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Anti- and pro-oxidant activity of *Cichorium* genus vegetables and effect of thermal treatment in biological systems

Adele Papetti [a], Maria Daglia [a], Pietro Grisoli [b], Cesare Dacarro [b], Cesarina Gregotti [c], Gabriella Gazzani [a,*]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy*
[b] *Department of Experimental and Applied Pharmacology, Faculty of Pharmacy, University of Pavia, Via Taramelli 14, 27100 Pavia, Italy*
[c] *Department of Internal Medicine and Therapeutics, Faculty of Medicine, University of Pavia, Piazza Botta 10, 27100 Pavia, Italy*

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Abstract

The antiradical activity of water soluble components in six vegetables belonging to the *Cichorium* genus, i.e., three cultivars of red *intybus* species var. *silvestre* (Treviso, Chioggia, Verona red chicories), a white *intybus* species var. *foliosum* (Belgian chicory), and two vegetables of the *endivia* species var. *latifolium* (escarole chicory) and var. *crispum* ("crispa" chicory), were studied using two biological systems consisting of: (1) microsome membrane rat hepatocyties in which oxidative damage was induced by CCl_4; (2) gram-positive bacterium, *Staphylococcus aureus* cultures, subjected to damage with cumene hydroperoxyde. The obtained results show that in both systems the red vegetables possess the strongest antioxidant properties and contain different antioxidant compounds whether at a low or high molecular weight, but only those of high molecular-weight (MW > 3500 Da) are able to act as antioxidants in all the used systems. The lower MW fraction (MW < 3500 Da) showed itself to be pro-oxidant in the microsome system. The effects of thermal treatments such as boiling, freezing and freeze-drying were also investigated.

Keywords: *Cichorium intybus*; Cichoriaceae; Vegetables; Biological anti-and pro-oxidants; Thermal treatment

1. Introduction

Aerobic organisms have developed a complex system of antioxidants in order to inactivate reactive oxygen (ROS) and nitrogen substances (RNS) formed in physiological conditions and accumulated in particular conditions within living organisms and, therefore, producing noxious effects (Gutteridge & Halliwell, 2000). Non enzymatic antioxidants such as albumin, GSH, ascorbic acid, α-tocopherol, β-carotene, uric acid, bilirubin and flavonoids are important junctions of the antioxidant system for protecting biological molecules. Some of these antioxidants are dietary vegetable components (Kaur & Kapoor, 2001). Dietary antioxidants become very important when oxidative stress in living organisms causes endogen antioxidant depletion (especially glutathione) and they appreciably contribute to the improvement of antioxidant status in the various biological fluids (Record, Dreosti, & McInereney, 2001). The importance of these antioxidants has been proven by several epidemiological studies which have pointed out the protective effects deriving from the consumption of vegetables and fruit against a number of chronic diseases, such as neoplastic, cardiovascular, inflammatory, neurodegenerative pathologies, cataracts, diabetes as well as during the aging process (Diaz, Frei, Vita, & Keaney, 1997; Floyd, 1999; Renaud & de Lorgeril, 1992; Schramm, Donovan, Kelly, Waterhouse, & German, 1998). Such protective effects are due to vegetable

* Correspondig author. Tel.: +39 0382 987863; fax: +39 0382 422975.
E-mail address: gabriella.gazzani@unipv.it (G. Gazzani).

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antioxidant compounds which are able to contrast the effects of oxidative stress recognised as an important concause in initiating and promoting the progression of the cited chronic pathologies. However much explained, the usefulness of knowing how each food can contribute to the total antioxidant action of dietary antioxidants is clear. For this reason, over recent years a number of studies were carried out to find and characterize the particular vegetable diet components able to inactivate the reactive compounds formed in vivo (Caldwell, 2003; Kuti & Konuru, 2004; Moridani, Pourahmad, Bui, Siraki, & O'Brien, 2003). Several methods are used to assess the activity of an antioxidant, or rather a mix of antioxidants, as it presents itself in a vegetable. These methods are either chemical or biological; the latter are generally more complex, so generally an initial screening investigation is carried out using simple chemical systems and later using biological systems where the components and the conditions of the medium (electrolytes, enzymes, pH, ionic strength, etc.), better mimic the conditions in which the antioxidants act in humans and which could interfere and change the activity of a compound.

A number of vegetables commonly used in the Italian diet, particularly in northern Italy, and considered to be traditional Italian food belong to the *Cichorium* genus. Some of them are grown in the winter season too because of their resistance to cold temperatures. These vegetables may consequently be considered an important crop because of their availability throughout the entire year, providing an advisable source of micronutrients during the cold season when fresh plant foods are far more scarce. These vegetables contains several polyphenols as reported by Mulinacci et al. (2001), but no investigations have been carried out about their antioxidant activity.

In our previous studies, we investigated within chemical systems the antioxidant activity of water soluble components in several *Cichorium* genus vegetables commonly used in the Mediterranean diet. Such activity was evaluated as antiradical activity against three different radical species namely DPPH$^{\cdot}$ (DPPH assay), peroxyl (linoliec acid-β-carotene system) and hydroxyl (deoxyribose assay) radicals, which all showed different reactivity (Papetti, Daglia, & Gazzani, 2002). The results of this study have indicated that water soluble *Cichorium intybus* var. *silvestre* components are particularly active as an antioxidant, even if they contain lipoxygenase enzymes detectable in a linoleic acid system which a study still under way has shown as a specific substrate of the enzyme.

The aim of this research was to evaluate the antiradical activity of the water soluble components of six vegetables belonging to the *Cichorium* genus by using two different biological systems in which oxidative damage was induced by radical promoters. We have studied three different cultivars of red *intybus* species var. *silvestre* (Treviso, Chioggia, and Verona red chicories), a white *intybus* species var. *foliosum* (Belgian chicory), and two vegetables belonging to the *endivia* species var. *latifolium* (escarole chicory) and var. *crispum* (''crispa'' chicory). The first biological system consists of microsome membrane rat hepatocyties in which oxidative damage was induced by CCl_4 giving off a $CCl_3^{\cdot}$ radical capable of initiating a radical reaction yielding lipid peroxidation. For the second bio-system: gram-positive bacterium, *Staphylococcus aureus* cultures, subjected to damage through lipid peroxidation of the membranes and a break on the DNA single strand with cumene hydroperoxyde, were used. The effects of thermal treatments such as boiling, freezing and freeze-drying on the antioxidant and antiradical activities of juices were also investigated.

2. Materials and methods

2.1. Chemicals

Cumene hydroperoxide (CumOOH), ethylenediaminetetraacetic acid (EDTA), nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G6P), trichloroacetic acid (TCA), phenol red were purchased from Sigma–Aldrich S.r.l. (Italy). Sodium chloride, sodium phosphate buffer, carbon tetrachloride (CCl_4), thiobarbituric acid (TBA), methemoglobin (MetHb) were purchased from Carlo Erba (Italy). *S. aureus* culture was purchased from ATCC (USA). Tryptone soya broth and Iso sensitest broth double strength were purchased from Oxoid, Basingstoke (England).

2.2. Vegetable samples

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

2.3. 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 juice was then filtered through a paper filter (Carlo Erba, diameter = 150 mm) and then through Millipore membranes of cellulose acetate/cellulose nitrate mixed esters (0.45 μm). The juice was subdivided into six batches. 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

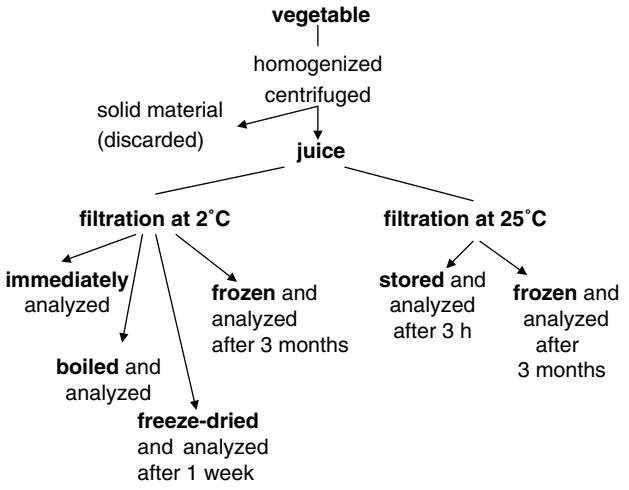

Fig. 1. Schematic diagram describing the sample preparation.

frozen for 3 months at −20 °C before analysis. Filtration resulted in a loss of most of the juice color. The first batch, (6 ml) filtered at 2°C, was immediately analyzed; the second batch (6 ml) was heated to a boil in 2 min and was then 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 (6 ml) was freeze-dried and stored in the dark at room temperature for 1 week. Afterwards, the residue was dissolved in distilled water to the initial volume. The last batch (6 ml) was frozen and kept at −20 °C for 3 months before analysis (Fig. 1).

2.4. Biological lipid peroxidation assay (protective activity, PA%)

Liver microsomes were prepared from male Wistar rats weighing 200–250 g following the method of Horie et al. (1989) with some modification (Daglia, Papetti, Gregotti, Bertè, & Gazzani, 2000; Gazzani, Papetti, Daglia, Bertè, & Gregotti, 1998). The microsomal pellets obtained were suspended either in 0.1 M sodium phosphate buffer, pH 7.4 (control sample), or in vegetable juice solutions (sample) to make a total volume of 6 ml, respectively. An aliquot (0.1 ml) of the obtained suspension was immediately removed and used for determination of microsomal proteins (Wang et al., 1996).

The remaining preparation was added to NaCl (1 ml, 140 μM), EDTA (1 ml, 50 μM), and sodium phosphate buffer (1 ml, 0.1 M, pH 7.4) and then subdivided into two aliquots of 4 ml, respectively. All test tubes containing samples were stoppered, and N_2 was bubbled through the solutions at 37 °C for 15 min to obtain anaerobic conditions for the following induction of the lipid peroxidation. To one group of samples were then added NADP (0.5 ml, 250 μM), and CCl_4/EtOH (20 μl, 50% v/v). An equivalent amount of buffer was instead added to second group. Both samples were placed in a shaking water bath at 37 °C for 30 min, and then the equal volumes of 30% TCA at 0 °C and 0.75% TBA were added (Lowry, Rosenbrough, & Randall, 1951).

The reaction mixtures were heated in boiling water for 15 min, kept in ice for 5 min, and then centrifuged for 10 min at 3000 rpm to separate corpusculate particles.

Absorbance of supernatants was read in a spectrophotometer (λ = 545 nm) using the second series of samples treated as above but without coenzymes to bring the spectrophotometer to zero. This was done to correct for interference due to color and TBA reacting substances (TBA-RS) naturally occurring in vegetable juice solutions.

The protective activity was expressed as the percentage decrease of TBA-RS relative to the control using the equation

$$PA\% = (a - b)/a \times 100,$$

where a is the TBA-RS in control sample and b represents the TBA-RS in sample.

2.5. Assay for antioxidant activity based on bactericidal action of ROO· (Mun'im, Negishi, & Ozawa, 2003)

The assay for antioxidant activity used *S. aureus* ATCC 25923. The bacteria were cultured overnight in tryptone soya broth and were washed three times with phosphate-buffered saline (PBS, pH 7.3) before being used in the cytotoxicity assay. Alkyl peroxyl radicals were generated via a heme-iron-catalyzed decomposition of cumene hydroperoxide (CumOOH). To test for any antioxidant activity within various vegetable juices (or their fractions), *S. aureus* (final concentration 1×10^6 CFU/ml) was treated in the reaction mixture of heme iron plus CumOOH (5.0 mM) in PBS (pH 7.3) in the presence or absence of the antioxidants. MetHb (100 μg/ml, 1.5 μM) was used as a catalyst for CumOOH. The antioxidant activity of various vegetable juices (or their fractions) was assessed by determining their ability to prevent the killing of *S. aureus* in the presence of ROO· generated by the reaction of MetHb plus CumOOH. The addition of a series of antioxidants and CumOOH was done as previously described by Akaike (Akaike, Ijiri, Sato, Katsuki, & Maeda, 1995). The reaction mixture was composed of the following: 0.1 ml of *S. aureus* (1×10^7 CFU/ml), 0.5 ml PBS, 0.1 ml of the various vegetable juices (or their fractions), 0.1 ml MetHb, and 0.1 ml of CumOOH. The mixture (total volume 0.9 ml) was incubated at 37 °C for 30 min. After the bacteria were exposed to the ROO· generating system, 0.9 ml of Iso Sensitest Broth double strength was added to the bacterial suspension, followed by its incubation at 37 °C for 20 h. The bacterial growth

was quantified by a turbidity measurement of the bacterial medium with a wave-length of 655 nm by using spectrophotometer (Jasco model Uvidec-320 Japan Spectroscospic Co., LTD, Tokyo, Japan). The percentage of bacterial growth was calculated by comparing the absorbance of the cultures obtained from bacteria exposed to the $ROO^{\cdot}$ generating system in presence of the antioxidants and the absorbance of the cultures obtained from bacteria exposed only to the antioxidants (maximal bacterial growth).

The percentage increase of bacteria-growth (BG%) can be expressed using the equation:

$$BG\% = \frac{A_{570}\ \text{control} - A_{570}\ \text{sample}}{A_{570}\ \text{control}} \times 100.$$

2.6. Dialysis

Dialysis was performed in Spectra/Por Biotech cellulose ester membrane with 3500 Da molecular weight cut-off. A 6 ml aliquot of vegetable juices was fractionated by dialysis in 600 ml of Millipore grade distilled water for 6 h at 4 °C. The standards used to test the dialysis membrane were recovered at a percentage higher than 80%. The retentates and the dialysates were brought up to the corresponding volume of juice before analysis.

2.7. Gel filtration chromatography (GFC)

The MW > 3500 Da fraction of Treviso red chicory, Belgian chicory, and of escarole chicory were separated into sub-fractions by preparative gel filtration chromatography (GFC) using superformance universal glass cartridge system 300×10 mm i.d. column (Götec-Labortechnik GmbH, Germany) with Toyopearl HW 65 (F) packing (molecular weight separation range: 10–1000 KDa) (Tosoh Corporation, Japan). The system was equipped with a Waters 490E UV–Vis detector (Waters Chromatography Division, Italy) and a Hitachi-Merck D 2500 integrator. Mobile phase was Millipore grade distilled water and flow rate was 0.5 ml min^{-1}. UV detection was at 210 nm.

2.8. Statistical analysis

The values represent a mean value of at least 10 replications. Data were analyzed using the analysis of variance test (ANOVA) with the statistical package Statgraphics Plus (1998). Means were separated with the LSD method at a confidence level of 99%.

3. Results and discussion

Six vegetables of the *Cichorium* genus belonging to different species and varieties were analyzed: three red cultivars of *C. intybus* var. *silvestre* from three different areas of production (Treviso red chicory, Chioggia red chicory, and Verona red chicory), *C. intybus* var. *foliosum* (Belgian chicory), *C. endivia* var. *latifolium* (escarole chicory), and *C. endivia* var. *crispum* (“crispa”chicory).

The antioxidant activity was determined both in a U:/ES/DTD501/Foch/4726bacterial culture where fatal bactericidal action against *S. aureus* was induced by adding cumene hydroperoxyde (CumOOH), as well as in a rat hepatocyte microsomal system in which lipid oxidative damage was induced by CCl_4. In the former assay, antiradical activity was expressed as the percentage increase of growth of bacteria in the presence of the vegetable juice sample relative to the control; in the latter assay, the antiradical activity was expressed as protective activity against lipid peroxidation induced in microsomes and resulted as the percentage decrease of hydroperoxide degradation products reacting with thiobarbituric acid.

For each vegetable the juices obtained at 2 °C, and the same juices either boiled for 30 min at 102 °C or frozen at −20 °C and then stored for 3 months, were analyzed by applying the *S. aureus* bioassay. In the microsome assay, the freeze-dried juices were reconstituted a week later, the juices filtered and then stored for 3 h at 25 °C, and the same juices were also frozen at −20 °C and then stored for 3 months, before analysis. Furthermore, vegetable juice components fractionated using dialysis with a 3500 Da cut-off membrane (which permits the separation of lower molecular components such as monomeric and polymeric polyphenols from the higher MW polymers) and by applying gel filtration chromatography technique, were analyzed.

As shown by the percentage of bacterial growth (BG%), shown in Fig. 2, all juices are able to appreciably protect bacteria from damage induced by CumOOH, which in the control culture (without vegetable juice) resulted as fatal. In particular, red vegetables showed very strong antiradical activities, significantly higher ($p < 0.01$) than those of the green vegetables. Regarding thermal treatments, the boiling of juices for 30 min only caused a measurable decrease in BG mean values for all the tested juices. As standard antioxidants, Trolox, BHT and ascorbic acid were tested. Ascorbic acid only showed antiradical activity in this system with a BG% = 60% for a 700 μM solution.

The juices of Treviso, Chioggia, Belgian and “crispa” chicories were fractionated according to their different molecular weight components by using a dialysis membrane with a cut-off at 3500 Da. For all juices, the obtained fractions tested in the *S. aureus* culture were both active and a noticeably higher activity always showed up in the higher molecular weight fractions (MW > 3500 Da) (Fig. 3) which showed values close to those of the corresponding juice.

The histograms shown in Fig. 4 show the results obtained when all juices were tested in the microsome

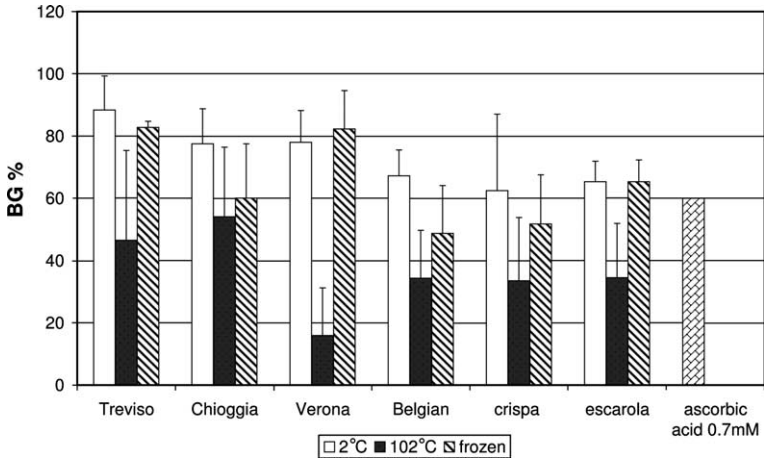

Fig. 2. Percentage increase of bacteria-growth (BG%) of 2, 102 °C, and frozen *Cichorium* genus tested juice vegetables.

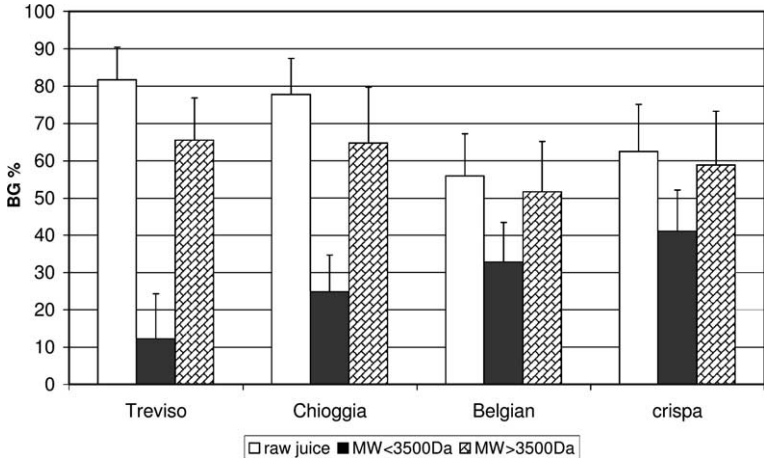

Fig. 3. Percentage increase of bacteria-growth (BG%) of Treviso, Chioggia, Belgian and ''crispa'' raw juices, dialysates and retentates.

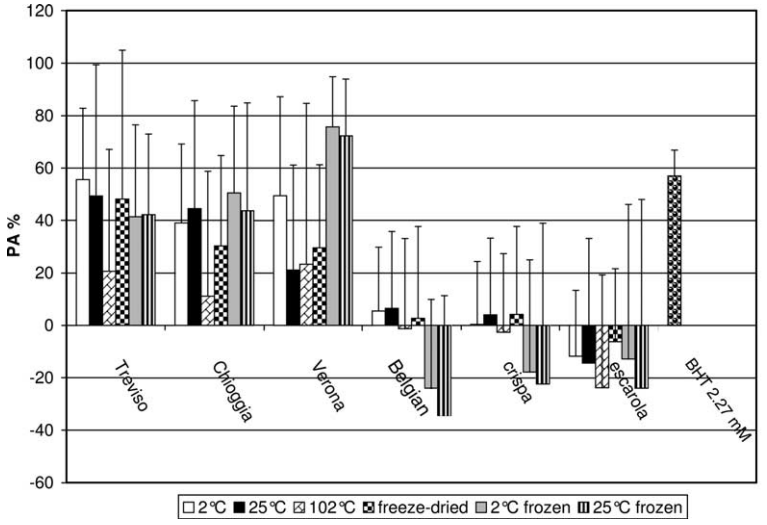

Fig. 4. Protective activity percentage (PA%) of 2, 25, 102 °C, freeze-dried, 2 °C frozen, and 25 °C frozen *Cichorium* genus tested juice vegetables.

membrane rat hepatocyties system. In this case also, red vegetables showed a notable activity, while the green vegetables showed no activity or were pro-oxidant. RSD values related to these results are very high, indicating that PA variability of all varieties and cultivars was very high. In fact, more than one sample with pro-oxidant activity in every group of samples was found. For this reason, in order to better understand these vegetables' behaviour, it seemed useful, especially for red vegetables, to consider not only the average activity detected for the samples of each varieties and cultivars, but also the percentage of samples with protective activity higher than any particular value. So, we have seen that Treviso red chicory showed the best protective activities: in fact, 89% of samples showed protective activity and 61% showed PA values higher than 50%. Regarding green vegetables, all juices showed poor activity: in fact only 46% of sample of "crispa" chicory, which showed the best protective activity, had antioxidant activity, and only 15% had PA values over 30%. Escarole chicory showed rather pro-oxidant activity: in fact, 58% of samples had PA negative values and only 7% of the remaining samples showed values higher than 30%.

The histograms shown in Fig. 4 also show the results obtained using the thermal treated juices (juices filtered and subsequently stored for 3 h at 25 °C, juices boiled for 30 min at 102 °C, juices freeze-dried and then reconstituted after a week, juices frozen at −20 °C and then stored for 3 months). Storage at 25 °C and freeze-drying showed a generally weak decrease in PA% mean-values, with the exception of Verona red chicory. On the contrary, freezing and storing at −20 °C for 3 months does not affect the PA% mean values of red chicories, again with the exception of Verona red chicory. Conversely, the freezing of green vegetable juices produced a marked pro-oxidant activity or, when already present as in the case of escarole chicory, it increased. Thermal treatment at 102 °C of red vegetable juices induced a very substantial decrease in PA% mean values, while the other juices, after boiling, became pro-oxidant or increased their pro-oxidant activity (escarole).

The highest activity demonstrated in red vegetables suggests the presence of water soluble compounds able to act as very strong antioxidants under the conditions of the microsomial system. Such compounds are probably absent or present in low concentration in green vegetables, including Belgian chicory which belongs to *intybus* species as do red chicories.

The results obtained by the analysis of dialysis fractions of all the vegetable juices are shown in histograms in Fig. 5. Regarding red vegetables, in this system the MW < 3500 Da fraction showed high pro-oxidant activity, while the MW > 3500 Da fraction possessed so high a protective activity that, in their presence, TBA-RS deriving from lipid peroxidation were suppressed by at least 75%. Regarding green chicories, dialysis techniques have provided the MW < 3500 Da fractions with a lower pro-oxidant activity than the other *intybus* specie vegetables, and a higher molecular weight fraction with weak protective activity. Therefore, all the red chicories showed a low molecular weight fraction with strong pro-oxidant properties and a high molecular weight fraction with strong antioxidant properties. Conversely, the dialysis fractions of the green vegetables showed poor activity in both, whether pro- or antioxidant.

Table 1 shows the dry residues given by the raw juices or their dialysis fractions. The results showed that the dry residues of whole *intybus* specie juice are significantly higher than the other ones, including Belgian chicory, which generally did not possess protective activity. However, if we considered the dry residue of the

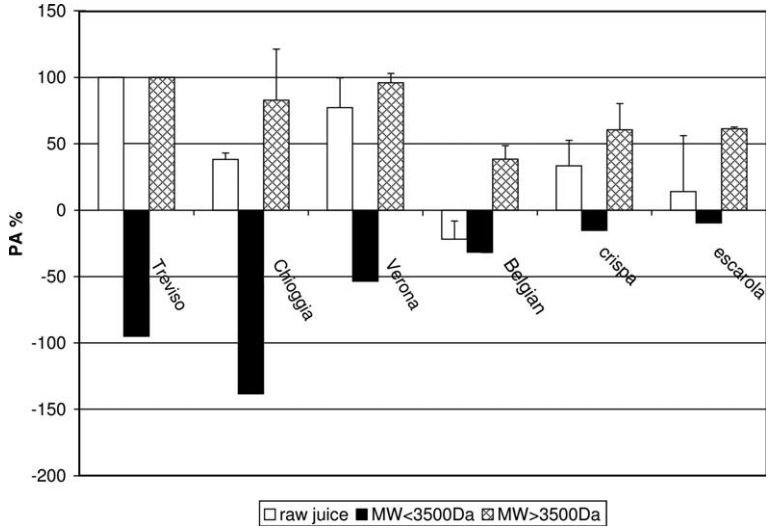

Fig. 5. Protective activity percentage (PA%) of *Cichorium* genus tested raw juice vegetables, dialysates and retentates.

Table 1
2 °C juice, MW < 3500 Da, and MW > 3500 Da fractions dry residue (mg ml^{-1}) of the tested vegetables

Vegetable	Dry residue (mg ml^{-1})		
	2 °C	MW < 3500 Da	MW < 3500 Da
Treviso	42.11 ± 1.51	35.68 ± 1.15	6.38 ± 0.45
Chioggia	40.96 ± 1.42	35.75 ± 1.18	5.01 ± 0.32
Verona	36.55 ± 1.63	28.61 ± 0.99	7.37 ± 0.28
Belgian	43.47 ± 1.96	36.73 ± 1.38	2.81 ± 0.11
Crispa	32.29 ± 1.12	28.06 ± 0.94	3.96 ± 0.26
Escarola	31.15 ± 1.07	27.28 ± 0.89	3.41 ± 0.16

highest molecular weight antioxidant fractions (MW > 3500 Da), it is more scarce in all green vegetables, including Belgian chicory. These data confirmed by GFC analysis of the dialysis fractions of Treviso red chicory and Belgian and escarole chicory indicate that red var. *silvestre* vegetables contain higher concentrations of antioxidant soluble components than the green vegetables (Fig. 6).

The fractions with MW higher than 3500 Da, newly dialysed with cut-off membranes 300 KDa, gave the higher MW fraction which, regarding red vegetables, seem to be mainly responsible for antioxidant properties of the vegetables. In fact, these properties resulted as having a PA = 100% out of 95% of the examined samples.

The results obtained showed that all water soluble components of the vegetables under consideration are active in the biological systems used, nevertheless, they have different properties depending on the assay used for the analysis, and within the same system, on the type of vegetable and on the molecular weight of its components.

In the system using *S. aureus* bacteria, all of the vegetables were always antioxidant showing BG% significantly higher for red *C. intybus* var. *silvestre* vegetables. Regarding thermal treatments, only boiling significantly decreased BG%. In this system, all the vegetables showed that MW > 3500 Da components were the main ones responsible for the activity, while the lower MW components were shown to be less protective against radical action. On the contrary, the results obtained within the microsome membrane hepatocyte system showed significant differences between the vegetable component activities. On the whole, only red vegetable juices showed as strong a protective activity as in the previous system. Such activity, however, was quite variable from sample to sample, even within the same type of cultivar, with the result that some samples were pro-oxidant in every series of sample including the red vegetables. In this system, too, green vegetable juices always showed poor antioxidant activity, or were even pro-oxidant, and again, only the MW > 3500 Da fraction of the red vegetables demonstrated antioxidant activity;

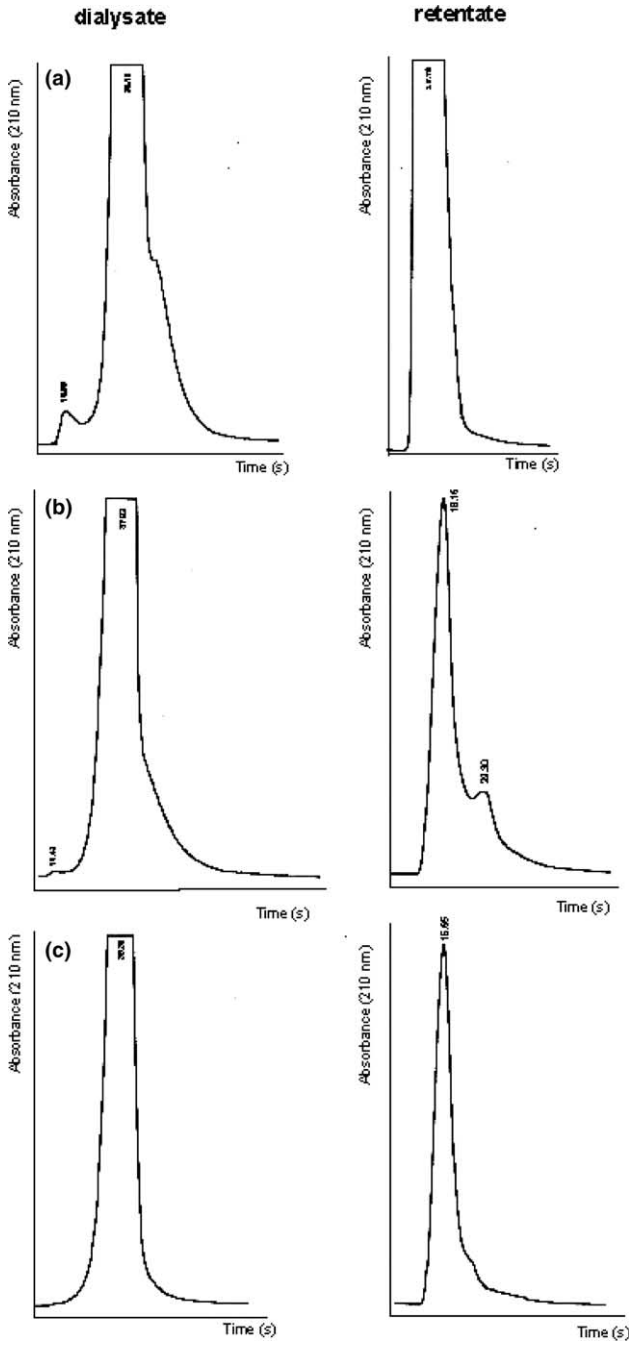

Fig. 6. (a) GFC of Treviso red chicory dialysate and retentate. (b) GFC of Belgian chicory dialysate and retentate. (c) GFC of escarole dialysate and retentate.

in fact, this fraction, after the separation of the MW < 3500 Da fraction, which was pro-oxidant, showed higher activity than the corresponding raw juice, and often, especially in the case of var. *silvestre* vegetables, this fraction was able to completely inhibit lipid peroxidation in microsomes. GFC analysis of dialysis fractions and their dry residues showed that they were very abundant in red vegetables. On the contrary, green vegetables, which in both the systems had low activity,

were less rich in both soluble antioxidant and pro-oxidant components. All these results seem to indicate that raw juices' protective activity is the result of a different compound action with an opposite activity, i.e., the MW < 3500 Da fraction with pro-oxidant activity and the MW > 3500 Da fraction with antioxidant activity. In regard to red vegetables, among the different components of this fraction, the brown polymers with MW > 300 KDa were found to be responsible for the strong antioxidant activity revealed. The higher or lower activity presented by each sample of the same cultivar could depend on the relative concentration of different components with their different properties. The expression of these components in a vegetable is probably due to the climatic or growing conditions.

Again, the pro-oxidant activity of the MW < 3500 Da fraction is unexpected because it contains monomeric and polymeric polyphenols (investigations under way showed the presence of at least three different chlorogenic acid derivatives), which are currently considered as acting as an antioxidant in vivo.

The results obtained using the biological systems regarding the juices "in toto" are in agreement with the results obtained in our previous study (Papetti et al., 2002) where the pro- and antiradical activity were determined in chemical systems against the DPPH$^{\cdot}$ stable radical, the scarcely reactive peroxyl radical, and the highly reactive hydroxyl radical. In all these systems, too, the activity was on the whole, stronger in the red *C. intybus* vegetables than in the *C. endivia* ones. However, during a more thorough study, done in order to find the components responsible for the activities registered for all the juices in the different assays, big differences emerged regarding the fractions responsible for the antioxidant activity in the chemical systems and in the biological systems; the latter ones are generally considered of greater importance because they reproduce the conditions where xenobiotics can act "in vivo" better than in chemical systems. In the present investigation, high molecular weight components were responsible for most of the antiradical activity, conversely in linoleic acid-β carotene chemical system, the higher molecular weight fractions were found to be pro-oxidant. Such activity was found to be unstable after boiling, which makes the juice antioxidant. Data from an investigation, not yet published, demonstrate that pro-oxidant activity, noticeable in the chemical system containing linoleic acid, is due to the presence in raw juice and in the higher molecular weight fraction of lipooxygenase enzyme, whose activity is not noticeable in the biological systems. This fact suggests that in such systems the enzyme component is efficiently inactivated by the protection mechanisms with which living beings are endowed in order to oppose to the oxidant agent actions.

4. Conclusions

The results obtained from the investigations regarding all the cited vegetables seem to indicate that they contain different antioxidant compounds both with low and high molecular weight, though only high molecular-weight ones are able to act as antioxidants in all the chemical and biological systems that we have used for our investigations.

Investigations are under way to characterize the anti-pro-oxidant components in the red vegetables, and to explain their different behaviour within different systems.

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