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Antioxidant potency of white (*Brassica oleracea* L. var. *capitata*) and Chinese (*Brassica rapa* L. var. *pekinensis* (Lour.)) cabbage: The influence of development stage, cultivar choice and seed selection

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ABSTRACT

The accumulation of total phenols (TP, Folin-Ciocalteu method) and total flavonoids (TF, colorimetric assay with $AlCl_3$) and the evolution of antioxidant capacity (FRAP assay, DPPH and ABTS radical scavenging assays) have been monitored in juices of Croatian white cabbage (*Brassica oleracea* var. *capitata*) cultivars Varaždinski and Ogulinski, as well as Chinese cabbage (*Brassica rapa* var. *pekinensis*), at various developmental stages. Measurements were performed every four weeks, starting from planting to full maturity, throughout the course of eight months. In the first 8–12 weeks, the TP and TF contents as well as antioxidant capacity increased significantly in all analyzed juice samples and in most even doubled. This rapid increase was followed by a gradual decrease in values derived from all assays, over the course of 12–30 weeks, to the final values which were in all cases lower than the values measured at week 4. The results also point to significant variability in TP and TF contents and antioxidant capacity at the fully mature stage between white and Chinese cabbage juices and between juices extracted from cultivars Ogulinski and Varaždinski.

1. Introduction

A diet rich in fruits and vegetables has been attributed protective properties against Alzheimer's disease (Dai et al., 2006), various cardiovascular pathological conditions and several common cancers (Bazzano et al., 2002). Plant foods with apparent anticancer (Fowke et al., 2000, 2003) and cardioprotective properties include varieties of *Brassica oleracea* (Beecher, 1994), which have exhibited genotoxic properties (Kassie et al., 1996) and high antioxidant and antimicrobial activities (Roy et al., 2007; Ayaz et al., 2008) in earlier studies. One of 20 *Brassicaceae* varieties is white cabbage (*B. oleracea* L. var. *capitata*) whose cultivars are native to the Mediterranean region and southwestern Europe, however, today *Brassicaceae* forms are grown for food everywhere in the world. Cruciferous vegetables are among the most important dietary vegetables consumed in Europe, owing to their availability in local markets, affordability and consumer preference.

Due to its anti-inflammatory and antibacterial properties, cabbage has widespread use in traditional medicine, in alleviation of symptoms associated with gastrointestinal disorders (gastritis, peptic and duodenal ulcers, irritable bowel syndrome) as well as in treatment of minor cuts and wounds and mastitis. Fresh cabbage juice, prepared either separately or mixed with other vegetables such as carrot and celery, is often included in many commercial weight-loss diets (Greenly, 2004), diets that improve bioavailable content of nonheme iron (Chiplonkar et al., 1999), as well as alternative therapies for cancer patients (Maritess et al., 2005). Clinical research has shown positive effects of cabbage juice consumption in healing peptic ulcers (Cheney, 1949), and facilitating the reduction of serum LDL levels (Suido et al., 2002). Chemical components analysis has shown that white cabbage is rich in phytochemicals including phenolic compounds (Kusznierewicz et al., 2008), carotenoids (Nilsson et al., 2006) and glucosinolates (Song and

Abbreviations: ABTS, 2,2′-azinobis (3-ethylbenz-thiazoline-6-sulphonic acid); CE, catechin equivalents; CHIN, Chinese cabbage; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing/antioxidant power; GAE, gallic acid equivalents; TF, total flavonoids; TP, total phenols; TEAC, trolox equivalent antioxidant capacity; Vž-DOM, white cabbage cultivar Varaždinski seed producer from Istria; Vž-EU, white cabbage cultivar Varaždinski seed producer from Italy; Vž-LO, white cabbage cultivar Varaždinski local seed producer; OG, white cabbage cultivar Ogulinski.

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Thornalley, 2007). Cabbage also belongs to the group of vegetables high in vitamin C (Gould et al., 2006) and antioxidant potential (Kusznierewicz et al., 2008; Nilsson et al., 2006).

Among more than 10 white cabbage varieties grown in Croatia the most important one is cultivar Varaždinski, which is widespread in the Varaždin county and accounts for 65% of total cabbage production in Croatia. This cultivar is commonly fermented due to its robust and compact head, with soluble dry matter content above 3% (Dobričević et al., 2006). White cabbage cultivar Varaždinski is of invaluable agricultural and economic importance for the Varaždin region, where about 50% of cabbage manufacturers grow this variety using seed from different seed producers. To the best of our knowledge, there are no published studies characterizing the differences in phytochemical composition and antioxidant properties between Varaždinski cabbage seeds in circulation. Also, there are no published studies aimed at monitoring the changes in polyphenol composition and antioxidant capacity of cabbage juice extracted from different developmental stages of white cabbage cultivars.

In the study reported here we monitored the total phenol (TP, Folin-Ciocalteu method) and total flavonoid (TF, colorimetric assay with $AlCl_3$) contents as well as antioxidant capacity of water-soluble cabbage juice components during maturation of white cabbage cultivars Varaždinski (seed produced in the European Union – Italy, Croatia – Istra, and by a local producer in Varaždin), Ogulinski and Chinese cabbage (*Brassica rapa* cv. *pekinensis*). Chinese cabbage (*B. rapa* cv. *pekinensis*) was included in the study because of the more recent introduction of this species among Croatian cabbage growers and the established market that Chinese cabbage has throughout Asia, and also North America. Antioxidant capacity was evaluated using commonly employed spectrophotometric assays (Ferric Reducing/Antioxidant Power (FRAP), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) and 2,2′-azinobis (3-ethylbenz-thiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) assays). We also report the results of antimicrobial activity testing of studied cabbage varieties at full maturation.

2. Materials and methods

2.1. Chemicals, bacterial strains and instruments

Ferrous sulphate hepta hydrate ($FeSO_4{\cdot}7H_2O$) was purchased from Kemika (Zagreb, Croatia) and sodium nitrate from ($NaNO_2$) Laphoma (Skopje, Macedonia). Sigma Chemical Co. (St. Louis, MQ, USA) supplied the remaining analytical-grade reagents. The following bacterial strains from the culture collection of the Faculty of Food Technology and Biotechnology were used as test microorganisms: *Staphylococcus aureus* 3048, *Escherichia coli* 3014, *Salmonella enterica* serovar Typhimurium FP1 and *Bacillus subtilis* ATCC 6633. UV–vis spectrophotometer Bio-Spec-1601(Shimadzu Corporation, Kyoto, Japan) was used for absorbance measurements.

2.2. Growing conditions and extraction

White cabbage cultivars Ogulinski and Varaždinski from different seed producers (Table 1), as well as Chinese cabbage were planted in the greenhouse in the suburban Varaždin region on February 16th, 2009. Until May 20th, 2009, all cabbage varieties were grown in the greenhouse under identical growing conditions, after which seedlings were transplanted to the field. Every four weeks after planting (beginning on March 16th), and at full maturation (on September 15th), the cabbage heads were harvested and transported to the laboratory for immediate processing. The ripening stage and full maturity were determined according to the following parameters: length of vegetation, compactness, hardness and size/weight of the cabbage head. The samples, taken as cross-sections that incorporated an equal share of inner and outer leaves, from three separate heads (5 g each) were shredded in liquid nitrogen, placed in plastic vials, centrifuged in a Multifuge 3S-R centrifuge (Kendro, Germany) at 10,000 g for 45 min, and filtered at room temperature in order to completely separate juice from each sample.

For antimicrobial testing, fully mature fresh cabbage samples were freeze-dried for 72 h on Lyovac GT 2 (STERIS GmbH, Hűrth, Germany) and extracted in 80% methanol (2 g in 40 ml), by shaking (1 h) and sonication (15 min). The extracts were dried using a rotary evaporator at 30 °C (Bűchi, Switzerland) and any remaining liquid was removed by freeze-drying.

2.3. Phytochemicals content

The TP of juices was determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965), in a total reaction volume of 2 mL. To 20 μL of cabbage juice, 100 μL of the Folin-Ciocalteu phenol reagent was added, followed by 300 μL of saturated sodium carbonate solution. The final reaction volume was made up to 2 mL with distilled water. Absorbance was read at 765 nm after 2 h. Gallic acid was used as a standard and the results were expressed as milligrams of gallic acid equivalents per milliliter of juice (mg GAE/mL).TF of cabbage juice was determined according to the $AlCl_3$ colorimetric assay (Zhishen et al., 1999) in a total reaction volume of 2 mL. To 200 μL of cabbage juice, 800 μL of distilled water and 60 μL of (5%, w/v) $NaNO_2$ were added. 5 min later, 60 μL of (10%, w/v) $AlCl_3$ were added. After additional 6 min, 400 μL of 1 M solution of NaOH were added and the final reaction volume was adjusted to 2 mL with distilled water. Absorbance of the mixture was determined at 420 nm. The results were expressed as milligram of catechin equivalents per milliliter of juice (mg CE/mL).

2.4. Antioxidant capacity

The FRAP assay was used to estimate the antioxidant potential of tested extracts, according to the original method of Benzie and Strain (1999). 50 μL of properly diluted cabbage juice was mixed with 950 μL of the FRAP reagent and absorbance was read at 593 nm after 4 min reaction time. A calibration curve was constructed for ferrous sulphate $FeSO_4{\cdot}7H_2O$ and the results were expressed as μM Fe^{2+}.

DPPH and ABTS radical scavenging capacities was determined according to the methods outlined by Brand-Williams et al. (1995) and Re et al. (1999), respectively. In the DPPH radical scavenging assay, 20 μL of cabbage juice was mixed with 980 μL of 0.0094 mmol/L DPPH methanolic solution. After 30 min of reaction at 20 °C absorbance was read at 515 nm. In the ABTS radical scavenging assay, 20 μL of the tested cabbage juice was added to 2.0 mL of $ABTS^{\bullet+}$ solution, and the absorbance readings were taken after exactly 6 min at 734 nm. A calibration curve for DPPH and ABTS assays was prepared using Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and the results were expressed as mM Trolox.

2.5. Antimicrobial activity

Antimicrobial activity of freeze-dried extracts dissolved in a nutrient broth to a final concentration of 100 g/L with 2% DMSO addition, was tested against test microorganisms. Antimicrobial activity testing was performed according to the agar-well (Šušković et al., 1993) and agar-disc assays, as well as the turbidimetric microdilution assay (Tolonen et al., 2004). *Staphylococcus aureus* 3048, *Escherichia coli* 3014, *Salmonella enterica* serovar Typhimurium, *Bacillus subtilis* ATCC 6633 were grown at 37 °C

Table 1
White cabbage and Chinese cabbage samples and extraction yields.

Common name	Latin name	Variety	Cultivar/hybrid	Seed producer	Region of origin	Country of origin	Yield (%)
White cabbage	*B. oleracea*	*capitata*	Varaždinski	Local seed producer (Vž-LO)	Varaždin	Croatia	28.5
				Poljovrt (Vž-HR)	Istra	Croatia	37.5
			Ogulinski	Miagra (Vž-EU)	Italy	EU	36.7
				Local seed producer (OG)	Varaždin	Croatia	25.5
Chinese cabbage	*B. rapa*	*pekinensis*	Richi* estoril F1	Sakata (CHI)	–	South Korea	18.4

Vž-DOM: white cabbage cultivar Varaždinski seed producer from Istria; Vž-EU: white cabbage cultivar Varaždinski seed producer from Italy; Vž-LO: white cabbage cultivar Varaždinski local seed producer; OG: white cabbage cultivar Ogulinski, CHI: Chinese cabbage*Hybrid.

Table 2a
Total phenol concentrations in cabbage juices at different stages of maturity.

Weeks	Total phenols mg/mL GAE				
	Vž-EU	Vž-HR	Vž-LO	OG	CHI
4	$2.95 \pm 0.23^{c\,C}$	$2.67 \pm 0.05^{b\,C}$	$2.84 \pm 0.04^{bc\,B}$	$2.84 \pm 0{,}10^{bc\,C}$	$1.72 \pm 0.10^{a\,A}$
8	$3.96 \pm 0.12^{bc\,D}$	$3.51 \pm 0.23^{ab\,E}$	$4.47 \pm 0.28^{cd\,C}$	$4.50 \pm 0.18^{d\,E}$	$3.34 \pm 0.02^{a\,B}$
12	$3.96 \pm 0.11^{b\,D}$	$4.01 \pm 0.02^{b\,F}$	$5.18 \pm 0.23^{d\,D}$	$4.62 \pm 0.10^{c\,E}$	$3.44 \pm 0.13^{a\,B}$
16	$3.23 \pm 0.17^{b\,C}$	$2.85 \pm 0.06^{a\,C}$	$2.77 \pm 0.10^{a\,B}$	$3.19 \pm 0.15^{b\,D}$	$3.39 \pm 0.13^{b\,B}$
20	$3.63 \pm 0.11^{d\,D}$	$3.28 \pm 0.04^{c\,D}$	$2.65 \pm 0.05^{b\,B}$	$3.10 \pm 0.04^{c\,CD}$	$1.92 \pm 0.02^{a\,A}$
24	$1.05 \pm 0.04^{a\,AB}$	$1.46 \pm 0.05^{c\,B}$	$1.19 \pm 0.01^{b\,A}$	$1.09 \pm 0.01^{a\,B}$	$1.88 \pm 0.04^{d\,A}$
28	$0.89 \pm 0.02^{a\,A}$	$1.13 \pm 0.01^{b\,A}$	$0.94 \pm 0.03^{a\,A}$	$1.07 \pm 0.02^{b\,B}$	
30	$1.03 \pm 0.04^{c\,B}$	$1.03 \pm 0.01^{c\,A}$	$0.94 \pm 0{,}02^{b\,A}$	$0.67 \pm 0.00^{a\,A}$	

Different letters next to the values denote significiant differences ($p < 0.05$) between cultivars (a–e) and between different developmental stages of the same cultivar (A–F).
Vž-DOM: white cabbage cultivar Varaždinski seed producer from Istria; Vž-EU: white cabbage cultivar Varaždinski seed producer from Italy; Vž-LO: white cabbage cultivar Varaždinski local seed producer; OG: white cabbage cultivar Ogulinski; CHI: Chinese cabbage.

overnight in nutrient broth (beef extract 3 g/L; peptone 5 g/L) (Biolife, Milano, Italy).

2.6. Statistical and mathematical analyses

All presented numeric values are means obtained from triplicate extraction ± standard deviation (SD). One-way ANOVA and post hoc multiple mean comparison (Tukey's HSD test) were performed using the PAST (ver. 1.97) software package (Hammer et al., 2001). Differences at $p < 0.05$ were treated as significant.

3. Results and discussion

3.1. Phytochemicals content at different developmental stages

The TP and TF contents of cabbage juice extracted from cabbage heads collected at different developmental stages are shown in Tables 2a and 2b. All cultivars of white cabbage showed significantly higher TP (1.6-fold) and TF (2.7-fold) contents 12 weeks after planting compared to the initial values observed at 4 weeks. This rapid increase was followed by a gradual decrease in all assays, over the course of weeks 12–30, to the final values which were in all cases lower than the values measured at week 4. In general, white cabbage varieties showed two major drops in the TP content – on the average, a 1.5-fold decrease after 12 and a 2.6-fold decrease after 20 weeks. After the period of 24 weeks, the TP content of juice extracted from all white cabbages stabilized around 1 mg/mL GAE, while there were no significant differences in the TF values. Compared to measurements at 16 weeks, at 20 weeks Chinese cabbage juice showed a 1.8-fold drop in the TP and a 3.5-fold drop in the TF value. These findings are in accordance with Pandjaitan et al. (2005) who studied the phenolic content of spinach harvested at three different maturity stages (immature, mid-mature and mature stage) and found that there are much higher levels of TP and TF in mid-mature than in immature and mature leaves. Higher levels of TP in intermediate stages are probably a consequence of a more active plant metabolism which accompanies active/rapid growth in the first few months.

Chinese cabbage developed faster compared to white cabbage varieties and reached full maturity 24 weeks after planting. The juice extracted at full maturation showed a TP content of 1.88 ± 0.04 mg GAE/mL and a TF content of 0.49 ± 0.01 mg CE/mL. These values are significantly higher than the TP and TF values observed for white cabbage juices at full maturation (30 weeks after planting) which exhibited the TP content in the range of 0.67–1.03 mg GAE/mL and the TF content in the range of

Table 2b
Total flavonoid concentrations in cabbage juices at different stages of maturity.

Weeks	Total flavonoids mg/mL CE				
	Vž-EU	Vž-HR	Vž-LO	OG	CHI
4	$1.04 \pm 0.02^{b\,B}$	$1.08 \pm 0.03^{b\,B}$	$1.02 \pm 0.02^{b\,B}$	$1.13 \pm 0.02^{b\,B}$	$0.48 \pm 0.02^{a\,AB}$
8	$2.02 \pm 0.07^{c\,CD}$	$1.77 \pm 0.03^{b\,C}$	$2.25 \pm 0.04^{d\,E}$	$2.61 \pm 0.02^{e\,D}$	$1.60 \pm 0.10^{a\,C}$
12	$2.66 \pm 0.04^{b\,E}$	$2.74 \pm 0.04^{cb\,F}$	$3.28 \pm 0.12^{d\,F}$	$3.02 \pm 0.11^{dc\,E}$	$1.61 \pm 0.17^{a\,C}$
16	$1.87 \pm 0.12^{cd\,C}$	$1.99 \pm 0.09^{d\,D}$	$1.39 \pm 0.06^{a\,C}$	$1.70 \pm 0.08^{cb\,C}$	$1.45 \pm 0.09^{a\,BC}$
20	$2.03 \pm 0.06^{c\,D}$	$2.32 \pm 0.04^{d\,E}$	$1.87 \pm 0.04^{b\,D}$	$2.57 \pm 0.01^{e\,D}$	$0.42 \pm 0.07^{a\,A}$
24	$0.18 \pm 0.00^{a\,A}$	$0.33 \pm 0.01^{b\,A}$	$0.20 \pm 0.02^{a\,A}$	$0.19 \pm 0.01^{a\,A}$	$0.49 \pm 0.01^{c\,A}$
28	$0.13 \pm 0.00^{b\,A}$	$0.20 \pm 0.01^{a\,A}$	$0.13 \pm 0.01^{b\,A}$	$0.12 \pm 0.01^{b\,A}$	
30	$0.15 \pm 0.00^{b\,A}$	$0.25 \pm 0.01^{c\,A}$	$0.15 \pm 0.00^{b\,A}$	$0.08 \pm 0.00^{a\,A}$	

Different letters next to the values denote significiant differences ($p < 0.05$) between cultivars (a–e) and between different developmental stages of the same cultivar (A–F).
Vž-DOM: white cabbage cultivar Varaždinski seed producer from Istria; Vž-EU: white cabbage cultivar Varaždinski seed producer from Italy; Vž-LO: white cabbage cultivar Varaždinski local seed producer; OG: white cabbage cultivar Ogulinski; CHI: Chinese cabbage.

0.08–0.25 mg CE/mL. The observed TP values are in line with those of Roy et al. (2007) who found 623.3 ± 44.5 μg GAE/mL in white cabbage and 543.3 ± 84 μg GAE/mL in Chinese cabbage juice. In our experiment, at the fully ripe stage, Chinese cabbage juice showed a 2.1 times higher TP content than white cabbage juice (average value 0.97 g GAE/mL) which is not in accordance with some earlier studies where white cabbage was reported to have higher TP content than Chinese cabbage (Roy et al., 2007; Lee et al., 2007). The TP contents of all our analyzed juices at full maturation were higher than 15.06 ± 4.7 mg GAE/100 ml reported for cabbage juice in a study by Swatsitang and Wonginyoo (2008) who determined the TP content of 20 vegetable juices.

3.2. Antioxidant capacity of cabbage juice at different developmental stages

In our experiment we utilized three methods, FRAP, DPPH and ABTS tests, in order to assay the contribution of both lipophillic and hydrophilic antioxidants to the overall antioxidant capacity of cabbage juice (Prior et al., 2005). The results obtained from all three assays are shown in Fig. 1a–c. Our antioxidant activity testing parallels the results obtained in the TP and TF assays which is evident from high correlation factors determined between TP content and antioxidant assay results (0.963, 0.921, 0.934 for FRAP, ABTS and DPPH, respectively), and TF content and antioxidant assay results (0.931, 0.915, 0.890 for FRAP, ABTS and DPPH, respectively). We observed an increase in the antioxidant capacity of cabbage juice in the first 8 or 12 weeks, depending on the assay and individual sample (Fig. 1a–c). The highest antioxidant capacity measured by FRAP (19.09 ± 0.08 μM Fe^{2+}) and ABTS (11.52 ± 0.81 mM Trolox) methods was determined for juice extracted from cultivar Ogulinski at 8 weeks, while 12 weeks after planting Vž-LO juice exhibited the highest antioxidant capacity measured by the DPPH assay (4.75 ± 0.04 mM Trolox). At 12 weeks, analyzed cabbage juices exhibited DPPH radical scavenging capacity (2.28–4.75 mM Trolox) comparable to the capacity of commercial dark fruit juices analyzed earlier which ranged between 3.25 mM Trolox and 5.68 mM Trolox (Piljac-Žegarac et al., 2009). After achieving the maximum value, the antioxidant capacity of white cabbage as evaluated by all three assays, assumed a gradual decreasing trend, with individual fluctuations within a sample. The sharpest drop in the antioxidant capacity in all analyzed white cabbage juices was observed from weeks 20 to 24. The only exception was the juice extracted from Vž-EU cabbage whose antioxidant capacity significantly increased from weeks 28 to 30.

The juice extracted from Chinese cabbage (FRAP: 5.02 ± 0.05 μM Fe^{2+}; ABTS: 5.29 ± 0.10 mM Trolox; DPPH: 1.82 ± 0.01 mM Trolox) at full maturity exhibited a higher antioxidant capacity in all three assays (2.5× for FRAP, 2.4× for ABTS, 2.8× for DPPH) than the juice extracted from white cabbage cultivars (averages for FRAP: 1.99 ± 0.46 μM Fe^{2+}; ABTS: 2.21 ± 0.57 mM Trolox; DPPH: 0.65 ± 0.01 mM Trolox). These findings are in line with those of Lee et al. (2007) who also confirmed higher DPPH radical scavenging capacity of Chinese cabbage in comparison to white cabbage. Among 20 tested vegetable juices (Swatsitang and Wonginyoo, 2008) white cabbage juice showed the lowest antioxidant capacity in the DPPH assay, while at the same time showing a higher value than tomato and lime juice in the 2-deoxyribose assay. Among white cabbage cultivars analyzed at full maturity, Vž-EU showed a significantly higher antioxidant capacity in the FRAP (2.50 ± 0.06 μM Fe^{2+}) and ABTS assays (2.84 ± 0.22 mM Trolox); however, in the DPPH assay, Vž-HR exhibited the highest value (0.99 ± 0.01 mmol Trolox/mL). All cabbage juices analyzed at full maturity in our experiment showed higher antioxidant capacities in the ABTS assay than white cabbage juices (0.54 ± 0.02 TE μmol/mL) analyzed by Kusznierewicz et al. (2008). However, the

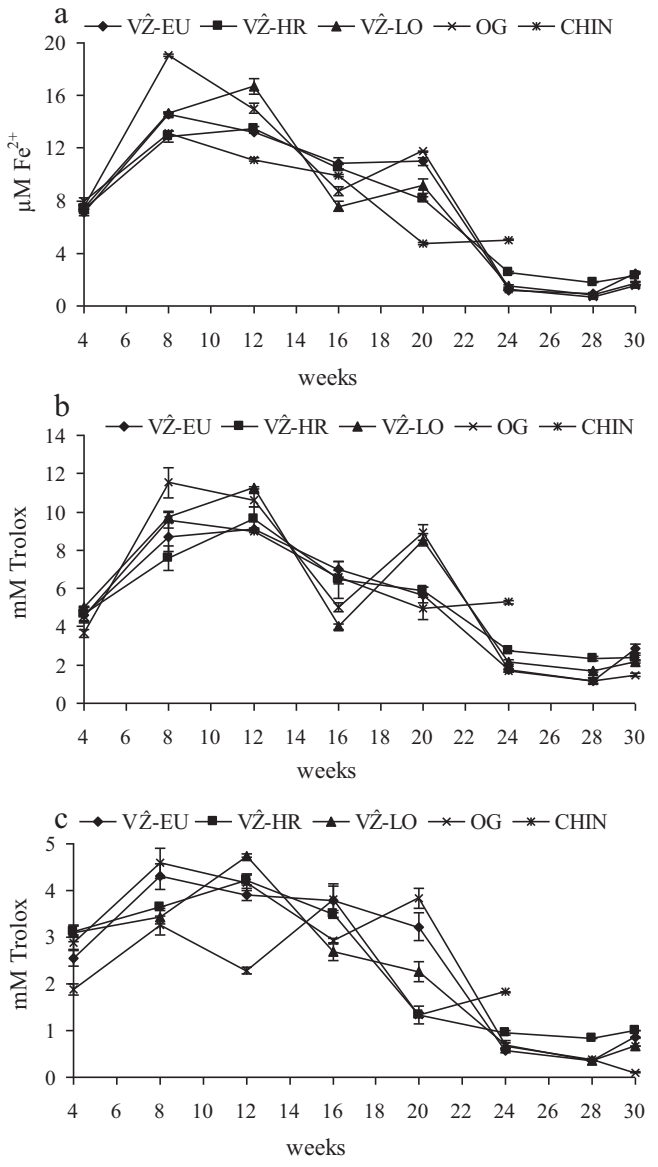

Fig. 1. Antioxidant activity of tested cabbage juices at different stages of maturity measured by (a) FRAP, (b) ABTS and (c) DPPH assays. Abbreviations refer to the following: Vž-DOM: white cabbage cultivar Varaždinski seed producer from Istria, Vž-EU: white cabbage cultivar Varaždinski seed producer from Italy, Vž-LO: white cabbage cultivar Varaždinski local seed producer, OG: white cabbage cultivar Ogulinski, CHI: Chinese cabbage.

ABTS assay results for cabbage juices in our experiment were still significantly lower than the value for broccoli (2.2 ± 0.4 mmol/L Trolox) vegetable juice (Sun et al., 2007).

3.3. Phytochemicals content and antioxidant capacity between different cultivars

Chinese cabbage juice showed a significant difference in the TP and TF contents compared to the juice extracted from white cabbage cultivars at various growing stages, except at weeks 8 and 16, when it exhibited the same TP and TF contents, respectively, as cultivar Ogulinski (Tables 2a and 2b). These results confirm the findings of previous authors who reported significantly different TP and TF contents between Chinese and white cabbage cultivars (Roy et al., 2007; Lee et al., 2007). The TP and TF contents as well as antioxidant capacity also significantly differed between the juices extracted from different growing stages of white cabbage

cultivars and between juices extracted from Varaždinski cultivars originating from different seed producers. At the fully ripe stage among white cabbages, the juice extracted from Vž-HR showed the highest TF (0.25 ± 0.01 mg CE/mL) content and DPPH radical scavenging capacity (0.99 ± 0.00 mM Trolox), while the juice extracted from Vž-EU showed the highest antioxidant capacity measured by FRAP (2.50 ± 0.06 μM Fe^{2+}) and ABTS (2.84 ± 0.22 mM Trolox) assays. At the fully ripe stage identical TP contents were measured for juices extracted from Vž-HR and Vž-EU (1.03 mg GAE/mL). This corresponds with the data found in earlier studies which reported significantly different TP content (Singh et al., 2006) and antioxidant capacity (Nilsson et al., 2006) among cultivars of white cabbage. Singh et al. (2006) quantified antioxidant phytonutrients in 18 white cabbage varieties and hybrids and observed significant variability among the cultivars in the content of phytonutrients such as vitamin C, β-carotene, lutein, DL-α-tocopherol and phenolics. Varying contents of polyphenolic compounds and antioxidant capacity among cultivars were also demonstrated in broccoli (Martinez-Villaluenga et al., 2010).

3.4. Antimicrobial activity at full maturity

Extracts were prepared in 80% methanol for antimicrobial activity testing, with extraction yields ranging from 18% (Chinese cabbage) to 37.5% (Vž-HR), as shown in Table 1. Methanol was used earlier as an extraction solvent in antimicrobial activity testing of different cruciferous vegetables (Hu et al., 2004) and kale (*B. oleraceae* L. var. *acephala* DC.) (Ayaz et al., 2008).

At tested concentrations, cabbage extracts analyzed in our study did not show antimicrobial activity against test-microorganisms as revealed by the agar-well and agar-disc assays. Ayaz et al. (2008) tested antimicrobial activity of kale (*B. oleraceae* L. var. *acephala* DC.) methanol extracts using the agar well diffusion method and confirmed activity against *S. aureus*, *B. subtilis* and *E. coli*, at 10 times higher extract concentrations (1 g/L) than those utilized in our study. Thus, our negative results for antimicrobial activity might be due to low extracts concentrations utilized. Absorbance measurement of samples dissolved in nutrient broth with 2% DMSO did not give relevant results in the turbidimetric microdilution assay since extracts precipitated during incubation due to their weak solving ability.

4. Conclusions

Significant variation in the content of phenolic phytochemicals and antioxidant capacity of cabbage juice extracted from white and Chinese cabbage varieties was observed at different stages of maturity indicating that developmental processes within the plant influence the synthesis of polyphenolic compounds during maturation of cabbage. In addition, differences in the content of phenolic antioxidants and antioxidant capacity were observed among cultivars of Varaždinski cabbage grown from different seed producers, indicating that intracultivar variation should not be disregarded when identifying cabbage seed with the potential for generating a plant with optimal phytochemical content. The monitoring of total phenol, total flavonoid content, and antioxidant capacity towards the last stages of maturation could be useful to producers who are trying to optimize the phytochemical content of their final products.

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