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Anti- and Pro-oxidant Water Soluble Activity of *Cichorium* Genus Vegetables and Effect of Thermal Treatment

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Both the pro- and antiradical water soluble activity, toward DPPH•, ROO•, OH• radicals found in seven diet vegetables belonging to the *Cichorium* genus, and the effects of boiling, freezing, and freeze-drying on such activities were investigated. The vegetables were three red cultivars of *Cichorium intybus* var. *silvestre* from three different areas of production, that is, chicory from Chioggia, Treviso, and Verona, *C. intybus* var. *foliosum* (Belgian chicory), *C. endivia* var. *latifolium* (escarole), *C. endivia* var. *crispum* ("crispa"), and a hybrid vegetable obtained by the cross between *C. intybus* var. *silvestre* and *C. endivia* var. *latifolium* (chicory from Castelfranco). The juices obtained by simple centrifugation of vegetables operating at 2 or 25 °C and submitted to the thermal technological treatments were assessed for antiradical activity using the DPPH• assay, the linoleic acid−β-carotene system, and the deoxyribose assay. In all three assays used, each vegetable juice was shown to possess antiradical activity; there was a significant level in the *C. endivia* and the Belgian chicories and higher levels in the red *C. intybus* vegetables and the hybrid vegetable. All juice behaviors in the linoleic acid−β-carotene system indicate that they also contain a thermally unstable component, which in a cold medium promptly promoted and accelerated linoleic acid peroxidation, therefore masking the presence of any thermally stable antiperoxyl radical components. The presence of these components, which efficiently protect linoleic acid from peroxidation, can be singled out only after inactivation by heating, or separation by dialysis, of the pro-oxidant components. Dialysis fractions showed that the pro-oxidant component has MW > 50000 Da and that the juices contain a number of antioxidant components which contribute to their antiradical activity.

KEYWORDS: Vegetables; antioxidants; pro-oxidants; lipid peroxidation; thermal treatment

INTRODUCTION

Free radicals initiate and accelerate lipid peroxidation, which damages the flavor of fat- and oil-rich products in foods and as well as many important biological molecules in living organisms. Plant materials are known to contain many different components that can act as antioxidants because they act as free radical scavengers, singlet oxygen quenchers, or metal chelators (*1*−*5*). These actions make plant foods of particular importance in our diet because it has been demonstrated that consumption of the antioxidant compounds occurring in fruits and vegetables protects living organisms from oxidative damage, resulting in the prevention of various chronic pathologies such as neoplastic, cardiovascular, inflammatory, and neurodegenerative diseases as well as aging in general (*6*−*8*).

A number of vegetables commonly used in the Italian diet, particularly in northern Italy, considered to be traditional Italian food, belong to the *Cichorium* genus. Some of them are grown in the winter season because of their resistance to cold temperatures. Therefore, these vegetables may also be considered an important crop because of their availability throughout the entire year, providing an important source of micronutrients during the cold season when fresh plant foods are far more scarce.

In this study we investigate the anti- and pro-oxidant activity of the water soluble components in seven common diet vegetables of the *Cichorium* genus belonging to different species (*intybus* and *endivia*), varieties, and cultivars. The pro- or antiradical activity was determined in comparison to the DPPH• stable radical, the scarcely reactive peroxyl radical, and the highly reactive hydroxyl radical, to ascertain in what measure the vegetable scavenger activity depends on the nature of the considered radical and then to verify an eventual antioxidant potential contained in each diet vegetable belonging to both spices.

The effects of thermal treatments such as boiling, freezing, and freeze-drying on the water soluble component activities and the possibility of interaction among their different fractions that, at least in chemical system, may greatly influence the activity of the whole juice were also investigated.

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Table 1. Description of Tested Vegetables[a]

name	mL g^{-1} [b]	mg mL^{-1} [c]
C. intybus var. *silvestre* (chicory of Chioggia)	0.51 ± 0.11	38.60 ± 4.11
C. intybus var. *silvestre* (chicory of Treviso)	0.57 ± 0.05	39.11 ± 3.98
C. intybus var. *silvestre* (chicory of Verona)	0.41 ± 0.04	37.93 ± 4.05
C. intybus var. *foliosum* (Belgian chicory)	0.68 ± 0.11	40.77 ± 5.08
cross between *C. intybus* and *C. endivia foliosum* (chicory of Castelfranco)	0.47 ± 0.10	44.26 ± 5.28
C. endivia var. *crispum* (crispa chicory)	0.56 ± 0.09	31.47 ± 3.76
C. endivia var. *latifolium* (escarole chicory)	0.59 ± 0.06	34.25 ± 3.95

[a] Values represent means of 10 replications. [b] Milliliters of raw juice obtained from 1 g of tested vegetables. [c] Dried residue of tested vegetables.

MATERIALS AND METHODS

Vegetable Samples. The vegetables were purchased from a local supermarket: Treviso red chicory, Chioggia red chicory, and Verona red chicory (*Cichorium intybus* var. *silvestre*), Castelfranco chicory (cross between *C. intybus* from Treviso and *Cichorium endivia* var. *foliosum*), Belgian chicory (*C. intybus* var. *foliosum*), escarole chicory (*C. endivia* var. *latifolium*), and "crispa" chicory (*C. endivia* var. *crispum*).

Sample Preparation. The vegetables were washed, weighed, cut into small pieces, homogenized, and then centrifuged at 5000 rpm for 4 min to completely separate the juices from each vegetable. The volume and the dried residue (**Table 1**) of each vegetable juice were measured, and then the juice was subdivided into six batches. The juice was filtered through Ruudfilter Schleicher Schuell 1573 (no. 314709, diameter = 190 mm) and then by Millipore membranes of cellulose acetate/cellulose nitrate mixed esters (0.45 μm). Four lots of each juice were filtered in an ice bath (2 °C), and two lots were filtered at room temperature (25 °C) and stored for 3 h or frozen for 3 months at −20 °C before analysis. Filtration resulted in a loss of most of the juice color. The first batch filtered at 2 °C was immediately analyzed; the second batch (5 mL) was heated to reach boiling in 2 min and then was boiled for 30 min, the time commonly used in home cooking of most vegetables. The temperature during boiling was experimentally measured and was found to be 102 ± 0.5 °C. The third batch (5 mL) was freeze-dried and stored at room temperature and in the dark for 1 month, and then the residue was dissolved in distilled water to the initial volume. The last batch was frozen and kept at −20 °C for 3 months before analysis.

Deoxyribose Assay. The scavenger activity of the vegetable juices, based on the inhibition of the deoxyribose degradation caused by the attack of hydroxyl radicals, was evaluated using the Aruoma et al. (*9*) method and included some modifications. The hydroxyl radical was induced in the system by Fenton reaction.

In a final volume of 1.2 mL, the reaction mixture contained the following reagents at the final concentrations: $FeCl_3$ (25 μM) premixed with EDTA (100 μM) in KH_2PO_4/KOH buffer (pH 7.4); 2-deoxy-D-ribose (2.8 mM); H_2O_2 (2.8 mM); ascorbic acid as the promoter of the reaction reducing Fe(III) to Fe(II) (100 μM); and 12 μL vegetable juice (sample); or all the same volumes of KH_2PO_4/KOH buffer (control sample). Both samples were placed in a water bath at 37 °C for 1 h, and then 1 mL of 1% thiobarbituric acid and 1 mL of 2.8% trichloroacetic acid were added. The reaction mixtures were heated in a water bath at 80 °C for 20 min, kept in ice for 5 min, and then centrifuged for 5 min at 3000 rpm to separate the particles. The absorbance values of the samples's supernatant and of the control sample were read in a spectrophotometer at 532 nm against the relative solutions prepared as described but without ascorbic acid (ΔAbs) to correct for interference due to the juice color and thiobarbituric acid-reactive substances (TBARS) that might naturally occur in vegetable juices.

The scavenger activity was expressed as the percent of inhibitory activity (IA%) of deoxyribose degradation in the presence of the vegetable juice (sample), relative to the control sample (without the vegetable juice), using the equation

$$\text{IA\%} = 100 - \frac{\Delta\text{Abs sample}}{\Delta\text{Abs control sample}} \times 100 \quad (1)$$

The scavenger activity was also determined for an aqueous solution of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox C), which was assayed at three final concentrations of 50, 100, and 200 μM.

DPPH$^\bullet$ Assay (*10*). The antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) as a free radical. A 100 μL aliquot of vegetable juice (sample) or a 100 μL aliquot of KH_2PO_4/NaOH buffer (pH 7.4) (control sample) was added to 3.9 mL of a 6×10^{-5} mol L^{-1} methanol/KH_2PO_4/NaOH buffer (50:50 v/v) DPPH$^\bullet$ solution. The decrease in absorbance was determined at 515 nm when the reaction reached a plateau (after 20 min of reaction).

The percent scavenger activity (ARA) against DPPH$^\bullet$ was calculated according to the following equation

$$\text{ARA\%} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad (2)$$

The scavenger activity was also determined for a methanolic solution of Trolox C, which was assayed at two final concentrations of 50 and 200 μM.

Linoleic Acid—β-Carotene Assay. The antioxidant activities of the vegetable juices, based on coupled oxidation of linoleic acid and β-carotene, were evaluated following the method of Taga et al. (*11*) with some particular modifications (*12*). β-Carotene (5 mg) (Merck) was dissolved in 50 mL of chloroform solution. A 3 mL aliquot of the β-carotene chloroform solution was added to a conical flask along with 40 mg of linoleic acid (Merck) and 400 mg of Tween 20 (Merck). The chloroform was evaporated until dry under reduced pressure at low temperature (<30 °C). Distilled water (100 mL) was added to the dried mixture, and the mixture was shaken. Five aliquots (400 μL) of vegetable juices were added to 5 mL of β-carotene emulsion in test tubes, and the mixture was mixed well (samples). In preliminary tests, the adding of juices showed no significant change in the sample's pH. One sample's absorbance was immediately measured using the spectrophotometer at 470 nm, and the other samples' absorbances were measured after 5, 10, 20, and 30 min of incubation in a water bath at 50 °C. Each sample was read against an emulsion prepared as described but without β-carotene (blank). To correct for any influence due to juice color in the calculation of the β-carotene degradation rate (dr), four aliquots (400 μL) of each juice were added to 5 mL of blank (blank samples). These mixtures for each time point were read spectrophotometrically, and the absorbance measured was subtracted from that of the corresponding sample. The dr of β-carotene was calculated by first-order kinetics

$$(\ln(A_0/A_t))/t = \text{dr of sample} \quad (3)$$

where A_0 = absorbance of the sample − absorbance of blank sample at time 0 (absorbance was read immediately after the addition of juice), A_t = absorbance of the sample − absorbance of blank sample at time t, and t = 5, 10, 20, or 30 min of incubation at 50 °C.

$$(\ln(a_0/a_t))/t = \text{dr of control sample} \quad (4)$$

where 400 μL of distilled water was added to 5 mL of β-carotene emulsion and treated as the corresponding sample, a_0 = absorbance of the control sample at time 0, and a_t = absorbance of the sample at time t.

Antioxidant activity (AA) was expressed as the percent of inhibition relative to the control using the following equation:

$$\text{AA\%} = \frac{\text{dr control sample} - \text{dr sample}}{\text{dr control sample}} \times 100 \quad (5)$$

A Trolox C (Sigma) solution (100 μM) was also assayed for AA.

Statistical Analysis. The values represent mean values of at least 10 replications, carried out on 10 different samples of each vegetable. Data were analyzed by analysis of variance (ANOVA) with the

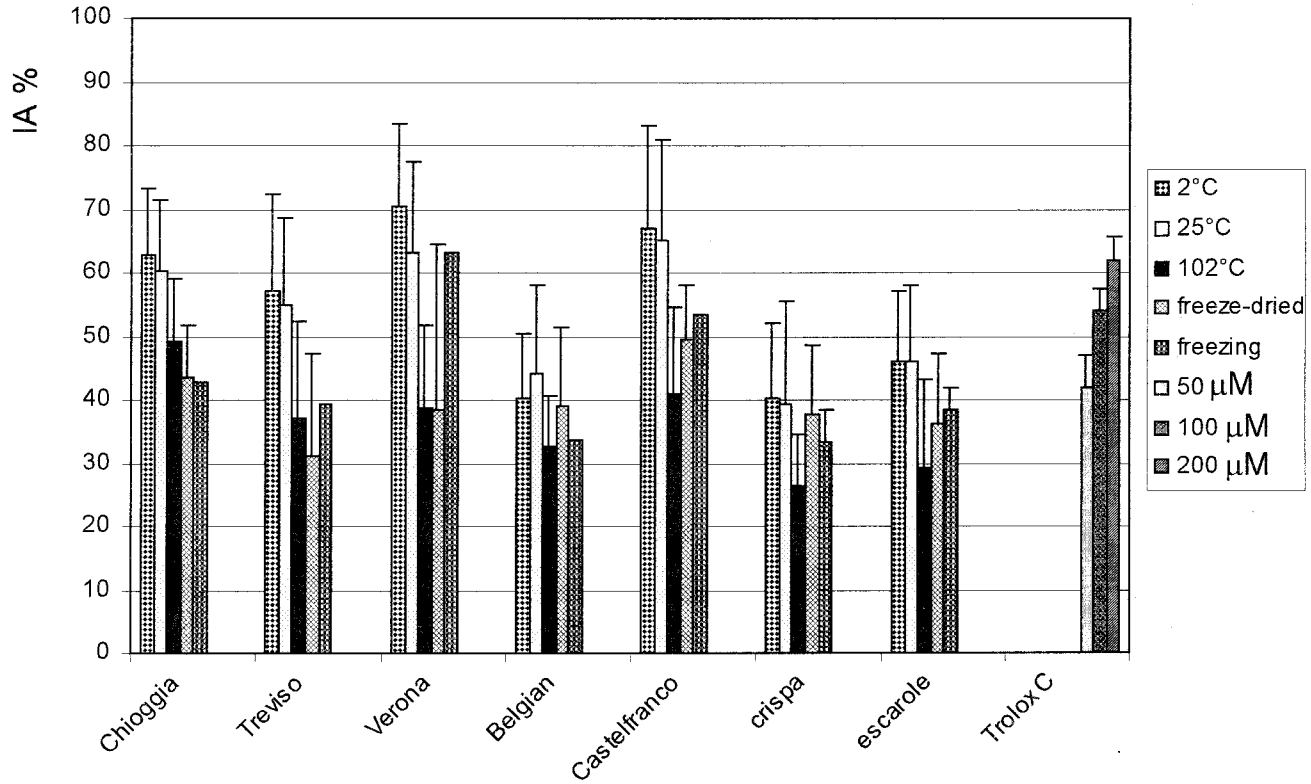

Figure 1. Inhibitory activity percentage (IA%) of tested vegetable juices obtained by different thermal/technological treatments and of 50, 100, and 200 μM Trolox C solutions against hydroxyl radical in the deoxyribose assay.

statistical package Statgraphics Plus (1998). Means were separated with the LSD method at a confidence level of 95%.

RESULTS AND DISCUSSION

Table 1 reports some botanical characteristics of the tested vegetables, the volume of raw juice obtained from 1 g of each vegetable, and the dry residue given by 1 mL of raw juice. The results showed that the Verona chicory had the least amount of juice, whereas the Belgian chicory had the highest content. The different cultivars of the red *C. intybus* var. *silvestre* (Chioggia, Treviso, and Verona chicories), the var. *foliosum* (Belgian chicory), and the hybrid vegetable (Castelfranco chicory) had a higher content of water soluble components in the juices than the *C. endivia* var. *crispa* and var. *latifolium* vegetables.

The antioxidant activity was determined using three different chemical assays, which are the deoxyribose assay, the DPPH$^{\bullet}$ assay, and the linoleic acid−β-carotene assay.

For each vegetable, the juices obtained by working at 2 °C, the same juices also boiled for 30 min at 102 °C or frozen at −20 °C and then stored for 3 months, freeze-dried and then reconstituted after a month, and the juices filtered and then stored for 3 h at 25 °C were analyzed by applying the three mentioned assays.

With regard to the deoxyribose assay (**Figure 1**), the raw juices obtained from the three red vegetables of the *C. intybus* species var. *silvestre* showed very strong deoxyribose degradation inhibitory activity (IA). Chioggia, Treviso, and Verona red chicories reached IA values of 62.80, 57.10, 70.50%, respectively. The white *C. intybus* var. *foliosum* vegetable (Belgian chicory) and the *C. endivia* vegetable juices had remarkable, although lower, antihydroxyl radical activity (IA = 40.40, 40.20, and 46.00% for Belgian, crispa, and escarole chicories, respectively). The hybrid vegetable showed an IA value close to that of the red *C. intybus* var. *silvestre* one (IA = 67.10%). In this assay, therefore, the Belgian and the *C. endivia* vegetables had activities similar to that shown by a 50 μM Trolox C solution (IA = 41.97%), a water soluble analogue of vitamin E, whereas the red *C. intybus* and the hybrid vegetable juices showed IA values closer to those given by a 200 μM Trolox C solution (IA = 61.81%).

Preparation and storage at 25 °C did not significantly influence the IA values, whereas the other applied thermal treatments such as boiling, freeze-drying, and freezing generally strongly decreased the IA mean values for all of the vegetable juices. Nevertheless, due to the high standard deviation (SD) values, no significant differences were found when compared to the corresponding raw juice IA values.

A linear dose−response relation was shown (**Figure 2**) either by *C. intybus* or by *C. endivia* raw vegetable juices. Considering the dried residue obtained by the same volume of each vegetable juice, the same IA values are presented for a lower concentration of dried residue in the system for the red *C. intybus* vegetables with respect to the green *C. intybus* or *C. endivia* vegetables. The green chicories reached the plateau (IA = 85%) at the concentration of 20 mg mL^{-1}, whereas the red chicories and the hybrid vegetable reached the highest activity (IA = 90−100%) at a concentration of 16 mg mL^{-1}, indicating that they contain at least one compound that is more active against the hydroxyl radical. This seems to be confirmed by the different slope of the linear dose−response relationship shown by the red and green tested vegetables.

The same trend in the behavior of the two considered spices was observed in the DPPH$^{\bullet}$ assay (**Figure 3**). Higher antiradical activity (ARA) was shown by the red *C. intybus* vegetable juices (ARA = 69.63, 84.45, and 69.63% for Chioggia, Treviso, and Verona red chicories, respectively), whereas weaker activity was registered for the *C. intybus* var. *foliosum* and the *C. endivia* var. *crispum* and var. *latifolium* vegetables (ARA = 56.59,

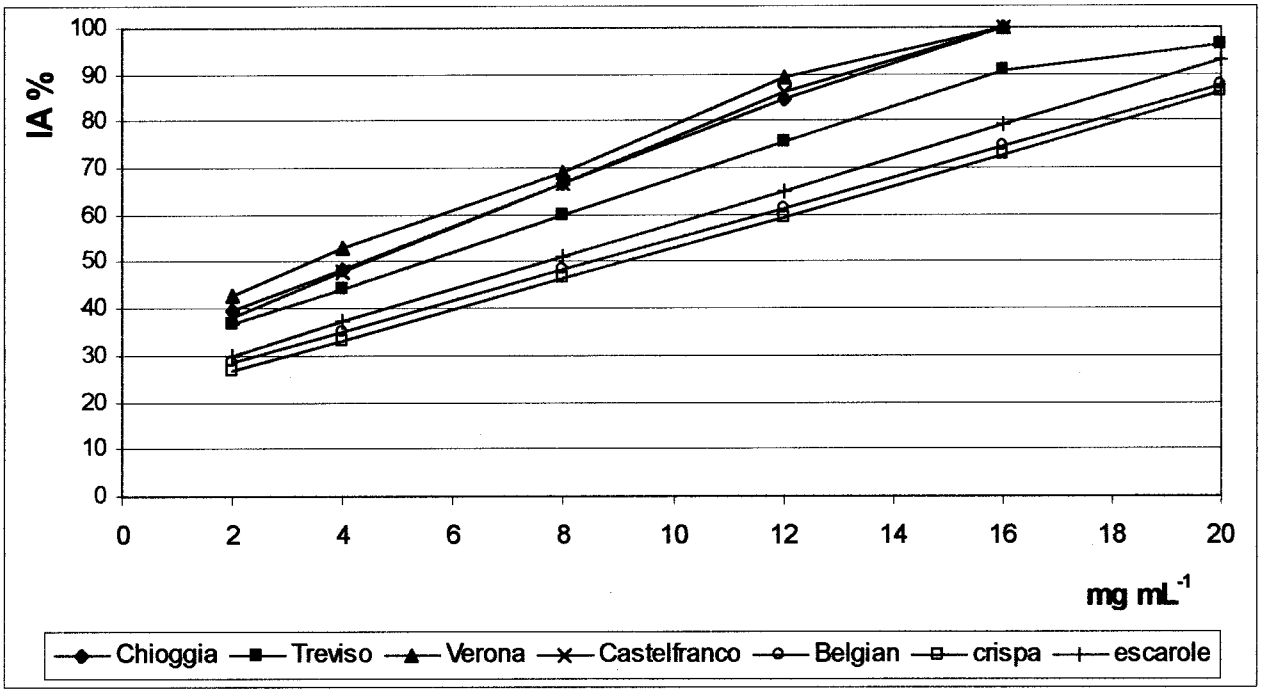

Figure 2. Inhibitory activity percentage (IA%) versus concentration of vegetable juice dried residues.

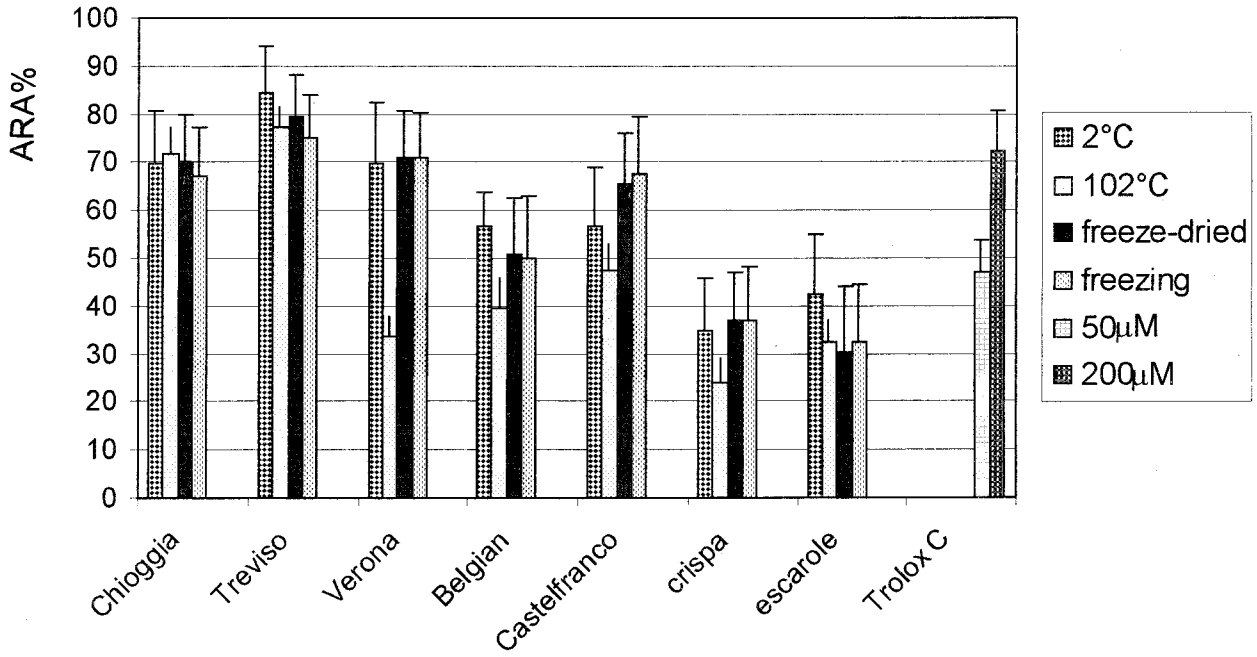

Figure 3. Antiradical activity percentage (ARA%) of tested vegetable juices obtained by different thermal/technological treatments and of 50 and 200 μM Trolox C solutions in the DPPH$^{\bullet}$ assay.

34.94, and 42.39%, respectively). Also in this case, the hybrid vegetable showed an antiradical activity close to those of the *C. intybus* chicories (ARA = 56.77%). The ARA values of 50 and 200 μM Trolox C solutions were 47.25 and 72.15%, respectively. The latter value is near the plateau found in the dose−response relationship for Trolox C (data not shown).

The histograms reported in **Figure 3** show that in this case only the boiling treatment generally decreased the ARA values for the tested chicories, with significant differences only for Verona and Belgian chicories ($p < 0.05$).

In this case, too, a linear dose−response relationship was found for all of the raw vegetable juices (**Figure 4**). The linear relationship was found in the range of 1.5−4 mg for the *C. intybus* and the hybrid vegetables and in the range of 1.5−6 mg of dried residue mL^{-1} for the *C. endivia* vegetables. No other perceptible increase in the ARA values was found above these ranges. The highest ARA values were 80−85% for the red chicories, 78% for the hybrid vegetable, and 50−65% for *C. endivia* chicories. Also, in this case the slopes of the linear dose−response relationship were different for the *C. endivia* and *C. intybus* vegetables.

With regard to the antiperoxyl radical activity, the linoleic acid−β-carotene system showed very strong differences in the properties of each vegetable depending upon the time and temperature of the reaction and the thermal treatments applied to the vegetable juices.

Inhibition of the β-carotene degradation rate due to the vegetable juices was determined after 10, 20, and 30 min of

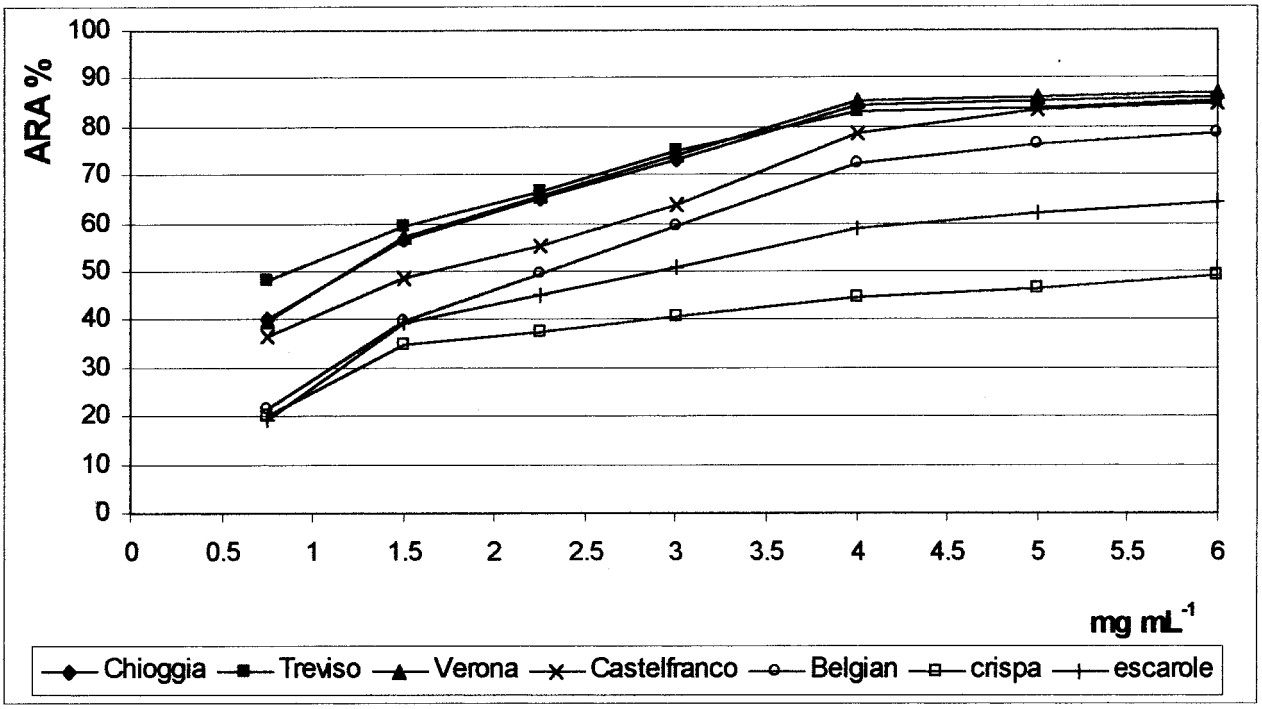

Figure 4. Antiradical activity percentage (ARA%) versus concentration of vegetable juice dried residues.

reaction at 50 °C for each juice obtained at the different temperatures (2 and 25 °C) or submitted to the different treatments (boiling, freezing, or freeze-drying). The results, expressed as antioxidant activity (AA), are reported in **Figure 5** for the *C. intybus* vegetables and in **Figure 6** for the *C. endivia* and hybrid vegetables and for a 100 μM Trolox C standard solution.

All of the *C. intybus* vegetable juices initially (after 10 min of reaction) showed, on average, rather weak antioxidant activity (**Figure 5a**) that increased with time (**Figure 5b**), reaching values >70% at the end of the monitoring period (**Figure 5c**) (AA_{30} = 79.14, 77.57, 70.80, and 80.44% for Chioggia, Treviso, Verona, and Belgian chicories, respectively).

Conversely, *C. endivia* var. *latifolium* and var. *crispum* raw juices initially (**Figure 6a**) revealed high pro-oxidant activity (AA_{10} = −86.50 and −47.50% for crispa and escarole, respectively; the negative AA values showed that these vegetables accelerate the degradation rate of β-carotene), which decreased with time, and after 20 min of reaction, these vegetables also were found to possess antioxidant activity (**Figure 6b**). At the end of the monitoring period, however, they had values lower (**Figure 6c**) than those of all the *C. intybus* vegetables (AA_{30} = 59.00 and 43.00% for crispa and escarole, respectively). In this case, too, the raw juice of the hybrid vegetable showed the same behavior of the *C. intybus* vegetables (AA_{10} = 7.11% and AA_{30} = 74.64%) (**Figure 6**).

In this system, the analysis of different volumes of raw juices showed no dose−response relationship.

The preparation and storage of juices at 25 °C weakly influenced the juice AA%, whereas thermal treatment at 102 °C had a strong positive effect, in particular, on the AA values obtained after 10 min of reaction for all of the juices. In fact, the boiled juices, also those initially strongly pro-oxidant of the *C. endivia* species, initially showed high antioxidant activity (AA > 60%), which at the end of the reaction time was higher than that of the corresponding raw juices and close to that of the standard antioxidant (AA_{30} = 90%). It should be noted that boiling heavily decreased the variability of the AA values, which was particularly high for the raw juices at the beginning of the monitoring period, as shown by the standard deviation values.

With regard to freeze-drying, it was observed that this treatment initially made the antioxidant properties of the *C. intybus* vegetables (even of the hybrid vegetable) pro-oxidant, or even annihilated them and strongly increased the pro-oxidant activity of the *C. endivia* var. *latifolium*. Nevertheless, at the end of the monitoring period all of the vegetables were found to be antioxidant but with average values sometimes lower than those of the corresponding raw juices.

With regard to freezing, its effect was similar to that described for freeze-drying. All juices frozen and stored at −20 °C for 3 months before the analysis were found to be initially pro-oxidant, but at the end of the monitoring period they also became antioxidant. Nevertheless, their AA_{30} values were perceptively lower than those obtained for the corresponding raw juices (with the exception of Verona chicory), so much so that for the *C. endivia* juices the values were close to 0.00%. This behavior was also confirmed for the juices that had been prepared at 25 °C and then submitted to the same treatment of freezing and stocking at −20 °C for 3 months.

To verify whether pro-oxidant activity was destroyed by boiling also after freezing, aliquots of Treviso, Belgian, and escarole chicory juices, frozen and stored at − 20 °C for 3 months, were subjected to boiling for 30 min at 102 °C and then analyzed. The results (**Figures 5** and **6**) showed that they had lost their pro-oxidant activity and, as in the case of the raw juices after boiling, showed even initially very high AA values.

The juices of these same three vegetables were also tested using the linoleic acid−β-carotene model system without heating to accelerate the peroxidation of the linoleic acid and measuring the β-carotene degradation percentage every minute for the first 5 min and then every 5 min for an hour (**Figure 7**). The β-carotene degradation percentage at 20 °C was higher in the presence of the juices compared to the control sample also after 1 min of reaction (AA_{10} = −192, −167, and −172% for Treviso, Belgian, and escarole chicories, respectively), indicating

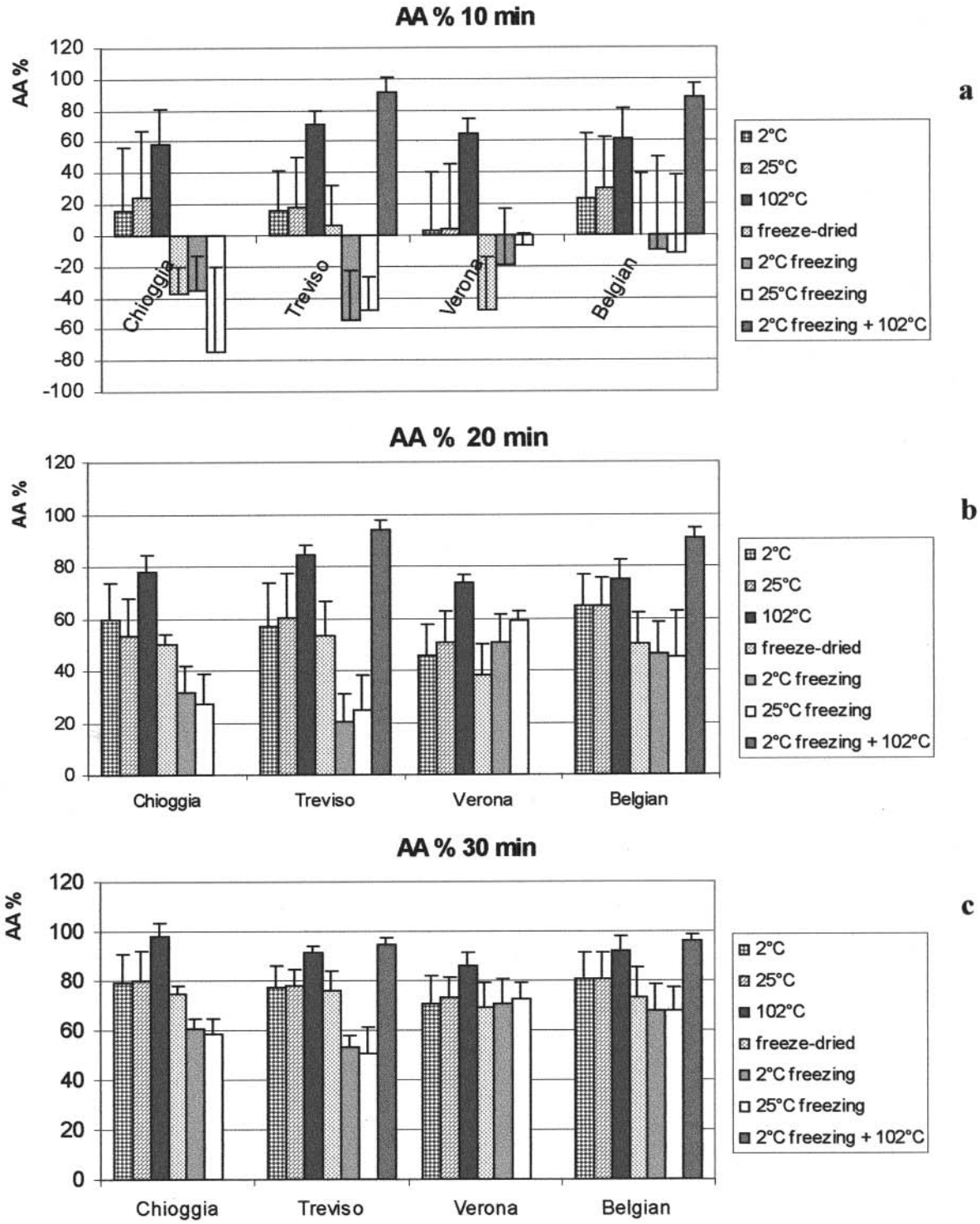

Figure 5. Antioxidant activity percentage (AA%) of *C. intybus* vegetable juices obtained by different thermal/technological treatments against peroxyl radical in the linoleic acid–β-carotene assay.

there was no induction period in the peroxidation of linoleic acid, as when an enzyme is in action, and a strong pro-oxidant activity lasted for 60 min (the monitoring period). Negative AA values were also registered after 30 min ($AA_{30} = -275$, -400, and -375% for Treviso, Belgian, and escarole chicories, respectively). Conversely, the boiled juices always were anti-oxidant in these conditions, too (**Figure 7**).

The fact that in the linoleic acid−β-carotene system no dose−response relationship was found could probably be due to the simultaneous presence in each vegetable juice of different compounds with opposite activities on linoleic acid peroxidation.

To obtain preliminary information about the molecular weight of the components responsible for the anti- and pro-oxidant activities and eventual interactions between them, the juices of Treviso, Belgian, and escarole chicories chosen among the samples with initial high pro-oxidant activity in the linoleic acid−β-carotene system were fractioned by dialysis membrane (cutoff = 3500 Da). The obtained fractions were tested in the deoxyribose assay and in the linoleic acid−β-carotene system (**Table 2**). In the deoxyribose assay both fractions obtained from each vegetable possessed antihydroxyl radical activity and showed similar IA values, giving altogether an IA value slightly

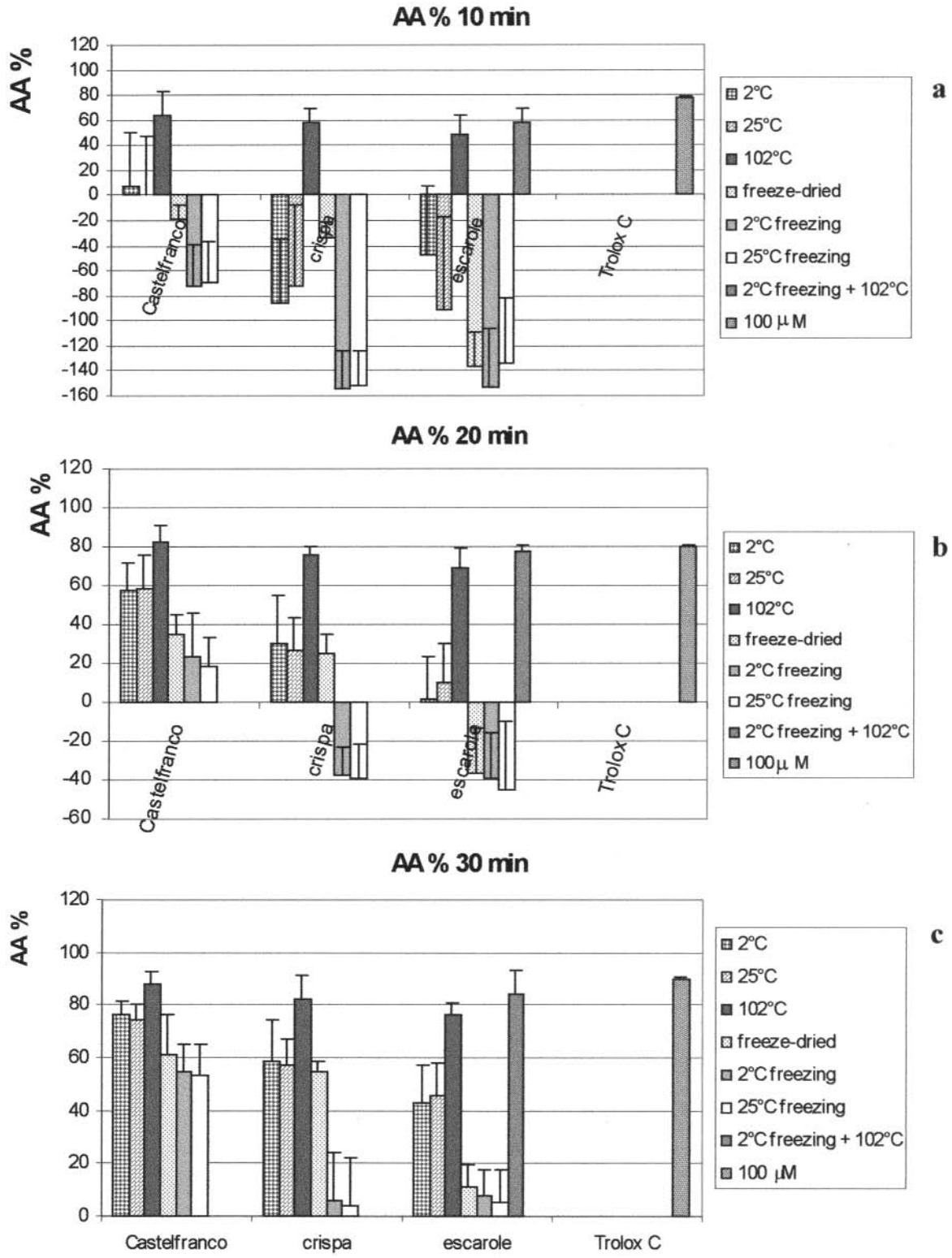

Figure 6. Antioxidant activity percentage (AA%) of Castelfranco chicory and of *C. endivia* vegetable juices obtained by different thermal/technological treatments and of a 100 μM Trolox C solution against peroxyl radical in the linoleic acid–β-carotene assay.

higher than that of the corresponding raw juice, indicating weak interactions among the compounds of the two fractions. This was conversely so in the linoleic acid−β-carotene system: the two fractions showed opposite initial activities. All of the fractions of each vegetable with MW <3500 Da were always antioxidant with values higher than those of the corresponding whole juices, whereas all of the fractions with MW >3500 Da were initially pro-oxidant (with AA_5 values higher than those of the corresponding raw juices for escarole and Belgian chicories, whereas Treviso chicor's AA_{10} was similar to that of its raw juice); however, at the end of the reaction period, they were antioxidant, also.

The two fractions obtained by dialysis from each vegetable juice were recombined and tested again for their AA%. The results showed that the raw juice, the higher MW fraction, and the recombined juice of Treviso chicory had similar initial pro-oxidant activity values, which indicate that the pro-oxidant components are stable and no interactions occurred among the

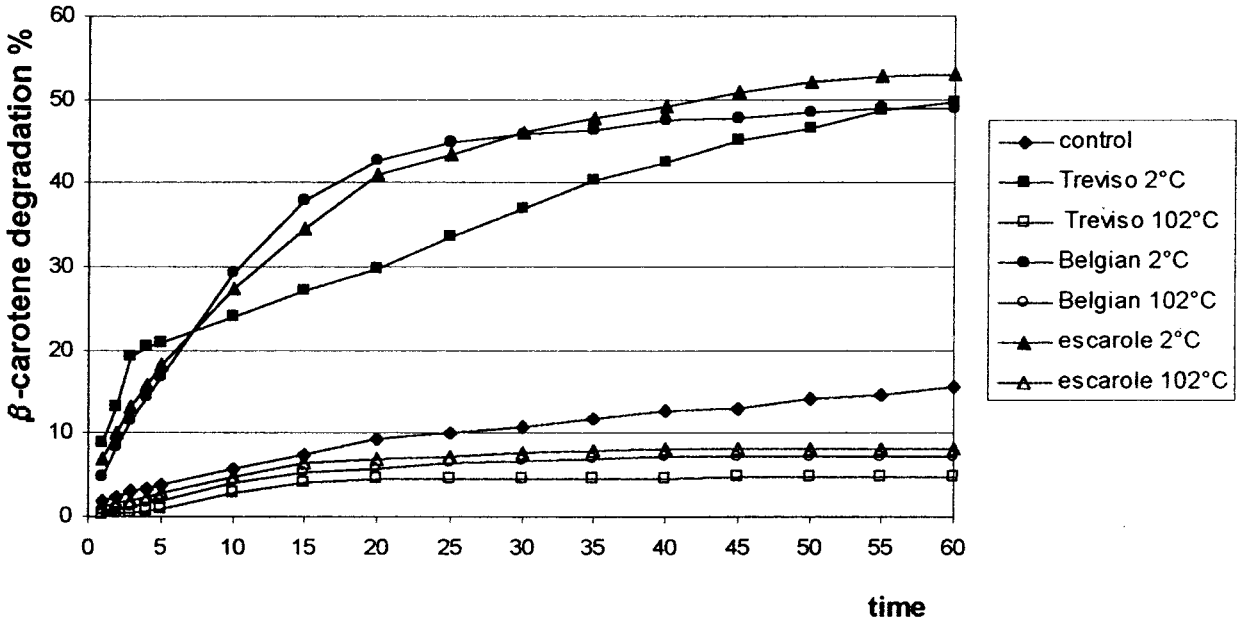

Figure 7. β-Carotene degradation percentage in the absence and presence of raw and boiled Treviso, Belgian, and escarole chicory juices at 20 °C.

Table 2. IA% and AA% Values of Vegetable Juices (2 and 102 °C) and of Fractions Obtained by Dialysis Cutoff at 3500 Da[a]

chicory sample	sample	IA%	AA% 5 min	AA% 10 min	AA% 20 min	AA% 30 min
Treviso	raw juice	54.49	–220	24	65	79
	MW < 3500 Da[b]	37.13	65	85	96	96
	MW > 3500 Da[b]	31.14	–235	–110	54	65
	recombined juice		–183	–90	79	94
	boiled juice		80	87	91	94
	MW < 3500 Da[c]		83	93	93	95
	MW > 3500 Da[c]		81	90	91	94
Belgian	raw juice	47.80	–176	–105	7	76
	MW < 3500 Da[b]	26.04	68	86	90	95
	MW > 3500 Da[b]	24.11	–25	28	52	69
	recombined juice		–5	41	63	81
	boiled juice		50	64	75	79
	MW < 3500 Da[c]		50	61	70	81
	MW > 3500 Da[c]		65	91	97	97
escarole	raw juice	41.17	–225	–79	26	35
	MW < 3500 Da[b]	27.08	25	46	88	92
	MW > 3500 Da[b]	16.20	–68	21	54	73
	recombined juice		–31	52	79	87
	boiled juice		39	53	69	74
	MW < 3500 Da[c]		75	91	96	96
	MW > 3500 Da[c]		67	79	87	93

[a] Values represent means of three replications. [b] Fractions obtained by dialysis of raw juices. [c] Fractions obtained by dialysis of boiled juices.

higher and lower MW fractions with regard to pro-oxidant activity.

For escarole and Belgian chicories, the recombined juices showed pro-oxidant activity lower than those of the corresponding whole juices and even of the isolated pro-oxidant fractions, indicating partial inactivation of the pro-oxidant component over time and with handling.

Newly carried out dialysis on the initially pro-oxidant fractions with MW >3500 Da, using a membrane with a cutoff of 50000 Da, showed that the pro-oxidant activity is maintained by the retentate components (MW > 50000 Da).

The AA values of the fractions with MW < 3500 Da, higher than those of the raw juices, seem to demonstrate that the pro-oxidant fractions can contrast or completely mask the action of the strong antioxidant compounds also occurring in all of the tested vegetables.

Dialysis separation (cutoff = 3500 Da) was also carried out on the same three vegetable juices treated at 102 °C. In this case, both fractions obtained from each boiled juice were always highly antioxidant (**Table 2**). Until now we cannot say if the higher MW fraction also contains antioxidants, or if the pro-oxidant component when treated by heating becomes a powerful antioxidant agent.

CONCLUSION

The obtained results showed that all of the considered *Cichorium* vegetables contain water soluble components which possess scavenger activity against the three different considered radicals. Such activity, in all three assays used, is on the whole stronger in the red *C. intybus* vegetables than in the *C. endivia* ones. The hybrid vegetable generally showed activity and behavior closer to that of *C. intybus* than to *C. endivia* vegetables. Nevertheless, it is clear that in all of the considered vegetables there is at least a component which can promote linoleic acid peroxidation to also occur. Pro-oxidant components are able to act promptly and efficiently for a long time in cold media masking the antioxidant components but are quickly inactivated at 50 °C and completely lose their activity after boiling, revealing the presence of some heat stable strongly antioxidant components that also occur in vegetable juices.

The dialysis of juices gave rise to two fractions, one with MW < 3500 Da and the other one with MW > 3500 Da. Both fractions given by each vegetable were found to possess about half of the whole juice scavenger activity in the deoxyribose assay. Conversely, in the linoleic acid−β-carotene system the fractions with the lower MW were shown to be strongly antioxidant and to maintain their activity after high-temperature treatment. The higher MW dialysis fractions, on the contrary, were found to be pro-oxidant and to maintain their pro-oxidant activity after elimination of the compounds with MW < 50000 Da. Therefore, this activity can be ascribed to one or more components with MW > 50000 Da. The pro-oxidant activity of these fractions was unstable toward heating, whereas their activity after freeze-drying and freezing increased. These features indicate that lipoxygenase enzymes, widely occurring

in plants, may be responsible for the pro-oxidant activity revealed only in the presence of linoleic acid. The increase of pro-oxidant activity registered after either freeze-drying or freezing could be perhaps ascribed to the denaturation of the lipoxygenase inhibitors that attend these enzymes in plant materials.

In conclusion, our findings seem to indicate that a number of water soluble components possessing antioxidant activity in all of the chemical assays used occur in all of the tested diet vegetables. In particular, the high and stable activity of the red chicories and the hybrid vegetable suggests they contain very efficient radical scavenger compounds.

With regard to the lipid pro-oxidant components occurring in all of the tested vegetables, preliminary experimental data seem to indicate that they are inactivated in biological systems in which the only antioxidant activity was registered for the red *C. intybus* and the hybrid vegetable water soluble components and their MW > 50000 Da fractions. This fact support the possibility of a potential antioxidant within these vegetables that will protect against damaging free radicals and in doing so actually contribute to the beneficial effects of the Mediterranean diet on human health.

Investigations are currently under way to better isolate the pro- and antioxidant compounds occurring in the vegetables of the *Cichorium* genus, to better characterize their features and chemical structures, and to evaluate their activity in biological systems.

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