

## Physico-chemical Evaluation of *Rosmarinus officinalis* L. Essential Oils

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### ABSTRACT

Nineteen samples of *Rosmarinus officinalis* were extracted by steam distillation in a pilot plant and evaluated in terms of chemical compositions and physico-chemical characteristics. The volatile oil yields ranged from 0.37% (1999 harvest) to 0.49% (1998 harvest). Twenty components were identified in the oils. The major components were alpha-pinene (40.55 to 45.10%), 1,8-cineole (17.40 to 19.35%), camphene (4.73 to 6.06%) and verbenone (2.32 to 3.86%). The physico-chemical parameters averaged 0.8887 g/cm<sup>3</sup> for specific gravity, 1.4689 for refractive index, and +11.82° for optical rotation, and there were no significant variations in either the chemical or physico-chemical data in the different years.

**Key words:** *Rosmarinus officinalis*, rosemary, essential oil composition, alpha-pinene, 1,8-cineole, physico-chemical parameters

### INTRODUCTION

*Rosmarinus officinalis* L. (Lamiaceae) is a small evergreen which grows wild in most Mediterranean countries (Hèthelvi et al., 1987), reaching a height of 1.5 m. The main producers are Italy, Dalmatia, Spain, Greece, Turkey, Egypt, France, Portugal and North Africa (Svoboda and Deans, 1992). The botany of rosemary is rather complex, as there are several species within the Genus *Rosmarinus*, with a range of varieties and forms (Ghenther, 1985). Essential oils of *R. officinalis*, known as rosemary oils, are obtained by steam distillation of the fresh leaves and twigs, and the yields range from 0.5 to 1.0% (Tewari and Virmani, 1987). It is an almost colorless to pale

yellow liquid with a characteristic, refreshing and pleasant odor. Major constituents described for the oil are  $\alpha$ -pinene, 1,8-cineole and camphor (Bauer et al., 1997).

Significant variations in the chemical composition of oil have been reported with relation to the geographic origin (Tuker and Maciarelo, 1986; Tewari and Virmani, 1987; Mizrahi et al., 1991; Svoboda and Deans, 1992; Chalchat et al., 1993; Lawrence, 1995; Rao et al., 1997; Dellacassa et al., 1999). Time of harvest, condition of the twigs and leaves, distillation equipment and management have also been reported to have an important role in the overall quality of the oil (Tewari and Virmani, 1987).

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In this paper we report the chemical composition and the associated physico-chemical properties of rosemary essential oils extracted on a pilot scale installation during the years 1998, 1999 and 2000, with the aim of establishing the quality and chemical stability of the essential oils produced in the Southern region of Brazil.

## MATERIAL AND METHODS

### *Plant Material*

*Rosmarinus officinalis* was harvested in Campestre da Serra (Rio Grande do Sul State) during the years 1998, 1999 and 2000. The plants were identified by the Drs. R. Wasun and M. Rossato (Herbarium of the Museu de Ciências Naturais da Universidade de Caxias do Sul), and voucher specimens were deposited under catalogue number 8389.

### *Essential Oil Extraction*

Essential oils were distilled from the aerial parts of the plants in the pilot plant. The amount of fresh plant used varied from 60 to 310 Kg. A total of 6 extractions were completed in 1998, 7 in 1999 and 6 in 2000. The plant material was loaded in the still and disposed in layers to allow suitable distribution of the steam. The lid was hermetically closed and the process of distillation began with the injection of steam in the bottom of the still. The pressure employed during distillation was 3.6Kgf/cm<sup>2</sup>, and the duration was 90 min. Steam and essential oils were condensed and collected in the florentine flask. The oils were dried over sodium sulphate and stored in clean brown glass bottles, and kept in a controlled temperature chamber at 20°C, until the time of their analyses.

### *Physico-chemical analyses*

*Specific gravity (SG)* was measured with an Anton Paar Modelo DMA23<sub>N</sub> densimeter. *Viscosity (V)* was measured with a Brookfield DV-II Viscosimeter, at 20°C. *Optical Rotation (OR)* was measured with a CETI Polaris polarimeter. Solutions of 40g/L of the essential oil samples in analytical grade chloroform (Merck). The readings were made with 1 dm tubes at 20°C. *Refractive index (RI)* was measured with a CETI Quartz refractometer, at 20°C.

*Solubility in ethanol (SE)*: The tests were made by complete solubilization of 1 mL of essential oil in 85% dilute ethanol, at 20°C.

*Residue on evaporation (RE)*: The evaporation residue was measured by adding 3 g of essential oil sample in a Petry dish, evaporating in a boiling water bath, cooling in a desiccator, until two successive weights gave a difference of less than 0,1%.

*Boiling point (BP)*: 0.5 mL of sample was used to fill a wide bore capillary tube with an inversed thin capillary inside. This set up was fixed to the stem of a thermometer. The thermometer was then partially immersed in an glycerol bath, at a temperature dependent on the sample of essential oil used. The continuous and rapid flow of bubbles in the wide capillary was taken as boiling point.

### *Chemical Identification*

Gas chromatographic (GC) analysis were performed with a Hewlett Packard 6890 Series equipped with a HP-Chemstation data processor, fitted with a HP-Innowax bonded phase capillary column (30 m x 0.32 mm i.d., 0.50 µm film thickness, Hewlett Packard, Palo Alto, USA); column temperature, 40°C (8 min) to 180°C at 3°C/min, 180°–230°C at 20°C/min, 230°C (20 min); injector temperature 250°C; detector temperature 250°C; split ratio 1:50; carrier gas H<sub>2</sub> (34KPa). Volume injected 1 µL diluted in hexane (1:10). The GC/MS analysis were performed in a HP 6890 GC using a mass selective detector Hewlett Packard 6890/MSD5973, equipped with HP Chemstation software and Wiley 275 spectra data. A fused silica capillary column HP-Innowax (30 m x 0.25 mm), 0.25µm film thickness (Hewlett Packard, Palo Alto, USA) was used. The temperature program was the same used in the gas chromatography (GC) analysis: interface 280°C; split ratio 1:100; carrier gas He (56 KPa); flow rate: 1.0 mL/min; ionization energy 70 eV; mass range 40-350; volume injected 0.4µL diluted in hexane (1:10). Identification of the individual components was based on comparison of their GC retention indices (RI) on polar columns and comparison with mass spectra of components by GC/MS.

The data obtained for the chemical and physico-chemical parameters were submitted to a variance analysis (ANOVA) and a test of mean comparison

(Tukey 5%) using the computer program SPSS 10.0. 6 repetitions each were used for the samples of 1998 and 2000, and 7 for 1999.

## RESULTS AND DISCUSSION

The essential oil of *R. officinalis* L. gave an average yield of 0.49% v/w in 1998, 0.37% v/w in 1999 and 0.45% in 2000. The components, the percentage of each constituent and their retention indexes were summarized in Table 1. To simplify

the presentation the mean for each year are used. The components are arranged in order to GC elution on the Innowax columns. The main compounds were  $\alpha$ -pinene (40.55 to 45.10%), 1,8-cineole (17.40 to 19.35%), camphene (4.73 to 6.06%), verbenone (2.32 to 3.86%) and borneol (2.24 to 3.10%). Other components were found in less quantity (< 2%). The main components in this chemotype were different from those quoted by Price and Price (1999), identified as QT pinene-cineole.

**Table 1-** Percentual chemical composition (average of years) of Brazilian rosemary essential oils

Compounds	1998	1999	2000	Retention Indices
$\alpha$ -pinene	40.55	45.10	41.63	1010
camphene	5.17	6.06	4.73	1032
$\beta$ -pinene	2.70	2.86	2.40	1062
sabinene	0.74	0.67	0.84	1079
myrcene	1.39	1.59	1.71	1141
$\alpha$ -phelandrene	0.50	0.43	0.45	1155
limonene	3.41	3.81	3.33	1188
1,8-cineole	17.40	17.59	19.35	1200
$\gamma$ -terpinene	1.11	0.75	0.82	1243
para-cymene	0.99	1.56	1.23	1267
$\alpha$ -terpinolene	0.92	0.69	0.68	1278
chrysanthenone	0.65	0.51	0.53	1509
camphor	2.13	1.63	2.42	1516
linalool	2.02	1.71	2.16	1528
bornyl acetate	1.39	1.02	0.80	1538
$\beta$ -caryophyllene	2.80	2.11	2.16	1543
terpinen-4-ol	0.75	0.51	0.70	1551
verbenone	3.83	2.32	3.86	1595
borneol	2.97	2.24	3.10	1598
geraniol	2.68	2.07	2.61	1750
Non oxygenated	60.28	65.63	59.98	
Oxygenated	33.82	29.60	35.53	
Total	94.10	95.23	95.51	

The statistical treatment of chemical data (Table 2) showed that the differences in terms of concentration of the main constituents were non-significant. There was no influence of the year of harvest in the chemical composition or other characteristics of the oils. Compared with other rosemary oils, Brazilian oils were more similar to those of French origin due to their 1,8-cineole and camphor contents (Tewari and Virmani, 1987; Chalchat et al., 1993; Lawrence, 1995). There was also similarity between Brazilian oils and oils of North American cultivars (Tucker and Maciarello, 1986).

In Table 3, the mean values for some physico-chemical properties are shown. Their statistical treatment (Table 4) shows small variations among the samples, which were non-significant, with the exception of the Residue on Evaporation. Those for 2000 were lower than those for 1998 and 1999. Taking the data for these properties, Brazilian rosemary oils showed a mean refractive index of 1.4689, an average optical rotation of +11.82°, and an average specific gravity of 0.8887 g/cm<sup>3</sup>. In relation to samples from other world zones, Brazilian oils were closer to those from France and

Italy (Tewari and Virmani, 1987; Chalchat et al., 1993; Lawrence, 1995).

These results are an initial contribution to the establishment of average values of chemical and physico-chemical parameters for a regional

product of interest to industry. The stability of its composition and characteristics over several years is also relevant for its commercial utilization, as it is the basis of a consistent product.

**Table 2** - Variance analysis (ANOVA), yearly mean values and general area mean (pA\*S) of the main chemical constituents in rosemary oils - <sup>ns</sup> Non significant

	$\alpha$ -pinene	canfene	$\beta$ -pinene	limonene	1,8-cineole	camphor	linalool	verbenone	borneol	geraniol
F	0,620 <sup>ns</sup>	2,51 <sup>ns</sup>	1,20 <sup>ns</sup>	1,18 <sup>ns</sup>	0,519 <sup>ns</sup>	2,041 <sup>ns</sup>	1,56 <sup>ns</sup>	2,68 <sup>ns</sup>	2,19 <sup>ns</sup>	2,32 <sup>ns</sup>
1998	6116,26	786,46	404,03	513,80	2603,84	320,02	300,14	565,68	441,07	399,60
1999	6641,58	891,11	417,85	561,25	2583,01	240,39	250,97	341,90	327,95	301,50
2000	6023,41	673,75	343,92	483,58	2876,90	363,77	325,06	598,02	469,33	386,68
Average	6280,48	789,42	390,14	521,73	2682,40	304,50	289,89	493,45	408,32	359,37

**Table 3** - Physico-chemical properties (average of years) of Rosemary oils from Brazil

	Harvest year		
	1998	1999	2000
Refractive index	1.4697	1.4682	1.4689
Optical rotation (°)	+11.75	+12.13	+11.58
Solubility in 85°ethanol (v:v)	0.85	0.82	0.74
Viscosity (cP)	4379	4080	4756
Specific gravity (g/cm <sup>3</sup> )	0.8891	0.8858	0.8911
Evaporation index (%)	0.78	0.60	0.13
Boiling point (°C)	122	118	130

**Table 4** - Variance analysis (ANOVA), mean comparison test (Tukey 5%), mean annual values and general average of the physico-chemical parameters for Brazilian rosemary essential oils

	RI	OR (°)	SE (mL)	V (cP)	SG (g/cm <sup>3</sup> )	RE (%)	BP (°C)
F	1,316 <sup>ns</sup>	1,249 <sup>ns</sup>	0,646 <sup>ns</sup>	1,765 <sup>ns</sup>	3,493 <sup>ns</sup>	16,856 *	1,158 <sup>ns</sup>
1998	1,4696	10,0817	0,8500	4379,00	0,88911	0,7850 a	122
1999	1,4638	12,1286	0,8286	4080,42	0,88580	0,6042 a	118
2000	1,4689	11,5814	0,7429	4585,66	0,89112	0,3457 b	130
Mean	1,4673	11,3230	0,8050	4334,26	0,88866	0,5680	123,3

<sup>ns</sup> non significant; \* significant at 5%. Mean values followed by the same letters do not differ by Tukey 5%. RI- refraction index; OR- optical rotation; SE- solubility in ethanol; V- viscosity; SG- specific gravity; ER- residue on evaporation; BP- boiling point.

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## RESUMO

Dezenove amostras de *Rosmarinus officinalis* foram extraídas por destilação por arraste a vapor em uma planta piloto e os óleos essenciais foram

avaliados quanto a composição química e características físico-químicas. Os rendimentos de óleo essencial variaram de 0,37% (média de 1999) a 0,49% (média de 1998). Vinte componentes foram identificados nos óleos essenciais. Os componentes majoritários foram  $\alpha$ -pineno (40,55 a 45,10%), 1,8-cineol (17,40 a 19,35%), canfeno (4,73 a 6,06%) e verbenona (2,32 a 3,86%). A média por safra dos parâmetros físico-químicos avaliados resultaram em 0,8887g/cm<sup>3</sup> para densidade específica, 1,4689 para índice de refração e +11,82° para rotação óptica, sendo que

as variações encontradas para os parâmetros químicos e físico-químicos dos óleos essenciais não variaram significativamente em função do ano de produção.

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