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Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweets consumed in Italy assessed by three different *in vitro* assays

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With the aim to expand the Italian total antioxidant capacity (TAC) database, the TAC values of 11 spices, 5 dried fruits, 7 sweets, 18 cereal products, 5 pulses, and 6 nuts were determined using three different assays and considering the contribution of bound antioxidant compounds in fiber-rich foods (*i. e.* cereals, legumes, and nuts). Among spices, saffron displayed the highest antioxidant capacity, whereas among dried fruits, prune exhibited the highest value. The TAC values of all the chocolates analyzed were far higher than the other sweet extracts measured. Among cereal products, whole meal buckwheat and wheat bran had the greatest TAC. Among pulses and nuts, broad bean, lentil and walnuts had the highest antioxidant capacity, whereas chickpeas, pine nuts and peanuts were less effective. The contribution of bound phytochemicals to the overall TAC was relevant in cereals as well as in nuts and pulses. The complete TAC database could be utilized to properly investigate the role of dietary antioxidants in disease prevention.

Keywords: Cereals / Herbs / Nuts / Pulses / Total antioxidant capacity

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1 Introduction

Consumption of fruits and vegetables, as well as grains and nuts, has been associated with reduced risk of chronic diseases [1, 2]. Among food components with protective effect on chronic diseases, phytochemicals, a class of plant-derived molecules endowed of strong antioxidant properties, have received great attention. The additive and synergistic effects of such bioactive molecules present in plant food are responsible for their potent antioxidant properties. Thus, in order to properly investigate the role of dietary antioxidants in disease prevention, a complete database of antioxidant-rich foods is required.

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Abbreviations: ABAP, 2,2′-azobis-(2-amidinopropane) dihydrochloride; **ABTS**, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic) acid; **ABTS$^{\bullet+}$**, ABTS radical cation; **FRAP**, ferric reducing-antioxidant power; **R-PE**, R-phycoerythrin; **TAC**, total antioxidant capacity; **TEAC**, Trolox equivalent antioxidant capacity; **TPTZ**, 2,4,6-tripyridyl-s-triazine; **TRAP**, total radical-trapping antioxidant parameter; **Trolox**, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

The measure of total antioxidant capacity (TAC) may be an appropriate approach to assess the cumulative antioxidant properties of plant foods [3]. Moreover, the importance of TAC as a novel tool to investigate the association between diet and oxidative stress-induced disease is further suggested by recent studies [4, 5] showing respectively a negative association between dietary TAC and the incidence of gastric cancer or the levels of C-reactive protein in observational studies. In order to evaluate the overall intake of TAC in population studies, the TAC of 34 vegetables, 30 fruits, 34 beverages and 6 vegetable oils of the varieties most commonly consumed in Italy has been previously analyzed using three different assays [3]. In the present study, the TAC of spices, dried fruits, sweets, cereals, pulses, and nuts was determined with the aim to complete the Italian TAC database. Moreover, in fiber-rich foods where phenolics are present in both free and bound forms, such as cereals, legumes, and nuts, we assessed the contribution of bound antioxidant compounds to the TAC value.

2 Materials and methods

2.1 Chemicals

The 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis(3-ethylbenzothiazoline-6-sul-

fonic acid) diammonium salt (ABTS), and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, MO). R-phycoerythrin (R-PE) was purchased from Prozyme (San Leandro, CA); 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP) was purchased from Waco Chemicals (Richmond, VA).

All chemicals and solvents used were of HPLC-grade and were purchased from Carlo Erba (Carlo Erba, Milan, Italy). High-purity water was produced in the laboratory by using an Alpha-Q system (Millipore, Marlborough, MA).

2.2 Samples

The selection of the samples was based on food consumption data of the EPIC cohort of Varese province (Italy) kindly provided by the Epidemiology Unit of the National Cancer Institute based in Milan and developed on the basis of 24-h recalls recorded in the North Italy area (Dr. V. Krogh, Department of Epidemiology, National Cancer Institute, Milan, Italy, personal communication). The main putative contributors of antioxidants in the Italian diet have been analyzed and presented in our previous publication [3]. In this study, the remaining foods containing antioxidant compounds are analyzed. For each item, three samples were purchased according to the three cultivars and/or brands with the highest sales in the Italian market. Like other nutrients, the estimation of the overall dietary intake of TAC does not require an estimate of the variance for any single food item if the value of the given food as consumed by the responder is sufficiently close to the average value. The same approach was used previously to generate food TAC data [3, 6].

2.2.1 Fresh herbs, spices and dried fruits

Fresh herbs (n = 8, basil, bay leaf, mint, oregano, parsley, rosemary, sage, and thyme), dried powder spices (n = 3, paprika, black pepper, and saffron) and dried fruits (n = 5, apricot, chestnut, fig, prune, and raisin) were purchased in local supermarkets. Cleaned fresh herbs and dried fruits were cut, and then equal amounts of each food were pooled, mixed and homogenized under nitrogen in a high-speed blender. A precisely weighed amount of the homogenized sample (~0.5 g) was extracted with 4 mL of water under agitation for 15 min at room temperature, centrifuged at $1000 \times g$ for 10 min and the supernatant collected. The extraction was repeated with 2 mL of water twice for fresh herbs and once for the dried fruits and the supernatants were combined. The residue was re-extracted by the addition of 4 mL of acetone under agitation for 15 min at room temperature, centrifuged at $1000 \times g$ for 10 min and the supernatant collected. The extraction was repeated with 2 mL of acetone and the two supernatants combined. In the case of dried powder spices, a precisely weighed amount of sample (between 0.3 and 0.5 g) was extracted following the same procedure described above for fresh herbs.

2.2.2 Sweets

Gianduja (hazelnut chocolate), dark and milk chocolates, acacia honey, fruit (strawberry) and vanilla ice creams and cherries jam were purchased in local supermarkets. For fruit and vanilla ice creams and for cherries jam, equal amounts of each food were pooled, mixed and homogenized under nitrogen in a high-speed blender. A precisely weighed amount of the homogenized sample (~1 g) was extracted with 4 mL of methanol under agitation for 15 min at room temperature, centrifuged at $1000 \times g$ for 10 min and the supernatant collected. The extraction was repeated twice with 2 mL of methanol and the supernatants were combined. Honey (0.2 g) was diluted with 5 mL of methanol and directly tested for TAC.

Chocolate (0.5 g) was defatted with 5 mL n-hexane for 5 min in an ultrasonic bath at 30°C and subsequently centrifuged for 10 min at $1000 \times g$. Antioxidants were then extracted with 5 mL of a mixture of acetone/water (70 : 30, v/v) under agitation for 10 min at 30°C in the ultrasonic bath, centrifuged at $1000 \times g$ for 10 min and the supernatant collected. The extraction was repeated with 2 mL of the same mixture and the supernatants were combined.

2.2.3 Nuts, pulses, cereals and breakfast cereals

Nuts (n = 6, almonds, hazelnuts, peanuts, pine nuts, pistachios, and walnuts), dried pulses (n = 5, bean, broad bean, chickpeas, lentil, and pea), grains (n = 4, barley, white and whole meal rice, and spelta kernels), flours (n = 7, whole meal buckwheat, corn, whole meal oat, whole meal rye, white and whole meal wheat, durum wheat), cereal products (n =2, white and whole meal pastas), and breakfast cereals [n = 5, barley (puffed), cornflakes, oat (whole meal, puffed with honey), rice (white, puffed), wheat bran (extruded)] were purchased in local supermarkets. Before analyses, all samples were milled in a laboratory mill and then stored at −20°C. For the preparation of free TAC soluble extracts, a precisely weighed amount of the milled sample (between 0.2 and 1 g) was extracted by the addition of 4 mL of methanol under agitation for 15 min at room temperature, centrifuged at $1000 \times g$ for 10 min and the supernatant collected. The extraction was repeated three times (only two times for the cereal group) with 2 mL of methanol and the supernatants were combined. The extraction residue was further used to extract the bound phenolic compounds. For this purpose, the residue was digested with 1.5 mL of 2 M sodium hydroxide at room temperature for 1 h with shaking under nitrogen gas. The mixture was brought to pH 3 with an appropriate amount of 3 M acetic acid. The supernatant

was extracted with 3.75 mL of ethyl acetate under agitation for 15 min at room temperature. After centrifugation at $1000 \times g$ for 10 min the supernatant was removed. The extraction was further repeated for twice. The supernatants were combined, centrifuged at $1000 \times g$ for 5 min to remove solids and the solvent evaporated to dryness. Phenolic compounds were reconstituted with water before analysis.

All food extracts were adequately diluted in the appropriate solvent (depending on their activity) and immediately analyzed in triplicate for their antioxidant capacity. For the analyses of total radical-trapping antioxidant parameter (TRAP) of cereals, the methanolic free soluble extract was evaporated to dryness and reconstituted with water. The variation in the Trolox equivalent antioxidant capacity (TEAC), TRAP and ferric reducing-antioxidant power (FRAP) values for replicate analysis was always between 3 and 10% RSD. When the RSD was higher than 10%, the analyses were repeated to confirm the value.

2.3 TEAC assay

The method is based on the ability of antioxidant molecules to quench the long-lived ABTS radical cation ($ABTS^{\bullet+}$), a blue-green chromophore with characteristic absorption at 734 nm, compared with that of Trolox, a water-soluble vitamin E analog [7]. The addition of antioxidants to the preformed radical cation reduces it to ABTS, determining a decolorization. A stable stock solution of $ABTS^{\bullet+}$was produced by reacting a 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 – 16 h before use [7]. At the beginning of the analysis day, an $ABTS^{\bullet+}$ working solution was obtained by the dilution in ethanol of the stock solution to an A of 0.70 ± 0.02 AU at 734 nm, verified by a Hewlett Packard 8453 diode array spectrophotometer (HP, Waldbronn, Germany), and used as mobile phase in a flow-injection system, according to Pellegrini *et al.* [7]. Results were expressed as TEAC in mmol of Trolox/kg.

2.4 TRAP assay

The TRAP was determined according to the method of Ghiselli *et al.* [8] based on the protection provided by antioxidants on the fluorescence decay of R-phycoerythrin (R-PE, lag-phase) during a controlled peroxidation reaction. Briefly, 120 μL of diluted sample were added to 2.4 mL of phosphate buffer (pH 7.4), 375 μL of bi-distilled water, 30 μL of diluted R-PE and 75 μL of 2,2′-azobis(2–amidinopropane) dihydrochloride (ABAP); the reaction kinetics at 38°C were recorded for 45 min by an LS-55 luminescence spectrometer (Perkin Elmer Corporation, Wellesley, MA). TRAP values were calculated from the length of the lag-phase due to the sample compared to that of Trolox and expressed as mmol of Trolox/kg.

2.5 FRAP assay

The FRAP was assessed according to Benzie and Strain [9] using a Hewlett Packard 8453 diode array spectrophotometer (HP). The method is based on the reduction of Fe^{3+}-2,4,6-tripyridyl-s-triazine (TPTZ) complex to the ferrous form at low pH. This reduction is monitored by measuring the change of A at 593 nm. Briefly, 3 mL of working FRAP reagent prepared daily was mixed with 100 μL of diluted sample, the reagents mixed and the A at 593 nm was recorded after 30-min incubation at 37°C. FRAP values were obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mmol of Fe^{2+} equivalents/kg.

3 Results and discussion

It is well known that the TAC values of food items vary considerably from variety to variety and are affected by the cultivation and storage conditions [10]. However, the major application of this database will be in epidemiological studies for exploring the putative protective role of dietary TAC in selected groups of population on chronic-degenerative diseases such as cancer and cardiovascular disorders. In such studies, the accuracy in recording a dietary item by the means of food frequency questionnaires or others food record methods (*e.g.* 24-h recall) is not high enough for providing information regarding the food seasonality and variety. Thus, each item was obtained by mixing three samples purchased according to the three cultivars and/or brands with the highest sales in the Italian market in order to minimize the influence of such factors.

3.1 TAC of fresh herbs and spices

The TAC values of fresh herb and spice extracts and the ranking order for each assay are shown in Table 1. As already reported [11], some spices and fresh herbs had extremely high TAC values. Of the fresh herbs tested, bay leaf, rosemary and oregano all exhibited very high level of TAC values, regardless of the method applied. This observation is consistent with Zheng and Wang [12], who analyzed the antioxidant capacities of several culinary herbs by the oxygen radical absorbance capacity. The high antioxidant capacity of oregano and rosemary is likely due to the extremely high content of polyphenols [12], such as rosmarinic acid and other hydroxycinnamic acids that possess strong antioxidant activity [13]. Regarding the bay leaf, the

Table 1. Ferric reducing-antioxidant power (FRAP), total radical-trapping antioxidant parameter (TRAP) and Trolox equivalent antioxidant capacity (TEAC) of fresh herb and spice extracts[a, b)]

Herb and spice	FRAP (mmol Fe^{2+}/kg)		TRAP (mmol Trolox/kg)		TEAC (mmol Trolox/kg)	
	Value	Rank	Value	Rank	Value	Rank
Herb[c)]						
Basil	78.35	5	41.22	4	21.78	6
Bay leaf	204.49	1	89.31	1	47.93	1
Mint	38.61	7	11.81	7	8.84	7
Oregano	104.20	3	66.28	2	30.65	3
Parsley	26.05	8	9.55	8	7.91	8
Rosemary	104.56	2	56.32	3	43.95	2
Sage	77.50	6	35.20	6	23.43	5
Thyme	82.50	4	36.03	5	30.51	4
Spice[d)]						
Paprika	167.48	2	53.54	2	39.98	2
Pepper (black)	143.78	3	49.31	3	37.05	3
Saffron	739.43	1	374.05	1	53.04	1

a) Values are means, $n = 3$.
b) Values represent the sum of the water- and lipid-soluble extracts.
c) Values refer to fresh weight.
d) Values refer to dry weight.

Table 2. Ferric reducing-antioxidant power (FRAP), total radical-trapping antioxidant parameter (TRAP) and Trolox equivalent antioxidant capacity (TEAC) of dried fruit extracts[a, b)]

Dried fruit	FRAP (mmol Fe^{2+}/kg)		TRAP (mmol Trolox/kg)		TEAC (mmol Trolox/kg)	
	Value	Rank	Value	Rank	Value	Rank
Apricot	36.64	2	13.33	2	12.87	2
Chestnut	20.63	4	12.85	3	4.40	5
Fig	14.43	5	1.96	5	5.02	4
Prune	60.54	1	23.00	1	14.82	1
Raisin	23.26	3	6.17	4	6.63	3

a) Values are means, $n = 3$.
b) Values represent the sum of the water- and lipid- soluble extracts.

presence of polyphenols including isoquercitrin [14] as well as that of α- and γ-tocopherols [15] could explain its observed high antioxidant capacity.

Thyme, basil and sage exhibited intermediate TAC values in agreement with Zheng and Wang [12], whereas mint and parsley had the lowest antioxidant capacity, regardless of the assay used. Among the ground spices analyzed, saffron displayed the highest antioxidant capacity for all methods applied.

3.2 TAC of dried fruits

The overall antioxidant capacity of dried fruits and the ranking order for each assay are displayed in Table 2. Among the dried fruits analyzed, prune exhibited the highest value, in agreement with Wu *et al.* [16] and Karakaya *et al.*[17], followed by apricot. Conversely, among the dried fruits analyzed by Halvorsen *et al.* [18] the most effective was apricot followed by prune. The higher antioxidant capacity of prune is probably due to its higher phenolic content than that of dried apricot [17]. The principal phytochemicals in dried plums are phenolics, which include hydroxycinnamoylquinic acids and their derivates, flavonoids and coumarins [19].

3.3 TAC of sweets

TAC values of sweets are shown in Table 3. As expected, the TAC of all chocolates analyzed were far higher than the other sweets measured (*i.e.* honey, ice creams and jam). Chocolate containing cocoa is a rich source of flavonoids, particularly flavan-3-ols, mainly epicatechin, catechin and traces of other compounds, and procyanidins [20]. Procyanidins, mostly constituted by a variable number of flavan-

Table 3. Ferric reducing antioxidant power (FRAP), total radical-trapping antioxidant parameter (TRAP) and Trolox equivalent antioxidant capacity (TEAC) of sweet extracts[a)]

Sweet	FRAP (mmol Fe^{2+}/kg)		TRAP (mmol Trolox/kg)		TEAC (mmol Trolox/kg)	
	Value	Rank	Value	Rank	Value	Rank
Chocolate (Gianduja)	108.12	2	48.65	2	50.34	2
Chocolate (dark)	182.18	1	91.60	1	94.81	1
Chocolate (milk)	42.13	3	11.55	3	36.16	3
Honey (acacia)	0.90	7	N.D.[b)]	7	0.80	6
Ice cream (fruit)	9.89	5	2.23	5	2.17	5
Ice cream (vanilla)	2.41	6	0.46	6	0.25	7
Jam (cherries)	17.32	4	5.19	4	3.93	4

a) Values are means, $n = 3$.
b) N.D.; not detectable.

3,4-diol units, may be present in chocolate in a mixture consisting of dimers, trimers and polymers of up to 10 units [20]. In agreement with the literature [21, 22], dark chocolate showed the highest TAC value for all the three assays followed by gianduja and milk chocolate. The differences among chocolate TAC values were probably due to the different composition in term of cocoa polyphenol content. In fact, comparing dark to milk chocolate, it has been reported that the former showed higher content of catechins (dark 48 – 137 mg/100 g, milk 15 – 16 mg/100 g) [21] and of procyanidins, which have been found to be highly correlated to the antioxidant potential [22].

Regarding honey, the antioxidant capacity appeared to be a result of the combined activity of a wide range of compounds; phenolics (like *p*-hydroxybenzoic, *p*-coumaric, *cis*, *trans*-abscisic, and cinnamic acids, pinobanksin, pinocembrin, and chrysin), peptides, organic acids, Maillard reaction products, and possibly other minor components [23]. It has been claimed that honey is comparable to fruits and vegetables in term of antioxidant capacity on a fresh weight basis [24]. In disagreement with this statement, the acacia honey analyzed in the present study showed very low values of TAC, even not detectable when analyzed by TRAP assay, probably due to its floral source. In fact, in two studies [23, 24], honeys from various floral sources showed different TAC values: among all the honey analyzed, acacia honey was the less effective. However, the acacia honey is among the most consumed honey in the Italian population.

Finally, considering the other sweet products, fruit ice cream showed higher TAC value than vanilla ice cream, due to the presence of the added fruit purée, and cherry jam showed an appreciable value of TAC similar to that of cherry fruits [3]. This is not surprising, as the effect of processing to obtain jams, evaluated on raspberry, demonstrated that the main raspberry phenolics are not much affected by thermal processing under the specific conditions used by the industries [25].

3.4 TAC of cereals

Grain phytochemicals may exist both in free, soluble conjugates, and in insoluble bound forms [26]. Most are in insoluble bound forms: in fact, cereals are rich in cinnamic acids, mainly ferulic acid and its oxidized forms, esterified to arabinose residues in primary cell wall and to arabinoxylan and arabinogalactan in the aleurone layer and pericarp [27]. However, the content of phytochemicals in grains has been commonly underestimated in the literature [26, 28], because bound phytochemicals were not included in the determination. It has been demonstrated that bound phytochemicals are the major contributors to the total antioxidant capacity of cereals: 90% in wheat, 87% in corn, 71% in rice and 58% in oats [26]. Thus, in order to measure the complete TAC of cereal products, we utilized methanol for the extraction of soluble phytochemicals followed by an alkaline hydrolysis of the residue to complete the extraction of bound phenolic compounds. The TAC values of cereals extracts, obtained by methanol and after alkaline hydrolysis, and the ranking order for each assay are shown in Table 4.

For grains, in the case of TEAC and FRAP assays, barley exhibited the highest total antioxidant capacity, obtained by the sum of soluble and bound compound extraction, followed by brown rice for FRAP assay and spelta for TEAC assay. Conversely, spelta and brown rice had the highest TAC values in the TRAP assay. As expected, white rice exhibited the lowest TAC value, regardless of the applied method, since the milling process removes many antioxidant components concentrated in the outer layers of grains and in the germ [29]. In agreement with the literature [26, 30], for all the grains analyzed the bound phytochemicals were the major contributors to the total antioxidant capacity, ranging from 50% in barley, brown rice and spelta analyzed by TEAC assay and barley analyzed by TRAP assay to 100% in white rice, where the value of methanolic extract was not detectable in the TRAP assay.

Table 4. Ferric reducing antioxidant power (FRAP), total radical-trapping antioxidant parameter (TRAP) and Trolox equivalent antioxidant capacity (TEAC) of cereal extracts[a)]

Cereal	FRAP (mmol Fe^{2+}/kg)				TRAP (mmol Trolox/kg)				TEAC (mmol Trolox/kg)			
	Free[b)]	Bound[c)]	Total	Rank	Free[b)]	Bound[c)]	Total	Rank	Free[b)]	Bound[c)]	Total	Rank
Grains												
Barley	7.54	11.43	18.97	1	1.88	2.28	4.16	2	2.34	2.25	4.59	1
Rice (white)	0.54	7.37	7.91	4	N.D.[d)]	3.74	3.74	3	0.26	1.94	2.20	4
Rice (brown)	5.28	11.55	16.83	2	0.90	3.74	4.64	1	1.83	2.02	3.85	3
Spelta	4.80	9.36	14.16	3	1.71	2.93	4.64	1	1.96	2.05	4.01	2
Flours												
Buckwheat (whole meal)	35.39	19.93	55.32	1	7.60	8.69	16.29	1	19.92	6.30	26.22	1
Corn (white)	1.73	9.79	11.52	6	0.31	2.38	2.69	4	0.65	2.36	3.01	4
Durum wheat (white)	5.95	7.14	13.09	4	0.67	1.42	2.09	6	1.08	1.62	2.70	6
Oat (whole meal)	5.99	6.19	12.18	5	0.83	1.71	2.54	5	1.10	1.69	2.79	5
Rye (whole meal)	7.81	15.58	23.39	2	1.37	7.14	8.51	2	5.19	6.45	11.64	2
Wheat (white)	3.87	6.58	10.45	7	0.26	0.84	1.10	7	0.37	1.56	1.93	7
Wheat (whole meal)	7.47	12.76	20.23	3	0.88	3.26	4.14	3	1.99	2.59	4.58	3
Cereal products												
Pasta (white)	2.38	7.46	9.84	2	0.31	1.24	1.55	2	0.38	1.61	1.99	2
Pasta (whole meal)	5.20	18.00	23.20	1	0.95	7.77	8.72	1	2.13	3.01	5.15	1
Breakfast cereals												
Barley (puffed)	7.14	15.26	22.40	2	1.77	6.09	7.86	2	2.44	2.60	5.04	2
Cornflakes	2.35	11.41	13.76	4	0.46	2.33	2.79	4	0.63	1.56	2.19	4
Oat (whole meal, puffed, with honey)	6.13	8.36	14.49	3	1.10	2.80	3.90	3	1.53	2.52	4.05	3
Rice (white, puffed)	2.27	9.94	12.21	5	0.21	2.33	2.54	5	0.49	1.58	2.07	5
Wheat bran (extruded)	14.90	37.06	51.96	1	3.84	18.08	21.92	1	5.22	5.97	11.19	1

a) Values are means, $n = 3$.
b) Values of methanolic extracts.
c) Values of extracts after hydrolysis.
d) N.D.; not detectable.

Regarding flours, buckwheat displayed the highest TAC values for all the assays, followed by rye and whole meal wheat. White wheat showed the lowest TAC value, regardless of the applied method, being the most refined flour analyzed and confirming that most phenolic phytochemicals of whole-wheat grains are present in the bran/germ fraction [30] and removed during the refining process. As already stated for grains, most of the TAC was in the bound fraction with exception of buckwheat, where the contribution of soluble extract was more relevant. It has already been demonstrated that methanol is the most effective solvent in the extraction of buckwheat antioxidant compounds, such as flavonoids including rutin and quercetin and traces of cinnamic and benzoic acids [31]. Conversely, the very high contribution of bound extract observed in corn, between 78 and 88% for TEAC and TRAP assays, respectively, is consistent with the very high content of bound ferulic acid reported by Adom *et al.* [26]. Finally, the higher TAC value of durum wheat flour, measured by all the methods applied, with respect to that of common wheat flour is likely due to its higher content of ferulic acid [30].

Moving to cereal products, as expected, whole meal pasta exhibited higher TAC values if compared with the refined one for all the assays, due to the distribution of antioxidants in grains. It is worth noting that the breakfast cereals analyzed showed the same ranking order for all the applied methods: extruded wheat bran had the highest TAC value, following by puffed barley, puffed whole meal oat with honey. In agreement with Miller *et al.* [32], cornflakes, made from degerminated grains, and white puffed rice were the least effective. Baublis *et al.* [33] found an increased antioxidant capacity of aqueous extracts from cereal subjected to simulated gastrointestinal pH treatments with respect to untreated aqueous extracts. The authors justified this behavior by an increased extractability of phenolics esterified to carbohydrates from wheat bran following the acidic treatment [33]. This observation demonstrates again that the bound antioxidant components should be considered also in determining the TAC of processed cereal products.

3.5 TAC of pulses

To date, the total antioxidant capacity of pulses has been less investigated than that of cereals and other antioxidant-rich foods, such as vegetables and fruit, and information on

Table 5. Ferric reducing antioxidant power (FRAP), total radical-trapping antioxidant parameter (TRAP) and Trolox equivalent antioxidant capacity (TEAC) of pulse extracts[a)]

Pulse	FRAP (mmol Fe^{2+}/kg)				TRAP (mmol Trolox/kg)				TEAC (mmol Trolox/kg)			
	Free[b)]	Bound[c)]	Total	Rank	Free[b)]	Bound[c)]	Total	Rank	Free[b)]	Bound[c)]	Total	Rank
Bean	3.60	5.99	9.59	3	1.13	1.46	2.58	3	1.51	1.78	3.30	4
Broad bean	10.38	6.94	17.32	2	6.14	N.D.[d)]	6.14	2	7.70	5.56	13.26	1
Chickpeas	2.10	3.40	5.50	5	0.24	0.21	0.45	5	0.73	2.17	2.90	5
Lentil	8.58	33.07	41.65	1	1.32	9.36	10.68	1	3.68	5.62	9.30	2
Pea	3.56	5.04	8.59	4	N.D.[d)]	0.94	0.94	4	1.31	2.42	3.73	3

a) Values are means, $n = 3$.
b) Values of methanolic extracts.
c) Values of extracts after hydrolysis.
d) N.D.; not detectable.

their phytochemical composition is limited. Moreover, to our knowledge, the contribution of insoluble bound forms of phytochemicals to the TAC of legume seeds has not yet been investigated. In the non-lignified primary wall, the formation of ester and ether linkages between cell wall polysaccharides and feruloyl residues both in monocotyledons (*e.g.* cereals) and dicotyledons (*e.g.* legumes) has been suggested [34]. Moreover, Lozovaya *et al.* [34] postulated the possibility of the existence of ether links between ferulic acid and wall proteins found in room temperature alkali extractable fraction of several dicotyledons plant tissues. In addition, other ferulic acid ether-linked to yet unidentified wall component, different from lignin, cannot be excluded [34]. With the aim of taking into account the contribution of bound phytochemicals to TAC of pulses, the same procedure of cereal products was applied. For pulses, the TRAP, FRAP and TEAC values of methanolic and alkaline hydrolyzed extracts, the sum of these two values, and their ranking order are shown in Table 5. Considering the TAC obtained by the sum of soluble and bound compound extracts of all the legume seeds analyzed, lentil and broad bean had the highest TAC values; in particular, lentil was the most effective in the case of TRAP and FRAP assays, whereas broad bean exhibited the greatest antioxidant capacity when analyzed by TEAC assay. Conversely, chickpeas showed the lowest TAC values regardless of the assay applied. However, when methanolic extract, able to extract all the soluble and free phytochemicals, was considered broad bean resulted the legume seed with the highest TAC values in all the assays. Legumes contain a wide range of polyphenolic compounds, including flavonols, flavone glycosides, flavanols and oligomeric and polymeric proanthocyanidins [35], located essentially in their seed coat which can contribute to their antioxidant capacity. Among pulses, broad bean presents a very high content of free flavanols (average concentration of 154.5 mg total flavanols/100 g fresh weight), as reported by de Pascual-Teresa *et al.* [36], who analyzed the content of catechins and proanthocyanidins in 56 Spanish foodstuffs. Moreover, it is worth noting that among the pulses analyzed, the contribution of bound phytochemicals of broad bean was lower (or not detectable) than that of methanolic extracts. On the contrary, for all the other legume seeds analyzed, the TAC values of residues, obtained after alkaline hydrolysis at room temperature, were remarkable (Table 5). In fact, the TAC values of these extracts were higher than those of methanolic extracts, ranging from 46% in chickpeas analyzed by TRAP assay to 100% in pea, where the value of methanolic extract was not detectable in the TRAP assay.

Finally, pulses contain phytoestrogens, such as isoflavons (*i.e.* formononetin, daidzein, genistein, biochanin A, and coumesterol) and the precursors of mammalian lignans (*i.e.* matairesinol and secoisolariciresinol) [37], which could contribute to their antioxidant capacity.

3.6 TAC of nuts

Following the same analytical approach used with cereals and legumes, the alkaline hydrolysis was also carried out on nuts. In Table 6, the FRAP, TRAP and TEAC values of methanolic and alkaline hydrolyzed extracts and the sum of those for all the nuts analyzed and their ranking order are shown. In all the three assays, walnuts displayed the highest TAC values followed by pistachios. At the bottom of the ranking, there were pine nuts for FRAP and TRAP assays, and peanuts for TEAC assay. The extremely high TAC values of walnuts are not surprising considered the high phenolic content. The walnut polyphenols are principally of the non-flavonoid type (*i.e.* ellagitannins), present in the thin tan-brown skin that lines the meat of the nut, and most of these compounds have demonstrated to possess high antioxidant activity [38]. As far as pistachio antioxidant capacity is concerned, the presence of anthocyanins obtained by a solvent-soluble extraction, which have demonstrated to exhibit strong antioxidant activities [39], could explain its high antioxidant capacity [40].

Table 6. Ferric reducing-antioxidant power (FRAP), total radical-trapping antioxidant parameter (TRAP) and Trolox equivalent antioxidant capacity (TEAC) of nut extracts[a)]

Nut	FRAP (mmol Fe^{2+}/kg)				TRAP (mmol Trolox/kg)				TEAC (mmol Trolox/kg)			
	Free[b)]	Bound[c)]	Total	Rank	Free[b)]	Bound[c)]	Total	Rank	Free[b)]	Bound[c)]	Total	Rank
Almonds	23.57	17.77	41.34	4	1.78	4.55	6.33	4	9.25	4.12	13.36	3
Hazelnuts	19.39	22.92	42.31	3	2.45	4.45	6.90	3	6.69	5.33	12.02	4
Peanuts	3.11	12.36	15.46	5	0.46	2.84	3.30	5	1.76	3.00	4.76	6
Pine nuts	8.32	5.10	13.42	6	0.95	0.60	1.54	6	2.10	3.15	5.25	5
Pistachios	102.72	89.95	192.67	2	9.13	16.78	25.92	2	37.39	24.07	61.46	2
Walnuts	412.29	41.65	453.94	1	27.58	4.27	31.85	1	119.91	17.11	137.01	1

a) Values are means, $n = 3$.
b) Values of methanolic extracts.
c) Values of extracts after hydrolysis.

It is worth noting that regardless of the assay applied the high TAC value of walnuts is mainly due to the soluble extract, which contributed for almost 90% of the TAC value. Conversely, in other nuts analyzed the contribution of bound antioxidants was high ranging from 31% in almonds analyzed by TEAC assay to 86% in peanuts determined by TRAP assay. Moreover, in the case of TRAP assay the contribution of bound antioxidants was higher than that observed in the case of TEAC and FRAP assays. This different behavior among the TAC assays applied can be explained considering the fact that the TRAP assay is less able to detect liposoluble antioxidants usually present in free form such as tocopherols, of which nuts are a good source [41]. Thus, the TRAP assay resulted more affected by the water-soluble antioxidants, most of them present in bound form.

Finally, it is of interest to notice the wide range of the TAC values measured for all the assays: in fact, the TEAC values ranged from 4.76 to 137.01 mmol Trolox/kg, the FRAP values from 13.42 to 453.94 mmol Fe^{2+}/kg and the TRAP values from 1.54 to 31.85 mmol Trolox/kg. This huge difference in the TAC values is likely due to the phenolic compounds, which are high in walnuts and pistachios but very low in pine nuts, as well as to the tocopherol content, which is high in pistachios and almonds and lower in peanuts and pine nuts [41].

Our data demonstrate that the contribution of bound phytochemicals to the overall TAC is relevant not only in cereals, as already reported, but also in nuts and pulses. The fate of these phytochemicals is to reach the colon in an undigested form. Unabsorbed phytochemicals can be modified by the microflora, yielding different compounds that may be absorbed. It has been demonstrated that microbial esterase present in the mammalian intestine can release hydroxycinnamic acids bound to plant wall cell into the lumen and that free acids and their metabolites can be absorbed into the circulatory system [42]. Moreover, the unabsorbed phytochemicals may act also extracellularly in the protection of the gastrointestinal tract suggesting a potential role in colorectal cancer prevention [43, 44].

4 Concluding remarks

The data reported in the present study are needed to integrate the Italian TAC database with categories of foods such as spices, dried fruits, nuts, pulses, cereals and sweets, which are important contributors to the total TAC intake, thus allowing a more correct estimation of TAC intake in epidemiological studies. Moreover, the additional information concerning the antioxidant capacity associated with non-extractable polyphenols will allow distinguishing the protective effect linked to these compounds from that of soluble free polyphenols.

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