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Comparison of the Bioactive Compounds and Antioxidant Potentials of Fresh and Cooked Polish, Ukrainian, and Israeli Garlic

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Garlic (*Allium sativum* L.) is an essential part of Polish, Ukrainian, and Israeli cuisine. The aim of this investigation was to compare the changes in bioactive compounds, proteins, and antioxidant potentials in fresh Polish, Ukrainian, and Israeli garlic samples after subjection to cooking temperature. Dietary fiber and essential trace elements were comparable. The antioxidant potentials were determined by four scavenging methods using β-carotene, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO), and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) radical cation with $K_2S_2O_8$ or MnO_2 assays. Polyphenols, tocopherols, proteins, and antioxidant potentials were higher in Polish garlic, but not significantly ($P > 0.05$). The SDS- and native-PAGE electrophoretic patterns of all three fresh garlic samples were without significant differences. Most of the proteins were in the molecular mass range of 24−97 kDa, and the more intensive major bands were concentrated at 50 and 12 kDa. The 50 kDa protein nearly disappears and the intensity of the 12 kDa lectin bands slightly decreases during cooking. It was observed that the bioactive compounds, antioxidant potential, and proteins in garlic decrease significantly after 20 min of cooking at 100 °C ($P < 0.05$). In conclusion, (a) the bioactive compounds, electrophoretic patterns, and antioxidant potential of fresh Polish, Ukrainian, and Israeli garlic samples are comparable; (b) garlic samples subjected to 100 °C during 20 min preserve their bioactive compounds, antioxidant potential, and protein profile and are comparable with fresh garlic; and (c) fresh garlic should be added to dishes cooked at 100 °C in the last 20 min of the cooking process.

KEYWORDS: Fresh and cooked garlic; bioactive compounds; antioxidant potential

INTRODUCTION

Garlic (*Allium sativum* L.) is one of the bulbous rooty vegetables, which has been used for many centuries both as flavoring and as folk herbal medicine (*1*, *2*). In the past this vegetable was mostly valued as a source of aroma compounds (*1*). It was shown that the garlic aroma character is determined by the presence of di-2-propenyl disulfide. However, garlic contains also a wide spectrum of bioactive compounds, which include dietary fiber and trace elements, especially Se and phenolics. Selenomethionine is the major selenocompound in Se accumulator plants and some plants of economic importance such as garlic exposed to excess Se. Se incorporation into garlic significantly increases activities of garlic in cancer prevention and inhibition (*3*, *4*).

No doubt, the successful use of garlic in folk medicine is connected to the above-mentioned compounds (*5*). At present, the health-promoting properties of this vegetable have attracted

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considerable interest also from scientific medical investigators (*6*), claiming that garlic plays a certain role even in cancer prevention (*3*, *7*). However, by far the most studied and reported health-promoting effect of garlic is cardioprotection (*1*). Our review of the recent published papers shows that garlic possesses plasma lipid-lowering, anticoagulant, and antioxidant properties (*8*−*10*).

Garlic is widely used in Poland, Ukraine, and Israel as an obligatory part of many cooked dishes. It is common knowledge that vegetables exposed to temperature lose a certain part of their bioactivity. However, we did not find publications describing changes in the bioactivity and protein spectrum of garlic subjected to cooking temperature.

Therefore, we decided (a) to determine in vitro the content of the essential bioactive compounds, the antioxidant potential, and the protein profile of fresh garlic and Polish, Ukrainian, and Israeli garlic subjected to cooking and (b) to find the optimal cooking regimen, which preserves intact the bioactive compounds, antioxidant potential, and protein composition of garlic.

As far as we know there have been no such investigations.

MATERIALS AND METHODS

Chemicals. 6-Hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid (Trolox), β-carotene, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Griess reagent, sodium nitroprusside, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Folin−Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO), and ABTS was purchased from Fluka Chemie (Buchs, Switzerland). All reagents were of analytical grade.

Garlic Samples. Fresh Polish garlic samples were from the Cracow region, Ukrainian samples were from Zoluchev of the Lviv region, and Israeli samples were from Galilee and were purchased from local growers at the peak of their maturity by the respective participants of this international project.

The garlic samples were harvested in the period of 2002−2003. Harvest time covered 2 weeks around the following dates: for Poland and Ukraine about October and for Israel in February. Garlic was harvested when the bulbs were well mature: the tops had fallen and were very dry. The garlic bulbs were clean, white, and well cured (dried neck and outer skins). The cloves were firm to the touch. Cloves from mature bulbs had a high dry weight and soluble solids content (>35% in both cases). Each preparation was carried out with a separate batch of garlic bulbs from each location. The bulbs were cracked to separate the cloves. The defective and smallest ones were discarded, and the rest were peeled and immediately subjected to three different temperature regimens in a common oven (Davo) at 100 °C for 20, 40, or 60 min. After the thermal treatment, the samples were immediately prevented from oxidizing by placing them under liquid nitrogen, lyophilized, and analyzed.

Dietary Fiber. Dietary fiber in the selected samples was analyzed according to the method of Prosky et al. (*11*). The results are given in grams per kilogram of fresh weight (FW).

Trace Elements. The contents of cobalt, copper, iodine, manganese, selenium, and zinc were determined as previously described (*12*).

Total Polyphenols. One hundred milligrams of lyophilized sample was deproteinated with acetonitrile and washed with dichloromethane. Then a 50 mg aliquot of deproteinated lyophilized sample was accurately weighed in a screw-capped tube. The total phenols were extracted with 5 mL of 1.2 M HCl in 50% methanol/water. The samples were vortexed for 1 min and heated at 90 °C for 3 h with vortexing every 30 min. After the samples had cooled, they were diluted to 10 mL with methanol and centrifuged for 5 min at 4000*g* with a benchtop centrifuge to remove solids. The phenols were measured at 750 nm after reacting for 10 min, using the Folin−Cocialteu reagent, and diluted 5-fold before use, with gallic acid as standard (*13*). The results are given in milligrams per 100 g of FW of gallic acid equivalent (GAE).

Total Tocopherols. Total tocopherols were determined following the IUPAC standard method as previously described (*14*). The results are given in micrograms per 100 g of FW.

Total Antioxidant Potentials. There are many methods for total antioxidant potential determination; each has its limitations, and some give different antioxidant activity trends (*15*, *16*). Therefore, we decided to determine the antioxidant potential of fresh garlic and Polish, Ukrainian, and Israeli garlic samples subjected to cooling temperature by four different tests:

1. Antioxidant Assay Using β-Carotene Linoleate Model System (β-Carotene). To an emulsion of β-carotene (0.2 mg) in 0.2 mL of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) was added 40 mL of oxygenated water, which initiated this assay as an oxidant. Four milliliter aliquots of this emulsion were added to test samples. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching β-carotene, measuring the absorbance at 470 nm, during $t = 180$ min at an interval of 15 min: $AA = 100\,[1 - (A_0 - A_t)/(A°_0 - A°_t)]$, where A_0 and $A°_0$ are the absorbance values measured at zero time and A_t and $A°_t$ are the absorbance values measured in the test sample and control, respectively, after incubation for 180 min (*17*).

2. Radical Scavenging Activity Using DPPH Method. Five milliliters of a 0.1 mM methanolic solution of DPPH was added to 100 μL of studied samples. Changes in the absorbance of the samples and standards were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated as follows: % radical scavenging activity = (control OD − sample OD/control OD) × 100 (*17*).

3. Scavenging Activity against Nitric Oxide (NO). A 0.5 mL portion of a mixture (0.4 mL of garlic extract and 0.1 mL of sodium nitroprusside solution) was diluted with 0.3 mL of Griess reagent. The absorbance of the chromophore formed during the diazotination of nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 570 nm and referred to the absorbance of standard solutions of sodium nitrite salt treated in the same way with Griess reagent. The nitrite concentration was calculated by referring to the absorbance of standard solutions of potassium nitrite. Results were expressed as percentage nitrite production with respect to control values (*18*).

4. $ABTS^{•+}$ Radical Cation Decolorization Assay with $K_2S_2O_8$ or MnO_2. (A) $ABTS^{•+}$ radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). After the addition of 990 μL of $ABTS^{•+}$ solution to 10 μL of different extracts (0.2 mg/mL) or Trolox standards (final concentration of 0−20 μM) in ethanol or phosphate-buffered saline (PBS), the absorbance was monitored exactly 1 and 6 min after the initial mixing.

(B) $ABTS^{•+}$ was prepared as well by passing a 5 mM aqueous stock solution of ABTS through manganese dioxide on a Whatman no. 5 filter paper. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2 μM Whatman PVDF syringe filter. This solution was then diluted in a 5 mM phosphate-buffered saline, pH 7.4, to an absorbance of 0.70. The percentage decrease of the absorbance at 734 nm in both the $K_2S_2O_8$ and MnO_2 assays was calculated and plotted as a function of the concentration of the extracts and of Trolox for the standard reference data (*19*). Trolox, BHT, and BHA were used as standards in these methods.

Determination of Proteins. Five milligrams of each sample was used for the determination of protein content according to the method of Bradford (*20*).

SDS-PAGE Protein Extraction. All garlic samples (80 mg of each) were dissolved in 1 mL of sample buffer (2% SDS, 10% glycerol, 2%-mercaptoetanol, 0.002% bromophenol blue, and 0.62 M Tris-HCl, pH 6.8). The extracts were kept overnight at room temperature. Samples then were boiled for 5 min and then centrifuged at 18000*g* for 15 min at 15 °C. SDS-PAGE was performed with the Hoeffer SE 600 vertical unit (Hoeffer Scientific Instruments, San Francisco, CA) according to the method of Laemmli (*21*) using polyacrylamide gels (resolving gel $T = 13.7\%$, $C = 1.7\%$, stacking gel $T = 3.8\%$, $C = 1.8\%$) with a gel size of 180 × 160 × 1.5 mm. Sample size was 5 μL. The run was carried out at 25 mA per gel until the end of electrophoresis. Gels were stained with 0.25% Coomassie Brillant Blue R in methanol/water/glacial acetic acid (5:5:1 v/v) and destained in water.

Native-PAGE Protein Extraction. The meal (160 mg for each sample) was transferred to a 1.5 mL Eppendorf tube. To each tube was added

Table 1. Dietary Fiber Content (Grams per Kilogram) in Edible Parts of Fresh Polish, Ukrainian, and Israeli Garlic Samples[a]

	Polish	Ukrainian	Israeli
total	23.1 ± 2.1a	22.9 ± 2.1a	22.8 ± 3.1a
soluble	8.2 ± 0.6a	8.2 ± 0.7a	8.1 ± 1.1a
insoluble	14.9 ± 1.2a	14.7 ± 1.2a	14.7 ± 1.6a

[a] Values are means ± SD of five measurements. Means in rows without letters in common differ significantly ($P < 0.05$).

Table 2. Content of Essential Trace Elements in Polish, Ukrainian, and Israel Garlic (Micrograms in 100 g of Fresh Weight)[a]

trace element	Polish	Ukrainian	Israeli
manganese	446.9 ± 21.4a	435.8 ± 21.0a	460 ± 21.3a
copper	43.3 ± 7.7a	144.1 ± 7.5a	145.1 ± 7.6a
zinc	556.1 ± 27.1a	555.7 ± 27.1a	554.9 ± 27.1a
iodine	2.5 ± 0.1a	2.4 ± 0.1a	2.6 ± 0.1a
selenium	5.5 ± 0.2a	5.4 ± 0.2a	5.4 ± 0.2a
cobalt	traces	traces	traces

[a] Data are means ± standard deviations of five measurements. Means in rows with different letters differ significantly ($P < 0.05$).

1 mL of the solvent (3 M urea, 1% 2-Me, methyl green 0.025%). The tubes were vortexed and allowed to stand overnight at room temperature, then vortexed again, and centrifuged at 18000*g* for 5 min [resolving gel (7%): 15.4 mL of monomers solution, 53.05 mL of water, 1.54 mL of glacial acetic acid]. The solution was degassed and kept cool (prior to freezing), and then 50 μL of 3% hydrogen peroxide was added (for four gels of 180 mm × 160 mm × 0.75 mm) [stacking gel (5%): 1.56 mL of monomer solution, 89 μL of glacial acetic acid, 8.32 mL of water, 11.2 μL of 3% hydrogen peroxide]. The monomers solution consisted of 30.4 g of acrylamide, 1.6 g of bisacrylamide, 247 mg of ascorbic acid, 10 mg of $FeSO_4 \cdot 7H_2O$, water to 100 mL. The upper (+) electrode solution consisted of 186 mL of 0.005 M K_2SO_4 and 808 μL of 88% HCOOH made up with water to 800 mL. The lower (−) electrode buffer consisted of 19.9 mL of 88% HCOOH made up with water to 4.2 L. A total of 18−24 μL of protein extract was loaded per well. Electrophoresis was carried out at a constant voltage of 100 V for 15 min and then 500 V and terminated after the green dye had left the bottom of the cassette. The temperature was maintained at 15 °C. Scanning in transmission light with Agfa SnapScan 1236 (Agfa-Gevaert N.V Belgium, Agfa SnapScan 1236s color image scanner) was done. The following molecular mass markers (kDa) were used: 205, myosin; 116, β-galactosidase; 97, phosphorylase *b*; 66, bovine albumin; 45, ovalbumin; 36, glyceraldehyde-3-phosphatase; 29, carbonic anhydrase; 20, trypsin inhibitor; 14, α-lactalbumin.

Statistics. To verify the statistical significance of the studied parameters means (M) of five time analyzed samples ± standard deviation (SD) were defined. When appropriate, differences between groups were tested by two-way ANOVA. The *P* values of <0.05 were considered to be significant.

RESULTS

Significant differences in the content of total, soluble, and insoluble dietary fiber of the Polish, Ukrainian, and Israeli garlic samples were not found (**Table 1**).

The contents of the studied trace elements in the samples of Polish, Ukrainian, and Israeli garlic are comparable (**Table 2**). The studied garlic samples originated from three different geographical regions and did not differ with respect to their selenium content. Probably the selenium soil contents in the studied regions of Poland, Ukraine, and Israel are comparable. The results of selenium in the investigated samples were similar to the data of others (*3*, *22*).

No significant changes in dietary fibers and trace elements were observed in the cooked garlic samples.

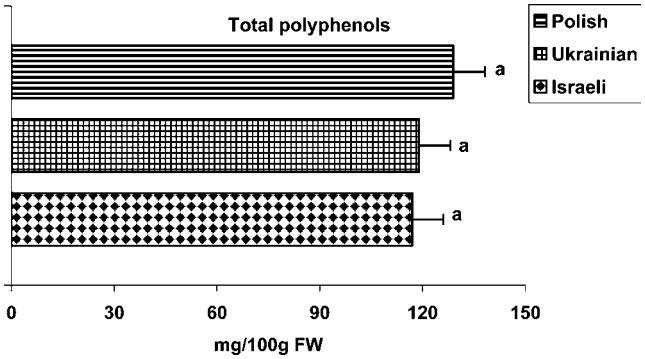

Figure 1. Polyphenols in fresh Polish, Ukrainian, and Israeli garlic samples. Mean values ± SD of five measurements (horizontal lines). Figures with different letters are significantly different ($P < 0.05$).

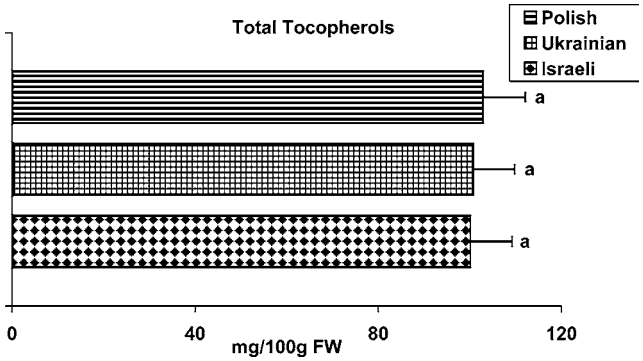

Figure 2. Tocopherols in fresh Polish, Ukrainian, and Israeli garlic samples. Mean values ± SD of five measurements (horizontal lines). Figures with different letters are significantly different ($P < 0.05$).

The content of total polyphenols in fresh Polish garlic was higher than in the Ukrainian and Israeli samples, but the difference was not significant (**Figure 1**).

As can be seen (**Figure 2**), the content of total tocopherols in fresh Polish garlic was higher than in Ukrainian and Israeli samples, but as in the case of total polyphenols the difference was not significant. No significant changes in the content of total tocopherols were observed in the cooked garlic samples. The mean value of total tocopherols in raw garlic (100−103 μg/100 g of FW) was lower than shown by others (*23*, *24*).

As was mentioned, after cooking, the amount of total tocopherol did not change significantly, and this result corresponds also with the data of others. This is very important because all tocopherols are known to be strong antioxidants, especially α- and γ-tocopherols (*25*).

The amount of proteins in the investigated samples varied from 2.96 to 2.64% and during processing had decreased by ∼15%.

Total polyphenols and total antioxidant potential in fresh garlic and samples subjected to cooking temperature of 100 °C for 20 min were comparable ($P > 0.05$). No significant changes in fresh and cooked garlic samples among Polish, Ukrainian, and Israeli were observed (**Table 3**).

The changes in total polyphenols and total antioxidant potential in the samples subjected to two other temperature regimens (40 and 60 min) were significant ($P < 0.05$).

The percentage of inhibition using the ABTS scavenging radical (**Figure 3A**) with $K_2S_2O_8$ (K) and with (**Figure 3B**) MnO_2 (M) depends on the time of assay (1−6 min), as well as the treated garlic sample. As can be seen, the results are very similar using these two methods and have shown the highest inhibition with fresh garlic and garlic treated during 20 min

Table 3. Total Polyphenols and Total Antioxidant Potential of Fresh Garlic and Samples Subjected to Three Different Temperature Regimes (100 °C for 20, 40, and 60 min)[a]

sample	total polyphenols (mg/100 g of FW)	DPPH[b] (% RSA)	NO[c] AA[d] (%)	β-carotene[e] AA (%)
fresh	47.3 ± 5.1a	68.9 ± 5.8a	61.7 ± 5.5a	71.8 ± 7.1a
100 °C, 20 min	43.7 ± 5.0a	67.9 ± 5.7a	59.2 ± 5.3a	70.6 ± 6.7a
100 °C, 40 min	33.2 ± 3.9b	48.1 ± 5.1b	42.7 ± 4.9b	54.5 ± 5.2b
100 °C, 60 min	31.7 ± 3.8b	46.9 ± 5.1b	40.9 ± 4.1b	52.6 ± 5.2b

[a] Values are means ± standard deviations of five measurements. Means in columns with different letters differ significantly ($P < 0.05$). [b] 1,1-Diphenyl-2-picrylhydrazyl. [c] Scavenging activity against nitric oxide. [d] Antioxidant activities,%. [e] β-Carotene linoleate model system.

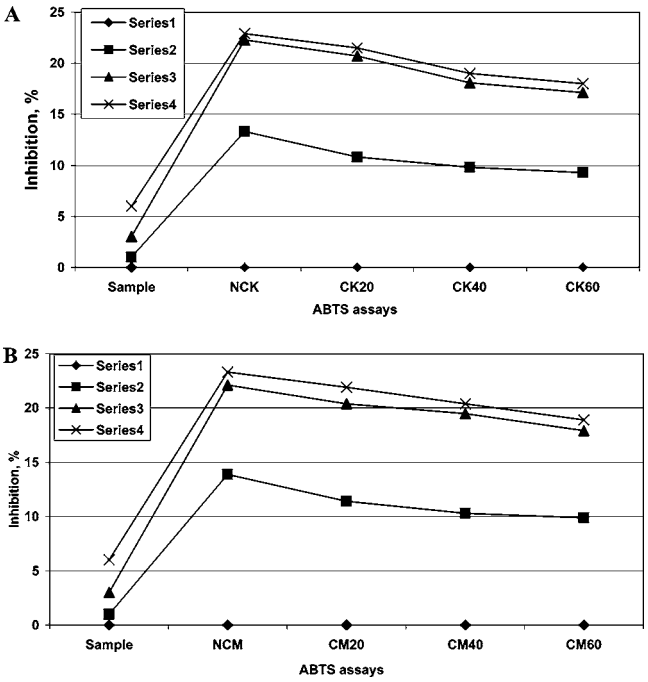

Figure 3. Kinetics of ABTS scavenging effect of garlic extracts. The concentration of the samples was 0.25 mg/mL. (**A**) ABTS was produced by reaction with potassium persulfate (K): noncooked (NCK), cooked for 20 min (CK20), cooked for 40 min (CK40), and cooked for 60 min (CK60) garlic samples. (**B**) ABTS radical cation was produced by reaction with manganese dioxide (M): noncooked (NCM), cooked for 20 min (CM20), cooked for 40 min (CM40), and cooked for 60 min (CM60) garlic samples. Series 1, 0 min; series 2, 1 min; series 3, 3 min; series 4, 6 min time of ABTS assay determination.

(series 4, NCK and CK20, **Figure 3A**; and series 4, NCM and CM20, **Figure 3B**). The decrease of the inhibition ability by ABTS between the fresh and cooked garlic samples during 20 min was ~6−8%.

Figure 4 shows the relationship between the percentage of inhibition determined by ABTS and DPPH and the time of thermal treatment. Fresh samples had the highest antioxidant activity followed by samples cooked for 20 min. Then the antioxidant activity was diminished followed by 40 min of cooking, and the lowest was determined at 60 min of cooking.

The electrophoretic patterns of the Polish, Ukrainian, and Israeli fresh garlic samples are presented in the **Figure 5**. On the basis of the SDS-PAGE system, sample buffer-soluble proteins have been separated into numerous components (37 bands). The molecular mass of detected components ranged from

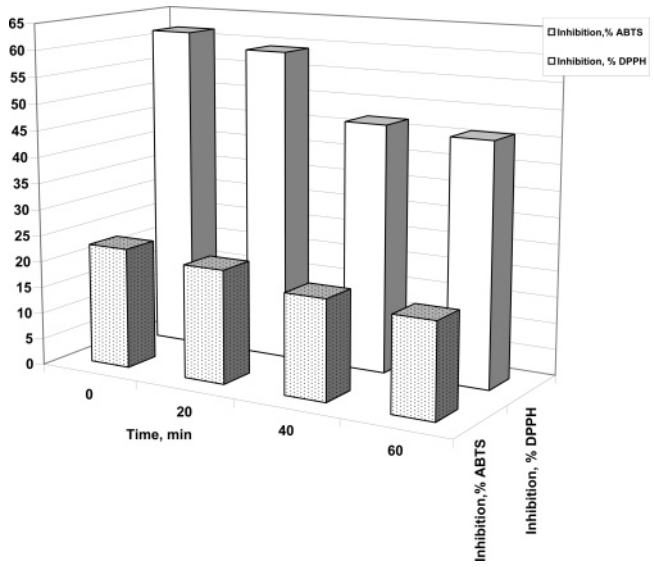

Figure 4. Percentage of inhibition determined by ABTS and DPPH scavengers in garlic samples followed by thermal treatment during 0, 20, 40, and 60 min.

10 to 205 kDa. The majority of the protein bands were in the molecular mass range of 24−97 kDa. In all examined patterns two duplicated, more intensive major components (50 and 12 kDa) were detected. Quantitative and qualitative differences between samples were found. Samples 2 and 3 differed from sample 1 by a higher density of 12 kDa bands and the presence of a band at 8 kDa (arrows in **Figure 5A**). Other bands were very weak and thin.

Figure 5B presents protein bands of fresh and cooked samples. About 20 bands were detected, mostly in the molecular mass range of 12−20 kDa. The bands of <8 kDa were diffused. The intense major components were concentrated between 16 and 12 kDa. Quantitative and qualitative differences in density of bands between protein patterns were found. An effect of cooking time on protein patterns was detected. The bands of all treated samples (2, 3, and 4) are clear, and the band density is lower than for corresponding bands of nontreated sample (**Figure 5B**, lane 1).

The phenomenon of decreasing intensity in almost all bands after 60 min of cooking can be explained by the effect of more advanced dissociation of proteins to subunits and polypeptides. Both lectins (12 kDa) and alliinase (50 kDa) are the predominant proteins in the bulbs, but the lectins are far more abundant than alliinase, as shown in the electrophoregram (**Figure 5B**).

DISCUSSION

Over the past 20 years the use of herbs and natural products has gained popularity, and now they are widely consumed (*6*, *8*, *10*). Garlic is one such herb, which has been used throughout the history of civilization for treating a wide variety of ailments (*1*, *2*, *5*). Garlic and garlic extracts are potent cardiovascular and anticancer reagents (*1*, *2*, *5*, *7*), which exercise hypocholesterolemic, thrombolitic, and antioxidant effects.

Therefore, garlic is a proper supplement to antiatherosclerotic diets. However, the garlic bioactive values could differ in different countries, and when subjected to cooking temperatures, garlic looses part of the bioactivity. Therefore, we decided to compare the contents of some bioactive compounds and the antioxidant potential of fresh garlic and Polish, Ukrainian, and Israeli garlic samples subjected to cooking temperature.

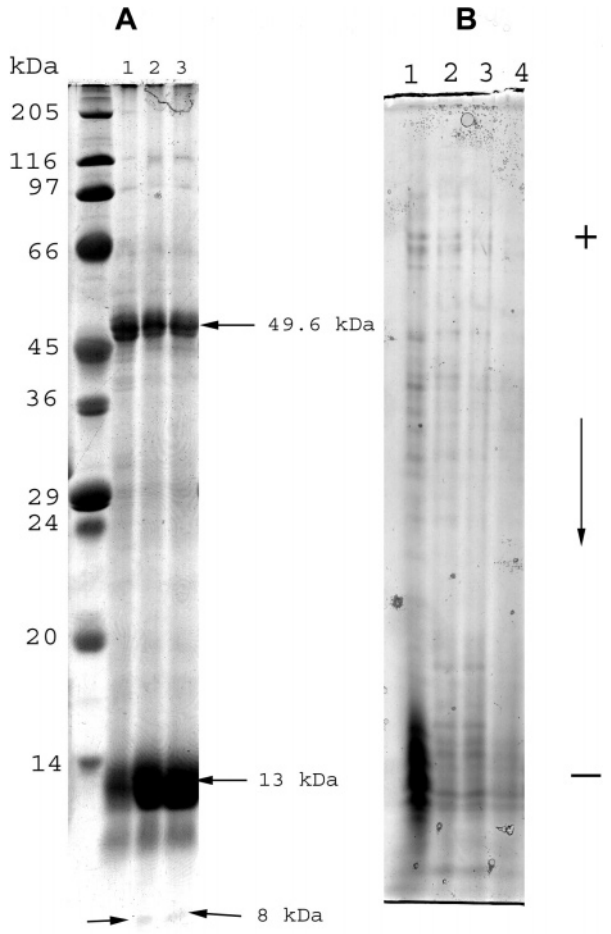

Figure 5. Comparison of the band intensity of proteins extracted from garlic samples and separated by SDS-PAGE (**A**) [lanes 1, 2, and 3, Polish, Ukrainian, and Israeli samples from the left-side molecular markers (kDa): 205, myosin; 116, β-galactosidase; 97, phosphorylase *b*; 66, albumin; 45, ovalbumin; 36, glyceralaldehyde-3-phosphate dehydrogenase; 29, carbonic anhydrase; 24, trypsinogen, PMSF-treated; 20, trypsin inhibitor; 14, α-lactalbumin] and by native-PAGE (**B**) (lane 1, fresh; and lanes 2, 3, and 4, treated at 100 °C for 20, 40, and 60 min, respectively).

Fruits and vegetables contain many different classes of individual antioxidants. Garlic is not an exception. The determination of only individual antioxidants could not be enough for assessment of the antioxidant potential of garlic. Therefore, we have determined also its total antioxidant potential by four different, complementary assays, based on different chemical mechanisms, which reflect the cumulative capacity to scavenge free radicals in order to show the real potential of garlic extracts in vitro studies (*17*−*19*).

We have found that the contents of dietary fibers and trace elements are comparable in all three studied garlic samples. Total polyphenols and total tocopherols were higher in Polish garlic, but the differences were statistically not significant. Some differences in the contents of selenium, total tocopherols, and proteins shown in this paper and data published by others may be due to the growing conditions. As was shown, the nutritional value of plants depends on environmental conditions such as temperature, season, location, moisture, soil fertility, and irrigation (*26*).

We also found that fresh garlic, subjected to a cooking regimen of 100 °C during 20 min, preserves its bioactivity: the decrease in the contents of the studied compounds and the decrease in the total antioxidant potential were statistically not significant ($P > 0.05$). The level of the antioxidant potential of the studied samples corresponds with the data of others who have determined the radical scavenging effect of garlic extracts in aqueous and lipophilic fractions and DPPH (*27*).

The electrophoretic patterns of fresh garlic and garlic subjected to a cooking temperature of 100 °C showed some differences. We cannot compare our data with the results of others: no data have been reported of the effect of thermal treatment on electrophoretic patterns in sample-buffer-soluble proteins.

The use of native-PAGE on gradient gels and stained by Coomassie Briilant Blue (CBB) has shown the presence of only a few bands in the range of 12 and 70 kDa. Combination of proteomics and analytical methods has allowed identification of these polypeptides as lectins and allinase, respectively (*28*−*31*). Malik et al. (*32*) identified in garlic the 26S proteasome (multicatalytic protease complex, MPC) on polyacrylamide gel under denaturing conditions. According to Mochizuki et al. (*28*) CBB staining of polyacrylamide gels was unsuitable for detecting protein bands in garlic as well in garlic products. In this case silver staining was successfully used, and many distinct bands between 12 and 200 kDa were obtained. Our results have shown that the use of 13.7% polyacrylamide gel, followed by conventional staining (CBB), allowed detection in fresh samples of a high number of bands (up of 37 in totals) too, but not clear, very thin ("weak") bands. Only two more distinct double bands (called earlier "major bands")—12 and 50 kDa—were detected. We suppose that 50 kDa double bands correspond with alliinase subunits and 12 kDa bands with lectins, respectively, which is supported by other investigations (*29*−*31*).

The process of cooking at a temperature of 100 °C for more than 20 min caused an increase of distinctness in almost all bands in comparison to fresh sample. This phenomenon is noticeable especially in the case of minor bands. The density of 50 kDa bands has nearly disappeared, and the intensity of 12 kDa lectin bands has slightly decreased. We suppose that lectins are more thermostable in such conditions. Alliinase is a dimeric protein composed of identical 50 kDa subunits (*29*−*31*). Both lectins and alliinase are the predominant proteins in the bulbs, but the lectins are far more abundant than alliinase (*30*). Maybe ϵ-*N*-pyrrolylnorleucine in garlic protein food products is directly responsible for antioxidant potentials from the protein profile (*29*). This compound seemed to be a natural antioxidant in many species of vegetables and marine and other food products.

In addition to the optimal treatment process, we can propose a simple, cheap method of the identification of numerous garlic bands for screening of a number of garlic samples. It can be very cost-effective when compared with both silver staining and ready-to-use gradient gels. Lectins from legume seeds are considered to be harmful from the nutritional point of view, thus requiring inactivation before consumption (*33*). Maybe in our case the cooking process of garlic is also important for the inactivation of lectins, but this is only a speculation and has to be investigated.

According to the electrophoretic patterns presented in this paper the same molecular weights were detected in nearly all garlic samples, but the main patterns were in the range of 12 and 50 kDa, as shown in the already cited literature data.

We were interested in answering the following question: Are there relationships between proteins (on the basis of its electrophoretic patterns) and antioxidant potential of garlic after cooking? It was found that the decrease of the total antioxidant potential is related to the decrease in the qualitative and qualitative properties of the protein spectra. The main antioxi-

dants are polyphenols, but proteins also play a part in this system. During cooking the soluble proteins were partially lost. Therefore, the total antioxidant potential of the garlic samples was decreased.

The following conclusions can be drawn: (a) The contents of the bioactive compounds, the electrophoretic protein pattern, and the antioxidant potential of the studied fresh Polish, Ukrainian, and Israeli garlic samples are comparable. (b) Fresh garlic subjected to a cooking temperature of 100 °C for 20 min preserves its bioactive compounds, antioxidant potential, and protein profile. (c) To preserve the bioactivity of fresh garlic, it should be added to cooked dishes in the last 20 min of the cooking process.

ABBREVIATIONS USED

β-carotene, β-carotene linoleate model system; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FW, fresh weight; GAE, gallic acid equivalent; NO, scavenging activity against nitric oxide; SD standard deviation.

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