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Influence of Processing on Antioxidant Activity and Phenolic Content of Swiss Chard (*Beta Vulgaris* L. Subsp. *Cicla*)

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ABSTRACT: Swiss chard (*Beta vulgaris* L. Subsp. *Cicla*) is a good source of different bioactive compounds highly used in different diets all over the world, especially in Mediterranean. The leaves and stems are edible parts used in traditional dishes. The aim of this study was to determine the effect of different food processing treatments on total phenolic content and antioxidant activity of Swiss chard using two methods of extraction. Total phenolic content of raw, boiled, and frozen Swiss chard was determined by Folin-Ciocalteu method. ABTS radical scavenging capacity assay and 1,1-diphenil-2-picrylhydrazyl assay (DPPH) were used to determine the antioxidant activity. The highest phenolic content and antioxidant activity was found in raw extracts of Swiss chard (34.47±3.67 mg GA/g DW). Freezing and boiling led to an equal reduction of total phenolic content of investigated samples. The antioxidant activity was not significantly affected by boiling procedure, while freezing led to decrease of the antioxidant activity of Swiss chard.

Keywords: *polyphenols; antioxidant activity; boiling; freezing*

Utjecaj procesiranja na antioksidativnu aktivnost i ukupni sadržaj fenola blitve (*Beta vulgaris* l. Subsp. *Cicla)*

SAŽETAK: Blitva (*Beta vulgaris* L. Subsp. *Cicla*) je dobar izvor različitih bioaktivnih spojeva koji se često koriste u različitim dijetama širom svijeta, posebno na Mediteranu. Listovi i stabljike su jestivi dijelovi koji se koriste u tradicionalnim jelima. Cilj ovog istraživanja bio je utvrditi efekat različitih postupaka prerade hrane na ukupan sadržaj fenola i antioksidativnu aktivnost blitve pomoću dvije metode ekstrakcije. Ukupan sadržaj fenola u sirovoj, kuhanoj i smrznutoj blitvi određen je Folin-Ciocalteu metodom. Za određivanje antioksidantne aktivnosti korišteni su ABTS test i 1,1-difenil-2-pikrilhidrazil test (DPPH). Najveći ukupni sadržaj fenola i antioksidativne aktivnosti su utvrđeni u sirovim ekstraktima blitve (34,47 ± 3,67 mg GA/g DW). Zamrzavanje i kuhanje doveli su do jednakog smanjenja ukupnog sadržaja fenola u ispitivanim uzorcima. Procedura kuhanja nije značajno utjecala na antioksidativnu aktivnost, dok je smrzavanje dovelo do smanjenja antioksidativne aktivnosti blitve.

Ključne riječi: *polifenoli, antioksidativna aktivnost, kuhanje, zamrzavanje*

INTRODUCTION

Plants are natural sources of bioactive compounds that are produced in plants as secondary metabolites. Plant secondary metabolites are receiving a lot of attention due to their therapeutic, preventing, toxicological and immunostimulant activity (Anulika *et al.*, 2016).

Protective effects and health benefits of fruit and vegetable consummation has been shown in numerous studies (Block et al., 1992; Yahia *et al.*, 2010; Boeing *et al.*, 2012). These health benefits have been attributed to the presence of various bioactive compounds, vitamins, and phytochemicals such as carotenoids, ascorbic acid, and polyphenols. Among these, a prominent role is occupied by phenolic compounds with their ability to act as potent antioxidants and scavengers of reactive oxygen species. Antioxidants can be defined as substances that effectively delays or inhibits oxidation of the substrate (Halliwell *et al.*, 1989). Different studies have shown that antioxidant activity is significantly correlated with the content of bioactive compounds.

Swiss chard (*Beta vulgaris* L. subsp. *cicla*) is a biennial vegetable cultivated in many parts of the world. The leaves can be used in salads or cooked like spinach. Significant concentrations of nutrients were determined in leaves in different samples of Swiss Chard (Vitamins A, C and B, calcium, iron, phosphorus, sodium, potassium, calcium, magnesium) (Trifunović *et al.* 2015). Phenolic content and antioxidant activity of Swiss chard have been more studied in last two decades. Antioxidant activity of Swiss chard was high in comparison with other vegetables consumed in Mediterranean diet (Bolkent *et al.*, 2000). Several techniques that use different mechanisms have been used to determine *in vitro* antioxidant activity. In some of them hydrogen atom transfer is involved (HAT) while others involve electron transfer reactions (ET) (Shalaby and Shanab, 2013). Therefore, it is important to use different methods to obtain a more thorough assessment of the antioxidant potential of a sample. High correlation was observed between the total phenolic content and antioxidant activity (Sacan and Yanardağ, 2010). Effects of processing on flavonoids and vitamin C content were also analyzed (Gil *et al.*, 1998).

Although the antioxidant analysis of vegetables has been extensively investigated worldwide, very few studies have been carried out to assess Swiss chard grown in Bosnia and Herzegovina and consumed locally. This study evaluated the total phenol content and antioxidant activity of fresh and processed samples of Swiss chards grown organically in at one location in Mostar. Antioxidant activity was determined using techniques with different mechanisms of action, one with ET reactions involved (DPPH (2,2-diphenyl-1-picrylhydrazyl radical)) and other with HAT mechanism (ABTS (2,2'-azinobis-3-ethylbenzotiazoline-6-sulfonic acid radical)).

EXPERIMENTAL

Sample preparation

Fresh Swiss chard was collected at one location in Mostar. One kilogram of sample was cleaned, stems were removed, and the green tissue was sliced into almost same small pieces. Samples was mixed and divided into three equal parts. One part was used as raw, and other parts were boiled or frozen before analysis. Sample was frozen at -20ºC for a day. For boiling we used 500 ml of tap water to boil 250 g of Swiss chard for 8 minutes. After boiling sample was separated from water. All samples were homogenized, and aliquots were weighted.

Extraction

Two types of extraction were used: ultrasound and agitation extraction using orbital shaker. Sample of Swiss chard (1 g) was extracted with 10 ml of 80% ethanol for 30 minutes at 200 rpm at room temperature using orbital shaker (Agitador orbital, Optic Ivymen System). For ultrasound extraction 1 g of sample was extracted with 10 ml of 80% ethanol for 30 minutes at 40ºC. All samples were filtrated after extraction.

Chemical analysis

Chemicals

2,2´ - azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was purchased from Sigma-Aldrich (Germany) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Across organics (USA). Potassium chloride was purchased from Lach:ner (Czech Republic). All other chemicals were purchased from Semikem (BiH).

Determination of total phenolic content

The total phenolic content was determined with Folin–Ciocalteu method described by Singleton *et al.* (1990) and as previously described by Kazazic *et al.* (2016). Gallic acid was used to prepare the standard curve, and the results were expressed as milligrams of gallic acid equivalent per g dry weight (mg GAE/g DW). All measurements were done in triplicate. Data are presented as means ± standard deviation.

Antioxidant activity using ABTS•+ assay

Determination of the antioxidant activity with ABTS•+ reagent (2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) was carried out following the method of Re *et al.* (1999), and as described in work by Kazazic *et al.* (2016). The results were expressed as µmol of Trolox equivalent per g dry weight (µmol TE/g DW).

DPPH free radical scavenging assay

Another method that was used for determination of the antioxidant activity using 1,1-diphenil-2-picrylhydrazyl assay (DPPH) was carried out following procedure described by Brand-Williams *et al.* (1995) and as described in work by Kazazic *et al.* (2016). The results were expressed as µmol of Trolox equivalent per g dry weight (µmol TE/g DW).

RESULTS AND DISCUSSION

Total phenolic content (TPC) found in our samples is shown in Table 1. Higher TPC is detected in the extracts obtained by ultrasound than by agitation extraction.

The highest value of TPC was found in extract of raw Swiss chard (34.47 mg GA/g DW) and the lowest concentration in boiled Swiss chard (12.62 mg GA/g DW). After boiling, the TPC of Swiss chard decreased as previously reported by Natella *et al.* (2010) (raw 70.5 mg GAE/g DW; boiled 53.5 mg GAE/g DW).

Table 1. Total phenolic content of raw, boiled, and frozen Swiss chard

	TP (mg GA/g DW)
	Agitation extraction (AE)
Raw	28.60±2.08
Boiled	12.62±3.12
Frozen	13.60±1.71
	Ultrasound extraction (UE)
Raw	34.47±3.67
Boiled	15.38±2.50
Frozen	15.70 ±2.07

The application of heat during cooking covers different methods such as boiling, steaming, baking, frying. Many vegetables are prepared simply by boiling or steaming. Heat cause changes in physical and chemical characteristic of vegetables (van Boekel *et al.*, 2010). Many studies have shown TPC reduction after thermal processing of vegetables in comparison to fresh food. Natella *et al.* (2010) reported that in all seven vegetables which were analyzed TPC decreased after boiling. Ismail *et al.* (2004) detected a loss of TPC for boiled spinach. Agüero *et al*. (2005) reported that longer blanching of Swiss chard results in losses of ascorbic acid and chlorophyll pigments. Effects of heat treatment of food on phytochemicals depends on the treatment parameters, structure of food matrix, and chemical nature of the specific compound (Sikora *et al.* 2008; Natella *et al.*, 2010; van Boekel et al., 2010; Palermo *et al.*, 2013). Reduction of phenols during cooking can be explained by transmission of phenols into the cooking water.

Freezing is a common process for the long-term preservation of vegetables. Freezing of Swiss chard caused reduction of TPC to 47.55% and 45.55% in samples extracted by agitation and ultrasound extraction, respectively. In literature, very few data are available on TPC of frozen green vegetables. Ninfali *et al*. (2003) detected higher concentration of TPC in raw samples of beet greens and spinach than in frozen one. Also, Leahu *et al*. (2015) reported that TPC in raw samples (cauliflower, broccoli, carrots) was higher than in frozen samples. Freezing causes cell breakage, allowing enzymatic reactions to occur. Phenolic compounds can be degraded during freezing process and more extensively during thawing because of their interaction with oxidative enzymes. Therefore, different pre-freezing treatments can be applied to fruits and vegetables to retain their quality (Khattab *et al*., 2015).

Antioxidant activity (AOA) depend on the method of extraction and method used for their determination. AOA in samples was determined using two different methods of extraction: ultrasound extraction. and extraction by agitation. For the determination of antioxidant activity, we used ABTS and DPPH method (Table 2).

The highest level of AOA was found in raw samples (ABTS 1.88 μmol TA/g DW; DPPH 0.49 μmol TA/g DW). Antioxidant activity did not change after boiling which is comparable with results in Natella *et al*. (2010) and Jiménez - Monreal (2009) work. For determination of antioxidant activity Natella *et al*. (2010) used the Crocin test and reported 49.8±4.2 mmol TA/g DW in raw and 46.7±4.6 mmol TA/g DW in boiled samples (6% reduction of AOA after boiling). High amount of antioxidants can be found after thermal processing due to destruction of the cell walls and inactivation of oxidative enzymes (Jiménez – Monreal *et al*., 2009). Products of several reactions (Maillard reaction, Strecker degradation, hydrolysis of esters) that occur during thermal processing of food at high temperatures can increase or retain antioxidant activity. These new compounds produced could be compensating the overall antioxidant activity of processed foods although the content of naturally occurring antioxidants decreased due to thermal processing.

Table 2. Antioxidant activity of raw, boiled, and frozen Swiss chard

	ABTS (μmol TA/g DW)	**DPPH (μmol TA/g DW)**
	Agitation extraction	Agitation extraction
Raw	1.88±0.01	0.49±0.01
Boiled	1.81±0.01	0.47±0.01
Frozen	1.24±0.01	0.33±0.01
	Ultrasound extraction	Ultrasound extraction
Raw	1.84±0.04	0.49±0.01
Boiled	1.81±0.02	0.47±0.01
Frozen	1.23 ±0.01	0.32±0.01

Freezing produced the highest losses of antioxidant activity. Maurcia *et al*. (2009) used ABTS method to determine AOA in Swiss chard samples that were frozen one day. He reported slight losses of AOA which can be explained by the different preparation method used. Also freezing rate influences the crystal size of the ice and the food texture. Therefore, content of bioactive compounds may be lost due to leaching upon thawing (Neri *et al*., 2020). The preservation of AOA of vegetables depends on the vegetable and pre-freezing treatments (Martínez *et al.,* 2013).

CONCLUSION

The results of this study showed that different processing treatments affected the total phenolic content and the antioxidative activity of Swiss chard. The treatment processes used had a greater impact on the reduction of the total phenol content. Antioxidant activity decreased after freezing, while boiling had almost no impact on antioxidant activity of Swiss chard. These changes should be considered when calculating dietary intake for boiled and frozen Swiss chard. Further investigations are required to optimize thermal and non-thermal food processing operations to retain bioactive compounds in the processed foods.

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