

Raw and boiled garlic enhances plasma antioxidant activity and improves plasma lipid metabolism in cholesterol-fed rats

Shela Gorinstein^{a,1,*}, Hanna Leontowicz^b, Maria Leontowicz^b, Jerzy Drzewiecki^c,
Katarzyna Najman^b, Elena Katrich^a, Dinorah Barasch^a,
Kazutaka Yamamoto^d, Simon Trakhtenberg^e

^a Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University, Hadassah Medical School, Jerusalem, Israel

^b Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland

^c Plant Breeding and Acclimatization Institute, Radzikow, Poland

^d National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan

^e Kaplan University Medical Center, Rehovot, Israel

Received 19 February 2005; accepted 16 May 2005

Abstract

In the present study the effect of garlic, in a form more similar to how most people eat garlic, on lipid and antioxidant metabolism in rats was investigated. The antioxidant activity was determined by the efficacy to scavenge 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) derived radicals in garlic samples. The highest results were estimated in aqueous fraction in comparison with other extracts divided on the basis of polarity. Wistar male rats were randomly divided into 10 diet groups, each with seven animals. The groups were named: Control, RG (raw garlic), BG (boiled garlic for 20 min), AERG (aqueous extract of raw garlic), AEBG (aqueous extract of boiled garlic), Ch (Cholesterol), Ch/RG, Ch/BG, Ch/AERG and Ch/AEBG. All experimental diets were supplemented with 25 mg of lyophilized garlic/kg body weight obtained from raw, boiled and their aqueous extracts over a period of 30 days.

Serum lipid (total cholesterol, LDL-cholesterol and triglycerides) concentrations were higher in all groups fed cholesterol (Ch); however, the increase was significant only in Ch group, without garlic supplementation. In groups of rats fed diets with cholesterol, garlic samples significantly hindered the rise of TC and LDL-C ($P < 0.05$). A significant increase ($P < 0.05$) in the plasma antioxidant activity was registered in experimental groups of rats fed cholesterol-free diets supplemented with garlic; oppositely, a significant decrease was only in group of rats given food containing cholesterol without garlic.

The protein spectra has shown that during short boiling some proteins change their functional properties such as solubility and mobility, resulting in a number of protein bands in SDS-electrophoresis.

Conclusions: Raw and boiled garlic improved plasma lipid metabolism and plasma antioxidant activity in an experiment on rats. Thus, dietary hypolipidemic garlic was effective in reducing the oxidant stress, which was indicated by an increase of antioxidant activity and a decrease of lipids in the rats' blood. It was found that garlic boiled for 20 min has the same bioactivity as raw garlic in its antioxidant and protein spectra. Therefore it should be added at this time to foods. The selenium and copper content of raw garlic is not altered by boiling. The protein electrophoretic pattern of raw garlic is altered by boiling.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Raw and boiled garlic; Rats; Lipids; Proteins; Antioxidant activity; Plasma

Introduction

Garlic is well known for its medicinal benefits, especially in helping to prevent cancer and cardiovascular diseases (Ou et al., 2003). Alliins (*S*-alk(en)yl-L-cysteine sulfoxides) are sources of major active compounds in allium plants.

* Corresponding author. Tel.: +972 2 6758690; fax: +972 2 6757076.

E-mail address: gorin@cc.huji.ac.il (S. Gorinstein).

¹ Affiliated with David R. Bloom Center for Pharmacy at the Hebrew University of Jerusalem, Jerusalem, Israel.

Alicin (diallylthiosulfinate) is the main biologically active component of freshly crushed garlic (*Allium sativum* L.) cloves (Vimal and Devaki, 2004). Garlic activity was compared with dietary curcumin and capsaicin (Kempaiah and Srinivasan, 2004).

The chemopreventive effect of *S*-allylcysteine (SAC), a water-soluble garlic constituent, against gastric carcinogenesis induced in male Wistar rats by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) and saturated sodium chloride (S-NaCl) was studied (Velmurugan et al., 2003). The modulatory effects of garlic on hepatic and blood oxidant–antioxidant status may play a key role in preventing cancer development at extrahepatic sites (Arivazhagan et al., 2004). The antiatherogenic effects of egg yolk-enriched garlic powder, which has been used as a traditional health-promoting food in southern Japan since ancient times, on LDL oxidation and oxidant stress-induced cell injury models were reported (Yamaji et al., 2004).

Garlic was studied in different forms of extracts: aqueous, ethanol, dried powders (Shin and Kim, 2004; Tsao et al., 2003; Ameen et al., 2003). It was shown that aqueous garlic extract alleviates ischaemia–reperfusion-induced oxidative hepatic injury in rats (Sener et al., 2005). In vitro and in vivo experiments conducted mainly with cultures of rat hepatocytes showed the inhibitory effects of garlic on important enzymes in the biosynthesis of cholesterol and fatty acids. The garlic antiatherogenic effects decreased lipid plaques in the arteries of hypercholesterolemic animals and decreased accumulation of cholesterol in the walls of their blood vessels. The reports were based on garlic extracts and their frozen fractions on cholesterol plasma levels and vascular reactivity in cholesterol-fed rats (Slowing et al., 2001). Similar studies were conducted on alloxan-induced diabetic rats (El-Demerdash et al., 2005).

Garlic oil possesses antioxidant properties and provides protection against ethanol induced gastric injury (Khosla et al., 2004). Recently, there have been studies showing that garlic extract attenuates hyperhomocysteinemia caused by folic acid deficiency in rats (Yeh et al., 2005) or gentamicin-induced renal damage and oxidative stress in rats (Maldonado et al., 2003).

The importance of garlic proteins has not been studied intensively. There are few reports about a novel protein with antimicrobial and antiproliferative activities from multiple-cloved garlic bulbs (Xia and Ng, 2005).

Trace elements such as Se and Cu are very important components of garlic. Selenium in garlic significantly increases the ability of garlic to prevent and inhibit cancer. The major selenocompounds found in selenized garlic are Se-methylselenocysteine and gamma-glutamyl-Se-methylselenocysteine (Auger et al., 2004; Ogra and Suzuki, 2005).

There are published reports that heat has an effect on the bioactive compounds present in garlic. It has been shown that the antimicrobial activity of garlic decreased as the heating temperature increased. This fact suggests that alliinase may be the most critical factor producing activity when garlic is heated (Gazzani et al., 1998; Kim et al., 2002).

Most recent research on garlic has used garlic in the form as tablets, flesh, raw, boiled, cooked and dried. In spite of the

numerous reports on garlic bioactive compounds and changes during heat treatment we have decided to study garlic in a form that is more similar to how most people consume garlic. The protein spectra were not studied intensively, because in numerous reports, garlic was investigated in other forms than in this study.

In order to show the lipid-lowering effect of garlic, the experiments were carried out on rats treated with and without cholesterol and garlic in its raw and boiled forms and its corresponding aqueous extracts. The antioxidants, proteins and minerals were investigated in these samples. During different types of processing, garlic loses some of its efficacy, so it was important to find the optimal boiling time during which garlic retains its cardioprotection.

Materials and methods

Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, Folin-Ciocalteu reagent, β -carotene, gallic acid, butylated hydroxyanisole (BHA), and sodium dodecyl sulfate (SDS) were obtained from Sigma Chemical Co., St. Louis, MO, USA. All reagents were of analytical grade. Deionized and distilled water were used throughout.

Sample and extract preparations

Raw garlic samples were from the Warsaw region and purchased at the peak of their maturity at the local market. The edible parts were weighed. The raw garlic was boiled at 100 °C for 20 min. Water from cooked samples was removed by filtration. The samples were cooled, crushed and frozen at a temperature of –20 °C, and 500 mg of raw and boiled garlic samples were equal to 25 mg of lyophilized garlic.

Raw and boiled garlic samples were treated with solvents of different lipophilicity to obtain fractions for testing the antioxidant activity (Betancor-Fernández et al., 2003). To 10–100 mg of garlic powder, either 1 mL of water, methanol/water (70:30), or ethanol was added. After 24 h of incubation under agitation at 4 °C in the dark, all suspensions were centrifuged at 2570×*g* for 10 min and the supernatants collected. The pellets were washed with 0.5 mL solvent, left for 2 h at 4 °C in the dark and centrifuged. Supernatants from the same solvent were combined. The extracts were designated as fraction A (water), fraction B (methanol/water (70:30)), and fraction C (ethanol). The clear supernatants and the solutions of the pure compounds were stored at 80 °C until use. Storage time was less than 7 days. A highly lipophilic fraction (fraction D) was prepared from 1 g of garlic powder in 50 mL acetone/water (75:25 v/v) for 2 h in the dark and vacuum-filtered through a Büchner funnel. The residue was extracted again until the filtrate was colorless. The filtrates were transferred to a decanting funnel; 150 mL of diethyl ether was added. The upper phase, which contained the lipophilic compounds, was

washed several times with water. The ether solution was filtered through a solid bed of Na_2SO_4 and dried. The residue was dissolved in acetone. Aqueous garlic extracts from raw and boiled garlic were prepared as described below on the basis of 25 mg of lyophilized garlic. The extracts were lyophilized and then used as a dietary supplement.

Determination and separation of proteins

Garlic samples were dissolved in two different sample buffers: (a) 2% SDS, 10% glycerol, 2%-mercaptoethanol, 0.002% bromophenol blue and 0.62 M Tris-HCl, pH 6.8; and (b) 2% SDS, 3 M Urea, 10% glycerol, 2%-mercaptoethanol, 0.002% bromophenol blue and 0.62 M Tris-HCl, pH 6.8. Electrophoresis was performed with the Hoeffer SE 600 vertical unit (Hoeffer Pharmacia Biotech Inc., San Francisco, CA 94107, USA) according to Laemmli (1970), using polyacrylamide gels (resolving gel T=13.7%, C=1.7%, stacking gel T=3.8%, C=1.8%) with gel size of 180160×1.5 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 Brilliant Blue R in methanol/water/glacial acetic acid (5:5:1 v/v), destained in water and scanned in transmission light with an Agfa SNAPSCAN 1236 (Agfa-Gevaert N.V Belgium, Agfa SnapScan 1236 s Color image scanner). With the use of software (BIO-GENE[®] v.98 Vilbert Lourmat, France), two densitometry protein profiles of electrophoregrams extracted with sample buffer were created. Background subtraction was made (for maximum spot size 35) before determination of the peak volume quantification. Volume of peaks was calculated as the sum of all intensities included in the defined area, where the band separation was automatically made according to the peak top detection. The number of defined areas and sum of all peak volumes (total volume) for each profile was calculated. The ratio between total volumes of both profiles was determined.

Determination of the bioactive compounds

Minor minerals, Se and Cu, were determined as previously described (Gorinstein et al., 2001).

Total polyphenols

Total polyphenols were determined by Folin-Ciocalteu method and measured at 765 nm. The results are given in mg of gallic acid (Singleton et al., 1999) equivalent per g dry weight (DW).

Determination of the total antioxidant potential

The antioxidant potential of raw and boiled garlic was determined using 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($\text{ABTS}^{\text{U}+}$) with $\text{K}_2\text{S}_2\text{O}_8$. $\text{ABTS}^{\text{U}+}$ radical cation was generated by the interaction of ABTS (250 μM) and $\text{K}_2\text{S}_2\text{O}_8$ (40 μM). After addition of 990

μL of $\text{ABTS}^{\text{U}+}$ solution to 10 μL of Trolox standards (final concentration 0–20 μM) in phosphate buffered saline (PBS), the absorbance was monitored exactly 1, 6 and 9 min after the initial mixing. The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the samples and of Trolox for the standard reference data (Miller et al., 1996). When the samples were dissolved in ethanol, hexane or dichloromethane, the $\text{ABTS}^{\text{U}+}$ solution was diluted with ethanol. For samples dissolved in water, acetone, or methanol/water (70:30), dilution was with water. Standards such as β -carotene, BHA and glutathione were dissolved in dichloromethane, and Trolox in both water and ethanol. This method was applied for determination of antioxidant potential of garlic samples and plasma antioxidant activity.

Rats and diets

The Institute of Animal Physiology and Nutrition of Polish Academy of Science (Jablonna, Poland) provided male Wistar rats ($n=70$) with a mean weight of 150 g at the beginning of the experiment. These rats were randomly divided into 10 diet groups of 7: Control, RG (raw garlic), BG (boiled garlic for 20 min), AERG (aqueous extract of raw garlic), AEBG (aqueous extract of boiled garlic), Ch (Cholesterol), Ch/RG, Ch/BG, Ch/AERG and Ch/AEBG.

The Control group was fed standard diet, which comprised wheat starch, casein, soybean oil, cellulose and mineral and vitamin mixtures.

Lyophilized 25 mg of raw garlic (equivalent of 500 mg raw or boiled garlic)/kg body weight (RG), AERG (aqueous extract of raw garlic), the same quantity of boiled garlic for 20 min (BG) and AEBG (aqueous extract of boiled garlic), 1% of cholesterol (Ch), 1% of cholesterol and 25 mg of lyophilized raw garlic (Ch/RG), 1% of cholesterol and the same quantity of boiled garlic for 20 min for Ch/BG and Ch/AEBG, respectively, were used for experimental groups. The dose of 500 mg (25 mg of lyophilized garlic) on 1 kg body weight was chosen as the most effective (Banerjee et al., 2002). As it was described in the section of extract preparation the aqueous garlic extracts were prepared from the same amount of garlic (25 mg/kg body weight), then lyophilized and used as diet supplementation. These diets contained as percentages of energy: 66% carbohydrates, 25% protein, and 9% fat. The calculated energy of the used diets was from 396.2 to 400.1 kcal/100 g, and the difference was not significant.

All rats were fed once a day at 10:00 h ad libitum, having unrestricted access to drinking water. Garlic or extracts were added to the food before feeding.

The food was removed from the cages at 6 p.m. the day before and the samples were collected at 9 a.m. the next day. Before the experiment, the blood samples were drawn from the tail vein. At the end of the experiment, the rats were anesthetized using diethyl ether. Blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests. The weight gain of the rats was recorded on a weekly basis. Two time points were used in this experiment: before and after

30 days of feeding. At these points wide range of laboratory tests was performed. Plasma total cholesterol (TC) was determined with Randox kit reagents No. Cat. CH 280, Appl. No. 7 (Allain et al., 1974), low-density lipoproteins (LDL-C) according to Friedewald et al. (1972), high-density lipoprotein cholesterol (HDL-C) according to Miida et al. (2002) and triglycerides (TG) with Randox kit reagents No. Cat. 1697, Appl. No. 8 (Gorinstein et al., 2002). The Animal Care Committee of Warsaw Agricultural University approved this study.

Statistical analysis

To verify the statistical significance of the studied parameters, means (M) \pm S.D. of five times analyzed samples were defined. When appropriate, differences among groups were tested by one-way ANOVA. In the assessment the antioxidant

potential Spearman correlation coefficient (R) was used. Linear regressions were also calculated. The P values of <0.05 were considered significant.

Results

Determination and separation of proteins

Buffer-soluble (Fig. 1) and urea-soluble (Fig. 2) garlic proteins were separated into numerous components (respectively, up to 42 and 37 bands). The molecular weight range of detected components was from 10 to 205 kDa (Figs. 1 and 2). The pattern obtained for proteins extracted by both solutions was similar. The majority of the protein bands fall into the MW range 24–116 kDa. The bands with MW less than 12 kDa were diffused. Intensive major components were concentrated

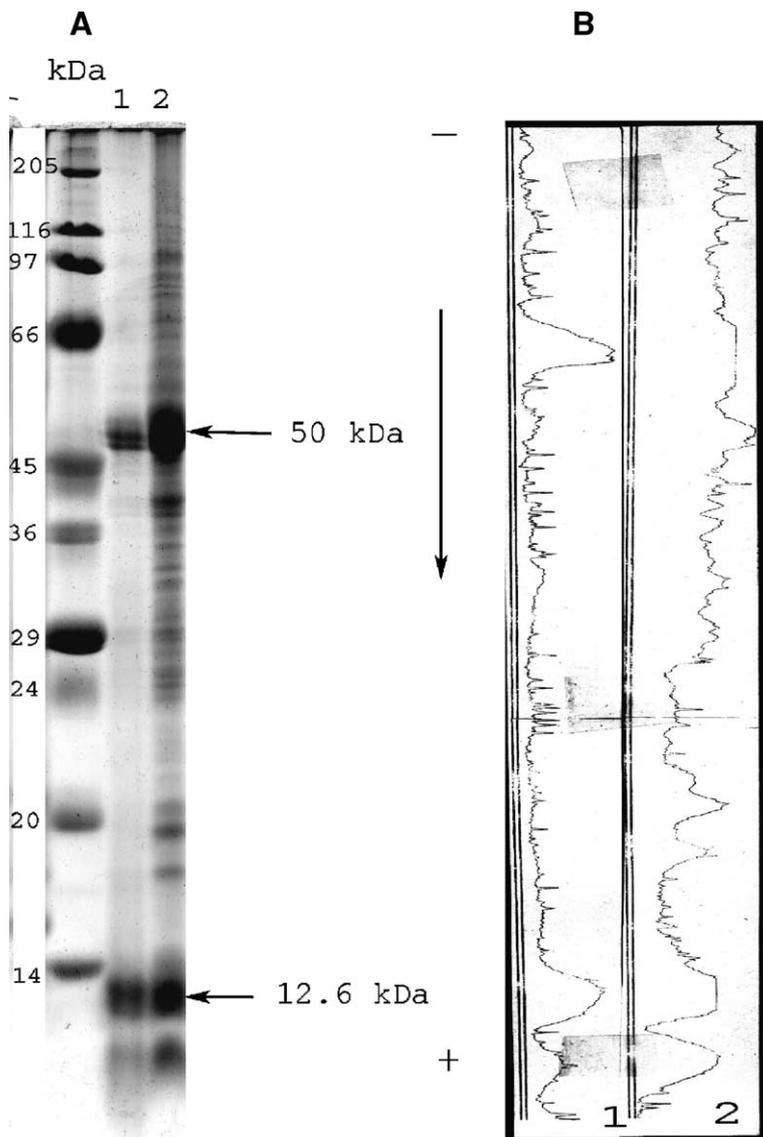


Fig. 1. Comparison of the band intensity of garlic proteins extracted with sample buffer containing SDS and 2-ME and separated by SDS-PAGE. (A) Molecular markers (kDa): 205—myosin; 116— β -galactosidase; 97—phosphorylase b; 66—albumin; 45—ovalbumin; 36—glyceralaldehyde-3-phosphate dehydrogenase; 29—carbonic anhydrase; 24—trypsinogen, PMSF treated; 20—trypsin inhibitor, 14— α -lactalbumin; lane 1—raw garlic (RG); lane 2—boiled garlic (BG). (B) Densitograms of lanes 1 and 2.

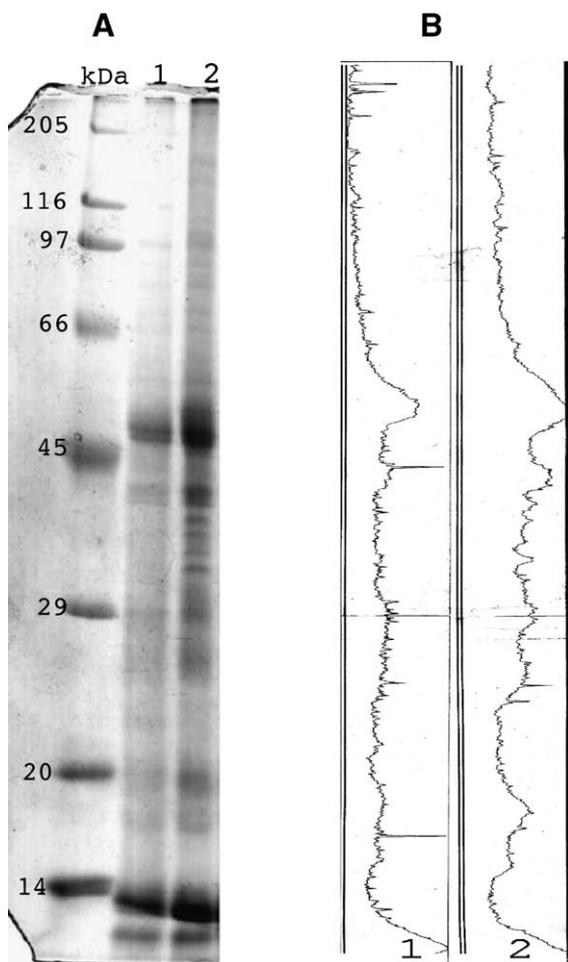


Fig. 2. Comparison of the band intensity of garlic proteins extracted with sample buffer containing 2% SDS, 3 M Urea and 2-ME and separated by SDS-PAGE. (A) Molecular markers in kDa with the same molecular weights as in Fig. 1; lane 1—raw garlic (RG); lane 2—boiled garlic (BG). (B) Densitograms of lanes 1 and 2.

between protein bands of 50 kDa and 12.6 kDa (arrows in Fig. 1A). The treatment process increased the intensity of all detected bands, which became clearer and more visible. In the case of two major, double bands, the increase of intensity was less visible, especially for 12 kDa bands; those remained nearly unchanged (see arrows, Fig. 1).

Quantitative and qualitative differences in band density between protein patterns were found. The effect of boiling on protein patterns was detected. The bands of boiled sample (Fig. 1, lane 2) were clear and the band density was higher than for corresponding bands of raw samples, and the bands of raw garlic (Fig. 1A, lane 1) were very weak and thin compared to the boiled ones. The intensity of the bands in densitograms between the raw and boiled garlic (Fig. 1B, lanes 1 and 2) was determined as volumes and by their ratio. Proteins extracted with urea gave a slightly different electrophoretic separation (Fig. 2A). These results were in correlation with others (Kao et al., 2004) and our recent results (Gorinstein et al., 2005) and made it possible to identify these polypeptides as lectins and allinase. The buffer-soluble raw and boiled garlic densitometry profiles of proteins were divided, respectively, among 10 (Fig. 1B, lane 1) and 34 (Fig. 1B, lane 1) areas. The total volume of peaks for the raw

sample was 1,906,729 (Fig. 1B, lane 1) and for boiled garlic was 4,071,095 (Fig. 1B, lane 2). The ratio between total peaks volume was 2.135, twice that in the non-treated one.

Minor minerals

The results of copper ($\mu\text{g}/100\text{g FW}$) in raw (RG), boiled (BG), aqueous extracts of raw (AERG) and boiled (AEBG) garlic were as follows: 143.3 ± 7.6 ; 143.7 ± 7.2 ; 144.1 ± 6.9 ; and 144.1 ± 6.9 . Selenium ($\mu\text{g}/100\text{g FW}$) showed the following order: for RG (5.5 ± 0.2); BG (5.4 ± 0.1); AERG (5.5 ± 0.1); and AEBG (5.4 ± 0.2). There were no significant changes in the contents of Cu and Se in raw and boiled garlic and their extracts.

Polyphenols and antioxidant potential

The efficacy of the boiled garlic and its aqueous extract to scavenge ABTS was lower by about 15% than that for the raw sample (Fig. 3A, B). Each point on the curve was the average of five determinations of the assay procedure: for RG— 25.5 ± 2.5 ; 37.2 ± 3.6 ; 57.3 ± 5.2 ; 66.3 ± 6.3 and BG— 17.6 ± 1.7 ; 30.8 ± 3.3 ; 47.5 ± 5.1 and 56.2 ± 5.9 (Fig. 3A). For the extracts (Fig. 3B) similar results were obtained. A small decrease in the antioxidant capacity of boiled garlic and its extract was not significant ($P > 0.1$).

The highest TEAC ($\mu\text{mol TE/g}$) in raw garlic was estimated in fraction A, corresponding to 26.1 ± 2.4 ; followed by fraction B: 21.4 ± 1.8 , then fraction C: 20.1 ± 1.6 and then D: 0.93 ± 0.7 . Corresponding fractions from boiled garlic were about 13% to 17% lower than the raw one (Fig. 4). For comparison, the radical scavenging effect of β -carotene, Trolox, BHA and

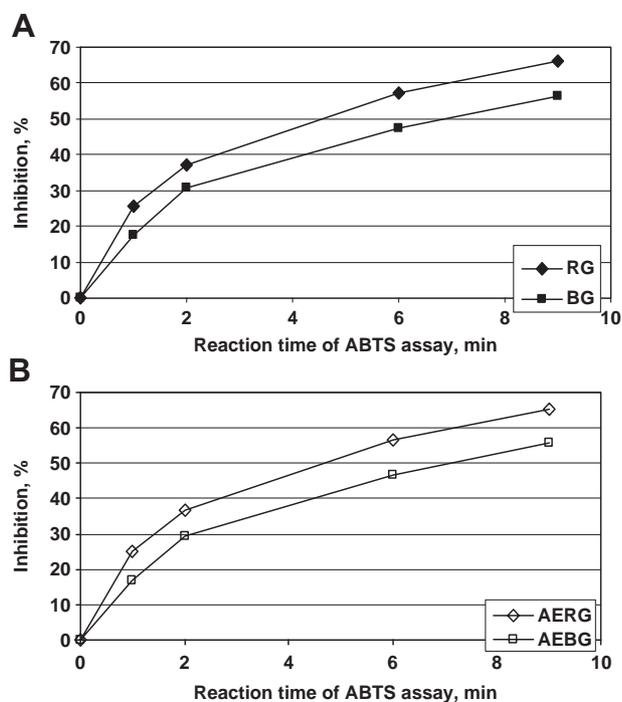


Fig. 3. Inhibition of (A) raw (RG) and boiled (BG) garlic; (B) aqueous extracts of raw (AERG) and boiled (AEBG) garlic against ABTS radical scavenger. Each point of measurements is the average of five determinations.

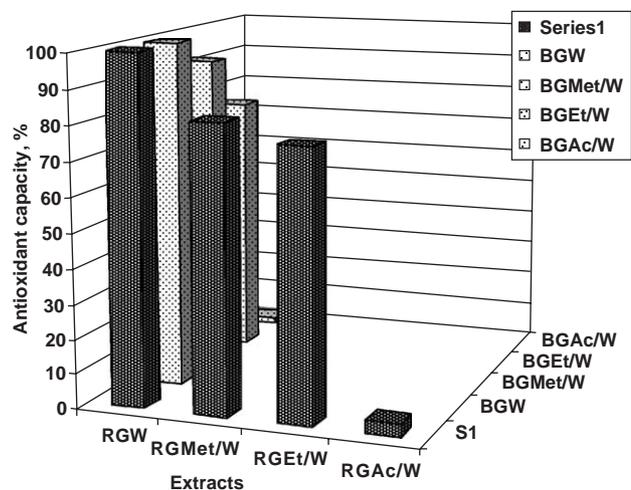


Fig. 4. Antioxidant activity (relative percentage) of raw garlic (RG) extracts with different polarity: RGW, RGMet/W, RGEt/W and RGAc/W, water, methanol, ethanol and acetone, respectively; of boiled (BG) extracts: BGW, BGMet/W, BGEt/W, BGAc/W.

glutathione was measured and compared with that of garlic extracts. β -Carotene showed an antioxidant activity superior to Trolox in the TEAC assay. Total phenols (mg GAE/g DW) were determined in all fractions: A— 11.42 ± 1.45 ; B— 10.00 ± 1.23 ; C— 5.73 ± 0.62 ; D— 2.19 ± 0.3 . The amount of

polyphenols in boiled samples was about 13% less than in the raw samples. The contribution of total polyphenols to the antioxidant potential of raw and boiled garlic was high.

Rats

All diet groups had the same plasma lipid concentration at the beginning of the experiment. No significant change was found in the lipid levels after completion of the experiment in all groups of rats fed cholesterol-free diets (Fig. 5A, $P > 0.05$). In group of rats fed cholesterol-containing diets (Fig. 5B), only raw and boiled (at 100°C for 20 min) garlic samples (Ch/RG, Ch/AERG, Ch/BG and Ch/AEBG) significantly hindered the rise of TC and LDL-C ($P < 0.05$). Garlic samples and/or cholesterol added to the diets did not affect food intake, body weight gains or feed efficiencies (data not shown).

A significant increase in the plasma antioxidant activity (Table 1) of the rats in RG, AERG, BG and AEBG groups was found ($P < 0.05$). As can be seen, after the trial the plasma antioxidant activity in the groups of rats fed cholesterol-free diets supplemented with garlic had increased significantly ($P < 0.05$). The plasma antioxidant activity in the groups of rats fed diets with cholesterol decreased. However, the decrease was significant ($P < 0.05$) only in group of rats (Ch) whose diet was not supplemented with garlic. Therefore, garlic supple-

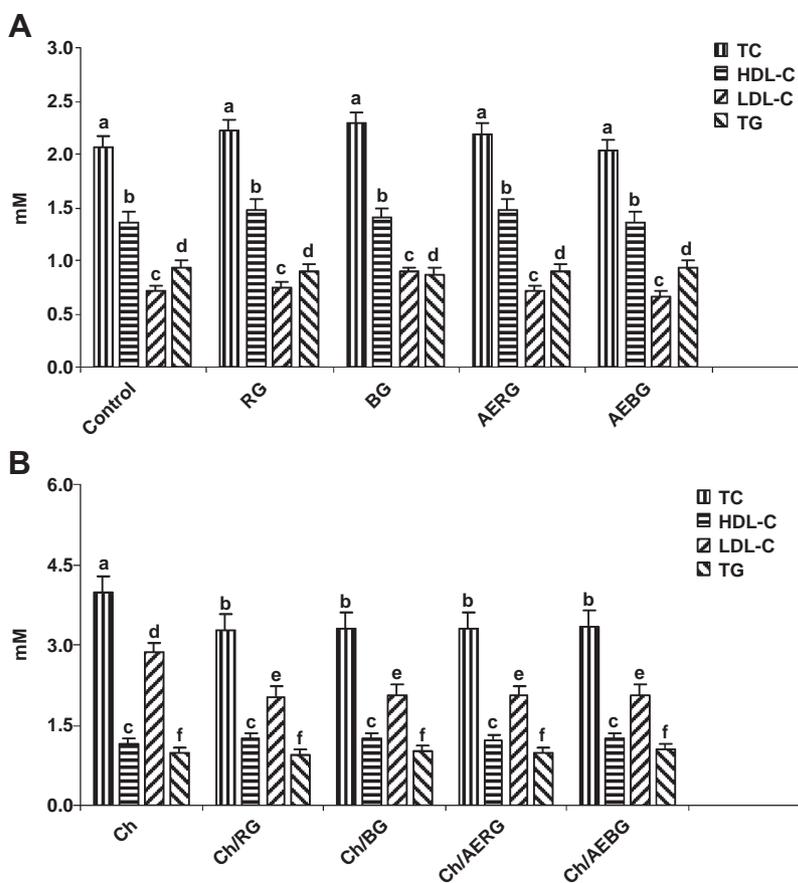


Fig. 5. Changes in serum lipid levels after completion of the trial: (A) groups of rats fed cholesterol-free diets; (B) groups of rats fed cholesterol-containing diets. $M \pm S.D.$, vertical lines; $n = 7$. Means with different letters are significantly different ($P < 0.05$). Abbreviations, RG (raw garlic), BG (boiled garlic for 20 min), AERG (aqueous extract of raw garlic), AEBG (aqueous extract of boiled garlic), Ch (Cholesterol), Ch/RG, Ch/BG, Ch/AERG and Ch/AEBG.

Table 1
Changes in the plasma antioxidant activity (mM) after completion of the trial

Diet groups	Before	After
Control	1.20±0.1 ^a	1.21±0.1 ^a
RG	1.22±0.1 ^a	1.39±0.1 ^b
BG	1.21±0.1 ^a	1.38±0.1 ^b
AERG	1.20±0.1 ^a	1.38±0.1 ^b
AEBG	1.21±0.1 ^a	1.37±0.1 ^b
Ch	1.22±0.1 ^a	0.97±0.1 ^b
Ch/RG	1.20±0.1 ^a	1.12±0.1 ^a
Ch/BG	1.22±0.1 ^a	1.11±0.1 ^a
Ch/AERG	1.21±0.1 ^a	1.10±0.1 ^a
Ch/AEBG	1.20±0.1 ^a	1.09±0.1 ^a

Means±S.D., *n* = 7.

Means with different letters in rows are significantly different (*P* < 0.05). Abbreviations, RG (raw garlic), BG (boiled garlic for 20 min), AERG (aqueous extract of raw garlic), AEBG (aqueous extract of boiled garlic), Ch (Cholesterol), Ch/RG, Ch/BG, Ch/AERG and Ch/AEBG.

ments for diets containing cholesterol hindered the decrease of the plasma antioxidant activity.

Discussion

Common available garlic preparations in the form of garlic oil, garlic powder and pills are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile (Elkayam et al., 2003; Abdel-Wahhab and Aly, 2003; Stevinson et al., 2000). Despite the impressive effects of garlic, most studies are limited by lack of controlled methods and by the use of preparations with unknown amounts and chemical identification of the active ingredient. Therefore this study was designed to examine the effects of raw and boiled garlic and their aqueous extracts on lipid, antioxidant and protein status in serum of rats. For this purpose, Wistar rats were fed diets with garlic and cholesterol supplements.

In this research, the effect of garlic *in vitro* and *in vivo* was studied by a mechanism related to its ability to scavenge free radicals such as ABTS; and in decrease of oxidative stress which was characterized by the elevated antioxidant activity in the serum of rats. It was found that aqueous garlic extracts showed higher antioxidant activity than those extracted with different polarity solvents. Aqueous extracts were used as a supplementation to diet-treated rats. This verifies other studies showing that the water-soluble *S*-allylcysteine reduces the extent of lipid peroxidation and significantly enhances antioxidant activities *in vitro* and *in vivo* (Velmurugan et al., 2003; Betancor-Fernández et al., 2003; Perez-Severiano et al., 2004; Almaca, 2004).

The changes in total polyphenol content and in total antioxidant potential of samples of raw garlic and garlic boiled at three different temperature regimes (20, 40 and 60 min) were summarized in our recent publication (Gorinstein et al., 2005). The decrease in polyphenols and in related antioxidant potential was significant only after boiling the raw garlic samples for 40 and 60 min (*P* < 0.05).

Our results, like others of vegetable juices, showed a linear correlation between antioxidant activity and cooking time using different cooking methods such as boiling, frying,

steaming, baking and canning (Gazzani et al., 1998; Kim et al., 2002). It was shown that garlic samples both raw and boiled for 20 min were similar in their biological activity. As mentioned above, the radical scavenging activities also correlated positively with the total phenolics of the extracts in all fractions. The aqueous fraction possessed the highest activities and, respectively, had the highest amount of phenolics. These results correspond with others showing that allicin is the main active compound in extracted with water from garlic (Vimal and Devaki, 2004; Tsao et al., 2003).

We have found little information about the comparison of the antioxidant activity of raw and boiled garlic similar to eating conditions, because in very recent studies, garlic was investigated in different forms such as oil (Khosla et al., 2004). It is difficult to discuss the results of the influence of garlic extracts, because the preparations (a), amounts (b) and applications (c) were different in each report: (a) homogenization in water of 100 mg/kg/day (Baluchnejadmojarad and Roghani, 2003); (b) 500 mg/kg (Ameen et al., 2003); 250 mg/kg (Arivazhagan et al., 2004); 2.0–4.0% dry powder or curcumin (0.2%)/capsaicin (0.015%)/garlic (2.0% dry powder) added to the diets (Kempaiah and Srinivasan, 2004; Pedraza-Chaverri et al., 2000; Yeh et al., 2005); (c) intragastric intubation; administration in saline; injection of 1.2 mL/kg body weight/24 h/6 days subcutaneously and intraperitoneally; dried powders as supplementation to diets (Arivazhagan et al., 2004; El-Demerdash et al., 2005; Maldonado et al., 2003; Shin and Kim, 2004).

Our results showed that garlic juices were applied and exerted antioxidant and antihyperglycemic effects in their different forms and amounts. The results also depend on the experimental protocols performed on extract-treated rats and different technologies used in the preparation and processing of garlic samples.

After 30 days of this experiment, *in vivo* plasma lipids (total cholesterol, LDL and triglycerides) concentrations were higher in all groups fed cholesterol; however, the increase was significant only in Ch group, without garlic supplements. Therefore, the addition of garlic hindered the rise of plasma lipids in rats fed cholesterol. Diets supplemented with garlic in rats that were not fed cholesterol did not affect the lipid levels (*P* > 0.05). Our results showed that plasma total cholesterol decreased in all groups treated with raw and boiled garlic. This effect was higher in rats fed raw garlic and its aqueous fraction. LDL decreased significantly with respect to the hypercholesterolemic groups treated with raw garlic and boiled for 20 min. Others have also demonstrated the hypolipidemic effect of garlic when added to diets of rats fed cholesterol (Banerjee et al., 2002; Ou et al., 2003; Slowing et al., 2001).

At the end of the trial, a significant increase in the plasma antioxidant activity in groups of rats fed cholesterol-free diets was registered (*P* < 0.05). A decrease in the plasma antioxidant activity was found in all groups of rats fed cholesterol-containing diets, however the decrease was significant only in Ch group, which diet was not supplemented with garlic. Such results were expected: a cholesterol-supplemented diet decreased the blood antioxidant activity.

We suggest that organosulfur compounds derived from garlic are potent agents for protecting LDL against oxidation and glycation and that they may bring benefit even to patients with diabetes mellitus or cardiovascular diseases by preventing complications. Our results were similar to those of Pedraza-Chaverri et al. (2004), who reported that the ability of raw garlic to prevent Cu²⁺-induced LDL oxidation is not affected by boiling. The effect of a 2% garlic diet on acute and chronic experimental nephrotic syndrome induced by puromycin aminonucleoside showed that consuming garlic can prevent the increase in cholesterol-LDL induced in an experimental model of nephrotic rats and diminished significantly total-cholesterol and triglycerides, but not HDL-cholesterol (Pedraza-Chaverri et al., 2000). There was also congruence with other studies showing that garlic extracts and diallyl sulfides significantly decreased the malondialdehyde level and liver antioxidant enzyme levels, thus offering antioxidant protection (Ameen et al., 2003; Vimal and Devaki, 2004; Tsao et al., 2003; Kempaiah and Srinivasan, 2004).

Trace elements Se and Cu, which were in the accepted range in garlic samples, did not change drastically during boiling. These data well correspond with others that found Se and Cu, which are essential trace nutrients and sulfur analogs, have high chemical activity. We suppose that these components are a part of seleno- and copper-proteins and some enzymes (glutathione peroxidase, peroxidases, blood and tissue proteins) and marked copper-chelating capability of organosulfur compounds derived from garlic (Ou et al., 2003).

As food components, Se and Cu may offer some protection from atherosclerosis, coronary ischemic disease and cancer (Baraboi and Shestakova, 2004).

We propose that major bands of 50 kDa correspond with allininase and 12 kDa bands with lectins bands, respectively. The major double bands also occur in gels after treatment. As shown on the electrophoregrams, the raw sample also resolved in many minor bands, which became more intense after boiling. The density of 50 kDa bands has increased, but 12 kDa bands of lectins remained unchanged. Lectins are apparently more thermostable during the garlic treatment. The phenomenon of increasing intensity of almost all the bands can be explained by the effect of more advanced dissociation of protein to subunits and polypeptides. Garlic bulb proteins are located in bodies-complexes, and in this case the boiling causes the disengagement of proteins. The protein spectra obtained by electrophoretic separation can be compared with those in a few studies: antifungal activity of alliumin of 13 kDa remained after boiling for 1 h and also after treatment with trypsin or chymotrypsin (1:1, w/w) for 30 min at room temperature (Xia and Ng, 2005).

Our study found a connection between antioxidant activity and protein composition. When the sample was boiled for a short time, the proteins became more soluble and possibly more digestible.

Conclusions

Our results show that in vitro and in vivo studies, boiled garlic and its aqueous extract are strong antioxidants. The

protein spectra changed during boiling. The antioxidant ability of garlic samples in vivo can be explained by the improved lipid and antioxidant metabolic indices in rat serum. The protective effect of garlic samples was linked to the decrease in the oxidative stress in the animal model. Raw and boiled garlic brought about improvement in serum spectra.

Acknowledgement

The authors are thankful to Mrs. Judy Siegel-Itzkovich, the Jerusalem Post's Health and Science Reporter and Software Reviewer, Jerusalem, Israel, for editing this manuscript.

References

- Abdel-Wahhab, M.A., Aly, S.E., 2003. Antioxidants and radical scavenging properties of vegetable extracts in rats fed aflatoxin-contaminated diet. *Journal of Agricultural and Food Chemistry* 51 (8), 2409–2414.
- Allain, C.C., Poon, L.S., Chen, C.S.G., Richmond, W., Fu, P.C., 1974. The enzymatic determination of total serum cholesterol. *Clinical Chemistry* 20 (4), 470–475.
- Almaga, G., 2004. Antioxidant effects of sulfur-containing amino acids. *Yonsei Medical Journal* 45 (5), 776–788.
- Ameen, M., Musthapa, M.S., Abidi, P., Ahmad, I., Rahman, Q., 2003. Garlic attenuates chrysotile-mediated pulmonary toxicity in rats by altering the phase I and phase II drug metabolizing enzyme system. *Journal of Biochemical and Molecular Toxicology* 17 (6), 366–371.
- Arivazhagan, S., Velmurugan, B., Bhuvaneswari, V., Nagini, S., 2004. Effects of aqueous extracts of garlic (*Allium sativum*) and neem (*Azadirachta indica*) leaf on hepatic and blood oxidant-antioxidant status during experimental gastric carcinogenesis. *Journal of Medicinal Food* 7 (3), 334–339.
- Auger, J., Yang, W., Arnault, I., Pannier, F., Potin-Gautier, M., 2004. High-performance liquid chromatographic-inductively coupled plasma mass spectrometric evidence for Se-“alliins” in garlic and onion grown in Se-rich soil. *Journal of Chromatography. A* 1032 (1–2), 103–107.
- Baluchnejadmojarad, T., Roghani, M., 2003. Garlic extract attenuates time-dependent changes in the reactivity of isolated aorta in streptozotocin-diabetic rats. *Life Sciences* 73 (18), 2281–2289.
- Banerjee, S.K., Maulik, M., Mancahanda, S.C., Dinda, A.K., Gupta, S.K., Maulik, S.K., 2002. Dose-dependent induction of endogenous antioxidants in rat heart by chronic administration of garlic. *Life Sciences* 70 (13), 1509–1518.
- Baraboi, V.A., Shestakova, E.N., 2004. Selenium: biological role and antioxidant activity. *Ukrains'kii Biokhimičnii Zhurnal* 76 (1), 23–32.
- Betancor-Fernández, A., Pérez-Gálvez, A., Sies, H., Stahl, W., 2003. Screening pharmaceutical preparations containing extracts of turmeric rhizome, artichoke leaf, devil's claw root and garlic or salmon oil for antioxidant capacity. *Journal of Pharmacy and Pharmacology* 55 (7), 981–986.
- El-Demerdash, F.M., Yousef, M.I., Abou El-Naga, N.I., 2005. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. *Food and Chemical Toxicology* 43 (1), 57–63.
- Elkayam, A., Mirelman, D., Peleg, E., Wilchek, M., Miron, T., Rabinkov, A., Oron-Herman, M., Rosenthal, T., 2003. The effects of allicin on weight in fructose-induced hyperinsulinemic, hyperlipidemic, hypertensive rats. *American Journal of Hypertension* 16 (12), 1053–1056.
- Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clinical Chemistry* 18 (6), 499–502.
- Gazzani, G., Papetti, A., Massolini, G., Daglia, M., 1998. Anti- and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *Journal of Agricultural and Food Chemistry* 46 (10), 4118–4122.
- Gorinstein, S., Zachwieja, Z., Foltá, M., Barton, H., Piotrowicz, J., Zemser, M., Weisz, M., Trakhtenberg, S., Martín-Belloso, O., 2001. Comparative

- content of dietary fiber, total phenolics, and minerals in persimmon and apples. *Journal of Agricultural and Food Chemistry* 49 (2), 952–957.
- Gorinstein, S., Leontowicz, H., Lojek, A., Leontowicz, M., Číž, M., Krzeminski, R., Gralak, M., Czerwinski, J., Jastrzebski, Z., Trakhtenberg, S., Grigelmo-Miguel, N., Soliva-Fortuny, R., Martin-Belloso, O., 2002. Olive oils improve lipid metabolism and increase antioxidant potential in rats fed diets containing cholesterol. *Journal of Agricultural and Food Chemistry* 50 (21), 6102–6108.
- Gorinstein, S., Drzewiecki, J., Leontowicz, H., Leontowicz, M., Najman, K., Jastrzebski, Z., Zachwieja, Z., Barton, H., Shtabsky, B., Katrich, E., Trakhtenberg, S., 2005. Comparison of the bioactive compounds and antioxidant potentials of fresh and cooked Polish, Ukrainian and Israeli garlic. *Journal of Agricultural and Food Chemistry* 53 (7), 2726–2732.
- Kao, S.-H., Hsu, Ch.-H., Su, S.-N., Hor, W.-T., Chang, W.-H., Chow, L.-P., 2004. Identification and immunologic characterization of an allergen, alliin lyase, from garlic (*Allium sativum*). *Journal of Allergy and Clinical Immunology* 113 (1), 161–168.
- Kemppaiah, R.K., Srinivasan, K., 2004. Influence of dietary curcumin, capsaicin and garlic on the antioxidant status of red blood cells and the liver in high-fat-fed rats. *Annals of Nutrition & Metabolism* 48 (5), 314–320.
- Khosla, P., Karin, R.S., Baraga, V.K., 2004. Effect of garlic oil on ethanol induced gastric ulcers in rats. *Phytotherapy Research* 18 (1), 87–91.
- Kim, J.Y., Lee, Y.C., Kim, K.S., 2002. Effect of heat treatments on the antimicrobial activities of garlic (*Allium sativum*). *Journal of Microbiology and Biotechnology* 12 (2), 331–335.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of bacteriophage T4. *Nature* 227 (5259), 680–685.
- Maldonado, P.D., Barrera, D., Medina-Campos, O.N., Hernández-Pando, R., Ibarra-Rubio, M.E., Pedraza-Chaverri, J., 2003. Aged garlic extract attenuates gentamicin induced renal damage and oxidative stress in rats. *Life Sciences* 73 (20), 2543–2556.
- Miller, N.J., Sampson, J., Candeias, L.P., Bramley, P.M., Rice-Evans, C.A., 1996. Antioxidant activities of carotenes and xanthophylls. *FEBS Letters* 384 (3), 240–242.
- Miida, T., Nakamura, Y., Mezaki, T., Hanyu, O., Maruyama, S., Horikawa, Y., Izawa, S., Yamada, Y., Matsui, H., Okada, M., 2002. LDL-cholesterol and HDL-cholesterol concentrations decrease during the day. *Annals of Clinical Biochemistry* 39 (3), 241–249.
- Ogra, Y., Suzuki, K.T., 2005. Speciation of selenocompounds by capillary HPLC coupled with ICP-MS using multi-mode gel filtration columns. *Journal of Analytical Atomic Spectrometry* 20 (1), 35–39.
- Ou, C.C., Tsao, S.M., Lin, M.C., Yin, M.C., 2003. Protective action on human LDL against oxidation and glycation by four organosulfur compounds derived from garlic. *Lipids* 38 (3), 219–224.
- Pedraza-Chaverri, J., Medina-Campos, O.N., De los Angeles Granados-Silvestre, M., Maldonado, P.D., Olivares-Corichi, I.M., Hernandez-Pando, R., 2000. Garlic ameliorates hyperlipidemia in chronic aminonucleoside nephrosis. *Molecular and Cellular Biochemistry* 211 (1–2), 69–77.
- Pedraza-Chaverri, J., Gil-Ortiz, M., Albarrán, G., Barbachano-Esparza, L., Menjívar, M., Medina-Campos, O.N., 2004. Garlic's ability to prevent in vitro Cu²⁺-induced lipoprotein oxidation in human serum is preserved in heated garlic: effect unrelated to Cu²⁺-chelation. *Nutrition Journal* 3 (1), 10.
- Perez-Severiano, F., Rodriguez-Perez, M., Pedraza-Chaverri, J., Maldonado, P.D., Medina-Campos, O.N., Ortiz-Plata, A., Sanchez-Garcia, A., Villeda-Hernandez, J., Galvan-Arzate, S., Aguilera, P., Santamaria, A., 2004. S-Allylcysteine, a garlic-derived antioxidant, ameliorates quinolinic acid-induced neurotoxicity and oxidative damage in rats. *Neurochemistry International* 45 (8), 1175–1183.
- Sener, G., Sehirli, O., Ipci, Y., Ercan, F., Sirvanci, S., Gedik, N., Yegen, B.C., 2005. Aqueous garlic extract alleviates ischaemia–reperfusion-induced oxidative hepatic injury in rats. *Journal of Pharmacy and Pharmacology* 57 (1), 145–150.
- Shin, S.H., Kim, M.K., 2004. Effect of dried powders or ethanol extracts of garlic flesh and peel on lipid metabolism and antithrombotic capacity in 16-month-old rats. *Hanguk Yongyang Hakhoechi* 37 (7), 515–524.
- Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology* 299, Oxidants and Antioxidants, Part A, 152–178.
- Slowing, K., Ganado, P., Sanz, M., Ruiz, E., Tejerina, T., 2001. Study of garlic extracts and fractions on cholesterol plasma levels and vascular reactivity in cholesterol-fed rats. *Journal of Nutrition* 131 (3S), 994S–999S.
- Stevinson, C., Pittler, M.H., Ernst, E., 2000. Garlic for treating hypercholesterolemia—a meta-analysis of randomized clinical trials. *Annals of Internal Medicine* 133 (6), 420–429.
- Tsao, Sh.-M., Hsu, Ch.-C., Yin, M.-C., 2003. Garlic extract and two dually sapphires inhibit mythically-resistant *Staphylococcus aureus* infection in BALB/c mice. *Journal of Antimicrobial Chemotherapy* 52 (6), 974–980.
- Velmurugan, B., Bhuvaneshwari, V., Nagini, S., 2003. Effect of S-allylcysteine on oxidant–antioxidant status during N-methyl-N'-nitro-N-nitrosoguanidine and saturated sodium chloride-induced gastric carcinogenesis in Wistar rats. *Asia Pacific Journal of Clinical Nutrition* 12 (4), 488–494.
- Vimal, V., Devaki, T., 2004. Hepatoprotective effect of allicin on tissue defense system in galactosamine/endotoxin challenged rats. *Journal of Ethnopharmacology* 90 (1), 151–154.
- Yamaji, K., Sarker, K.P., Abeyama, K., Maruyama, I., 2004. Antiatherogenic effects of an egg yolk-enriched garlic supplement. *International Journal of Food Sciences and Nutrition* 55 (1), 61–66.
- Yeh, Y.-Y., Lim, H.-S., Yeh, Sh.-M., Picciano, M.F., 2005. Garlic extract attenuates hyperhomocysteinemia caused by folic acid deficiency in the rat. *Nutrition Research* 25 (1), 93–102.
- Xia, Li., Ng, T.B., 2005. Isolation of alliumin, a novel protein with antimicrobial and antiproliferative activities from multiple-cloved garlic bulbs. *Peptides* 26 (2), 177–183.