Gabriela Natalia Mezza*, Ana V. Borgarello, Jorge D. Daguero, and Maria C. Pramparo

Obtention of Rosemary Essential Oil Concentrates by Molecular Distillation and Free Radical Scavenging Capacity Analysis

Abstract: Rosemary essential oil is used in pharmaceutical, cosmetic and food industries. Due to its constituents' chemical activity, it is also used as antioxidant to preserve foods and as antibacterial and antifungal agents.

The most abundant components of rosemary essential oil used in this work are α-pinene, myrcene, 1,8-cineole and camphor, which respond to 1,8-cineole chemotype. Two sets of molecular distillation experience were conducted. Antioxidant power of distillates and residues obtained was quantified, and the residues obtained from molecular distillation have more antioxidant power than distillates and rosemary essential oil.

The results of this study show that it is feasible to use molecular distillation operation to obtain concentrates of rosemary essential oil. Residues collected present higher antioxidant power than rosemary essential oil, probably due to the presence of camphor, linalool, linalyl acetate and α-terpineol, which are present in higher proportion in the residues.

Keywords: antioxidants, rosemary essential oil, molecular distillation, DPPH*

***Corresponding author: Gabriela Natalia Mezza**, State University of Río Cuarto, Río Cuarto, Córdoba, Argentina,
E-mail: gmezza@ing.unrc.edu.ar
Ana V. Borgarello: E-mail: aborgarello@ing.unrc.edu.ar, **Jorge D. Daguero**: E-mail: jdaghero@ing.unrc.edu.ar, **Maria C. Pramparo**: E-mail: mpramparo@ing.unrc.edu.ar, State University of Río Cuarto, Río Cuarto, Córdoba, Argentina

Introduction

Rosemary belonging to the Labiatae family is a woody and evergreen aromatic shrub. It has an intense and pleasant smell reminiscent of pine wood as noted by Szumny *et al.* [1]. It is an herb that has several uses both as fresh or dried leaf and as oil or oleoresin.

Since ancient times, aromatic herbs and spices have been added to different types of food to improve flavor and organoleptic properties. Herbs are also used as nutraceuticals, due to the different activities of their secondary biomolecules that confer antimicrobial, spasmolytic, carminative, hepatoprotective, antiviral and anticarcinogenic properties as Bozin *et al.* [2] claim.

It is possible to obtain essential oil from these herbs by hydrodistillation or extraction by steam distillation, because they contain volatile components feasible to be removed by this technology.

Rosemary essential oil is used in pharmaceutical and cosmetic industries as a perfume additive in lotions and fragrances and in food industry as seasoning. Moreover, due to the chemical activity of its constituents, it is also used as an antioxidant to preserve foods and as antibacterial and antifungal agents as Socani *et al.* [3] claim. Rosemary essential oil is one of the most commonly used essential oils due to its antibacterial and/or antifungal activity. It can kill or inhibit the development of pathogens (fungi, bacteria or viruses) that may be involved in pathological processes that affect the skin and mucous membranes as noted by López Luengo [4].

Another important use of rosemary essential oil is in the medical–pharmaceutical field; the aroma-therapy as an alternative to conventional treatments, both in curing diseases and in promoting health. There are cosmetics to tone the body that include in its composition orange, rosemary or lemon essential oils. Rosemary essential oil is used in anti-wrinkle creams and anti-cellulite gels and prevents the formation of blackheads and pimples. Torelló *et al.* [5] claim that it is also use to stimulate hair growth.

There are different species of rosemary, *Rosmarinus officinalis*, *Rosmarinus erriocaly*, *Rosmarinus laxiflorus* and

Rosmarinus lavandulaceus. The specie *R. officinalis* is the only one that grows naturally in the Mediterranean region. Chemotaxonomy studies suggest the classification of rosemary essential oils in chemotypes, whose name corresponds to the name of the major component. Of experimental measurements performed on multiple samples, three major chemotypes were found: α-pinene, camphor and 1,8-cineole as Varela *et al.* [6] claim.

Not all components of an essential oil have similar structures and/or features. Among the components present in rosemary essential oil, there are monoterpenes hydrocarbons and oxygenated monoterpenes. Monoterpene hydrocarbons are less valuable than oxygenated compounds in terms of their contribution to the fragrance of essential oils. Bousbia *et al.* [7] noted that oxygenated compounds are highly odoriferous and hence are more valuable as raw material in the cosmetic industry.

To obtain products concentrated in these compounds of important nutraceutical interest, different techniques can be used (fractional distillation under vacuum, hydrodistillation, solvent extraction, etc.). Given the thermosensitive characteristics of the compounds identified in essential oil, a viable technology to be used is molecular distillation, however, has not yet been sufficiently studied to separate and concentrate fractions with a high content of the active ingredients mentioned.

Concentrates from rosemary essential oil can be obtained by molecular distillation. Molecular distillation is based on the evaporation of the components of a mixture and its immediate condensation on a surface nearby. The operation is carried out at very high vacuum in an equipment built, so that the distance traveled by molecules between the surfaces of evaporation and condensation is shorter than the mean free path of evaporated molecules as Pramparo *et al.* [8] claim. Under these conditions of high vacuum, the volatility of the components increases and the operating temperature decreases, allowing the separation of the components at low temperature. Martinello *et al.* [9] and Pramparo *et al.* [10] claim that because of this, thermal decomposition of the components is reduced and separation occurs at acceptable velocities

Some components present in rosemary essential oil have antioxidant power. The antioxidant activity is defined as the ability of a compound to inhibit oxidative degradation. One of the methods used to determine the antioxidant activity is the analysis of 2.2-diphenyl-1-picryl-hydrazyl (DPPH*). This analysis is based on the ability of free radical DPPH* to react with H donors. The DPPH* radical shows a great intensity to absorb in the visible region, so it can be determined by UV–vis spectroscopy.

The aim of this work was to analyze the antioxidant power of rosemary essential oil concentrates obtained by molecular distillation, and to identify and quantify their major components. The chemical composition of the different concentrates was related to their antioxidant power.

Materials and methods

Materials

All solvents used in the experiments were HPLC grade and were purchased from Cicarelli. The radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH*) was purchased from Sigma–Aldrich.

Rosemary (Rosmarinus officinalis) essential oil from plantations in the province of San Juan was donated by Platario S.A.

The standard compounds were acquired by Sigma–Aldrich and Fluka.

Molecular distillation

Molecular distillation was performed in UIC KDL4 equipment (falling film, evaporation surface: 4 dm^2 and condensing surface: 2 dm^2). The distiller is equipped with variable speed rotating rollers. Figure 1 shows a scheme of the distillation equipment.

In the first set of experiments, operating conditions were varied in order to obtain different yields, so that the relation $\%D/F$, where D is mass of distillate and F is mass of essential oil fed, varies from 0 to 100%, this relation will be defined as the yield of the distillation stage. Operation pressure was varied from 10.5 to 1.3 kPa, evaporation temperature was set at 27°C and feeding flow was varied between 0.014 and 0.019 ml s^{-1}. The feed was kept at room temperature, the condenser temperature was set at 2°C and rotor speed was constant at 200 rpm. Different fractions of distillate and residue were obtained. These fractions were analyzed by gas chromatography, and the percentage yields (η) and concentration index (CI) of the main components were determined, according to the following equations:

$$\eta_i^{j,k} = \frac{x_i^{j,k}\, w^{j,k}}{x_i^F\, w^F} 100 \qquad [1]$$

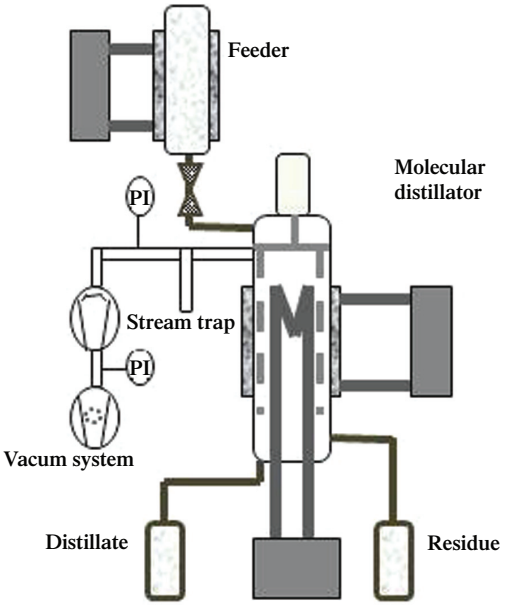

Figure 1 Scheme of molecular distillation equipment.

$$CI_i^{j,k} = \frac{x_i^{j,k} - x_i^{F,k}}{x_i^{F,k}} 100 \quad [2]$$

where
η: percentage yields;
CI: concentration index;
w^j: mass in g;
x: mass composition;
i: main components;
j: distillate (D), residue (R);
k: number of experience;
F: feed.

After verifying that the molecular distillation is a feasible operation for separating and concentrating the compounds present in rosemary essential oil, a second set of experiences was performed under the methodology of distillation in series. Three stages of distillation were performed. In the first stage, rosemary essential oil was fed, and two fractions were obtained: distillate and residue. In subsequent steps, feeding flow consisted in the residue from the previous stage. This method of operation is schematically represented in Figure 2. Stages 1 and 2 were performed under the same operating conditions; in stage 3, operation pressure was modified. The operation pressure of stages 1 and 2 was 7.8 kPa, and in stage 3, the pressure was set at 6.5 kPa. In all stages, the evaporation temperature was maintained at 12°C, and feeding flow was around 0.02 ml s^{-1}. The feed was kept at room temperature, the condenser temperature was set at 2°C and rotor

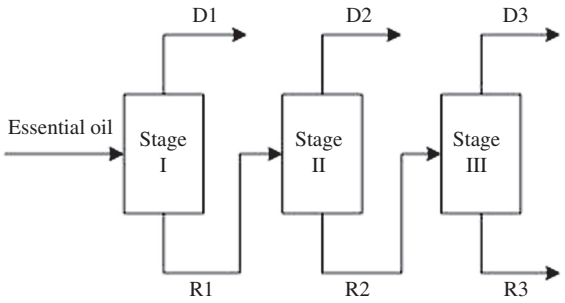

Figure 2 Scheme representing the molecular distillation operating in series.

speed was constant at 200 rpm. The composition of all the obtained fractions was analyzed by gas chromatography, and their antioxidant power was determined.

Gas chromatography

Gas chromatography was performed using a Hewlett Packard HP 6890 chromatograph equipped with a flame ionization detector and a HP INNOWax column. The oven temperature program was 2°C min^{-1} from 60 to 250°C and 10 min isothermal at 250°C. The carrier gas flow (N_2) was 0.01 ml s^{-1}. The injector temperature was 250°C, and detector temperature was 350°C. The sample was diluted with dichloromethane, and the injected volume was 1 µl. The identification of the components present in the samples was performed by comparing the retention times of sample peaks with the retention time of standards. The relative concentrations were calculated according to peak area given by a HP3398A integrator.

Free radical scavenging capacity

To evaluate the antioxidant activity of rosemary essential oil and the fractions obtained by molecular distillation, a method was selected based on the capacity of free radical scavenging. The DPPH* radical was used to determine the free radical scavenging capacity of the samples, because in the presence of an antioxidant it is reduced to DPPH-H form, decreasing its spectrophotometric absorbance. The technique used was based on the method used by Brand-Williams *et al.* [11] with modifications. Different hexane solutions of rosemary essential oil and distillates and residues obtained from the molecular distillation were prepared. Two milliliters of these solutions of different concentrations (0.1–50 mg ml^{-1}) were added to 2 ml of a solution of DPPH* in hexane (~0.9 AU at 515 nm). The

reaction time was determined following the reaction of rosemary essential oil and DPPH* in hexane. The decrease in absorbance values during the reaction was measured every 15 s until constant values were reached, determining this as the "plateau" of the reaction. A plotting of the absorbance of the reaction against time was constructed. The concentration of the reaction sample was 12.9 mg ml^{-1}. The time needed to reach the "plateau" was ~7,200 s. The absorbance was measured at room temperature with a Metrolab 330 spectrophotometer. The blank consisted of hexane, and control solution consisted of 2 ml of DPPH* solution and 2 ml of hexane.

The free radical scavenging capacity percentage (%RSC) was calculated according to:

$$\%\text{RSC} : ((\text{ACAC} - A_S)/A_C) \times 100$$

where A_S is the absorbance of the sample solution containing antioxidant and A_C is the absorbance of control solution.

To determine the free radical scavenging capacity, EC_{50} value was defined as the amount of sample, in mg/ml, that produces a decrease of the initial concentration of DPPH* to 50%. A high EC_{50} value indicates a weak free radical scavenging capacity, while a low EC_{50} value indicates a high free radical scavenging capacity.

Results and discussion

Identification and quantification of the main components of rosemary essential oil

Chromatographic determinations made raw materials; it was found that the most abundant components of essential oil are α-pinene, myrcene, 1,8-cineole and camphor. Table 1 shows the main compounds identified in rosemary essential oil with their concentrations calculated according to peak area given by the HP3398A integrator. Figure 3 shows the chromatogram of rosemary essential oil.

According to the composition of rosemary essential oil, it is determined that corresponds to 1,8-cineole chemotype.

Oxygenated compounds contribute to the fragrance of essential oils. In essential oil studied, the components responsible for aroma, according to their chemical structure, are: 1,8-cineole, camphor, linalool, linalyl acetate and α-terpineol. Among these compounds, 1,8-cineole and camphor are the most abundant. Therefore, the characteristic fragrance of this essential oil is attributed to these compounds.

Table 1 Main volatile compounds present in the rosemary essential oil.

Designation	Compound	Chemical structure	Concentration (g per 100 g)
A	α-Pinene	Monoterpene hydrocarbons	14.33
B	β-Pinene	Monoterpene hydrocarbons	1.44
C	Myrcene	Monoterpene hydrocarbons	6.43
D	Limonene	Monoterpene hydrocarbons	2.89
E	**1,8-Cineole**	Cyclic ether – monoterpenoid	*25.26*
F	γ-Terpinene	Monoterpene hydrocarbons	3.83
G	Camphor	Terpenoid	18.22
H	Linalool	Alcohol acyclic monoterpenoid	2.43
I	Linalyl acetate	Acyclic ester	2.54
J	α-Terpineol	Alcohol monocyclic monoterpenoid	2.44

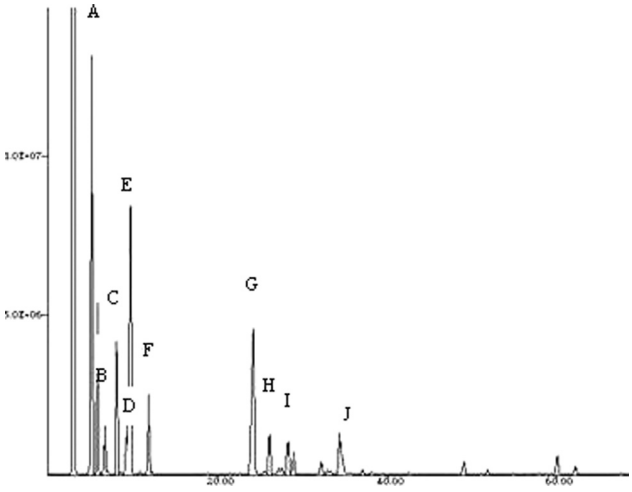

Figure 3 Chromatogram of rosemary essential oil.

Analysis of the results of the concentration by molecular distillation

Table 2 shows conditions (pressure, temperature and feed flow) of the first set of experiments of molecular distillation and the results $\%D/F$ (yields of the distillations stages) and $\%R/F$.

Table 2 Results of the molecular distillation tests (rotor speed: 200 rpm, $T_{evaporation}$: 27°C and $T_{condenser}$: 2°C).

No. assays	Pressure (kPa)	Feed flow (ml s^{-1})	%D/F^a	%R/F^b
I	10.5	0.015	22.30	77.70
II	3.9	0.019	44.90	55.10
III	7.9	0.014	54.20	45.80
IV	1.3	0.018	80.22	19.78

Notes: [a]$\%\frac{D}{F} = \%\frac{\text{g distillate}}{\text{g feed}}$ of the respective stage and [b]$\%\frac{D}{F} = \%\frac{\text{g residue}}{\text{g feed}}$ of the respective stage.

The values of percentage yield (η) and concentration index (CI) of the main components in distillates and residues are shown in Table 3.

Table 3 Percentage yield (η) and concentration index (CI) of the main components.

		DI	RI	DII	RII	DIII	RIII	DIV	RIV
α-Pinene	η_A	41.46	58.57	68.79	31.21	79.49	20.51	86.08	13.92
	CI_A	0.86	−0.25	0.53	−0.43	0.47	−0.55	0.07	−0.30
Myrcene	η_C	29.40	70.61	56.99	43.01	65.21	34.79	93.42	6.58
	CI_C	0.32	−0.09	0.27	−0.22	0.20	−0.24	0.16	−0.67
1,8-Cineole	η_E	11.68	88.30	49.22	50.78	47.95	52.05	93.24	6.76
	CI_E	−0.48	0.14	0.10	−0.08	−0.12	0.14	0.16	−0.66
Camphor	η_G	10.35	89.64	24.23	75.77	25.86	74.14	83.09	16.91
	CI_G	−0.54	0.15	−0.46	0.38	−0.52	0.62	0.04	−0.14

By analyzing the amount of distillate and residue from each molecular distillation, it is observed that by manipulating the more influent operating conditions (they are pressure, evaporating temperature and feed rate) different quantities of distillate and residue can be obtained.

The concentration indexes and the yields of main components are shown to be dependent on the relative amounts of residue and distillate obtained in the molecular distillation operation.

When analyzing the percentage yields of distillates, it is observed that, in general, compounds that have a higher yield (more volatile compounds) are those with lower retention time in the chromatographic column.

Molecular distillation operation is feasible to be used to separate components of rosemary essential oil with a certain level of concentration; this is shown with this set of experiences.

Table 4 shows the results of the second set of experiences using molecular distillation under the distillation methodology of three stages in series. Conditions (pressure, temperature and feed flow) and results %D/F (yields of the distillations stages) and %R/F are shown in the table.

Table 4 Results of the second set of experiences using molecular distillation (rotor speed: 200 rpm, $T_{evaporation}$: 12°C and $T_{condenser}$: 2°C).

No. stages	Feed	Feed flow (ml s^{-1})	Pressure (kPa)	%D/F^a	%R/F^b
1	Rosemary essential oil	0.018	7.8	7.60	92.40
2	R1	0.018	7.8	6.81	93.20
3	R2	0.024	6.5	5.30	94.70

Notes: [a]$\%\frac{D}{F} = \%\frac{\text{g distillate}}{\text{g feed}}$ of the respective stage and [b]$\%\frac{D}{F} = \%\frac{\text{g residue}}{\text{g feed}}$ of the respective stage.

Analysis and identification of major components in the fractions of distillate and residues obtained from the molecular distillation

Chromatographic determinations were made to the raw material, residues and distillate, products of molecular distillation assays. Table 5 shows the main volatile compounds identified in the rosemary essential oil and the distillate and residue fractions, with their relative concentrations calculated according to peak area given by the HP3398A integrator.

Table 5 Main volatile compounds present in distillates and residue fractions and their EC_{50} values.

	R3	R2	R1	D1	D3	D2
EC_{50}	**2.5**	**3.5**	**4.2**	**11.3**	**23.2**	**33.2**
Compound	**Concentration (g per 100 g)**					
α-Pinene	9.80	10.96	11.81	25.98	22.92	23.48
β-Pinene	1.07	1.16	1.24	2.29	2.18	2.24
Myrcene	5.05	5.34	5.62	8.87	9.28	9.40
Limonene	2.51	2.58	2.68	3.65	4.01	4.01
1,8-Cineole	22.56	23.36	23.99	29.29	32.52	32.50
γ-Terpinene	3.39	3.50	3.60	4.43	5.05	5.00
Camphor	21.36	20.53	19.90	8.30	12.15	11.23
Linalool	3.10	2.93	2.78	0.00	0.84	0.75
Linalyl acetate	3.59	3.44	3.21	0.00	0.29	0.00
α-Terpineol	3.26	3.05	2.89	0.00	0.73	0.65

Determination of the free radical scavenging capacity

The reaction time between each sample tested and the solution of DPPH* was 2 h to consider that the reaction after that time reached a plateau, this can be seen from

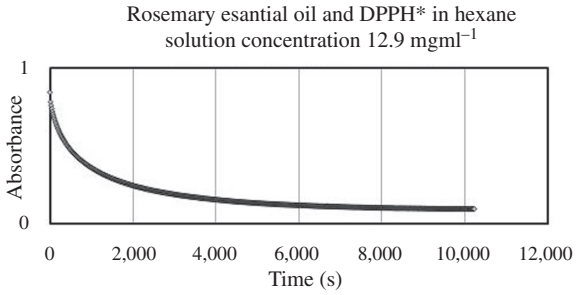

Figure 4 Absorbance of the reaction sample against time in seconds.

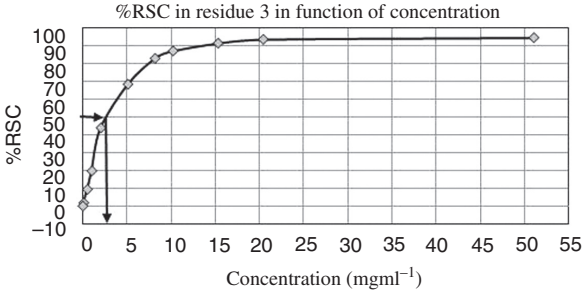

Figure 5 Determination of EC_{50} value for residue 3.

Figure 4, where the absorbance of the reaction sample against time in seconds is plotted.

Figure 5 shows the graphical location EC_{50} where %RSC is plotted as a function of the sample concentration and determining the concentration of 50 %RSC.

EC_{50} values of the samples assess (distillates and residues) are shown in Table 5 with compounds identified in each of these samples.

Relationship between the free radical scavenging capacity and the components present in the samples

EC_{50} values of tested samples increased in the following order: R3 < R2 < R1 < D1 < D3 < D2. This indicates that R3 is the one with greater free radical scavenging capacity, while D2 is the one with lower capacity of free radical scavenging. The EC_{50} value for rosemary essential oil is 4.1, intermediate value between the EC_{50} values of residues and distillates.

The comparison between the free radical scavenging capacity and gas chromatography analysis reveals the following: free radical scavenging capacity increases with the concentration of compounds like camphor, linalool, linalyl acetate and α-terpineol and decreases with the concentration of compounds like α-pinene, β-pinene, myrcene, limonene, 1,8-cineole and γ-terpinene.

According to previous analysis, it was conclued that the extracts enriched in compounds camphor, linalool, linalyl acetate and α-terpineol have a high antioxidant power. Bozin *et al.* [12], Çelik and Özkaya [13], Shalaby *et al.* [14] and Yan and White [15] mentioned the possible antioxidant power of these compounds.

The residues obtained from the molecular distillation have more antioxidant power than distillates and rosemary essential oil.

It was concluded that molecular distillation operation is feasible to obtain extracts enriched in antioxidant power.

Conclusions

It was determined that the most abundant components of rosemary essential oil obtained from *R. officinalis* variety grown in the province of San Juan are α-pinene, myrcene, 1,8-cineole and camphor. This variety responds to 1,8-cineole chemotype.

Concentrations and yields of the major components were shown to be dependent on the relative amounts of residue and distillate obtained in the molecular distillation operation.

When analyzing the percentage yields of distillates, it is observed that the more volatile compounds are those with lower retention time in the chromatographic column.

It was found that the method for measuring antioxidant activity through the free radical scavenging capacity can be used to determine the antioxidant power of rosemary essential oil. The results showed that rosemary essential oil has considerable antioxidant capacity.

The molecular distillation is an effective operation to separate and concentrate rosemary essential oil in order to obtain fractions with higher antioxidant activity than the raw material. The residue of the last stage of molecular distillation operating in series, rich in camphor, linalool, linalyl acetate and α-terpineol exhibits the highest antioxidant capacity.

These residues, with more antioxidant power, have greater economic value for the food industry than rosemary essential oil. On the other hand, distillates, with higher concentrations of 1,8-cineol and α-pinene, have greater economic value for the cosmetic and pharmaceutical industries for its contribution to the fragrance of final product due to 1,8-cineol and for its antibacterial power due to concentration in α-pinene, respectively.

References

1. Szumny A, Figiel A, Gutiérrez-Ortíz A, Carbonell-Barrachina AA. Composition of Rosemary essential oil (*Rosmarinus officinalis*) as affected by drying method. J Food Eng 2010;97:253–60.
2. Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. Antimicrobial and antioxidant properties of Rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., *Lamiaceae*) essential oils. J Agric Food Chem 2007;55:7879–85.
3. Socani SA, Tofana M, Socaciu C, Varban D, Muste S. Comparative study of different rosemary essential oil. Bulletin USAMV-CN 2007;63.
4. López Luengo MT. Plantas medicinales de aplicación en dermofarmacia. OFFARM 2003;22:122–5.
5. Torelló M, Viscasillas A, Del Pozo A. Aromacosmética (I), Concepto y Aplicaciones. OFFARM 2003;22:178–80.
6. Varela F, Navarrete P, Cristobal R, Fanlo M, Melero R, Sotomayor JA, et al. Variability in the chemical composition of wild *Rosmarinus officinalis* L. Acta Horticulturae 2009;826:167–74.
7. Bousbia N, Abert Vian M, Ferhat MA, Petitcolas E, Meklati BY, Chemat F. Comparison of two isolation methods for essential oil from rosemary leaves: hydrodistillation and microwave hydrodiffusion and gravity. Food Chem 2009;114:355–62.
8. Pramparo M, Prizzon S, Martinello MA. Estudio de la composición de ácidos grasos, tocoferoles y esteroles a partir del destilado de desodorización. Grasas y aceites 2005;56:228–34.
9. Martinello M, Villegas M, Pramparo M. Retaining maximum antioxidative potency of wheat germ oil refined by molecular distillation. J Sci Food Agric 2007;87:1559–63.
10. Pramparo M, Leone I, Martinello MA. Simulation of deacidification process by molecular distillation of deodorizer distillate. Latin Am Appl Res 2008;38:299–304.
11. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT – Food Sci Technol 1995;28:25–30.
12. Bozin B, Mimica-Dukic N, Bogayac M, Suvajdzic L, Simin N, Samojlik I, et al. Chemical composition, antioxidant and antibacterial properties of *Achillea collina becker ex heimerl s.l.* and *A. pannonica scheele* essential oils. Molecules 2008;13:2058–68.
13. Çelik S, Özkaya A. Effects of intraperitoneally administered lipoic acid, vitamin E, and linalool on the level of total lipid and fatty acids in guinea pig brain with oxidative stress induced by H_2O_2. J Biochem Mol Biol 2002;35:547–52.
14. Shalaby EA, Nasr NF, El Sherief SM. An in vitro study of the antimicrobial and antioxidant efficacy of some nature essential oils. J Med Plants Res 2011;5:922–31.
15. Yan PS, White PJ. Linalyl acetate and other compounds with related structures as antioxidants in heated soybean oil. J Agric Food Chem 1990;38:1904–8.