

Comparative control of the bioactivity of some frequently consumed vegetables subjected to different processing conditions

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ABSTRACT

The main aim of this investigation was to find processing conditions and to control them, which maximally preserve bioactive compounds and antioxidant activity of garlic and onions. Garlic, white and red onions were subjected to bleaching and boiling. The contents of polyphenols, flavonoids, flavanols, tannins, corresponding antioxidant activities and their correlation coefficients were determined in various methanol and acetone extracts. The antioxidant activity was determined by 2, 2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric-reducing/antioxidant power (FRAP) and Cupric reducing antioxidant capacity (CUPRAC) antioxidant assays. It was found that bleaching for 90" most fully preserves polyphenols (8.25, 9.75 and 11.98 vs. 9.00, 10.52 and 15.87 mg GAE/g DW and the level of antioxidant activity – 8.82, 22.50 and 23.90 vs. 9.00, 23.05 and 24.30 μM TE/g DW of DPPH in extracts of treated samples with 100% of methanol vs. raw garlic, white and red onions, respectively. In conclusion, comparative control shows that bleaching for 90" of all studied vegetables most fully preserves contents of bioactive compounds and the level of antioxidant activity. Extraction of bioactive compounds with 100% methanol was more effective than with 50% methanol and 100% acetone.

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1. Introduction

It was shown that consumption of fruits and vegetables prevent many diseases (Banerjee & Maulik, 2002; Corzo-Martinez, Corzo, & Villamiel, 2007; Vainio & Weiderpass, 2006). These health properties of fruits and vegetables depend on their antioxidants, mainly phenolics (Bahorun, Luximon-Ramma, Crozier, & Aruoma, 2004; Halvorsen et al., 2002; Kevers et al., 2007; Miesan & Mohamed, 2001; Moreno, Corzo-Martinez, Dolores del Castillo, & Villamiel, 2006; Nencini et al., 2007; Pellegrini et al., 2007).

Garlic (*Allium sativum* L.), white and red onions (*Allium cepa* L.) are consumed in everyday cooking all over the world. The use of these vegetables goes to the ancient time (Banerjee & Maulik, 2002). Moreover, recently was reported that garlic and onion ex-

tracts are effective in prevention of cardiovascular disease, because of their hypocholesterolemic, hypolipidemic, anti-hypertensive, anti-diabetic, antithrombotic and anti-hyperhomocysteinemia effects (Corzo-Martinez et al., 2007; Kaur & Kapoor, 2002; Potter, 2005; Rahman & Lowe, 2006). These biological activities have been reviewed, indicating the compounds responsible for each one of them (Bahorun et al., 2004; Gorinstein, Leontowicz, Leontowicz, Drzewiecki, et al., 2006; Kim, Ye, Lim, Ha, & Kwon, 2005; Miesan & Mohamed, 2001; Nencini et al., 2007; Stratil, Klejdus, & Kuban, 2006).

In addition, the influence of the processing on the bioactivity and the adverse effects and interactions with different medications has also been considered (Aoyama & Yamamoto, 2007; Corzo-Martinez et al., 2007; Gorinstein, Leontowicz, Leontowicz, Jastrzebski, et al., 2006; Kawamoto, Sakai, Okamura, & Yamamoto, 2004).

The stimulatory effects on mouse splenocyte proliferation of total phenolics and flavonoid contents of onions was described (Lin & Tang, 2007).

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However, the comparative effect of garlic and onion extracts, which was obtained after cooking and the use of various solvents are less studied (Irkin & Korukluoglu, 2007; Kim et al., 2005).

Some reports demonstrated the influence of thermal treatment on onions in humid heat (boiled and vapor), dry heat (oven) and high frequency microwaves (Agostini, Jimenez, Ramon, & Gomez, 2004; Amin & Lee, 2005; Woo et al., 2007). The assessment of the results is connected to the use of the extraction procedures (Kim et al., 2005). These authors found that physiological activities of Korean- and Chinese-grown garlic (GKG) and (GCG) extracted with water or with either 50% or 100% ethanol are different. Nitrite-scavenging activity (NSA) was the highest in water and 50% ethanol extracts of both origins. Superoxide dismutase (SOD)-like activity of GKG extracts was higher than those of GCG, and those of water extracts were the highest. In another report it was shown that antifungal activity of garlic (*A. sativum* L.), onion (*Allium cepa* L.) and leek (*Allium porrum* L.) aqueous, ethyl alcohol and acetone extracts against *Aspergillus niger* (*A. niger*) is different: onion extract with ethyl alcohol [275 mg/mL minimal fungicidal concentration (MFC)], aqueous garlic extract (325 mg/mL MFC) and aqueous leek extract (900 mg/mL MFC) were the most inhibitory (Irkin & Korukluoglu, 2007). In recent reports different extracts of raw vegetables were compared, showing the amounts of polyphenols and their antioxidant activities (Santas, Carbo, Gordon, & Almajano, 2008). Pellegrini et al., 2007, showed that the total antioxidant capacity is strongly affected by the solvents used during extraction. It was also shown that processing of garlic and onions can change their composition (Aoyama & Yamamoto, 2007; Gorinstein, Leontowicz, Leontowicz, Drzewiecki, et al., 2006; Kawamoto et al., 2004; Roy, Takenaka, Isobe, & Tsushida, 2007; Xu, Wei, Guo, Yang, & Wu, 2007).

Therefore, it is very important to find the best way to preserve the contents of bioactive compounds and the antioxidant activities of processed vegetables.

In this research garlic, white and red onions were subjected to bleaching for 90' and boiling for 10' and then polyphenols, flavonoids, flavanols, tannins and the antioxidant activities were determined in their methanol and acetone extracts and compared with the data before the treatment. 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric-reducing/antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) antioxidant assays were applied in this investigation.

We did not find published data of such comprehensive investigations.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), lanthanum (III) chloride heptahydrate, Folin-Ciocalteu reagent (FCR), FeCl₃·6H₂O, CuCl₂·2H₂O, 2,9-dimethyl-1, 10-phenanthroline (neocuproine), and butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co., St. Louis, MO, USA. 2, 4,6-tripyridyl-s-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionized and distilled water was used throughout.

2.2. Samples

Raw garlic (*A. sativum* L.), and white and red onions (*Allium cepa*) were obtained from Polish Company "Elena" in 2008. The fol-

lowing steps of treatments were applied: bulbs of garlic, and white and red onions were washed, cleaned, peeled and cut with plastic knife (garlic for halves, onions for pieces) before heat treatment. The studied vegetables were processed under different heat treatment: blanched and boiled. Blanching was done for all samples in water at 100 °C for 90' (s). Boiling was similar to blanching, but the time of this treatment was different from that of blanching, starting from 10 min and increasing till 60 min. The data of boiling after 10 min are not shown, and were discussed in the previous reports (Gorinstein, Leontowicz, Leontowicz, Drzewiecki, et al., 2006). The samples were lyophilized and then grounded for fine particles under cooling system. This protocol is applied in the present study, because it is similar for everyday food cooking. Different solvents (methanol and acetone) were used for the maximum extraction of bioactive compounds. The used 27 garlic and onion samples were named as following: GAR (50%Me), polyphenols extracted from raw garlic with 50% methanol at 90°C; GA90' (50%Me), bleached garlic for 90'; GA10' (50%Me), boiled for 10', WOR (50%Me), polyphenols extracted from raw white onion with 50% methanol at 90°C; WO90' (50%Me), bleached for 90'; WO10' (50%Me), boiled for 10'; ROR (50%Me), polyphenols extracted from raw red onion with 50% methanol at 90°C; RO90' (50%Me), bleached for 90' and RO10' (50%Me), boiled for 10'; GAR (100%Me), polyphenols extracted with 100% methanol from raw garlic at room temperature; GA90' (100%Me), bleached for 90'; GA10' (100%Me), boiled for 10', WO (100%Me), polyphenols extracted with 100% methanol from raw white onion at room temperature; WO90' (100%Me), bleached for 90'; WO10' (100%Me), boiled for 10'; ROR (100%Me), polyphenols extracted with 100% methanol from raw red onion at room temperature; RO90' (100%Me), bleached for 90' and RO10' (100%Me), boiled for 10'; GAR (100%Ac), polyphenols extracted with 100% acetone from raw garlic at room temperature; GA90' (100%Ac), bleached garlic for 90'; GA10' (100%Ac), boiled for 10', WOR (100%Ac), polyphenols extracted with 100% acetone from raw white onion at room temperature; WO90' (100%Ac), bleached for 90'; WO10' (100%Ac), boiled for 10'; ROR (100%Ac), polyphenols extracted with 100% acetone from raw red onion at room temperature; RO90' (100%Ac), bleached for 90' and RO10' (100%Ac), boiled for 10'.

2.3. Preparation of extracts

The shredded garlic, white and red onions were freeze-dried (Alpha 2-4 Christ) and then ground to powder. The powder was stored at -20 °C until extraction of antioxidant phytochemicals. Defatted lyophilized vegetable samples were extracted from a 50 mg aliquot with 5 mL of 50% methanol/water with heating at 90 °C for free polyphenols. The samples were cooled, diluted to 10 mL with methanol, and centrifuged for 5 min at 4000g with a benchtop centrifuge to remove solids (50%Me). Portions of 1 g of all freeze-dried samples were extracted three times with methanol (4 mL). The extracts portions were combined and centrifuged at 10,000g for 5 min at room temperature (100% Me). Freeze-dried samples (1 g) were extracted three times with acetone (4 mL). The extracts portions were combined and centrifuged at 10,000g for 5 min at room temperature (100% Ac). These extracts were used for determination of antioxidant activity and the bioactive compounds (Vinson, Hao, Su, & Zubik, 1998).

2.4. Determination of the contents of the bioactive compounds

The studied bioactive compounds were determined as previously described (Gorinstein, Leontowicz, Leontowicz, Drzewiecki, et al., 2006; Gorinstein, Leontowicz, Leontowicz, Jastrzebski, et al., 2006). To determine the total amount of polyphenols in the studied extracts, the Folin-Ciocalteu reagent (FCR) was used,

and the measurement was performed at 765 nm with gallic acid as the standard. Results were expressed as mg of gallic acid equivalent (GAE). The UV spectra of methanol extracts were measured from 200 to 300 nm.

Flavonoids, extracted with 5% NaNO₂, 10% AlCl₃·6H₂O and 1 M NaOH, were measured at 510 nm.

The total flavanols amount was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read.

The extracts of condensed tannins (procyanidins) with 4% methanol vanillin solution were measured at 500 nm. (+)-Catechin served as a standard for flavonoids, flavanols, and tannins, and the results were expressed as catechin equivalents (CE).

2.5. Determination of the antioxidant activity

The following four tests were used:

- (1) Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric-tripiridyltriazine (Fe³⁺-TPTZ) to a ferrous form (Fe²⁺), which absorbs light at 593 nm (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002; Ozgen, Reese, Tulio, Scheerens, & Miller, 2006).
- (2) 2,2-Azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt (ABTS⁺): ABTS⁺ radical cation was generated by the interaction of ABTS (7 mM/L) and K₂S₂O₈ (2.45 mM/L). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm (Ozgen et al., 2006).
- (3) 1,1-Diphenyl-2-picrylhydrazyl method (DPPH): DPPH solution (3.9 mL, 25 mg/L) in methanol was mixed with the samples extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable (Ozgen et al., 2006).
- (4) Cupric reducing antioxidant capacity (CUPRAC): This assay is based on utilizing the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent. The absorbance at 450 nm was recorded against a reagent blank (Apak, Guclu, Ozyurek, & Karademir, 2004).

2.6. Statistical analysis

The results of this investigation are means ± SD of five measurements. Differences between samples were tested by two-way ANOVA using GraphPad Prism, version 2.0. (GraphPad Software, San Diego, CA), following by Duncan's new multiple range test to assess differences groups means. The *P* values of <0.05 were considered significant.

3. Results

3.1. Bioactive compounds

In order to determine the content of polyphenols, the main bioactive compounds of both garlic and onions the UV spectra was applied. The received data proved that the highest amount of polyphenols was extracted from red onions followed by white onions and garlic (Fig. 1A–C). It was very interesting which of the used processing most fully preserve the polyphenols content. According to the value of absorbances the highest preservation of bioactive compounds remained after bleaching of 90'.

The comparison of the two onions with standard (Fig. 1C) have shown that OR90' has exactly the same maximum wavelength of

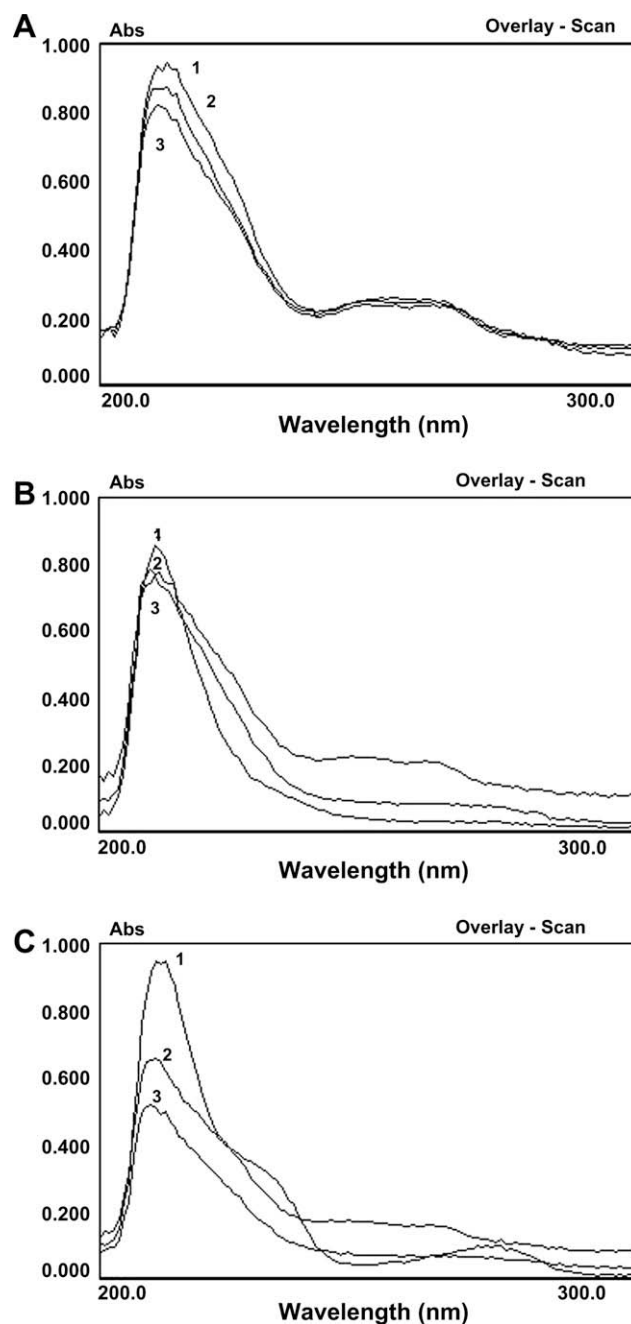


Fig. 1. UV spectra of polyphenols extracted with 100% methanol from vegetables: (A) 1, 2 and 3, ROR (100%Me), WOR (100%Me) and GAR (100%Me). Absorbances (AI): 0.942, 0.880 and 0.832; maximum of wavelengths (nm): 208.7; 208.5 and 206.7. (B) 1, 2 and 3, OR90' (100%Me), OW90' (100%Me) and Garlic 90' (100%Me). Absorbances (AI): 0.851, 0.784 and 0.764; maximum of wavelengths (nm): 207.3; 205.9 and 206.3. (C) 1, 2 and 3, catechin 0.01 M, OR10' (100%Me) and OW10' (100%Me). Absorbances (AI): 0.951, 0.654 and 0.514; maximum of wavelengths (nm): 207.7; 206.3 and 205.5. Concentration of the samples was 0.5 mg DW/mL. Abbreviations: DW, dry weight; ROR (100%Me), WOR (100%Me) and GAR (100%Me), polyphenols, extracted with 100% methanol from raw red and white onions and garlic samples; OR90' (100%Me), OW90' (100%Me) and GA90' (100%Me), 100% methanol from bleached for 90' red and white onions and garlic; OR10' (100%Me) and OW10' (100%Me), extracted with 100% methanol from boiled for 10' red and white onions.

207.3 and absorbance of 0.851 as catechin (207.7) and slightly higher absorbance of 0.951. These results are similar to Yilmaz and Toledo (2006), who indicate that aqueous solutions of methanol or acetone were better than a single-compound solvent system for extraction of total phenols from Muscadine seed powder.

Our results show that methanol solvent was more effective in extraction of bioactive compounds than acetone. The data of the changes in the contents of the studied bioactive compounds in 50% and 100% methanol and 100% acetone extracts of the studied vegetables after technology treatment are summarized in Table 1. It was found that the significant highest content of polyphenols was in the 100% methanol extracts of 90' bleached vs. raw vegetables (8.25, 9.75 and 11.98 vs. 9.00, 10.52 and 15.87 mg GAE/g DW, respectively). Also the other studied bioactive compounds (flavonoids, flavanols and tannins) have changed in the same way as polyphenols in both onions and garlic. Tannins were not detected in garlic (Table 1).

3.2. Antioxidant activity

It is known that the contents of individual bioactive compounds not always predicted the level of the antioxidant activity. Therefore, four different assays [2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric-reducing/antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC)] were applied in this investigation in order to receive reliable data. The changes of the level of the antioxidant activity in methanol and acetone extracts of garlic and white and red onions after technology treatment showed that the highest level of the antioxidant activity by DPPH was in 100% methanol extracts of raw red onion in comparison with bleached for 90': (24.30 ± 2.1 vs. 23.90 ± 2.71 μM TE/g DW, respectively), however the difference was not significant [$P > 0.05$] (Table 2).

Bleaching of garlic, white and red onions for 90' maximally preserved the contents of bioactive compounds and the level of the antioxidant activity (Tables 1 and 2). Fig. 2 shows the correlation coefficients between the polyphenols and all antioxidant assays in raw and treated garlic, white and red onions samples in different extracts (A), in 50% methanol, (B), 100% methanol and (C), 100% acetone. The best correlation was between polyphenols in garlic samples, extracted with 50% methanol, and lower in white and red onions.

4. Discussion

It is known that allicin and its derivative compounds are the main active substances responsible for the hypolipidemic and hypocholesterolemic effects of garlic and onions in both human and animal studies (Corzo-Martinez et al., 2007; Rahman & Lowe, 2006). In this investigation the bioactive compounds of the polyphenol family and also their connection to antioxidant activities of garlic and onions are studied. In the discussion we show the results of our investigation of the main bioactive compounds (polyphenols and flavonoids) and antioxidant activity.

Literature data demonstrate a wide variability of bioactive compounds and the antioxidant activity of vegetables. Our results showed that polyphenols extracted with 100% methanol were 9.00, 10.52 and 15.87 mg GAE/g DW for raw garlic, white and red onions, respectively.

The comparison with other reports showed that white onions contained different amounts of polyphenols, depending on the sol-

Table 1
Bioactive compounds in various extracts of garlic and white and red onions

Samples	Polyphenols, mg GAE/g	Flavonoids, mg CE/g	Flavanols, μg CE/g	Tannins, mg CE/g
GAR(50%Me)	6.36 ± 0.5 ^c	0.41 ± 0.03 ^b	17.45 ± 1.56 ^c	–
GA90' (50%Me)	5.43 ± 0.4 ^c	0.32 ± 0.02 ^b	17.00 ± 1.71 ^c	–
GA10' (50%Me)	3.65 ± 0.3 ^b	0.28 ± 0.02 ^b	15.10 ± 1.23 ^c	–
WOR (50%Me)	7.48 ± 0.6 ^c	0.76 ± 0.05 ^c	65.01 ± 0.5 ^e	1.20 ± 0.1 ^b
WO90' (50%Me)	7.00 ± 0.5 ^c	0.70 ± 0.06 ^c	50.11 ± 0.4 ^e	1.00 ± 0.1 ^b
WO10' (50%Me)	6.73 ± 0.5 ^c	0.73 ± 0.06 ^c	37.21 ± 0.3 ^d	0.84 ± 0.1 ^b
ROR (50%Me)	11.96 ± 0.9 ^d	0.98 ± 0.08 ^c	72.61 ± 0.6 ^f	1.59 ± 0.1 ^b
RO90' (50%Me)	11.51 ± 0.9 ^d	0.90 ± 0.08 ^c	58.92 ± 0.5 ^f	1.40 ± 0.1 ^b
RO10' (50%Me)	10.90 ± 0.9 ^d	0.80 ± 0.07 ^c	40.04 ± 0.3 ^d	1.28 ± 0.1 ^b
GAR (100%Me)	9.00 ± 0.8 ^d	0.56 ± 0.04 ^b	18.15 ± 1.60 ^c	–
GA90' (100%Me)	8.25 ± 0.7 ^d	0.41 ± 0.03 ^b	17.76 ± 1.58 ^c	–
GA10' (100%Me)	6.23 ± 0.5 ^c	0.32 ± 0.02 ^b	16.11 ± 1.52 ^c	–
WOR (100%Me)	10.52 ± 0.9 ^d	1.04 ± 0.10 ^c	91.94 ± 0.8 ^g	1.69 ± 0.1 ^b
WO 90' (100%Me)	9.75 ± 0.8 ^d	0.89 ± 0.08 ^c	86.52 ± 0.7 ^g	1.48 ± 0.1 ^b
WO10' (100%Me)	8.06 ± 0.7 ^d	0.79 ± 0.07 ^c	73.44 ± 0.6 ^f	1.41 ± 0.1 ^b
ROR (100%Me)	15.87 ± 1.3 ^e	1.31 ± 0.10 ^c	16.71 ± 1.4 ^c	2.55 ± 0.2 ^c
RO90' (100%Me)	11.98 ± 0.9 ^d	1.09 ± 0.10 ^c	13.43 ± 1.2 ^b	1.95 ± 0.2 ^b
RO10' (100%Me)	11.03 ± 0.9 ^d	0.91 ± 0.08 ^c	12.15 ± 1.0 ^b	1.21 ± 0.1 ^b
GAR (100%Ac)	2.65 ± 0.2 ^a	0.17 ± 0.01 ^b	1.45 ± 0.1 ^a	–
GA90' (100%Ac)	1.75 ± 0.1 ^a	0.10 ± 0.010 ^a	1.11 ± 0.1 ^a	–
GA10' (100%Ac)	1.58 ± 0.1 ^a	0.08 ± 0.010 ^a	1.09 ± 0.1 ^a	–
WOR (100%Ac)	1.65 ± 0.1 ^a	0.04 ± 0.004 ^a	1.40 ± 0.1 ^a	0.16 ± 0.01 ^a
WO 90' (100%Ac)	1.56 ± 0.1 ^a	0.04 ± 0.004 ^a	1.10 ± 0.1 ^a	0.14 ± 0.01 ^a
WO10' (100%Ac)	1.53 ± 0.1 ^a	0.03 ± 0.003 ^a	1.00 ± 0.1 ^a	0.11 ± 0.01 ^a
ROR (100%Ac)	2.20 ± 0.2 ^a	0.17 ± 0.010 ^b	1.90 ± 0.2 ^a	0.22 ± 0.02 ^a
RO90' (100%Ac)	2.11 ± 0.2 ^a	0.15 ± 0.01 ^b	1.50 ± 0.1 ^a	0.19 ± 0.02 ^a
RO10' (100%Ac)	1.58 ± 0.1 ^a	0.08 ± 0.01 ^a	0.9 ± 0.1 ^a	0.13 ± 0.01 ^a

Values are means ± SD of five measurements. Means in columns without superscript letters in common differ significantly ($P < 0.05$). The samples were named as following: GAR (50%Me), polyphenols extracted from raw garlic with 50% methanol at 90 °C; GA90' (50%Me), bleached garlic for 90'; GA10' (50%Me), boiled for 10', WOR (50%Me), polyphenols extracted from raw white onion with 50% methanol at 90 °C; WO90' (50%Me), bleached for 90'; WO10' (50%Me), boiled for 10'; ROR (50%Me), polyphenols extracted from raw red onion with 50% methanol at 90 °C; RO90' (50%Me), bleached for 90' and RO10' (50%Me), boiled for 10'; GAR (100%Me), polyphenols extracted with 100% methanol from raw garlic at room temperature; GA90' (100%Me), bleached for 90'; GA10' (100%Me), boiled for 10', WO (100%Me), polyphenols extracted with 100% methanol from raw white onion at room temperature; WO90' (100%Me), bleached for 90'; WO10' (100%Me), boiled for 10'; ROR (100%Me), polyphenols extracted with 100% methanol from raw red onion at room temperature; RO90' (100%Me), bleached for 90' and RO10' (100%Me), boiled for 10'; GAR (100%Ac), polyphenols extracted with 100% acetone from raw garlic at room temperature; GA90' (100%Ac), bleached garlic for 90'; GA10' (100%Ac), boiled for 10', WOR (100%Ac), polyphenols extracted with 100% acetone from raw white onion at room temperature; WO90' (100%Ac), bleached for 90'; WO10' (100%Ac), boiled for 10'; ROR (100%Ac), polyphenols extracted with 100% acetone from raw red onion at room temperature; RO90' (100%Ac), bleached for 90' and RO10' (100%Ac), boiled for 10'.

Table 2Antioxidant activity ($\mu\text{M TE/g DW}$) in various extracts of garlic and white and red onions after technology treatment

Samples	DPPH	FRAP	CUPRAC	ABTS
GAR (50%Me)	7.00 \pm 0.6 ^c	6.90 \pm 0.6 ^c	15.13 \pm 1.2 ^e	23.71 \pm 2.0 ^c
GA90'' (50%Me)	6.96 \pm 0.6 ^c	6.37 \pm 0.6 ^c	10.07 \pm 0.8 ^d	19.76 \pm 1.7 ^c
GA10' (50%Me)	6.03 \pm 0.5 ^c	3.49 \pm 0.2 ^b	7.00 \pm 0.6 ^c	12.78 \pm 1.1 ^b
WOR (50%Me)	21.44 \pm 1.9 ^e	14.60 \pm 1.2 ^d	23.21 \pm 2.0 ^f	26.50 \pm 2.2 ^c
WO90'' (50%Me)	20.56 \pm 1.8 ^e	14.00 \pm 1.2 ^d	21.86 \pm 1.9 ^f	20.27 \pm 1.8 ^c
WO10' (50%Me)	14.52 \pm 1.2 ^d	13.45 \pm 1.2 ^d	19.34 \pm 1.8 ^f	14.58 \pm 1.2 ^b
ROR (50%Me)	22.00 \pm 1.9 ^e	19.20 \pm 1.7 ^e	39.66 \pm 3.5 ^g	49.68 \pm 3.8 ^d
RO90'' (50%Me)	21.85 \pm 1.9 ^e	16.37 \pm 1.3 ^d	38.82 \pm 3.5 ^g	47.14 \pm 3.7 ^d
RO10' (50%Me)	14.71 \pm 1.2 ^d	16.00 \pm 1.3 ^d	29.14 \pm 2.5 ^f	43.68 \pm 3.6 ^d
GAR (100%Me)	9.00 \pm 0.7 ^c	8.12 \pm 0.7 ^c	24.00 \pm 2.1 ^f	25.00 \pm 2.2 ^c
GA90'' (100%Me)	8.82 \pm 0.7 ^c	7.48 \pm 0.6 ^c	20.43 \pm 1.8 ^f	23.04 \pm 2.1 ^c
GA10' (100%Me)	7.76 \pm 0.6 ^c	5.37 \pm 0.4 ^c	15.20 \pm 1.3 ^e	13.25 \pm 1.1 ^b
WOR (100%Me)	23.05 \pm 2.0 ^e	15.00 \pm 1.2 ^d	26.90 \pm 2.2 ^f	24.14 \pm 1.9 ^c
WO90'' (100%Me)	22.50 \pm 2.0 ^e	14.12 \pm 1.2 ^d	22.69 \pm 2.0 ^f	23.85 \pm 1.8 ^c
WO10' (100%Me)	16.00 \pm 1.3 ^d	14.05 \pm 1.1 ^d	21.44 \pm 2.0 ^f	21.11 \pm 1.9 ^c
ROR (100%Me)	24.30 \pm 2.1 ^e	21.20 \pm 1.8 ^e	40.65 \pm 3.6 ^g	49.42 \pm 3.8 ^d
RO90'' (100%Me)	23.90 \pm 2.1 ^e	18.95 \pm 1.6 ^e	36.48 \pm 3.4 ^g	46.81 \pm 3.7 ^d
RO10' (100%Me)	14.00 \pm 1.2 ^d	16.58 \pm 1.3 ^d	34.51 \pm 3.3 ^g	44.68 \pm 3.6 ^d
GAR (100%Ac)	3.21 \pm 0.2 ^b	2.64 \pm 0.2 ^a	3.75 \pm 0.3 ^b	5.03 \pm 0.4 ^a
GA90'' (100%Ac)	2.99 \pm 0.2 ^b	1.85 \pm 0.1 ^a	3.54 \pm 0.3 ^b	4.75 \pm 0.4 ^a
GA10' (100%Ac)	1.48 \pm 0.1 ^a	1.43 \pm 0.1 ^a	1.44 \pm 0.1 ^a	3.85 \pm 0.3 ^a
WOR (100%Ac)	2.40 \pm 0.2 ^b	2.94 \pm 0.2 ^b	2.32 \pm 0.2 ^b	5.83 \pm 0.5 ^a
WO90'' (100%Ac)	2.18 \pm 0.1 ^a	2.41 \pm 0.2 ^b	2.00 \pm 0.1 ^a	4.68 \pm 0.4 ^a
WO10' (100%Ac)	1.60 \pm 0.1 ^a	1.83 \pm 0.1 ^a	1.39 \pm 0.1 ^a	4.09 \pm 0.3 ^a
ROR (100%Ac)	2.68 \pm 0.2 ^b	3.05 \pm 0.2 ^b	3.12 \pm 0.2 ^b	6.21 \pm 0.5 ^a
RO90'' (100%Ac)	2.34 \pm 0.2 ^b	2.75 \pm 0.2 ^b	3.00 \pm 0.2 ^b	5.81 \pm 0.5 ^a
RO10' (100%Ac)	1.70 \pm 0.1 ^a	2.13 \pm 0.1 ^a	1.90 \pm 0.1 ^a	4.89 \pm 0.4 ^a

Abbreviations: Values are means \pm SD of five measurements. Means in columns without superscript letters in common differ significantly ($P < 0.05$). The samples were named as following: GAR (50%Me), polyphenols extracted from raw garlic with 50% methanol at 90°C; GA90'' (50%Me), bleached garlic for 90'; GA10' (50%Me), boiled for 10'; WOR (50%Me), polyphenols extracted from raw white onion with 50% methanol at 90°C; WO90'' (50%Me), bleached for 90'; WO10' (50%Me), boiled for 10'; ROR (50%Me), polyphenols extracted from raw red onion with 50% methanol at 90°C; RO90'' (50%Me), bleached for 90' and RO10' (50%Me), boiled for 10'; GAR (100%Me), polyphenols extracted with 100% methanol from raw garlic at room temperature; GA90'' (100%Me), bleached for 90'; GA10' (100%Me), boiled for 10'; WO (100%Me), polyphenols extracted with 100% methanol from raw white onion at room temperature; WO90'' (100%Me), bleached for 90'; WO10' (100%Me), boiled for 10'; ROR (100%Me), polyphenols extracted with 100% methanol from raw red onion at room temperature; RO90'' (100%Me), bleached for 90' and RO10' (100%Me), boiled for 10'; GAR (100%Ac), polyphenols extracted with 100% acetone from raw garlic at room temperature; GA90'' (100%Ac), bleached garlic for 90'; GA10' (100%Ac), boiled for 10'; WOR (100%Ac), polyphenols extracted with 100% acetone from raw white onion at room temperature; WO90'' (100%Ac), bleached for 90'; WO10' (100%Ac), boiled for 10'; ROR (100%Ac), polyphenols extracted with 100% acetone from raw red onion at room temperature; RO90'' (100%Ac), bleached for 90' and RO10' (100%Ac), boiled for 10'.

vent used, which ranged from 2.57 in acetone extract to 6.53 mg GAE/g DW in 75% ethanol (Santas et al., 2008). Our data were higher than those reported by Bahorun et al. (2004), of 8211 $\mu\text{g GAE/g DW}$ for methanol extracts.

According to Vinson et al. (1998), the phenol content for garlic was 2.56, and red and white onions – 5.63 and 2.32 in comparison with our already cited data of 9.00, 10.52 and 15.87 mg GAE/g DW for raw garlic, white and red onions, respectively. As can be seen, our result are higher; however the highest as in the investigation Vinson et al., 1998, are of red onions.

Reported data by Stratil et al. (2006), showed the phenol content in red onion is 12.58 in comparison with 15.87 mg GAE/g DW of our results. As can be seen, the result of Stratil et al. (2006), is higher, however the difference is not significant.

Kevers et al. (2007) indicated that polyphenols in garlic and white onion were 4.07 and 6.87, respectively, in comparison with our data of 6.36 and 7.48 mg GAE/g DW, extracted with 50% methanol (Table 1). Also the results of these authors and our data differ not significantly. The next investigators found that polyphenols in white and red onions were about 26.11 and 25.27 mg GAE/g DW

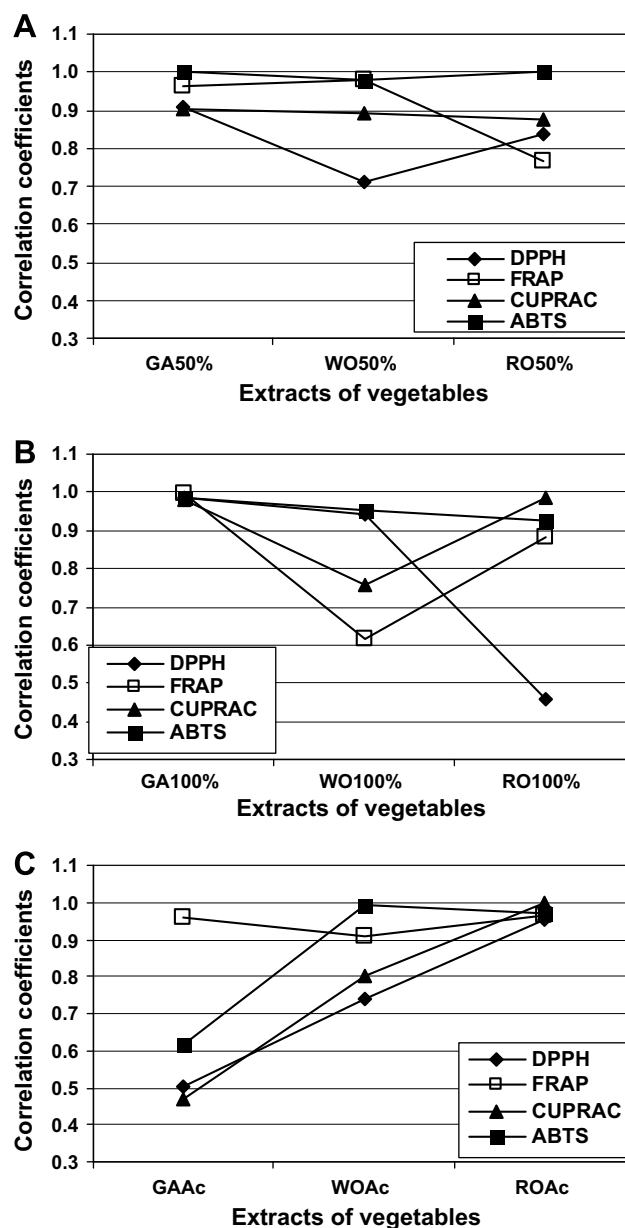


Fig. 2. Relationship between total phenolics and antioxidant activities determined by DPPH, FRAP, CUPRAC and ABTS assays expressed as correlation coefficients in different extracts of polyphenols: A, 50% methanol; B, 100% methanol; C, 100% acetone. **Abbreviations:** DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric-reducing/antioxidant power; CUPRAC, cupric reducing antioxidant capacity ABTS, 2,2-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt; GA50%, WO50%, RO50%, polyphenols extracted from garlic samples, and white and red onions with 50% methanol; GA100%, WO100%, RO100%, polyphenols extracted from garlic samples, and white and red onions with 100% methanol; GAAc, WOAc, ROAc, polyphenols extracted from garlic samples, and white and red onions with 100% acetone.

(Lin & Tang, 2007) and our data were as much as twice lower. The above cited data about the content of polyphenols in studied vegetables are different and it is connected mainly to the use of different extraction methods.

Flavonoids as shown by the above mentioned authors were lower than ours: for garlic 41 and for onions 76 mg CE/100 g DW (Table 1). The next cited authors found flavonoids in water and acetone extracts of red onions 1734.1 and 23.6–450.4, and different methanol and acetone extracts in the range of 980–720 and 13 mg/kg [22] and 3302.24 $\mu\text{g QE/g}$ (Bahorun et al., 2004).

However, flavonoids found in white and red onions were 7.34 and 5.32–3.44 mg CE/g DW, respectively. Lin and Tang (2007) were higher than our results. The data of other investigators (Miean & Mohamed, 2001) show that flavonoids were for onions about 12,175 mg QE/kg DW, four times higher than our results. In our opinion, the cited different results of the flavonoids content are mostly connected by use of different extraction methods.

Some authors show that higher phenolic content was associated with higher antioxidant activity (Santas et al., 2008).

So, they reported that white onion extracts with 100% methanol had the highest antioxidant activity (μM Trolox/g DW) of 81.1 and 24.9 for TEAC and FRAP assays, respectively. When another solvent such as 50% methanol was used probably the extraction of bioactive compounds was lower than for the 100% methanol and the antioxidant activity (μM Trolox/g DW) was 62.7 and 19.3 for TEAC and FRAP, respectively (Santas et al., 2008). Our results are lower (Table 2): the highest antioxidant activity was in 50% methanol extract of red onion by ABTS (49.68 ± 3.8) and in 100% methanol by DPPH (24.30 ± 2.1) μM Trolox/g DW, respectively.

Halvorsen et al. (2002) have found that FRAP values for red onion was about 5.4–5.7 mmol Fe^{+2} /100 g DW and for garlic 0.4–0.9 mmol Fe^{+2} /100 g DW, which are higher than our data for red onion and close to our data for garlic (Table 2). Also the results of Baborun et al. (2004), Nencini et al. (2007), and Ou et al. (2002) for white and purple onions are in accordance with our data (Table 2). In raw and processed garlic extracts, FRAP gave significantly lower values than CUPRAC. Garlic is rich in thiol-type antioxidants, and it has been well established in literature that FRAP is not as reactive to $-\text{SH}$ antioxidants as CUPRAC. The obtained data showed the advantage of CUPRAC over FRAP.

Moreno et al. (2006) showed that the antioxidant activity of onions was as much as twice higher than for garlic. These results are in accordance with ours. However, others suggest (Pellegrini et al., 2007) a mean concentration of antioxidants assayed by ABTS method in water and acetone extracts of red onions 14.31 and 4.39 mM TE/kg DW, respectively, which is higher in 50% methanol extract and lower in 100% acetone extract than our results. The TEAC by ABTS assay was 29.02 μM TE/g DW in comparison with our data of 49.68 μM TE/g DW in 50% methanol extract (Table 2). Also the data for red onions by Wu et al. (2004), concerning ORAC, were 127.2 μM TE/g DW and the lipophilic fraction -1.27 μM TE/g DW, respectively, correspond with our findings of acetone extract by DPPH of 2.68 μM TE/g DW (Table 2).

The linear relationship between total phenolics and antioxidants activity was not very high in all studied antioxidants tests (Fig. 2), and these results were different from others, which showed the correlation coefficient of 0.96 (Baborun et al., 2004; Kevers et al., 2007; Santas et al., 2008; Stratil et al., 2006; Vinson et al., 1998). The processing of vegetables decreased the content of their bioactive compounds and the level of the antioxidant activity (Gorinstein, Leontowicz, Leontowicz, Drzewiecki, et al., 2006; Gorinstein, Leontowicz, Leontowicz, Jastrzebski, et al., 2006; Roy et al., 2007). Roy et al. (2007) subjected raw extracts of *Allium* vegetables to thermal treatment at 75 °C or 100 °C for 30 and 60 min. Measuring the total phenolics by the Folin-Ciocalteu method, these authors observed that thermal treatment significantly decreased their contents in leek and garlic extracts. Only Woo et al. (2007) claimed that in onion heated at various temperatures (110–150 °C) for various times (1–5 h), the contents of total polyphenol, flavonoid, free sugar and antioxidant activity were increased. Therefore, all authors agree that extraction procedures affect the contents of bioactive compounds and the antioxidant activity in one or another way (Pellegrini et al., 2007). The changes after processing of vegetables are reported also by Agostini et al. (2004). They show that after boiling of fresh onions the antioxidant capacity decreases from 0.223 to 0.146 μM TE/g, respectively. Xu et al.

(2007) reported that various cooking methods exerted different effects on the content of flavonoids. These data were similar to our results found during the heating treatment. Therefore, the aim of this investigation was not only to find processing technique, which maximally preserves bioactive compounds and antioxidant activity of garlic and onions but also an extraction method, which most fully extracts their bioactive compounds. We found that bleaching for 90" most fully preserves the bioactive compounds and the antioxidant activity of the studied vegetables. Also others prefer this method (Amin & Lee, 2005; Kaur & Kapoor, 2002).

We also found that only the 100% methanol extracts of all studied vegetables possess higher contents of the studied phenolic compounds and higher level of the antioxidant activity. Also other authors found that different extract procedure gave different results (Irkin & Korukluoglu, 2007; Kim et al., 2005).

As was mentioned, the data about the content of bioactive compounds and antioxidant activity in studied vegetables are different: it is connected mainly by the use of vegetables from different geographical regions, grown in different climatic and different storing conditions. This can be an explanation of different results shown by many investigators.

It was very interesting to know the possible reasons and mechanisms why bioactive compounds and antioxidant activity changed after the different heat treatments (Ou et al., 2002). These authors studied a total of 927 freeze-dried vegetable samples, including 111 white cabbages, 59 carrots, 51 snap beans, 57 cauliflower, 33 white onions, 48 purple onions, 130 broccoli, 169 tomatoes, 25 beets, 88 peas, 88 spinach, 18 red peppers, and 50 green peppers, using the oxygen radical absorption capacity (ORAC) and ferric reducing antioxidant capacity (FRAP) methods. They found that the two antioxidant assays ORAC and FRAP, give different antioxidant activity trends. The above cited authors explain that the discrepancy in the results is based on the chemistry principles upon which these methods are built: the ORAC method is chemically more relevant to chain-breaking antioxidants activity, while the FRAP has some drawbacks such as interference, reaction kinetics, and quantitation methods. On the basis of the ORAC results, green pepper and red onion are the leading sources of antioxidant activities against the peroxy radicals. The results of the determination of the antioxidant activity of the studied vegetables using ORAC are in accordance with our data: red onion is one of the vegetables with high content of phenolics and flavonoids and high level of antioxidant activity. The possible reasons of the change of the bioactive compounds in vegetables after various heat treatments can be explained by their physical properties (texture, color, matrix softening, increased extractability). The mechanism of the change in proteins is based on the degradation and denaturation during heating process. The percentage of denaturation depends on the time of treatment. It was determined in this report that all bioactive compounds and their antioxidant activities have differently changed during blanching and boiling.

5. Conclusion

In conclusion, comparative control shows that bleaching for 90" of raw garlic, white and red onions most fully preserves their bioactive compounds (polyphenols, flavonoids, flavanols and tannins) and the level of antioxidant activity. In the 100% methanol extracts of the studied vegetables most fully were detected the above mentioned bioactive compounds and the level of antioxidant activity. Onions and garlic should be consumed for the potential of their biological activities. The attention should be paid for the processing methods in order to preserve the bioactive properties of these vegetables.

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