensory and chemical profile

A total of 31 compounds were found for 4 types of CEO (grapefruit, lemon, mandarin, and orange) from AR, the USA and commercial (Table 1). The number of compounds found for each plant EO varied between 5 for grapefruit and 25 for lemon. Limonene and myrcene were the only two compounds found in all species from Argentina and the USA; whereas, there was 14 compounds that were only found in lemon EO (Table 1).

The CEO extracted were all transparent. These CEO were characterized by their persistent and penetrating aroma.

Grapefruit EO showed a similar chemical profile between the different origins, with myrcene being the second major compound behind limonene.

The limonene content in lemon was usually lower than in the other citrus fruits. The monoterpenes γ-terpinene and β-pinene were observed in higher amounts. Lemon EO was high in other types of monoterpenes such as alcohols, aldehydes, ester monoterpenes, and sesquiterpenes. In the case of CEO CM, all these compounds were found in lower amounts.

The GC-MS of mandarin EO showed a large difference between AR and the other EO because the content of limonene was significantly lower than the mandarin EO from the USA and CM. The content of γ-terpinene was higher in the AR EO.

The orange EO was high in limonene, with myrcene and linalool as minor components.

These observations are consistent with previous results (Adukwu et al., 2012; Bustamante et al., 2016; Luciardi et al., 2016; Perdones et al., 2016). A cluster analysis (Figure 1) showed that there were two well-defined clusters that separated the three types of lemon and the mandarin EO AR from the rest of the CEO. This is because the limonene content in that group was significantly lower than the rest (Table 1).

3.2. Antioxidant capacity

ABTS, FRAP, DPPH and CUPRAC assays were carried out to determine the antioxidant capacity of CEO (Figure 2).

ABTS assay. Mandarin samples showed the highest antioxidant capacity, more specifically the CM type. On the other hand, grapefruit AR showed the lowest activity. Only orange showed similar values between the three origins.

FRAP assay. Grapefruit and lemon EO showed a similar antioxidant capacity. The highest value of this assay was for mandarin EO, AR and CM types. Their chemical profiles showed higher values of hydrocarbon monoterpenes, meanwhile, the USA EO had alcohol monoterpenes and hydrocarbon sesquiterpenes (Table 1).

DPPH assay. TEAC values showed different antioxidant trends compared to ABTS, leading to the conclusion that each assay has a different mechanism of action. Lemon EO showed the three highest values, regardless of origin. On the other hand, the lowest values were from grapefruit and orange EO (Table 1).

CUPRAC assay. Some similarities could be observed between the TEAC values from this assay if compare with TEAC values of the DPPH assay. Although the values were lower in the DPPH assay, the lemon EO again showed the best antioxidant capacity and grapefruit EO AR and USA showed the lowest values (Table 1).

Given the complexity of these mixtures and the different principles of these tests, it is not unexpected that relative activities with these antioxidant tests will vary. López-Alarcón and Denicola (2013) explained that this may be because each assay is affected by several factors. For example, a compound may have a good reducing power of iron (FRAP), but not against copper (CUPRAC). In addition, the role of minor components or the synergy between these may be the cause of the increase in the antioxidant potential of a mixture compound.

3.3. Antimicrobial activity

The inhibition zone (IZ) and MIC and MBC values were determined. A significant variation in the antimicrobial properties of the EO was observed. The diameter of the IZ .is shown in Figure 3.

237 Grapefruit and lemon EO showed consistently strong antimicrobial activity against all tested
238 bacteria. Both grapefruit and lemon EO were more effective at inhibiting *E. coli* than other bacteria.
239 Mandarin EO showed consistently moderate activity against all tested bacteria although the highest
240 antimicrobial activity was also observed with *E. coli*. Similar results were found by Guo et al.
241 (2018) who studied different CEO from China and found an *E. coli* antimicrobial resistance against
242 lemon EO and mandarin EO consistent with the results. The action of CEO against pathogenic
243 bacteria had been already reported by Cuca et al. (2009), for EO from the peel of Bingtang sweet
244 orange (*Citrus sinensis Osbeck*), which was high in limonene and was effective in the inhibition of
245 *E. coli* ATCC 25922. Likewise Fisher and Phillips (2008) reported a strong antibacterial activity of
246 CEO from sweet orange (*Citrus sinensis*), bergamot (*Citrus bergamia*), and lemon (*Citrus limon*),
247 which contained limonene (45–95%) against *E. coli* O157, *S. aureus*, and *B. cereus*. Orange EO
248 was weak (*L. plantarum* ES147, *L. plantarum ATCC* 8014, *E. coli*) or failed to inhibit the growth of
249 *L. mesenteroides* MS1 (Table 2). These results were consistent with Fernández-López et al. (2005)
250 who also found orange EO ineffective against *L. mesenteroides*. Ambrosio et al. (2017) observed
251 similar results with orange EO against *L. plantarum.*
252 Limonene was also found as a major compound in all CEO (Table 1) but a high variation was
253 observed in the amount of this compound in the oils (98.2% for grapefruit EO CM and 60.0% for
254 lemon EO USA). The antibacterial activity of these CEO and the content of limonene were not
255 correlated, suggesting that the antibacterial activity of both EO was due to the presence of minor
256 compounds and not limonene. Similar results were observed by several authors: Serra et al. (2018)
257 studied *L. mesenteroides* MS1 inhibition against CEO and concluded that limonene did not shown a
258 bactericidal effect; Fisher and Phillips (2006) showed that limonene, had no antibacterial activity,
259 while linalool had high antibacterial activity against *S. aureus*, *B. cereus*, *E. coli* O157 and *C.*
260 *jejuni*.
261 EO mechanistically should be more effective against Gram-positive bacteria due to the direct
262 interaction of the cell membrane with hydrophobic components of the EO and the presence of

lipoproteins and lipopolysaccharides that form a barrier to restrict entry of hydrophobic compounds (Sokovicx́ et al., 2010). On the other hand, Gram-negative bacteria should be more resistant to plant EO because they have a hydrophilic cell wall (Kim et al., 2011). However, a higher inhibition diameter on Gram-negative bacteria (*E. coli*) than Gram-positive bacteria (*L. plantarum* ES147, *L. plantarum* ATCC 8014, *L. mesenteroides* MS1) for grapefruit and lemon EO was obtained while orange EO did not inhibit both types of bacteria equally and mandarin EO inhibited both types of bacteria moderately. This may be explained by the existence of different cellular targets on bacteria that specifically bind with different compounds in the EO by various modes, which enabled some Gram-negative bacteria to be more sensitive than Gram-positive bacteria (Klein et al., 2013). These results are consistent with Deans and Ritchie (1987) who concluded that Gram-positive and Gram-negative bacteria were equally sensitive to CEO and their components from lemon, mandarin, and orange.

CEO from different origins showed varying degrees of antibacterial activity against all strains (Table 2). Grapefruit EO from the USA showed statistically higher activity with all bacteria than the AR EO and CM EO. No inhibition was observed against any bacteria for grapefruit CM EO. Lemon EO from the USA showed significantly higher activity than the AR EO or CM EO only against *L. mesenteroides* MS1. No inhibition was found against *L. plantarum* AATC 8014 and *E. coli* for lemon EO CM. Mandarin EO from different origins acted differently on each bacterium; mandarin EO AR showed higher inhibition zones with *L. plantarum* ES147 than mandarin EO USA. Mandarin EO USA statistically showed the highest inhibition zone against *E. coli* ATCC 2592, while no differences between origins were observed with *L. plantarum* ATCC 8014. No inhibition with CM and grapefruit EO origin was observed for any bacteria. Different geographic locations where plants were grown, harvest time, genotype, and weather conditions during growth and harvest (Celiktas et al., 2007; Oussalah et al., 2007) can account for these differences, and therefore, the composition and the activity of EO obtained from plants growing in different locations should be characterized.

289 The CEO, which showed the best antimicrobial activity in the paper disk diffusion assay (grapefruit
290 EO USA and lemon EO AR and USA), were selected to determined the MIC and MBC against the
291 *E. coli*. Among them, EO USA showed lower MIC and MBC than EO AR. Lemon EO MIC and
292 MBC results were 0.55 and 0.95 mg/mL for AR type and 0.33 and 0.42 mg/mL for USA type,
293 respectively. On the other hand, grapefruit EO USA type showed 0.35 and 0.48 mg/mL for MIC
294 and MBC respectively. The strong antibacterial activity of grapefruit EO which gave the highest
295 inhibition diameters (20 to 24 mm) was confirmed by the lowest MIC and MBC values observed
296 against *E coli*.
297 *3.4. Principal Component Analysis*
298 To assess the variability between the different CEO and the results of antioxidants assays and the
299 antimicrobial analysis, a multivariate analysis was used on the principal components (PC) (Figure
300 4). Results represent the biplot of different CEO studied with points, using the antioxidants assays
301 (ABTS, FRAP, DPPH and CUPRAC) and the antimicrobial activities (against *L. plantarum* ATCC
302 8014, *L. plantarum* ES147*, E. coli* and *L. mesenteroides* MS1) as variables. Two reduced
303 dimensions were used, representing 71.8% of the samples. The PC1 and PC2 accounted for 45.3
304 and 26.5% of the variability, respectively. The PC1 included the antimicrobial analysis (*L.*
305 *plantarum* ATCC 8014, *L. plantarum* ES 147*, E. coli* and *L. mesenteroides* MS1) and the
306 antioxidant assays (ABTS and FRAP) because they were the variables with greatest projection on
307 the positive and negative PC1 semi-axis, respectively. The weights of the antimicrobial analysis had
308 a strong positive relationship between them, suggesting a similar contribution for each CEO. FRAP
309 and ABTS assays were located at the negative PC1 semi-axis indicating an opposite correlation
310 with the antimicrobial variables. This could be associated with a positive value of PC1 as indicating
311 a lower value of limonene, and this could indicate that limonene is responsible for the antioxidant
312 capacity of CEO. From the data dispersion, grapefruit EO USA, lemon EO AR and USA types
313 located on the positive PC1 semi-axis are similar between them, but different from the CEO located
314 on the PC1 negative semi-axis. A strong association between the antimicrobial activity and these

315 CEO was found, consistent with the high IZ (Table 2). The variability of PC2 was represented by
316 the DPPH and CUPRAC assays. These assays showed a weak correlation with the other
317 antioxidants assays and had no association with any other variable.
318
319 **4. Conclusions**
320 Minimum differences were found between the chemical profile of AR and USA CEO and were not
321 significant. Lemon EO showed strong antioxidant capacity in terms of DPPH and CUPRAC assays,
322 which might be used as a potential natural preservative to prevent product oxidation. The present
323 study of 4 different CEO from 3 different origins showed a potential selective antibacterial activity
324 of grapefruit and lemon EO against pathogenic bacteria (*E. coli*) and beneficial bacteria (*L.*
325 *plantarum* ATCC 8014 and *L. plantarum* ES147), with a diminished antibacterial activity on
326 beneficial bacteria which can be positive since bacteria such as *L. plantarum* have significant
327 biological roles in the human gastrointestinal tract. Diversified behavior was observed between the
328 4 CEO of the 3 different origins. EO from citrus species from AR and USA can provide additional
329 bioactivities that might be used by the cosmetic fragrance, nutraceutical and pharmaceutical
330 industries.
331
332 **Conflict of interest**
333 The authors confirm that they have no conflicts of interest with respect to the work described in this
334 manuscript.
335
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456 *paradisi*) and orange (*Citrus sinensis*) essential oils. *Journal of Food Safety*, 28(2008), 567–
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458 **Table 1.** Relative composition of grapefruit, lemon, mandarin and orange essential oils from Argentina (AR), United States (USA) and commercial
459 (CM) using GC-MS (HP-5 column) [a]
460

RI	Compounds	Grapefruit			Lemon			Mandarin			Orange		
		AR	USA	CM	AR	USA	CM	AR	USA	CM	AR	USA	CM
924	α-thujene						0.4	0.6 ± 0.1	0.1 ± 0.1				
939	α-pinene				0.9 ± 0.6	1.5 ± 0.0	2.1	1.7 ± 0.2	0.7 ± 0.2				0.5
974	sabinene		0.2 ± 0.3			1.3 ± 0.0	1.1	0.2 ± 0.0	0.1 ± 0	0.3	0.6 ± 0.1		
979	β-pinene				5.3 ± 1.1	11 ± 2	11.0	1.3 ± 0.0	0.3 ± 0				1.3
994	myrcene	0.7 ± 1.0	1.1 ± 0.2	0.9	1.0 ± 0.2	0.7 ± 0.2	0.9	1.2 ± 0.0	1.4 ± 0.0		1.3 ± 0.1	1.3 ± 0	1.1
1001	δ-carene	0.3 ± 0.2								1.1			
1015	α-terpinene				0.3 ± 0.5	0.3 ± 0.1		0.3 ± 0.0	0.1 ± 0				
1024	p-cymene				1.6 ± 1.4	0.4 ± 0.1	0.9	1.8 ± 0.3	0.2 ± 0.3	2.4			
1025	β-phellandrene	0.3 ± 0.2	0.1 ± 0.1		0.9 ± 0.3							0.1 ± 0.1	
1029	1,8-cineole				0.8 ± 0	0.3 ± 0					0.5 ± 0		
1038	limonene	98 ± 2	95 ± 1	98.2	72 ± 4	60 ± 9	69.7	72 ± 2	94 ± 4	90.3	96.1 ± 0.4	91.5 ± 0	97.3
1044	β-ocimene		0.2 ± 0.2	0.4	0.1 ± 0							0.3 ± 0	
1068	γ-terpinene				8.8 ± 0.4	10.5 ± 0.5	10.1	19 ± 2	1.9 ± 2.4	3.9	0.2 ± 0		
1088	isoterpinolene				0.3 ± 0.2								
1086	α-terpinolene				0.2 ± 0.2	0.4 ± 0.1		0.8 ± 0.0	0.2 ± 0	0.1			
1095	linalool		0.1 ± 0						0.4 ± 0.2		0.6 ± 0	0.3 ± 0	0.1
1174	terpinen-4-ol				0.3 ± 0.2				0.1 ± 0				
1186	α-terpineol				0.4 ± 0.3								
1227	nerol				0.1 ± 0	0.3 ± 0.2							
1235	neral				1.8 ± 0	2.5 ± 1.7							
1249	geraniol				0.1 ± 0	0.3 ± 0.2							
1254	linalyl acetate				0.1 ± 0	0.4 ± 0.3							
1264	geranial				2.0 ± 0.9	3.2 ± 2.1	1.4						
1359	neryl acetate				0.9 ± 0.8	0.3 ± 0.3							
1379	geranyl acetate				0.3 ± 0.3	0.6 ± 0.0	0.4						
1410	*trans*-caryophyllene	0.2 ± 0.2			0.7 ± 0								
1503	α-farnesene				0.7 ± 0.6	0.5 ± 0			0.1 ± 0				
1505	β-bisabolene				1.1 ± 1.0	0.9 ± 0							

461

462 [a] expressed as the mean of two samples ± SD, except for CM origin which corresponds to one sample.

463 **Table 2.** Inhibition zone (mm) showing antibacterial activity of the measured essential oils against
464 beneficial bacterium (*L. plantarum* ATCC 8014, *L. plantarum* ES 147), food-borne bacteria (*L.*
465 *mesenteroides* MS1) and pathogenic bacterium (*E. coli*) [a, b]

Citrus essential oil	Origin	Inhibition zone /mm*			
		L. plantarum ES 147	***L. plantarum ATCC 8014***	***L mesenteroides MS1***	***E. coli***
Grapefruit	Argentina	5.0 ± 0.1 a	7.7 ± 1 b	ND	5.8 ± 1 a
	USA	8 ± 1 b	10 ± 1 c	7.0 ± 0.9 b	21 ± 2 b
	Commercial	ND	ND	ND	ND
	Average	6.1	7.6	5.7	10.7
Lemon	Argentina	10 ± 3 b	7 ± 2 a	7.0 ± 2 a	15 ± 3 b
	USA	8.0 ± 0.6 b	9 ± 2 a	10 ± 2 b	16 ± 1 b
	Commercial	ND	6 ± 0 a	ND	8 ± 0 a
	Average	7.7	7.3	7.5	13.0
Mandarin	Argentina	7.7 ± 0.5 c	6.7 ± 0.8 b	ND	6.5 ± 0.5 a
	USA	6.3 ± 0.5 b	6 ± 1 b	ND	9 ± 4 b
	Commercial	ND	ND	ND	ND
	Average	6.3	6.0	ND	6.7
Orange	Argentina	7.2 ± 0.9 b	ND	ND	5.5 ±0.6 a
	USA	7.0 ± 0.9 b	ND	ND	6.0 ±0.5 a
	Commercial	ND	ND	ND	6 ±0 a
	Average	6.4	5.0	5.0	5.8

466

467 * Inhibition area including 5 mm disc diameter, expressed as the mean of three replicates ± SD. ND
468 no inhibition.
469 a Means followed by the same letter in the same column for each essential oil are not significantly
470 different ($p<0.05$).
471 b Average value expressed as the mean of three replicates on each origin (12 replicates).

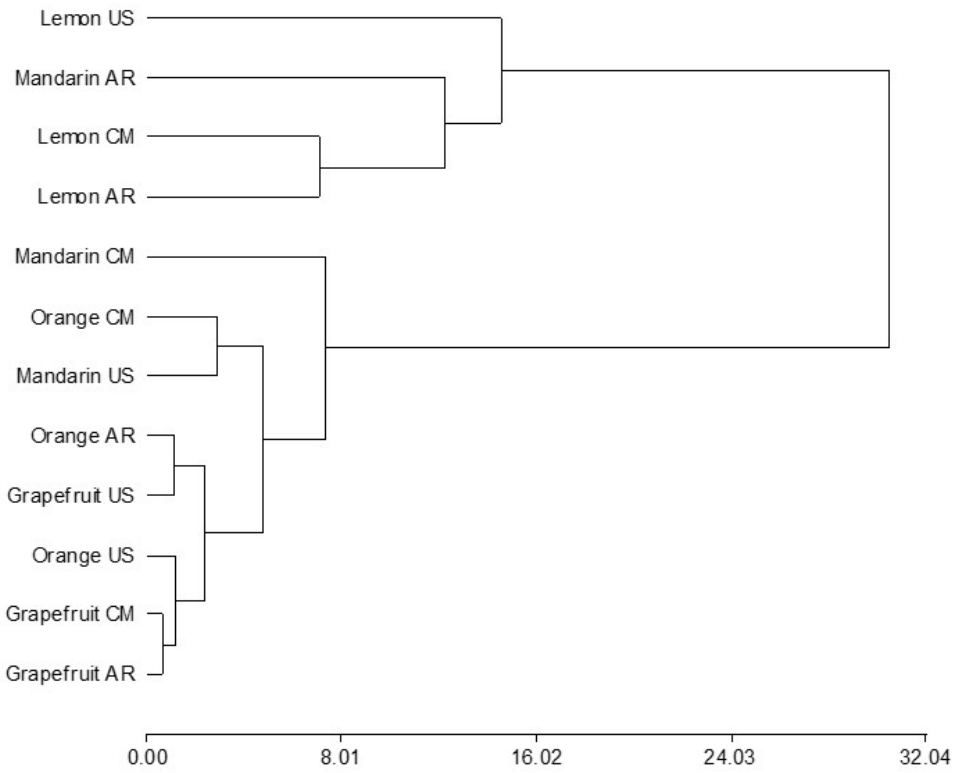
Figures Legends

Figure 1. Essential oils clustering of different citrus species and origins (AR: Argentina, USA: United States, CM: Commercial) obtained by Euclidean distance and average linkage method.

Figure 2. Antioxidant capacity of citrus essential oil of Argentina (white), United States (light grey), and commercial (dark grey) determined using ABTS (a), FRAP (b), DPPH (c) and CUPRAC (d) assays. Bars are the positive standard deviation (SD) obtained from two different samples at each CEO and origin. * For a given origin of CEO, averages with the same letter do not have significant differences (α=0.05, DGC)

Figure 3. Representative picture of broad inhibitory zones of essential oils against bacteria. (a) Grapefruit EO USA against *Escherichia coli.* (b) Lemon EO USA against *Leuconostoc mesenteroides* MS1. (c) Grapefruit EO AR against *Lactobacillus plantarum* ATCC 8014.

Figure 4. Principal Component Analysis (PCA) biplot obtained from the first (PC1) and second (PC2) principal components. Euclidean distance variables: ABTS, FRAP, DPPH and CUPRAC as antioxidant's assays, and L147 (*Lactobacillus plantarum* ES147), L8014 (*L. plantarum* ATCC 8014), LEUCO (*Leuconostoc mesenteroides* MS1) and ECOLI (*Escherichia coli*) as antimicrobial assays. Treatments: 4 essential oils (grapefruit, lemon, mandarin and orange) from three different origins: AR (Argentina), USA (United States) and CM (commercial).



493

494 **Figure 1**

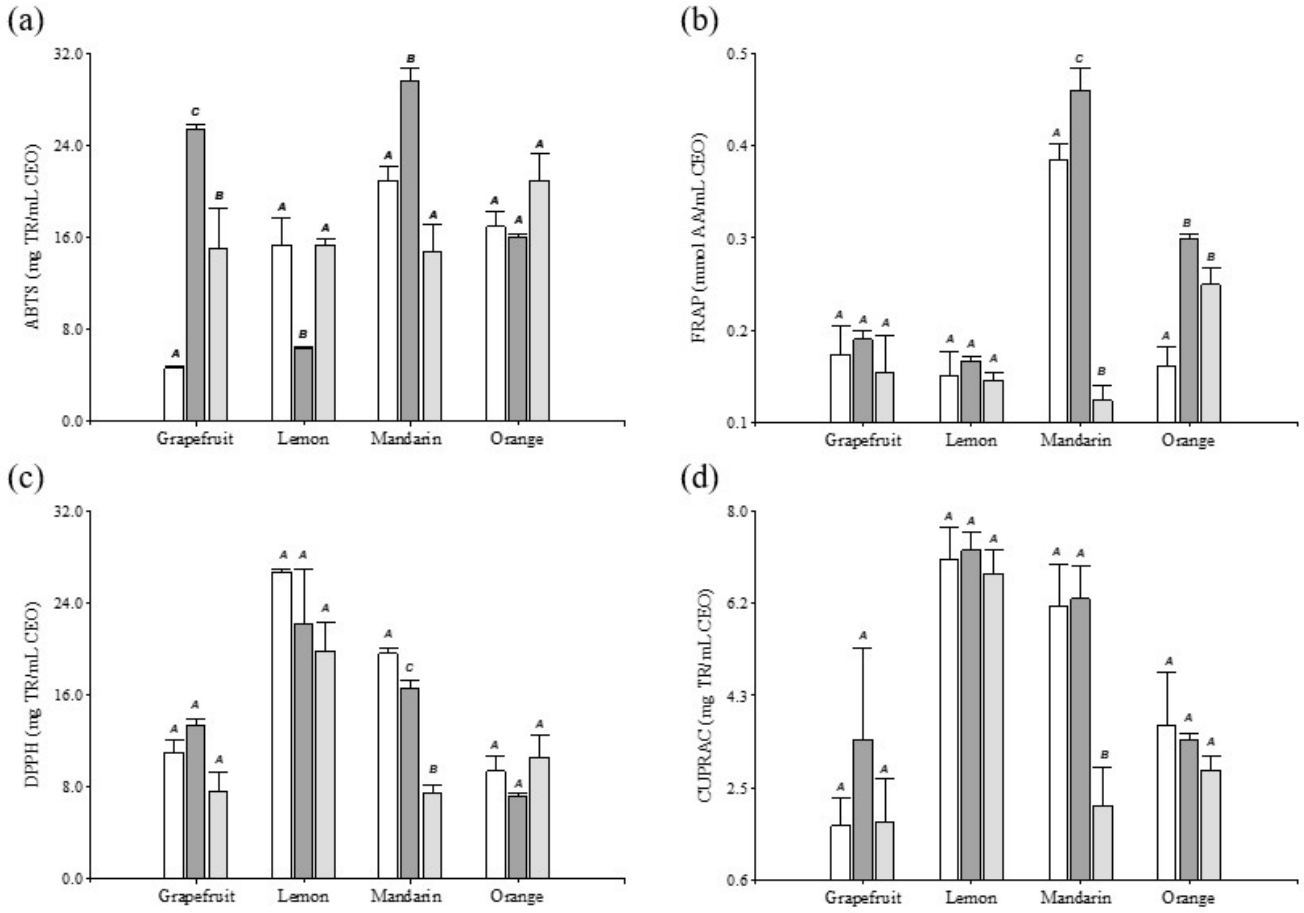
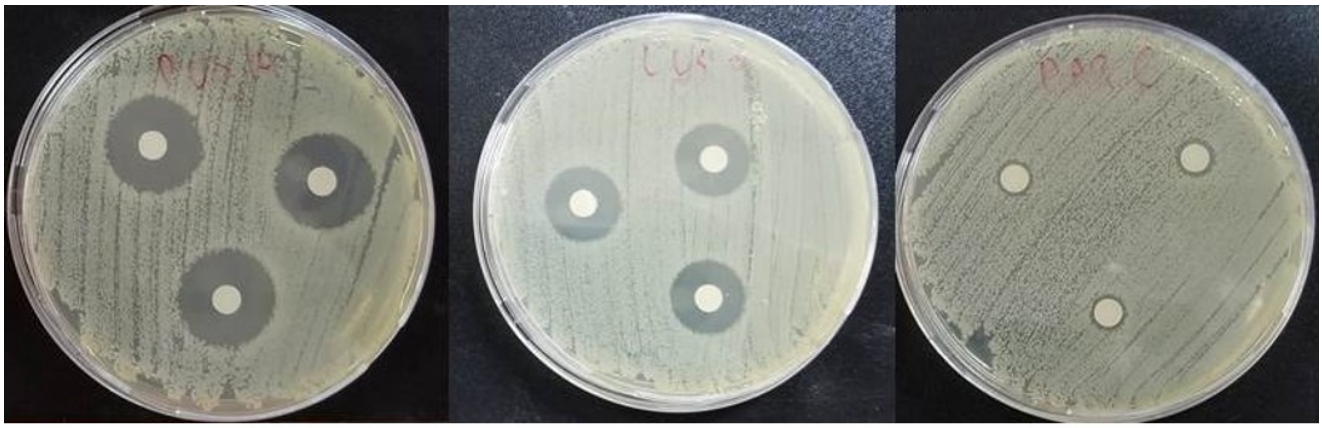
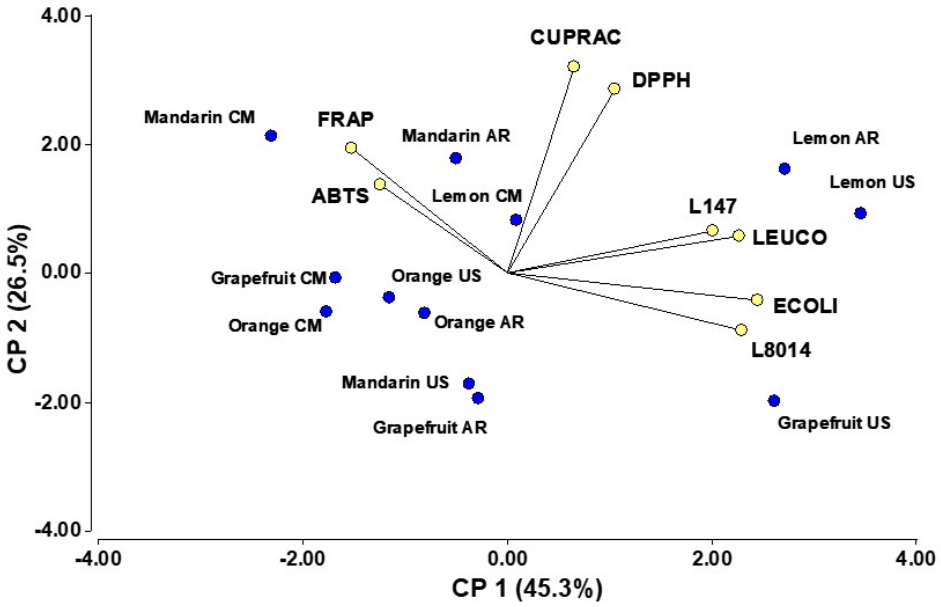

(a)
ABTS (mg TR/mL CEO)
32.0
24.0
16.0
8.0
0.0
Grapefruit
Lemon
Mandarin
Orange
(b)
FRAP (mmol AA/mL CEO)
0.5
0.4
0.3
0.2
0.1
(c)
DPPH (mg TR/mL CEO)
(d)
CUPRAC (mg TR/mL CEO)
8.0
6.2
4.3
2.5
0.6

Figure 2



a) b) c)

Figure 3



499

500 **Figure 4**

The authors: M.A Raspo, M. B Vignola, A.E Andreatta and H.R Juliani declare there are no conflicts of interest regarding the publication of this article. All the authors have read and approved the guide for authors and are aware of its submission to Food Bioscience.

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