Influence of Cultivar and Storage of Chicory (*Cichorium intybus* L.) Plants on Polyphenol Composition and Antioxidative Potential

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Abstract

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We determine the total polyphenol content (TPC) and antioxidative potential (AOP) in external and internal leaves of different cultivars of chicory, both before and after storage. We analysed the red cultivars Leonardo, Trevisio, Mesola, Verona, and Chioggia, the red-spotted cultivar Castelfranco, and the sweet cultivars Jupiter, Uranus, and Mercurius. The chicories were stored at temperatures from 0.1°C to 0.8°C and relative humidity from 90% to 95%. Cultivar and leaves significantly influenced TPC and AOP, while storage influenced AOP only. The outer leaves showed significantly higher TPC and AOP. The TPC in chicory ranged from 20 mg/100 g to 400 mg/100 g fresh weight and the AOP ranged from 0.20 μmol/g to 0.85 μmol/g fresh weight.

Keywords: antioxidants; AOP; DPPH; leaves; storage

Chicory (*Chicorium intybus* L.) is a typical Mediterranean vegetable that belongs to the family *Asteraceae* (Innocenti *et al.* 2005). Chicory has a long history of herbal use and is of great value due to its tonic effects upon the digestive tract. In Europe, chicory has been consumed for centuries as a leafy vegetable, and it is nowadays ranked as a functional vegetable of the 21st century (Wang & Cui 2011). Many chicory varieties are relatively important as agricultural crops, with red chicory, which is also known as radicchio, having a greater commercial value (Poli *et al.* 2002). Chicory is mostly consumed in raw salads in the winter time, when many fresh vegetables are not available. Chicory is a vegetable that is particularly resistant to low temperatures (Rossetto *et al.* 2005), and its red coloration increases when it is grown at low temperatures (Žnidarčič *et al.* 2004).

Salad vegetables are relevant as dietary sources of natural antioxidants. The traditional recommendation for a healthy diet includes the consumption of raw vegetables, which has been associated with a lower risk of cardiovascular diseases and cancer (Llorach *et al.* 2004; Sulaiman *et al.* 2011). From the nutritional point of view, antioxidants such as polyphenols and dietary fibre are the most important compounds (Faller & Fialho 2010), because they can have protective effects against many chronic pathologies (Papetti *et al.* 2002). Polyphenols, like flavonols and anthocyanins, have been reported to be the compounds that have the greatest antioxidant activities (Llorach *et al.* 2008). Anthocyanins are present at higher levels in red varieties of chicory, and these are thus responsible for their higher antioxidant activities (Lavelli *et al.* 2009).

The polyphenol content and antioxidant activities of some chicory varieties were studied previously (Papetti *et al.* 2002; Innocenti *et al.* 2005). The present study focused mainly on a few commercial varieties of chicory that are widely grown in Europe and are used for salads. The aim of our study was to evaluate nine different chicory cultivars (red, red spotted and sweet green) that are commonly produced in Slovenia, for their total polyphenol content (TPC), and their antioxidative potential (AOP). These

analyses were performed both for the internal and external leaves and before and after chicory storage.

MATERIAL AND METHODS

Plant material. Nine commercial cultivars of radicchio (*Chicorium intybus* L.) were studied: five red coloured (Leonardo, Trevisio, Mesola, Verona, Chioggia), one red spotted (Castelfranco), and three sweet green (Jupiter, Uranus, Mercurius). The chicories were cultivated in the Posavje region (Slovenia) on a moderate soil, with their planting following lucerne (*Medicago sativa*). Before ploughing, 500 kg/ha Multicomb 13-11-20 + microelements were used. The chicory plantlets were transplanted to open fields in August. Additional nutrition was supplied by 400 kg/ha Multi K (12-0-42 + 2% MgO) and Multi Cal (15.5-0-0 + 19% Ca) at a ratio of 3:5. After the harvesting at the end of November, the vegetables were transported to a cold store at a temperature of 0.1–0.8°C and relative humidity of 90–95%. Samples for analysis were taken after harvest and after storage for 2 to 6 weeks, depending on the variety.

Sample preparation. Internal and external leaves of three randomly chosen plants of each cultivar were used for the experimental analysis. For each sample, 10 g of fresh leaves were chopped using a ceramic knife, and extracted in a plastic vial with 15 g 2% metaphosphoric acid. The tissue was homogenised using an Ultraturax T 25 (20 500 rpm) for 5 minutes. The samples were stored at –20°C until analysed, as described below.

Total polyphenol content. TPC was determined using the Folin-Ciocalteu method, as described by Singleton and Rossi, and slightly modified (Roura *et al.* 2006). Samples were centrifuged at 14 000 rpm for 5 min (Eppendorf centrifuge 5415 D; Eppendorf AG, Hamburg, Germany). The supernatant was filtered (17 mm syr filter CA, 0.45 µm) directly in vials (PK 100 1.5 ml ABC vial; clear glass; W/PATCH 6 mm *i.d.*; 11.6 × 32 mm). The samples of the sweet green varieties were not diluted, while the other samples were diluted with deionised water at a ratio of 3:1 (v/v). Gallic acid solution (500 mg/l; Merck, Darmstadt, Germany) was used for the construction of the calibration curve. Briefly, 1 ml of each methanol fraction from the sample

Table 1. Total polyphenol content (mg/100 g) in the internal and external leaves before and after harvest for the different varieties of chicory, with their full statistical parameters

Cultivar	Internal leaves					External leaves				
	$\bar{x}$	min	max	SD	*CV* (%)	$\bar{x}$	min	max	SD	*CV* (%)
Before storage										
Leonardo	170.7	167.0	175.9	4.64	2.7	235.3	231.9	239.8	4.06	1.7
Trevisio	237.1	229.7	241.9	6.50	2.7	276.4	273.4	279.1	2.89	1.0
Mesola	147.5	136.9	153.9	9.22	6.3	171.4	169.2	173.7	2.25	1.3
Verona	242.2	241.8	242.9	0.64	0.3	239.4	337.3	242.4	2.05	0.9
Chiogga	253.6	249.2	256.4	3.88	1.5	396.1	391.0	401.1	5.05	1.3
Castelfranco	183.2	178.9	191.6	7.30	4.0	96.9	93.5	100.2	3.35	3.5
Jupiter	42.5	41.1	43.6	1.28	3.0	41.7	39.3	45.7	3.47	8.3
Uranus	42.1	38.8	44.7	3.00	7.1	33.8	31.8	35.5	1.86	5.5
Mercurius	42.3	35.6	52.1	8.68	20.5	37.6	34.3	42.8	4.56	12.1
After storage										
Leonardo	143.4	137.6	150.1	6.29	4.4	239.2	232.8	250.1	9.46	4.0
Trevisio	229.3	217.2	240.3	11.55	5.1	143.7	142.0	145.0	1.53	1.1
Mesola	172.4	172.1	173.1	0.58	0.3	208.1	200.1	212.1	6.93	3.3
Verona	221.1	218.4	223.5	2.56	1.6	250.6	245.1	254.3	4.84	1.9
Castelfranco	175.5	169.5	181.6	6.05	3.5	107.0	104.4	110.5	3.16	3.0
Jupiter	50,3	49.4	51.7	1.21	2.4	38.9	37.5	40.0	1.28	3.3
Uranus	59,6	55.2	62.1	3.85	6.5	20.1	19.1	20.8	0.91	4.5
Mercurius	79,1	74.4	84.2	4.91	6.2	39.3	38.6	40.3	0.91	2.3

n = 3 for all samples; , $\bar{x}$ – mean; min – minimal value; max – maximal value; SD – standard deviation; *CV* (%) – coefficient of variation

was mixed with 60 ml deionised water and 5 ml diluted Folin-Ciocalteu reagent in a 100-ml flask. The solution was well mixed, and after 30 s and before 8 min, 15 ml of a 20% solution of Na_2CO_3 was added. The solution was then incubated at 20°C for 2 h (Jackson *et al.* 1978). The absorption was measured at 765 nm, with each determination repeated 3 times. TPC is expressed as gallic acid equivalents (GAE; mg gallic acid/100 g fresh weight – FW) using the calibration curve of gallic acid which ranged from 50 mg/l to 500 mg/l (R^2 = 0.9994).

Antioxidative potential. AOP was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazil radical) assay (Nakajima *et al.* 2004). The samples were centrifuged and filtered (17 mm syr filter CA 0.45 µm) into vials. For the reference value (RF), 60 µl methanol and 1.5 ml DPPH solution (4 mg/20 ml methanol) were mixed in an Eppendorf tube, with the samples in triplicates. For the sweet green cultivars and the red cultivar Verona chicory extracts, 180 µl was then mixed with 1.5 ml of DPPH solution. For the other cultivars, only 60 µl of the chicory extracts were mixed with the same volume of DPPH. For the blank, 60 µl of the extraction solution was mixed with 1.5 ml methanol. Absorbance was measured at 517 nm after 15 min at room temperature. AOP was calculated according to the following equations:

$$\Delta A = RF - sample + blank$$

$$n\ (mol) = \Delta A/\varepsilon \times ((V = 0.00156) \times L)$$

$$\varepsilon = 12000\ (1 \times cm)/mol,\ L = 0.4\ cm$$

$$AOP = M_{DPPH}\ (nmol/l) = n \times 1 \times 10^6 \times 1\ 0^3/60$$

Statistical analysis. The data were analysed according to the least squares method, using a general linear model (GLM) procedure (SAS Software 1999). The statistical model for TPC and AOP included the effects of the internal and external leaves. The full statistical parameters were also calculated: minimum, maximum, standard deviation (SD) and coefficient of variation. Differences at $P < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Total polyphenol content. The mean TPCs for the different varieties for the internal and external leaves are shown in Table 1, along with the full statistical parameters. The red and red spotted varieties had higher TPCs than the sweet green chicories. The coefficients of variability were not high, which is an indicator of the TPC homogeneity. These values are in agreement with previous studies that investigated the green and red cvs Catalogna, Chioggia, and Trevisio (Innocenti *et al.* 2005).

Table 2 reports the comparison of TPCs between the internal and external leaves before and after storage. The red and red spotted varieties had higher TPCs in the internal leaves, as compared to the external leaves, both before and after storage. In previous studies, *Chicorium intybus* L. was investigated for TPC in leaves and roots generally (Conforti *et al.* 2009). The highest TPCs in the internal leaves before storage were in cvs Chioggia, Verona, and Trevisio (up to 250 mg/100 g FW). The sweet green cvs Jupiter, Uranus, and Mercurius had much lower TPCs, by up to 6-fold (about 40 mg/100 g FW). Similar levels of TPCs were found in the outer leaves after harvest (i.e. before storage). Cultivars Castelfranco and Verona and all of the sweet cultivars had significantly higher

Table 2. Comparison of total polyphenol contents in the chicory cultivars between the internal and external leaves before and after storage, as analysed using Duncan's test (α = 0.5)

Cultivar	Internal vs. external leaves		Before storage vs. after storage	
	before storage	after storage	internal leaves	external leaves
Leonardo	****	****	**	ns
Trevisio	***	***	ns	****
Mesola	*	***	**	***
Verona	ns	***	***	*
Chioggia	****	/	/	/
Castelfranco	****	****	ns	*
Jupiter	ns	***	**	ns
Uranus	*	****	**	***
Mercurius	ns	***	**	ns

ns – not significantly different; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$

Table 3. Differences in antioxidative potentials (AOP) and total polyphenols (TPC) in all the chicory cultivars for the internal and external leaves

Parameter	Internal leaves	External leaves
AOP (μmol/g)	0.570 ± 0.20[a]	0.683 ± 0.16[b]
TPC (mg/100 g)	146.59 ± 77[a]	151.50 ± 109[a]

[a,b]groups with different letters across rows are significantly different ($P \leq 0.05$)

TPCs in their internal leaves. This is in agreement with data reported in previous studies (Vanzani *et al.* 2011; Ferioli & D'Antuono 2012).

After storage, the highest TPCs were in cvs Trevisio and Verona (up to 230 mg/100 g FW). All of the sweet cultivars had lower TPCs (50–80 mg/100 g FW). After storage, the highest TPCs in the external leaves were in cvs Verona, Leonardo, and Mesola (up to 250 mg/100 g FW). TPCs in the external leaves were also the lowest in the sweet green chicory varieties (20–40 mg/100 g FW). There were significantly higher TPCs in the external leaves of cvs Leonardo, Mesola, and Verona after storage, as compared to those after harvest. There were significantly higher TPCs in the internal leaves of all the other cultivars. The highest TPC (that was statistically significant) was in cvs Verona and Trevisio. The TPCs of the internal and external leaves after storage decreased in cvs Leonardo, Verona, and Trevisio. The other cultivars showed small increases in TPCs after storage. This is due to a partial drying of the outer leaves of these cultivars during storage, in general TPCs are expected to decrease.

Antioxidative potential. The AOPs of the extracts were evaluated using the DPPH free radical scavenging assay. In all of the cultivars, the external leaves showed significantly higher AOP as compared to the internal leaves (Table 3). Similarly, TPC was higher in the external leaves, although this difference did not reach statistical significance (Table 3). The mean AOPs for the different cultivars in the internal and external leaves and the calculated statistical parameters are shown in Table 4. AOP was higher in the red cultivars, with lower AOPs in the red spotted and sweet green cultivars. Llorach *et al.* (2004)

Table 4. Antioxidative potential (μmol/g) in the internal and external leaves before and after harvest for the different varieties of chicory, with their full statistical parameters

Cultivar	Internal leaves					External leaves				
	$\bar{x}$	min	max	SD	*CV* (%)	$\bar{x}$	min	max	SD	*CV* (%)
Before storage										
Leonardo	0.74	0.68	0.77	0.05	6.7	0.85	0.81	0.91	0.05	6.2
Trevisio	0.73	0.59	0.83	0.13	17.1	0.78	0.77	0.80	0.02	2.0
Mesola	0.71	0.69	0.72	0.02	2.4	0.72	0.66	0.75	0.05	7.2
Verona	0.55	0.47	0.69	0.12	22.7	0.59	0.49	0.76	0.15	25.2
Chiogga	0.87	0.86	0.89	0.02	2.0	0.85	0.84	0.86	0.01	1.4
Castelfranco	0.38	0.36	0.40	0.02	5.3	0.75	0.62	0.86	0.12	16.2
Jupiter	0.54	0.39	0.68	0.15	27.0	0.59	0.49	0.73	0.13	21.6
Uranus	0.47	0.40	0.53	0.07	13.9	0.48	0.41	0.53	0.06	12.8
Mercurius	0.43	0.31	0.57	0.13	30.1	0.36	0.32	0.42	0.05	14.7
After storage										
Leonardo	0.77	0.73	0.79	0.03	4.2	0.60	0.53	0.67	0.07	11.7
Trevisio	0.48	0.39	0.56	0.09	17.8	0.74	0.68	0.78	0.05	7.0
Mesola	0.59	0.57	0.60	0.02	3.6	0.80	0.78	0.85	0.04	5.0
Verona	0.82	0.79	0.84	0.03	3.1	0.84	0.77	0.88	0.06	7.5
Castelfranco	0.41	0.37	0.45	0.04	9.8	0.68	0.64	0.71	0.04	5.3
Jupiter	0.75	0.72	0.78	0.03	4.1	0.86	0.82	0.89	0.04	4.1
Uranus	0.23	0.21	0.26	0.03	3.1	0.48	0.37	0.58	0.11	22.0
Mercurius	0.24	0.21	0.26	0.03	11.0	0.63	0.54	0.72	0.09	14.2

$n = 3$ for all samples, $\bar{x}$ – mean; mean, min – minimal value, max – maximal value, SD – standard deviation, *CV* (%) – coefficient of variation

Table 5. Comparison of antioxidative potentials in the chicory cultivars between the internal and external leaves before and after storage, as analysed using Duncan's test (α = 0.5)

Cultivar	Internal vs. external leaves		Before storage vs. after storage	
	before storage	after storage	internal leaves	external leaves
Leonardo	ns	*	ns	**
Trevisio	ns	*	*	ns
Mesola	ns	**	**	ns
Verona	ns	ns	*	ns
Chioggia	ns	/	/	/
Castelfranco	**	***	ns	ns
Jupiter	ns	*	ns	*
Uranus	ns	*	**	ns
Mercurius	ns	**	ns	*

ns – not significantly different; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001

also reported higher TPCs and AOPs in red-coloured chicory, as compared to iceberg lettuce.

After harvest, and before storage, the highest AOPs were in cvs Chiogga, Leonardo, Trevisio, and Mesola (0.7–0.9 μmol/g FW). The lowest AOP was in cv. Castelfranco, almost twice lower than the highest AOP. There were high AOPs after storage in the internal leaves of cvs Verona, Leonardo, and Jupiter (0.75–0.82 μmol/g FW). The sweet chicory cvs Mercurius and Uranus had up to 3-times lower AOPs (0.24 μmol/g FW). The red spotted cv. Castelfranco showed a significantly higher AOP, as compared to the internal leaves. All of the cultivars, with the exception of cvs Leonardo and Verona, had significantly higher AOPs after storage in the external leaves, as compared to the internal ones (Table 5).

Comparisons of AOPs in the chicory cultivars between the internal and external leaves before and after storage are reported in Table 5. Significantly higher AOPs were seen for cvs Leonardo, Mesola, and Trevisio (0.48–0.85 μmol/g FW) as compared to cvs Uranus and Mercurius (0.23–0.63 μmol/g FW). After storage, AOPs decreased in most cultivars, with the exception of cvs Verona and Jupiter.

Several studies have reported a relationship between AOPs measured with DPPH and TPCs (Llorach *et al.* 2004; Kevers *et al.* 2007; Conforti *et al.* 2009; Sulaiman *et al.* 2011). Polyphenols are compounds that have high AOP, so the correlation between TPCs and AOPs is positive and statistically significant (r = 0.46). These levels of antioxidants in chicory can have important contributions to the recommended daily intake of antioxidants (Rossetto *et al.* 2005).

CONCLUSIONS

Recent studies have shown that many polyphenol compounds and flavonoids contribute significantly to the total AOP. The data presented in this study demonstrate that the varieties of chicories analysed, which are mostly produced in Slovenia, have different polyphenol and antioxidative activities. TPCs were evaluated in the internal and external leaves before and after storage in nine different chicory varieties. There were higher TPCs measured in the red and red spotted chicory cultivars (100–400 mg/100 g FW), as compared to the sweet yellow-green cultivars (20–80 mg/100 g FW).

After harvest, there were small differences in TPCs between the internal and external leaves in the sweet cultivars. The highest TPCs were found in cvs Verona and Trevisio. The coloured cultivars showed TPCs up to several times higher as compared to the sweet green chicories. Due to the presence of substantial amounts of polyphenols in the red chicories, a single serving of this vegetable (100 g) can contribute up to 400 mg total polyphenols to the human diet. These amounts represent a significant contribution to the daily intake of 1 g/day of polyphenols, as reported in the previous studies (Scalbert & Williamson 2000; Rossetto *et al.* 2005).

There were higher AOPs in red chicories (0.40 to 0.85 μmol/g FW), as compared to the red spotted and sweet cultivars (0.2–0.6 μmol/g FW). Taking into account all the cultivars, significantly higher AOP was seen for the external leaves, as compared to the internal leaves. A slight decrease in AOP was found in most chicory cultivars during storage, due to senescence and oxidation processes.

Based on our data, the chicory variety and the leaf position have a significant influence on TPC and AOP, while storage influenced the AOP only. The outer leaves have significantly higher TPC and AOP in most cultivars. In conclusion, chicory represents a relatively cheap food and an important vegetable from the nutritional aspect.

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