

Antioxidant activity of sage (*Salvia officinalis* and *S fruticosa*) and oregano (*Origanum onites* and *O indercedens*) extracts related to their phenolic compound content

Lorena Pizzale,* Renzo Bortolomeazzi, Stefania Vichi, Eva Überegger and Lanfranco S Conte

Food Science Department, Udine University, Via Marangoni 97, I-33100 Udine, Italy

Abstract: In this study the antioxidant activity of methanolic extracts of oregano and sage samples was tested. Samples of oregano belonged to *Origanum onites* and *O indercedens* species, whilst samples of sage belonged to *Salvia officinalis* and *S fruticosa* species. Two methods were used to evaluate the antioxidant activity of sage and oregano extracts: the crocin test and the Rancimat test. The methanolic extracts were also analysed by HPLC for the qualitative/quantitative determination of phenolic compounds. The total phenolic compound content of oregano samples showed no significant differences between the two species, but rosmarinic acid was present in higher amount in *O indercedens*. Carvacrol content sharply differentiated flowers from leaves. Samples of *O indercedens* had a higher antioxidant activity evaluated by the crocin test, whereas no differences were evidenced by the Rancimat test. For sage samples, carnosic acid and methyl carnosate showed a significant difference between the two species, with *S fruticosa* samples having a higher content than *S officinalis* samples. Samples of *S fruticosa* also had a higher antioxidant activity evaluated by the crocin test. The antioxidant activities of sage samples were, on average, higher than those of oregano samples. Some samples of sage had a very high antioxidant activity, with induction times more than 10-fold higher than that of lard used as the reference sample.

© 2002 Society of Chemical Industry

Keywords: sage (*Salvia officinalis* and *S fruticosa*); oregano (*Origanum onites* and *O indercedens*); antioxidant activity; phenolic compound

INTRODUCTION

Oxidative degradation of lipids is one of the main factors limiting the shelf-life of food products. Oxidation of unsaturated lipids leads to the formation of compounds that are unacceptable from the point of view of both organoleptic characteristics and toxicological aspects.^{1–3} In order to control lipid oxidation, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate have been used. Nevertheless, toxicological effects^{3–5} together with consumer preference for natural products have resulted in increased interest in the use and research of natural antioxidants.

Spices and their alcoholic extracts belong to the more studied vegetal species because of their antioxidant activity. In recent years, spice extracts have appeared on the market as antioxidants for food industry use. The antioxidant capacity of some of these compounds has been proved to be comparable to and sometimes higher than that of synthetic antioxidants.^{6,7} In particular, the Labiatae family includes

a large number of plants that are well known for their antioxidant properties. Among these, rosemary and sage have been widely studied and most of their antioxidant components have been identified. It has been established that the antioxidant effects are mainly due to phenolic compounds.^{6,8,9}

The antioxidative effect of these compounds is the result of various possible mechanisms: free radical-scavenging activity, transition metal-chelating activity and/or singlet oxygen-quenching capacity.^{3,8,9}

The main phenolic compounds identified in rosemary and sage samples are rosmarinic acid, carnosic acid, carnosol, methyl carnosate, rosmanol, epirosmanol and rosmadial.^{9–12}

The antioxidant activity of several natural products of plant origin has been studied recently. Carnosol and carnosic acid possess good peroxy and hydroxyl radical-scavenging activity, since they inhibit the formation of hydroxyl radicals and chelate metals, while only carnosic acid appears to scavenge H₂O₂.¹⁰

Oregano is another plant of the Labiatae family that

* Correspondence to: Lorena Pizzale, Food Science Department, Udine University, Via Marangoni 97, I-33100 Udine, Italy
E-mail: lorena.pizzale@dsa.uniud.it

(Received 10 May 2001; revised version received 8 March 2002; accepted 19 March 2002)

has been studied for its antioxidant activity. Kikuzaki and Nakatani¹³ isolated five different phenolic compounds from the methanolic extract of leaves of *Origanum vulgare*: protocatechuic acid, caffeic acid, rosmarinic acid, a phenyl glycoside and 2-caffeoyloxy-3-[2-(4-hydroxybenzyl)-4,5-dihydroxyphenyl]propionic acid. All five showed antioxidative activity. Carvacrol and thymol have been isolated from essential oils of oregano, and their antioxidative effects have been reported by Deighton *et al.*¹⁴

In this work the antioxidant activities of methanolic extracts of sage and oregano samples were tested by means of the Rancimat and crocin tests, and the results were related to the phenolic content determined by HPLC analysis.

EXPERIMENTAL

Reagents

All solvents were of analytical or HPLC grade. Rosmarinic acid was purchased from Extrasynthese (Genay, France) and α -tocopherol from Merck (Darmstadt, Germany). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich (St Louis, MO, USA). 2,2'-Azobis(2,4-dimethylvaleronitrile) (AMVN) was provided by Wako Chemicals GmbH (Neuss, Germany). Lard was purchased from Unibon (Modena, Italy).

Samples

Samples used in this study were oregano leaves, oregano flowers and sage leaves. Oregano samples belonged to two species, *Origanum onites* (four samples of leaves (L) and four samples of flowers (F)) and *O. indicedens* (four samples of leaves (L) and four samples of flowers (F)), whilst sage species were *Salvia officinalis* (12 samples) and *S. fruticosa* (15 samples). Areas of production were Northern Italy for *S. officinalis* samples and Greece (Crete) for oregano and *S. fruticosa* samples. All samples were dried before they arrived at the laboratory for analysis.

Extraction

The dried sample was chopped into a pot and 5g exactly was weighed into a 250ml Erlenmeyer flask; 100ml of methanol was added and the sample was left to infuse overnight. The sample was then refluxed for 1h. After cooling to room temperature, the mixture was filtered through Whatman No 4 filter paper in a Buchner funnel. The filtered solution was evaporated at reduced pressure (Rotavapor, $T < 40^\circ\text{C}$) and the extract was further dried in a desiccator, under vacuum, to constant weight. The extract was then weighed, dissolved in 50ml of methanol and transferred to a 50ml volumetric flask. The solution was stored at -18°C .

HPLC analysis

A Varian 9010 HPLC pump, connected to a Varian

9050 UV-vis detector, and a Rheodyne 7125 injection valve with a 20 μl loop were used. The column was a Spherisorb ODS 2 (C18), 4.6mm \times 250mm, 5 μm particle size (Alltech, Deer Field, IL, USA).

The binary mobile phase comprised aqueous 5% (v/v) acetic acid/acetonitrile 85:15 (solvent A) and methanolic 5% (v/v) acetic acid (solvent B) with a linear gradient from 100% solvent A to 100% solvent B in 90min at a flow rate of 1ml min⁻¹. The UV spectra of phenolic compounds were obtained by a Shimadzu SPP-H6A photodiode array detector (DAD) connected to a Shimadzu LC-64 HPLC.

Rancimat test

The Rancimat test was carried out using lard as the matrix supplemented with methanolic extracts of oregano and sage at concentrations of 0.4 and 1g kg⁻¹ (corresponding to a phenolic compound concentration range of about 80–200 $\mu\text{g g}^{-1}$). Two replicate tests were carried out for each sample. The apparatus used was a Rancimat Metrohm 679 (Herisau, Switzerland) at a temperature 100 $^\circ\text{C}$ and an air flow rate of 20lh⁻¹.

Crocini-bleaching inhibition test

The method developed by Tubaro *et al.*¹⁵ was used. The procedure adopted was briefly as follows: crocin was extracted from saffron using methanol, and the concentration of the solution was measured in methanol at 443nm ($\epsilon = 1.33 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$); a 0.25M solution of AMVN in toluene was used as the radical source; an amount of methanolic extract was reduced to dryness and re-dissolved in toluene/dimethylformamide 1:4 (v/v) to give a final concentration of about 2000 $\mu\text{g ml}^{-1}$. Bleaching reactions at five different antioxidant concentrations (80, 160, 320, 480 and 640 $\mu\text{g ml}^{-1}$) and a blank (containing no antioxidants) were tested in parallel by following the decrease in absorbance at 443nm and a temperature of 40 $^\circ\text{C}$. The reference bleaching rate was determined using a solution of α -tocopherol standard (70 $\mu\text{g ml}^{-1}$) under the same conditions. The crocin-bleaching inhibition test gives an assessment of the overall antioxidant activity of a pure substance as well as of a complex mixture. By this method the ability of a compound to quench peroxy radicals is measured in terms of α -tocopherol equivalents (by weight) by analysing the kinetics of competitive inhibition of a parallel reaction where peroxy radicals bleach the carotenoid crocin (C). The crocin-bleaching rate by a peroxy radical ($-\Delta A_0$), corresponding to V_0 , decreases in the presence of an antioxidant (A) that competes for the peroxy radical, and the new bleaching rate V is less than V_0 . By plotting the ratio $[A]/[C]$ versus V_0/V , the slope K_A/K_C or $K_{\text{pseudo } \alpha\text{-tocopherol}}/K_C$ is obtained (where $[A]$ in the case of extracts is the theoretical concentration of α -tocopherol by considering the whole amount of sample as α -tocopherol). The $K_A/K_{\text{pseudo } \alpha\text{-tocopherol}}$ ratio (where K_A is obtained from the α -tocopherol standard and $K_{\text{pseudo } \alpha\text{-tocopherol}}$ from samples) indicates

the grams of extract having the same antioxidant activity as 1 g of α -tocopherol standard.

Statistical analysis

The *t*-test and correlation analysis were used as statistical methods for the analysis of the results. The software used was Statistica for Windows 2.1.

RESULTS AND DISCUSSION

Extraction yield

The extraction yields with methanol for the oregano and sage samples are reported in Tables 1 and 2 respectively.

No significant differences were observed between the average extraction yields of *O onites* and *O indercedens* samples considered as a whole (leaves plus flowers). Considering the yields of leaves and flowers separately, there was a significant difference in the case of *O onites*, while no differences were present for *O indercedens*.

In the case of sage samples the extraction yields differed significantly between the two species. The extraction yield of *S fruticosa* (25.5%) was higher than that of *S officinalis* (20.1%).

HPLC analysis of extract

The HPLC chromatogram of a methanolic extract of oregano was characterised by the presence of about 17 main peaks. Among them, three have been identified as caffeic acid, rosmarinic acid and carvacrol by

Table 1. Yields of extraction of oregano samples (F, flowers; L, leaves)

Sample	Yield (%)
<i>O indercedens</i>	
1.1 F	17.8
1.12 F	18.4
1.16 F	20.0
1.4 F	14.7
Ave \pm SD	17.7 \pm 2.2a
1.1 L	18.3
1.12 L	19.0
1.16 L	18.0
1.4 L	18.2
Ave \pm SD	18.4 \pm 0.4a
<i>O onites</i>	
1.11 F	17.0
1.15 F	18.9
1.19 F	14.8
1.20 F	17.4
Ave \pm SD	17.0 \pm 1.7a
1.11 L	20.3
1.15 L	22.4
1.19 L	22.3
1.20 L	22.5
Ave \pm SD	21.9 \pm 1.0b

Values followed by the same letter are not significantly different ($p < 0.05$).

Table 2. Yields of extraction of sage samples

Sample	Yield (%)
<i>S officinalis</i>	
DAJTI	21.8
TN 600	21.7
SKODRA	21.1
VALDA Y	20.7
TN 75	20.2
VALDA α	20.0
Fi 1	19.9
TN 191	19.8
SYN 1	19.3
VALDA X	18.8
POP.PART	18.8
Fi 12	18.7
Ave \pm SD	20.0 \pm 1.0a
<i>S fruticosa</i>	
2714	27.8
1213	27.2
719	26.7
717	26.5
709	26.5
1204	26.1
1206	25.6
720	25.4
2710	25.3
1207	25.2
2712	25.1
1216	24.7
2715	24.1
2711	23.3
2720	22.9
Ave \pm SD	25.5 \pm 1.4b

Values followed by the same letter are not significantly different ($p < 0.05$).

comparison of their retention times, UV absorption spectra and mass spectra with those of the corresponding standard compounds.

Few literature references are available for phenolic compounds of oregano,^{13,14} this fact, together with the lack of available standards, has strongly reduced the number of identified compounds. Nevertheless, all the compounds were considered to be of phenolic type on the basis of their UV spectra, characterised by absorption maxima between 260 and 330 nm. The quantitative analysis was carried out using the response factor of rosmarinic acid for all the compounds. The quantitative results (g kg^{-1} extract) for the phenolic compounds of oregano samples are reported in Tables 3 and 4.

The three identified compounds (caffeic acid, rosmarinic acid and carvacrol) amounted, on average, to 55% of the total phenolic compound content of oregano samples.

Among the identified compounds the only difference between the two species was in the rosmarinic acid content, which was about double in *O indercedens*.

Considering the botanical parts (flowers and leaves),

Table 3. Phenol contents (g kg⁻¹ extract) of *Origanum onites* samples (F, flowers; L, leaves)

Sample	Caffeic acid	Rosmarinic acid	Carvacrol	Other phenols	Total phenols	% of identified phenols
1.1 F	3.5	22.6	42.0	42.0	110.2	58.5
1.4 F	4.7	26.9	45.0	54.2	130.9	61.9
1.12 F	2.8	33.4	51.4	38.8	126.4	62.9
1.16 F	3.2	22.5	42.9	54.1	122.7	61.3
Ave±SD	3.6±0.8a	26.4±5.1a	45.3±4.2a	47.3±8.0	122.6±8.9a	
1.1 L	3.8	26.0	16.2	29.1	75.2	69.3
1.4 L	5.1	21.6	15.2	29.7	71.5	55.9
1.12 L	4.4	65.4	28.5	35.8	135.3	73.3
1.16 L	1.9	39.5	20.4	36.4	99.4	58.6
Ave±SD	3.8±1.4a	38.1±19.7a	20.1±6.1b	32.7±3.9	95.4±29.4a	

Values within a column followed by the same letter are not significantly different ($p < 0.05$).

Table 4. Phenol contents (g kg⁻¹ extract) of *Origanum onites* samples (F, flowers; L, leaves)

Sample	Caffeic acid	Rosmarinic acid	Carvacrol	Other phenols	Total phenols	% of identified phenols
1.11 F	3.0	10.5	32.4	52.8	98.7	45.2
1.15 F	2.4	18.3	31.7	55.5	108.7	44.4
1.19 F	3.0	19.1	39.2	54.4	115.6	39.7
1.20 F	4.2	16.6	28.9	38.4	88.0	44.8
Ave±SD	3.2±0.8a	16.1±3.9a	33.1±4.4a	50.3±8.0	102.8±12.0a	
1.11 L	3.4	15.9	13.2	40.1	72.5	56.4
1.15 L	4.0	14.7	15.4	51.7	85.7	53.0
1.19 L	3.0	12.0	22.6	47.1	84.5	48.6
1.20 L	2.9	8.0	22.9	41.0	75.5	46.5
Ave±SD	3.3±0.5a	12.7±3.5a	18.5±5.0b	45.0±5.5	79.6±6.5b	

Values within a column followed by the same letter are not significantly different ($p < 0.05$).

there were no differences in the content of caffeic acid and rosmarinic acid, whereas the carvacrol content sharply differentiated flowers from leaves, with the former having the higher concentration.

The two oregano species showed no significant differences with respect to the average total phenolic content (leaves plus flowers) (Table 5).

In the sage extracts, rosmarinic acid, carnosol, carnosic acid and methyl carnosate were identified among 18 peaks obtained by HPLC analysis.

Rosmarinic acid was identified by comparison with a standard sample, while the identification of carnosol, carnosic acid and methyl carnosate was based on comparison of their UV and mass spectra with those reported in the literature.¹⁶

The concentrations of phenolic compounds (g kg⁻¹ extract) in sage samples are reported in Table 6. The total phenolic compound content in sage extracts ranged from a minimum of 46.4 g kg⁻¹ for a sample of *S officinalis* to a maximum of 113.4 g kg⁻¹ for a sample

of *S fruticosa*, with no significant difference between the two species.

Among the identified compounds, only the average content of carnosic acid and methyl carnosate showed a difference between the two species, while in the case of rosmarinic acid and carnosol the average content was practically the same.

Cuvelier *et al*¹⁷ reported that methyl carnosate could be an artefact formed from carnosic acid during the extraction step with methanol. The higher amount of methyl carnosate observed in *S fruticosa*, together with the lower level of carnosic acid, could be explained according to these authors.

On the other hand, the methyl ester of carnosic acid has also been isolated by Al-Hazimi¹⁸ from *S lanigera* using petroleum ether as the extraction solvent.

Antioxidant activity

Tables 7–9 show the results of the Rancimat test as the ratio of the induction time of the sample (IT_{sample}) and

Table 5. Average (leaves + flowers)±SD values of phenol content (g kg⁻¹ extract) of oregano samples

Species	Caffeic acid	Rosmarinic acid	Carvacrol	Total phenols
<i>O. onites</i>	3.7±1.1a	32.2±14.7a	32.7±14.3a	109.0±24.8a
<i>O. onites</i>	3.2±0.6a	14.4±3.9b	25.8±8.9a	91.2±15.3a

Values within a column followed by the same letter are not significantly different ($p < 0.05$).

Table 6. Phenol contents (g kg⁻¹ extract) of sage samples

Sample	Rosmarinic acid	Carnosol	Carnosic acid	Methyl carnosate	Other phenols	Total phenols	% of identified phenols
<i>S officinalis</i>							
DAJTI	21.9	5.3	3.7	5.9	9.6	46.4	79.3
Fi 1	39.4	1.9	0.8	6.1	15.0	69.5	72.1
Fi 12	41.5	1.1	0.2	3.6	16.1	60.0	70.9
POP.PART	27.7	3.7	3.4	4.9	13.5	56.0	77.3
SKODRA	26.3	9.0	7.1	13.0	21.3	69.7	69.4
SYN 1	30.3	3.0	1.3	4.0	14.2	53.5	79.5
TN 191	45.1	4.0	3.1	5.1	18.2	75.5	75.9
TN 600	55.1	1.4	1.0	3.7	16.8	87.8	79.2
TN 75	58.6	2.0	2.0	2.7	26.3	88.7	69.7
VALDA α	57.3	1.3	nd	6.1	23.2	81.7	73.6
VALDA X	81.2	2.2	1.3	2.2	16.3	103.2	84.2
VALDA Y	81.8	1.9	1.8	6.2	15.6	107.4	85.4
Ave \pm SD	47.2 \pm 20.2a	3.1 \pm 2.3a	2.1 \pm 2.0a*	5.3 \pm 2.8a	17.2 \pm 4.54	75.0 \pm 19.5a	
<i>S fruticosa</i>							
709	56.2	2.7	0.7	3.2	8.0	91.0	86.5
717	50.4	4.1	2.8	8.9	20.9	95.8	66.6
719	42.5	2.4	0.9	8.8	19.7	81.2	69.4
720	56.4	2.8	0.9	9.6	20.1	98.7	70.4
1204	37.5	3.3	0.5	6.0	23.0	67.2	67.3
1206	45.8	2.5	0.6	5.2	28.1	79.6	61.1
1207	36.0	2.0	nd	6.2	25.4	72.3	68.0
1213	40.9	3.2	nd	8.7	27.2	80.0	66.0
1216	37.6	3.3	0.7	5.7	26.7	70.3	67.2
2710	31.4	3.4	0.8	8.8	21.4	64.0	74.9
2711	52.0	3.1	1.0	7.7	28.3	85.2	69.0
2712	44.4	2.3	0.6	5.7	29.6	61.3	69.1
2714	49.9	4.6	0.9	11.5	29.9	96.9	69.0
2715	76.6	3.6	0.6	7.7	28.8	113.3	70.6
2720	27.8	3.4	1.5	9.2	24.6	62.9	78.1
Ave \pm SD	45.7 \pm 12.1a	3.1 \pm 0.7a	0.8 \pm 0.7b	7.5 \pm 2.1b	24.1 \pm 5.68	81.3 \pm 15.4a	

Values within a column followed by the same letter are not significantly different ($p < 0.05$).

* $p < 0.10$.

the induction time of lard (IT_{lard}). As reference values, lard had an IT of 2h, whereas a 1:1 (w/w) mixture of BHA and BHT at a total concentration of 200 $\mu\text{g g}^{-1}$ had an IT of 12h, corresponding to an IT ratio of 6.

There was a high variability among the samples analysed, both for *O. inderecedens* as well for *O. onites*, and the statistical analysis of the results did not allow any discrimination between the two species of oregano

Table 7. Antioxidant activities of *Origanum inderecedens* samples (F, flowers; L, leaves)

Sample	Crocic test ^a	Rancimat test ^b	
		0.4g kg ⁻¹	1g kg ⁻¹
1.1 F	28.6	2.2	3.5
1.4 F	27.4	2.4	3.0
1.12 F	37.0	2.5	3.5
1.16 F	33.4	2.1	3.1
Ave \pm SD	31.6 \pm 4.45a	2.3 \pm 0.16a	3.3 \pm 0.29a
1.1 L	40.7	2.6	6.3
1.4 L	23.7	1.0	1.4
1.12 L	22.1	3.8	5.9
1.16 L	19.1	1.7	3.2
Ave \pm SD	26.4 \pm 9.73b	2.3 \pm 1.22a	4.2 \pm 2.34a

Values within a column followed by the same letter are not significantly different ($p < 0.05$).

^a $K_A/K_{\text{pseudo } \alpha\text{-tocopherol}}$

^b $IT_{\text{sample}}/IT_{\text{lard}}$

Table 8. Antioxidant activities of *Origanum onites* samples (F, flowers; L, leaves)

Sample	Crocic test ^a	Rancimat test ^b	
		0.4g kg ⁻¹	1g kg ⁻¹
1.11 F	64.7	1.4	1.9
1.15 F	26.9	2.4	2.4
1.19 F	25.8	1.4	2.1
1.20 F	58.2	2.2	2.8
Ave \pm SD	43.1 \pm 20.48a	1.84 \pm 0.52a	2.28 \pm 0.38a
1.11 L	39.4	2.2	4.0
1.15 L	38.7	1.5	2.0
1.19 L	40.3	2.4	3.2
1.20 L	55.1	2.3	2.8
Ave \pm SD	43.4 \pm 7.85a	2.1 \pm 0.41a	3.0 \pm 0.81a

Values within a column followed by the same letter are not significantly different ($p < 0.05$).

^a $K_A/K_{\text{pseudo } \alpha\text{-tocopherol}}$

^b $IT_{\text{sample}}/IT_{\text{lard}}$

Table 9. Average (leaves+flowers)±SD values of antioxidant activity of oregano samples

Species	Crocini test ^a	Rancimat test ^b	
		0.4 g kg ⁻¹	1 g kg ⁻¹
<i>O. inderecedens</i>	29.0±7.5a	2.3±0.80a	3.7±1.62a
<i>O. onites</i>	45.6±14.4b	2.0±0.45a	2.6±0.70a

Values within a column followed by the same letter are not significantly different ($p < 0.05$).

^a $K_A/K_{\text{pseudo } \alpha\text{-tocopherol}}$

^b $IT_{\text{sample}}/IT_{\text{lard}}$

or between flowers and leaves at a confidence level higher than 90%.

At an extract concentration of 0.4 g kg⁻¹, both flowers and leaves had, on average, an IT about twice that of lard, while at 1 g kg⁻¹ the IT ratio was about 3.

The results of the crocin-bleaching inhibition test for the oregano samples are reported in Tables 7–9. Considering both flowers and leaves, the average value of 29.0 g equiv obtained for *O. inderecedens* was significantly different ($p < 0.05$) from the average value of 43.6 g equiv obtained for *O. onites*, with the former having the higher antioxidant activity (Table 9). No significant differences were observed between the botanical parts of the two species (Tables 7 and 8).

The results of the Rancimat test for the sage extracts are reported in Table 10. Also in this case there were no significant differences between the two species analysed.

The antioxidant activities of sage samples were, on average, higher than those of oregano samples, particularly at the concentration of 1 g kg⁻¹. Some samples of sage had a very high antioxidant activity at 1 g kg⁻¹, with IT values more than 10-fold higher than that of lard.

Also in the case of sage samples a significant difference resulted from the analysis of the data of the crocin test (Table 10) between *S. officinalis* and *S. fruticosa*. The latter, with a value about half that of the former, had the higher antioxidant activity, also compared with the oregano samples (Table 10).

The extracts of *O. inderecedens* and *O. onites* had an antioxidant activity lower than that of the 1:1 (w/w) mixture of BHA and BHT in the Rancimat test. On the contrary, the extracts of sage presented interesting features, with antioxidant activities comparable to that of BHA + BHT, although at higher concentration.

The two methods used to test the antioxidant activities gave different results. While the Rancimat test did not discriminate between the two oregano species nor between the two sage species, the crocin test differentiated both the oregano as well as the sage species.

These findings confirm the difficulty of comparing the results of the many different methods used to test antioxidant activities, as well as the difficulty of assessing the antioxidant activity of a product on the basis of a single method.

The results of the Rancimat and crocin tests were then related to the phenolic content determined by HPLC analysis.

For oregano samples the correlation analysis highlighted that crocin and Rancimat test data were significantly correlated with rosmarinic acid content ($r = -0.54$, $p < 0.05$, $r = 0.57$, $p < 0.05$ and $r = 0.58$, $p < 0.05$ respectively for the crocin test, the Rancimat test at 0.4 g kg⁻¹ and the Rancimat test at 1 g kg⁻¹). Under the conditions adopted in our antioxidant tests, rosmarinic acid seems to be the main compound responsible for the antioxidant activity.

Lindberg Madsen *et al*¹⁹ reported that the antioxidant activity of oregano is due to a variety of components, including hydrophilic and lipophilic compounds, and they found good agreement between antioxidant activity and total phenolic content (detected spectrophotometrically) of spice extract. Our results are in accordance with these literature data.

For sage samples the correlation analysis highlighted that crocin test data were significantly correlated with

Table 10. Antioxidant activities of sage samples

Sample	Crocini test ^a	Rancimat test ^b	
		0.4 g kg ⁻¹	1 g kg ⁻¹
<i>S. officinalis</i>			
DAJTI	33.9	2.7	7.4
Fi 1	34.7	3.4	9.1
Fi 12	53.3	2.6	4.3
POP.PART	39.2	4.3	10.1
SKODRA	29.0	8.2	18.1
SYN 1	51.3	1.1	3.9
TN 600	40.2	5.4	10.1
TN 191	36.0	2.7	5.7
TN 75	29.2	2.3	5.9
VALDA α	29.7	3.3	8.6
VALDA X	27.7	2.7	7.8
VALDA Y	28.3	1.8	7.4
Ave±SD	36.0±8.7a	3.4±1.90a	8.2±3.75a
<i>S. fruticosa</i>			
709	20.4	2.6	8.1
717	13.4	1.9	5.8
719	13.3	1.6	3.7
720	17.4	3.7	7.7
1204	23.4	4.3	9.0
1206	23.6	1.3	2.4
1207	19.8	3.2	5.9
1213	18.4	2.9	11.1
1216	22.9	2.6	7.7
2710	17.9	1.7	11.8
2711	17.8	2.1	11.1
2712	21.0	1.5	6.9
2714	17.1	4.2	13.9
2715	16.5	1.7	3.3
2720	19.6	2.4	8.3
Ave±SD	18.8±3.16b	2.5±0.97a	7.8±3.28a

Values within a column followed by the same letter are not significantly different ($p < 0.05$).

^a $K_A/K_{\text{pseudo } \alpha\text{-tocopherol}}$

^b $IT_{\text{sample}}/IT_{\text{lard}}$

methyl carnosate content ($r = -0.55$, $p < 0.01$) and total phenol content ($r = -0.46$, $p < 0.05$). Data of the Rancimat test at 0.4 g kg^{-1} were correlated with carnosol and carnosic acid content ($r = 0.64$, $p < 0.01$ and $r = 0.63$, $p < 0.01$ respectively). Data of the Rancimat test at 1 g kg^{-1} were correlated with carnosol content ($r = 0.65$, $p < 0.01$), carnosic acid content ($r = 0.49$, $p < 0.01$) and methyl carnosate content ($r = 0.56$, $p < 0.01$). No significant correlation was found between data of the Rancimat test at 1 g kg^{-1} and total phenol content.

These results are in good accordance with literature data^{17,20,21} showing carnosic acid, carnosol and methyl carnosate as the most effective antioxidants present in rosemary and sage extracts.

REFERENCES

- 1 Frankel EN, Lipid oxidation: mechanisms, products and biological significance. *J Am Oil Chem Soc* **61**:1908–1915 (1984).
- 2 Shahidi F, Janitha PK and Wanasundara PD, Phenolic antioxidants. *Crit Rev Food Sci Nutr* **32**:67–103 (1992).
- 3 Namiki M, Antioxidants/antimutagens in food. *Crit Rev Food Sci Nutr* **29**:273–300 (1990).
- 4 Barlow SM, Toxicological aspects of antioxidants used as food additives, in *Food Antioxidants*, Ed by Hudson BJJF. Elsevier Applied Science, London, pp 253–307 (1990).
- 5 Bermond P, Biological effects of food antioxidants, in *Food Antioxidants*, Ed by Hudson BJJF. Elsevier Applied Science, London, pp 193–251 (1990).
- 6 Pokorny J, Natural antioxidants for food use. *Trends Food Sci Technol* **9**:223–227 (1991).
- 7 Cuvelier M-E, Berset C and Richard H, Use of a new test for determining comparative antioxidative activity of butylated hydroxyanisole, butylated hydroxytoluene, alpha- and gamma-tocopherols and extracts from rosemary and sage. *Sci Alim* **10**:797–806 (1990).
- 8 Das NP and Pereira TA, Effects of flavonoids on thermal autoxidation of palm oil: structure–activity relationships. *J Am Oil Chem Soc* **67**:255–258 (1990).
- 9 Schwarz K and Ternes W, Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis*. II. Isolation of carnosic acid and formation of other phenolic diterpenes. *Z Lebensm Untersuch Forsch* **195**:99–103 (1992).
- 10 Aruoma OI, Halliwell B, Aeschbach R and Loligers J, Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid. *Xenobiotica* **22**:257–268 (1992).
- 11 Cuvelier M-E, Berset C and Richard H, Antioxidant constituents in sage (*Salvia officinalis*). *J Agric Food Chem* **42**:665–669 (1994).
- 12 Riccheimer SL, Bernart MW, King GA, Kent MC and Bailey DT, Antioxidant activity of lipid-soluble phenolic diterpenes from rosemary. *J Am Oil Chem Soc* **73**:507–514 (1996).
- 13 Kikuzaki H and Nakatani N, Structure of a new antioxidative phenolic acid from oregano (*Oreganum vulgare* L.). *Agric Biol Chem* **53**:519–524 (1989).
- 14 Deighton N, Glidewell SM, Deans SG and Goodman BA, Identification by EPR spectroscopy of carvacrol and thymol as the major sources of free radicals in the oxidation of plant essential oils. *J Sci Food Agric* **63**:221–225 (1993).
- 15 Tubaro F, Micossi E and Ursini F, The antioxidant capacity of complex mixtures by kinetic analysis of crocin bleaching inhibition. *J Am Oil Chem Soc* **73**:173–179 (1996).
- 16 Cuvelier M-E, Richard H and Berset C, Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *J Am Oil Chem Soc* **73**:645–652 (1996).
- 17 Cuvelier M-E, Berset C and Richard H, Antioxidant constituents in sage (*Salvia officinalis*). *J Agric Food Chem* **42**:665–669 (1994).
- 18 Al-Hazimi HMG, The isolation of methyl carnosate from *Salvia lanigera*. *Phytochemistry* **25**:1238–1239 (1986).
- 19 Lindberg Madsen H, Nielsen BR, Bertelsen G and Skibsted LH, Screening of antioxidative activity of spices. A comparison between assays based on ESR spin trapping and electrochemical measurement of oxygen consumption. *Food Chem* **57**:331–337 (1996).
- 20 Huang S-W, Frankel EN, Schwarz K, Aeschbach R and German JB, Antioxidant activity of carnosic acid and methyl carnosate in bulk oils and in oil-in-water emulsion. *J Agric Food Chem* **44**:2951–2956 (1996).
- 21 Frankel EN, Huang S-W, Aeschbach R and Prior E, Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. *J Agric Food Chem* **44**:131–135 (1996).