Food Chemistry 242 (2018) 9–15

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant activity of rosemary essential oil fractions obtained by molecular distillation and their effect on oxidative stability of sunflower oil

Gabriela N. Mezza[a], Ana V. Borgarello[a], Nelson R. Grosso[b], Héctor Fernandez[c], María C. Pramparo[a], María F. Gayol[a,*]

[a] Facultad de Ingeniería, Universidad Nacional de Río Cuarto, Río Cuarto, Argentina
[b] Química Biológica, Facultad de Ciencias Agropecuarias (UNC), IMBIV-CONICET, CC 509, 5000 Córdoba, Argentina
[c] Facultad de Ciencias Exactas, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina

ARTICLE INFO

Keywords:
Antioxidant activity
Molecular distillation
Oxidative stability
Rosemary essential oil
cis-Sabinene hydrate
α-Terpineol

ABSTRACT

The objective of this study was to evaluate the antioxidant activity of rosemary essential oil fractions obtained by molecular distillation (MD) and investigate their effect on the oxidative stability of sunflower oil. MD fractions were prepared in a series of low-pressure stages where rosemary essential oil was the first feed. Subsequently, a distillate (D1) and residue (R1) were obtained and the residue fraction from the previous stage used as the feed for the next. The residue fractions had the largest capacity to capture free radicals, and the lowest peroxide values, conjugated dienes and conjugated trienes. The antioxidant activity of the fractions was due to oxygenated monoterpenes, specifically α-terpineol and *cis*-sabinene hydrate. Oxidative stability results showed the residues (R1 and R4) and butylated hydroxytoluene had greater antioxidant activity than either the distillate fractions or original rosemary essential oil. The residue fractions obtained by short path MD of rosemary essential oil could be used as a natural antioxidants by the food industry.

1. Introduction

Conventional sunflower oil (SO) is the most frequently consumed oil in Argentina. However, SO is susceptible to oxidation because it contains large amounts of unsaturated fatty acids, particularly polyunsaturated fatty acids, such as linoleic acid (18:2 ω-6). Lipid oxidation produces rancid odors, unpleasant flavors, and discoloration. It also decreases the nutritional quality and safety of foods due to secondary oxidation products that have harmful effects on human health (Lercker & Rodriguez-Estrada, 2002).

Natural and synthetic antioxidants are added to edible oils to delay oxidative deterioration, thereby maintaining the quality and prolonging the shelf-life of food product. The use of natural compounds as additives is in increasing demand. Rosemary (*Rosmarinus officinalis* L.) essential oil (REO) is considered a natural antioxidant, but the antioxidant compounds present have not yet been established fully.

REO composition is affected by various factors, such as weather, soil humidity, extraction method, distance between plants, harvest time, and drying method. Therefore, researchers have sought to obtain information about the yield, composition, and chemical properties of REO following a variety of extraction methods, harvesting times, and plant parts (Bousbia et al., 2009; Elamrani, Zrira, & Benjilali, 2000; Peter, 2004; Socaci, Tofana, Socaciu, Varban, & Muste, 2007; Szumny, Figiel, Gutiérrez-Ortíz, & Carbonell-Barrachina, 2010). REO chemical composition differed significantly but the major chemical constituents are α-pinene, 1,8-cineole, camphor, myrcene, and camphene (Elanrami et al., 2000; Flamini, Cioni, Morelli, Macchia, & Ceccarini, 2002; Romero Márquez, 2004; Varela et al., 2009).

Several studies have reported the antioxidant activity of REO (Beretta, Artali, Maffei Facino, & Gelmini, 2011; Bozin, Mimica-Dukic, Samojlik, & Jovin, 2007; Kadri et al., 2011; Ojeda-Sana, Van Baren, Elechosa, & Juárez, 2013; Sacchetti et al., 2005). Some authors reported that the antioxidant activity was related to the presence of compounds, such as verbenone and borneol (Sacchetti et al., 2005), but others indicate that constituents, like oxygenated monoterpenes and sesquiterpene hydrocarbons (Bozin et al., 2007), alcoholic ethers and phenolic compounds (Beretta et al., 2011), 1,8-cineol, α-pinene, β-pinene, α-thujene, trans-caryophyllene, β-thujone, borneol, and camphor (Kadri et al., 2011), or myrcene (Ojeda Sana et al., 2013), were responsible for these characteristics.

Molecular distillation (MD) is a separation technique often used to purify thermolabile substances and low volatility compounds (Pramparo, Prizzon, & Martinello, 2005; Fregolente et al., 2007; Pramparo, Leone, & Martinello, 2008; Shao, Jiang, & Ying, 2007).

* Corresponding author at: Facultad de Ingeniería, Universidad Nacional de Río Cuarto, Road 36, km 601, CP: X5804BYA, Río Cuarto, Córdoba, Argentina.
E-mail address: mgayol@ing.unrc.edu.ar (M.F. Gayol).

http://dx.doi.org/10.1016/j.foodchem.2017.09.042
Received 22 September 2015; Received in revised form 21 August 2017; Accepted 8 September 2017
Available online 09 September 2017

However, few studies have used MD to separate essential oil fractions.

In a previous study, MD was used to concentrate methyl chavicol from basil essential oil and the conditions were optimized by response surface methodology. The results showed that it is possible to increase the concentration of methyl chavicol from 83.81 to 89.79% (Martins et al., 2012). Borgarello, Mezza, Soltermann, and Pramparo (2014) separated fractions from oregano essential oil by MD with greater antioxidant activity. Free radical scavenging capacity (RSC) was increased in residue fractions with higher concentrations of thymol and carvacrol, terpinen-4-ol and γ-terpinene (Olmedo, Nepote, & Grosso, 2014). Other research has reported the antioxidant properties of aguaribay raw oil and its fractions prepared by vacuum distillation. Residues with the most terpinen-4-ol and germacrene D had the greatest antioxidant activity (Guala, Elder, Perez, & Chiesa, 2009).

REO has separated and concentrated previously using a three-stage MD process to produce residues with the greatest antioxidant activity possible (Mezza, Borgarello, Daguero, & Pramparo, 2013). However, it was not demonstrated clearly which compounds were responsible for these antioxidant properties. The current study focused on preparation of REO fractions by short path MD using high-pressure separation, and evaluated antioxidant activity in SO to identify the compounds responsible for the protective effect in this food product.

2. Materials and methods

2.1. Materials

REO was donated by Platario SA (Buenos Aires, Argentina). The essential oil was derived from *Rosmarinus officinalis* L. that had been harvested in August 2011. The REO was produced in Barreal, San Juan, Argentina, located at 31° 40′ S latitude and 69° 29′ W longitude, at 1650 m above sea level. The essential oil was obtained by hydrodistillation by Platario SA. The essential oil was dried over anhydrous sodium sulfate, preserved in sealed flasks, and stored at 4–6 °C until analysis.

Refined SO (Natura brand, from Aceitera General, Córdoba, Argentina) was used for the storage assays to evaluate the antioxidant properties of the molecularly distilled fractions, based on deterioration using lipid oxidation indicators.

2.2. Methods

2.2.1. MD process description

The MD was performed in four stages using a DCC4 falling film distiller (Ingeniería Bernoulli SA, Buenos Aires, Argentina) equipped with a 0.04 m^2 evaporation surface, a condensing surface of 0.02 m^2, and variable speed rotating rollers. In the first stage, REO was used and two fractions, a distillate (D1) and residue (R1), were obtained. In subsequent steps (2–4), residue fractions obtained from the previous stage was used as the feed.

MD operating conditions are presented in Table 1. For all stages, the condenser temperature was set at −2.1 °C; the feed was kept at room temperature; the evaporation temperature was maintained at 26 ± 1 °C; the feed flow was around 1.10 ± 0.05 mL/min; and the rotor speed was kept constant at 200 rpm. The operation pressure was reduced by 50% for each successive stage.

2.2.2. Chemical composition of REO and MD fractions

The chemical composition of REO and MD fractions was determined using a gas chromatography-mass spectrometry equipped with a flame ionization detector and a Carbowax capillary column (60 m × 0.25 mm, 0.25 μm). The oven temperature was held at 60 °C for 5 min and then increased 5 °C/min up to 240 °C. The carrier gas flow (He) was 1 mL/min. The injector and detector temperatures were 250 and 350 °C, respectively. The samples were diluted in *n*-hexane (1/100 μL) and 1 μL was injected. Identification of the compounds was performed by comparing the peak mass spectrum with the mass spectrum of pure standards. Relative concentrations were calculated according to peak area normalization using TurboMass 5.4.2 software.

2.2.3. Antioxidant activity – RSC

The method of Mezza et al. (2013) was used to evaluate the antioxidant activity of REO and MD fractions. Sample-hexane solutions (2 mL) prepared at 0.1 and 50 mg/mL were added to 2 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in hexane. After 120 min, the absorbance was measured at 517 nm. The blank was hexane and the control solution was prepared with 2 mL DPPH solution and 2 mL hexane. The RSC percentage was calculated as: ((AC–AS)/AC)*100, where AS is the absorbance of the sample solution containing antioxidant and AC is the absorbance of control solution.

IC_{50} was defined as the amount of sample (μL/mL) that produced a 50% decrease in the initial DPPH concentration. Lower IC_{50} values indicate higher free RSC.

2.2.4. Oxidative stability of SO

Samples of refined SO were supplemented with REO and MD fractions that exhibited a high free RSC. Sunflower oil without any additive (SO) was used as a control sample. SO supplemented with butylated hydroxytoluene (BHT) was prepared to compare its antioxidant activity against the natural antioxidants (REO and various fractions). REO and various MD fractions were added at 0.1 g/100 g while 0.02 g/100 g BHT was added, based on the maximum amount allowed in edible oils according to the Argentine Food Code (2012). Samples were placed in test tubes and stored uncovered in a dark place at 23 °C (room temperature). Samples of each product were removed from storage for chemical analyses at 0, 5, 15, 26, 46, 65, and 115 days.

The sample identifications were assigned as follows: SOREO, SO supplemented with REO; SOR1, SO supplemented with the stage R1 fraction; SOR4, SO supplemented with the stage R4 fraction; SOD4: SO supplemented with the stage D4 fraction; and SOBHT, SO enriched with BHT.

Peroxide value (PV) and conjugated dienes and trienes (CD and CT, respectively) were used as indicators to evaluate the oxidation of the stored samples. PV was analyzed according to the AOAC method and expressed as active oxygen milliequivalents (meqO_2/kg) (AOAC, 1980). CD and CT were measured in a UV–vis spectrophotometer (Biotraza

Table 1
Pressure for operating condition and distillates and residues percentages obtained by molecular distillation.

Stage N°	F[A]	Pressure (kPa)	g D/100 g F	g R/100 g F	g D/100 g REO	g R/100 g REO
1	REO	6.00	19.67 ± 0.30	80.33 ± 0.30	19.67 ± 0.30	80.33 ± 0.30
2	R1	3.00	20.24 ± 1.06	79.76 ± 1.06	16.27 ± 0.91	64.07 ± 0.62
3	R2	1.50	16.26 ± 0.42	83.74 ± 0.42	10.42 ± 0.37	53.65 ± 0.24
4	*R3*	0.75	14.89 ± 3.31	85.12 ± 3.32	7.99 ± 1.81	45.66 ± 1.57
Σ D/REO					54.35	

[A] Abbreviations. D = distillate, F = feeding, R = residue, REO = rosemary essential oil, R1 n = residue fraction of stage 1, R2 = residue fraction of stage 2, R3 = residue fraction of stage 3.