

Effect of Food Preparation Technique on Antioxidant Activity and Plant Pigment Content in Some Vegetables Species

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Abstract Effect of food preparation technique on antioxidant activity and plant pigment content in broccoli, Brussels sprouts, white cabbage, kale, chard, spinach and garden patience were studied. The highest content of chlorophyll a was detected in garden patience (0.837 mg/g f.v.) and the lowest was in brussels sprout (0.047 mg/g f.v.), while chlorophyll b and carotenoid content was lower. Boiling had influence on pigment content in selected vegetables. Different food preparation technique showed influence on antioxidant activity of vegetables extracts. Boiled vegetables extracts showed highest antioxidant activity, which indicates that valuable phytochemicals remains in water during cooking process. Highest reducing power is detected for frozen garden patience extract (16.775 AAE/10 g f.v.), while DPPH, ABTS and FRAP assays showed highest antioxidant activities for boiled and microwave cooked vegetables. Correlations among antioxidant activities based on ABTS, DPPH, FRAP and TRP assays were positively high and r ranged between 0.68 and 0.99.

Keywords: antioxidant activity, leafy green vegetables, chlorophyll, carotenoids, correlation

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1. Introduction

Vegetables are one of the best known sources of vitamins, nutrients and fibers. This makes them an essential part of a healthy nutrition. Leafy green vegetables are well known for their characteristic color, flavor, and therapeutic value [1,2]. Significance of vegetables in a human diet is shown in numerous studies. Epidemiological studies have shown that fruit and vegetable rich diet helps to prevent cancer and cardiovascular diseases [3]. Leafy green vegetables are low in calories and fat, high in dietary fibers, calcium and iron, and very high in phytochemicals such as vitamin C, carotenoids, lutein and folate. Foliates reduce risk of cardiovascular disease and memory loss, and helps human organism to produce serotonin. Antioxidants, such as vitamin C and lutein from leafy greens may help reduce your risk of cataract and macular degeneration.

Antioxidants are molecules that can inhibit or delay the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions [4,5]. Antioxidant activities of plant extracts are correlated with their phenolic content. Phenolic compounds showed higher antioxidant activity than antioxidant vitamins and carotenoids [4,6]. Antioxidant effectiveness of phenolic compounds in food depends on the number and location of

hydroxyl groups, interaction with other food components, and cooking process [7]. Vegetables could be eaten fresh, cooked by boiling in water or prepared in microwave oven. These processes have effect on plant secondary metabolites, such as antioxidants and pigments. [7]. In broccoli boiled for 5 minutes, significant reductions of 72%, 66% and 65% for total phenols, L-ascorbic acid and antioxidant activity, respectively were found. Thermal treatment decreased the total phenolic content in kale, spinach, cabbage and shallots, and antioxidant activity in some of them [8]. Turkmen et al. (2005) showed that there is no significant loss in antioxidant activity, with the exception of some losses of phenolics in squash, peas and leek.

The present study was aimed to determine effect of food preparation technique on antioxidant activity *in vitro* and plant pigment content in green leafy vegetables. We have chosen broccoli (*Brassica oleracea* var. *silvestris*), Brussels sprouts (*Brassica oleracea* var. *gemmifera*), white cabbage (*Brassica oleracea* var. *capitata*), kale (*Brassica oleracea* var. *sabauda*), chard (*Beta vulgaris*), spinach (*Spinacia oleracea*) and garden patience (*Rumex patientia*) because of their essential importance in Serbian traditional cuisine. Antioxidant activities were determined for fresh, frozen, boiled and microwave processed vegetables. We chose water as solvent because it is used in everyday food preparation. To the best of our knowledge, this study is the first one treating antioxidant

properties of vegetables listed above, taking into consideration different ways of their storage and preparation.

2. Materials and Methods

2.1. Plant Material

Fresh broccoli, brussels sprouts, white cabbage, kale, chard, spinach and garden patience were purchased from local market in Niš – Serbia. A part of plant material was stored in plastic bags and kept frozen until extraction.

2.2. Preparation of Vegetable Extracts

Vegetables were washed, dried on paper towel and cut into small pieces. A 10g portion of vegetables was taken for each food preparation technique. All vegetables samples were extracted with water. For raw vegetables extracts, 10g of fresh plant material was mixed with water and the extraction was performed twice in an ultrasonic bath for 15 minutes (extract 1). Vegetables sample of 10g was added to water and cooked for 10 minutes. Vegetables were separated from water for the further treatment, while water from boiled vegetables was one, separated extract (extract 2). Cooked vegetable was mixed with water and extracted twice in ultrasonic bath for 15 minutes (extract 3). Frozen vegetables (10 g) was thawed, cut into pieces and boiled for 10 minutes in water. Water from boiled vegetables was used as extract (extract 4). Microwave vegetables extract was prepared as following: 10 g of vegetable was placed in a glass dish, 50 ml of distilled water was added and then it was cooked in a commercial – 1000 W microwave oven. Cooking took 5 minutes and then vegetable was separated from water (extract 5). All of prepared mixtures were filtered and stored in a refrigerator at 5 °C until they were analyzed.

2.3. Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and FeCl₃, were purchased from Sigma Co. St. Louis, Missouri, USA.

All other chemicals and reagents used K₃[Fe(CN)₆], phosphate buffer (NaH₂PO₄-Na₂HPO₄), CCl₃COOH, ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), CH₃COONa, K₂S₂O₈, methanol were purchased from Merck, Darmstadt, Germany. All the chemicals and reagents were of analytical grade.

2.4. Methods

The contents of chlorophyll and carotenoids were determined spectrophotometrically. Vegetables were cut into almost equal small pieces or slices and portions of 0.5000 g were weighed, mixed with acetone and homogenised in a mortar. To prevent chlorophyll pheophytinization, MgCO₃ was added before vegetable homogenization. Mixtures were filtered, mortar and pestle were washed several times with acetone and the content was quantitatively transferred to the filter. The filter was washed with acetone so that the rest of the filter was completely white. Filtrate was diluted with acetone to a total volume of 25 ml. Absorbance of prepared mixtures

was recorded at 662, 644 and 440 nm using acetone as blank and pigment content was calculated using the formula of Holm and Wetstein:

$$\text{Chlorophyll } a = 9.784 \cdot A_{662} - 0.990 \cdot A_{644} \quad (1)$$

$$\text{Chlorophyll } b = 21.426 \cdot A_{644} - 4.650 \cdot A_{662} \quad (2)$$

$$\text{Chlorophyll } a + b = 5.134 \cdot A_{662} + 20.436 \cdot A_{644} \quad (3)$$

$$\text{Carotenoids} = 4.695 \cdot A_{440} - 0.268 \cdot (a + b) \quad (4)$$

A = absorbency at corresponding wave length, values 9.784, 0.990, 21.426, 4.650 and 0.288 is the molar absorptivity coefficient according to Holm (1954) and Wetstein (1957) for acetone (absorption of 1 cm). After calculating the concentrations, the amounts of pigment per g of fresh matter were calculated applying the formula:

$$c = \frac{c_1 V r}{m} \quad (5)$$

c = content of pigment (mg/g) of fresh matter;

c₁ = the concentration of pigment calculated by the previous formula (mg/l);

v = the starting volume of extract (ml);

r = dilution;

m = the weighed fresh plant (g).

Determination of ferric reducing power: The reducing power was determined according to the method of [10]. Mixtures of appropriate amounts of the extract, 1 ml of 1% K₃[Fe(CN)₆] solution and 1 ml phosphate buffer (pH 6.6), were incubated at 50 °C for 30 minutes. After cooling 1 ml of 10% trichloroacetic acid, 0.6 ml of FeCl₃ were added and diluted with water to a total volume of 5 ml. Absorbance of obtained mixtures was measured at a wavelength of 700 nm. Increased absorbance of reaction mixture indicates higher reducing capacity. Phosphate buffer was used as blank solution. The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations. Total reducing power of extracts was calculated using ascorbic acid calibration curve and expressed as mg of ascorbic acid equivalent per 10 g of vegetables (mg AAE/10 g f.v.).

DPPH "scavenging" radical capacity of samples was determined using DPPH radical [11]. Reaction mixtures of samples were prepared by mixing appropriate amounts of extract, 1.5 ml of DPPH and methanol to a total volume of 4 ml. Prepared solutions were left in the dark for 60 minutes and then the absorbance was measured at 515 nm. All determinations were performed in triplicate. Methanol was used to zero spectrophotometer. Scavenging radical capacity was calculated via trolox calibration curve and expressed as mg of trolox equivalent per 10 g of fresh vegetables (mgTE/10 g f.v.).

ABTS radical "scavenging" activity was measured using a modification of the method of [6]. ABTS radical was produced by reaction of ABTS stock solution with potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 - 16 h before use. The solution was then diluted by mixing 7 ml ABTS⁺ solution with 12 ml methanol to obtain an absorbance of 0.7 ± 0.02 units at 734 nm using the spectrophotometer. An aliquot of each extract was mixed with 1,8 mL of diluted ABTS radical cation solution and diluted with methanol to a total volume of 4 ml. After reaction at room temperature for 6

min, the reduction in absorbance was measured at 734 nm. Results are expressed as mg of trolox equivalents per 10 g of fresh sample (mgTE/10 g f.v.).

Ferric reducing antioxidant power assay was performed using method of Wong et al. (2005). Appropriate amount of extract were added with 1 ml of FRAP reagent, that was prepared with mixture of 300 mM sodium acetate buffer (pH 3,6), 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution and 20 mM FeCl₃ x 6H₂O (10:1:1), and diluted with water to a total volume of 4 ml. The reaction mixture was incubated in a water bath at 37° C for 30 min. The increase in absorbance was measured using spectrophotometer at 595 nm. Results are expressed in μM Fe/ 10 g fresh mass.

2.5. Statistical Analysis

The evaluation of the obtained analytical data was performed by statistical means. The elimination of outliers was done by Grubb’s test, for each method the arithmetic mean, the standard deviation and the coefficient of variation and correlations were calculated applying software Statistica 7.

3. Results

Chlorophyll and carotenoid content in green leafy vegetables was measured in fresh and boiled vegetables and the results were presented in Figure 1, Figure 2 and Figure 3.

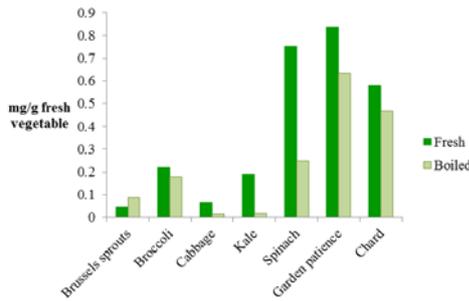


Figure 1. Chlorophyll a content in fresh and boiled vegetables

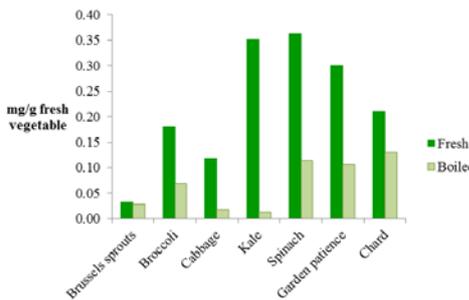


Figure 2. Chlorophyll b content in fresh and boiled vegetables

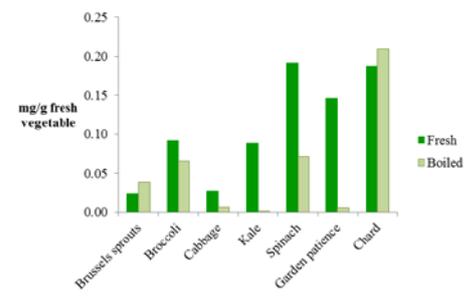


Figure 3. Carotenoid content in fresh and boiled vegetables

The highest content of chlorophyll a was detected in garden patience (0.837 mg/g f.v.), while the lowest was in brussels sprout (0.047 mg/g f.v.). In fresh vegetables chlorophyll b ranking was spinach > kale > garden patience > chard > broccoli > cabbage e>Brussels sprout. Values for carotenoids in selected vegetables varied between 0.024 mg/g fresh vegetables for brussels sprout to 0.191 mg/g fresh vegetables for spinach.

The reducing power ability of the extracts was determined using ascorbic acid as standard and results were expressed as mg of ascorbic acid equivalent per 10 grams of fresh vegetables (mg AAE/10 g f.v.). Ferric reduction power of vegetables extracts are presented in Figure 4. Highest reducing power is detected for frozen garden patience extract (16.775 AAE/10 g f.v.).

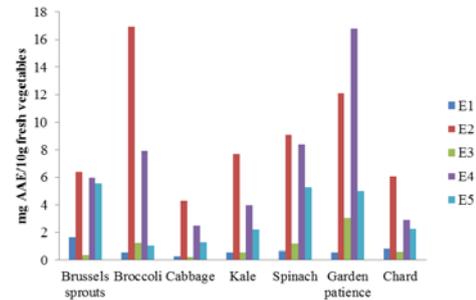


Figure 4. Reducing power of vegetables extracts

The DPPH radical scavenging activity of vegetables extracts are presented in Figure 5. DPPH radical scavenging activity depended on the type of vegetables and sample preparation technique. Liquor from boiled vegetables (E2) showed highest antioxidant activity in most cases. Only frozen garden patience liquor showed higher radical scavenging activity than boiled vegetables liquor.

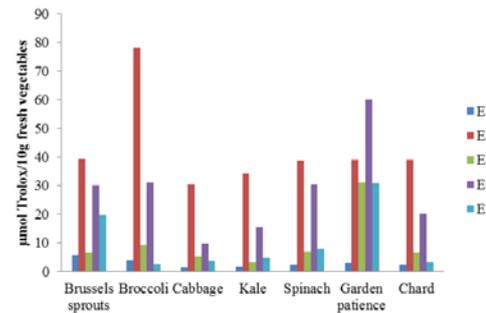


Figure 5. DPPH radical scavenging activity of vegetables extracts

Antioxidant activity of vegetables samples, determined by ABTS assay, is shown in Figure 6. Highest amount of antioxidants was found in microwave oven treated vegetables liquor.

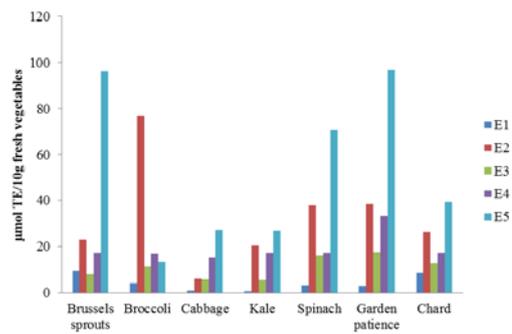


Figure 6. ABTS radical scavenging activity of vegetables extracts

Results obtained by FRAP assay are presented in Figure 7. Broccoli boiled extract has shown highest antioxidant activity (341.291 $\mu\text{mol Fe}/10\text{g f.v.}$).

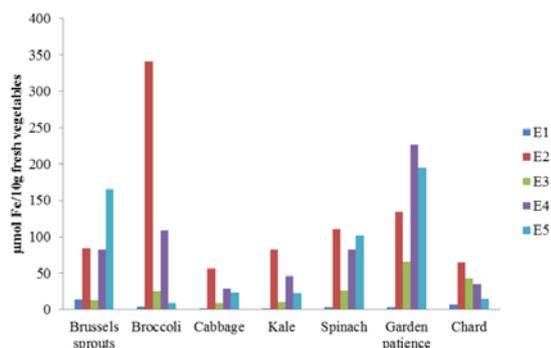


Figure 7. Total antioxidant activity measured by FRAP assay

Statistical analyses was used for investigating correlation between antioxidant activities determined by different assays. Strongest correlation was found between total reducing power and FRAP assays ($r = 0.985823$). Correlation between all pairs of antioxidant activity assays were positively high ($0.682412 \leq r \leq 0.985823$). Surprisingly weak correlation was found between ABTS and DPPH assays, which is not expected, considering their similar mechanism.

Table 1. Correlations among the different characteristics of broccoli (*Brassica oleracea* var. *silvestris*), Brussels sprouts (*Brassica oleracea* var. *gemmifera*), white cabbage (*Brassica oleracea* var. *capitata*), kale (*Brassica oleracea* var. *sabauda*), chard (*Beta vulgaris*), spinach (*Spinacia oleracea*) and garden patience (*Rumex patientia*)

		FRAP	DPPH	ABTS
TRP	E ₁	0.972256	0.836981	0.826958
	E ₂	0.937311	0.867283	0.955064
	E ₃	0.849137	0.946380	0.797492
	E ₄	0.985823	0.974061	0.899106
	E ₅	0.904508	0.747296	0.954018
FRAP	E ₁		0.845920	0.917516
	E ₂		0.974987	0.951302
	E ₃		0.858017	0.847151
	E ₄		0.976006	0.916587
	E ₅		0.949023	0.970951
DPPH	E ₁			0.715640
	E ₂			0.937686
	E ₃			0.682412
	E ₄			0.891101
	E ₅			0.881685

E1 - Raw vegetables extract, E2 - Boiled vegetables liquor, E3 - Boiled vegetables extract, E4 - Frozen vegetables liquor, E5 - Microwave oven extract

4. Discussion

The colour of leafy green vegetables is related to chlorophyll content, as this compound is the main pigment of green vegetables and masks the bright colour of carotenoids [13]. The amount of chlorophylls and carotenoids in green leafy vegetables are important and are sensitive indices of their physiological state and its changes during growth and when affected by thermal treatment. Amount of chlorophyll a was higher than chlorophyll b and carotenoids in all selected vegetables, which is in agreement with literature [14]. The total

carotenoid, chlorophyll a and b contents varied between species.

Most of vegetables are cooked by boiling in water before consumed and this process has influence on chemical composition of vegetables. Our result for pigments content in boiled vegetables showed that amounts of chlorophyll a, b and carotenoid decreased compared with the values for the fresh ones. Data presented in Figure 1 showed that content of chlorophyll a in boiled kale was about 90% lower than in fresh kale. As we can see, boiling had lowest influence on chlorophyll content in chard, with chlorophyll a reduction of 19.36%. Results for other analysed vegetables, except brussels sprout, showed lower chlorophyll a content in boiled than in fresh vegetables. Boiling caused significant loss of chlorophyll b content, especially in kale, where amount of this pigment is lower for 96%. Decrease in carotenoid content varied between species, and we can see higher amount of it in boiled Brussels sprout and garden patience, than in fresh one. This study revealed that cooking reduced pigment content in all selected vegetables, except Brussels sprout and garden patience.

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. These methods differ in terms of their assay principles and experimental conditions. In different methods particular antioxidants have varying contributions to total antioxidant potential [15]. Each fresh vegetable was extracted using five different methods, using water as solvent.

In present study we used determination of ferric reducing power assay because of its simplicity and reproducibility. Compounds with reducing power are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as antioxidants [16].

Fresh vegetables extracts (E1) showed no significant antioxidant activity. Brussels sprout contained 1.679 mg AAE/10 g f.v. and the rest of vegetables had similar reducing power. Distilled water was found as the most inefficient solvent in extracting phenolic compounds [17]. Weak antioxidant activity of fresh vegetables extracts can be explained by low solubility of phenolic compounds in water, so we can claim that most of valuable antioxidants remain in vegetables. Regardless of this fact, we decided to use water as solvent, because it is used in everyday food preparation. Boiled vegetables extracts (E2), prepared by boiling appropriate amounts of fresh vegetables in distilled water showed greatest antioxidant activity. The rankings were broccoli > garden patience > spinach > kale > Brussels sprout > chard > cabbage. Boiled vegetables were further treated in an ultrasonic bath and extracts prepared like this (E3) showed lower reducing power than E2. Among all these tested vegetables, garden patience showed highest reducing power (3.037 mg AAE/10 g f.v.), while cabbage showed lowest reducing power (0.225 mg AAE/10 g f.v.). Regarding reducing power ability (2.499 mg AAE/10 g f.v.) among vegetables extracts studied, cabbage had the poorest properties. Vegetables were also analyzed after treatment in microwave oven (E5), and results varied between 1.058 mg AAE/10 g f.v. for broccoli to 5.565 mg AAE/10 g f.v. for Brussels sprout. Various sample preparation methods showed differences between reducing ability of

vegetables. Highest reducing ability was recorded for boiled broccoli extract (E2), while the lowest was recorded for cabbage boiled/ultrasound extract (E3). We found that liquor from boiled vegetables contain higher amount of antioxidants than all other extracts. Obviously, high temperature extract antioxidants from vegetables better than ultrasound bath and microwave oven.

The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from antioxidant to form the reduced DPPH-H.

Antioxidant activity of fresh vegetables extracts (E1) determined by the DPPH radical scavenging method decreased in the order: garden patience > Brussels sprout > spinach > kale > cabbage > radish > broccoli. In boiled vegetables water, antioxidant activity varied from 78.085 $\mu\text{mol TE}/10\text{ g f.v.}$ in broccoli to 30.349 $\mu\text{mol TE}/10\text{ g f.v.}$ in cabbage. There was differences in the contents of antioxidant components and antioxidant activity between various cooking methods. Liquor from boiled vegetables (E2) showed highest antioxidant activity in all vegetables, except garden patience, where liquor from frozen vegetables had highest amount of antioxidants. Comparing different extract preparation methods, we concluded that DPPH radical scavenging activity was similar for fresh vegetables extracts and microwave oven cooked vegetables, so the best method for vegetables preparation is microwave oven cooking, because most of the antioxidants remain in vegetable. Highest amount of antioxidants was found in boiled vegetables liquor (E2), which indicates loss of antioxidants in vegetables during cooking process. The earlier study by Zhang and Hamazu (2004) showed that there was no significant differences in the contents of antioxidant components and antioxidant activity between conventional and microwave cooking. Our study showed differences in microwave and conventional cooking. Broccoli boiled extract (78.085 $\mu\text{mol TE}/10\text{ g f.v.}$) showed 29 times higher antioxidant activity, compared to broccoli microwave cooked extract (2.700 $\mu\text{mol TE}/10\text{ g f.v.}$).

ABTS free radical is another synthetic radical, and the ABTS assay can be used to assess the scavenging activity for both the polar and non-polar samples [6]. Standard ABTS assay use 734 nm as the absorption maximum. Decolorization of $\text{ABTS}^{\bullet+}$ reflects the capacity of antioxidant species to donate electrons or hydrogen atoms to inactivate this radical cation.

Results obtained by ABTS method showed deviation from results of other methods. As we can see in Figure 6, highest amount of antioxidants was found in microwave oven treated vegetables liquor. Values for ABTS scavenging activity varied between 13.305 $\mu\text{mol TE}/10\text{ g f.v.}$ in broccoli to 96.326 $\mu\text{mol TE}/10\text{ g f.v.}$ for Brussels sprout. Fresh vegetables liquor showed no significant antioxidant activity, compared to other preparation technique, which means that all valuable nutrients remained in vegetables. In boiled vegetables extract (E2), broccoli proved to be the best antioxidant. Compared with

cabbage (6.022 $\mu\text{mol TE}/10\text{ g f.v.}$), broccoli demonstrated 12 times higher antioxidant capacity (76.730 $\mu\text{mol TE}/10\text{ g f.v.}$). Broccoli and spinach are available throughout the year as deep-frozen foods. Levels of hydro-soluble antioxidants in liquor slightly decreased when vegetables were frozen and then boiled in water for 10 minutes, comparing to fresh boiled vegetables. Antioxidant activity of vegetables that was previously boiled, and then extracted with water decreased in the order: kale > cabbage > Brussels sprout > broccoli > chard > spinach > garden patience. Among all tested vegetables Brussels sprout and garden patience showed highest scavenging activity.

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay of [18]. This method was initially developed to assay plasma antioxidant capacity, but can be used for plant extracts too. At low pH, reduction of ferric tripyridyl triazine complex to ferrous form can be monitored by measuring the change in absorption at 593 nm. FRAP assay is simple, rapid to perform and shows highest correlation with both ascorbic acid and total phenolics [19]. Therefore, it is an appropriate technique for determining antioxidant activity in plant extracts.

FRAP assay confirmed that simple ultrasound water extraction gives poor results. Antioxidant activity in these extracts (E1) decreases in the order: Brussels sprout > chard > broccoli > spinach > garden patience > kale > cabbage. Szeto et al. (2002) reported that raw broccoli extract antioxidant activity determined by FRAP assay is 2940 $\mu\text{mol Fe}/\text{kg f.v.}$ However, in the present study it was found that raw broccoli antioxidant activity is 878.1 $\mu\text{mol Fe}/10\text{ g f.v.}$, which is consequence of different sample preparation technique. In most cases, boiled vegetables extracts (E2) showed highest amount of antioxidants. Broccoli boiled extract has shown highest antioxidant activity (341.291 $\mu\text{mol Fe}/10\text{g f.v.}$). Microwave prepared broccoli liquor contain 39 times less antioxidants (8.781 $\mu\text{mol Fe}/10\text{g f.v.}$) than boiled broccoli extract. Compared to fresh vegetables extract, boiled ultrasound vegetables extract (E3) showed higher amount of antioxidants. Among all tested vegetables chard showed highest antioxidant activity (65.592 $\mu\text{mol Fe}/10\text{g f.v.}$), whereas cabbage had lowest activity (9.455 $\mu\text{mol Fe}/10\text{g f.v.}$). Garden patience showed deviation from results for other vegetables. Frozen garden patience liquor showed highest antioxidant activity (226.187 $\mu\text{mol Fe}/10\text{g f.v.}$), whereas fresh extract showed lowest antioxidant activity (2.918 $\mu\text{mol Fe}/10\text{g f.v.}$). Antioxidant activity of fresh vegetables determined by FRAP method decreased in the order: garden patience > Brussels sprout > spinach > cabbage > kale > chard > broccoli.

Numerous methods have been applied for the assessment of free radical scavenging capacity of antioxidant compounds, natural and commercial products.

The most commonly used antioxidant methods. $\text{ABTS}^{\bullet+}$ DPPH \cdot are characterized by excellent reproducibility under certain assay conditions, but they also show significant differences in their response to antioxidants. The DPPH free radical (DPPH \cdot) does not require any special preparation, while the ABTS radical cation ($\text{ABTS}^{\bullet+}$) must be generated by enzymes or chemical reactions [21]. Another important difference is that ABTS can be dissolved in aqueous and organic media, in which

the antioxidant activity can be measured, due to the hydrophilic and lipophilic nature of the compounds in samples. In contrast, DPPH can only be dissolved in organic media, especially in ethanol, this being an important limitation when interpreting the role of hydrophilic antioxidants [22].

On the other side, the reaction used in FRAP assay is non specific. So, any half reaction that has lower redox potential, under reaction conditions, than the Fe (III)/Fe (II)-TPTZ half-reaction, will drive Fe (III)-TPTZ reduction.

Differences among results obtained by different methods are caused by different reaction mechanism and condition (FRAP does not measure thiol antioxidants, DPPH might be interfered by carotenoids having absorbance at 515 nm).

Correlations among antioxidant activities based on ABTS, DPPH, FRAP and TRP assays were positively high and ranged between 0.68 and 0.99; the highest correlation was between TRP and FRAP (0.99) and the lowest correlation was between DPPH and ABTS (0.68). FRAP and TRP assays measure the ability of antioxidant to reduce ferric Fe(III) ions to lower valency state, so strong correlation among them is expected. Weak correlation between ABTS and DPPH assays may be result of different extracts preparation techniques. Previous studies have shown that the ABTS assay underestimate antioxidant activity of plant extracts, while DPPH radical, due to steric effects, reacts easily only with small molecules [23].

5. Conclusion

Dependence of antioxidant abilities of leafy green vegetables' extracts on various cooking methods was evaluated. Different food preparation technique showed influence on antioxidant activity of vegetables' extracts. In most cases, boiled vegetables extracts showed highest antioxidant activity, which indicates that valuable phytochemicals remains in water during cooking process. Antioxidant capacity varied widely among selected vegetables, although all of selected vegetables are important sources of antioxidants. Chlorophyll and carotenoid concentration indicates physiological status of the plant. Obtained results for pigment content agree with those reported in the literature. Leafy green vegetables usually consumed in Serbia contain significant levels of nutrients, essential for human health. Quantity of these nutrients in studied vegetables and their antioxidant properties are dependent on the way of processing, as well as the type of vegetables.

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