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**Simultaneous extraction of rosemary and spinach leaves and its
effect on the antioxidant activity of products**

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Abstract

Mixed vegetal extracts are interesting target of new products as nutraceuticals, superior ingredients for the design of functional food, singular ingredients for cosmetics, etc. In this work the extraction of a mixture of spinach and rosemary leaves (50 % weight of each plant) was investigated in terms of its antioxidant activity, and compared with the extraction of the separate species. Phenolic diterpenes of rosemary and carotenoids of spinach were target compounds due their recognized biological activities. Two different extraction techniques were applied, namely pressurized liquid extraction using hexane at two different temperatures (100 and 150°C) and supercritical fluid extraction with pure carbon dioxide at 40°C and two different pressures (20 and 30 MPa). For each extraction technique and conditions three different raw materials were employed: spinach leaves, rosemary leaves and the mixture 50:50 of spinach and rosemary leaves.

The antioxidant activity of the samples produced was studied in terms of the ABTS assay and showed to be enhanced when the species are simultaneously extracted, with TEAC values around 20% higher than the values corresponding to mixing the extracts obtained by separate. A possible synergic effect between carotenoids and phenolic diterpenes was studied, although no specific synergic activity could be observed. However, the enhanced antioxidant activity could be attributed to a definite increase of the concentration of carnosic acid, which was observed in the samples produced by the simultaneous extraction.

Keywords: Supercritical fluid extraction; Pressurized liquid extraction; Antioxidants; Phenolic diterpenes; Carotenoids; Spinach; Rosemary.

1. Introduction

Antioxidants play a very important role in the food, cosmetic and pharmacy industries [1]. Both phenolic compounds and carotenoids have been identified as important antioxidant compounds present in natural matter. Furthermore, it has been reported that some antioxidants may act synergistically, thus being much more effective response against oxidation. The most studied synergism between antioxidants is between β -carotene and vitamins C and E [2-5].

Numerous plants and herbs have been recognized as a source of natural antioxidants. Among them, rosemary (*Rosmarinus officinalis* L.) is one of the *Lamiaceae* plants with large antioxidant activity. The substances related with its antioxidant activity are phenolic diterpenes such as carnosol, rosmanol, carnosic acid, methyl carnosate, and phenolic acids such as rosmarinic and caffeic acids. Particularly, carnosic acid and carnosol are the most abundant antioxidants present in rosemary extracts [6-10].

On the other side, spinach (*Spinacia oleracea*) is an edible flowering plant (*Amaranthaceae* family) native to central and southwestern of Asia, now cultivated all over the world, which is renowned for its high content of carotenoids. Numerous studies about its anti-carcinogenic, antimicrobial and antioxidant activity of spinach have been reported in recent years [11-13]. Besides carotenoids (mainly lutein and β -carotene) [14], other bioactive substances identified in spinach are phenolic compounds, such as flavonoids and phenolic acids (p-cumaric, galic and ferulic acids) [12, 15] and fatty acid derivative compounds, such as glycoglycerol lipids [16] and lipoic acid [17].

The extraction of antioxidants from plant matrix could be accomplished by different techniques. Solid-liquid extraction is a traditional and much utilized technology in which varying the solvent the recovery of target molecules could be attained. For example, carotenes are readily extracted using non-polar solvents (hexane, pentane, and petroleum ether) or moderate polar solvents (dichloromethane); phenolic compounds are usually extracted using water [12] and glycoglycerol lipids using ethanol or methanol [16]. As it is well-known, one of the main drawbacks of solid-liquid extraction is the large consumption of organic solvents. In this respect, pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE) are intensively investigated as more efficient extraction technologies.

Several works were reported about the extraction of carotenoids of spinach using conventional solid-liquid extraction with different solvents. For example, Bunea et al. [14] determined the content of carotenoids in fresh, stored and processed spinach by using a

1 solvent mixture comprised by methanol, ethyl acetate and petroleum ether, Pellegrini et al.
2 [18] extracted carotenoids of fresh spinach with acetone, and Simonovska et al. [19]
3 quantified lutein in spinach extracts obtained using water and triethylammonium acetate.
4 However, there is no bibliographic information, according to our knowledge, about the
5 extraction of carotenoids of spinach by SFE or PLE. The latter has been used to extract
6 flavonoids from spinach but no carotenoids were investigated [15] although this technique is
7 readily used to extract these compounds from other vegetal matrix, such as algae or carrot by-
8 products [20-23].

14 With respect to the extraction of the phenolic diterpenes of rosemary many publications could
15 be cited. The reader is referred to the works of García-Risco et al. [18, 24], Fornari et al. [25],
16 Herrero et al. [26, 27] or Hossain et al. [28] in which the most important contributions
17 regarding the SFE or PLE of rosemary are discussed.

22 Mixed vegetal extracts are of high interest as target of new products due to the synergic
23 effects among certain phytochemicals that could produce a much more active response. In
24 this respect, the simultaneous extraction of a mixture of the different vegetal species is of
25 high interest from a processing point of view, since manufacture costs may be considerable
26 reduced. Thus, the product obtained from the extraction of the mixture of species should be
27 of similar (or better) quality than the product obtained by mixing the separate extracts.

32 In this work, the PLE and SFE of a mixture of spinach and rosemary leaves (50 % weight of
33 each plant) was investigated and compared with the extraction of the separate species, with
34 the target of assess the effect on the antioxidant quality of the products obtained. To our
35 knowledge, this is the first time that the simultaneous extraction of spinach and rosemary
36 leaves is studied. Carotenoids and phenolic diterpenes, due to their lipid affinity, can be
37 readily extracted using non-polar paraffinic solvents, such as pentane, hexane or heptane
38 fractions, so as CO₂, which at supercritical conditions has a solvent power similar to that of
39 pentane. Thus, hexane was employed in PLE assays and pure supercritical CO₂ in the SFE
40 experiments.

49 The extraction yield and recovery of selected antioxidant substances, namely β -carotene and
50 lutein in spinach, and carnosic acid and carnosol in rosemary, were studied in terms of the
51 composition of the plant matter employed as raw material. Additionally, the antioxidant
52 activity of the different extracts was evaluated in order to determine potential synergic effects
53 among these main antioxidants present in these vegetal species.

2. Materials and methods

2.1 Chemicals and reagents

Carnosic acid ($\geq 96\%$) and Carnosol were purchased from Alexis Biochemical (Madrid, Spain). β -carotene (95 %) were purchased from Sigma-Aldrich (Madrid, Spain). Lutein ($\geq 95\%$) was purchased from Extrasynthese (Genay Cedex, France). Ethanol and phosphoric acid (85 %) were HPLC grade from Panreac. Acetonitrile, methanol and methyl-tert-butyl ether were HPLC grade from Lab Scan (Gliwice, Poland). Triethylamine was purchased from Sigma-Aldrich (Madrid, Spain). CO_2 (N38) was supplied from Air Liquid. Washed sea sand (particle size 0.25-0.30 mm) was purchased from Panreac (Barcelona, Spain).

2.2 Preparation of samples

Plant material consisted of dried leaves obtained from an herbalist's producer (Murcia, Spain). Water content in the spinach and rosemary samples was, respectively, 4.9 % weight and 8.3 % weight. The samples were ground in a cooled mill and were sieving to the appropriate size (between 200 and 600 μm). Thus, similar particle size was obtained for each batch of plant matrix. The 50:50 mixture of spinach and rosemary was obtained by homogenization of same amounts of ground rosemary and spinach.

2.3 Extraction methods

Pressurized liquid extraction (PLE): extractions with liquid hexane were carried out in an ASE 350 system from Dionex Corporation (Sunnyvale, CA, USA) equipped with a solvent controller unit. Hexane was selected as PLE solvent due to the good solubility that carotenoids and carnosic acid exhibit in this solvent.

Each extraction cell (10 ml capacity) was filled with 1 g of solid sample and 1 g of sea sand as a sandwich, and then placed into an oven. Then, the cell was filled with hexane up to a pressure of 1500 psi (which ensures the liquid state of the solvent at both temperatures studied) and was heated-up to the desired temperature. Static extractions were performed at 100 and 150°C during 10 minutes. After extraction the cell was washed with the solvent and subsequently the solvent was purged from cell using N_2 gas until complete depressurization was accomplished. The extracts were recovered in glass vials and the solvent was eliminated by evaporation under vacuum and then dried in a stream of N_2 . All experiments were carried out by duplicate. The dried samples obtained were stored at 4 °C in the dark until analysis.

1 *Supercritical fluid extraction (SFE)*: trials were carried out in a pilot-plant scale supercritical
2 fluid extractor (Thar Technology, Pittsburgh, PA, USA, model SF2000) comprising a 2 L
3 cylinder extraction cell with automatic control of temperature and pressure. A detail
4 explanation of the experimental device can be found elsewhere [29].
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7 For each experiment, the cell was filled with 0.4-0.5 kg of plant raw material. The extractions
8 were performed at 40°C and two different pressures (20 and 30 MPa) were employed. No
9 cosolvent was employed since both carotenoids and phenolic diterpenes can be satisfactory
10 extracted using pure CO₂. The extraction time was 5 h, no fractionation of the extract was
11 performed and the supercritical solvent (CO₂) flow rate was set to 60 g/min in all
12 experiments.
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16 Ethanol was used to wash out the collector vessel and ensure a complete recovery of the
17 material precipitated in the cell. Ethanol was eliminated by evaporation and the homogeneous
18 solid samples obtained were kept at 4°C in the dark until analysis. All experiments were
19 carried out by duplicate.
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25 26 27 **2.4 HPLC analysis**

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29 *Quantification of carnosic acid and carnosol*: samples were analyzed employing a HPLC
30 (Varian Pro-star) equipped with a MICROSORB-MV-100 C18 column (Varian) of 250 mm
31 × 4.6 mm and 5 µm particle size. The mobile phase consisted of acetonitrile (solvent A) and
32 0.1 % of phosphoric acid in water (solvent B) applying the following gradient: 0–8 min, 23 %
33 A and 8-25 min, 77 % A. This last composition was kept for 15 minutes and initial conditions
34 were gained in 5 min. Total time analysis was 45 minutes. The flow rate was constant at 0.7
35 mL/min. Injection volume was 20 µL and the detection was accomplished by using a diode
36 array detection system Varian storing the signal at a wavelength of 230 and 280 nm.
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40 *Quantification of β-carotene and lutein*: samples were analyzed employing a HPLC model
41 Agilent 1260 Infinity (Agilent, Santa Clara, USA) equipped with a KROMASIL 100 C18
42 column (Scharlab, Barcelona, Spain) of 25 mm × 4.6 mm and 3.5 µm particle size. The
43 mobile phase is constituted by solvent A, MeOH:H₂O:triethylamine (90:10:0.1, % vol) and
44 solvent B, methyl-tert-butyl ether: MeOH:H₂O:triethylamine (90:6:4:0.1, % vol). The
45 gradient started with 93 % A to 0 % A from 0 to 34 min and recovers the initial conditions of
46 the method in 4 min. Total time analysis was 38 minutes. During analysis the column was
47 maintained at 25°C. The flow rate was constant at 1 mL/min and the injection volume was 20
48 µL. For detection were assigned the wavelength of 450, 470, 550, 660 nm.
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2.5 Determination of antioxidant activity

ABTS^{•+} assay. The ABTS^{•+} assay described by Re et al. [30] was used to measure the antioxidant activity of the extracts. Briefly, ABTS^{•+} radical cation was generated by reacting 7 mmol/l ABTS with 2.45 mmol/l potassium persulfate after incubation at room temperature for 16 h in the dark. The ABTS^{•+} radical solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. 10 μ l of each extract (previously dissolved) at four different concentrations was added to 0.990 ml of diluted ABTS^{•+} radical solution. The reaction was allowed to stand until the absorbance reached a plateau, and the absorbance was recorded at 734 nm. Trolox was used as reference standard, and results were expressed as TEAC values (mmol TE/g extract). All analyses were done in triplicate.

Synergy assays. Synergy assays were done between carotenoids (β -carotene and lutein) and phenolic compounds (carnosic acid and carnosol) at three different levels. Results were compared with the estimated values calculated to each mixture. Moreover, this synergy assay was also carried out with a mixture of spinach and rosemary extracts.

3. Results and discussion

3.1 Extraction yield

The extraction techniques and conditions investigated are summarized in Table 1. For experiments 1 to 6 of Table 1, the raw materials extracted were (i) spinach leaves, (ii) rosemary leaves and (iii) the mixture comprising 50:50 weight spinach and rosemary leaves.

The extraction yields obtained in the different extractions are given in Table 2 and represented in Figure 1: S denotes spinach leaves (0% rosemary), SR the mixture 50% spinach and 50% rosemary, and R represents 100% rosemary leaves. As expected, PLE produced higher yields than CO₂-SFE. The temperature increase in PLE produces a significant increase in yield because higher temperature promotes higher analyte solubility, decreases the viscosity and surface tension of solvents, thus improving extraction rate. On the other hand, increasing the pressure from 200 to 300 bar in SFE does not produce a significant change in terms of total yield.

As can be observed in Figure 1, for both extraction methods applied and for all conditions employed, a linear correlation between the composition of the raw material and the extraction yield was obtained. Lineal regression coefficients (R^2) were higher than 0.97 in all cases and

thus, it can be stated that the influence of extracting mixed species on extraction yield is not noteworthy. That is, the extraction yields obtained experimentally when processing the mixed leaves (Y_{SR}^{exp}) are very close to the yields calculated as the mean values of the yields obtained in the extraction of the separate plants ($Y_{SR}^{cal} = (Y_S^{exp} + Y_R^{exp}) / 2$).

Figure 2 shows the Y_{SR}^{exp} and Y_{SR}^{cal} values together with the corresponding (experimental and calculated) standard deviations; for all type of extractions and solvents differences between both values are not significant. Then, despite the extraction procedure or conditions applied, the results obtained suggest that yield is not significantly enhanced or reduced when the mixture spinach/rosemary is extracted in comparison with processing the separate species.

3.2 Antioxidant activity and chemical analysis of samples

The antioxidant activity of the samples obtained by extraction of the mixture of species (SR) was compared with the antioxidant activity of the pure extracts (S and R) and their mixture (S+R).

As can be observed in Table 3 for extractions 1 and 4 of Table 1, rosemary extracts (R) are much more active than spinach extracts (S). Additionally, the sample denoted as S+R in Table 3, which was obtained by mixing equal amounts of S and R, presents TEAC values very close to the corresponding calculated mean value (differences lower than 2.3%). Nevertheless, the product indicated as SR in Table 3, which was obtained by the simultaneous extraction of spinach and rosemary leaves (50:50), resulted in noteworthy higher antioxidant activity. That is, the TEAC values of SR samples are around 20% higher than the TEAC values of the S+R samples. This effect could be attributed to small modifications in the composition of the extracts, due to the presence of both raw materials in the extraction cell, or even to synergistic effect between the antioxidant substances present in spinach and rosemary. In this regard, Hait-Dashan et al. [5] reported a synergistic activity between a spinach extract rich in aromatic polyphenols [31] and some phenolic compounds such as ferulic acid, caffeic acid and epigallocatechin-3-gallate. In order to elucidate whether small increase of certain components or synergistic effect between them explain the higher TEAC values of SR in comparison to S+R mixture, chemical characterization of the extracts was done, the main antioxidant substances in the extracts were identify and the potential synergistic effect between them was investigated using standards.

The concentrations (mg compound / g extract) of phenolic diterpenes (carnosic acid and carnosol) and carotenoids (β -carotene and lutein) were determined for all extracts obtained and are reported in Table 4. As expected, carnosic acid and carnosol were not detected to be present in spinach extracts and only very low concentrations of carotenoids were determined in rosemary extracts. Furthermore, taking into account the low polarity of solvents employed, no phenolic acids, such as rosmarinic acid in rosemary or ferulic acid in spinach, were detected in the samples.

The higher concentrations of carnosic acid were obtained using hexane as solvent (R and SR extracts) and carnosol was found in very low concentrations in all samples studied. About the quantification of carotenoids, significant higher concentrations of β -carotene were obtained in the SFE extracts and, according to the higher polarity of lutein in comparison with β -carotene, higher CO₂ density were required to obtain significant concentration of lutein in the SFE extracts (Extraction 4).

Table 4 also reports the expected concentration of antioxidant compounds in SR extracts, calculated as the mean value of the concentrations obtained in the extraction of the separate plants (S and R samples). In the case of β -carotene, it can be clearly stated that its extraction is reduced when the mixed raw material is processed, with experimental concentrations around 1.5 times lower in the SFE extracts, and ca. 2.5 times lower in the hexane PLE extracts. Figure 3 shows a comparison of the experimental β -carotene concentrations obtained in SR extracts and the corresponding mean values. Also represented in the figure are the standard deviations obtained, which indicate that differences are significant and thus, it could be accepted the observed decrease of β -carotene extraction in SR samples. In general, this behavior was also observed for lutein, particularly in the case of the SFE extractions.

On the contrary, according to the results given in Table 4 for carnosic acid, it could be argued that the extraction of carnosic acid is enhanced when the mixed material is processed (see Figure 4).

Among target compounds studied, β -carotene and lutein were the main carotenoids identified in spinach extracts, and carnosic acid and carnosol were the main phenolic diterpenes quantified in rosemary extracts. Thus, synergistic assays between β -carotene or lutein and carnosic acid or carnosol were carry out. The TEAC values obtained for the mixtures of carnosic acid + β -carotene or lutein are given in Table 5 and those corresponding to carnosol + β -carotene or lutein mixtures are reported in Table 6. According to the TEAC values

obtained the order of antioxidant capacity of the standards is as follows: carnosic acid > β -carotene > carnosol \approx lutein. As can be observed in these tables, for all phenolic compound + carotenoid mixtures studied no synergic enhancement of the antioxidant activity was observed when comparing the experimental TEAC value of the mixture with the corresponding calculated mean (linear) TEAC value. On the contrary, it was obtained a general decrease of the TEAC value of the phenolic compound + carotenoid mixture with respect to the corresponding mean theoretical value in certain cases.

As an example, Figure 5 shows the comparison between (a) the variation of TEAC values in carnosic acid + β -carotene mixtures and (b) the TEAC values obtained in the samples produced by extracting spinach, rosemary and a mixture 50:50 of spinach and rosemary leaves (SR) (Extractions 1 and 4 in Table 1). As can be observed in Figure 5, the mixtures of carnosic acid + β -carotene show similar TEAC values than the expected mean values, moreover, TEAC values of S+R showed a similar behavior, whereas the antioxidant activity of SR was significant enhanced in comparison with the expected mean value. Therefore, taking into account the analysis of the composition of the extracts given in Table 6, it could be stated that the observed increase of the antioxidant activity of the SR extracts could be a consequence of an enhancement of the extraction of carnosic acid, produced when both raw materials (spinach and rosemary) are simultaneously extracted, and synergistic effects between carotenoids from spinach and phenolic diterpenes from rosemary could be discarded. Furthermore, Figure 6 shows that the TEAC values of all extracts obtained (S, R, SR and S+R) could be satisfactory correlated with the concentration of carnosic acid present in the sample.

Conclusions

The product obtained from the simultaneous extraction of spinach and rosemary leaves was investigated to ascertain an enhancement of antioxidant activity, due to presumed potential synergic effects between carotenoids from spinach and phenolic diterpenes from rosemary. PLE using hexane and SFE with pure CO₂ were utilized as extraction technologies; these solvents were selected due to their good affinity to extract carotenoids and carnosic acid. The product obtained from the extraction of a mixture 50:50 spinach and rosemary leaves (SR) was compared with the extraction of solely spinach (S) and rosemary (R), and with the sample obtained by mixing equal amounts of S and R (S+R sample).

1 The antioxidant activities of the SR extracts were 20% higher than the antioxidant activities
2 of the S+R samples, which is a very attractive result in order to target new spinach-rosemary
3 mixed products. This effect could be explained by an increase in the concentration of
4 carnosic acid observed in the SR extracts, which was around 10-20% greater than the
5 expected mean values, as not synergic effects between carotenoids (β -carotene and lutein) of
6 spinach and phenolic diterpenes (carnosic acid and carnosol) of rosemary were found.
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10 11 12 13 14 **Acknowledges** 15

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Table 1. Methods and conditions employed in the extraction of spinach, rosemary and mixed spinach/rosemary (50:50) leaves.

Extraction number	Extraction method	Solvent	T (°C)	P (MPa)	Extraction time	solvent / raw material ratio (kg/kg)
1	ASE	hexane	100	10	10 min	18
2	ASE	hexane	150	10	10 min	18
3	SFE	CO ₂	40	20	5 h	33-54
4	SFE	CO ₂	40	30	5 h	33-54

Table 2. Yields obtained (%) in extractions 1 to 4 of Table 1. S: spinach leaves; SR: spinach/rosemary (50:50) leaves; R: rosemary leaves.

Extraction number	Plant matrix		
	S	SR	R
1	4.26	6.65	9.87
2	7.16	10.91	15.63
3	1.75	2.66	3.14
4	1.82	2.76	4.45

Table 3. TEAC values of the samples obtained by extraction of spinach leaves (S), rosemary leaves (R), the mixture of species (SR) and by mixing the pure extracts (S+R).

	Experimental value* (mmol Trolox/g)	Calculated mean value (mmol Trolox/g)
PLE, hexane, 100°C (Ext. 1 in Table 1)		
S	0.229 ± 0.005	
R	0.721 ± 0.018	
SR	0.565 ± 0.004	
S+R	0.475 ± 0.008	0.475
SFE, 30 MPa, 40°C (Ext. 4 in Table 1)		
S	0.109 ± 0.001	
R	0.578 ± 0.009	
SR	0.420 ± 0.006	
S+R	0.352 ± 0.002	0.344

*Mean ± S.D.

Table 4. Composition (mg of compound / g of extract) of antioxidant compounds identified in the extracts obtained from experiments 1 to 4 of Table 1. S: spinach leaves; SR: spinach/rosemary (50:50) leaves; R: rosemary leaves. EXP: experimental value; MV: calculated mean value.

Extraction number	Plant matrix	Phenolic compounds				Carotenoids			
		carnosic acid		carnosol		β -carotene		lutein	
		EXP	MV	EXP	MV	EXP	MV	EXP	MV
1	S	n.i.		n.i.		7.52		1.49	
	SR	101.13	80.75	3.93	6.82	1.47	3.88	1.01	0.75
	R	161.49		13.64		0.23		n.i.	
2	S	n.i.		n.i.		4.57		0.88	
	SR	58.13	52.10	1.81	4.81	0.97	2.37	0.64	0.45
	R	104.20		9.62		0.17		0.01	
3	S	n.i.		n.i.		11.38		0.58	
	SR	30.19	26.44	n.i.		4.10	6.01	n.i.	0.29
	R	52.88		n.i.		0.64		n.i.	
4	S	n.i.		n.i.		13.28		4.70	
	SR	54.34	47.42	0.00	3.41	5.08	7.10	0.53	2.35
	R	94.84		6.81		0.92		n.i.	

n.i.: non-identified

Table 5. TEAC values of carnosic acid, β -carotene, lutein and their mixtures.**(a) Carnosic acid + β -carotene**

Carnosic acid (%)	β -carotene (%)	TEAC value (mmol Trolox/g)	
		Experimental value	Calculated mean value
100	0	5.548 ± 0.076	-
63	37	5.057 ± 0.192	5.079
36	64	4.710 ± 0.101	4.737
15	85	4.513 ± 0.061	4.479
0	100	4.296 ± 0.104	-

(b) Carnosic acid + lutein

Carnosic acid (%)	Lutein (%)	TEAC value (mmol Trolox/g)	
		Experimental value	Calculated mean value
100	0	5.722 ± 0.154	-
57	43	4.821 ± 0.463	4.911
30	70	4.144 ± 0.142	4.423
13	87	4.049 ± 0.060	4.093
0	100	3.859 ± 0.084	-

Table 6. TEAC values of carnosol, β -carotene, lutein and their mixtures.

(a) Carnosol + β -carotene

Carnosol (%)	β -carotene (%)	TEAC value (mmol Trolox/g)	
		Experimental value	Calculated mean value
100	0	3.724 ± 0.058	-
75	25	3.542 ± 0.093	3.862
50	50	3.837 ± 0.116	4.000
25	75	3.860 ± 0.137	4.138
0	100	4.276 ± 0.044	-

(b) Carnosol + lutein

Carnosol (%)	Lutein (%)	TEAC value (mmol Trolox/g)	
		Experimental value	Calculated mean value
100	0	3.884 ± 0.071	-
69	31	3.770 ± 0.182	3.807
43	57	3.589 ± 0.058	3.741
20	80	3.676 ± 0.299	3.683
0	100	3.633 ± 0.040	-

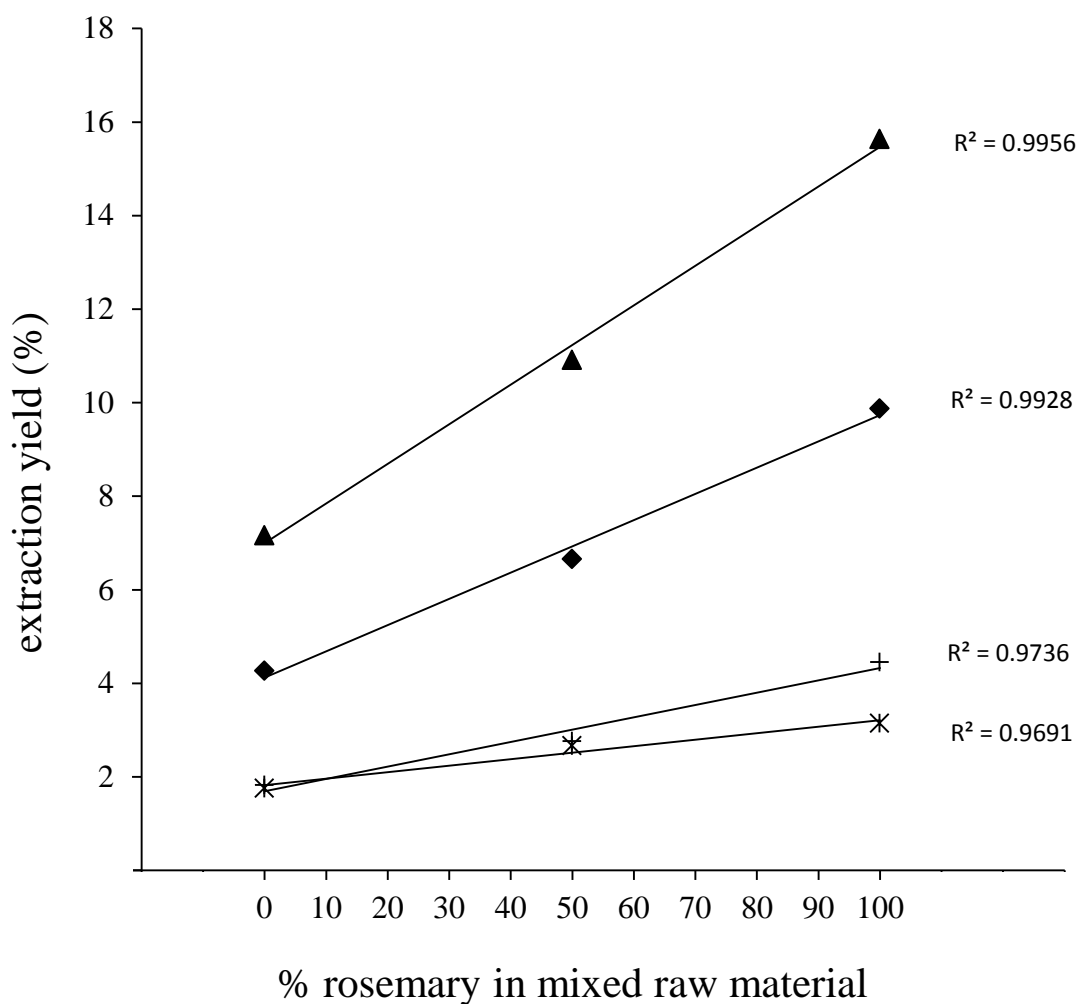


Figure 1. Extraction yield as a function of the percentage of rosemary leaves present in plant raw material: (◆) Hexane ASE at 100°C (Ext. 1); (▲) Hexane ASE at 150°C (Ext. 2); (*) CO₂ SFE at 20 MPa (Ext. 3); (+) CO₂ SFE at 30 MPa (Ext. 4).

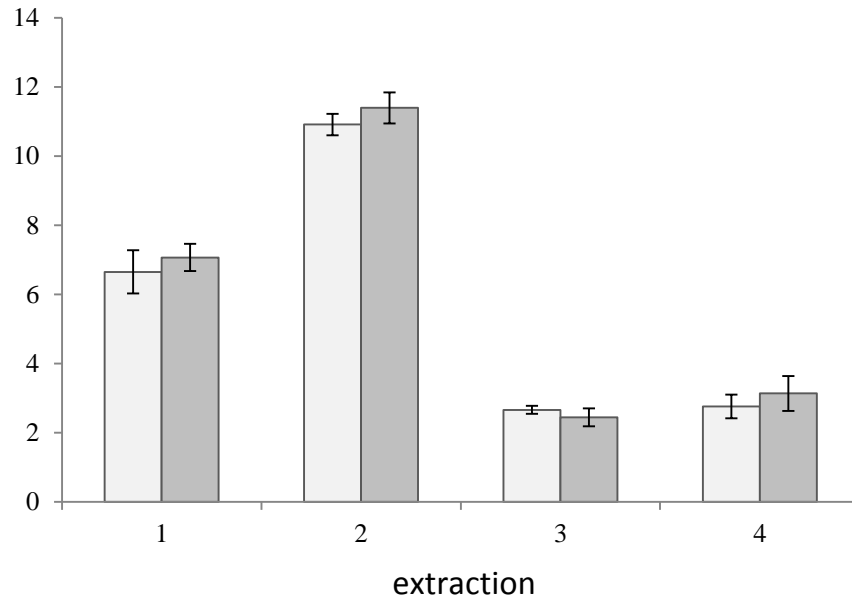




Figure 2. Extraction of spinach/rosemary leaves mixture (50 weight % of each plant): comparison between () experimental yields Y_{SR}^{exp} and () yields calculated as the mean values of the yields obtained in the extraction of the separate plants ($Y_{SR}^{\text{cal}} = (Y_S^{\text{exp}} + Y_R^{\text{exp}}) / 2$). 1 and 2: PLE; 3 and 4: SFE.

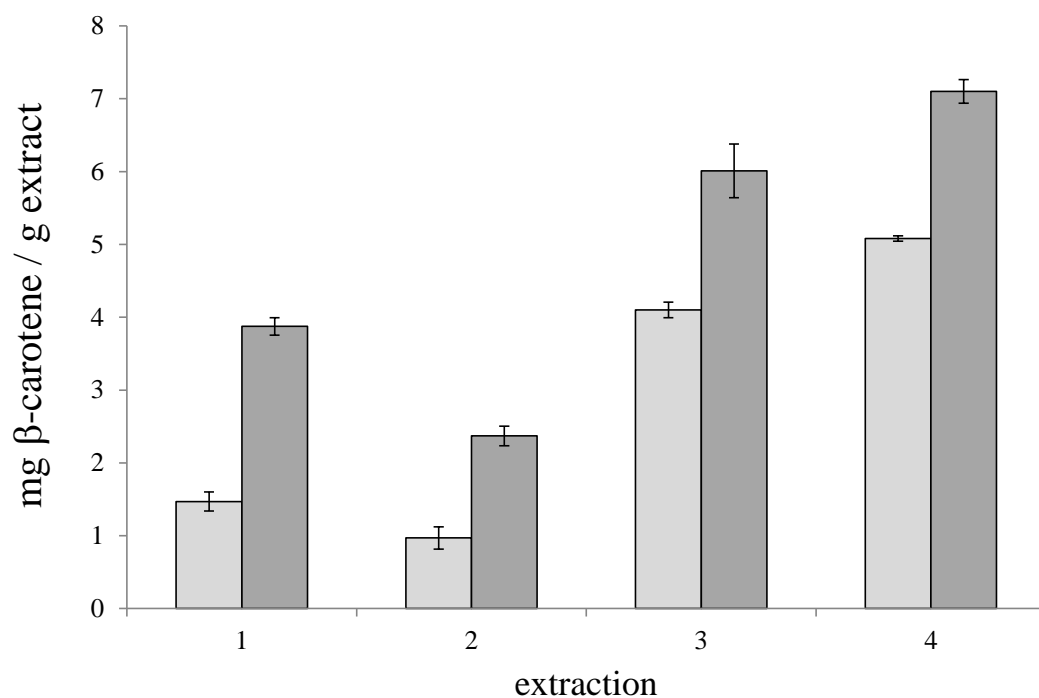


Figure 3. Spinach/rosemary leaves mixture (50 weight % of each plant): comparison between () experimental β -carotene concentrations and () values calculated as the mean values of the concentrations obtained in the extraction of the separate plants. 1 and 2: PLE; 3 and 4: SFE.

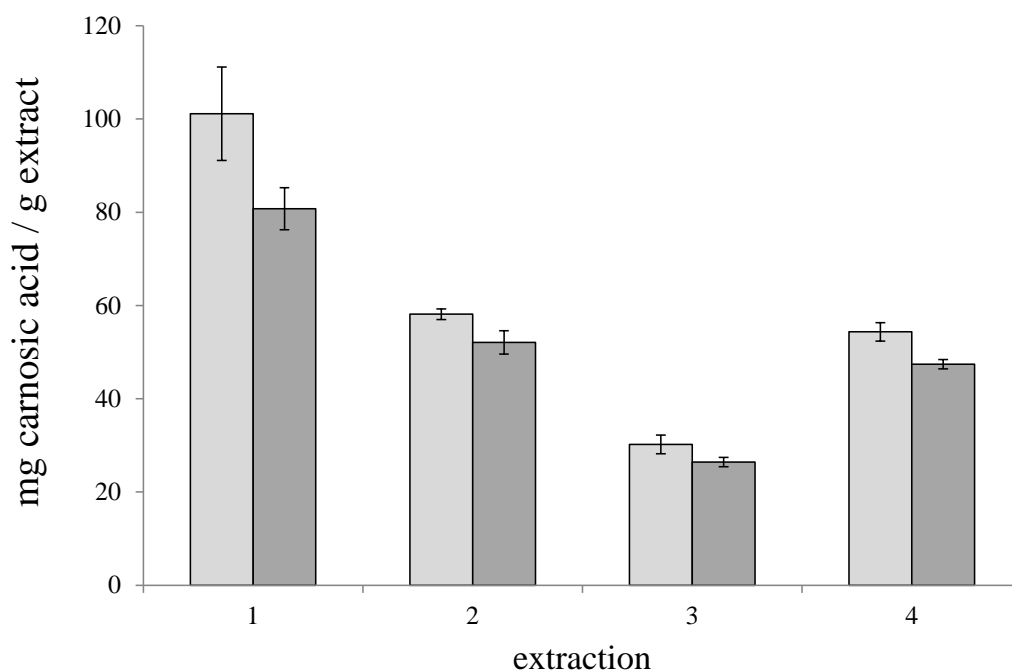


Figure 4. Spinach/rosemary leaves mixture (50 weight % of each plant): comparison between (□) experimental carnolic acid concentrations and (■) values calculated as the mean values of the concentrations obtained in the extraction of the separate plants. 1 and 2: PLE; 3 and 4: SFE.

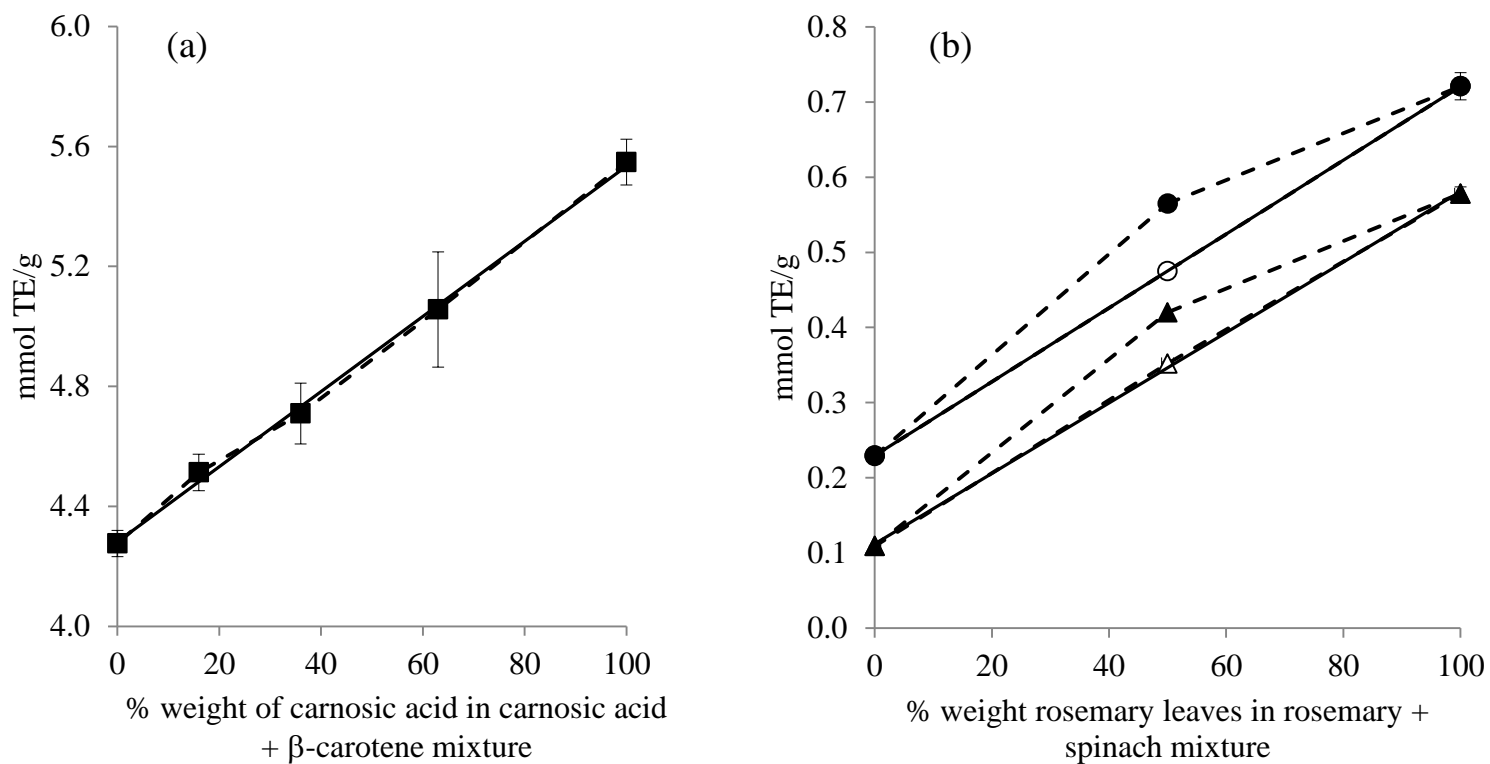


Figure 5. Comparison between (a) the variation of TEAC values in carnosic acid + β -carotene mixtures and (b) the TEAC values obtained in the samples produced by extracting spinach leaves (S), rosemary leaves (R) and a mixture 50:50 of spinach and rosemary leaves (SR). (●) PLE with hexane at 100°C (Ext. 1 in Table 1); (▲) SFE at 30 MPa and 40°C (Ext. 4 in Table 1). Empty symbols represent the mixture of spinach and rosemary extracts (S + R).

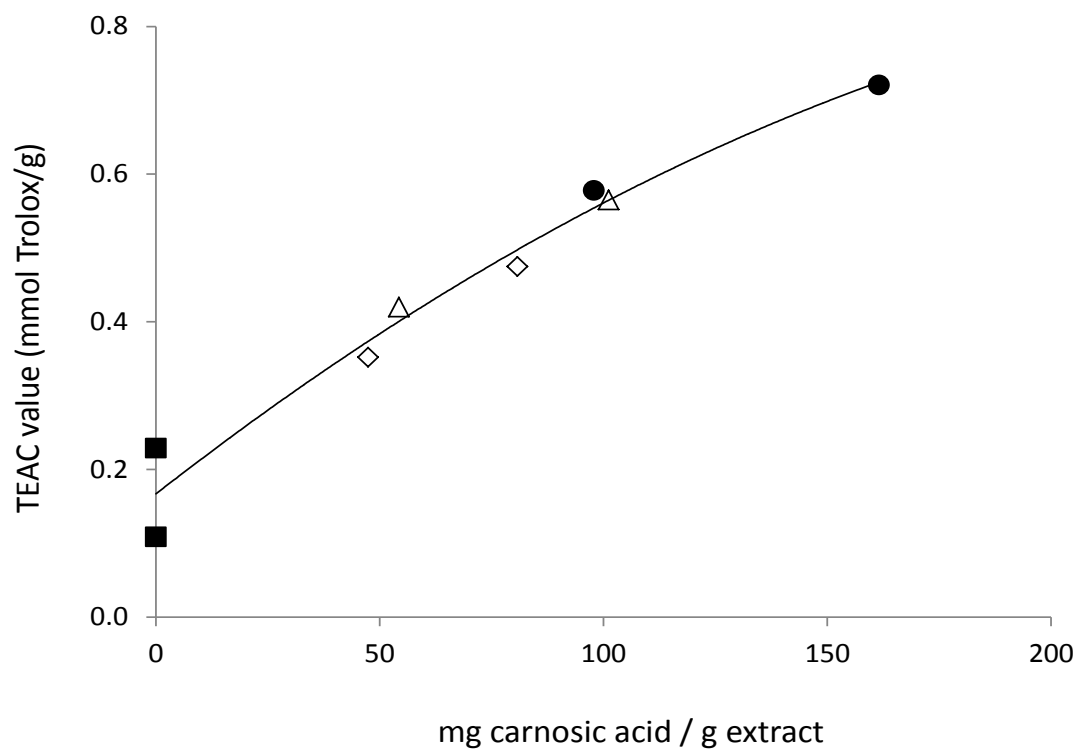
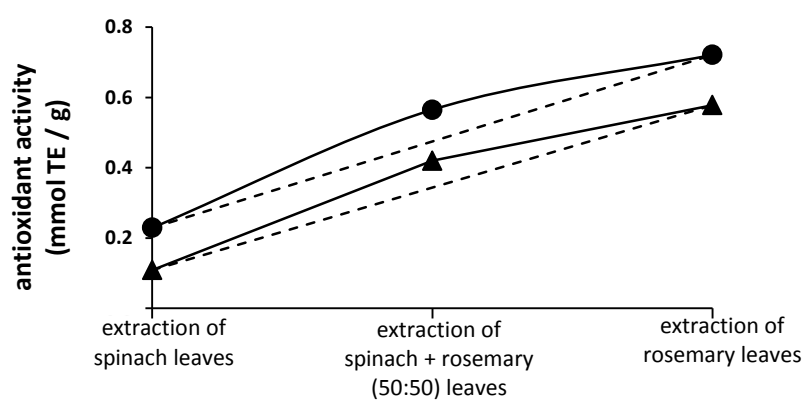


Figure 6. Variation of the TEAC value of the (■) S, (●) R, (△) SR and (◇) S+R extracts obtained from experiments 1 (PLE) and 4 (SFE) of Table 1. Solid line: general trend.



Highlights

- Simultaneous PLE and SFE of spinach and rosemary leaves was accomplished
- Extracts with improved antioxidant activity were obtained
- No synergic effect between spinach carotenoids and rosemary phenolics were sensed
- Antioxidant improvement could be related to enhancement of carnosic acid extraction