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Comparison of the Main Bioactive Compounds and Antioxidant Activities in Garlic and White and Red Onions after Treatment Protocols

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Polish garlic and white and red onions were subjected to blanching, boiling, frying, and microwaving for different periods of time, and then their bioactive compounds (polyphenols, flavonoids, flavanols, anthocyanins, tannins, and ascorbic acid) and antioxidant activities were determined. It was found that blanching and frying and then microwaving of garlic and onions did not decrease significantly the amounts of their bioactive compounds and the level of antioxidant activities ($P > 0.05$). The HPLC profiles of free and soluble ester- and glycoside-bound phenolic acids showed that *trans*-hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic, and sinapic) were as much as twice higher in garlic than in onions. Quercetin quantity was the highest in red onion among the studied vegetables.The electrophoretic separation of nonreduced garlic and onion proteins after boiling demonstrated their degradation in the range from 50 to 112 kDa.

KEYWORDS: Garlic; onions; bioactive compounds; antioxidant activity; boiling; blanching, frying; microwaving

INTRODUCTION

Increasing consumption of fruits and vegetables is due to consumer belief in potential health benefits, increasing availability and higher quality, and lifestyle changes (*1, 2*). Among the widely consumed vegetables are garlic (*Allium sativum* L.) and white and red onions (*Allium cepa L*). Garlic and onions were important medicines to the ancient Egyptians. There is evidence that during the earliest Olympics in Greece, garlic was fed to the athletes as perhaps one of the important "performance-enhancing" agents. References to the medical and culinary use of garlic were discovered on Sumerian clay tablets dating from 2600−2100 B.C. (*3*). Increasing attention has been paid in Europe to the medical use of garlic and onion in the past up to the present. In recent decades were published results of investigations which showed that garlic and onions possess antioxidant, anti-inflammatory, and antimicrobial properties (*4*). These properties allow raw garlic and onions and their preparations to successfully treat and prevent a wide range of diseases, such as cardiovascular, cancer, and others (*5, 6*). The published literature deals with the organosulfur compounds of garlic (*4, 7–10*), although there is an interest in fructans and anthocyanin pigments (*11, 12*). The highest quantity of fructans is found in garlic, artichoke, shallots, leek bulb, and onions and ranged from 1.2 to 17.4 g/100 g of fresh weight (FW) (*11*). In the case of onions both organosulfur compounds and flavonoids such as quercetin are significant bioactive compounds (*1, 6, 13, 14*). Despite low amounts of polyphenols in investigated vegetables, especially in garlic (*4, 7, 10, 15, 16*), the current study focused on the antioxidants, which are mostly composed of polyphenols.

Nowadays most consumed vegetables are subjected to different technological treatments, such as blanching, boiling, baking, frying, and microwaving (*8–10, 12, 14–17*). Some studies suggest that heating destroys the formation of the active allyl sulfur compounds in garlic, which may be involved in its anticancer properties (*8–10*). It was shown that the processing of garlic, onions, and other vegetables can markedly influence their effectiveness, changing the antioxidant activity and the bioactive substances (*12, 14–17*). Therefore, it is important to

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find technologies that preserve the amounts of bioactive compounds and their antioxidant activities in the treated vegetables. The protein profile of garlic and onions had not been studied intensively (*15, 18, 19*); therefore, the changes in proteins during the treatment of garlic and white and red onions were studied as well.

Our recent research (*15, 16*) showed the comparison of the antioxidant properties of garlic during different times of boiling. The present paper differs from the previous ones because it describes different vegetables, their protein and HPLC profiles, and changes during applied heat treatment. To determine the optimal regimen of the technological treatment the studied vegetables were subjected to blanching, boiling, frying, and then microwaving for different periods of time and their bioactive compounds (polyphenols, flavonoids, flavanols, anthocyanins, tannins, and ascorbic acid); HPLC phenolic and electrophoretic protein profiles and antioxidant activities were compared with the results after treatment.

Four antioxidant activity tests, 2,2-azinobis(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, which allow the most reliable data to be obtained.

As far as we know there are no published results of such comprehensive investigations.

MATERIALS AND METHODS

Chemicals. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), lanthanum(III) chloride heptahydrate, Folin−Ciocalteu reagent (FCR), sodium dodecyl sulfate (SDS), β-mercaptoethanol (β-ME), acrylamide, polyacrylamide, Coomassie Brilliant Blue R, molecular mass markers (14−97 kDa), $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, 2,9-dimethyl-1,10-phenanthroline (neocuproine), and butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co., St. Louis, MO. 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. Authentic reference compounds were obtained from Sigma-Aldrich, Prague, Czech Republic. All reagents were of analytical grade. Deionized and distilled water was used throughout.

Samples. Raw Polish garlic (*Allium sativum* L.) and white (Armstrong) and red (Red Baron) onions (*Allium cepa*) were obtained from Elena Co., Warsaw, Poland. The following steps of treatments were applied: bulbs of garlic and white and red onions were washed, cleaned, peeled, and cut with a plastic knife (halves for garlic and pieces for onions) before heat treatment. The studied vegetables were blanched, boiled, and fried. Blanching was done for all samples in water at 100 °C for 90 s (90″). Boiling was similar to blanching, but the time of this treatment was different from that of blanching, starting from 10 min and increasing to 60 min. The data of boiling after 10 min are not shown and were discussed in previous paper (*15, 16*). Frying was carried out without fat in a pan at 100 °C during 10 min. All garlic and onions, treated according to the above-mentioned protocol, were divided in two parts: one was analyzed immediately, and the second was frozen until use. Then the vegetables of the second part were thawed and microwaved during 6 min for garlic samples and 5 min for onions. The microwaving time for garlic and onions depended on their different structures with controlled temperature of 65−70 °C and a power of 500 W for all investigated vegetables. The samples were lyophilized and then ground to fine particles under a cooling system. This protocol is applied in the present study because it is similar to everyday food cooking.

The 21 garlic and onion samples were as follows. Garlic samples: lyophilized raw garlic, named garlic; blanched for 90 s, garlic BL90″; boiled for 10 min, garlic BO10′; fried for 10 min, garlic F10′; blanched and microwaved (M), garlic BL90″+M; boiled for 10 min and M, garlic BO10′+M; fried for 10 min and M, garlic F10′+M. White onion samples: lyophilized raw white onion, named white onion; blanched for 90 s, white onion BL90″; boiled for 10 min, white onion BO10′; fried for 10 min, white onion F10′; blanched for 90″ and then subjected to microwaving (M), white onion BL90″+M; boiled for 10 min and M, white onion BO10′+M; fried for 10 min and M, white onion F10′+M. Red onion samples: lyophilized raw red onion, named red onion; blanched for 90 s, red onion BL90″; boiled for 10 min, red onion BL10′; fried for 10 min, red onion F10′; blanched for 90 s and microwaved (M), red onion BL90″+M; boiled for 10 min and M, red onion BO10′+M; fried for 10 min and M, red onion F10′+M.

Preparation of Extracts. Defatted lyophilized vegetable samples were extracted from a 50 mg aliquot with 5 mL of 1.2 M HCl in 50% methanol/water with heating at 90 °C for total polyphenols. The samples were cooled, diluted to 10 mL with methanol, and centrifuged for 5 min at 4000*g* with a benchtop centrifuge to remove solids. These extracts were used for the determination of antioxidant activity and the bioactive compounds (*15, 20, 21*).

HPLC Polyphenol Profile. Free, ester-bound (released after alkaline hydrolysis), and glycoside-bound (released after acid hydrolysis) phenolic acids were obtained from a methanol extract of garlic and white and red onion lyophilized dry matter ground in liquid nitrogen. 2,6-Di-*tert*-butyl β-cresol was added as antioxidant, and nitrogen was immediately bubbled through the sample after NaOH addition to minimize the oxidation of phenolic acids during alkaline hydrolysis. Phenolic acids were eluted using acetic acid gradient in methanol. Flavonoid compounds were extracted with 80% methanol according to the method of glycoside-bound phenolic acids extraction (released after acid hydrolysis). Flavonoid compounds were eluted using a gradient of acetonitrile with phosphoric acid. Phenolic acids and flavonoids (quercetin and kaempferol) were determined by monitoring of their absorption maxima at 260 nm using authentic reference compounds (*22–24*).

Determination of Bioactive Compounds. The studied bioactive compounds were determined as previously described (*15, 20, 21, 23, 24*). To determine the total amount of polyphenols in the studied extracts, FCR was used, and the measurement was performed at 765 nm with gallic acid as the standard. Results are expressed as milligrams of gallic acid equivalent (GAE).

Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \cdot 6H_2O$, and 1 M NaOH, were measured at 510 nm. The total flavanol amount was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read (*25*). To ensure the presence of flavanols on the nuclei (*25*), subsequent staining with the DMACA reagent resulted in an intense blue coloration in onion (*A. cepa* L).

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 are expressed as catechin equivalents (CE).

The absorbances for total anthocyanins were measured for two pH values (1.0 and 4.5) in a Beckman spectrophotometer at 510 nm, using the pH differential method (*12*). Results are expressed as milligrams of cyanidin-3-glucoside equivalent (CGE).

Total ascorbic acid was determined by using the CUPRAC assay. The water extract was prepared from 100 mg of lyophilized sample and 5 mL of water, then mixed, stirred for 24 h, and centrifuged. The extract (1 mL) was mixed with 2 mL of 3.0×10^{-3} M of lanthanum(III) chloride heptahydrate. Ethyl acetate (EtAc) was used for extraction to avoid the interference of flavonoids. For determination of ascorbic acid by the CUPRAC assay the aqueous phase was examined. One milliliter of Cu(II)−neocuproine (Nc), in ammonium acetate-containing medium at pH 7, was mixed with 1 mL of the obtained extract, and the absorbance of the formed bis(Nc)−copper(I) chelate was measured at 450 nm (*26*).

Determination of the Antioxidant Activity. The following four tests were used:

(1) The ferric reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 593 nm (*27*).

(2) The 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) radical cation was generated by the interaction

Table 1. Amounts of Methanol-Soluble Individual Phenolic Acids (Represented by the Sum of Free and Ester- and Glycoside-Bound Forms), Quercetin, and Kaempferol (Milligrams per 100 g of Dry Matter)[a]

compound	white onion	red onion	garlic
protocatechuic acid	0.12 ± 0.01 a	5.08 ± 0.04 c	0.36 ± 0.03 b
p-hydroxybenzoic acid	4.40 ± 0.3 b	0.62 ± 0.05 a	0.61 ± 0.05 a
vanillic acid	0.63 ± 0.05 c	0.10 ± 0.01 a	0.27 ± 0.02 b
caffeic acid	0.01 ± 0.001 a	0.03 ± 0.002 b	0.86 ± 0.07 c
p-coumaric acid	0.04 ± 0.003 b	0.06 ± 0.005 c	0.02 ± 0.002 a
ferulic acid	0.02 ± 0.002 a	0.25 ± 0.02 b	0.03 ± 0.003 b
sinapic acid	0.26 ± 0.02 a	0.20 ± 0.02 a	0.05 ± 0.004 b
quercetin	48.80 ± 3.3 b	110.60 ± 8.1 c	8.06 ± 0.6 a
kaempferol	0.50 ± 0.04 c	traces a	0.10 ± 0.01 b

[a] Values are means of two independent experiments with two replicates ± SD. Values in rows with different letters are significantly different ($P < 0.05$).

of ABTS (7 mM/L) and $K_2S_2O_8$ (2.45 mM/L). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm (*27, 28*).

(3) A 1,1-diphenyl-2-picrylhydrazyl method (DPPH) solution (3.9 mL, 25 mg/L) in methanol was mixed with the sample extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable (*27*).

(4) The cupric reducing antioxidant capacity (CUPRAC) 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 (*26*).

Protein Extraction and Electrophoresis. Total proteins from defatted lyophilized vegetable samples (80 mg each) were extracted with 1 mL of sample buffer [0.0625 M Tris-HCl, pH 6.25, containing 2% SDS, 10% glycerol, 2% mercaptoethanol (β-ME), and 0.001% bromophenol blue] (*15*).

The protein samples were extracted with and without reduction (β-ME was not applied in this case). A Hoeffer SE-600 apparatus (Hoeffer Pharmacia Biotech Inc., San Francisco, CA) was used for sodium dodecyl sulfate−polyacrylamine gel electrophoresis (SDS-PAGE), according to Laemmli method (*29*).

Statistical Methods. 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), followed by Duncan's new multiple-range test to assess differences in group means. Differences of $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Bioactive Compounds. As described above, the raw fruits and vegetables with high quantities of bioactive compounds possess antioxidant, anti-inflammatory, and antimicrobial properties (*1, 4, 7, 15, 30–33*). However, most of the consumed vegetables are subjected to different technological treatments (*8–10, 12, 14, 17*), which influence their effectiveness.

Phenolic acids (protocatechuic, *p*-hydroxybenzoic, vanillic, and ferulic) in garlic and onions were present in three fractions: F1, free phenolic acids; F2, methanol-soluble ester-bound phenolic acids (soluble phenolic esters); and F3, methanol-soluble glycoside-bound phenolic acids (soluble phenolic glycosides). Caffeic and anisic acids were in very low concentrations in free forms only, and *p*-coumaric and sinapic acids predominantly were found in ester-bound forms (*7, 22, 23*). Protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic, and sinapic acids were comparable in the investigated vegetables (**Table 1**; **Figure 1**). The total concentration of phenolic acids [mg/100 g of dry weight (DW), **Table 1**] was higher in red onion (6.34 ± 0.06) than in white onion (5.48 ± 0.05) and in garlic (2.20 ± 0.01). Protocatechuic acid was the major component in red onion, *p*-hydroxybenzoic acid in white onion, and caffeic acid in garlic. The sum of the four hydroxy-

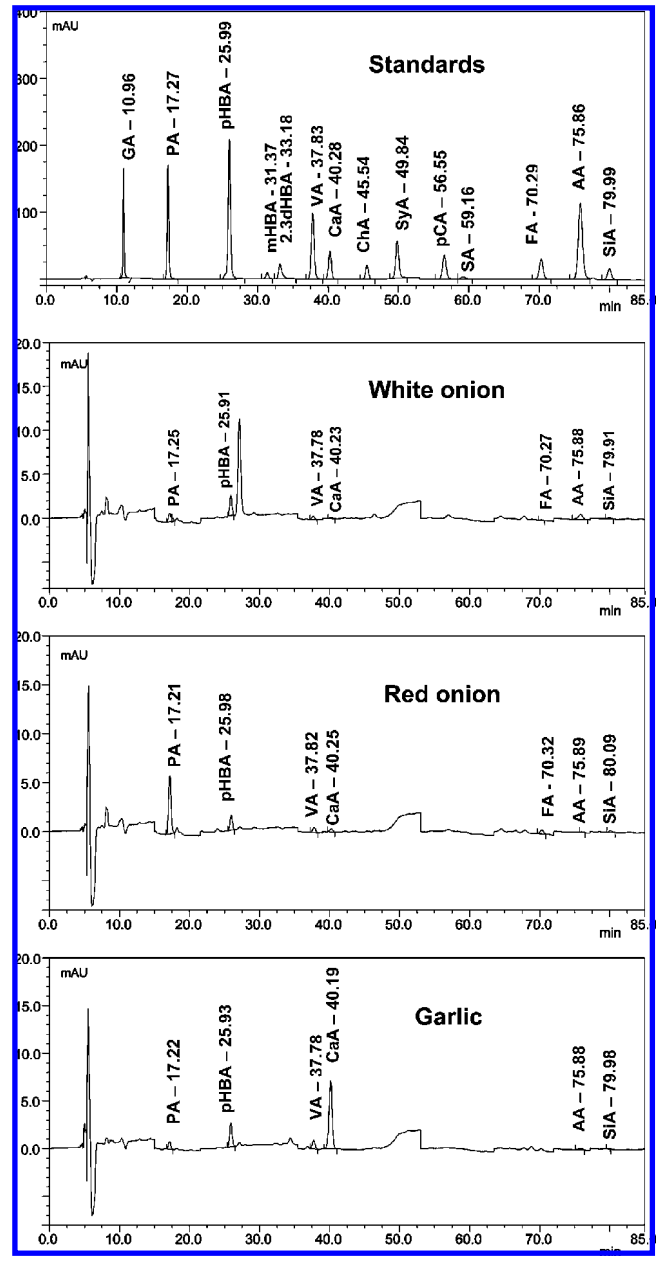

Figure 1. HPLC analysis of free phenolic acids extracted from white and red onions and garlic. Each profile represents an equivalent amount of extract, normalized on a volume of extract per 5 mg of tissue basis. Retention times [t_R (min)] are presented above the peaks of individual acids. GA, gallic acid; PA, protocatechuic acid; pHBA, *p*-hydroxybenzoic acid; mHBA, *m*-hydroxybenzoic acid; 2.3dHBA, 2,3-dihydroxybenzoic acid; VA, vanillic acid; CaA, caffeic acid; ChA, chlorogenic acid; SyA, syringic acid; pCA, *p*-coumaric acid; SA, salicylic acid; FA, ferulic acid; AA, anisic acid; SiA, sinapic acid.

cinnamic acids (mg/100 g of DW, **Table 1**) was higher in garlic (0.96 ± 0.01) than in red (0.54 ± 0.01) and white onions (0.33 ± 0.01). The highest quantity of ferulic acid was detected in red onions. Sinapic acid is almost equal in the two onions and lower in garlic. The ratios between the concentrations of ferulic and sinapic acids and that of caffeic and *p*-coumaric acids for garlic and white and red onions were about 0.1, 5.6, and 5.0, respectively, and may be a simple parameter for estimation of the nutritional value of these vegetables. In onions and garlic were found only quercetin and kaempferol from the tested standard compounds: quercetin, kaempferol, apigenin, esculetin, and L-epicatechin (**Table 1**; **Figure 2**). These two flavonoids

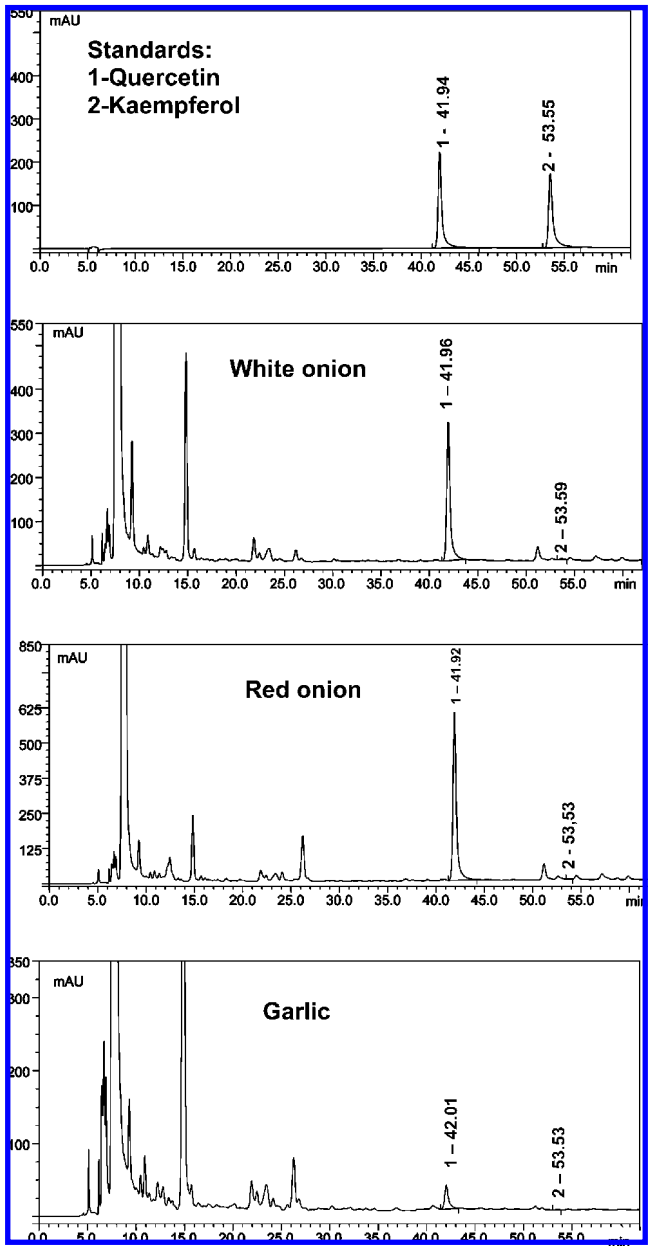

Figure 2. HPLC analysis of quercetin and kaempferol extracted from white and red onions and garlic. Each profile represents an equivalent amount of extract, normalized on a volume of extract per 10 mg of tissue basis. Retention times [t_R (min)] are presented above the peaks of quercetin and kaempferol.

were separated and identified in the following order: quercetin and kaempferol with retention times of 41.94 and 53.55 min, respectively. The data were slightly different from the literature reported retention times of 43.26 and 52.82 min for kaempferol and quercetin, respectively (*31*). The difference in the results depends on the separation conditions and the preparation and extraction of the samples. Quercetin was the highest in red onion: 14 times higher than in garlic and twice that in white onion. Flavonoids (kaempferol and quercetin, mg/100 g of DW) indicated in the literature for white and red onions were 13.26−23.86 and 92.79−558.16, respectively (*31*), and 0.96 and 41 (*33*), which are similar to our results (**Table 1**).

The amounts of the studied bioactive compounds in the investigated vegetables are relatively high (**Table 2**). The quantities of polyphenols (mg of GAE/g of DW) and tannins (mg of CE/g of DW) in raw garlic and white and red onions were 19.40 ± 1.2, 23.23 ± 0.9, and 29.72 ± 1.2 and 2.40 ± 0.1, 2.64 ± 0.1, and 4.88 ± 0.1, respectively, being significantly higher in raw red onion than in raw garlic and raw white onion ($P < 0.05$). Our results were comparable with those of other authors cited below. Therefore, the polyphenols (mg of GAE/g of DW) reported in the literature were in the following range: for not differentiated onions, 10.6 (*24*); red onions, 8.2 (*30*); different cultivars of red onions, from 10.6 to 21.2; and white onions, 7.3 (*31*); and for garlic, 18.9 (*20, 32*). Flavonoids (mg of CE/g of DW) in raw garlic and white and red onions were 3.37 ± 0.3, 3.99 ± 0.3, and 3.84 ± 0.3, respectively (**Table 2**). Flavonoids [mg of quercetin equivalents (QE)/g of DW] in red and white onions showed 4.18 (*30*) and 0.77−4.63 (*31*), respectively, which are close to our data (**Table 2**).

The amount of flavanols (mg of CE/100 g of DW) in garlic and white and red onions (**Table 2**) was estimated as 6.71 ± 0.4, 4.58 ± 0.4, and 5.93 ± 0.4, respectively, which was lower than the reported values of 6.1 for garlic and 3.37−1.71 for onions (*2*). The total flavanols varied from nondetectable in most of the vegetables to 184 mg/100 g of DW, found in a sample of broad bean (*34*); therefore, it was difficult to provide a comparison with obtained data. Anthocyanins (mg of CGE/kg of DW) in raw white and red onions were 28.34 ± 1.3 and 460.22 ± 10.9, respectively, and in raw red onion were significantly higher than in raw white onion ($P < 0.05$). This is in agreement with the cited data (*1*): anthocyanins in red and white onions were in the ranges of 317−1951 and 5.69−333, respectively, and their value was about 10% of the total flavonoids. In garlic, anthocyanins were not detected (**Table 2**).

Ascorbic acid (μg of AA/g of DW) among the studied samples (**Figure 3**) showed 735.7 ± 50.2, 1382.1 ± 90.1, and 1994.1 ± 99.9 for garlic and white and red onions, respectively. The significantly highest quantity of ascorbic acid ($P < 0.05$) was found in raw red onion.

The highest antioxidant activities (**Table 3**) were registered in raw red onion: 41.33 ± 3.9, 31.05 ± 1.5, 59.17 ± 3.1, and 69.88 ± 3.1 μM TE/g of DW for DPPH, FRAP, CUPRAC, and ABTS, respectively. It was significantly higher than in raw garlic and white onion ($P < 0.05$). Others have measured the antioxidant activity (ABTS, μM TE/g of DW) of different onions as 14.31 (*36*), 15.61 (*31*), 29.02 (*30*), and 64.11 (*24*). Antioxidant activity (FRAP, μM Fe^{2+}/g of DW) was 96.75 for garlic, 52.10 for onions (*28*), and 68.86 for red onions (*30*). This is in agreement with our results (**Table 3**).

The reported data showed the amount of bioactive compounds and their antioxidant activities in the studied raw garlic and onions to be greatly variable, depending on the extraction procedure (*35*). The composition of vegetables is changing during their treatment, and there are limited data for such comparison. What are the technologies that prevent the decrease of the bioactive compounds and the level of antioxidant activities in garlic and onions? To answer this question, raw Polish garlic and white and red onions were investigated in vitro and then subjected to blanching, boiling, frying, and microwaving for different periods of time.

The best preservation of all bioactive compounds (**Table 2**) after blanching, boiling, frying, and microwaving was observed in the garlic samples BL90″ and F10′, red onions F10′ and BL90″, and white onions F10′ and BL90″. Good preservation of the bioactive compounds was also found in the same samples subjected to microwaving.

The calculated correlation coefficients for polyphenols in garlic and white and red onions and their antioxidant activities

Table 2. Changes in the Amounts of the Studied Bioactive Compounds in Garlic and White and Red Onions after Processing (Dry Weight)[a]

sample	polyphenols (mg of GAE/g)	flavonoids (mg of CE/g)	flavanols (mg of CE/100 g)	anthocyanins (mg of CGE/kg)	tannins (mg of CE/g)
garlic	19.40 ± 1.2 c	3.37 ± 0.3 c	6.71 ± 0.4 e	nd	2.40 ± 0.1 b
garlic BL90″	16.11 ± 1.1 b	3.15 ± 0.2 c	5.16 ± 0.4 d	nd	2.13 ± 0.1 b
garlic BO10′	13.52 ± 0.8 a	1.84 ± 0.1 a	3.99 ± 0.2 b	nd	1.50 ± 0.05 a
garlic F10′	15.98 ± 0.9 b	2.94 ± 0.2 c	4.29 ± 0.3 c	nd	2.00 ± 0.1 b
garlic BL90″+M	15.53 ± 0.9 b	2.37 ± 0.2 b	5.06 ± 0.4 d	nd	2.00 ± 0.1 b
garlic BO10′+M	12.05 ± 0.7 a	1.76 ± 0.1 a	3.06 ± 0.2 a	nd	1.42 ± 0.05 a
garlic F10′+M	15.11 ± 0.8 b	2.29 ± 0.2 b	3.86 ± 0.3 b	nd	1.94 ± 0.1 b
white onion	24.49 ± 1.2 d	3.99 ± 0.3 c	4.58 ± 0.4 c	28.34 ± 1.3 b	2.64 ± 0.1 b
white onion BL90″	23.90 ± 1.1 d	3.32 ± 0.2 c	3.71 ± 0.3 b	22.11 ± 1.1 b	2.56 ± 0.1 b
white onion BO10′	19.43 ± 0.8 c	2.69 ± 0.2 b	3.30 ± 0.3 a	14.14 ± 0.9 a	1.87 ± 0.05 a
white onion F10′	23.23 ± 0.9 d	3.12 ± 0.2 c	3.62 ± 0.3 a	21.14 ± 1.0 b	2.48 ± 0.1 b
white onion BL90″+M	22.64 ± 0.9 d	3.02 ± 0.2 c	3.51 ± 0.3 a	20.89 ± 1.0 b	2.36 ± 0.1 b
white onion BO10′+M	18.23 ± 0.7 b	2.56 ± 0.2 b	2.90 ± 0.2 a	12.09 ± 0.7 a	1.50 ± 0.05 a
white onion F10′+M	22.15 ± 0.8 c	2.98 ± 0.2 c	3.42 ± 0.3 a	20.09 ± 0.8 b	2.24 ± 0.1 b
red onion	29.72 ± 1.2 d	3.84 ± 0.3 c	5.93 ± 0.4 d	460.22 ± 10.9 e	4.88 ± 0.1 d
red onion BL90″	24.35 ± 1.1 d	2.75 ± 0.2 b	5.73 ± 0.4 d	352.18 ± 10.3 d	3.44 ± 0.1 c
red onion BO10′	22.04 ± 0.8 c	2.54 ± 0.1 b	5.44 ± 0.4 d	281.09 ± 11.8 c	3.29 ± 0.05 c
red onion F10′	23.90 ± 0.9 d	2.69 ± 0.2 b	5.51 ± 0.4 d	358.18 ± 10.7 b	3.36 ± 0.1 c
red onion BL90″+M	24.19 ± 0.9 d	2.63 ± 0.2 b	5.46 ± 0.4 d	337.41 ± 11.2 d	3.40 ± 0.1 c
red onion BO10′+M	21.44 ± 0.7 c	2.52 ± 0.2 b	5.10 ± 0.4 d	231.18 ± 11.2 c	2.28 ± 0.1 b
red onion F10′+M	23.01 ± 0.8 c	2.46 ± 0.2 b	5.29 ± 0.4 d	318.14 ± 11.9 c	3.30 ± 02 c

[a] Values are means ± SD of five measurements. Means in columns without letters in common differ significantly ($P < 0.05$). Garlics: lyophilized raw garlic (garlic), blanched for 90 s (garlic BL90″), boiled for 10 min (garlic BO10′), fried for 10 min (garlic F10′), blanched and microwaved (M) (garlic BL90″+M), boiled for 10 min and M (garlic BO10′+M), fried for 10 min and M (garlic F10′+M). White onions: lyophilized white onion (white onion), blanched for 90 s (white onion BL90″), boiled for 10 min (white onion BL10′), fried for 10 min (white onion F10′), blanched for 90 s and M (white onion BL90″+M), boiled for 10 min and M (white onion BO10′+M), fried for 10 min and microwaved (white onion F10′+M). Red onions: lyophilized raw red onion (red onion), blanched for 90 s (red onion BL90″), boiled for 10 min (red onion BL10′), fried for 10 min (red onion F10′), blanched for 90 s and microwaved (M) (red onion BL90″+M), boiled for 10 min and M (red onion BO10′+M), fried for 10 min and M (red onion F10′+M). The power was 500 W and the microwaving process lasted 6 min for garlic samples and 5 min for onions. CGE, cyanidin-3-glucoside equivalent; CE, catechin equivalent; DW, dry weight; nd, not detected. Polyphenols are expressed as milligrams of gallic acid equivalent (GAE).

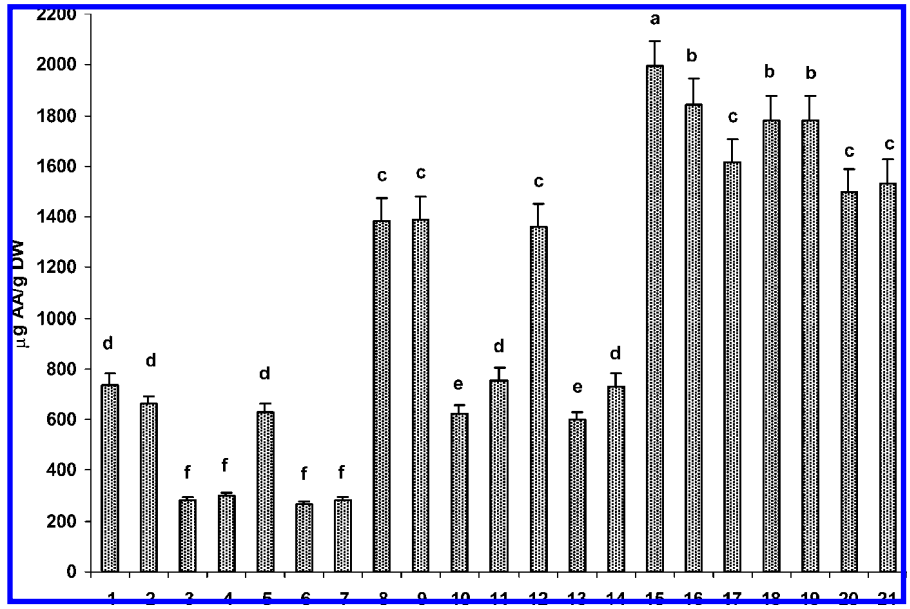

Figure 3. Changes in the amounts of ascorbic acid in garlic and white and red onions after processing (means ± SD). Bars with different letters are significantly different ($P < 0.05$). 1, garlic; 2, garlic BL90″; 3, garlic BO10′; 4, garlic F10′; 5, garlic BL90″+M; 6, garlic BO10′+M; 7, garlic BO10′+M; 8, white onion; 9, white onion BL90″; 10, white onion BO10′; 11, white onion F10′; 12, white onion BL90″+M; 13, white onion BO10′+M; 14, white onion F10′+M; 15, red onion; 16, red onion BL90″; 17, red onion BO10′; 18, red onion F10′; 19, red onion BL90″+M; 20, red onion BO10′+M; 21, red onion F10′+M.

during the heat treatment were the highest for white onions, moderate for garlic, and relatively low for red onions (**Figure 4**; **Tables 2** and **3**). Oppositely, other papers (*2*) have shown relatively low amounts of polyphenols for garlic and onions and high antioxidant activity by oxygen radical absorbance capacity (ORAC). The linear relationship between total phenolics and type of cooking was high. This can be explained by the involvement of additional bioactive compounds in the overall antioxidant activity of the studied vegetables (*6–8*). Changes in flavonoid content [mg of quercetin equivalents (QE)/g of DW] of onions were reported for raw onions (4.94): blanching, 3.01; boiling for 3 min, 2.65; and microwaving, 2.89 (*33*). Our data calculated on QE (**Table 2**) were similar to those reported. Minimal changes in the amount of ascorbic acid were registered in the samples, which were subjected to minimal time of different treatments: garlic BL90″ and garlic F10′; white onion

Table 3. Changes in the Antioxidant Activity of the Studied Vegetables Samples (Dry Weight), Subjected to Different Technology Treatments As Shown by All Four Used Tests[a]

sample	DPPH (μM TE/g)	FRAP (μM TE/g)	CUPRAC (μM TE/g)	ABTS (μM TE/g)
garlic	34.86 ± 3.4 d	11.95 ± 0.8 b	29.00 ± 1.4 b	43.73 ± 1.7 c
garlic BL90″	29.05 ± 2.9 c	10.55 ± 0.8 a	21.65 ± 1.0 b	38.77 ± 1.6 b
garlic BO10′	16.86 ± 1.2 a	7.40 ± 0.4 a	13.40 ± 0.9 a	30.43 ± 1.2 a
garlic F10′	30.83 ± 1.2 a	10.87 ± 0.4 a	21.85 ± 0.9 b	38.92 ± 1.2 b
garlic BL90″+M	27.21 ± 2.8 b	10.01 ± 0.8 a	20.08 ± 1.4 b	35.01 ± 1.5 b
garlic BO10′+M	14.81 ± 0.9 a	6.63 ± 0.3 a	12.66 ± 0.9 a	27.16 ± 1.2 a
garlic F10′ +M	27.67 ± 1.1 c	10.11 ± 0.3 a	20.65 ± 0.9 b	37.76 ± 1.2 b
white onion	24.65 ± 2.4 b	23.22 ± 1.2 c	37.92 ± 2.9 c	50.94 ± 3.0 d
white onion BL 90″	21.11 ± 2.2 b	22.10 ± 1.1 c	35.21 ± 2.9 c	48.15 ± 3.0 c
white onion BO10′	14.21 ± 1.8 a	16.49 ± 1.0 b	25.94 ± 2.8 b	36.08 ± 2.8 b
white onion F10′	22.98 ± 1.9 b	22.87 ± 1.0 c	35.21 ± 2.8 c	49.54 ± 2.8 d
white onion BL90″+M	20.48 ± 2.0 b	23.02 ± 1.1 c	34.25 ± 2.9 c	47.04 ± 3.0 c
white onion BO10′+M	12.42 ± 1.7 a	15.44 ± 1.0 b	24.87 ± 2.7 b	34.25 ± 2.9 b
white onion F10′ +M	20.87 ± 1.8 b	23.39 ± 1.1c	34.79 ± 2.7 c	47.32 ± 2.9 c
red onion	41.33 ± 3.9 e	31.05 ± 1.5 d	59.17 ± 3.1 d	69.88 ± 3.1 f
red onion BL90″	36.61 ± 3.4 d	27.24 ± 1.4 d	57.20 ± 3.0 d	59.78 ± 3.0 e
red onion BO10′	28.15 ± 2.9 c	22.75 ± 1.2 c	54.16 ± 2.8 d	50.11 ± 2.9 d
red onion F10′	36.97 ± 3.0 d	27.78 ± 1.3 d	57.48 ± 2.9 d	59.95 ± 2.9 e
red onion BL90″+M	34.36 ± 3.0 d	26.14 ± 1.4 d	56.89 ± 3.0 d	56.56 ± 2.9 e
red onion BO10′ +M	26.84 ± 2.9 c	20.17 ± 1.2 c	53.32 ± 2.8 d	48.78 ± 2.8 c
red onion F10′ +M	34.65 ± 2.9 d	26.81 ± 1.3 d	56.91 ± 2.8 d	57.87 ± 2.9 e

[a] Values are means ± SD of five measurements. Means in columns without letters in common differ significantly ($P < 0.05$). Garlics: lyophilized raw garlic (garlic), blanched for 90 s (garlic BL90″), boiled for 10 min (garlic BO10′), fried for 10 min (garlic F10′), blanched and microwaved (M) (garlic BL90″+M), boiled for 10 min and M (garlic BO10′+M), fried for 10 min and M (garlic F10′+M). White onions: lyophilized white onion (white onion), blanched for 90 s (white onion BL90″), boiled for 10 min (white onion BL10′), fried for 10 min (white onion F10′), blanched for 90 s and microwaved (white onion BL90″+M), boiled for 10 min and microwaved (white onion BO10′+M), fried for 10 min and microwaved (white onion F10′+M). Red onions: lyophilized raw red onion (red onion), blanched for 90 s (red onion BL90″), boiled for 10 min (red onion BL10′), fried for 10 min (red onion F10′), blanched for 90 s and microwaved (red onion BL90″+M), boiled for 10 min and microwaved (red onion BO10′+M), fried for 10 min and microwaved (red onion F10′+M). The power was 500 W, and the process of microwaving lasted for 6 min for garlic samples and for 5 min for onions. DPPH, 1,1-diphenyl-2-picrylhydrazyl method; FRAP, ferric reducing/antioxidant power; CUPRAC, cupric reducing antioxidant capacity; ABTS, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); TE, Trolox equivalent.

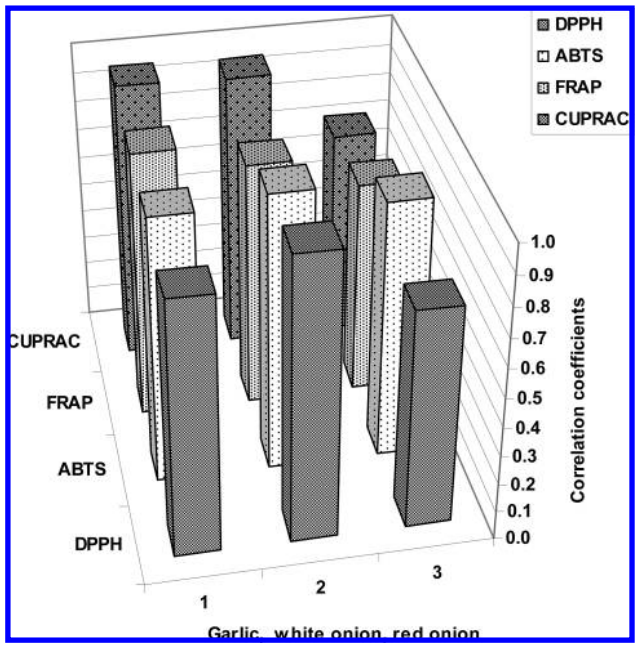

Figure 4. Calculated correlation coefficients between the polyphenols during the processing in garlic and white and red onions and antioxidants determined by different assays (DPPH, FRAP, CUPRAC, and ABTS). DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing/antioxidant power; CUPRAC, cupric reducing antioxidant capacity; ABTS, 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid).

BL90″ and white onion F10′; red onion BL90″ and red onion F10′. The reported data (μg of AA/g of DW) for white (*24*) and red onions (*30*) and garlic (*36*) were 723, 1520, and 332, respectively, in comparison with our data (**Figure 3**). Changes of ascorbic acid (μg of AA/g of DW) during treatment showed that raw garlic had 220.1, but after boiling only 138.6 remained, losing about 38% (*37*), and these results were lower than our data of raw garlic, 735, and 282 after boiling during 10 min (**Figure 3**).

Protein Extraction and Electrophoresis. The obtained electrophoretic patterns of white and red onions were similar; therefore, we present only the separations of red onion proteins with reduction, using β-ME, and without reduction (**Figure 5A**) and extracted from garlic and applied to the gel without reduction (**Figure 5B**). It is interesting that the spectra of raw garlic and red onion have specific bands in the range of molecular masses from 50 to 112 kDa that disappeared after heat treatment (**Figure 5A**, lane 8; **Figure 5B**, lane 1). The boiling procedure for more than 20 min caused degradation of the proteins in studied vegetables. The 14 kDa bands in raw garlic and onions during electrophoretic separation without reduction had more proteins than the treated samples. The 50, 70, 77, 100, 102, and 112 kDa bands disappeared after treatment, probably as less stable proteins in these vegetables. Literature data indicate that the superoxide dismutase (SOD) antioxidant activities during boiling, microwaving, and baking were diminished for garlic by about 86, 96, and 90% and for onions by about 60, 77, and 26%, respectively (*37*), describing the changes of amino acid composition during processing (*36*). This means that even minimal time of heating effects the protein pattern intensity, but it is visible only for nonreduced samples. Similar data were obtained in our previous studies (*15, 16*).

The onion, nucleolin-like protein, migrated as two clusters of acidic spots, 43 and 42 kDa, respectively, in molecular mass (*19*): the soluble nuclear fraction to identify plant cell phos-

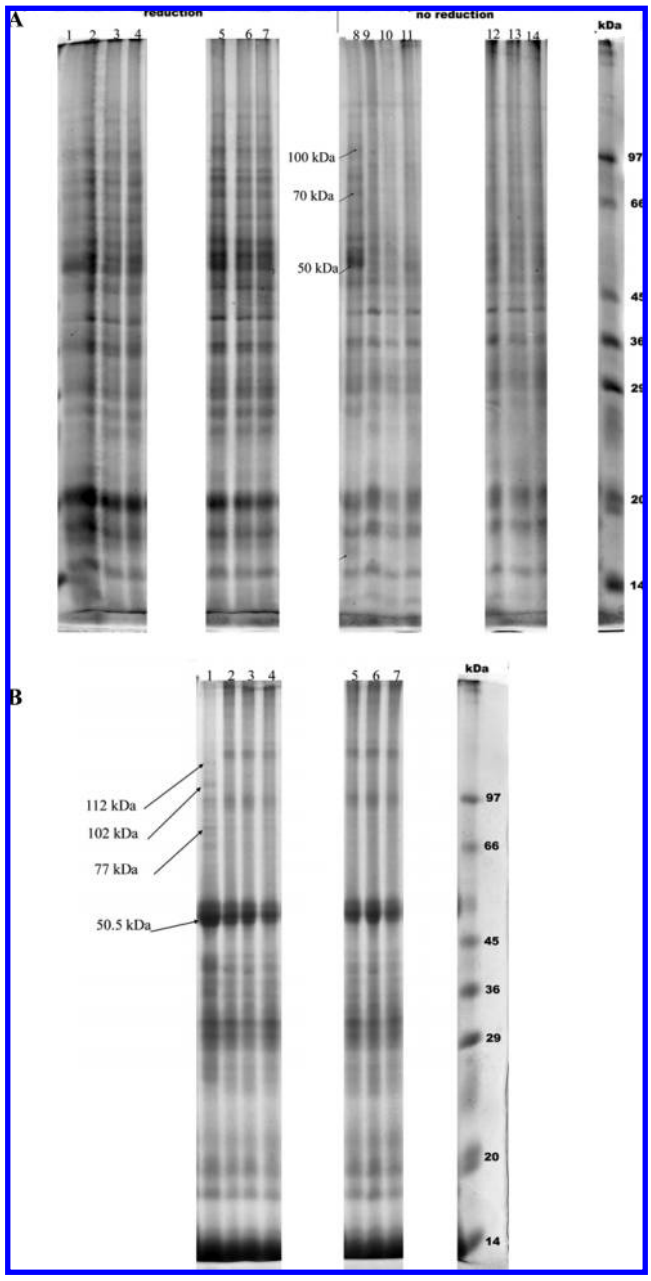

Figure 5. Comparison of the band intensity of proteins extracted from garlic samples and separated by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE). (**A**) Lanes: 1, red onion; 2, red onion BL90″; 3, red onion BO10′; 4, red onion F10′; 5, red onion BL90″+M; 6, red onion BO10′+M; 7, red onion F10′+M (lanes 1−7, with reduction; lanes 8−14, no reduction); 8, red onion; 9, red onion BL90″; 10, red onion BO10′; 11, red onion F10′; 12, red onion BL90″+M; 13, red onion BO10′+M; 14, red onion F10′+M (without reduction). (**B**) Lanes: 1, garlic; 2, garlic BL90″; 3, garlic BO10′; 4, garlic F10′; 5, garlic BL90″+M; 6, garlic BO10′+M; 7, garlic BO10′+M. Molecular markers (kDa): α-lactaalbumin (14 kDa), trypsin inhibitor (20 kDa), carbonic anhydrase (29 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), ovalbumin (45 kDa), bovine serum albumin (66 kDa), phosphorylase *b* (97 kDa). Abbreviations: lyophilized raw red onion (red onion), blanched for 90 s (red onion BL90″), boiled for 10 min (red onion BL10′), fried for 10 min (red onion F10′), blanched for 90 s and microwaved (red onion BL90″+M), boiled for 10 min and microwaved (red onion BO10′+M), fried for 10 min and microwaved (red onion F10′+M).; lyophilized raw garlic (garlic), blanched for 90 s (garlic BL90″), boiled for 10 min (garlic BO10′), fried for 10 min (garlic F10′), blanched and microwaved (M) (garlic BL90″+M), boiled for 10 min and M (garlic BO10′+M), fried for 10 min and M (garlic F10′+M). The power was 500 W, and the process of microwaving lasted 6 min for garlic and 5 min for onions.

phoproteins that are considered to be markers proliferation. In some cited reports (*38*) a protein of 289 amino acids with a calculated molecular mass of 30.6 kDa and a p*I* of 6.52 was isolated from onion (*A. cepa* L.). Such bands have appeared also in the investigated samples (**Figures 5A**, lanes 1−7; and **Figure 5B**, lanes 1−4). The 14 kDa pattern in raw garlic and onions was intensive, which corresponds to the data of others (*18*), where the 26S proteasome (multicatalytic protease complex, MPC) was purified from fresh garlic cloves (*A. sativum*). Polyacrylamide gel electrophoresis under denaturing conditions separated the garlic MPC into multiple polypeptides having molecular masses in the ranges of 21−35 and 50−112 kDa.

As was expected, the used technology treatment led to a decrease in the amount of bioactive compounds and the level of antioxidant activity. This decrease was connected with the kind of treatment and its duration. It was found that blanching and microwaving of garlic and onions for 90 s and frying did not decrease significantly the quantities of their bioactive compounds and the level of antioxidant activity ($P > 0.05$). These findings were comparable with the data of others (*8, 14*). The decreases in the bioactive compounds mostly based on polyphenols during boiling and microwaving were about 20% for garlic, 26% for white onions, and the lowest for red onions at 19% (**Table 2**).

These findings were supported by others (*12*); it was described that all thermal treatments, except microwave heating, reduced anthocyanin content in different vegetables by about 35−24% in comparison with our data of 30% (**Table 2**). Similar numbers were reported for steamed vegetables (*2*), which retained about 80% of phenolics, and for boiled vegetables, which retained only 30% of antioxidants. Boiling of onions leads to about 30% loss of quercetin glycosides (*37*), which transfers to the boiling water, but frying does not affect flavonoid intake (**Tables 2 and 3**).

The possible reasons why bioactive compounds, antioxidant activities, and proteins in garlic and onions changed after various cooking methods can be explained by their different physical properties (texture, color, matrix softening, increased extractability). As described under Materials and Methods, garlic was microwaved for 6 min, and for the same treatment onions required only 5 min. 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 work that all bioactive compounds and their antioxidant activities have differently changed during blanching, boiling, frying, and microwaving, and this is in agreement with others, who showed that during frying the antioxidant activity was higher in comparison with other methods (*12, 17, 37*). The increase in the antioxidant activities determined by ABTS and FRAP assays in the treated vegetables in comparison with the raw ones, which were explained by the conversion of antioxidants to higher amounts of antioxidant chemical species (*17*), does not agree with the results shown in the present paper. However, the correlation coefficients were from 0.96 to 0.87 for garlic, from 0.95 to 0.85 for white onions, and from 0.88 to 0.72 for red onions, showing that there is not a proportional decrease in the antioxidant activities and the bioactive compounds during processing.

In conclusion, the processing of garlic and onions leads to decreases in the amounts of their bioactive compounds, antioxidant activity, and some differences in the protein profile. This decrease was connected to the kind of treatment and its duration: only blanching and then microwaving of garlic and onions for 90 s did not decrease significantly the amounts of

their bioactive compounds and the level of the antioxidant activity ($P > 0.05$).

ABBREVIATIONS USED

ABTS, 2,20-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; CUPRAC, cupric reducing antioxidant capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl method; DW, dry weight; FCR, Folin−Ciocalteu reagent; FRAP, ferric reducing/antioxidant power; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; DMACA, *p*-dimethylaminocinnamaldehyde; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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