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# Comparative control of the bioactivity of some frequently consumed vegetables subjected to different processing conditions

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ABSTRACT

The main aim of this investigation was to find processing conditions and to control them, which maximally preserve bioactive compounds and antioxidant activity of garlic and onions. Garlic, white and red onions were subjected to bleaching and boiling. The contents of polyphenols, flavonoids, flavanols, tannins, corresponding antioxidant activities and their correlation coefficients were determined in various methanol and acetone extracts. The antioxidant activity was determined by 2, 2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric-reducing/antioxidant power (FRAP) and Cupric reducing antioxidant capacity (CUPRAC) antioxidant assays. It was found that bleaching for 90″ most fully preserves polyphenols (8.25, 9.75 and 11.98 vs. 9.00, 10.52 and 15.87 mg GAE/g DW and the level of antioxidant activity – 8.82, 22.50 and 23.90 vs. 9.00, 23.05 and 24.30 μM TE/g DW of DPPH in extracts of treated samples with 100% of methanol vs. raw garlic, white and red onions, respectively. In conclusion, comparative control shows that bleaching for 90″ of all studied vegetables most fully preserves contents of bioactive compounds and the level of antioxidant activity. Extraction of bioactive compounds with 100% methanol was more effective than with 50% methanol and 100% acetone.



## 1. Introduction

It was shown that consumption of fruits and vegetables prevent many diseases (Banerjee & Maulik, 2002; Corzo-Martinez, Corzo, & Villamiel, 2007; Vainio & Weiderpass, 2006). These health properties of fruits and vegetables depend on their antioxidants, mainly phenolics (Bahorun, Luximon-Ramma, Crozier, & Aruoma, 2004; Halvorsen et al., 2002; Kevers et al., 2007; Miean & Mohamed, 2001; Moreno, Corzo-Martinez, Dolores del Castillo, & Villamiel, 2006; Nencini et al., 2007; Pellegrini et al., 2007).

Garlic (*Allium sativum* L.), white and red onions (*Allium cepa* L.) are consumed in everyday cooking all over the world. The use of these vegetables goes to the ancient time (Banerjee & Maulik, 2002). Moreover, recently was reported that garlic and onion extracts are effective in prevention of cardiovascular disease, because of their hypocholesterolemic, hypolipidemic, anti-hypertensive, anti-diabetic, antithrombotic and anti-hyperhomocysteinemia effects (Corzo-Martinez et al., 2007; Kaur & Kapoor, 2002; Potter, 2005; Rahman & Lowe, 2006). These biological activities have been reviewed, indicating the compounds responsible for each one of them (Bahorun et al., 2004; Gorinstein, Leontowicz, Leontowicz, Drzewiecki, et al., 2006; Kim, Ye, Lim, Ha, & Kwon, 2005; Miean & Mohamed, 2001; Nencini et al., 2007; Stratil, Klejdus, & Kuban, 2006).

In addition, the influence of the processing on the bioactivity and the adverse effects and interactions with different medications has also been considered (Aoyama & Yamamoto, 2007; Corzo-Martinez et al., 2007; Gorinstein, Leontowicz, Leontowicz, Jastrzebski, et al., 2006; Kawamoto, Sakai, Okamura, & Yamamoto, 2004).

The stimulatory effects on mouse splenocyte proliferation of total phenolics and flavonoid contents of onions was described (Lin & Tang, 2007).

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However, the comparative effect of garlic and onion extracts, which was obtained after cooking and the use of various solvents are less studied (Irkin & Korukluoglu, 2007; Kim et al., 2005).

Some reports demonstrated the influence of thermal treatment on onions in humid heat (boiled and vapor), dry heat (oven) and high frequency microwaves (Agostini, Jimenez, Ramon, & Gomez, 2004; Amin & Lee, 2005; Woo et al., 2007). The assessment of the results is connected to the use of the extraction procedures (Kim et al., 2005). These authors found that physiological activities of Korean- and Chinese-grown garlic (GKG) and (GCG) extracted with water or with either 50% or 100% ethanol are different. Nitrite-scavenging activity (NSA) was the highest in water and 50% ethanol extracts of both origins. Superoxide dismutase (SOD)-like activity of GKG extracts was higher than those of GCG, and those of water extracts were the highest. In another report it was shown that antifungal activity of garlic (*A. sativum* L.), onion (*Allium cepa* L.) and leek (*Allium porrum* L.) aqueous, ethyl alcohol and acetone extracts against *Aspergillus niger* (*A. niger*) is different: onion extract with ethyl alcohol [275 mg/mL minimal fungicidal concentration (MFC)], aqueous garlic extract (325 mg/mL MFC) and aqueous leek extract (900 mg/mL MFC) were the most inhibitory (Irkin & Korukluoglu, 2007). In recent reports different extracts of raw vegetables were compared, showing the amounts of polyphenols and their antioxidant activities (Santas, Carbo, Gordon, & Almajano, 2008). Pellegrini et al., 2007, showed that the total antioxidant capacity is strongly affected by the solvents used during extraction. It was also shown that processing of garlic and onions can change their composition (Aoyama & Yamamoto, 2007; Gorinstein, Leontowicz, Leontowicz, Drzewiecki, et al., 2006; Kawamoto et al., 2004; Roy, Takenaka, Isobe, & Tsushida, 2007; Xu, Wei, Guo, Yang, & Wu, 2007).

Therefore, it is very important to find the best way to preserve the contents of bioactive compounds and the antioxidant activities of processed vegetables.

In this research garlic, white and red onions were subjected to bleaching for 90″ and boiling for 10′ and then polyphenols, flavonoids, flavanols, tannins and the antioxidant activities were determined in their methanol and acetone extracts and compared with the data before the treatment. 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric-reducing/antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) antioxidant assays were applied in this investigation.

We did not find published data of such comprehensive investigations.

## 2. Materials and methods

### 2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), lanthanum (III) chloride heptahydrate, Folin-Ciocalteu reagent (FCR), $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, 2,9-dimethyl-1, 10-phenanthroline (neocuproine), and butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co., St. Louis, MO, 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.

### 2.2. Samples

Raw garlic (*A. sativum* L.), and white and red onions (*Allium cepa*) were obtained from Polish Company "Elena" in 2008. The following steps of treatments were applied: bulbs of garlic, and white and red onions were washed, cleaned, peeled and cut with plastic knife (garlic for halves, onions for pieces) before heat treatment. The studied vegetables were processed under different heat treatment: blanched and boiled. Blanching was done for all samples in water at 100 °C for 90″ (s). Boiling was similar to blanching, but the time of this treatment was different from that of blanching, starting from 10 min and increasing till 60 min. The data of boiling after 10 min are not shown, and were discussed in the previous reports (Gorinstein, Leontowicz, Leontowicz, Drzewiecki, et al., 2006). The samples were lyophilized and then grounded for fine particles under cooling system. This protocol is applied in the present study, because it is similar for everyday food cooking. Different solvents (methanol and acetone) were used for the maximum extraction of bioactive compounds. The used 27 garlic and onion samples were named as following: GAR (50%Me), polyphenols extracted from raw garlic with 50% methanol at 90°C; GA90″ (50%Me), bleached garlic for 90″; GA10′ (50%Me), boiled for 10′, WOR (50%Me), polyphenols extracted from raw white onion with 50% methanol at 90°C; WO90″ (50%Me), bleached for 90″; WO10′ (50%Me), boiled for 10′; ROR (50%Me), polyphenols extracted from raw red onion with 50% methanol at 90°C; RO90″ (50%Me), bleached for 90″ and RO10′ (50%Me), boiled for 10′; GAR (100%Me), polyphenols extracted with 100% methanol from raw garlic at room temperature; GA90″ (100%Me), bleached for 90″; GA10′ (100%Me), boiled for 10′, WO (100%Me), polyphenols extracted with 100% methanol from raw white onion at room temperature; WO90″ (100%Me), bleached for 90″; WO10′ (100%Me), boiled for 10′; ROR (100%Me), polyphenols extracted with 100% methanol from raw red onion at room temperature; RO90″ (100%Me), bleached for 90″ and RO10′ (100%Me), boiled for 10′; GAR (100%Ac), polyphenols extracted with 100% acetone from raw garlic at room temperature; GA90″ (100%Ac), bleached garlic for 90″; GA10′ (100%Ac), boiled for 10′, WOR (100%Ac), polyphenols extracted with 100% acetone from raw white onion at room temperature; WO90″ (100%Ac), bleached for 90″; WO10′ (100%Ac), boiled for 10′; ROR (100%Ac), polyphenols extracted with 100% acetone from raw red onion at room temperature; RO90″ (100%Ac), bleached for 90″ and RO10′ (100%Ac), boiled for 10′.

### 2.3. Preparation of extracts

The shredded garlic, white and red onions were freeze-dried (Alpha 2-4 Christ) and then ground to powder. The powder was stored at −20 °C until extraction of antioxidant phytochemicals. Defatted lyophilized vegetable samples were extracted from a 50 mg aliquot with 5 mL of 50% methanol/water with heating at 90 °C for free polyphenols. The samples were cooled, diluted to 10 mL with methanol, and centrifuged for 5 min at 4000*g* with a benchtop centrifuge to remove solids (50%Me). Portions of 1 g of all freeze-dried samples were extracted three times with methanol (4 mL). The extracts portions were combined and centrifuged at 10,000*g* for 5 min at room temperature (100% Me). Freeze-dried samples (1 g) were extracted three times with acetone (4 mL). The extracts portions were combined and centrifuged at 10,000*g* for 5 min at room temperature (100% Ac). These extracts were used for determination of antioxidant activity and the bioactive compounds (Vinson, Hao, Su, & Zubik, 1998).

### 2.4. Determination of the contents of the bioactive compounds

The studied bioactive compounds were determined as previously described (Gorinstein, Leontowicz, Leontowicz, Drzewiecki, et al., 2006; Gorinstein, Leontowicz, Leontowicz, Jastrzebski, et al., 2006). To determine the total amount of polyphenols in the studied extracts, the Folin-Ciocalteu reagent (FCR) was used,