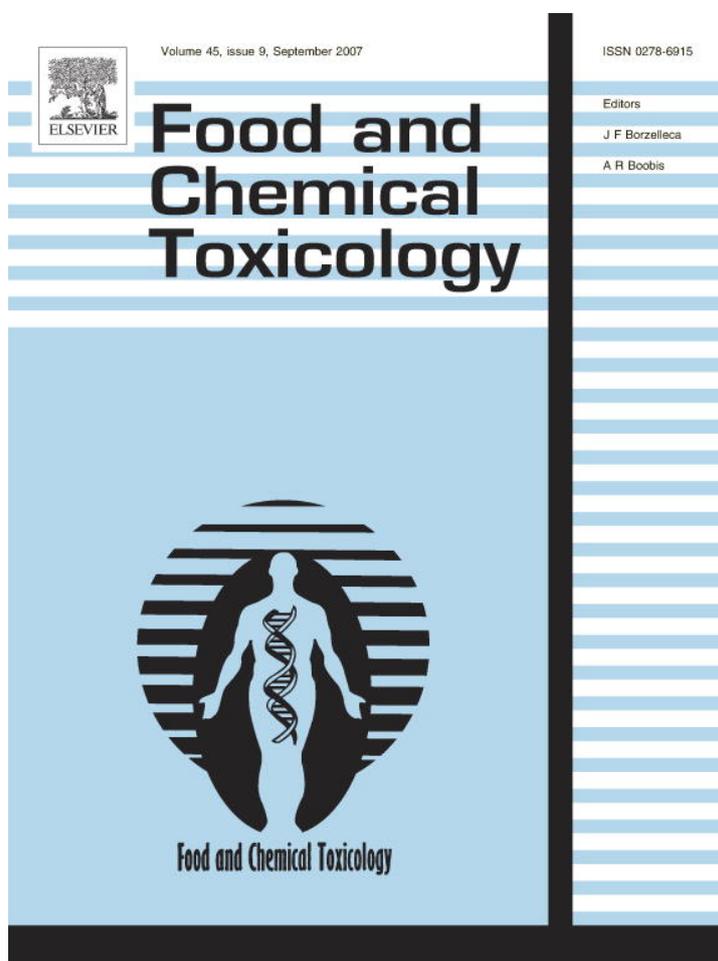


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## The bioactivity of processed garlic (*Allium sativum* L.) as shown *in vitro* and *in vivo* studies on rats

Zenon Jastrzebski <sup>a</sup>, Hanna Leontowicz <sup>b</sup>, Maria Leontowicz <sup>b</sup>, Jacek Namiesnik <sup>c</sup>,  
Zofia Zachwieja <sup>d</sup>, Henryk Barton <sup>d</sup>, Elke Pawelzik <sup>e</sup>, Patricia Arancibia-Avila <sup>f</sup>,  
Fernando Toledo <sup>f</sup>, Shela Gorinstein <sup>g,\*</sup>,<sup>1</sup>

<sup>a</sup> Department of Pharmacology, Institute of Public Health, Warsaw, Poland

<sup>b</sup> Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland

<sup>c</sup> Chemical Faculty, Gdansk University of Technology, Gdansk, Poland

<sup>d</sup> Department of Food Chemistry and Nutrition, School of Medicine, The Jagiellonian University, Cracow, Poland

<sup>e</sup> Institute of Agricultural Chemistry, Georg-August-University, Goettingen, Germany

<sup>f</sup> Department of Basic Sciences, Universidad del Bio-Bio, Chillan, Chile

<sup>g</sup> Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University – Hadassah Medical School, Jerusalem, Israel

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### Abstract

Garlic (*Allium sativum* L.) is widely used as an obligatory part in many cooked dishes losing during this process a certain part of its bioactivity. Antioxidant capacity measured by the ferric-reducing/antioxidant power (FRAP) method and by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay was the highest in raw and in a short time processed garlic samples by cooking.

70 Wistar rats were randomly divided into 10 diet groups, each of seven. They were named Control, NPG, PG1, PG2, PG3, Chol, Chol/NPG, Chol/PG1, Chol/PG2 and Chol/PG3. The rats of the Control group were fed basal diet (BD), which included wheat starch, casein, soybean oil, cellulose, mineral and vitamin mixtures. To the BD of the nine other groups were added 25 mg of lyophilized non processed garlic equivalent of 500 mg non processed garlic/kg body weight (NPG), the same quantity of processed garlic for 20, 40 and 60 min for PG1, PG2 and PG3, respectively, 1% of cholesterol (Chol), 1% of cholesterol and 25 mg/kg body weight of lyophilized non processed garlic (Chol/NPG), 1% of cholesterol and the same quantity of processed garlic for 20, 40 and 60 min for Chol/PG1, Chol/PG2 and Chol/PG3, respectively. The dose of 500 mg (25 mg of lyophilized garlic/kg body weight) was chosen as the most effective (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, pp. 1509–1518).

Plasma lipid profile and the total antioxidant capacity in rats significantly differed in diet groups with addition of garlic samples cooked for a long time. In summary, garlic cooked for a short time preserves a high bioactivity of non processed garlic. The diet supplemented with these samples and cholesterol improved lipid indices, decreased fibrinogen and increased antioxidant activity in plasma of rats. Therefore, for preservation of garlic bioactivity optimal regime has to be used.

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**Keywords:** Raw and processed garlic; Lipid metabolism; Antioxidants

### 1. Introduction

Health properties (Ichikawa et al., 2003; Tepe et al., 2005) of garlic (*Allium sativum* L.) depend on its bioactive compounds. Raw garlic is widely used, but this vegetable is also an obligatory part in many cooked dishes (Gorinstein

\* Corresponding author. Tel.: +972 2 6758690; fax: +972 2 6757076.  
E-mail address: gorin@cc.huji.ac.il (S. Gorinstein).

<sup>1</sup> Author is affiliated with David R. Bloom Center of Pharmacy.

et al., 2005; Gorinstein et al., 2006a and Gorinstein et al., 2006b). Some authors reported that garlic is effective in regulating plasma lipid levels (Steiner and Li, 2001) and increases plasma anticoagulant activity (Aritz-Castro et al., 1992; Ackermann et al., 2001). The wide range of ailments in which this vegetable is used (Bhagyalakshmi et al., 2005; Constenla and Lozano, 2005; Kabasakal et al., 2005; Lawson and Gardner, 2005; Pedraza-Chaverri et al., 2006) could create an impression that garlic is a remedy for all diseases. The most studied and reported health promoting effect of garlic is cardioprotective (Banerjee et al., 2002; Durak et al., 2002; Williams et al., 2005; Zahid et al., 2005).

Fruits and vegetables subjected to temperature or stored at higher temperature are losing certain part of their bioactivity (Cardelle-Cobas et al., 2005). However, the knowledge about the influence of temperature on bioactive properties of garlic *in vitro* and *in vivo* is very limited.

In spite of published reports on the influence of garlic on lipid metabolism (Stevenson et al., 2000; Lawson et al., 2001) there is a disagreement in the application of garlic as lipid-lowering agent (Kerckhoffs et al., 2002; Peleg et al., 2003). However, it is still a controversy about the plasma lipid regulating and antioxidant increasing properties of garlic.

Therefore, we decided to determine the optimal technological cooking regime, which preserves the bioactivity of raw garlic and in order to have our own opinion on this controversy to conduct an experiment on rats fed cholesterol-containing and cholesterol-free diets supplemented with garlic.

As far as we know, there are not such published papers.

## 2. Materials and methods

### 2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox); 2,4,6-tripyridyl-*s*-triazine (TPTZ);  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$ ; 1,1-diphenyl-2-picrylhydrazyl (DPPH); butylated hydroxyanisole (BHA); Folin-Ciocalteu reagent and nonoxidized cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Deionized and distilled water was used throughout.

### 2.2. Samples preparation

Raw garlic was purchased at the market from a local grower (Gorinstein et al., 2005; Gorinstein et al., 2006a and Gorinstein et al., 2006b). The garlic samples were prepared manually. The edible parts were weighted and divided into four parts: one of them was used as raw and three others were cooked at 100 °C for 20, 40 and 60 min, respectively. The samples were cooled, crashed and frozen under temperature of –20 °C. Water from boiled samples was removed and lyophilized with the cooked samples, respectively.

### 2.3. Extraction, determination, and UV spectra of the bioactive compounds

Extraction of total polyphenols was done as described previously (Gorinstein et al., 2005). Total polyphenols were determined by Folin-Ciocalteu method and measured at 765 nm. The results were given in  $\mu\text{g}$  of gallic acid equivalent (Singleton et al., 1999) per 100 g fresh weight (FW).

All spectra of extracts were measured on an Uvikon 930 (Bio-Teck-Kontron) and were recorded from 250 to 600 nm (Sarni-Manchado et al., 2000).

### 2.4. Determination of the total antioxidant activity

Combination of Ferric Reducing/Antioxidant Power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assays were used for determination of total antioxidant potential (Pellegrini et al., 2003; Katalinic et al., 2006).

#### 2.4.1. DPPH assay

The volume of different garlic extracts was adjusted to 100  $\mu\text{L}$  by adding MeOH. A 0.1 mM methanolic solution of DPPH was added (5  $\mu\text{L}$ ) to tubes, shaken vigorously and was standing at 27 °C for 20 min. The control was prepared as above without any extract, and MeOH was used for the baseline correction. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula: % radical scavenging activity =  $(\text{control OD} - \text{sample OD} / \text{control OD}) \times 100$ , where OD is optical density at 517 nm. BHA was used for comparison (Singh et al., 2002).

#### 2.4.2. FRAP assay

This method (Benzie and Strain, 1996) measures the ability of the antioxidants contained in the garlic samples to reduce ferric-tripyridyltriazine ( $\text{Fe}^{3+}$ -TPTZ) to a ferrous form ( $\text{Fe}^{2+}$ ) which absorbs light at 593 nm. The ferro- and ferric-iron form complexes with TPTZ reagent are the main products of this reaction. FRAP level was calculated by plotting a standard curve of absorbance against  $\mu\text{mol/L}$  or  $\mu\text{mol/g}$  concentration of  $\text{Fe}^{2+}$  standard solution or Trolox.

### 2.5. Animals and diets

The Animal Care Committee of University approved this study. The male Wistar rats ( $n = 70$ ) with a mean weight of 150 g rats were randomly divided into 10 diet groups, each of seven. They were named Control, NPG, PG1, PG2, PG3, Chol, Chol/NPG, Chol/PG1, Chol/PG2 and Chol/PG3. The rats of the Control group were fed basal diet (BD), which included wheat starch, casein, soybean oil, cellulose, mineral and vitamin mixtures. To the BD of the 9 other groups were added 25 mg of lyophilized raw garlic equivalent of 500 mg raw garlic/kg body weight (Raw), the same quantity of cooked garlic for 20, 40 and 60 min for PG1, PG2 and PG3, respectively, 1% of cholesterol (Chol), 1% of cholesterol and 25 mg/kg body weight of lyophilized non processed garlic (Chol/NPG), 1% of cholesterol and the same quantity of cooked garlic for 20, 40 and 60 min for Chol/PG1, Chol/PG2 and Chol/PG3, respectively. The dose of 500 mg (25 mg of lyophilized garlic/1 kg body weight) was chosen as the most effective (Banerjee et al., 2002).

During the experiment the dose of the used garlic was adjusted to the growing weight of every animal. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats. 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*. They had unrestricted access to drinking water. Garlic was added to the diets before feeding.

It is generally accepted that the most reliable data of the blood lipid metabolism can be obtained from fasting animals, 14–16 h after the last feeding. Therefore, the food was removed from the cages at 6 pm the day before. The samples were collected at 9 am the next day. The plasma was prepared and used for laboratory tests.

### 2.6. Plasma lipids, plasma fibrinogen and antioxidants

Two time points were in this experiment: before and after 30 days of different feeding. At these time points, a wide range of laboratory tests was

performed. Total cholesterol (TC, with Randox kit reagents, Catalog No. CH 280 Application No 7), low-density lipoproteins (LDL-C, Friedewald et al., 1972), high-density lipoprotein cholesterol (HDL-C, Izawa et al., 1997), and triglycerides (TG, with Randox kit reagents, Catalog No. 1697, Application No 8) were determined as previously described (Krzeminski et al., 2003).

Serum fibrinogen was precipitated with methanol, then purified by sequential DEAE anion-exchange chromatography, dialyzed against water for 72 h, and lyophilized.

The content of blood circulating fibrinogen (g/L) was determined by the classical method of Roche (Roche Diagnostics, Okopowa 58/72 01-042 Warszawa, Poland).

Antioxidant activity of plasma was determined by FRAP (Benzie and Strain, 1996).

### 2.7. Statistical methods

Values of the results *in vitro* experiment are given as means  $\pm$  SD of five measurements. Where appropriate, data were tested by two-way ANOVA using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA), following by Duncan's new multiple range test to assess differences between groups means. Differences of  $P < 0.05$  were considered significant.

## 3. Results

### 3.1. *In vitro* experiment

#### 3.1.1. Total polyphenols and antioxidant activities

Methanolic extracts of different garlic samples NPG, PG1, PG2 and PG3 (Fig. 1a, b) had maximum absorptions of their UV spectra in a broad range between 230 and 260 nm, which indicated the presence of phenolic compounds. All garlic extracts had nearly the same peaks and differ by the peak intensity and very slight shift of the peaks. *p*-Coumaric acid which was used as a standard (Fig. 1c) showed exactly the same maximum absorption of the first peak as all garlic extracts. In the same garlic extracts after UV measurements were estimated antioxidant activities by FRAP and DPPH.

The decrease in the content of total polyphenols and in related total antioxidant (by FRAP and DPPH) activities (Fig. 2) was significant only after cooking at 100 °C for 40 and 60 min ( $P < 0.05$ ). The contribution of total polyphenols to the antioxidant activities (Fig. 3) of raw and cooked garlic was high ( $R^2$  from 0.9971 for FRAP and to 0.9705 for DPPH).

### 3.2. *In vivo* experiment

#### 3.2.1. Feed intake, body weight gains and feed efficiencies

Addition of raw or cooked garlic and/or cholesterol to the diets did not affect feed intake, body weight gains or feed efficiencies (data not shown).

### 3.3. Plasma lipids and plasma fibrinogen

At baseline, the 10 diet groups did not differ from one another in plasma lipid and fibrinogen concentrations

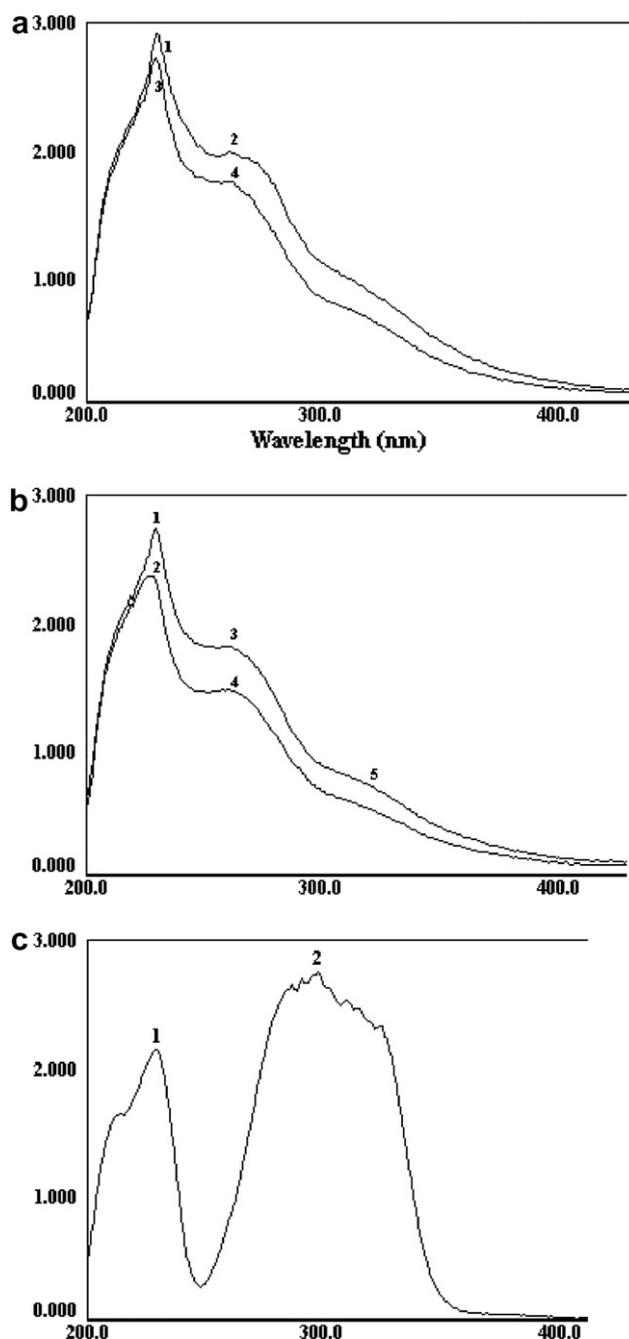


Fig. 1. A, B, UV-visible spectra of total polyphenol compounds detected at the corresponding wavelength: (a) polyphenols of garlic samples at  $0.5 \text{ mg mL}^{-1}$  with major peaks (nm): (1) 229.4, (2) 260.2; and (3) 228.6, (4) 259.4, corresponding to NPG and PG1. (b) Polyphenols of garlic samples at  $0.5 \text{ mg mL}^{-1}$  with major peaks (nm): (1) 228.7, (3) 258.0; and (2) 226.5, (4) 259.6, corresponding to PG2 and PG3. (c) *p*-Coumaric acid at 0.2 mM with the following major peaks (nm): (1) 228.5; and (2) 296.6. Abbreviations: Garlic (G) samples during processing: NPG, non processed; PG1, PG2, PG3, processed garlic at 100 °C for 20, 40 and 60 min.

(data not shown). After the experiment in the rats of two groups (Chol/NPG and Chol/PG1) a significant hindered of the rise of TC and LDL-C ( $P < 0.05$ ) and significant decrease in the concentration of plasma fibrinogen ( $P < 0.05$ ) were registered (Table 1).

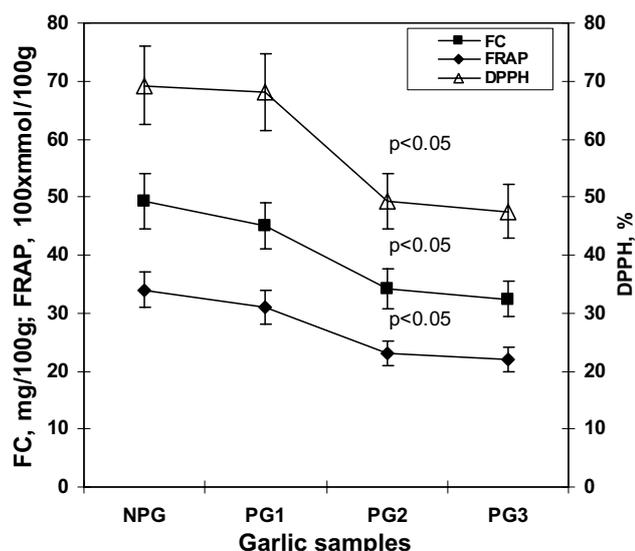


Fig. 2. Antioxidant capacities determined by different radical scavenging assays of garlic (G) samples during processing: NPG, non processed; PG1, PG2, PG3, processed garlic at 100 °C for 20, 40 and 60 min. (■) FC, polyphenols, µg/100 g FW: NPG = 49.3 ± 3.1<sup>a</sup>; PG1 = 45.1 ± 3.0<sup>a</sup>; PG2 = 34.1 ± 2.7<sup>b</sup>; PG3 = 32.4 ± 2.6<sup>b</sup>. (◆) FRAP, 100 × mmol/100 g: NPG = 34 ± 2.7<sup>a</sup>; PG1 = 31 ± 2.6<sup>a</sup>; PG2 = 23 ± 2.1<sup>b</sup>; PG3 = 22 ± 2.1<sup>b</sup>. (Δ) DPPH, %inhibition: NPG = 69.2 ± 5.1<sup>a</sup>; PG1 = 68.1 ± 5.0<sup>a</sup>; PG2 = 49.3 ± 3.1<sup>b</sup>; PG3 = 47.5 ± 3.1<sup>b</sup>. Values are means ± SD of five measurements. Values in the same method with different superscript letters are significantly different ( $P < 0.05$ ). Abbreviations: Folin-Ciocalteu (FC); Ferric Reducing/Antioxidant Power (FRAP); 1,1-diphenyl-2-picrylhydrazyl (DPPH).

### 3.4. Plasma antioxidant activity

At the end of the trial, a significant increase in the plasma antioxidant activity in the rats of NPG and PG1 groups was found ( $P < 0.05$ ): an increase in the FRAP values (Fig. 4). In rats of Chol, Chol/PG2 and Chol/PG3 groups the decrease in the antioxidant activity values (Fig. 4) was significant ( $P < 0.05$ ).

## 4. Discussion

As was mentioned in the Introduction, fruits and vegetables subjected to temperature are losing certain part of their bioactive substances. The knowledge about the influence of temperature on bioactive properties of cooked

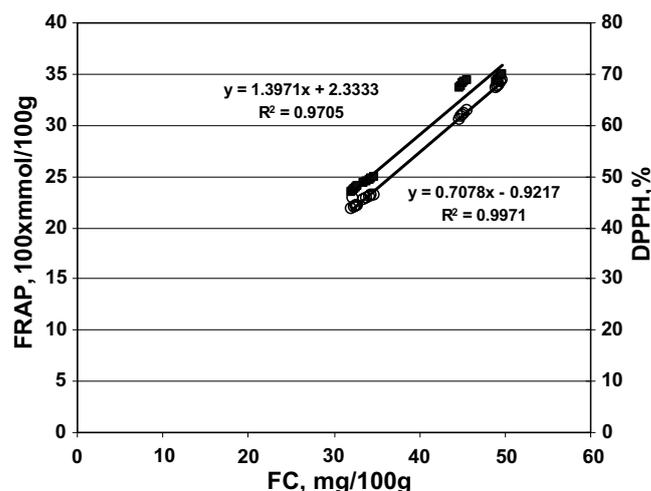


Fig. 3. Relationship, calculated by a linear regression analysis for processed garlic samples and their antioxidant capacities. (■) FC (µg/100 g, X): NPG = 49.3 ± 3.1<sup>a</sup>; PG1 = 45.1 ± 3.0<sup>a</sup>; PG2 = 34.1 ± 2.7<sup>b</sup>; PG3 = 32.4 ± 2.6<sup>b</sup> and FRAP (100 × mmol/100 g, Y<sub>1</sub>): NPG = 34 ± 2.7<sup>a</sup>; PG1 = 31 ± 2.6<sup>a</sup>; PG2 = 23 ± 2.1<sup>b</sup>; PG3 = 22 ± 2.1<sup>b</sup> to (○) FC (µg/100 g, X): NPG = 49.3 ± 3.1<sup>a</sup>; PG1 = 45.1 ± 3.0<sup>a</sup>; PG2 = 34.1 ± 2.7<sup>b</sup>; PG3 = 32.4 ± 2.6<sup>b</sup> and DPPH (%inhibition, Y<sub>2</sub>): NPG = 69.2 ± 5.1<sup>a</sup>; PG1 = 68.1 ± 5.0<sup>a</sup>; PG2 = 49.3 ± 3.1<sup>b</sup>; PG3 = 47.5 ± 3.1<sup>b</sup>. Values are means ± SD of five measurements. Values in the same method with different superscript letters are significantly different ( $P < 0.05$ ). Correlation coefficients are indicated. Abbreviations: Folin-Ciocalteu (FC); Ferric Reducing/Antioxidant Power (FRAP); 1,1-diphenyl-2-picrylhydrazyl (DPPH); NPG, non processed; PG1, PG2, PG3, processed garlic at 100 °C for 20, 40 and 60 min.

garlic is very limited (Constenla and Lozano, 2005; Dugo et al., 2005; Haciseferogullari et al., 2005). It was shown in some reports that health promoting effect of garlic is mostly cardioprotective (Banerjee et al., 2002; Zahid et al., 2005), and opposite data were described as well (Stevenson et al., 2000; Peleg et al., 2003).

Therefore, the aims of this investigation were: (a) to determine the optimal technological regime, which preserves the bioactivity of garlic and (b) to study the influence of raw and cooked garlic on plasma lipid levels and plasma antioxidant activity of rats fed cholesterol-containing and cholesterol-free diets. The optimal conditions for cooked garlic are very important.

The influence of different garlic processing and its antioxidant capacity which is reported in this study

Table 1  
Plasma lipids (mmol/L) and fibrinogen (g/L) in rats fed diets supplemented with non processed or processed garlic and 1% cholesterol

Diets	TC	LDL-C	HDL-C	TG	Fibrinogen
Chol	3.69 ± 0.21 <sup>a</sup>	2.08 ± 0.12 <sup>a</sup>	1.61 ± 0.07 <sup>a</sup>	0.88 ± 0.05 <sup>a</sup>	3.15 ± 0.19 <sup>a</sup>
Chol/NPG	2.81 ± 0.17 <sup>b</sup>	1.19 ± 0.05 <sup>b</sup>	1.62 ± 0.07 <sup>a</sup>	0.79 ± 0.05 <sup>a</sup>	2.51 ± 0.16 <sup>b</sup>
Chol/PG1	3.01 ± 0.18 <sup>b</sup>	1.37 ± 0.05 <sup>b</sup>	1.64 ± 0.07 <sup>a</sup>	0.91 ± 0.05 <sup>a</sup>	2.53 ± 0.16 <sup>b</sup>
Chol/PG2	3.60 ± 0.21 <sup>a</sup>	1.97 ± 0.05 <sup>a</sup>	1.63 ± 0.07 <sup>a</sup>	0.93 ± 0.05 <sup>a</sup>	3.09 ± 0.16 <sup>a</sup>
Chol/PG3	3.63 ± 0.21 <sup>a</sup>	1.99 ± 0.05 <sup>a</sup>	1.64 ± 0.07 <sup>a</sup>	0.95 ± 0.05 <sup>a</sup>	3.12 ± 0.16 <sup>a</sup>

Values are means ± SD,  $n = 7$ . Means in columns without letters in common differ significantly ( $P < 0.05$ ).

Abbreviations used: Chol, non oxidized cholesterol; Chol/NPG, group of rats which diet was supplemented with non processed garlic; Chol/PG1, Chol/PG2 and Chol/PG3, groups of rats which diet was supplemented with processed garlic for 20, 40 and 60 min, respectively; HDL cholesterol; LDL-C, LDL cholesterol; TC, total cholesterol; TG, triglycerides.

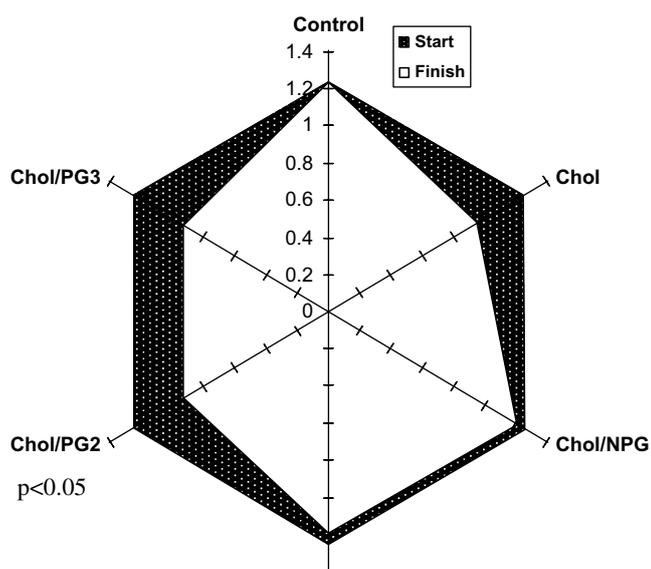


Fig. 4. Plasma antioxidant power after diets with cholesterol and garlic samples. (■) start of the experiment; (□) finish of the experiment. Abbreviations: Chol, cholesterol; NPG, PG1, PG2, PG3, non-processed, processed for 20, 40 and 60 min, respectively. At the finish of the experiment the following antioxidant values of plasma were obtained: Control ( $1.23 \pm 0.11^a$ ); Chol ( $0.94 \pm 0.09^b$ ); Chol/NPG ( $1.20 \pm 0.13^a$ ); Chol/PG1 ( $1.19 \pm 0.13^a$ ); Chol/PG2 ( $0.92 \pm 0.09^b$ ); Chol/PG3 ( $0.93 \pm 0.09^b$ ). Values are means  $\pm$  SD of five measurements. Values in the same method with different superscript letters are significantly different ( $P < 0.05$ ).

corresponds with recent data of Bhagyalakshmi et al. (2005) and Pedraza-Chaverri et al. (2006), where the ability of garlic extracts to scavenge different radicals was discussed. To measure the total antioxidant capacity of garlic extracts were employed FRAP and DPPH assays that utilize the same single electron transfer mechanism. These two methods were chosen for comparison because they are generally employed to evaluate plant materials (Ozgen et al., 2006; Schlesier et al., 2002). The limitations of these two methods are the non-physiologically low pH value and short time of the reaction which do not measure all antioxidants. Plant extracts contain different phenolic compounds, as it was shown by UV garlic extracts spectra. Real estimation or comparison of antioxidant capacity of the samples is complicated by the capacity for polymerization of these compounds. Single electron transfer reactions (FRAP and DPPH) can be relatively slow and measure relative percent decrease in product rather than kinetics or total antioxidant capacity. The inhibition of accumulation of colored radical reagents (DPPH) in the presence of antioxidants is not always linearly correlated to antioxidant concentration. FRAP generally do not measure all antioxidants in a complex matrix, and those antioxidants (reductants) giving slow reactions with the reagent may not be totally oxidized within the recommended time protocol of the method (Koleva et al., 2002; Apak et al., 2004).

Our results are in accordance with others (Ozgen et al., 2006), showing different values for the garlic extracts in

absolute terms but show the same relative ranking. A strong correlation was found for the total phenolic content and the FRAP and DPPH assays and this is in correspondence with Schlesier et al. (2002).

It was shown that the heating during specific time was unable to eliminate the capacity of the extracts to scavenge DPPH or the ability of the antioxidants in garlic extracts to reduce ferric-tripiridyltriazine ( $\text{Fe}^{3+}$ -TPTZ) to a ferrous form ( $\text{Fe}^{2+}$ ). Due to new innovations in instrumentation and processing technologies, the antioxidant properties of garlic can be preserved (Bhagyalakshmi et al., 2005; Lawson and Gardner, 2005).

In our recent studies (Gorinstein et al., 2006a) there were no significant changes in the dietary fibers contents, essential trace elements in the samples after cooking at  $100^\circ\text{C}$  for 20, 40 and 60 min. The selenium and copper content of raw garlic is not altered by boiling. The decrease in the content of total and  $\alpha$ -tocopherols was significant only in cooking samples at  $100^\circ\text{C}$  for 40 and 60 min ( $P < 0.05$ ).

In order to receive reliable data of the possible changes in the antioxidant potential a combination of FRAP and DPPH assays were used. As it was mentioned above we have to take into account the wide variety and range of action of antioxidant compounds presented in actual foods (Pellegrini et al., 2003). The results on polyphenols in this report were slightly higher than shown by others (Halvorsen et al., 2002; Szeto et al., 2002). The correlations between the total polyphenols and the two scavenging antioxidant assays were similar to others (Bahorun et al., 2004).

After 30 days of experiment *in vivo*, raw and cooked at  $100^\circ\text{C}$  for 20 min garlic samples significantly hindered the rise of plasma lipids in group of rats fed cholesterol (Chol/NPG and Chol/PG1). Diets supplemented with garlic in rats fed without cholesterol did not affect the lipid levels ( $P > 0.05$ ). Also others have demonstrated that hypolipidemic effect of fruits and vegetables is evident when they are added to diets of rats fed cholesterol (Kerckhoffs et al., 2002).

At the end of the trial, a significant increase in the plasma antioxidant activity in the rats of the NPG and PG1 groups and a decrease in the plasma antioxidant activity in rats fed with added cholesterol (Chol, Chol/NPG, Chol/PG1, Chol/PG2 and Chol/PG3 groups) were observed. However, the decrease in the plasma antioxidant activity in rats of the Chol/NPG and Chol/PG1 was significantly less than in rats of other groups fed cholesterol. Such results were expected: cholesterol supplemented diet decreases the blood antioxidant activity. Also Durak et al. (2002) reported that cholesterol-fed animals showed a significantly impaired antioxidant system.

It was found a dose-depending decrease (Gorinstein et al., 2006b) in the content of the blood circulating fibrinogen ( $P < 0.05$  in both cases).

Fibrinogen as well decreased of about 11% which is similar to the decrease of fibrinogen in patients with coronary heart disease (Chernyad'eva et al., 2003). The data of the previous investigation confirm that commercial garlic

possesses dose-dependent hypolipidemic, antioxidant and anticoagulation properties (Chernyad'eva et al., 2003). The fibrinogen content decreased significantly in Chol/PG2 and Chol/PG3 of about 15% in comparison with Chol/NPG and Chol/PG1.

Protein profile of plasma samples showed that in fibrinogen fraction of commercial garlic was detected less protein bands and lower intensity than in other groups. The main patterns were located in the range of 50–16 kDa, showing that the amount of fibrinogen has decreased during such diet (Gorinstein et al., 2006b).

Boiled garlic loses its anti-platelet activity. This may be because heating the garlic can destroy the enzyme, alliinase, which is responsible for converting alliin to allicin. Allicin is the anti-platelet component of the raw garlic extract. The results show that garlic can be beneficial for preventing thrombosis if taken in a raw rather than a boiled or processed form. Overall, boiled garlic has little or no effect on the activity of cyclooxygenase. These results show that garlic can be beneficial for preventing thrombosis if taken in a raw rather than a boiled or processed form. Therefore, an aqueous extract of fresh garlic in an experiment on rabbits had also showed that raw garlic has higher bioactivity than boiled samples (Ali, 1995).

Therefore, the aims of this investigation were achieved: we determined the optimal technological boiling regime, which preserves the bioactivity of raw garlic and it was found that garlic positively influences the plasma lipid levels and plasma antioxidant activity. The data of the present investigation confirm that garlic sample possesses hypolipidemic, antioxidant and anticoagulation properties. 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' plasma and a decrease in fibrinogen content. These results correspond to Apitz-Castro et al. (1992) and Ackermann et al. (2001). Therefore, it should be added at this time to foods.

The reported results support our recent publication (Gorinstein et al., 2006) where was shown the effect of garlic, in a form more similar to how most people eat garlic, on lipid and antioxidant metabolism in rats. 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 and in present study DPPH and FRAP were employed. The highest results (Gorinstein et al., 2006) were estimated in aqueous fraction in comparison with other extracts divided on the basis of polarity, but in the present report overall composition of non-fractionated garlic was used.

At the end of the trial changes in the level of the plasma lipids were recorded. The most dramatic changes were in the TC and LDL-C levels in rats of the Chol/NPG and Chol/PG1 diet groups. The decrease in the levels of the TC and LDL-C was significant ( $P < 0.05$ ). Therefore, diets supplemented with raw and cooked for 20 min garlic are bioactive and are decreasing the levels of TC and LDL-C. Opposite, the diets supplemented with garlic

cooked for 40 and 60 min are losing part of their bioactivity and therefore did not decrease the TC and LDL-C. The HDL-C and TG levels remained the same. According to our experience with other natural products only in experiments which duration is more than 60 days also significant changes in these two indices were observed. In our previous investigation *in vivo* we used powdered lyophilized garlic (Gorinstein et al., 2006b). The optimal results were received using 125 mg of powdered lyophilized garlic (a dose equal to 500 mg of raw garlic) per kg of animal weight. Therefore, for person of 70 kg weight is needed  $125 \text{ mg} \times 70 = 8750 \text{ mg}$  or 8.75 g per day, which could be taken with food in three divided doses. However, we did not recommend to apply automatically the results of the animal experiments to humans.

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.

In conclusion, this study indicates that raw and cooked at 100 °C for 20 min garlic samples contain high comparable quantities of bioactive compounds and possess high total antioxidant potential. Only these garlic samples positively influence plasma lipid levels and plasma antioxidant activity in rats fed cholesterol-containing diets. In order to preserve the important properties of garlic, this vegetable must be added to cooking dishes not early than 20 min before the end of the cooking process. However, the results of this experiment on animals could not be to automatically apply to humans.

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