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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