JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Flavonoid and Carbohydrate Contents in Tropea Red Onions: Effects of Homelike Peeling and Storage

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The content of anthocyanins, flavonols, and carbohydrates of Tropea red onions (*Allium cepa* L.) was determined by HPLC and HPLC-MS. Cyanidin derivatives constitute >50% of total anthocyanins, but delphinidin and petunidin derivatives, which have not been reported in red onions thus far, were also detected. The flavonoid distribution in the different layers of the bulbs indicates that, after homelike peeling, the edible portion contains 79% of the total content of quercetin 4′-glucoside but only 27% of the anthocyanins. Storage of onions for 6 weeks in different conditions, all of them mimicking home storage habits, resulted in a decrease to 64−73% of total anthocyanins. The same trend was verified for the total antioxidant activity, which was reduced to 29−36%. A decrease in glucose and fructose content correlated with anthocyanin degradation was also observed. Storage at low temperature seems to better preserve the onion anthocyanins.

KEYWORDS: Red onion (*Allium cepa* L.); anthocyanin; quercetin 4′-glucoside; delphinidin; carbohydrates; storage

INTRODUCTION

Numerous surveys provide convincing evidence of an inverse relationship between the intake of fruits and vegetables and the incidence of coronary heart disease and certain cancers. Phytochemical components, in particular, flavonoids, are considered to be important contributing factors to the overall antioxidant activity of the diet (*1*−*4*). Epidemiological studies about the major sources of antioxidant intake highlighted the importance of onions, both for specific sulfur-containing compounds and for the high levels of a specific class of flavonoids, the flavonols (*5*−*7*). White, yellow, and red onions are, in fact, known to contain a large amount of flavonols; the majority are glucose derivatives of quercetin and kaempferol (*8*). In red onions, due to the presence of anthocyanins, the flavonoid content is particularly high. Although the anthocyanin content of red onion is quite low compared to that of blackberry or red grape (*9*) and their level is ∼10% with respect to flavonols (*8*), the simultaneous intake of flavonols and anthocyanins could be regarded as particularly healthy.

In previous works regarding anthocyanins in red onions, cyanidin 3-glucoside, peonidin 3-glucoside, and cyanidin 3-laminariobioside have been identified (*10*, *11*). Terahara et al. (*12*) described malonylated anthocyanins in Japanese red onion, and Ferreres et al. (*13*) found in red onion cyanidin 3-glucoside and cyanidin 3-arabinoside, with their respective malonylated derivatives. Fossen and co-workers (*14*) reported that in five different cultivars of red onion, cyanidin 3-(6″-malonylglucoside) content, together with cyanidin 3-(6″-malonyl-3″-glucosylglucoside) and cyanidin 3-glucoside, constituted >95% of the total anthocyanins. Donner et al. (*15*) confirmed these data, also finding peonidin 3-glucoside in four red onion cultivars and, for the first time, peonidin 3-malonylglucoside as a minor anthocyanin.

Little information is reported in the literature about the absolute amount of anthocyanins in red onions. An indication of up to 250 mg/kg was reported by Clifford (*9*), and a similar value was found by Ferreres et al. (*13*). However, it is not clear if this value relates to the whole onion bulb or only to the edible part, which has the skin and the outer layer rich in the anthocyanins removed. Another study, in fact (*15*), reported values ranging from a maximum of 219 mg/100 g to a minimum of 109 mg/100 g, but analyses were performed only on dry skins. Flavonoids exist in a multiplicity of complex conjugates with sugars, so sugars are known to be very important for anthocyanin biosynthesis, but literature data about this matter are still disputed (*16*−*20*).

In the framework of a survey aimed at characterization of the typical Italian varieties, Tropea red onion (*Allium cepa* L.), produced in some areas of southern Italy (Calabria region), was

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10.1021/jf011102r CCC: $22.00
Published on Web 02/16/2002

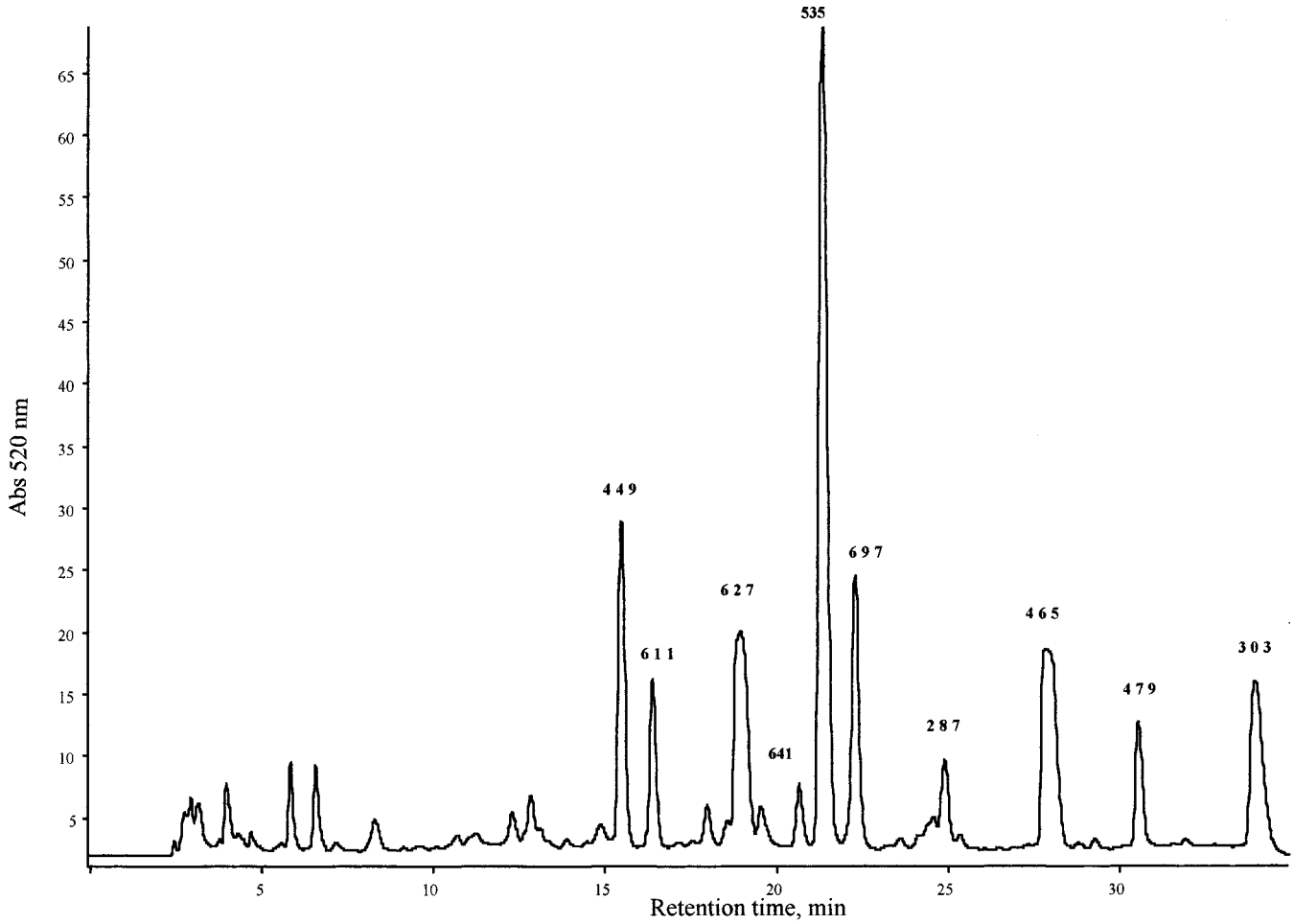

Figure 1. HPLC chromatogram recorded at 520 nm of the anthocyanins extracted from a representative sample of Tropea red onion. Coupling to electrospray MS allowed the determination of molecular mass highlighted on each peak. Molecular ion, mass fragments, and comparison with literature data suggested the following identifications: 449, cyanidin glucoside; 611, cyanidin (glucosylglucoside); 627, delphinidin glucosylglucoside; 641, petunidin (glucosylglucoside); 535, cyanidin (malonylglucoside); 697, cyanidin (malonyl glucosylglucoside); 287, cyanidin aglycon; 465, delphinidin 3-glucoside; 479, petunidin glucoside; 303, delphinidin aglycon.

investigated. This onion is known for its sweetness, and due to its crispness it is often consumed uncooked, so that the flavonoid compounds are totally preserved (*21*, *22*). After harvest and before marketing, these onions are cured to develop thick, tight outer scales and to reduce disease problems (*23*). Curing take place during a preliminary storage period of 4−8 weeks in ventilated hangars in order to remove surface moisture (*24*); the outer scales get drier and sugars migrate from leaves to bulbs (*25*).

The objective of this study was to identify and quantify by HPLC-MS the main anthocyanins present in Tropea red onions. The chemical composition of the bulbs (fructose, glucose, sucrose, anthocyanins, and other flavonoids) was investigated separately for the dry outer skin, the large fleshy layer that is usually removed in domestic preparation, and the edible portion that is actually eaten. Also, the variation of carbohydrates, anthocyanins, and antioxidant activity during storage in different conditions was studied.

MATERIALS AND METHODS

Sample Collection. Tropea red onions were obtained from the most widespread local cultivar in the typical area of production Vibo Valentia (Calabria, Italy). After harvesting, the onions were stored, still with their leaves, for 8 weeks at an average temperature of 25 °C. When bulbs were ready for marketing (end of July), 10 bulbs for each sample were selected to obtain samples as representative and uniform as possible, consisting of onions in the weight range of 95−155 g with a mean weight of ∼125 g. In a first experiment the analytical measurement were carried out on bulbs separately on three portions represented by (i) the outer dry skins, (ii) the outer fleshy layer, and (iii) the internal part representing the edible portion.

In a second experiment the bulbs were sampled as "ready for market" and stored under three conditions of temperature and relative humidity (RH) to simulate commonly used domestic storage. The three conditions were as follows: controlled temperature (5 °C; 30% RH); cool ambient conditions (average temperature of 25 °C; 66% RH); and warm ambient conditions (average temperature of 30 °C; 50% RH). At the beginning and after 2, 4, and 6 of storage under simulated domestic conditions, analyses were carried out on the edible portion. Analyses were performed separately on three groups of bulbs for each storage conditions, randomly chosen from the harvested onions.

Analytical Determinations. *Carbohydrates.* To determine fructose, glucose, and sucrose, 1 g of fresh onions was added to CH_3CN/H_2O (4:1) up to 10 mL and homogenized in an Ultra Turrax T25 blender at 8000*g* min^{-1} for 3 min. The samples were centrifuged at 3000 rpm for 10 min and filtered through 0.20 μm syringe cellulose filters before HPLC analysis. Samples were injected onto a 150 × 4.6 mm i.d. Hypersil 5 APS 2 (Chrompack), using CH_3CN/H_2O (4:1) as mobile phase, at a flow of 0.5 mL/min and a refractometric index detection (Knauer).

Flavonols. Flavonols were extracted according to the method of Hertog et al. (*26*). One gram of fresh onion was homogenized and

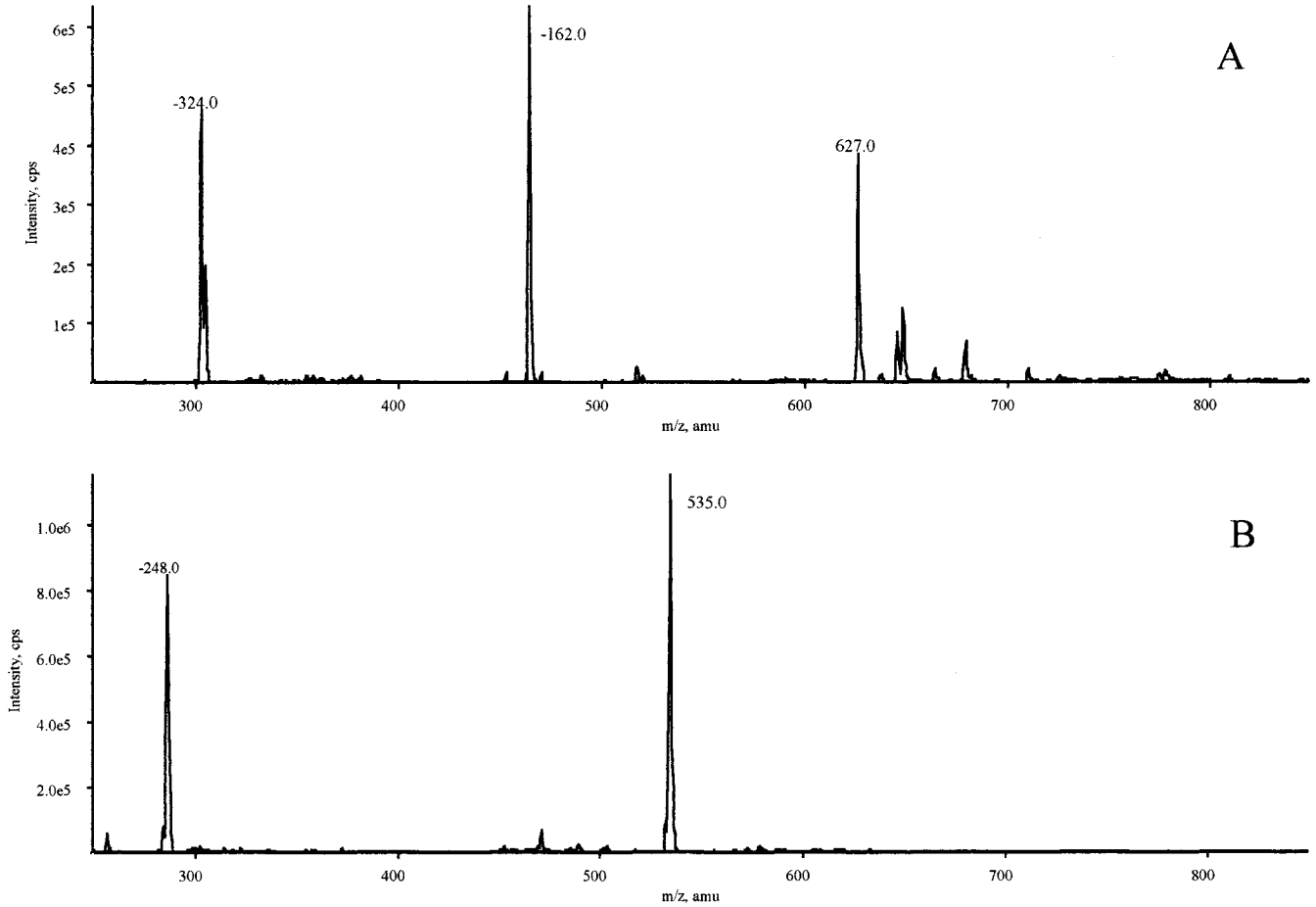

Figure 2. Mass fragmentation patterns of major anthocyanins in Tropea red onion: (A) mass spectrum of delphinidin 3-(glucosylglucoside), peaks at M − 162 and M − 324 uma are due to losses of one and two hexose units, respectively; (B) mass spectrum of cyanidin 3-(malonylglucoside), peak at M − 248 uma is due to loss of one hexose and one malonyl unit.

extracted with 25 mL of methanol stabilized with butylhydroxytoluene (BHT) and hydrolyzed with 6 M HCl at 90 °C for 2 h. After cooling, the extract was diluted to 100 mL with methanol and immediately analyzed by HPLC according to the method of Hertog et al. (*26*), with minor modifications. To measure the amount of the glycosylated form of flavonoids, acid hydrolysis was avoided. The chromatographic instrumentation consisted of a refrigerated ESA 540 autoinjector (4 °C), two ESA 580 pumps, an LC-10AD electrochemical detector with eight channels (Shimadzu), and software that controlled the instrumentation and carried out data processing (ESA). A Supelcosil LC-18 column (250 × 4.6 mm i.d., 5 μm) with a Spherisorb Supelguard LC-18 (Supelco) was used. Chromatography was performed at 30 °C, at a flow rate of 1 mL/min using the following solvent system: (A) 0.01 M sodium phosphate adjusted to pH 2.8 with 85% orthophosphoric acid; (B) methanol. The linear gradient used consisted of 87% of solvent A, decreased to 60% over 13.5 min and to 10% over 25.5 min, reaching the final condition of 0% over 3 min, then returned to 87% of solvent A over 3 min, and maintained at this condition for 4 min. The flow rate of the eluent was constant at 1 mL/min, and the setting potentials were 60, 120, 200, 340, 480, 620, 760, and 900 mV. Sample peaks were quantified with the external standard method. The precision and accuracy has been calculated on 10 standard curves and were <10% for all phenols tested.

Anthocyanins. The anthocyanins were extracted from onion tissues by suspending 1.5 g of homogenized tissue in 5 mL of methanol (0.1% HCl) at room temperature for 10 min. The extract was filtered and used for HPLC analyses. HPLC separation was carried out at a flow rate of 1 mL/min using a Prodigy ODS2 5 μm (250 × 4.6 mm i.d.) as described by Fossen et al. (*14*) with some modification. The column was equilibrated in 80% solvent A (10% formic acid) and 20% solvent B (methanol/water/formic acid, 50:40:10). Four minutes after injection, a linear gradient of 22 min up to 80% solvent B allowed the elution (monitored at 520 nm) of all anthocyanin compounds. Quantification of single compounds was achieved by a calibration curve obtained using pure malvidin as a standard (Extrasynthese).

Single anthocyanins were identified by splitting, after HPLC separation, 30 μL/min of the eluent into an API 100 (Sciex) MS single-quadrupole instrument, using an electrospray ion source (ESI) and a positive mode detection. Ionization was achieved using a probe voltage of 4.6 kV and a declustering potential of 50 V. The mass-to-charge ratio (*m*/*z*) scale was calibrated with the ions of ammonium adduct of polypropylene glycol. Full-scan spectra were acquired from 250 to 850 atomic mass units (amu) using a step size of 0.5 amu and a dwell time of 2 ms.

Antioxidant Activity. The antioxidant activity was performed by the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation method on the methanol extracts obtained as described for the anthocyanin analysis (*27*). One hundred microliters of the extract was added to 1 mL of chromogen solution. The Trolox equivalent antioxidant capacity (TEAC) was obtained by a calibration curve obtained with 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) under the same conditions. The antioxidant capacity was expressed in millimoles of Trolox per 100 g of fresh onion.

Statistical Analysis. In the figures vertical bars represent standard error. To evaluate the relationship between sugars and anthocyanins, the Pearson coefficient (*r*) was calculated and presented in a rectangular correlation matrix (see **Table 2**).

RESULTS AND DISCUSSION

The chromatogram of the anthocyanins present in Tropea red onions obtained by HPLC-MS is shown in **Figure 1**. This qualitative pattern is very similar in all samples analyzed in

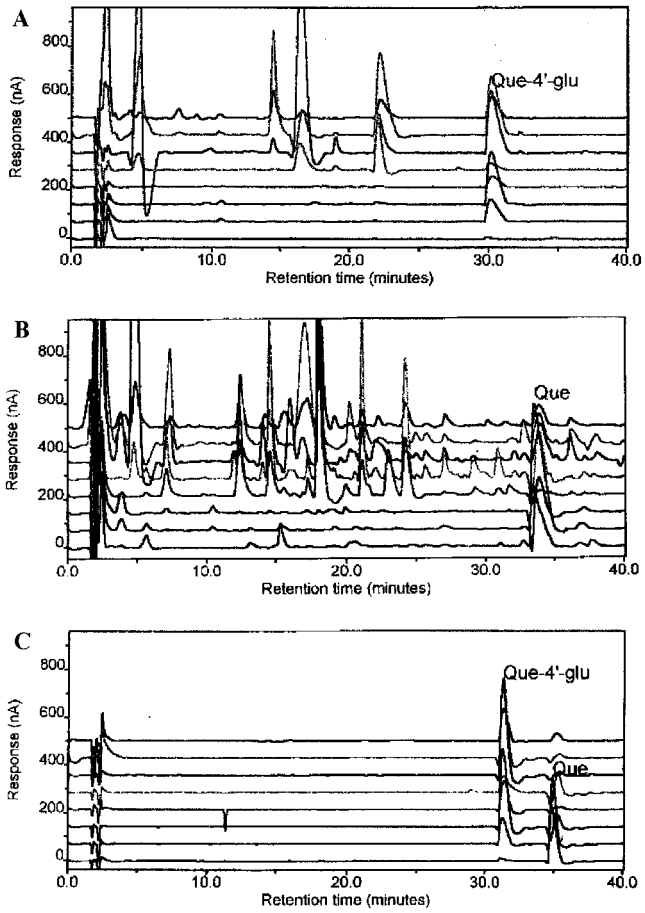

Figure 3. Chromatogram obtained by electrochemical detection on flavonols from Tropea red onion: (A) quercetin 4′-glucoside quantified in unhydrolyzed onion extract; (B) quercetin quantified in hydrolyzed onion extract; (C) pool of standards; the other peaks present in the extract are unknown. Traces represent the different potentials used as described under Materials and Methods. Que = quercetin; Que-4′-glu = quercetin 4′-glucoside.

this work. The different anthocyanins were identified according to the molecular ions as reported in the caption of **Figure 1**. The mass spectrometric fragmentation patterns of the two major compounds present in Tropea red onions are shown in **Figure 2**. The spectrum of delphinidin (glucosylglucoside) (**Figure 2A**) has a molecular ion at *m*/*z* 627, and the peaks at M − 162 and M − 324 formed as a consequence of the loss of one or two units of hexose, respectively. The spectrum of cyanidin (malonylglucoside) (**Figure 2B**) has a molecular ion at *m*/*z* 535 and a major fragment at *m*/*z* 249 corresponding to the loss of the malonylglucoside fragment.

The derivatives of mono- and diglucosylated cyanidin, with or without a malonyl moiety, account for >50% of total anthocyanin content. This partially confirms data on anthocyanin patterns from onions reported by Fossen et al. (*14*) and Donner et al. (*15*), who found that in five cultivars of red onion, including one indicated as Tropea (*14*), cyanidin 3-(6″-malonylglucoside) is the major anthocyanin compound, but cyanidin derivatives represent >95% of the total anthocyanins. The LC-MS analysis identified in the Tropea red onion samples investigated in this work a marked amount of delphinidin and petunidin derivatives, which were not previously found in red onions. In particular, delphinidin 3-glucosylglucoside and other delphinidin derivatives account for ∼30% of the total anthocyanin content of the edible portion of the bulbs. To our knowledge this is the first indication of delphinidin and petunidin

Table 1. Distribution of the Main Flavonoids and Carbohydrates in the Three Different Portions of Tropea Red Onion[a]

	dry skin		outer fleshy layer		edible portion	
flavonoid	fresh wt (mg/kg)	dry matter (mg/kg)	fresh wt (mg/kg)	dry matter (mg/kg)	fresh wt (mg/kg)	dry matter (mg/kg)
delphinidin 3-glucosylglucoside	3524 ± *75*	7831 ± *96*	47 ± *4*	582 ± *29*	65 ± *5*	815 ± *37*
cyanidin 3-(6″-malonylglucoside)	3303 ± *154*	7340 ± *197*	100 ± *6*	1250 ± *51*	15 ± *1*	188 ± *8*
cyanidin 3-(6″-malonyl-3″-glucosylglucoside)	2054 ± *65*	4565 ± *84*	50 ± *2*	625 ± *17*	10 ± *1*	125 ± *4*
quercetin 4′-glucoside	1887 ± *212*	4193 ± *271*	656± *1*	8200 ± *7*	598 ± *53*	7475 ± *383*

	dry skin		outer fleshy layer		edible portion	
sugar	fresh wt (g/kg)	dry matter (g/kg)	fresh wt (g/kg)	dry matter (g/kg)	fresh wt (g/kg)	dry matter (g/kg)
fructose	ND[b]		14.0 ± *0.1*	175.2 ± *0.1*	14.2 ± *0.1*	177.5 ± *0.3*
glucose	2.6 ± *0.1*	5.8 ± *0.2*	20.2 ± *0.2*	252.4 ± *1.8*	22.1 ± *0.1*	276.2 ± *0.1*
sucrose	ND		4.3 ± *0.01*	53.7 ± *0.3*	11.9 ± *0.1*	148.7 ± *0.6*

[a] Data represent the average of five determinations ± the standard error. [b] ND, not detected.

derivatives in red onions. Delphinidin and petunidin, in contrast to cyanidin, do not have malonyl derivatives in detectable amounts, indicating that the presence of malonylated derivatives is an exclusive feature of cyanidin derivatives as previously reported (*12*). Interestingly, in contrast to the cv. Morada de Amposta studied by Ferreres et al. (*13*), no arabinoside derivatives were found in Tropea red onions.

The flavonol pattern of Tropea red onion, shown in **Figure 3**, is similar to that reported for different onion cultivars studied by other authors (*28*). Quercetin and quercetin derivatives are by far the major compounds of this class; in particular, quercetin 4′-glucoside is the main compound identified in Tropea red onion. It is known that red onions contain higher levels of quercetin 4′-glucoside with respect to other varieties of yellow and white onions (*22*).

The effective intake of flavonoids is dependent on their distribution in the different parts of fruits and vegetables. Price and co-workers (*8*, *29*) have indicated that much of the data reported in the literature relates to the whole onion bulb and not to the edible part, which has the outer layers, rich in flavonoids, removed. Moreover, there is evidence of a decreasing trend in the content of some flavonoids from the dry skin to the inner rings (*28*).

Because the onion peeling for consumption results in some nutritional compounds being lost with the external layers, we have quantified the main flavonoids and carbohydrates present in the three parts of the bulbs (**Table 1**), namely, the dry skin, the outer fleshy layer, and the edible portion, separated as shown in **Figure 4**. The anthocyanins are heavily concentrated in the skin and in the outer fleshy layer, whereas in the edible tissue they are restricted to a single layer of cells in the epidermal tissue (*8*). Appreciable differences can be observed even in the anthocyanin content of the dry weight (**Table 1**).

The dry skin is quite rich in both anthocyanins and flavonols, with a high percentage of aglycon forms (data not shown). This part, corresponding to 2% of the total weight, cannot be eaten and it is discarded. It is noteworthy that ∼63% of the red onion anthocyanins are present in the dry skin. Also, the outer fleshy layer, which accounts for 15% of the total weight, is particularly rich in cyanidin derivatives. Unfortunately, it is common practice to discard also this layer together with the skin. This means that, after bulb peeling, only 27% of the total anthocyanins of

Figure 4. Different portions of Tropea red onion: (from left) skins, outer fleshy layer, and edible portion.

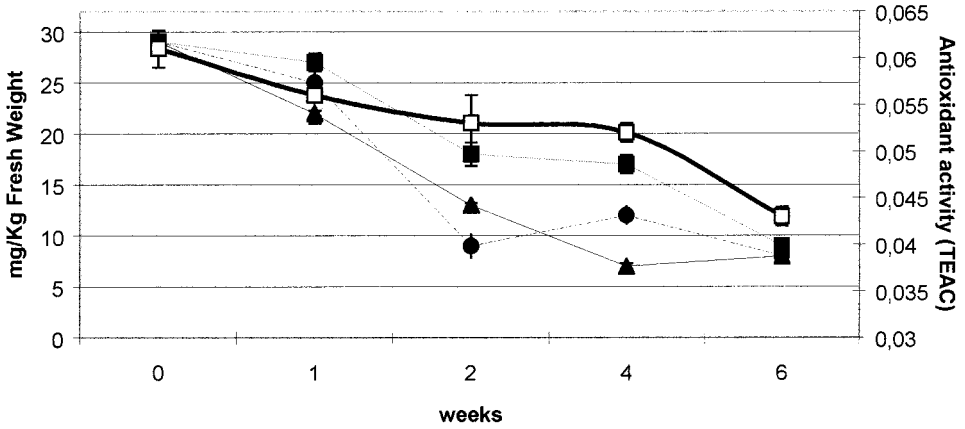

Figure 5. Variation of cyanidin 3-(6″-malonylglucoside) concentration and antioxidant activity during 6 weeks of storage in different conditions: [left axis, amount of cyanidin 3-(6″-malonylglucoside)]: ■, 5 °C, RH 30%; ▲, 25 °C, 66% RH; ●, 30 °C 50% RH; (right axis) □, Trolox equivalent antioxidant capacity (TEAC) of whole flavonoid extracts during 6 weeks of storage of the onions at 5 °C. Data represent the average of three determinations and are expressed as millimoles of Trolox/100 g of onion (fresh weight).

red onion are actually consumed. This figure is quite different for quercetin 4′-glucoside. In fact, 79% of the total amount of this compound is still present in the edible portion after peeling. The amount of quercetin 4′-glucoside found in Tropea red onions (598 mg/kg) is slightly higher than that reported in Red Baron onion by Rhodes and Price (*8*), who found ∼450 mg/kg, and by Price and Rhodes (*22*), who found a value of 394 mg/kg.

The carbohydrate content measured in the edible portion of Tropea red onion is similar to that reported in onion by Souci and Fachmann (*30*). Sucrose is slightly lower (11.9 vs minimum of 14.8 g/kg and maximum of 21.4 g/kg), whereas glucose is higher (22.1 vs minumum of 7.6 g/kg and maximum of 20.1 g/kg) and fructose are similar (14.2 vs minimum of 10.8 g/kg and maximum of 24.6 g/kg). Even if related to non-red onion samples, Tropea red onion seems to have a comparable free sugars level. In fact, values of free fructose ranging between 37.3 and 214.8 g/kg of dry matter, of free glucose between 34.9 and 248.6 g/kg, and of sucrose between 31.7 and 120.0 g/kg were reported for five different varieties of onions (*31*).

The data shown so far were obtained on onions stored for 8 weeks in uncontrolled conditions as happens in normal practice. In fact, the production period of Tropea red onion is from May to July, but it is possible to store the bulbs to consume them during the autumn, which is a very common practice for the bulbs of the July harvesting. Therefore, in a second experiment the anthocyanins and carbohydrates of the bulbs were analyzed in relation to three storage conditions, simulating domestic storage, and also the antioxidant activity was evaluated. In **Figure 5** the amount of the main red onion anthocyanin, cyanidin malonylglucoside, during storage is shown. The degradation is slower when onions are refrigerated at 5 °C in low humidity, particularly up to 4 weeks. However, it is noteworthy that, after 6 weeks of storage in all conditions, the whole anthocyanin content is reduced between 64 and 73%. The same trend was observed also for the total antioxidant activity. The antioxidant activity (TEAC) of the onions decreased during storage, paralleling the decrease of anthocyanins. TEAC was reduced by 29% after 6 weeks at 5 °C and by 36% when bulbs were stored in warm ambient conditions. These results can be discussed in light of similar work performed on flavonols, but comparison is hampered by the differences in the experimental conditions. Rhodes and Price (*8*), using homogenized onions, observed a decrease in diglucoside derivatives and a corresponding increase of monoglucosylated and

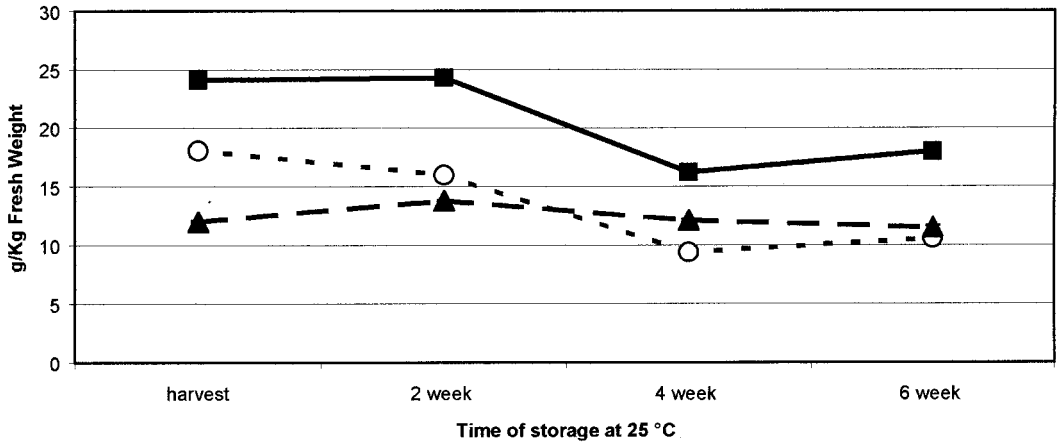

Figure 6. Amount of carbohydrates during storage in cool ambient conditions (25 °C; 66% RH): ■, glucose; ○, fructose; ▲, sucrose. Data represent the average of five determinations and are expressed in grams per kilogram. Standard error was covered by symbols.

Table 2. Correlations between Sugars and Anthocyanins[a]

	fructose	glucose	sucrose
cyanidin glucoside	0.64	0.74[b]	0.51
cyanidin diglucoside	–0.32	–0.12	0.77[b]
delphinidin diglucoside	0.10	0.10	0.10
petunidin diglucoside	0.39	0.28	–0.39
cyanidin malonylglucoside	0.70[b]	0.65	0.11
cyanidin malonyldiglucoside	0.61	0.63	0.35

[a] Pearson coefficients (r). [b] Significant at $p \leq 0.05$.

aglycon derivatives. Price et al. (*29*), using homelike storage, reported a 50% decrease of quercetin 4′-glucoside 2 weeks after harvest and little change in the following 6 months of storage. It may be supposed that flavonol glucosides are more resistant than anthocyanins during domestic storage.

A correlation analysis between the anthocyanins and the carbohydrates (**Table 2**) indicated a correlation between the carbohydrate depletion (**Figure 6**) and the degradation observed for some anthocyanins during storage. In the literature there are some results suggesting that sugars play a regulatory role in the synthesis of anthocyanins, but no data are available on their behavior during storage of the whole bulbs. Literature data demonstrate that the "in vitro" addition of carbohydrates to apple slices or berry skins causes an increase of anthocyanin biosynthesis (*32*). Anthocyanin production, by apple skin disks, was limited by carbon availability, and sucrose stimulates this production by providing increased substrate for the required synthetic pathways (*33*). Other studies claim that sugars play an important role in anthocyanin synthesis, chiefly in the synthesis of diglycosyl derivatives, but do not refer to any regulatory role (*17*−*19*).

The postharvest practice adopted for onions, favoring the migration of carbohydrates from leaves to bulbs, could lead to the biosynthesis of glycosylated anthocyanins. Total blockage of anthocyanin synthesis is in fact observed when the supply of sugar to Kyoho berries is cut off by defoliation at veraison (*34*). In this framework, it is possible to speculate that during storage under our conditions, with leaves already detached from the onions 20 days before the time 0 of our experiment, free carbohydrates as well as glycosylated metabolites have been used to supply energy to the onion tissue, thus resulting in the trend observed in **Figures 5** and **6**.

In conclusion, our data confirm that Tropea red onion is a rich source of quercetin 4′-glucoside, the concentration of this flavonol being higher than that reported for other red onions. On the other hand, the contribution to the overall anthocyanin intake in the diet is quite limited because a significant portion of it is eliminated during peeling. The anthocyanin pattern has some similarities, but also some differences, with respect to those described in previous works regarding red onions (*13*−*15*), delphinidin and petunidin being observed for the first time in red onion. The storage period is a critical step to determine the nutritional quality of the bulbs. In fact, the marked decrease of the total antioxidant activity during storage suggests that the overall antioxidant reservoir of the bulbs was reduced by onion metabolism. Storage at low temperature and in low-humidity conditions seems to better preserve the onion anthocyanins.

ABBREVIATIONS USED

RH, relative humidity; BHT, butyl hydroxytoluene; amu, atomic mass unit; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); TEAC, Trolox equivalent antioxidant capacity.

ACKNOWLEDGMENT

We are indebted to Elena Azzini, Aldo Bertone, and Dr. Alfredo Terra for technical assistance

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Received for review August 13, 2001. Revised manuscript received December 18, 2001. Accepted December 18, 2001. Financial assistance was received from POM Research Project A14, "Qualificazione dei prodotti tipici per migliorare la competitività della produzione agroalimentare meridionale". UE finance, allocated for developing regions, is handled from INEA on behalf of MiPAF.

JF011102R