British Journal of Nutrition (2005), **93**, 257–266
DOI: 10.1079/BJN20041327

Antioxidant capacity of vegetables, spices and dressings relevant to nutrition

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(Received 26 May 2004 – Revised 3 September 2004 – Accepted 14 October 2004)

Vegetables are the most important sources of phenolics in the Mediterranean diet. Phenolics, especially flavonoids, are suggested as being essential bioactive compounds providing health benefits. In this study, twenty-seven vegetables, fifteen aromatic herbs and some spices consumed in Central Italy (the Marches region) were studied to reveal total phenolic, flavonoid and flavanol content as well as their antioxidant capacity measured by the oxygen radical absorbance capacity (ORAC) method. A comparison in terms of antioxidant capacity was made between different salads, as well as between salads to which aromatic herbs had been added. Lemon balm and marjoram at a concentration of 1·5 % w/w increased by 150 % and 200 % respectively the antioxidant capacity of a salad portion. A 200 g portion of a salad enriched with marjoram corresponded to an intake of 200 (SD 10) mg phenolics and 4000 (SD 300) ORAC units (μmol Trolox equivalents). Olive oils and wine or apple vinegars were the salad dressings that provided the highest increase in antioxidant capacity. Among the spices tested, cumin and fresh ginger made the most significant contribution to the antioxidant capacity. The results are useful in surveying the antioxidant parameters of vegetables, herbs and spices produced and consumed in our geographical area as well as in quantifying the daily intake of phenolics and ORAC units. The results can be used in public health campaigns to stimulate the consumption of vegetables able to provide significant health protection in order to prevent chronic diseases.

Dietary counselling: Phenolic compounds: Antioxidant capacity: ORAC units: Antioxidant-rich foods

Chronic diseases are induced by metabolic alterations that are often correlated and contemporarily present in patients. A typical example is the plurimetabolic syndrome, characterized by the concurrent presence of obesity, diabetes, dyslipidaemia and hypertension (World Health Organization, 1985) in the same individual. Insulin resistance is a fundamental pathogenic factor in the ever-increasing incidence of this syndrome, which is associated with an elevated risk of cardiovascular morbidity (Reaven, 2001). Many scientific papers have focused on the relationship between free-radical-induced oxidative stress and the progressive increase in the risk of disease (World Health Organization, 1990, 2003; Willett, 2002).

The incidence of chronic diseases is higher in the elderly (Smith *et al.* 1999), but conditions that generate various pathologies start in childhood and adolescence (Stewart *et al.* 2002). In Europe, obesity is becoming a serious problem: in Italy, a large prevalence of overweight and obesity was observed in school subjects (Celi *et al.* 2003). In addition, children of normal weight could become obese if they did not follow a balanced nutritional regimen (American Dietetic Association, 1999). Most young people lead a sedentary life and make common nutritional errors, among which we highlight an insufficient consumption of fruits and vegetables (Ames, 1998).

Fruits and vegetables contain antioxidant compounds broadly called polyphenols that are known to reduce oxidative stress and prevent chronic diseases (Ames *et al.* 1993, 1995; Diaz *et al.* 1997; Giacosa *et al.* 1997; Gariballa & Sinclair, 1998; Miller *et al.* 1998; Muller *et al.* 1999). The antioxidant properties of these compounds are responsible for their anticancer, antiviral and anti-inflammatory properties (Cao *et al.* 1997). They can also prevent capillary fragility and platelet aggregation (Benavente-Garcia *et al.* 1997; Aviram, 1999).

To encourage vegetable consumption through nutritional counselling, among both the young and the elderly, it is important to bear in mind that not all vegetables have the same phenolic composition and not all phenolics have the same antioxidant capacity (Vinson *et al.* 2001; Ou *et al.* 2002; Ninfali & Bacchiocca, 2003). It is therefore important to recognize which vegetables have the highest antioxidant capacity and introduce them regularly into the diet (Halliwell, 1999) and equally important to know

Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane)dihydrochloride; ORAC, oxygen radical absorbance capacity.

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how much antioxidant capacity is lost by cooking vegetables compared with the levels in fresh vegetables (Gil *et al.* 1999; Nicoli *et al.* 1999).

Simple, practical data regarding the antioxidant properties of both raw and cooked vegetables commonly consumed in a geographical area can help health professionals to organize campaigns persuading the public to increase their vegetable intake (Arts *et al.* 2000; De Pascual-Teresa *et al.* 2000; Franke *et al.* 2004). It is also important to vary seasonings, suggesting salads with a good flavour and high antioxidant capacity, and making this kind of food more attractive, while concentrating significant amounts of beneficial compounds in limited quantities of vegetables.

This paper reports the phenolic, flavonoid and flavanol content as well as the antioxidant capacity of a number of fresh vegetables, herbs and spices consumed in Central Italy. These same parameters were also studied in cooked vegetables, salads and common Italian salad dressings.

Materials and methods

Chemicals

2,2′-Azobis(2-amidinopropane)dihydrochloride (AAPH) was purchased from Polyscience (Warrington, PA, USA), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) and fluorescein sodium salt were obtained from Aldrich (Milwaukee, WI, USA), Folin-Ciocalteu phenol reagent and all other reagents from Sigma Aldrich (Milan, Italy).

Vegetables

All vegetables, except *Brassicacea*, were obtained fresh from a local greengrocer in the spring and summer of 2003. *Brassicacea* were purchased, fresh, in the winter of 2002. Naturally occurring herbs were collected in the spring and early summer of 2003, whereas spices were obtained from a local herb supplier. Twenty-seven different types of vegetable and fifteen different types of herb were tested. In some cases, more than one cultivar was tested. For each vegetable cultivar and for each herb, at least four composite samples were tested, each in duplicate.

Salads, 200 g total weight each, were prepared using the following fresh vegetables: lettuce (Romana); tomato (Sarom); cucumber (Lungo verde degli ortolani); onion (Bianca di Maggio); carrot (Tancar). Ingredients were combined to obtain a balanced mixture of each component. Briefly, salad 1 was prepared with 76 g lettuce and 124 g tomato; salad 2 with 50 g lettuce, 80 g tomato and 70 g cucumber; salad 3 with 44 g lettuce, 72 g tomato, 63 g cucumber and 21 g onion; salad 4 with 39 g lettuce, 64 g tomato, 45 g cucumber, 18 g onion and 34 g carrot. Salads, 200 g each, used to evaluate possible advantages gained by the addition of fresh aromatic herbs, were prepared as follows: salad 1 by mixing 100 g lettuce and 100 g tomato; salad 2 with 98·5 g lettuce, 98·5 g tomato and 3 g lemon balm; salad 3 with 98·5 g lettuce, 98·5 g tomato and 3 g marjoram. For each salad, the experiment was repeated four times, and each salad was tested in duplicate.

Cooking regimens

Some vegetables (black cabbage, broccoli, cauliflower, green cabbage, savoy cabbage) were heat-treated by boiling or steaming. For boiling, 100 g vegetables were added to 500 ml boiling water; after 15 min, the vegetables were drained, cooled on ice and processed for the phenolic and oxygen radical absorbance capacity (ORAC) analysis. For steaming, 100 g vegetable were steamed for 30 min in a steam pan, then cooled on ice and immediately analysed for phenolics and ORAC. For each vegetable, the experiment was repeated four times and each regimen was tested in duplicate.

Dressings

We used an extra-virgin olive oil produced in the 2002/2003 season in the Marche region by a local producer; named-brand peanut oil and vinegars were purchased in a supermarket. Aromatic oils were prepared as follows. For the basil oil, 5 g basil inflorescence was added to 1 litre extra-virgin olive oil and maceration occurred over 10 d in the dark; then 5 g leaves were added and a further 30 d maceration were allowed before the assay. For the garlic oil, 6 g fresh garlic and 4 g seasoned garlic were partially slit with a knife, added to 1 litre extra-virgin olive oil and left in the dark for a 40 d maceration period. For the garlic aromatic oil, 2 g peeled garlic, 1 g rosemary, 2 g garden sage and 5 g red chilli pepper, cut into small pieces, were added to the oil and left in the dark for 40 d. For the parsley oil, 10 g parsley were cut into large pieces, added to extra-virgin olive oil, left for 40 d in the dark and then used for the assay. Vegetables and aromatic herbs were carefully cleaned and wiped before adding them to the extra-virgin olive oil; after maceration, the oils were filtered and used.

Phenolic extraction

Vegetables or aromatic herbs were cleaned, carefully washed with tap water, wiped by soft centrifugation and chopped in a food processor, whereas dry spices were processed. Ground materials were suspended (1:5, w/v) in 80:20 v/v acetone/perchloric acid 5 %, shaken for 30 min at 4 °C and then centrifuged for 10 min at 3000 ***g*** (Ninfali & Bacchiocca, 2003). The supernatant was collected and used for the assays.

For the olive and seed oil as well as the aromatic oils, the extraction of phenolics was performed using 80 % methanol as reported (Ninfali *et al.* 2002). The diluted phenolic extract was used for the assays.

Vinegars were diluted with 0·075 M-Na phosphate buffer pH 7·0 and then assayed directly for phenolics and ORAC.

Assay of phenolics, flavonoids and flavanols

Phenolic compounds were assayed according to the Folin-Ciocalteu method (Singleton *et al.* 1999). The total phenolic content was expressed as caffeic acid equivalents in mg/g fresh vegetable. Flavonoids were determined by the method of Eberhardt *et al.* (Eberhardt *et al.* 2000;

Liu *et al.* 2002) and flavanols according to that of Arnous *et al.* (2002).

Oxygen radical absorbance capacity assay

The original method of Cao *et al.* (1993), with a few modifications, was used (Ninfali & Bacchiocca, 2003). The final reaction mixture for the assay (2 ml) was prepared as follows: 1650 μl 0·05 μM fluorescein sodium salt in 0·075 M-sodium phosphate buffer, pH 7.0, 200 μl diluted sample or 50 μM Trolox. The control was 0·075 M-Na phosphate buffer, pH 7.0. Fluorescence was read every 5 min at 37 °C using an LS-5 spectrofluorometer (Perkin-Elmer, Norwalk, CT, USA) at 485 nm excitation, 520 nm emission. When stability was reached, the reaction was initiated with 150 μl 5·55 mM AAPH and fluorescence was read up to a value of zero. The ORAC value is calculated according to the formula:

$$\text{ORAC } (\mu\text{molTroloxequivalents/g}) = [(A_s - A_b)/(A_t - A_b)]\ kah$$

where A_s is the area under the curve (AUC) of fluorescein in the sample, calculated with the ORIGIN 2·8 integrating program (Microcal Software), A_t is the AUC of the Trolox, A_b is the AUC of the control, k is the dilution factor, a is the concentration of the Trolox in μmol/l, and h is the ratio between the litres of extract and the grams of vegetable or oil used for the extraction.

Statistics

Duplicate analysis for each measurement (total phenolics, flavonoids, flavanols and ORAC) were conducted for the twenty-seven vegetable samples, fifteen aromatic herbs, six spices and ten dressings. To establish the reproducibility of the analytical method, sample preparation was repeated four times. Differences between the means were evaluated with ANOVA, using the Origin 2·8 (Microsoft Software) statistics program. A multivariate analysis was performed to discover a possible relationship between the different parameters (ORAC, phenolics, flavonoids and flavanols). The significance of the model was evaluated by ANOVA. The significance of the regression coefficients was evaluated by Student's t test. The significance level was fixed at 0·05 for all the statistical analysis.

Results

Table 1 shows the total phenols, flavonoids, flavanols and ORAC values of forty vegetables. Pepper, radish, onion, lettuce, tomato, cucumber, aubergine and courgette from more than one cultivar were analysed. Each different cultivar causes vegetables, albeit of the same family, to possess significantly different phenolic, flavanol and ORAC values. This is clearly evident, for example, in lettuce, onion and pepper cultivars. Various lettuce cultivars have been analysed and compared with Chioggia red chicory, which is commonly used in Northern and Central Italy, mixed with or instead of lettuce, to prepare salads. The total phenolic values in Chioggia red chicory were higher than those found in any analysed lettuce cultivar. The lettuce Rossa di Trento is the cultivar with the highest phenolic content and antioxidant activity and its ORAC value is not significantly different from that of Chioggia red chicory. A second vegetable showing marked differences from one cultivar to another is cabbage. Green cabbage shows in fact one half of the phenolics, flavonoids and ORAC values of savoy or black cabbage; moreover, savoy cabbage has a higher flavonoid content and higher ORAC values than black cabbage. A similar discrepancy between the cultivars is observed for the radish.

In order to explore the relationship between the ORAC and the other considered variables (total phenols, flavonoids and flavanols), we applied a multiple linear regression model. The model was statistically significant ($P<0·001$). Table 2 shows the regression coefficient for each variable together with its significance value. The coefficients show that the ORAC values strictly depend on the total phenols ($P=0·048$) and flavanols ($P=0·001$), whereas the contribution of the flavonoids was not significant ($P=0·156$).

Table 3 lists total phenols, flavonoids, flavanols and ORAC values for selected aromatic herbs. Garden thyme, garden sage, rosemary and marjoram have the highest concentrations of phenolics and the highest ORAC values. The values are many-fold higher than those of the vegetables reported in Table 1.

Table 4 shows total phenols, flavonoids and ORAC values for four spices and two seasoned salts of different brands. A high phenolic content as well as noteworthy antioxidant activity was found in cumin and fresh ginger. Values of seasoned salt 1 are about the double those of salt 2.

Table 5 shows the phenolics and ORAC values of both pure or aromatized oils as well as of vinegars commonly used in seasoning vegetables. Beyond the marked difference in extra-virgin olive oil and seed oil, previously described (Ninfali *et al.* 2001), it is interesting to note the limited phenolics and ORAC values of flavoured oils. The maceration of vegetables, spices or herbs in extra-virgin olive oil caused a 40–60 % loss in phenolics and a 40–80 % loss in ORAC units.

Among the vinegars, red wine and apple vinegars are those with the highest phenolic and ORAC values; compared with extra-virgin olive oils, they have a higher concentration of phenolics, but their antioxidant activity is inferior.

Brassica vegetables have a strong flavour if eaten fresh and are therefore generally cooked. We compared the phenolic and ORAC values of some of them after cooking in boiling water or steaming (Fig. 1). Fig. 1(A) shows that boiled brassica vegetables lost almost 80 % of their phenolic content, whereas steamed vegetables lost only 20–30 %, compared with fresh vegetables. Fig. 1(B) shows that the ORAC values of the same vegetables showed a pattern very similar to that of the phenolic content. Fig. 2 shows the linear relationship between total phenolics and type of cooking; the linear correlation coefficients were higher than 0·960 for all vegetables.

Fig. 3 shows ORAC values of different salads. Fig. 3(A) shows ORAC values for four salads of 200 g serving size. The basic salad was prepared with lettuce and tomato; this

Table 1. Total phenol, flavonoid, flavanol and oxygen radical absorbance capacity (ORAC) values in the vegetables analysed

			Total phenols*		Flavonoids*		Flavanols*		ORAC*	
Vegetables	Cultivar	Botanical name	mg/100 g	SD	mg/100 g	SD	mg/100 g	SD	μmolTE/100 g	SD
Aubergine	Violetta lunga	*Solanum melongena*	64·8	5·9	25·70	2·31	0·73	0·08	1414	131
	Black beauty		57·4	6·0	28·40	2·61	0·35	0·03	1194	126
Artichoke	Violetto	*Cynara scolymus*	330·4	32	285·20	25·0	0·88	0·09	6552	650
Asparagus	Argenteuil	*Asparagus officinalis*	64·0	5·9	24·60	2·61	0·77	0·08	1288	130
Beet green	Sottile marchigiana	*Beta vulgaris*	53·0	6·2	47·00	4·17	2·41	0·21	2724	256
Beetroot	Tonda sanguigna	*Beta vulgaris* var Rubra	154·1	14·0	92·80	9·23	2·21	0·19	3632	37
	Cuor di bue grosso, green cabbage	*Brassica oleracea* conv. capita, cv capita	52·5	5·2	19·50	2·01	0·81	0·06	856	83
Cabbage	Testa di ferro, savoy cabbage	*Brassica oleracea* conv. Capita, cv Sabauda	105·2	92·0	45·70	4·23	0·66	0·06	2050	21
	Cabeza negra, black cabbage	*Brassica oleracea* conv. Acephala, cv viridis	108·6	10·0	38·60	3·86	0·79	0·08	1773	181
Broccoli	Ramoso calabrese	*Brassica oleracea* conv. Botrytis, cv Italica	109·5	10·0	60·10	6·11	0·64	0·07	3529	353
Carrot	Tancar	*Daucus carota*	14·6	2·1	12·80	1·30	0·53	0·05	107	10
Celery	L. var dulce	*Apium graveolens*	13·5	1·5	6·10	0·61	0·51	0·06	343	35
Cauliflower	Precoce di fano	*Brassica oleracea*, conv. Botrytis	62·3	6·1	32·00	3·51	0·72	0·08	925	90
Courgette	Verde di Milano	*Cucurbita pepo*	26·4	3·0	9·02	0·92	0·58	0·05	180	15
	Di faenza		32·4	3·1	7·20	0·81	0·47	0·04	344	26
Cucumber	Lungo verde degli ortolani	*Cucumis sativus*	18·9	2·0	4·71	0·47	0·41	0·03	182	15
	Verde lunghissimo		18·7	2·0	0·85	0·09	0·29	0·02	252	29
Fennel	Chiarino	*Foeniculum vulgare*	27·5	2·2	11·0	1·12	0·22	0·02	361	37
Garlic	Bianco	*Allium sativum*	81·2	7·9	12·4	1·09	1·69	0·14	5346	550
Green pepper	Midway	*Capsicum annuum*, cv Grossum	44·6	4·6	9·90	0·92	0·56	0·06	1059	110
Green chilli	Sigaretta di Bergamo	*Capsicum annuum*, (frutescens)	101·1	11·0	8·92	0·85	0·42	0·03	534	55
Leek	Atal	*Allium porrum*	41·6	4·0	10·10	1·02	1·01	0·09	490	43
	Rossa di Trento		88·2	8·5	28·00	3·09	0·53	0·04	3323	320
	Romana		54·7	5·1	38·70	4·01	0·98	0·08	910	89
Lettuce	Cappuccio estiva 'Kagnran'	*Lactuga sativa* L.	44·2	4·1	36·50	4·10	0·82	0·08	956	90
	Catalogna		55·6	4·2	47·60	5·01	1·26	0·13	1053	101
	Cocarde		66·2	6·2	25·90	2·26	0·54	0·06	2127	214
Onion	Bianca di maggio	*Allium cepa*	23·6	2·4	6·40	0·7	0·28	0·02	342	35
	Rossa di tropea		42·8	4·1	3·60	0·4	0·21	0·02	1521	138
Radish	Tondo	*Raphanus sativum*	61·4	6·9	10·90	1·10	1·25	0·15	3602	381
	Jolly		30·0	3·5	10·80	1·20	1·26	0·13	1240	147
Red chicory	Rosso di chioggia	*Cichorium intybus*	129·5	11·0	89·10	8·56	1·13	0·10	3537	349
Red chilli	Ciliegia piccante	*Capsicum annuum*, (frutescens)	158·1	12·0	15·30	1·60	0·66	0·06	509	54
Red pepper	Quadrato d'asti rosso	*Capsicum annuum*, cv Grossum	76·5	8·2	7·91	0·81	0·42	0·04	842	80
Spinach	America	*Spinacia oleracea*	89·4	8·1	32·50	3·26	1·34	0·12	2732	287
Squash	Butternut	*Cucurbita pepo*	23·2	2·1	9·22	0·98	0·33	0·03	396	41
	Miroo a grappolo		50·7	5·3	6·21	0·61	0·26	0·02	934	96
Tomato	S. Marzano	*Solanum lycopersicum* L.	32·3	2·9	6·12	0·61	0·48	0·03	697	70
	Sarom		31·3	2·9	7·06	0·81	0·15	0·02	395	40
Yellow pepper	Cuneo giallo	*Capsicum annuum*, cv Grossum	113·7	11·0	7·02	0·72	0·91	0·09	950	90

*Values are referred to mg/100 g fresh weight vegetable and are the means with standard deviations of four different determinations.

Table 2. Statistical coefficients obtained from the multiple linear regression model

Variables	Regression coefficients	*P* value
Total phenols	10·101	0·048
Flavonoids	8·659	0·156
Flavanols	1042·778	0·001

The test was performed using the values from Table 1. The significance of the regression coefficients was evaluated by the Student's *t* test.

salad was enriched with one ingredient in each step, while subtracting a proportional amount of previously present vegetables in such a way as to maintain the 200 g serving size. In practical terms, in salad 2 we added cucumber, in salad 3 onions and in salad 4 carrots. The phenolic content of the four salads did not show any significant difference (data not shown). On the contrary, ORAC values increased by 20 % in type 3 salads in comparison to type 1 salads, whereas in type 4 salads, reducing the other components to allow for the addition of carrots, the ORAC value decreased by 30 %.

Fig. 3(B) shows the ORAC values of salads to which fresh aromatic herbs had been added. The addition of 1·5 % w/w of lemon balm doubled the ORAC value of the basic salad, whereas the addition of 1·5 % w/w marjoram increased it 4-fold. The content of phenolics correlated with the antioxidant capacity in the three salads (data not shown). In practice, a 200 g portion of a salad enriched with 1·5 % fresh marjoram leaves gave an intake of 200 ± 10 mg phenolics and 4000 ± 300 μmolTrolox equivalents (ORAC units).

Discussion

The present study shows the phenolic content and antioxidant capacities of fresh and seasonally harvested vegetables. The phenolic content was matched with the concentration of two phenolic subgroups, flavonoids and flavanols. By a multiple linear regression model, we provided significant evidence that ORAC values are strictly dependent on the phenolics and that the contribution to ORAC of the flavanols is remarkable. We also show that the diversification and the combination into salads of different vegetables provides an opportunity to introduce a variety of phenolics with the possibility of markedly increasing the total antioxidant capacity of the vegetable portion.

The use of freshly harvested vegetables in human nutrition is of fundamental importance today as modern storage or transforming systems allow the conservation of fresh vegetables for long periods of time in refrigerated cells under a controlled atmosphere, although the long-storage

Table 3. Phenolic, flavonoid, flavanol and oxygen radical absorbance capacity (ORAC) values in selected herbs

		Total phenols*		Flavonoids*		Flavanols*		ORAC*	
Herbs	Botanical name	mg/100 g	SD	mg/100 g	SD	mg/100 g	SD	μmolTE/100 g	SD
Chive	*Allium schoenoprasum*	74·9	8·9	35·3	3·1	1·10	0·10	2094·2	196
Dill	*Anethum graveolens*	215·2	22·0	93·2	9·6	0·73	0·06	4392·1	403
Garden sage	*Salvia officinalis*	798·0	81·0	749·5	71·0	1·61	0·17	32 004·1	3095
Garden savory	*Santureja hortensis*	201·2	20·0	67·5	62·0	1·13	0·12	9645·2	871
Garden thyme	*Thymus vulgaris*	1537·0	140·0	1165·3	100·0	0·22	0·02	27 425·5	2501
Hyssop	*Hyssopus officinalis*	214·5	21·0	176·0	17·0	2·65	0·31	6050·2	600
Lemon balm	*Melissa officinalis*	434·0	40·0	289·0	26·0	1·91	0·21	5996·5	500
Marjoram	*Origanum majorana*	854·2	83·0	812·6	82·0	2·71	0·26	27 297·4	2611
Oregano	*Origanum vulgare*	435·1	41·0	361·0	31·0	1·14	0·12	13 970·2	1090
Parsley	*Petroselium hortensis*	67·9	6·1	52·2	4·2	0·90	0·06	1301·8	131
Peppermint	*Mentha piperita*	611·2	60·0	592·5	49·0	4·33	0·39	13 978·1	1100
Rocket	*Eruca sativa*	136·4	12·0	46·0	6·2	1·42	0·13	2373·3	230
Rosemary	*Rosmarinus officinalis*	1377·3	130·0	1321·2	131·0	2·41	0·21	290·32	2842
Sweet basil	*Ocymum basilicum*	234·0	28·0	230·0	20·0	0·93	0·09	4805·2	450
Tarragon	*Artemisia Dracunculus*	570·0	59·0	537·0	48·0	0·11	0·02	15 542·2	1206

* Values are referred to mg/100 g fresh weight vegetable and are means with standard deviations of four different determinations.

Table 4. Phenolic, flavonoid and oxygen radical absorbance capacity (ORAC) values in selected spices

		Total phenols*		Total flavonoids*		ORAC*	
Spices	Botanical name	mg/100 g	SD	mg/100 g	SD	μmolTE/100 g	SD
Cumin	*Cuminum cyminum*	750·0	69	740·1	76·0	76 800·2	7500
Cardamom	*Elettaria cardamomum*	148·3	15	19·3	2·3	2764·0	254
Coriander	*Coriandrum sativum*	134·2	14	94·6	9·2	5141·3	531
Fresh ginger	*Zingiber officinalis*	200·5	19	117·5	12·0	14 840·2	1060
Seasoned salt 1	–	274·2	16	255·0	23·0	1897·3	132
Seasoned salt 2	–	110·5	12	91·0	8·0	897·0	78

* Values are referred to mg/100 g of spices and are the means with standard deviations of four different determinations.

Table 5. Phenolic and oxygen radical absorbance capacity (ORAC) values in selected oils and vinegars

		Total phenols		ORAC	
	Oils and vinegars	mg/100 g*	SD	μmolTE/100 g*	SD
Oils	Seed oil (peanut)	0·13	0·02	106	9·5
	Extra-virgin olive oil	16·50	1·50	1150	103·0
	Extra-virgin olive oil with garlic	9·20	0·81	557	60·0
	Extra-virgin olive oil with garlic and red hot pepper	8·30	0·92	219	20·0
	Extra-virgin olive oil with parsley	7·60	0·63	766	71·0
	Extra-virgin olive oil with basil	7·50	0·84	684	63·0
Vinegars	Red wine vinegar	23·40	1·91	410	39·0
	Apple vinegar	20·20	1·75	564	52·0
	Honey vinegar	18·20	1·52	225	23·0
	Apple and honey vinegar	24·30	2·11	270	24·0

* Values are referred to mg/100 g oil or vinegar and are the mean ± SD of four different determinations.
Apple and honey vinegar were commercially prepared through the acetic fermentation of apple and honey in an unknown ratio. Extra-virgin olive oils with macerated vegetables were prepared by us as reported in the text. Seed oil was a named-brand peanut oil, whereas extra-virgin olive oil was locally produced by a minor brand company.

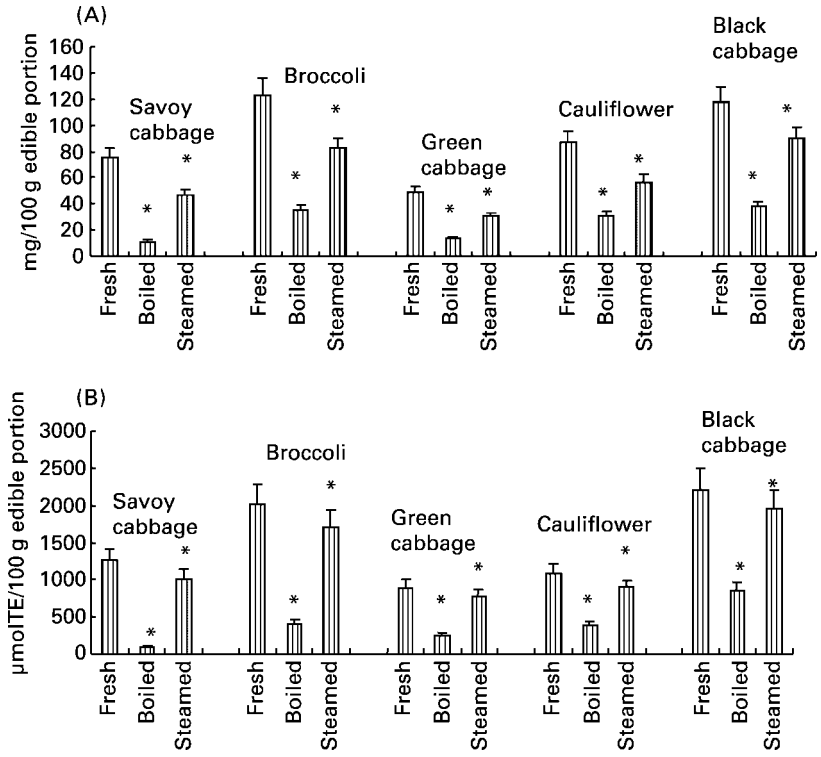

Fig. 1. Concentration of total phenols (A) and oxygen radical absorbance capacity (ORAC) values (B) in five different vegetables considered as fresh, boiled for 15 min in water or steamed for 30 min. All values are the means with standard deviations of four different determinations. *Significantly different from fresh vegetable by ANOVA with $P \leq 0{\cdot}05$.TE.

or mildly processed vegetables possess a significantly lower phenolic and ascorbic acid content than fresh vegetables (Kalt *et al.* 1998, 1999) and their antioxidant capacity decreases proportionally (Serafini *et al.* 2002; Ninfali & Bacchiocca, 2004). Only the raw and seasonally harvested vegetables are able to exhibit *in vitro* their maximal antioxidant capacity owing to their intact phenolic and flavanol contents.

The present results are obtained in *in vitro* conditions, but they can certainly have a relevance in *in vivo*. The bioavailability of the phenolics is one of the most studied aspects of the nutritional biochemistry of vegetables. The vast number of studies so far produced has provided the following evidence:

1. When the phenolic content of the consumed food is compared with the phenolic concentration present in plasma, part of the phenol glucoside moiety is shown to appear in the plasma as aglycones or as glucuronide or sulphate conjugates (Walle, 2004), which maintain their antioxidant capacity (Lu *et al.* 2003).
2. The pharmacokinetics of the phenolics depend on the food type since, for instance, in a vegetable diet containing citrus fruits, only quercetin, but not

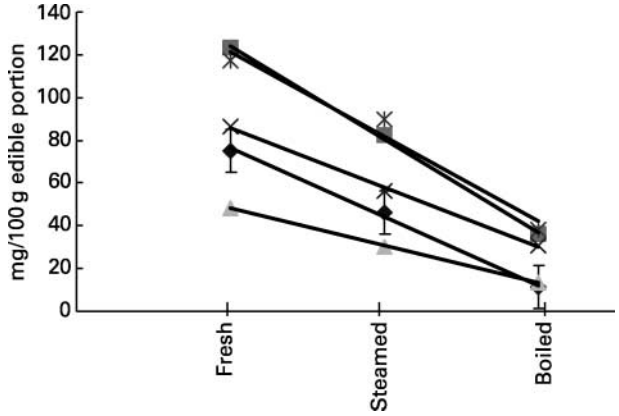

Fig. 2. Linear relationship between total phenolics and type of cooking. Cooking conditions were the same as reported in the legend to Fig. 1. The linear correlation coefficients were the following: broccoli, *r* 0·998; cauliflower, *r* 0·997; black cabbage, *r* 0·968; green cabbage, *r* 0·999; savoy cabbage, *r* 0·996.

hesperetin or naringenin, was considered to be a good biomarker of intake (Erlund *et al.* 2002), whereas after the consumption of cooked tomato paste, naringenin was found to be a good marker of bioavailability (Bugianesi *et al.* 2002).

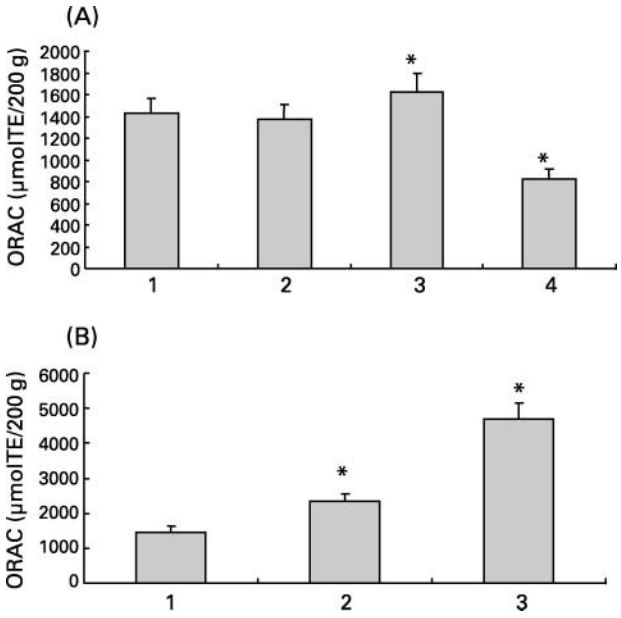

Fig. 3. Oxygen radical absorbance capacity (ORAC) values of (A) four different vegetable salads and (B) three salads different in terms of the addition of aromatic herbs. Each salad has a total weight of 200 g. In (A), the ingredients are as listed: 1: 76 g lettuce + 124 g tomato; 2: 50 g lettuce + 80 g tomato + 70 g cucumber; 3: 44 g lettuce + 72 g tomato + 63 g cucumber + 21 g onion; 4: 39 g lettuce + 64 g tomato + 45 g cucumber + 18 g onion + 34 g carrot. For cultivars of vegetables used for the salads see p. 2 of proofs. In (B), each salad has these ingredients. 1: 76 g lettuce + 124 g tomato; 2: 75 g lettuce + 122 g tomato + 3 g lemon balm; 3: 75 g lettuce + 122 g tomato + 3 g marjoram. For cultivars of vegetables used see p. 2 of proofs. Values are the mean ± SD of four different determinations. *Significantly different from salad 1 by ANOVA with $P \leq 0{\cdot}05$. TE.

In summary, this suggests that not all of the ingested phenolics can reach the plasma, but those that escape in the bloodstream lead to a significant increase in total plasmatic antioxidant capacity, both when they are in the free aglyconic form and when they are in the glucuronide or sulphate conjugated form (Cao *et al.* 1998; Lu *et al.* 2003; Prior *et al.* 2003). The interval of time during which the plasmatic antioxidant capacity remains significantly increased depends on the amount and type of the vegetable mixture consumed.

In this paper, we attributed a major importance to the phenolic family, whereas we did not evaluate specifically the contribution to the ORAC values of the vitamin C concentration, since the vitamin C antioxidant capacity can be better estimated using other methods of detection (Szeto *et al.* 2002). However, the contribution of vitamin C to the ORAC values of the vegetables reported in Table 1 can be derived, for example, for peppers and *Brassicacea*, through the following calculation. Peppers have an ascorbate value in the intracellular fluid of 15 (SD 2) μM, whereas for brassica vegetables this is about 5 (SD 1) μM vitamin C (www.inran.it). Since the ORAC value of 1 μM ascorbate solution is 0·95 (SD 0·02) μmol Trolox equivalents/l (Ou *et al.* 2001), the contribution of vitamin C to the ORAC can be obtained by the equation:

$$[(0{\cdot}95 \times \mu M \text{ ascorbate})/\text{ORAC value}] \times 100.$$

Using this method, the contribution of ascorbic acid to the total ORAC value was in the range 3–5 % for the peppers and 0·5–1·0 % for the brassicas.

A second aspect we consider in the present study is the choice of cultivar. The comparison of selected cultivars reveals impressive differences. Among the various types of lettuce, we found that the phenolic and ORAC values of Rossa di Trento lettuce were 3–4-fold higher than the values obtained from Romana lettuce, one of the most consumed lettuces in Italy, which was used as a reference. These data indicate that the choice of the cultivar increases both the quantity and the quality of phenolics and very likely also the health benefits. The lettuce Rossa di Trento and Chioggia red chicory, which in addition also contain anthocyanins, show high levels of phytochemicals so the consumption of these cultivars should be encouraged.

Our data on vegetable combinations show that the introduction of different vegetables into the same mixture does not significantly change the phenolic content but can change the ORAC value, which accurately represents the antioxidant capacity of the mixture. We have shown that the introduction of onions increased the ORAC value by 20 %, whereas carrots reduced it by 30 %. The antioxidant capacity can be increased by the opportune combination of vegetables. However, the norm of diversifying vegetable consumption should remain since a decline in the ORAC value is not necessarily a negative aspect if a large variety of dietary antioxidants is ingested. Moreover, the ORAC method measures only hydrosoluble antioxidants. Since we have applied it to salads including carrots, which contain carotenoids extractable by means of apolar solvents, we justify the carrot-induced decrease in the ORAC value considering that carotenoids express limited

antioxidant activity towards the peroxyl radicals utilized in the ORAC assay. However, carotenoids are strong antioxidants against the singlet oxygen, and we therefore need a sufficient intake of carrots and other yellow vegetables in the daily diet.

In this work, we have quantified the loss of phenolics and antioxidant capacity in brassica vegetables that need to be cooked. Steamed vegetables retained about 80 % of the phenolic and ORAC values of raw vegetables; boiled vegetables retained only 30 % of antioxidants, also losing flavour. From raw to boiled brassica vegetables, a linear negative relationship was found between phenolic content and type of cooking. Therefore, the preferred cooking process for vegetables should be steaming at the mildest temperature and for the least possible time in order to protect phenolics and vitamins. This aspect has been elucidated several times in terms of vitamin loss but only seldom in terms of phenolics and ORAC value. The present report contributes to quantifying the phenolic loss and definitively orientating the cooking process toward steaming (Rumm-Kreuter, 2001).

The contribution of aromatic herbs to phenolic and ORAC values is another interesting aspect of this work. Our comparative analysis gave very high ORAC values for garden sage, marjoram, rosemary and garden thyme. These aromatic herbs, utilized in many food products, have been shown to be rich in rosmarinic acid (Zheng & Wang, 2001), a very potent antioxidant. Aromatic herbs represent a reservoir of phenolic compounds concentrated in just a few grams of material and can represent one of the simplest ways to increase the phenolic content and antioxidant capacity of the daily diet, with possible health benefits (Zheng & Wang, 2001). Our data show that the introduction of aromatic herbs into the salads markedly increases the phenolic and ORAC values of the whole salad.

Spices and aromatized salts should be regarded as supplement seasonings capable of providing a marked increase in phenolic and antioxidant capacity. Among the selected spices, we revealed that cumin has the highest ORAC value, although its phenolic and flavonoid content is not the highest.

Two-herb seasoned salt showed very different phenolic and ORAC values; this could be due either to the different composition of the dehydrated herbs or to the different salt:herb ratio. The producers generally did not specify these data, although this information would represent a useful parameter of quality, highlighting their health benefits to consumers.

Finally, we would like to illustrate the importance of the use of salad seasonings. Extra-virgin olive oils, carefully produced using freshly gathered olives at the right degree of maturation, should be the principal condiment for their content of phenolic compounds, which, due to their marked antioxidant capacity, protect the cardiovascular system (Visioli & Galli, 1998; Visioli *et al.* 1998). On the contrary, we cannot support the use of aromatized oils in which the phenolic content has been dramatically reduced as a result of the activity of phenol oxidases contained in the vegetable, or the use of seed oil, which does not contribute in any way to the phenolic pool but furnishes only fatty acids. The presence of phenolics in wine (Miyagi *et al.* 1997; Mukamal *et al.* 2003) and apple vinegars can have positive health effects since these maintain a good portion of the phenolics present in the fruit expressing a significant antioxidant capacity (Burns *et al.* 2000).

On the basis of the results obtained from the evaluation of phenolic and flavonoid content and the ORAC values of several vegetables, herbs, spices and dressings, we conclude that it is important to educate consumers on the benefits of varying vegetable consumption, choosing those that have the highest antioxidant capacity in order to promote a healthy diet. We stress the need to introduce aromatic herbs as a seasoning supplement in the diet of every age group. The addition of aromatic herbs to salads, at a percentage compatible with palatability, markedly increases their antioxidant capacity. Salad dressings for normal, daily use, extra-virgin olive oil and wine or apple vinegar should be profitably integrated with spices and seasoned salts.

The analysis of the differences in phenolic content and antioxidant capacity of selected vegetables reported here provides a simple and compelling tool for nutrition professionals to guide family vegetable consumption.

Acknowledgements

The authors wish to thank the Association Legambiente of Urbino and the Regione Marche (Ambito Locale 4) for their support of the research on ORAC food analysis.

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