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The Influence of Raw and Processed Garlic and Onions on Plasma Classical and Non-classical Atherosclerosis Indices: Investigations *In Vitro* and *In Vivo*

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Garlic and white and red varieties of onion were subjected to processing by a variety of culinary methods, and bioactive compounds then determined. For *in vivo* studies, 84 male Wistar rats were randomly divided into 14 diet groups, each of six rats, including two control groups (one with no supplementation and one with cholesterol supplementation only). During the 30-day trial, the basal diets of the other 12 groups were supplemented with 1% cholesterol and raw or processed vegetables.

Both raw red onion and red onion subjected to blanching for 90 s hindered the rise in plasma lipids more than the other vegetables studied in the supplemented diets. The decrease in antioxidant activity compared to the cholesterol-supplemented control group was significantly less for the group fed with red onion subjected to blanching for 90 s.

No histological changes were detected in the studied organs of rats that had been fed cholesterol. In conclusion, blanching for 90 s most fully preserved the bioactive compounds and antioxidant potentials, and hindered the rise in plasma lipid levels and the decrease in plasma antioxidant activity of rats fed cholesterol. Alkaline phosphatase levels correlated with classical atherosclerosis indices, and determination of alkaline phosphatase is suggested as an additional index in atherosclerosis testing.

Keywords: vegetables; antioxidants; rats; plasma lipids; transaminases; CRP.

INTRODUCTION

It was shown that consumption of fruit and vegetables prevents some diseases (Dallongeville *et al.*, 2006; Knai *et al.*, 2006; Vainio and Weiderpass, 2006). Among vegetables, garlic and onions play a special role (Campos *et al.*, 2003; Gorinstein *et al.*, 2006; Corzo-Martínez *et al.*, 2007; Kung-chi *et al.*, 2007). It has been reported that garlic and onions are effective in preventing cardiovascular disease because of their hypocholesterolemic, hypolipidemic, antihypertensive, antidiabetic, antithrombotic and antihyperhomocysteinemia effects (Corzo-Martínez *et al.*, 2007; Faller and Fialho, 2009).

These vegetables also exercise antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory and prebiotic activities (Corzo-Martínez *et al.*, 2007; Omar *et al.*, 2007; Utesch *et al.*, 2008). Therefore, in recent decades, the role of garlic and onions in preventing cardiovascular diseases has been studied extensively (Kung-chi *et al.*, 2007). It has been shown that consumption of both vegetables indeed improves the atherosclerosis indices (Corzo-Martínez *et al.*, 2007; Liu *et al.*, 2007). The protective effects of these natural products are related to saturated (14:0, 15:0, 16:0, and 18:0) and unsaturated (7–16:1, 7–18:1, 9–18:1, 9,12–18:2, 9,12,15–18:3) acids, together with novel cyclic sulfur-containing fatty acids (Dembitsky *et al.*, 2007), their phenolic compounds and to a lesser extent to their dietary fiber (Martinez-Gonzalez *et al.*, 2002; Halvorsen *et al.*, 2006). The protective effect of garlic oil against fatty liver in rats chronically fed with a high-fat diet was investigated (Wang *et al.*, 2007).

Garlic and onions are mostly consumed after processing rather than raw which leads to a certain decrease in the contents of their bioactive compounds and their related antioxidant potential (Nicoli *et al.*, 1999; Kawamoto *et al.*, 2004).

The aim of this investigation was to find a kind of processing that best preserves the contents of their bioactive compounds and antioxidant potentials and *in vivo* studies to investigate the influence the processed vegetables had on plasma atherosclerotic indices. The contents of the bioactive compounds in natural

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products does not necessarily indicate the level of the antioxidant potential (Lotito and Frei, 2004). The synergetic effect, which could exist between individual bioactive compounds, means that individual antioxidant potential may be greater than their sum (Akila and Devaraj, 2008). Therefore, it was decided to compare also the total antioxidant potential of the studied vegetables.

It is known that some antioxidant assays result in different antioxidant activity trends (Ou *et al.*, 2002). Three assays which complement each other for determination of the total antioxidant potential were used: ABTS, FRAP and DPPH. In the last few decades, different enzymes that were not classical predictors of the atherosclerotic process have been recommended (Ridker, 2003). Among them were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and C-Reactive Protein (CRP). It was decided to use these non-classical indices to assess their values in predicting the status of the atherosclerotic process. The cholesterol-supplemented diets could trigger atherosclerotic changes in the aorta (Kwon *et al.*, 2003). Therefore, histological investigation of some organs of rats fed cholesterol was performed.

As far as we know, results of such investigations have not been published.

MATERIAL AND METHODS

Chemicals. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (FCR), $FeCl_3 \cdot 6H_2O$, and butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co. (St Louis, MO, USA). 2, 4, 6-tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie (Buchs, Switzerland). All reagents were of analytical grade. Deionized and distilled water was used throughout.

Samples. In this investigation, garlic (*Allium sati*V*um* L.) and white (Armstrong) and red (Red Baron) onions (*Allium cepa*) harvested in 2008 were used.

The unprocessed bulbs of garlic and white and red onions were washed, cleaned, peeled and cut with a plastic knife (halves for garlic and pieces for onions). The samples were divided into 14 groups before blanching, boiling or frying and named as following: 1. Garlic, lyophilized raw garlic; 2. Garlic90seconds, garlic blanched for 90 s; 3. Garlic10minutes, garlic boiled for 10 min; 4. Garlic10minutesF, garlic fried for 10 min; 5. WO, lyophilized raw white onion; 6. WO90 seconds, white onion blanched for 90 s; 7. WO10minutes, white onion boiled for 10 min; 8. WO10minutesF, white onion fried for 10 min; 9. RO, lyophilized raw red onion; 10. RO90seconds, red onion blanched for 90 s; 11. RO10minutes, red onion boiled for 10 min; 12. RO10minutesF, red onion fried for 10 min; 13. Control; 14 Control/Chol.

Animals and diets. The Animal Care Committee of the Warsaw Agricultural University approved this study. Male Wistar rats (84) with an average weight of 115 g at the onset of the experiment were used in this investigation. The animals were divided into 14 groups of six and housed in plastic cages.

These groups were named as follow: Control, Control/Chol, Garlic, Raw Garlic; Garlic90seconds, Garlic10minutes, Garlic10minutesF; WO, raw white onion, WO90seconds, WO10minutes, WO10minutesF; RO, raw red onion; RO90seconds, RO10minutes, and RO10minutesF. A five-day adaptation and the 24-hour starvation periods were used before the beginning of the study. The rats of Control group were fed a basal diet (BD). The BD of the Control/Chol group was supplemented with 1% of non-oxidized cholesterol (NOC) only.

Diets of all other 12 groups were supplemented with 1% of non-oxidized cholesterol (NOC) and with lyophilized raw or processed vegetables (blanched for 90 s, boiled for 10 min or fried for 10 min) in quantities of 25 mg at the onset of the experiment and 45 mg/day/rat at the end, respectively.

The changes in the quantities of the supplemented garlic and onions were connected to the growing weight of the animals. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats. Our prior experiments on laboratory animals have shown that cellulose does not have significant hypocholesterolemic effects. Therefore, cellulose was used as a control fiber. All rats were fed once a day at 10:00 h. They had unrestricted access to drinking water.

Laboratory tests. Garlic, and white and red onions were subjected to blanching for 90 s, boiling for 10 min or frying for 10 min and then the contents of polyphenols, flavonoids, flavanols, anthocyanins and tannins, and the antioxidant potentials were determined as previously described (Gorinstein *et al.*, 2008; 2009) and compared with the data using raw vegetables. The presence of polyphenols in the investigated vegetables was studied by Fourier Transform Infrared (FT-IR) spectroscopy. A Bruker Optic GMBH Vector FT-IR spectrometer (Bruker Optic GMBH, Attingen, Germany) was used to record IR spectra (Gorinstein *et al.*, 2009).

At the end of the experiment, the rats were anaesthetized using diethyl ether, and the blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests, which included determination of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and plasma antioxidant activity.

As was stated, the ABTS, FRAP and DPPH tests were adopted also for determination of the plasma antioxidant activity.

AST, ALT, ALP and CRP were determined with utilization of analyzer Siemens-Advia 1650 according to the principles of the Bayer Chemistry System.

The histology of the aorta, heart and brains were analyzed. After a formalin fixation, segments of these organs were processed by a common paraffin technique. Each sample was cut into 72 serial sections (thickness of 5 μm) with a transversally oriented cutting plane and stained with hematoxylin and eosin (HE), as well as with green trichrome (Baluchnejadmojarad and Roghani, 2003; Deepa and Varalakshmi, 2005;

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Valcheva-Kuzmanova *et al.*, 2007; Akila and Devaraj, 2008).

Statistical analysis. The results of this investigation *in vitro* are means ± SD of five measurements. Differences between groups were tested by two-way ANOVA. In the assessment of the antioxidant potential, Spearman correlation coefficient (R) was used. Linear regressions were also calculated. The P values of <0.05 were considered significant.

RESULTS

Figure 1(A–C) shows bands assignments for polyphenols in the investigated vegetable samples. The wavelengths numbers (cm^{-1}) of FTIR spectra were determined in our previous experiment for catechin 827, 1,039, 1,115, 1,143, 1,286, 1,478, 1,511 and 1,610 and were assigned to C–H alkenes, –C–O alcohols**,** C–OH alcohols, –OH aromatic, C–O alcohols, C–H, alkanes,

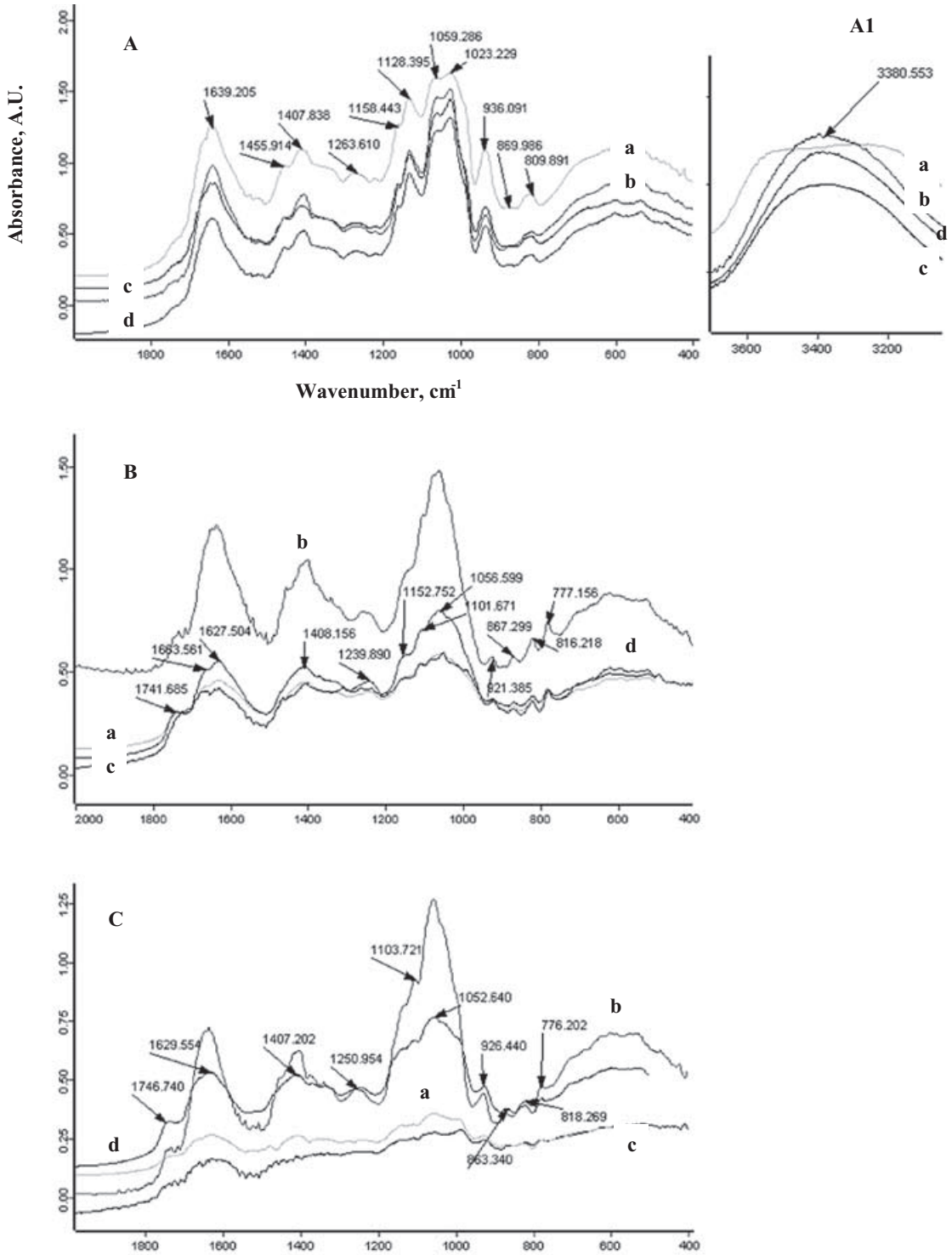

Figure 1. FTIR spectra of Garlic **(A) (AI)**; white onion (WO) **(B)**; red onion (RO) **(C)**. The following lines on A, AI, B and C: line **a**, Garlic, WO and RO, fried for 10 min; line **b**, Garlic, WO and RO, raw; line **c**, Garlic, WO and RO, boiled for 10 min; line **d**, Garlic, WO and RO, blanched for 90 s.

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C=C aromatic ring, and C=C alkenes (Gorinstein *et al.*, 2009). Gallic acid showed the following wavelength numbers (cm^{-1}) of 866, 1,026, 1,238, 1,450, 1,542, and 1,618 (Gorinstein *et al.*, 2009). The line **a** (Figs 1A, 1AI, 1B and 1C), according to the absorbance units, had the highest position for Garlic10minutesF (Fig. 1A). For WO10minutesF and RO10minutesF in the same range of wavelengths (1800–700 cm^{-1}) as for garlic and showed a lower position, similar to blanching for 90 s (Figs 1B and 1C). In FTIR (line **b**, Figs 1AI, B and C) spectra (3600–3200 cm^{-1}) for Raw Garlic, WO and RO showed the highest position. Line **c** of blanching for 90 s was similar to Raw Garlic (Fig. 1A), and in the spectra of onions was similar to WO10minutesF and RO10minutesF. The line **d** for boiled vegetables during 10 min was the lowest for Garlic, in WO and RO were placed under the line for the raw samples. The measured spectra in the range of 3450–3650 cm^{-1} for –OH (Fig. 1A) were equal in all investigated samples (spectra for WO and RO are not shown). An equal peak for all samples was shown at 1408 cm^{-1} in the wavelength range between 1310 and 1410 cm^{-1} for polyphenols. The range between 1500 and 1700 cm^{-1} for aromatic compounds was similar for all samples, showing a small shift (cm^{-1}) in the main peak around 1639 (Fig. 1A), 1628 and 1663 (Fig. 1B) and 1630 (Fig. 1C). FTIR spectroscopy can be used as an additional tool to screen vegetables for their content of phenolic compounds.

The results of the *in vitro* investigation of the contents of the bioactive compounds in raw and processed garlic and onions are summarized in the Table 1.

These results were compared with the data of our previous investigation (Gorinstein *et al.*, 2008). Some differences were found, but they were not significant ($P > 0.05$). As can be seen, vegetables subjected to blanching for 90 s most fully preserve the contents of bioactive compounds, and the highest amounts of these bioactive compounds were detected in red onion. However, these differences, excluding flavanols, were not significant ($P > 0.05$).

According to all three assays (DPPH, FRAP and ABTS) that were used, vegetables subjected to blanching for 90 s most fully preserve the antioxidant potential, and it was higher in red onion (Fig. 2). However, these differences, excluding FRAP, were not significant ($P > 0.05$).

Feed intake, gains in body weight and the feed-efficiency ratio are summarized in Table 2. The data were different: for example, the highest numbers of feed intake and body-weight gains (both in g) were in the group of white onion fried for 10 minutes (584.7 ± 24.4 and 178.8 ± 19.6, respectively), however the differences were not statistically significant ($P > 0.05$).

An increase in the levels of plasma lipids was registered in all groups fed diets containing cholesterol. However, the increase in the vegetable-supplemented groups *vs* Chol group was not significant ($P > 0.05$).

Only in diet groups supplemented with raw or blanched red onion was there hindering of the rise in

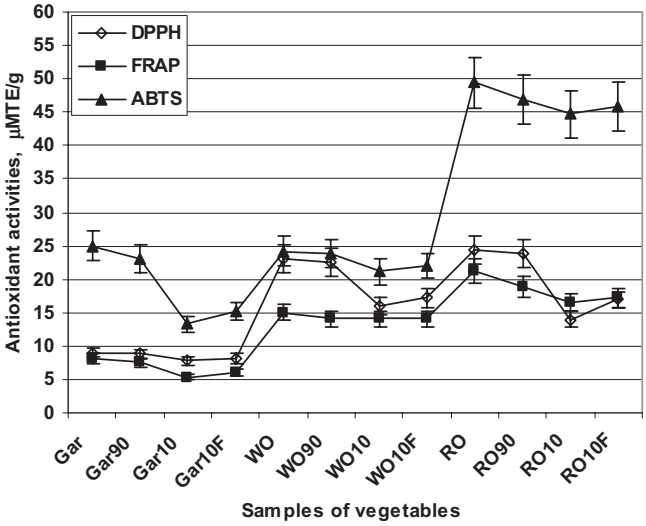

Figure 2. Antioxidant activities in raw garlic, white and red onions before and after their processing (μM TE/g DW). Abbreviations: DW, dry weight; Gar, raw garlic; Gar90, garlic blanched for 90 s; Gar10, garlic boiled for 10 min, Gar10F, garlic fried for 10 min; RO, raw red onion; RO90, red onion blanched for 90 s; RO10, red onion boiled for 10 min, RO10F, red onion fried for 10 min; WO, raw white onion, WO90, white onion blanched for 90 s; WO10, white onion boiled for 10 min, WO10F, white onion fried for 10 min. Values are means ± SD of 5 measurements.

Table 1. Contents of the studied bioactive compounds in raw garlic and in white and red onions and after their processing (DW)

Samples	Polyphenols mg $GAEg^{-1}$	Flavonoids, $mgCEg^{-1}$	Flavanols, μg CEg^{-1}	Anthocyanins, mg100 g^{-1}	Tannins, $mgCEg^{-1}$
Garlic	20.50 ± 1.3^{b}	3.73 ± 0.3^{c}	6.37 ± 0.4^{c}	0.68 ± 0.07^{b}	2.64 ± 0.2^{b}
Garlic90″	17.05 ± 1.1^{a}	2.66 ± 0.2^{b}	4.90 ± 0.3^{b}	0.60 ± 0.06^{b}	2.23 ± 0.2^{b}
Garlic10′	15.12 ± 0.9^{a}	1.98 ± 0.1^{a}	3.80 ± 0.2^{a}	0.40 ± 0.03^{a}	1.62 ± 0.1^{a}
Garlic10′F	16.84 ± 1.0^{a}	2.54 ± 0.2^{b}	4.08 ± 0.2^{a}	0.47 ± 0.04^{a}	2.11 ± 0.2^{b}
WO	23.66 ± 1.4^{c}	4.21 ± 0.4^{d}	7.55 ± 0.4^{c}	1.65 ± 0.2^{d}	2.76 ± 0.2^{b}
WO 90″	22.92 ± 1.4^{c}	3.52 ± 0.2^{c}	6.12 ± 0.3^{c}	1.49 ± 0.2^{c}	2.50 ± 0.2^{b}
WO 10′	19.92 ± 1.3^{b}	2.98 ± 0.1^{b}	5.44 ± 0.2^{b}	1.00 ± 0.1^{b}	1.82 ± 0.1^{a}
WO 10′F	22.54 ± 1.4^{c}	3.32 ± 0.3^{c}	5.98 ± 0.2^{b}	1.35 ± 0.1^{c}	2.38 ± 0.2^{b}
RO	30.12 ± 1.6^{d}	4.44 ± 0.4^{d}	8.72 ± 0.6^{d}	1.29 ± 0.1^{c}	4.99 ± 0.4^{d}
RO 90″	25.15 ± 1.5^{c}	2.95 ± 0.2^{b}	8.42 ± 0.6^{d}	1.00 ± 0.1^{b}	3.66 ± 0.3^{c}
RO 10′	22.24 ± 1.4^{c}	2.64 ± 0.1^{b}	8.00 ± 0.6^{d}	0.80 ± 0.08^{b}	3.12 ± 0.3^{c}
RO 10′F	24.92 ± 1.5^{c}	2.82 ± 0.2^{b}	8.10 ± 0.6^{d}	0.81 ± 0.08^{b}	3.34 ± 0.3^{c}

Values are means ± SD of 5 measurements. Means in columns without superscript letters in common differ significantly ($P < 0.05$). Abbreviations: DW, dry weight; Garlic, raw garlic; Garlic90″, blanched garlic for 90″; Garlic10′, garlic boiled for 10′, Garlic10′F, garlic fried for 10′; RO, raw red onion; RO 90″, red onion blanched for 90″; RO10′, red onion boiled for 10′, RO10′F, red onion fried for 10′; WO, raw white onion WO, WO 90″, white onion blanched for 90″; WO10′, white onion boiled for 10′, WO10′F, white onion fried for 10′; ″, seconds; ′, minutes.

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Table 2. Feed intake (FI), body weight gains (BWG) and feed efficiency ratio (FER)

Groups	FI, g	BWG, g	FI, day/g	BWG, day/g	FER
Garlic Raw	500.4 ± 62.5[a]	121.7 ± 41.4[a]	14.72 ± 1.90[a]	3.58 ± 0.32[a]	4.34 ± 0.84[a]
Garlic 90″	516.9 ± 43.4[a]	137.1 ± 27.0[a]	15.2 ± 1.30[a]	4.03 ± 0.80[a]	3.837 ± 0.4[a]
Garlic 10′	501.5 ± 50.8[a]	123.3 ± 32.6[a]	14.75 ± 1.50[a]	3.63 ± 1.00[a]	4.230 ± 0.77[a]
Garlic 10′ F	503.1 ± 54.6[a]	123.1 ± 36.3[a]	14.80 ± 1.60[a]	3.62 ± 1.10[a]	4.347 ± 1.07[a]
WO Raw	491.6 ± 44.0[a]	122.5 ± 20.6[a]	14.46 ± 1.30[a]	3.60 ± 0.60[a]	4.070 ± 0.49[a]
WO 90″	535.7 ± 40.2[a]	148.9 ± 26.0[a]	15.75 ± 1.20[a]	4.38 ± 0.80[a]	3.662 ± 0.49[a]
WO 10′	566.6 ± 26.0[a]	156.4 ± 27.5[a]	16.66 ± 0.80[a]	4.60 ± 0.80[a]	3.693 ± 0,50[a]
WO 10′ F	584.7 ± 24.4[a]	178.8 ± 19.6[a]	17.20 ± 0.70[a]	5.26 ± 0.60[a]	3.289 ± 0.23[a]
RO Raw	518.5 ± 68.2[a]	129,3 ± 53,6[a]	15.25 ± 2.00[a]	3.80 ± 1.60[a]	4.490 ± 1.42[a]
RO 90″	549.6 ± 43.5[a]	153.0 ± 36.2[a]	16.17 ± 1.30[a]	4.50 ± 1.10[a]	3.766 ± 0.91[a]
R 10′	543.3 ± 45.6[a]	147.2 ± 32.9[a]	15.98 ± 1.30[a]	4.33 ± 1.00[a]	3.801 ± 0.66
RO 10′F	528.0 ± 67.1[a]	138.5 ± 48.7[a]	15.53 ± 2.00[a]	4.07 ± 1.40[a]	4.114 ± 1.08[a]
Control	568.9 ± 64.3[a]	167.8 ± 24.8[a]	16.26 ± 1.80[a]	4.80 ± 0.7[a]	3.42 ± 0.42[a]
Control/Chol	533.1 ± 57.1[a]	153.1 ± 34.8[a]	15.68 ± 1.70[a]	4.50 ± 1.0[a]	3.582 ± 0.58[a]

Abbreviations: Garlic Raw, lyophilized raw garlic; Garlic90″, garlic blanched for 90″; Garlic10′, garlic boiled for 10′; Garlic10′F, garlic fried for 10′; WO, lyophilized raw white onion; WO90″, white onion blanched for 90″. WO10′, white onion boiled for 10′; WO 10′F, white onion fried for 10′; RO, lyophilized raw red onion; RO 90″, red onion blanched for 90″; RO 10′, red onion boiled for 10′; RO 10′F, red onion fried for 10′; ″, seconds; ′, minutes.

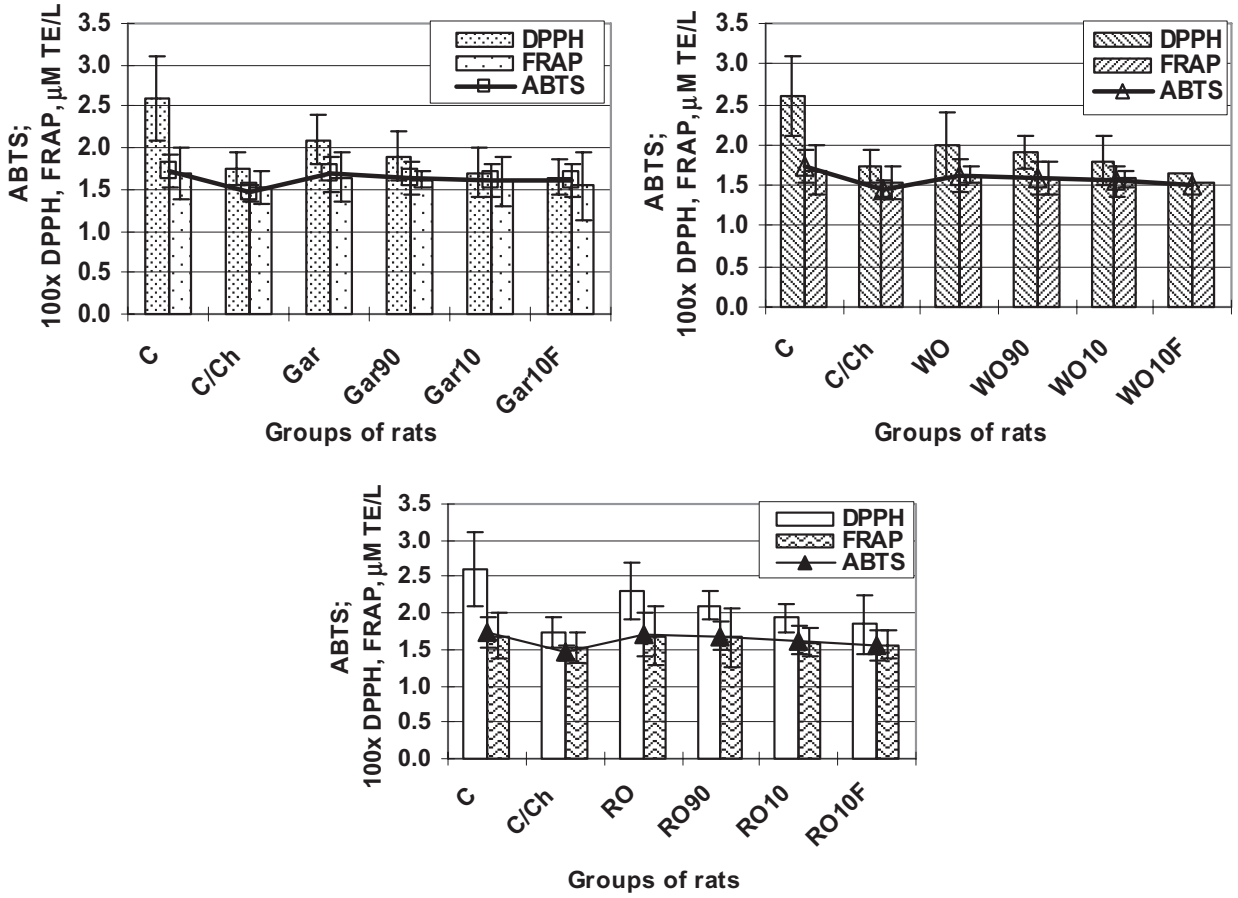

Figure 3. Plasma antioxidant activity of rats as influenced by raw garlic, white and red onions after their processing (μMTE/L). Abbreviations: C, Control, group of rats without additional treatment; C/Ch, group of rats on cholesterol containing diet only; All other groups: Gar, raw garlic; Gar90, garlic blanched for 90 s; Gar10, garlic boiled for 10 min, Garlic10F, garlic fried for 10 min; RO, raw red onion; RO90, red onion blanched for 90 s; RO10, red onion boiled for 10 min, RO10F, red onion fried for 10 min; WO, raw white onion, WO90, white onion blanched for 90 s; WO10, white onion boiled for 10 min, WO10F, white onion fried for 10 min were on cholesterol-containing diet supplemented with the studied raw or processed vegetables, respectively, AA, antioxidant activity. Values are means ± SD of 5 measurements.

plasma lipids vs. Chol group (TC – 11.2% and 10.7%, LDL-C – 11.0% and 6.5%, HDL-C – 12.6% and 8.6%, TG – 14.1% and 9.4%, respectively).

The data of the changes in plasma antioxidant activity in rats fed cholesterol-containing diets supplemented with garlic and onions are presented in Fig. 3. A significant decrease was registered in the plasma antioxidant activity *vs* Control Group ($P < 0.05$). However, the decrease in the antioxidant activity *vs* Chol Group was significantly less: by 15.8%, 20.7% and 9.2%, according to ABTS, DPPH and FRAP, respectively, for the Diet Group supplemented with blanched red onion.

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The determination of ALP, AST and ALT shows that only alkaline phosphatase correlates with the classic atherosclerosis indices. So, the content of the total cholesterol was in Control, Chol and CholRG groups 2.82, 3.69 and 3.02 mmol/L, respectively, and the level of ALP – 40.1, 44.7 and 41.9 IU/L, respectively. In our experiment, C-reactive protein (CRP) did not correlate with the data of plasma lipid and plasma antioxidant activity (data not shown). In our opinion, it could be connected with the short period of investigation.

No histological atherosclerotic changes in the studied organs (aorta, heart and brains) of rats fed cholesterol with and without vegetables supplementation were detected (Fig. 4).

DISCUSSION

As was stated, garlic and onions are usually eaten after processing, which according to some authors leads to a certain decrease in the contents of their bioactive compounds and their related antioxidant potential (Nicoli *et al.*, 1999; Kawamoto *et al.*, 2004; Sahlin *et al.*, 2004). Therefore, the aim of this investigation *in vitro* was to find a kind of processing that most fully preserves the contents of these indices in garlic and onions, followed by *in vivo* investigation of the influence of these processed vegetables on rat-plasma atherosclerosis data.

The differences in the vegetables were expected: it was shown by some authors that the contents of bioactive compounds in fruit and vegetables are influenced by geographical region, weather and storage conditions and their degree of ripeness (Geyas *et al.*, 1996; Proteggente *et al.*, 2002; Sun *et al.*, 2002).

As mentioned before, the investigated vegetables showed anticarcinogenic and antitumorigenic effects, but these properties were connected with both oil- and water-soluble allyl sulfur compounds (Omar *et al.*, 2007). The protective effect of organosulfur compounds, fatty acids and sulfur-containing fatty acids was demonstrated as well (Dembitsky *et al.*, 2007).

Our research is based on the antioxidant properties, mostly derived from polyphenols, of garlic and onions. Our results corresponded with Utesch *et al.* (2008), who showed that quercetin found in onions possesses antioxidant, anticarcinogenic, anti-inflammatory and cardioprotective properties. Some authors show that processing of fruit and vegetables decreases the contents of their bioactive compounds and antioxidant potential. Sahlin *et al.* (2004) investigated boiled, baked or fried tomatoes, and then total phenolics, ascorbic acid, lycopene and antioxidant potential using the ABTS assay were determined and compared with those in raw tomatoes. Boiling and baking had a relatively small effect on phenolics, ascorbic acid, lycopene and the antioxidant potential of tomatoes, while frying significantly reduced the total phenolics, ascorbic acid and lycopene ($P < 0.001$).

The quantification of polyphenols and the antioxidant capacity of fresh and processed onions were studied by Faller and Fialho (2009). Before the thermal processing, onions had a total of phenolics ranging from 16.76 to 21.57 mg GAE/g DW; antioxidant capacity determined by DPPH ranged from 17.97 to 22.32 μM TE/g DW. The cited numbers of hydrolysable polyphenols corresponded with our results (Table 1) for raw white onions of 23.66 mg GAE/g DW. The antioxidant activity was also within a similar range (Fig. 2, 23.05 μM TE/g DW by DPPH assay). The evaluation of boiling, microwaving and steaming on these parameters was performed. The antioxidant activity in onions decreased by about 40.8% during 8 min of boiling (Faller and Fialho, 2009), while in this report, the decrease in the DPPH assay after 10 min of boiling was 30.6% (Fig. 2). In general, cooking was found to reduce the antioxidant

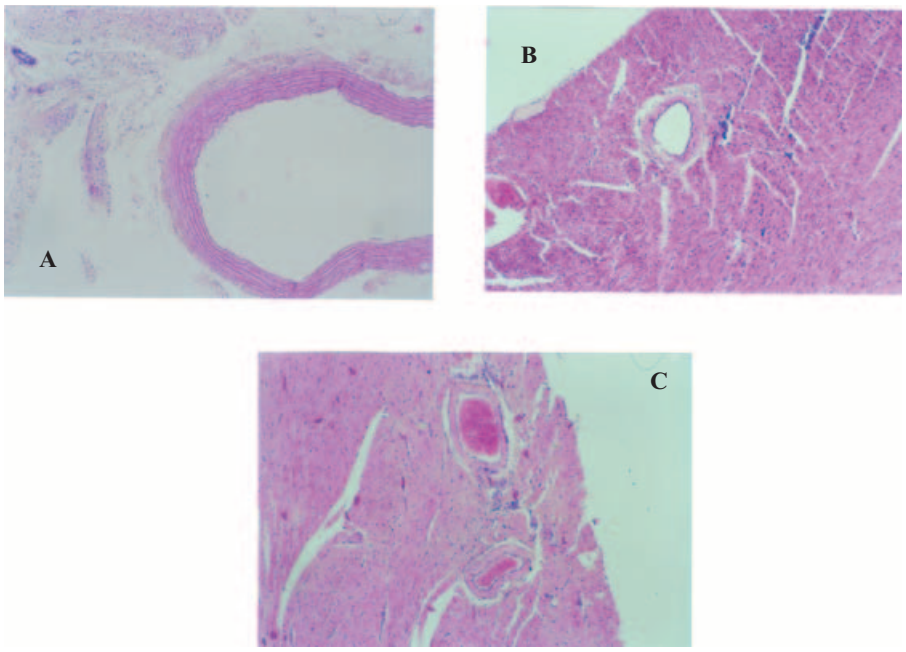

Figure 4. The histopathological examination of (A) microscopic image of the thoracic aorta wall without atherosclerotic lesions; (B) the coronary artery wall without atherosclerotic lesions; (C) the typical microscopic image of the brain arteries without atherosclerotic lesions.

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capacity in most vegetables, with small differences shown among the cooking methods. Polyphenols showed a positive correlation with the antioxidant capacity in raw and processed onions. However, not all investigators found that processing decreases the bioactivity of these natural products (Choi *et al.*, 2006; Randhir *et al.*, 2008). Choi *et al.* (2006) studied the effect of heat treatment on polyphenolic compounds and overall antioxidant activity of shitake mushrooms. Raw shitake was heated at 100 and 121°C for 15 or 30 min using an autoclave. After heat treatment, the free and bound polyphenolics and flavonoids in the mushroom extracts were analyzed.

ABTS and DPPH were measured to evaluate the antioxidant activity of the extracts. The polyphenolic contents and antioxidant activities in the extracts increased as heating temperature and time increased. The free polyphenolic content in the extract heated at 121°C for 30 min was increased by 1.9-fold compared to that in the extract from the raw sample. The ABTS and DPPH radical scavenging activities were increased by 2-fold and 2.2-fold compared to the raw sample, respectively. According to these investigators, the results showed that heat treatment significantly enhanced the overall antioxidant activities of shitake mushrooms. Randhir *et al.* (2008) have studied the effect of thermal processing via autoclaving on modifications of total phenolics, antioxidant activity and functionality of wheat, buckwheat, corn and oats sprouts and seedlings. Data concerning the changes in enzyme activity during the thermal processing of grains were compared.

Functionality for amylase and glucosidase inhibition related to Type 2 diabetes and levo-dihydroxy phenylalanine (l-DOPA) content, hypertension-related angiotensin converting enzyme 1 (ACE) inhibition and ulcer-related *Helicobacter pylori* inhibition were evaluated using *in vitro* assays. They reduced the cognitive function/diabetes-related l-DOPA content in all grains, sprouts and seedlings tested. It increased ACE inhibitory activity in buckwheat and oats, but decreased it in wheat and corn sprouts. Thermal processing in general resulted in tissue browning leading to higher total phenolic content and antioxidant activity linked to free radical scavenging. It increased amylase inhibitory activity in buckwheat and oats, but decreased in wheat and corn sprouts and seedlings, yet it increased glucosidase inhibitory activity in wheat, buckwheat and oats but decreased it in corn sprouts. It also improved the ulcer-related *H. pylori* inhibitory activity in all the grain sprouts and seedlings studied. These authors suggested that the changes in functionality were due to modifications in the total phenolic content and profile by phenolic oxidation or polymerization caused by thermal processing. We share the ideas of the above-cited authors. Our data show that blanching and frying for a short time only minimally decreases the bioactive compounds' content and the antioxidant potential of garlic and onions. We found that the influence of the studied raw and processed garlic and onions on plasma atherosclerosis indices was only partially significant. The changes in plasma lipid levels were not significant, whereas the relative decrease in the plasma antioxidant activity *vs* Chol Group was significant. This decrease in the plasma antioxidant activity was predictable. Other investigators have also observed that a cholesterol-supplemented diet leads to a decrease in plasma antioxidant activity (Mahfouz and Kummerow, 2000). Our data differed from Wang *et al.* (2007), whose rats receiving garlic oil were treated for eight weeks. The contents of TG, TC and LDL-C in liver and the contents of TC, TG and free fatty acids in serum decreased significantly in garlic-oil groups. Pathology showed that garlic oil markedly ameliorated fatty liver (Wang *et al.*, 2007). In our study, the changes were only in serum, and the pathology was not detected because the experimental period was only four weeks, and the percentage of fat was only 1%. As we stated above, we studied not only plasma lipids and antioxidant activity but also some other indices connected to the atherosclerosis process (Smith *et al.*, 2006). Among them was C-reactive protein (CRP), whose role in the risk for cardiovascular disease remains controversial (Smith *et al.*, 2006). Some authors claim that elevated CRP levels provided no further prognostic information beyond the traditional office-risk factor assessment to predict future major CVD and coronary heart disease (Wilson *et al.*, 2005). However, most of the investigators found that elevated C-reactive protein is a relatively moderate predictor of coronary heart disease (Ridker, 2003). According to the author cited above, in an attempt to improve global cardiovascular risk prediction, considerable interest has focused on CRP, a marker of inflammation that has been shown in multiple prospective epidemiological studies to predict incident myocardial infarction, stroke, peripheral arterial disease and sudden cardiac death.

Clinical data were supported by abundant laboratory and experimental evidence, which demonstrates that atherothrombosis, in addition to being a disease of lipid accumulation as in our research, also represents a chronic inflammatory process (Ridker, 2003). In the view of this author, CRP seems to be a stronger predictor of cardiovascular events than LDL cholesterol, and it adds prognostic information at all levels of calculated Framingham Risk and at all levels of the metabolic syndrome. Using widely available high-sensitivity assays, CRP levels of <1, 1 to 3, and >3 mg/L correspond to low-, moderate-, and high-risk groups for future cardiovascular events (Singh *et al.*, 2008). Individuals with LDL cholesterol below 130 mg/dL who have CRP levels >3 mg/L, represent a high-risk group often missed in clinical practice. The addition of CRP to standard cholesterol evaluation may thus provide a simple and inexpensive method to improve global risk prediction and compliance with preventive approaches (Ridker, 2003). According to these authors (Danesh *et al.*, 2004), CRP is a relatively moderate predictor of coronary heart disease. The results of our investigation do not support the data of the authors cited above (Ridker, 2003; Danesh *et al.*, 2004; Wilson *et al.*, 2005; Smith *et al.*, 2006). We did not find that the results of C-reactive protein investigation were in accordance with the data of the classical plasma atherosclerosis indices. No controversy exists about the diagnostic values of the three liver enzymes that were studied – ALT, AST and ALP (Ioannou *et al.*, 2006; Schindhelm *et al.*, 2007; Kain *et al.*, 2008). Schindhelm *et al.* (2007) found that ALT predicts coronary heart disease events. Also other investigators shared this view (Ioannou *et al.*, 2006; Kain *et al.*, 2008). In the United States, elevated serum ALT activity in the absence of viral hepatitis or excessive alcohol consumption is most commonly attributed to non-alcoholic fatty liver disease (NAFLD) (Ioannou *et al.*, 2006).

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NAFLD is related to predictors of coronary heart disease (CHD) such as insulin resistance and obesity. The association between elevated serum ALT activity and the 10-year risk of CHD using the Framingham Risk Score (FRS) was examined. A cross-sectional analysis was performed comparing participants in the Third National Health and Nutrition Examination Survey with normal and elevated ALT activity (>43 IU/L), which examined the mean levels of FRS. Elevated ALT activity was not associated with higher FRS among non-obese participants with viral hepatitis or excessive alcohol consumption. According to these authors, individuals with elevated serum ALT activity in the absence of viral hepatitis or excessive alcohol consumption and NAFLD have an increased calculated risk of CHD. This association is more prominent in women. ALT predicts the development of Type 2 diabetes mellitus and cardiovascular disease in Caucasian subjects (Kain *et al.*, 2008). Their study was aimed to determine the incidence of an elevated ALT and its relationship to metabolic and atherothrombotic risk factors in a healthy British South Asian population. They had a more adverse atherothrombotic profile, with higher tissue-type plasminogen activator and plasminogen activator inhibitor-1 (PAI-1) antigen. Kain *et al.* (2008) concluded that elevated levels of ALT is common in apparently healthy British South Asians and is significantly associated with an adverse metabolic and atherothrombotic risk profile. The results of our investigation do not support the claims that CRP and two of the studied transaminases (AST and ALT) are predictors of atherosclerosis. In our opinion, one of the reasons could be the short period in which this experiment was conducted.

No histopathological changes in the aortas, hearts and brains were established in our experiment. There are the same results from other diets such as that based on fruit (Leontowicz *et al.*, 2008). Obviously, the 30-day feeding of rats even with the 4% of cholesterol was not enough to induce morphological alterations (Valcheva-Kuzmanova *et al.*, 2007). The results of the influence of garlic application in the rats' diet (Baluchnejadmojarad and Roghani, 2003) showed that aortas prepared from eight-week but not four-week diabetic rats exhibited significantly increased contractile responses, which were partially attenuated by garlic extracts. In our case the amount of the cholesterol was lower than in other applications, and the duration of the experiment was relatively short.

CONCLUSION

Blanching of the studied vegetables for 90 s most fully preserves the contents of their bioactive compounds and related antioxidant potential. Diets, supplemented with red onion and to a lesser degree with white onion and garlic, significantly hindered the rise in plasma lipids levels and the decrease in the plasma antioxidant activity in cholesterol-fed rats. Determination of alanine aminotransferase could be recommended for use together with the classic atherosclerosis indices. The negative results of other tests are most probably connected with the shortness of this investigation and – in the case of negative histology – also with the low concentration of cholesterol in the diets.

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