Dearomatization of Antioxidant Rosemary Extracts by Treatment with Supercritical Carbon Dioxide

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Treatment with supercritical CO_2 is proposed for deodorizing antioxidant rosemary extracts obtained by steam distillation and Soxhlet extraction. The process conditions have been optimized by applying a Taguchi experimental design with the aim of obtaining, at minimum cost, a product with acceptable antioxidant activity as well as minimum rosemary aroma. Variables were selected for their effects on the selective extraction of the compounds responsible for the residual aroma of the rosemary extract. The optimized method allowed 90% dearomatization; no detrimental effects in antioxidant activity or color of the extracts have been observed after supercritical fluid processing.

Keywords: *Natural antioxidant; rosemary extract; supercritical fluid extraction; experimental design*

INTRODUCTION

The growing interest in natural foods has raised the demand for natural antioxidants, i.e., products of nonsynthetic origin. Used traditionally as spice (Chen et al., 1992), rosemary leaves have also been studied because of their antioxidant properties. An initial approach to the use of aromatic plants as natural antioxidants was described by Chipault et al. in 1952.

In 1955, Rac and Ostric-Matijasevic used a rosemary leaf extract as an antioxidant food additive. In 1966, Brieskorn et al. isolated and identified a compound, carnosol, from rosemary to which they attributed the antioxidant properties of the plant. In the 1960s and 1970s, more work was done in this field, mainly in Japan (Saito et al., 1976); in the late 1970s, Chang et al. patented a process to extract natural antioxidants from rosemary and sage. A complete review of this subject was published in 1985 by Kramer.

Rosemary antioxidant properties are mainly due to phenolic diterpenes (Schwarz and Ternes, 1992a,b; Schwarz et al., 1992) such as carnosol, rosmanol, 7-methyl-*epi*-rosmanol, isorosmanol, rosmadial, carnosic acid, methyl carnosate, rosmanol-9-ethyl ether (Cuvelier et al., 1994), and other phenolic acids such as rosmarinic and caffeic. These compounds are described in the literature and have been isolated and identified by numerous authors. Several studies have demonstrated that some of these compounds are more efficient than butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) (Chang et al., 1977; Inatani et al., 1983; Barbut et al., 1985; Chen et al., 1992; Iriarte et al., 1992).

Antioxidants must be efficient at low dosage levels, and changes in the aroma and color of the treated material must also be minimal. As a consequence, processes for obtaining antioxidants should provide extracts with high antioxidant activity but low aroma and color.

Several methods have been applied to extract antioxidants from labiatae herbs: solid−liquid extraction, aqueous alkaline extraction, and extraction with vegetable oils or glycerides. Steam distillation and molecular distillation are used for concentrating the active fraction or to remove the residual aroma. Some disadvantages related to these methods have been reported (Nguyen et al., 1994).

Solvent extraction can leave residues frequently prohibited in food by regulation. An additional drawback in antioxidant production using organic solvents is the oxidative transformations that the extract can suffer when the solvent residue is eliminated. Products obtained by using vapor stream purge have always a high aroma content that has to be eliminated to be useful to the food industry. Molecular distillation can cause dilution effects due to the presence of the distillation carrier.

To solve these problems, some processes based on supercritical fluid extraction (SFE) technology have been proposed lately (Djarmati et al., 1991; Nguyen et al., 1991, 1994; Muehlnikel, 1992; Gerard et al., 1995; Anonymous, 1995). Extraction with supercritical CO_2 has been used either to remove from the plant leaves the oleoresin containing the essential oil, prior to solvent extraction, or to obtain directly from the aromatic plants the antioxidant extract. Products obtained by SFE from rosemary herbs have in general a high antioxidant activity but are expensive and in some cases have a noticeable residual rosemary aroma.

As it has been widely described, the SFE process involves a high number of variables that can influence the quality of the final product. Usually the design of the SFE process operating conditions is based on solubility and mass transfer data. However, the necessary physicochemical data are not always available, and interactions among variables are difficult to deal with.

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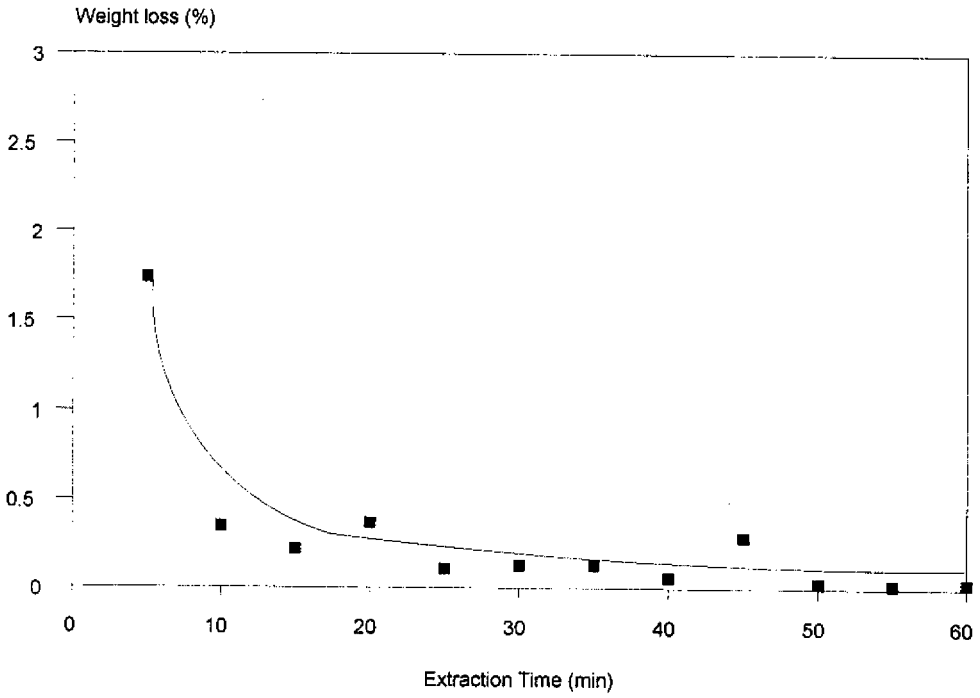

Figure 1. Graph representing weight loss (percent) measured after consecutive SFEs of a rosemary extract sample vs extraction time (minutes).

On the other hand, experimental design techniques have been used previously to improve extraction efficiency (Blanch et al., 1992) and chromatographic separation performance (Ibañez et al., 1995) and to select the optimum operating conditions of different processes (Oles and Yankovich, 1989; Billot and Pitard, 1992). Techniques of experimental design allow the study and optimization of many experimental variables, at various levels, with only a few experiments, considering interactions among variables and also the effect of noise factors.

In the present work a process for dearomatizing rosemary antioxidants obtained by solvent extraction of plant leaves is designed and optimized by applying a Taguchi experimental design (Taguchi, 1987). The proposed procedure involves the treatment of the plant extracts with supercritical CO_2 in conditions of selective extraction of the residues of essential oil and organic solvent. The goal of the present investigation was to obtain, at minimum cost, a product that has acceptable antioxidant activity and minimum rosemary aroma and is free of solvent residues. Negative effects on the antioxidant activity or the color of the plant extracts due to SFE treatment should be avoided.

EXPERIMENTAL PROCEDURES

Sample. The rosemary extract is obtained by using the following procedure: the rosemary leaves (*Rosmarinus officinalis*, from southwestern Spain) are first enzymatically hydrolyzed using hemicellulases. This way, cell walls are easily broken, liberating the highest possible amount of active products and increasing the extraction yield; after this treatment, the essential oil is first removed by using vapor stream purge and, after that, the extraction is performed by using the Soxhlet method with 2-propanol as solvent. The residual solvent content was ~7%.

Reactants. 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH, 95% purity) was obtained from Sigma-Aldrich (Madrid, Spain), acetone from Quimicen (Madrid, Spain), ethanol from Ferosa (Barcelona, Spain), and methanol (HPLC quality) from Lab Scan (Dublin, Ireland).

Extraction Method. A Suprex PrepMaster (Suprex Corp., Pittsburgh, PA) supercritical fluid extractor was used to perform all of the experiments. Sample [0.4 g (dry weight basis)] was introduced into a 5 mL stainless steel extraction cell. Supercritical CO_2 flow rate was controlled using a fixed restrictor, so flow rates between 3 and 4 mL/min were obtained at the experimental conditions tested.

Both, amount of sample and flow rate were selected, on the basis of previous work, to obtain a good mass transfer inside the extraction cell. At constant flow rate, the extraction time determines the total volume of CO_2 through the sample. To obtain the maximum extraction yield, experiments were done to determine the time in which the extraction is complete. Figure 1 shows the results obtained by representing weight loss (percent), measured after consecutive extractions of the same sample, at 5 min intervals. From a practical point of view the extraction can be considered sufficient after 30 min; an increase in the extraction time is not justifiable because it would provide scarce additional extraction yield.

Residual Aroma Analysis. Since the intensity of the rosemary aroma of the antioxidant can be related to the content of volatile compounds, the sum of peak areas of the chromatogram corresponding to solid-phase microextraction (SPME) was utilized for an objective evaluation of the residual aroma of the extracts. SPME was first introduced by Arthur and Pawliszyn in 1990. It is a solvent-free, inexpensive, rapid, and versatile method for the extraction of organic compounds and uses a fused-silica fiber coated with a polymeric stationary phase introduced into a liquid or gas sample. The method involves two processes: the partitioning of the analytes between the coating and the sample and the thermal desorption of the analytes into the gas chromatograph (Zhang et al., 1994). This technique, used as an isolation technique of volatile compounds, has been associated with separation techniques such as capillary gas chromatography.

An SPME holder (Supelco) equipped with a fused-silica fiber coated with a thin (100 μm) layer of poly(dimethylpolysiloxane) was chosen to extract the aroma compounds.

One-tenth gram of the product extracted by SFE was placed in a 10 mL vial and closed with Parafilm. The fiber was exposed to the headspace of the sample at 60 °C for 30 min.

A Perkin-Elmer model 8500 gas chromatograph equipped with a PTV injector and FID detector was used to perform the analysis. The system was coupled to a model 2600 chroma-

tography software system (Perkin-Elmer Nelson Analytical). A 50 m × 250 μm i.d. fused-silica capillary column (Chrompack, Middelburg, The Netherlands) coated with a 0.25 μm layer of CP-Sil-5 was used. Helium was the carrier gas. Thermal desorption of the compounds in the fiber took place in the GC injector at 200 °C for 15 min in splitless mode for 5 min. The detector operated at 250 °C. The oven temperature was programmed from 70 °C (3 min at constant temperature) to 180 °C at 5 °C/min. The final temperature was maintained for 15 min.

Identification by GC/MS. GC/MS analysis was carried out by coupling the gas chromatograph described above to a Perkin-Elmer ITD-50 ion trap detector (EI 70 eV). Capillary column and chromatographic program were used as mentioned previously. Compounds were identified by comparison of the spectra with those in a general purpose library. Moreover, the identity of the components was confirmed by matching their mass spectrometric data with those obtained from the same equipment and corresponding to authentic reference compounds.

Determination of Antioxidant Activity. The method used in the present investigation to measure the antioxidant activity of the rosemary extracts was based on the method described by Lamaison et al. (1988), i.e., the neutralization of the free radicals of DPPH by the antioxidant. Slight modifications have been introduced to correctly quantify the scavenger index of the rosemary extracts obtained by SFE. The method proposed is as follows: 0.014 g of DPPH was weighed and brought to 100 mL with methanol. The solution was sonicated for 10 min and then diluted 1:5 with methanol. The solution was prepared daily and kept under refrigeration until used. Rosemary extract solutions were prepared by weighing 0.05 g and adding 7 g of ethanol. Ten grams of DPPH solution was placed in test tubes, and 30 μL of rosemary extract solution was added (corresponding to 212 μg). Reaction was complete after 3 h at room temperature. Absorbance was measured at 516 nm in a Shimadzu UV-120-01 spectrophotometer (Shimadzu Corp., Kyoto, Japan). Methanol was used to adjust to zero. To determine the scavenger index, a previously described equation was utilized (Lamaison et al., 1988).

Color Measurement. Determination of the color was carried out according to the method developed by Gonzáles Cartagena (1990). This method is based on the measure of the absorbance of a solution between 200 and 780 nm. Values obtained allow the calculation of several parameters from which intensity (defined as the sum of absorbances) and dominant wavelength have been used in the present work.

A Beckmann model DU-2 spectrophotometer (Beckmann Instruments Inc., Fulerton, CA) was used to measure the color of the rosemary extract. Sample solutions were prepared with a concentration of 0.0025 g of rosemary extract/mL of acetone. After sonication for 2 min, the solutions were filtered through 0.22 μm Pro-X (Nylon-66) (Tecknokroma, Barcelona, Spain).

Experimental Design. Taguchi experimental design methodology (Taguchi, 1987) uses orthogonal arrays based on fractional factorial designs. Columns of the orthogonal array correspond to variables (control factors) or interactions, and rows correspond to the experiments to be carried out (Table 1). The size of the orthogonal array is selected depending on the degrees of freedom needed to study the intended number of variables and interactions. To assign each variable or interaction to a column, linear graphs, also developed by following Taguchi methods, are used. The selection of the linear graph and the assignment of control factors to its points must be performed in such a way that the interactions of interest become placed on lines connecting these points (Figure 2). This methodology considers also the robustness of the studied system, i.e. the attainment of the best performance with the lowest possible influence of uncontrollable (noise) factors. To this aim, the experiments are performed at different levels of selected noise factors (Taguchi, 1987; Peace, 1993). The Taguchi signal-to-noise ratio (S/N), a compromise between the extent and the variability of data obtained at each set of the control factors, is used as a response to evaluate experimental results.

Table 1. L_{27} (3^{13}) Taguchi Orthogonal Array[a]

run	*P*	*E*	*PE*	*PE*	*T*	*PT*	*PT*	*ET*	*S*	*W*	*ET*	Ac	*e*
1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	2	2	2	2	2	2	2	2	2
3	1	1	1	1	3	3	3	3	3	3	3	3	3
4	1	2	2	2	1	1	1	2	2	2	3	3	3
5	1	2	2	2	2	2	2	3	3	3	1	1	1
6	1	2	2	2	3	3	3	1	1	1	2	2	2
7	1	3	3	3	1	1	1	3	3	3	2	2	2
8	1	3	3	3	2	2	2	1	1	1	3	3	3
9	1	3	3	3	3	3	3	2	2	2	1	1	1
10	2	1	2	3	1	2	3	1	2	3	1	2	3
11	2	1	2	3	2	3	1	2	3	1	2	3	1
12	2	1	2	3	3	1	2	3	1	2	3	1	2
13	2	2	3	1	1	2	3	2	3	1	3	1	2
14	2	2	3	1	2	3	1	3	1	2	1	2	3
15	2	2	3	1	3	1	2	1	2	3	2	3	1
16	2	3	1	2	1	2	3	3	1	2	2	3	1
17	2	3	1	2	2	3	1	1	2	3	3	1	2
18	2	3	1	2	3	1	2	2	3	1	1	2	3
19	3	1	3	2	1	3	2	1	3	2	1	3	2
20	3	1	3	2	2	1	3	2	1	3	2	1	3
21	3	1	3	2	3	2	1	3	2	1	3	2	1
22	3	2	1	3	1	3	2	2	1	3	3	2	1
23	3	2	1	3	2	1	3	3	2	1	1	3	2
24	3	2	1	3	3	2	1	1	3	2	2	1	3
25	3	3	2	1	1	3	2	3	2	1	2	1	3
26	3	3	2	1	2	1	3	1	3	2	3	2	1
27	3	3	2	1	3	2	1	2	1	3	1	3	2

[a] *P*, pressure (bar); *E*, ethanol (modifier %); *T*, temperature (°C); *S*, % w/w residual extraction solvent; *W*, water (modifier %); Ac, acetic acid (modifier %); *e*, error.

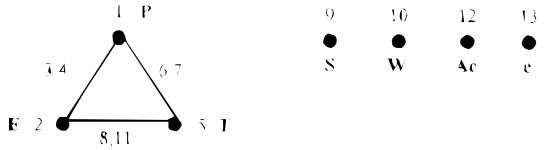

Figure 2. Linear graphs used to assign the control factors and interactions.

The experimental design applied in this work included several factors afecting supercritical extraction selectivity: CO_2 pressure and temperature, % w/w residual extraction solvent, and % of modifier. Three substances, ethanol, water, and acetic acid, were tested as modifier due to their different polarities and, therefore, their capabilities for modifying the supercritical fluid selectivity.

To investigate the above-mentioned factors at three different levels and considering three presumably important interactions (pressure−temperature, pressure−ethanol, and temperature−ethanol), the experimentation was designed according to an $L_{27}(3^{13})$ Taguchi orthogonal array. Table 1 shows the orthogonal array in the control factors (inner array) together with the respective experimental values at low (1), medium (2), and high (3) levels that correspond to the physical values shown in Table 2. Each factor and interaction was assigned to a column according to the linear graph selected (Figure 2). When factors are considered at three levels, the estimation of the interactions between two factors requires 4 degrees of freedom [$(3 - 1) \times (3 - 1) = 4$ df], i.e., two columns of the matrix from series 3. The quality of the CO_2 was considered as a noise factor with level 1 (high quality, 99.9999% purity) and level 2 (low quality, 99.998% purity). Therefore, the complete inner array was run once at each noise factor level.

In each experiment the total area of the chromatogram obtained by analyzing the residual aroma of the sample, the color, and the antioxidant activity was analyzed to evaluate the performance of the extraction process.

RESULTS AND DISCUSSION

The solvent-extracted rosemary antioxidant used as raw material in the present investigation had charac-

Table 2. Physical Values Corresponding to Taguchi Orthogonal Array in the Control Factors

run	*P*	*T*	*S*	*E*	*W*	Ac
1	100	40	100	0	0	0
2	100	60	50	0	5	5
3	100	80	0	0	10	10
4	100	40	50	5	5	10
5	100	60	0	5	10	0
6	100	80	100	5	0	5
7	100	40	0	10	10	5
8	100	60	100	10	0	10
9	100	80	50	10	5	0
10	200	40	50	0	10	5
11	200	60	0	0	0	10
12	200	80	100	0	5	0
13	200	40	0	5	0	0
14	200	60	100	5	5	5
15	200	80	50	5	10	10
16	200	40	100	10	5	10
17	200	60	50	10	10	0
18	200	80	0	10	0	5
19	350	40	0	0	5	10
20	350	60	100	0	10	0
21	350	80	50	0	0	5
22	350	40	100	5	10	5
23	350	60	50	5	0	10
24	350	80	0	5	5	0
25	350	40	50	10	0	0
26	350	60	0	10	5	5
27	350	80	100	10	10	10

Table 3. Results Obtained at the Experimental Conditions Tested (Noise Level 1; High-Quality CO_2, 99.9999% Purity)

run	SPME area	antioxidant activity	color intensity
1	224399	34.92	79.659
2	3819528	32.39	79.556
3	6906153	35.54	74.616
4	5041367	34.62	81.673
5	3115436	29.31	75.038
6	3496120	34.32	79.835
7	8046562	39.40	80.609
8	2027065	36.68	82.338
9	4035048	36.06	79.613
10	2275437	32.13	84.521
11	1672250	31.75	82.993
12	2141364	31.13	88.073
13	313648	32.77	89.522
14	1329091	32.13	89.024
15	1259663	33.43	84.629
16	2348758	31.46	86.855
17	3478895	33.73	85.242
18	6755626	28.78	93.891
19	5845004	31.34	83.122
20	3472311	36.74	94.872
21	90279	32.87	88.939
22	1496097	35.93	87.603
23	3637846	34.43	86.448
24	8688676	34.16	81.494
25	4984524	37.21	83.523
26	3435360	36.13	83.366
27	3189189	34.98	81.302

teristics, determined by using the analytical methodology described above, as follows: antioxidant activity, 44 μg/mL; residual aroma, 11 887 445 area counts; color residual, 76.4 (dominant wavelength, 574.1 nm).

Tables 3 and 4 show the results obtained at the experimental conditions tested. As can be seen, the antioxidant activities as well as the color intensity values display very small variation among the different experiments. Coefficients of variation ranged from 6 to 8% for both series of data.

The absolute areas obtained for residual aroma extracted by using SPME have a variation ~27% in the first series of data and ~35% in the second, the correlation coefficient between the two series being 0.65. Analysis of these results suggests that the only parameter that has enough variation is the residual aroma, so further analysis is performed.

Table 4. Results Obtained at the Experimental Conditions Tested (Noise Level 2; Low-Quality CO_2, 99.998% Purity)

run	SPME area	antioxidant activity	color intensity
28	894883	35.61	93.483
29	1176542	30.44	79.986
30	7777149	35.49	82.567
31	354878	33.16	94.738
32	3128642	37.35	79.457
33	3559468	35.49	81.786
34	7248376	37.84	83.167
35	86576	30.12	82.320
36	3494822	37.03	81.611
37	3192241	35.20	84.228
38	2358566	42.86	83.145
39	770539	36.39	89.311
40	4185512	36.84	89.046
41	268579	37.14	92.078
42	2337063	37.30	86.120
43	5038591	43.07	79.470
44	2536316	32.53	85.155
45	6363136	37.23	87.654
46	11070610	33.30	84.113
47	2766111	35.37	89.927
48	4342828	36.28	92.699
49	2126577	37.73	94.882
50	4157063	39.30	86.967
51	7726496	34.51	79.468
52	8337628	39.48	79.051
53	5958840	38.59	78.989
54	3496969	41.82	83.182

Table 5. Dearomatization Percentage of the Extracts Obtained at Each Experimental Condition[a]

run	dearomatization	run	dearomatization
1	98.1	28	92.5
2	67.9	29	90.1
3	41.9	30	34.6
4	57.6	31	97.0
5	73.8	32	73.7
6	70.6	33	70.1
7	32.3	34	39.0
8	82.9	35	92.7
9	66.1	36	70.6
10	80.9	37	73.1
11	85.9	38	80.2
12	82.0	39	93.5
13	97.4	40	64.8
14	88.8	41	97.7
15	89.4	42	80.3
16	80.2	43	57.6
17	70.7	44	78.7
18	43.2	45	46.5
19	50.8	46	6.9
20	70.8	47	76.7
21	99.2	48	63.5
22	87.4	49	82.1
23	69.4	50	65.0
24	26.9	51	35.0
25	58.1	52	29.9
26	71.1	53	49.9
27	73.2	54	70.6

[a] Runs 1−27 correspond to noise level 1 (high-quality CO_2, 99.9999% purity); runs 28−54 correspond to noise level 2 (low-quality CO_2, 99.998% purity).

Table 5 shows the percentage of dearomatization of the extracts estimated by taking into account the SPME total area of the corresponding experiments related to

the initial extract (results showed above). As can be observed, an almost complete deodorization was obtained in runs 1, 13, 21, 31, and 41. Among them, the conditions of run 1 are the most favorable since the energy consumption is the minimun among those tested and neither modifiers nor raw material drying is necessary. These conditions also provide residual solvent elimination because in the chromatograms of the extracts corresponding to the mentioned runs, no solvent peak was observed. In Figure 3 the chromatograms with the same *y*-axis scale, corresponding to the SPME extracts of the raw rosemary extract and the CO_2 treated in conditions of run 1, are compared. The identities of the peaks, obtained by GC/MS, confirm that the compounds removed by SFE from the rosemary antioxidants are typical components of rosemary aroma.

The response selected for process optimization was the Taguchi signal-to-noise ratio (S/N) obtained according to eq 1 (larger-the-better case), which implied an achievement of the maximum dearomatization at the maximal S/N ratio. The equation, expressed in decibels, is:

$$\mathrm{S/N} = -10 \log[(1/y_1^2 + 1/y_2^2)/2] \qquad (1)$$

where y_1 and y_2 are the response values of each inner array row at the two noise factor levels.

Table 6 shows the average of the Taguchi signal-to-noise ratio (S/N) at each level (levels 1−3) of the factor and also for each interaction considered in the design obtained by using the values presented in Table 5. So, for the pressure (*P* factor), the average of the S/N responses for all experiments performed at level 1 is 36.27 dB. δ is the effect of each factor and interaction referred to the response S/N and is calculated as the difference between the highest and the lowest value of the S/N.

The effects of the factors and interactions, presented in decreasing order of importance, are shown in Figure 4. This graph can be used to discriminate among strong and weak factors. As can be seen, there are three groups of factors clearly differentiated. One group contains the following: % (w/w) residual extraction solvent, pressure, and temperature (*S*, *P*, and *T*), and its effect can be considered strong. The second group is formed by water as modifier and the interactions pressure−temperature and ethanol−temperature (*W*, *PT*, and *ET*). As can be seen in Figure 4, this group is placed after the estimated error (*e*); usually the error differentiates between the strong and weak effects, but in the present case, since the difference between the error and the values of the effects for the second group is minimal, there is a reasonable doubt about considering or not the influence of this group of factors and interactions. Figure 5 shows the differences between consecutive effects; as can be seen, there is a peak in difference 7 (which corresponds to factor *W*), which suggests that the last effect to be considered strong should be *W* (water as modifier).

Taking into account the results shown previously, the factors and interactions that will be considered as important in the process of preparation of a natural antioxidant from rosemary extract are the following, ordered by decreasing importance: % (w/w) residual extraction solvent (*S*), pressure (*P*), temperature (*T*), the interaction pressure−temperature (*PT*), the interaction

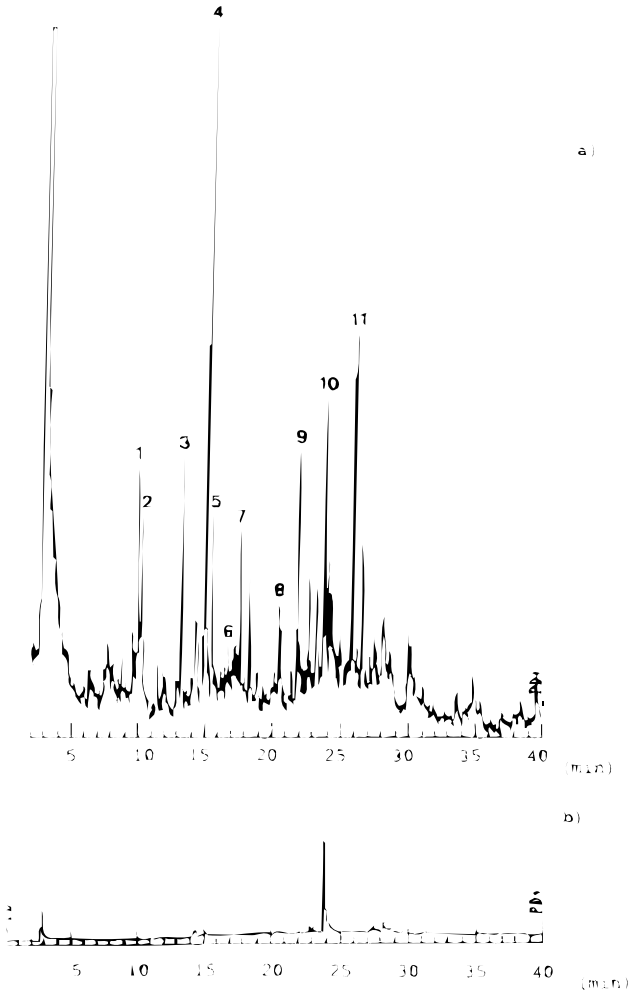

Figure 3. Chromatograms corresponding to (a) SPME of the raw rosemary extract and (b) SPME of rosemary extract treated with CO_2 at conditions of run 1. Peak identification: 1, α-pinene; 2, limonene; 3, linalool; 4, camphor; 5, borneol; 6. α-terpineol; 7, verberone; 8, nerol; 9, terpinolene; 10, *p*-cymen-8-ol; 11, caryophyllene.

ethanol−temperature (*ET*), and the use of water as modifier (*W*). The optimal conditions to obtain a product with an acceptable antioxidant activity and with a maximum dearomatization are the following: % w/w residual extraction solvent at level 1 (no drying necessary); pressure at level 2 (200 bar); temperature at level 2 (60 °C); ethanol at level 1 (0%); water at level 1 (0%); acetic acid had almost no influence in the process, so the easiest working conditions were selected that implied the use of 0% of acetic acid as modifier.

This set of conditions should yield maximum dearomatization; nevertheless, from the point of view of energy consumption, it is less favorable than the conditions of run 1, which has already provided an almost complete dearomatization.

In reference to the effect of the CO_2 quality, calculation of the differences between the average of dearomatization (percent) found in both series of data (with the two different qualities of CO_2) gave only a difference of 3.8, favoring the experiments performed with CO_2 of the highest quality. Taking into account the average value for both series, i.e., 72.3 and 90.8, the difference previously mentioned corresponds to 5%. Also, no negative effect could be detected in the antioxidant activity of the extracts prepared by using the CO_2 of the lowest quality. The average of the antioxidant activity values

Table 6. Average of Taguchi Signal-to-Noise Ratio (S/N) Obtained for the Factors and Interactions Considered in the Design

	P	*E*	*PE*	*PE*	*T*	*PT*	*PT*	*ET*	*S*	*W*	*ET*	Ac	*e*
level 1	36.2	35.3	35.5	36.4	34.3	36.3	36.3	34.8	38.1	36.6	35.2	36.2	37.5
level 2	37.4	36.8	36.6	34.7	37.5	36.6	34.8	37.0	36.7	34.4	35.2	36.3	34.7
level 3	33.6	35.2	35.1	36.2	35.5	34.4	36.2	35.5	32.5	36.2	36.9	34.8	35.0
δ	3.8	1.5	1.4	1.7	3.2	2.2	1.4	2.2	5.5	2.1	1.7	1.4	2.8

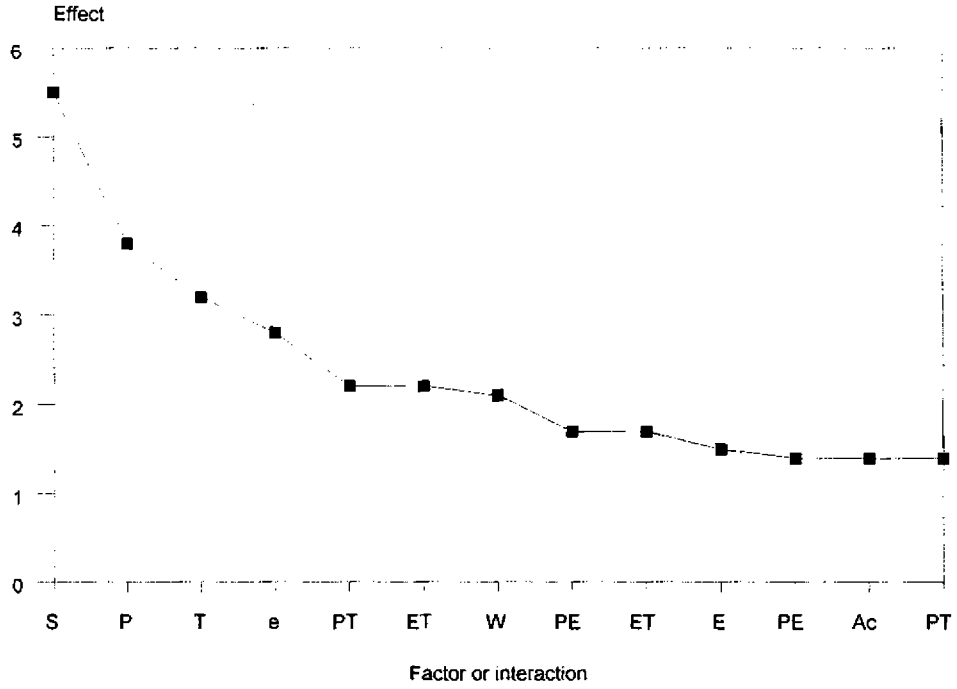

Figure 4. Graph representing the effects of factors and interactions presented in decreasing order of importance.

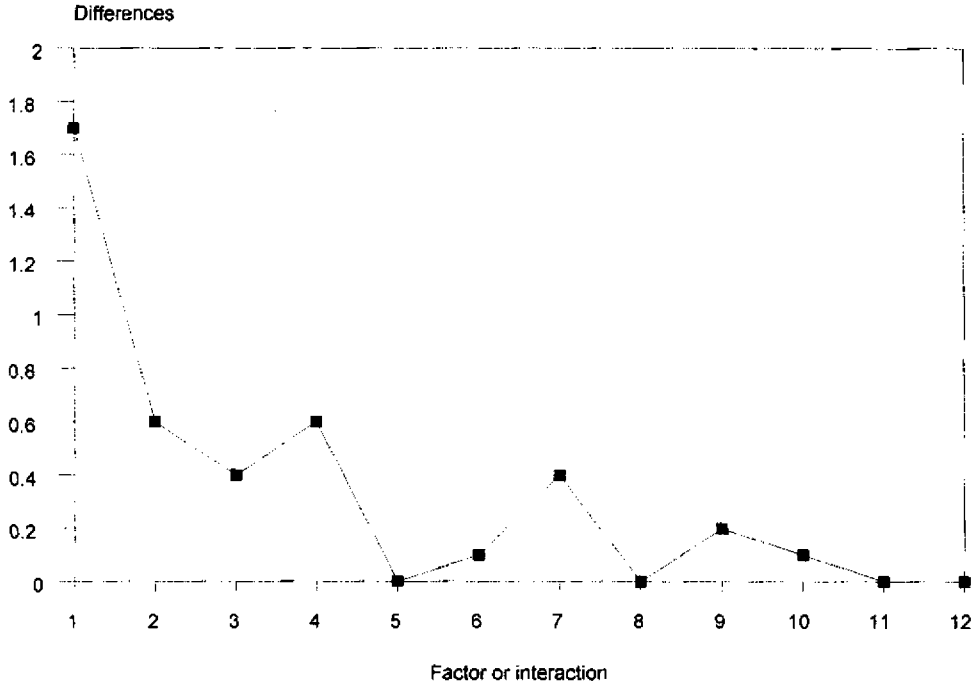

Figure 5. Graph showing the differences between the consecutive effects presented in Figure 4.

obtained by utilizing high-quality CO_2 was 33.8 (CV% = 7.3%), while the average for low-quality CO_2 was 36.5 (CV = 8.7%).

Since the present investigation has been performed at laboratory scale, the results obtained have to be considered as exploratory. Nevertheless, it is possible to conclude that by treatment with supercritical CO_2 of rosemary antioxidants obtained by solvent extraction, dearomatization close to 100% and complete solvent elimination are obtained. In such conditions the antioxidant activity of the raw materials remains constant as does the color of the sample. The proposed procedure yields products with medium antioxidant activity, but free of organic solvent residues and with very low

rosemary aroma, at an expected low cost at production scale due to the mild process conditions.

ABBREVIATIONS USED

BHA, butylhydroxyanisole; BHT, butylhydroxytoluene; SFE, supercritical fluid extraction; DPPH, 2,2-diphenyl-1-picrylhydrazyl hydrate; SPME, solid-phase microextraction; PTV, programmable temperature vaporizer; FID, flame ionization detector; S/N, Taguchi signal-to-noise ratio; *P*, pressure; *E*, ethanol; *T*, temperature; *S*, % w/w residual extraction solvent; *W*, water; Ac, acetic acid; *e*, error; *PE*, interaction pressure−ethanol; *PT*, interaction pressure−temperature; *ET*, interaction ethanol−temperature; CV, coefficients of variation.

LITERATURE CITED

Anonymous. Rosemary extract: effective against oxidation. *Lebensmitteltechnik* **1995**, *27* (3), 36−36.

Barbut, S.; Josephson, D. B.; Maurer, A. J. Antioxidant properties of rosemary oleoresin in turkey sausage. *J. Food Sci.* **1985**, *50*, 1356−1359.

Billot, P.; Pitard, B. Taguchi design experiments for optimizing the gas chromatographic analysis of residual solvent in bulk pharmaceuticals. *J. Chromatogr.* **1992**, *623*, 305−313.

Blanch, G. P.; Tabera, J.; Herraiz, M.; Reglero, G. Preconcentration of volatile components of foods: optimization of the steam distillation-solvent extraction at normal pressure. *J. Chromatogr.* **1992**, *628*, 261−268.

Brieskorn, C. H.; Fuchs, A.; Bredenberg, J. B.; McChesney, J. D.; Wenkert, E. The structure of carnosol. *J. Org. Chem.* **1966**, *29*, 2293−2297.

Chang, S. S.; Ostric-Matijasevik, B.; Hsieh, O. A. L.; Huang, C. L. Natural antioxidants from rosemary and sage. *J. Food Sci.* **1977**, *42* (4), 1102−1106.

Chen, Q.; Shi, H.; Ho, C. T. Effects of rosemary extracts and major constituents on lipid oxidation and soybean lipoxigenase activity. *J. Am. Oil Chem. Soc.* **1992**, *69* (10), 999−1002.

Chipault, J. R.; Mizumo, G. R.; Hawkins, J. M.; Lundberg, W. O. The antioxidant properties of natural spices. *Food Res.* **1952**, *17*, 46−55.

Cuvelier, M. E.; Berset, C.; Richard, H. Antioxidant constituents in sage. *J. Agric. Food Chem.* **1994**, *42*, 665−669.

Djarmati, Z.; Jankov, R. M.; Schwirtlich, E.; Djulinac, B.; Djordjevic, A. High antioxidant activity of extracts obtained from sage by supercritical CO_2 extraction. *J. Am. Oil Chem. Soc.* **1991**, *68* (10), 731−734.

Gerard, D.; Quirin, K.; Schwarz, E. CO_2 extracts from rosemary and sage. *Food Market. Technol.* **1995**, *9*, 46−55.

Gonzáles Cartagena, L. Contribución al estudio de la estabilidad fisico-química del vino. Ph.D. Dissertation. The University Polytechnic of Madrid at Madrid, 1990; pp 112−121.

Ibáñez, E.; Tabera, J.; Reglero, G.; Herraiz, M. Optimization of separation of fat-soluble vitamins by supercritical fluid chromatography using serial micropacked columns. *J. Agric. Food Chem.* **1995**, *43*, 2667−2671.

Inatani, R.; Nakatani, N.; Fuwa, H. Antioxidative effect of constituents of rosemary (*Rosmarinus officinalis* L.) and the derivatives. *Agric. Biol. Chem.* **1983**, *47*, 521−528.

Iriarte, J.; Villanueva, M. R.; Iturri, J. M. Estudio comparativo de antioxidantes en derivados cárnicos. Posible efecto sustitutivo del extracto de romero. *Aliment. Equipos Tecnol.* **1992**, April, 87−92.

Kramer, R. E. Antioxidants in clove. *J. Am. Oil Chem. Soc.* **1985**, *62* (1), 111−113.

Lamaison, J. L.; Petitjean-Freyret, C.; Carnat, A. P.; Carnat, A. Activities antiradicalaires in vitro d'huiles essentielles. *Plant. Med. Phytother.* **1988**, *22*, 231−234.

Muehlnikel, T. Extraction with carbon dioxide. Manufacture of dearomatized rosemary antioxidant. *Food Market. Technol.* **1992**, *6* (4), 37−38.

Nguyen, U.; Frakman, G.; Evans, D. A. Process for extracting antioxidants from Labiatae herbs. U.S. Pat. US5017397, 1991.

Nguyen, U.; Evans, G.; Frakman, G. Natural antioxidants produced by supercritical extraction. In *Supercritical Fluid Processing of Food and Biomaterials*; Rizvi, S. S. H., Ed.; Blackie Academic & Professional: Glasgow, U.K., 1994; 103 pp.

Oles, P. J.; Yankovich, A. Taguchi design experiments for optimizing the performance of a gas chromatograph and a mass selective detector. *LC-GC Int.* **1989**, *7* (7), 579−583.

Peace, G. S. *Taguchi methods. A Hands-on Approach to Quality Engineering*; Addison-Wesley: Reading, MA, 1993.

Rac, M.; Ostric-Matijasevic, B. The properties of rosemary as an antioxidant. *Rev. Fr. Corps Gras* **1955**, *2*, 796−803.

Saito, Y.; Kimura, Y.; Sakamoto, T. Studies on the antioxidant properties of spices II. The antioxidative effect of some spices. *J. Jpn. Soc. Food Nutr.* **1976**, *29* (9), 404−408.

Schwarz, K.; Ternes, W. Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis*. I. Determination of phenolic diterpenes with antioxidative activity amongst tocochromanols using HPLC. *Z. Lebensm. Unters. Forsch.* **1992a**, *195* (2), 95−98.

Schwarz, K.; Ternes, W. Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis*. II. Isolation of carnosic acid and formation of other phenolic diterpenes. *Z. Lebensm. Unters. Forsch.* **1992b**, *195* (2), 99−103.

Schwarz, K.; Ternes, W.; Schmauderer, E. Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis* III. Stability of phenolic diterpenes of rosemary extracts under termal stress as required for technological process. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 104−107.

Taguchi, G. *System of Experimental Design. Engineering Methods To Optimize Quality and Minimize Costs*; Kraus International Publications: White Plans, NY, 1987.

Zhang, Z.; Yang, M. J.; Pawliszyn, J. Solid-Phase Microextraction. *Anal. Chem.* **1994**, *66* (17), 844−853.

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