



## Bioactive polyphenolic compounds from white cabbage cultivars

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### ARTICLE INFO

#### Article history:

Received: February 2, 2018

Accepted: March 6, 2018

#### Keywords:

bioactive compounds

polyphenols

antioxidants

flavonoids

čepinski

### ABSTRACT

The aim was to analyse polyphenols and their antiradical activity in white cabbage cultivars Čepinski, Varaždinski, Ogulinski and Bravo from different geographical locations of Croatia. Polyphenols were analysed by using high-performance liquid chromatography (HPLC) and spectrophotometric methods (total polyphenols and total flavonoids), antiradical activity by using DPPH and ABTS methods. Caffeic, *p*-coumaric, ferulic and sinapic acids were identified. Total polyphenols, total flavonoids and polyphenols determined with HPLC (467 – 598, 104 – 166, 94 – 126 mg kg<sup>-1</sup> FW, respectively) and antiradical activity showed statistically significant variations. Varaždinski showed the highest polyphenol content, usually followed by Čepinski or Ogulinski while Bravo always showed the lowest polyphenol content. Principal component analysis showed clustering of all four cultivars. Čepinski, which was never analysed before, could be a valuable cultivar for further examination.

## Introduction

Cabbage belongs to the *Brassicaceae* family, *Brassica oleracea* species. Besides cabbage, the *Brassica oleracea* species include some edible crops such as broccoli, cauliflower and kale (Jacob et al., 2011). Cabbage (*capitata* group) is a biennial, dicotyledonous plant which can be classified as white and red. It has an economical and nutritional importance since it is consumed widely in different culinary forms (fresh or sour). Cabbage is available in local markets as a cheap and consumer preferred vegetable. White cabbage is light to green coloured, with thick leaves wrapped in round heads. Since white cabbage (*Brassica oleracea* var. *capitata* f. *alba*) is available the whole year, it represents a good source of polyphenolic compounds (Hounsome et al., 2009; Kusznierevicz et al., 2008; Martínez et al., 2010; Šamec et al., 2014).

Polyphenols have shown many potential bioactivities (Del Rio et al., 2010). It was shown that they possess potential activities against cancer (Del Rio et al., 2010; Rodriguez-Mateos et al., 2014), cardiovascular diseases (Hollman et al., 2011), and inflammation, (Del Rio et al., 2010) although the evidence is still not sufficient to be definitive. Interactions with other food constituents are also important because they affect their bioactivities, availability for absorption (bioaccessibility) and the amount absorbed in the organism (bioavailability) (Jakobek, 2015).

Phenolic acids (Heimler et al., 2006; Hounsome et al., 2009) and flavonols (Kaulmann et al., 2016) were identified in white cabbage. Bioactivities of cabbage polyphenols are also being studied like bioaccessibility (Kaulmann et al., 2016) and bioavailability in the human organism (Wiczowski et al., 2016) or their antiradical activity (Heimler et al., 2006; Kusznierevicz et al., 2008; Šamec et al., 2014). Sinapic acid, which is a characteristic feature of cabbage, has also shown various potential bioactivities (Lee et al., 2012;

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Pari and Jalalundeen, 2011; Roy and Prince, 2012, 2013; Silambarasan et al., 2015, 2016; Yoon et al., 2007). These bioactive polyphenolic compounds can vary depending on the cabbage cultivar, but papers about differences between cultivars are so far limited (Kusznierewicz et al., 2008; Park et al., 2014; Peñas et al., 2011).

The objective of this study was to investigate the polyphenolic compounds and antiradical activity of four cabbage cultivars; Varaždinski, Ogulinski, Čepinski and Bravo. Cultivars were grown in different geographical locations of Croatia, Varaždinski in Varaždin County, Ogulinski in Ogulin County, Čepinski and Bravo in Eastern Croatia in Osijek-Baranja County. Čepinski variety was not investigated before, to the best of our knowledge.

## Materials and methods

### Chemicals

Caffeic acid (C0625, 99%), *p*-coumaric acid (C9008,  $\geq 98\%$ ), ferulic acid (F3500, 99%), quercetin dihydrate (Q0125,  $\geq 98\%$ ), 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH, D9132), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, A1888, 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sinapic acid (A0348392) was purchased from Acros Organics (New Jersey, USA). Other chemicals were purchased from Fluka (Buchs, Switzerland) (orto-phosphoric acid 85%, HPLC grade; ammonium peroxodisulfate 09915), J. T. Baker (Deventer, Netherlands) (methanol HPLC grade) and Kemika (Zagreb, Croatia) (hydrochloric acid 36.2 %, sodium carbonate, Folin-Ciocalteu reagent, aluminium chloride, sodium nitrite).

### Samples and sample preparation

Four white cabbage cultivars (*Brassica oleracea*) from Croatia were studied – Bravo, Čepinski, Varaždinski and Ogulinski. Cabbages were harvested from local producers, Bravo and Čepinski (both in Osijek-Baranja County, near the town of Čepin, Croatia), Varaždinski from Studij Varaždin (Varaždin County, Croatia), Ogulinski from V. Sabljak (Ogulin County, Croatia). Each cabbage cultivar consisted of three cabbages. Each of three cabbages from one cultivar were cut into three equal pieces and the core was removed. Three pieces, one from each cabbage, were cut with a knife and pooled together to obtain one homogenous sample.

The second and the third piece were cut with the knife and pooled together to obtain the second and the third homogenous sample. These samples were ground with the use of a stick blender. In this way, three homogenous, pooled samples of cabbage from each cultivar were obtained. Extracts were prepared immediately after the homogenization.

### Polyphenol extraction

In the extraction procedure, the mass of the cabbage and the percentage of solvent that can produce the highest polyphenol concentration, were evaluated. To examine the cabbage mass, different masses of cabbage (0.2, 0.4 and 0.6 g) were weighed into plastic cuvettes. Polyphenols were extracted according to the procedure: 1.5 mL of 50% methanol in water was added, cuvettes were vortexed (Grant Bio, Cambridgeshire, England), placed into an ultrasonic bath RK 100 (Bandelin Sonorex, Berlin, Germany) for 15 minutes, and centrifuged for 10 min at 10000 rpm (Minispin Eppendorf, Germany). Extracts were separated from the residues and then the residues were extracted two more times with the same solvent. Extracts were combined. Furthermore, to evaluate the extraction solvent, cabbage samples (0.2 g) were extracted according to the same procedure by using three different solvents (50% methanol with water, 80% methanol with water and 100% methanol). Total polyphenols were determined in each extract with the Folin-Ciocalteu method.

After optimizing extraction protocol, polyphenols were finally extracted from all four cabbage cultivars by using 0.2 g of cabbage and 80% methanol as a solvent. Extracts were used for the determination of total polyphenols, total flavonoids, antiradical activity (DPPH and ABTS) and individual polyphenols with HPLC-PDA.

### Spectrophotometric method for the total polyphenol determination

Total polyphenols were determined by the Folin-Ciocalteu micro method (Waterhouse, 2016). The extract (100  $\mu\text{L}$ ) and distilled water (1500  $\mu\text{L}$ ) were mixed, Folin-Ciocalteu reagent (100  $\mu\text{L}$ ) and a sodium carbonate solution (200 g L<sup>-1</sup>) (300  $\mu\text{L}$ ) were added, and the mixture was incubated in a water bath at 40°C (30 min). The absorbance was read at 765 nm on an UV-Vis spectrophotometer UV 2005 (JP Selecta S.A., Barcelona, Spain). A calibration curve was constructed by measuring gallic acid solutions (0 to 500 mg L<sup>-1</sup>) with the same procedure.

The results were therefore expressed in mg of gallic acid equivalents (GAE) per kg of fresh cabbage weight (FW).

#### *Spectrophotometric method for the total flavonoid determination*

1080  $\mu\text{L}$  of distilled water, 400  $\mu\text{L}$  of extract and 60  $\mu\text{L}$  of  $\text{NaNO}_2$  (5%) were added into a glass cuvette and solution was mixed in a vortex. After 5 min, 60  $\mu\text{L}$  of  $\text{AlCl}_3$  (10%) was added, and after 6 min 400  $\mu\text{L}$  of  $\text{NaOH}$  (1 mol  $\text{L}^{-1}$ ). The solution was mixed again with a vortex. Absorbance was measured on the UV-Vis spectrophotometer UV 2005 (JP Selecta S.A., Barcelona, Spain) at 510 nm against the blank solution (which contained 400  $\mu\text{L}$  of distilled water instead of extract). A calibration curve was constructed by measuring (+)-catechin solutions (0 to 500 mg  $\text{L}^{-1}$ ) with the same procedure. Total flavonoids in the cabbage were expressed in mg of (+)-catechin equivalents (CE) per kg of fresh cabbage weight (FW).

#### *Reversed phase high-performance liquid chromatography with photodiode array detection (RP-HPLC-PDA)*

Analyses were performed on a Varian HPLC system (Varian Inc., Palo Alto, USA) (with a ProStar 230 solvent delivery module, a ProStar 330 PDA detector, an OmniSpher C18 column (250 x 4.6 mm, 5  $\mu\text{m}$ ), and a guard column (ChromSep 1 cm x 3 mm)). Mobile phases were 0.1% phosphoric acid in water (A) and 100% methanol (B). The gradient was developed: 3% B (0 min), 12% B (6 min), 15% B (12 min), 20% B (16 min), 24% B (21 min), 32% B (31 min), 58% B (60 min), 60% B (61 min), 80% B (65 min), continued at 80% B (65 to 66 min), and then decreased to 3% B (66 to 68 min). The flow rate was 1  $\text{mL min}^{-1}$  and the injection volume 50  $\mu\text{L}$ . Spectra were recorded from 190-600 nm, and all polyphenols were detected at 320 nm. Stock solutions of polyphenol standards (1000  $\text{mg}\cdot\text{L}^{-1}$ ) were prepared in methanol. Dilutions were prepared in methanol too and calibration curves were created by injecting various concentrations of polyphenol standards (0.9 to 180  $\text{mg L}^{-1}$  caffeic acid, *p*-coumaric acid, ferulic acid and quercetin, 1 to 200  $\text{mg L}^{-1}$  sinapic acid). All calibration curves were linear ( $r^2$  from 0.9919 to 0.9999). The limit of detection and the limit of quantification were as follows: caffeic acid 0.17, 0.5; *p*-coumaric acid 0.1, 0.15; ferulic acid 0.12, 0.38; sinapic acid 0.06, 0.2 and quercetin 0.08, 0.24  $\text{mg kg}^{-1}$  FW. Precision was estimated through the calculation of the coefficient of variation

of the individual polyphenol amount found in cabbage extracts (from 0.9 to 25%). In order to identify polyphenols from cabbage, polyphenol retention times and spectra were compared with those of standards. In this way, caffeic acid (maximum absorbance at 312 nm), *p*-coumaric acid (maximum absorbance at 301 nm), ferulic acid (maximum absorbance at 311 nm) and sinapic acid (maximum absorbance at 311 nm) were identified and then quantified based on the authentic calibration curves. Some sinapic acid derivatives, phenolic acids and flavonols could not be identified but they were recognized by their similar spectra maximums: sinapic acid derivatives at 311 nm, phenolic acids at 301 to 314 nm, and flavonols at 340 to 355 nm. Their peak areas were summed up, quantified by using sinapic acid, caffeic acid and quercetin calibration curves, respectively, and finally reported as unknown sinapic acid derivatives, unknown phenolic acids and unknown flavonols. The identifications were also compared to literature data (Ferrerres et al., 2006; Velasco et al., 2011).

#### *DPPH method*

The antiradical activity of cabbage extracts was determined by a DPPH $\cdot$  assay (Brand-Williams et al., 1995). The reaction solutions were prepared in glass cuvettes with 2650  $\mu\text{L}$  of methanol, 50  $\mu\text{L}$  of DPPH solution (1 mmol  $\text{L}^{-1}$ ) and 300  $\mu\text{L}$  of extract. The absorbance was measured at 517 nm (spectrophotometer UV 2005, JP Selecta S.A., Barcelona, Spain) against blank solution (which contained 300  $\mu\text{L}$  of extract and 2700  $\mu\text{L}$  of methanol) after one minute of reaction. The percentage of DPPH $\cdot$  radical inhibition was calculated as % inhibition =  $100 - A_t / (A_0 / 100)$  where % inhibition is a % of DPPH radical inhibition after one minute,  $A_t$  is the absorbance of the extract after one minute and  $A_0$  is the absorbance of DPPH $\cdot$  radical in zero minute. Finally, antiradical activity (AA) was expressed as  $\mu\text{mol}$  of inhibited DPPH radical per g of fresh weight cabbage according to the Eq. 1:

$$AA = \frac{n(\text{DPPH}) \times \text{inhibition}}{m(\text{cabbage})} \quad (1)$$

where  $n(\text{DPPH})$  is the amount of DPPH in  $\mu\text{mol}$  at the beginning of the reaction, % inhibition is % of DPPH radical inhibition in one minute,  $m$  (cabbage) is the cabbage mass in g, in the reaction solution. Higher results are representing a higher antiradical activity.

### ABTS method

The antiradical activity was also determined by using the ABTS<sup>•+</sup> method. The ABTS radical cation (ABTS<sup>•+</sup>) was produced by adding 200 µL of ammonium peroxodisulfate (65 mmol L<sup>-1</sup>) in 50 mL of ABTS solution (0.8 mmol L<sup>-1</sup> prepared in 0.1 mol L<sup>-1</sup> phosphate buffer solution pH 7.4) and kept in the dark at room temperature during 24 h before the analysis. The reaction solution consisted of 1600 µL of phosphate buffer pH 7.4 (0.1 mol L<sup>-1</sup>), 400 µL of cabbage extract and 500 µL ABTS<sup>•+</sup>. The absorbance was measured after one minute at 734 nm (spectrophotometer UV 2005, JP Selecta S.A., Barcelona, Spain).

Percent of ABTS<sup>•+</sup> radical inhibition was calculated as % inhibition = 100 -  $A_t / (A_0 / 100)$  where % inhibition is a % of ABTS<sup>•+</sup> radical inhibition after one minute,  $A_t$  is the absorbance of extract after one minute of reaction and  $A_0$  is the absorbance of ABTS<sup>•+</sup> radical in zero minute. Antiradical activity (AA) was expressed as µmol of inhibited ABTS<sup>•+</sup> radical per g of fresh weight cabbage according to the Eq. 2:

$$AA = \frac{\frac{n(\text{ABTS}) \times \text{inhibition}}{100}}{m(\text{cabbage})} \quad (2)$$

where  $n(\text{ABTS})$  is the amount of ABTS<sup>•+</sup> in µmol at the beginning of the reaction, % inhibition is % of ABTS<sup>•+</sup> radical inhibition in one minute,  $m(\text{cabbage})$  is the cabbage mass in g, in the reaction solution. Higher results are representing a higher antiradical activity.

### Statistical analyses

For the optimization of the extraction protocol, two cabbage extracts were made, and each was measured two times (a sample size of four). For measuring polyphenol content, three homogenous pooled samples of cabbage were prepared from every cultivar. For the RP-HPLC-PDA method, one extract was prepared from each pooled cabbage sample, and each was measured once (a sample size of three). For the total polyphenol, total flavonoid, DPPH and ABTS methods, three extracts were prepared from each pooled cabbage sample (9 extracts per variety) and each was measured

two times with methods for total polyphenols and total flavonoids (a sample size of eighteen) or once with DPPH and ABTS methods (a sample size of nine). Means ± standard deviation were reported. The results were analysed with post-hoc Tukey pairwise comparison tests which are auxiliary to one-way ANOVA. In order to visualize possible clustering of data and differences between varieties based on the polyphenol content and antiradical activity, principal component analysis (PCA) was applied. All the statistical analyses were carried out using analytical software Statistica (Statsoft, Tulsa).

### Results and discussion

Cabbage cultivars were described in Table 1. Čepinski and Bravo are round-headed, smooth-leaved cultivars, while Varaždinski and Ogulinski have flat heads but also smooth leaves. Figure 1 shows the results of the extraction procedure. Three different masses of the cabbage (0.2, 0.4 and 0.6 g) were extracted in a three step extraction procedure. Significantly higher polyphenol amounts were extracted with the lowest cabbage mass (0.2 g) (Figure 1 a). Three extraction solvents were also studied, 50 and 80% methanol in the water and 100% methanol (Figure 1 b). Although there were no statistically significant differences, it can be seen that similar polyphenol amounts were extracted with the use of 50 and 80% methanol, and the lowest with 100% methanol. According to these results, both 50 and 80% methanol could be a good choice. But higher percentages of methanol, like 80% methanol, could be a better choice due to the reduction of polyphenol oxidase activity by methanol (Jakobek et al., 2015). That is why for the further polyphenol extraction, the procedure included the extraction of 0.2 g of the cabbage with 80% methanol, in three extraction steps. Earlier studies also showed that although methanol (Kusznierewicz et al., 2008; Martínez et al., 2010) or acetone (Martínez et al., 2010) can be used for the polyphenol extraction, the use of aqueous methanol is usual (Hounsoume et al., 2009; Šamec et al., 2014) as in our study. Jaiswal et al. (2012 a, b) also showed that for the polyphenol extraction, 60% methanol is better in comparison to 60% acetone or water.

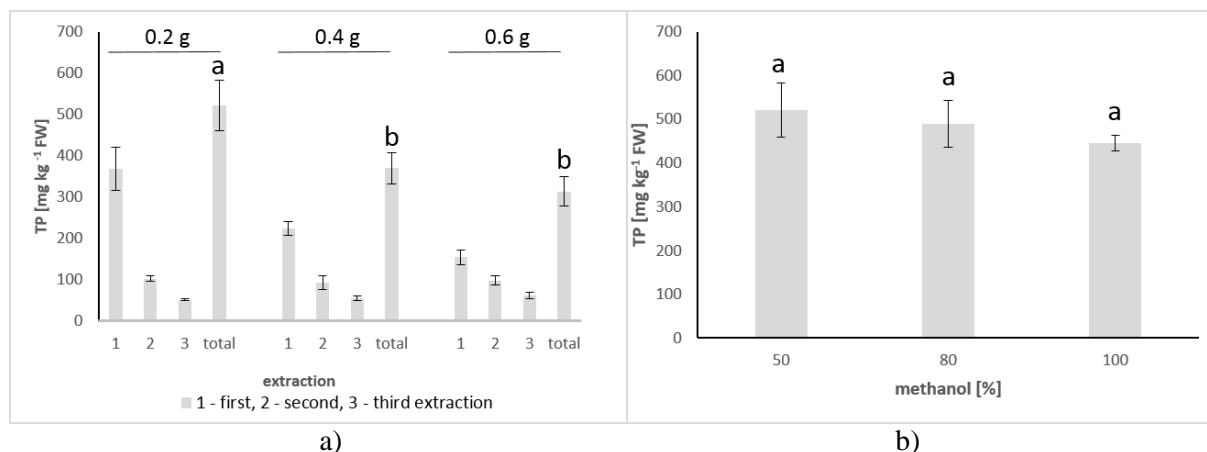
**Table 1.** The description of cabbage cultivars

Cabbage cultivar	Head			Leaves
	Shape	Longitudinal section	Colour	
Čepinski	round	firm headed	yellowish white	smooth, thin, light green
Varaždinski	flat	not compact	yellowish white	smooth, green
Ogulinski	flat	not compact	yellowish white	smooth, light green
Bravo	round	firm headed	yellowish white	smooth, light green

**Table 2.** The content of total polyphenols and total flavonoids in cabbage

Cabbage	total polyphenols*		total flavonoids*	
	[mg (GAE) kg <sup>-1</sup> FW]		[mg (CE) kg <sup>-1</sup> FW]	
Čepinski	564.9	±67.1 a,b	146.4	±19.9 b
Varaždinski	598.2	±74.7 a	165.8	±20.2 a
Ogulinski	538.3	±46.7 b	157.6	±17.8 a,b
Bravo	466.7	±35.6 c	103.9	±13.5 c

\*The means in columns with different letters are significantly different ( $p < 0.05$ ). GAE- gallic acid equivalents; CE – (+)-catechin equivalents; FW – fresh weight; Total polyphenols and total flavonoids determined spectrophotometrically, values are mean ± SD of 9 extracts each measured 2 times



**Fig. 1.** a) the effect of cabbage mass (0.2, 0.4, 0.6 g in 50% methanol) on the extraction of total polyphenols (TP) after three consecutive extractions. b) the effect of solvent type (0.2 g in 50%, 80% and 100% methanol) on the extraction of total polyphenols (TP). Each sample was prepared in two parallels and measured two times. The means with different letters are significantly different ( $p < 0.05$ ).

Table 2 shows the amount of total polyphenols and total flavonoids found in four cabbage cultivars. The amount of total polyphenols (from 467 to 598 mg kg<sup>-1</sup> FW) is in accordance with the literature data where 580 – 690 mg kg<sup>-1</sup> FW were reported (Martinez et al., 2010). Some data were expressed on the dry weight basis but if a cabbage contains 90% of the water, it is possible to recalculate the polyphenol amount which is again similar to our results (241 to 1194 mg kg<sup>-1</sup> FW) (Heimler et al., 2006; Kusznierevicz et al., 2008; Šamec et al., 2014). Total flavonoids (104 to 166 mg kg<sup>-1</sup> FW) were also in accordance with literature data (118 to 569 mg kg<sup>-1</sup> FW) (Heimler et al., 2006; Kusznierevicz et al., 2008; Šamec et al., 2014). The results for total polyphenols and total flavonoids showed good correlation ( $r^2 = 0.85$ ). Furthermore, statistically significant differences between four cabbage cultivars were found. The cultivars with the highest total polyphenols were Varaždinski and Čepinski, followed by Ogulinski and Bravo. The amount of total flavonoids was again the highest in Varaždinski, followed by Ogulinski, Čepinski and Bravo.

The amount of individual polyphenols determined with the RP-HPLC-PDA method is shown in Table 3. Caffeic, *p*-coumaric, ferulic and sinapic acid were identified. All cultivars contained low amounts of caffeic (0.6 to 1.2 mg kg<sup>-1</sup> FW), *p*-coumaric (0.2 to 0.8 mg kg<sup>-1</sup> FW) and ferulic acid (3.9 to 9.6 mg kg<sup>-1</sup> FW). Martinez et al. (2010) have found 2.5 to 3.1 mg kg<sup>-1</sup> FW caffeic acid, 1.2 to 1.5 mg kg<sup>-1</sup> FW *p*-coumaric acid, which is a little bit higher than in our study. Park et al. (2014) found an average of 1.56 mg kg<sup>-1</sup> FW of caffeic acid, 0.9 mg kg<sup>-1</sup> FW of *p*-coumaric acid and 0.97 mg kg<sup>-1</sup> FW of ferulic acid. In our study, sinapic acid was found in amounts from 43 to 51 mg kg<sup>-1</sup> FW, while Park et al. (2014) and Lee et al. (2011) reported 22.6 and 43 mg kg<sup>-1</sup>, respectively. Furthermore, samples contained unknown sinapic acid derivatives (4 to 10 mg kg<sup>-1</sup> FW), unknown phenolic acids (37 to 47 mg kg<sup>-1</sup> FW), and flavonols (3.1 to 7.4 mg kg<sup>-1</sup> FW). The results showed that Varaždinski had significantly higher amounts of almost all of individual polyphenols and that Čepinski and Varaždinski distinguished from other cultivars by the high amount of sinapic acid and derivatives. Furthermore, the differences in total

polyphenols between Varaždinski and other cultivars are statistically significant while between Čepinski, Ogulinski and Bravo are not.

Antiradical activity determined with the DPPH and ABTS methods is shown in Table 4. DPPH values were lower than ABTS values which is in accordance with literature data (Pérez-Jiménez and Saura-Calixto, 2008). The ability of polyphenols from cabbage to scavenge free radicals was shown in many earlier studies (Heimler et al., 2006; Jacob et al., 2011; Jaiswal et al., 2012a; Kuszneriewicz et al., 2008; Šamec et al., 2011; Šamec et al., 2014).

The results of the HPLC analysis were investigated with principal component analysis (PCA). This statistical tool enables the analysis of the experimental data and the recognition of possible patterns or classifications between cultivars. In this paper, the aim was to recognize possible

classification of cabbage cultivars or to see their differences based on the polyphenol content. The results for individual phenolic acids, unknown sinapic acids, unknown phenolic acids and total flavonols were analysed with the PCA (Figure 2). Together, factors 1 and 2 explained 85.62% of the differences between varieties (first factor 63.75 %, and second factor 21.87 %). The possible classification of cultivars can also be seen. All cultivars showed clustering according to the polyphenolic compound content.

Figure 3 shows the PCA plot of antiradical activity, total polyphenols and total flavonoids determined with the spectroscopic methods. This plot showed that factor 1 and 2 can explain 92.54% of differences between cultivars (factor 1: 68.93%, factor 2: 23.61%). Cultivars showed separation which also supports the thesis that they are different.

**Table 3.** The content of polyphenolic compounds\* obtained by using the HPLC analysis

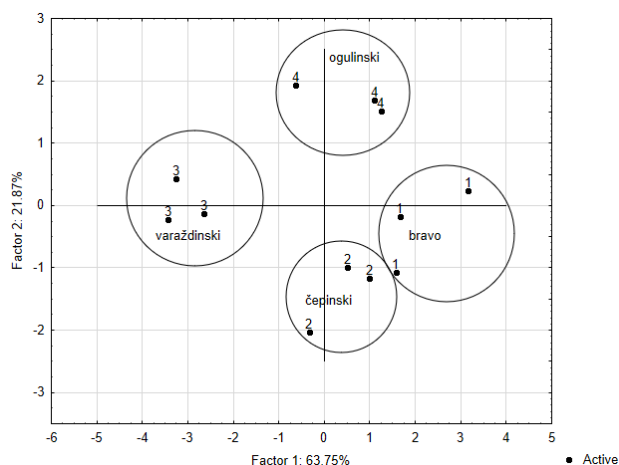
Compound	Cabbage cultivar							
	Čepinski [mg kg <sup>-1</sup> FW]		Varaždinski [mg kg <sup>-1</sup> FW]		Ogulinski [mg kg <sup>-1</sup> FW]		Bravo [mg kg <sup>-1</sup> FW]	
<b>Phenolic acids</b>								
caffeic acid	0.6	±0.1 b	1.1	±0.2 a	1.2	±0.2 a	0.7	±0.1 b
<i>p</i> -coumaric acid	0.4	±0.1 b	0.8	±0.1 a	0.3	±0.1 b	0.2	±0.0 b
ferulic acid	3.9	±1.6 b	9.6	±2.4 a	5.9	±0.8 a,b	4.2	±1.0 b
sinapic acid	49.3	±0.7 a,b	50.5	±1.3 a	42.8	±1.2 c	44.8	±3. 6 b,c
unknown sinapic acid derivatives <sup>a</sup>	6.7	±1.4 a,b	9.7	±1.4 a	9.5	±0.8 a	4.3	±1.0 b
unknown phenolic acids <sup>b</sup>	41.1	±1.0 b	46.8	±1.5 a	39.0	±2.0 b	36.7	±2.9 b
<b>total sinapic acid derivatives</b>	56.0	±2.1 a,b	60.2	±2.7 a	52.3	±2.0 b	49.1	±4.6 b
<b>total phenolic acids</b>	102.0	±4.9 b	118.5	±6.9 a	98.7	±5.1 b	90.9	±8.6 b
<b>Flavonols</b>								
total flavonols <sup>c</sup>	5.7	±2.4 a,b	7.4	±0.3 a	3.9	±1.2 a,b	3.1	±0.7 b
<b>total polyphenols</b>	107.7	±7.3 b	125.9	±7.2 a	102.6	±6.3 b	94.0	±9.3 b

Values are mean ± SD of three extracts each measured once. LOD and LOQ as follows: caffeic acid 0.17, 0.5; *p*-coumaric acid 0.1, 0.15; ferulic acid 0.12, 0.38; sinapic acid 0.06, 0.2 and quercetin 0.08, 0.24 mg kg<sup>-1</sup>. Coefficient of variation from 0.9 to 25 %. FW – fresh weight; \*The means in rows with different letters are significantly different ( $p < 0.05$ ); <sup>a</sup> all unknown sinapic acid derivatives calculated by using sinapic acid calibration curve; <sup>b</sup> all unknown phenolic acids calculated by using caffeic acid calibration curve; <sup>c</sup> all unknown flavonols calculated by using quercetin calibration curve

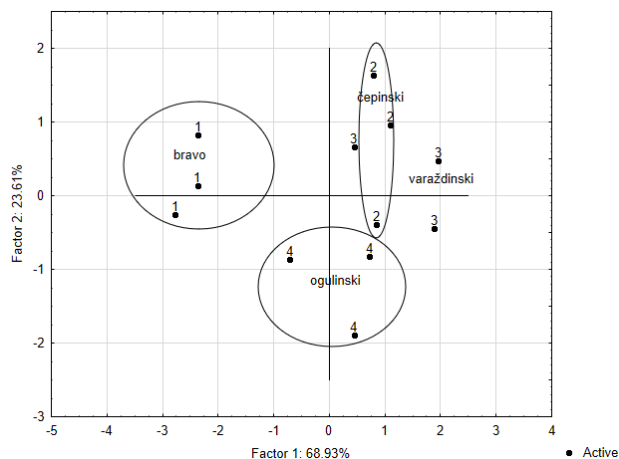
**Table 4.** Antiradical activity of cabbage by using DPPH and ABTS methods

Cabbage	DPPH*		ABTS*	
	[μmol DPPH /g cabbage]		[μmol ABTS /g cabbage]	
Čepinski	0.6	± 0.3 <sup>c</sup>	11.2	± 0.9 <sup>a</sup>
Varaždinski	0.8	± 0.3 <sup>b,c</sup>	10.8	± 0.9 <sup>a</sup>
Ogulinski	1.2	± 0.2 <sup>a</sup>	10.6	± 0.9 <sup>a</sup>
Bravo	1.0	± 0.2 <sup>a,b</sup>	8.4	± 0.7 <sup>b</sup>

Values are mean ± SD of 9 extracts each measured once. Antioxidant activity expressed as the amount of inhibited free radical (μmol) per g fresh weight cabbage, in one minute. \*The means in columns with different letters are significantly different ( $p < 0.05$ )



**Fig. 2.** Principal component analysis of four cabbage cultivars (1 - Bravo, 2 - Čepinski, 3 - Varaždinski, 4 - Ogulinski) using their polyphenolic compound concentrations obtained by the HPLC method (caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, unknown sinapic acid derivatives, unknown phenolic acids, total flavonol content).



**Fig. 3.** Principal component analysis of four cabbage cultivars (1 - Bravo, 2 - Čepinski, 3 - Varaždinski, 4 - Ogulinski) using their total polyphenol, total flavonoid, DPPH and ABTS values.

According to our results, the cultivar Varaždinski contained significantly higher amounts of polyphenols, followed by Čepinski and Ogulinski, while Bravo had the lowest polyphenol content (Table 2 and 3). These results can be compared to some earlier studies. Namely, Varaždinski (Šamec et al., 2011, 2014) and Ogulinski cultivars (Šamec et al., 2011) were analysed before. In these earlier studies it was shown that Varaždinski juices had higher polyphenol content than juices made of Ogulinski cabbage, which is similar to our study. Furthermore, our study also showed that Čepinski had reasonably high amount of polyphenols and usually followed Varaždinski (Table 2 and 3). Since this cultivar was never analysed before, it could be highlighted that this cultivar is also valuable with the

potential for bigger production and further examination. The lowest polyphenol compound content was always found in the Bravo cultivar. The differences found between cabbage cultivars could be assigned to (i) different cultivars, (ii) different geographical region and climate. Peñas et al. (2011) also showed that polyphenolic content of the white cabbage depended on cultivars. Furthermore, all four cultivars were grown in different regions and in different climate. Čepinski and Bravo were grown in Eastern Croatia, in the continental climate region. Varaždinski was grown in the north western part of Croatia with continental climate. Ogulinski originated from the mountain area with mountain climate. Peñas et al. (2011) showed that geographical location and climate conditions (temperature and radiation)

affected the polyphenol content and antiradical activity of white cabbage, similar to our study.

## Conclusion

In this study, polyphenol content and antiradical activity of four cabbage cultivars Čepinski, Varaždinski, Ogulinski and Bravo were analysed. Overall, the highest polyphenol content was found in cultivar Varaždinski, followed by Čepinski and Ogulinski, and the lowest amount was found in the Bravo cultivar. Polyphenols from the cabbage showed the possibility to scavenge free radicals. The PCA analysis of all the data showed that cultivars clustered into separate groups which means that all cultivars are potentially different. The differences in the polyphenol content and antiradical activity could be assigned to (i) cultivars and possibly (ii) different geographical regions and climate. Overall results showed that Čepinski, which was never analysed before, usually followed Varaždinski according to the polyphenol content and that could be considered as valuable for bigger production and further examination.

## Acknowledgements

We wish to express our gratitude to prof. Andrew R. Barron for his help in the statistical analysis. This work was supported by the Faculty of Food Technology Osijek.

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