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ScienceDirect
Nutrition Research 26 (2006) 362–368
NUTRITION RESEARCH
www.elsevier.com/locate/nutres

Supplementation of garlic lowers lipids and increases antioxidant capacity in plasma of rats

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Received 12 September 2005; revised 28 May 2006; accepted 7 June 2006

Abstract

The bioactivity of raw and boiled garlic (*Allium sativum* L.), comprising contributions from polyphenols, was determined by cupric-reducing antioxidant capacity and trolox equivalent antioxidant capacity assays. Boiling garlic at 100°C for 20 minutes preserves its bioactivity and makes it comparable with the raw sample. Wistar male rats were randomly divided into 10 diet groups with garlic supplementation. The control group was fed basal diet that included wheat starch, casein, soybean oil, cellulose, mineral, and vitamin mixtures. To the basal diet of the other groups, 25 mg of lyophilized garlic equivalent to 500 mg raw garlic/kg body weight (raw) was added. The same quantity of boiled garlic for 20, 40, and 60 minutes (Gar20, Gar40, and Gar60 groups), 1% of cholesterol (Chol) and 25 mg of lyophilized raw garlic (Chol/Raw), 1% of Chol, and the same quantity of boiled garlic for Chol/Gar20, Chol/Gar40, and Chol/Gar60 groups were added, respectively. After the trial in rats of Chol/Raw and Chol/Gar20 diet groups, the added garlic significantly hindered the rise in plasma lipids. A significant increase ($P < .05$) in plasma antioxidant activity was registered in Raw and Gar20 diet groups. In conclusion, raw and boiled garlic at 100°C for 20 minutes improved the plasma lipid levels in rats fed cholesterol-containing diets and increased the plasma antioxidant activity in groups of rats fed cholesterol-free diets. Garlic boiled for a short time can be used as an additive in cooking.

Keywords: Raw and boiled garlic; Rats; Plasma lipids; Antioxidant activity

1. Introduction

Garlic (*Allium sativum* L.) possesses many healthful properties that are related to its bioactive compounds [1-4]. It was reported that consumption of garlic is very helpful in regulating plasma lipid levels [5] as well as plasma anticoagulant activity [6,7] and in prevention of the atherosclerosis process [8] and even cancer [9]. It was

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doi:10.1016/j.nutres.2006.06.008

shown that garlic also provides protection against ethanol-induced gastric injury [10]. The most studied and reported health-promoting effect of garlic is cardioprotection [5,8]. However, a pilot study of garlic consumption shows no significant effect on markers of oxidation or subfraction composition of low-density lipoprotein (LDL) [11]. It was also reported that short-term garlic therapy in adults with mild to moderate hypercholesterolemia does not affect lipid levels [12]. Kerckhoffs et al [13] claim that it is still uncertain whether garlic or garlic preparations can be used as lipid-lowering agents.

These is no doubt that garlic and garlic preparations possess anticoagulant abilities [6,7]. However, there is still a controversy regarding the plasma lipid regulating and antioxidant increasing properties of garlic. Therefore, we decided to study the possible changes in the plasma lipid levels and antioxidant activity through an experiment on rats fed cholesterol-containing and cholesterol-free diets supplemented with raw or boiled garlic. Fruits and vegetables subjected to temperature lose a certain part of their bioactivity [14,15]. Garlic is widely used as an obligatory ingredient in many cooked dishes [1,3,5]. The knowledge about the influence of temperature on the bioactive properties of garlic is very limited; therefore, the optimal cooking regime that preserves the bioactivity of raw garlic was determined.

2. Methods and materials

2.1. Chemicals

6-Hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid (trolox); 2, 2′ -azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS); Folin-Ciocalteu reagent; $CuCl_2 \times 2H_2O$; gallic and caffeic acids; catechin; and neocuproine (2,9-dimethyl-1,10-phenanthroline) were purchased from Sigma Chemical Co (St. Louis, Mo, USA) and from Fluka Chemie (Buchs, Switzerland). All reagents were of analytical grade.

Deionized and distilled water were used throughout.

2.2. Samples preparation

Raw Polish garlic was purchased from a local grower in Warsaw from the harvest of 2004. The garlic samples were prepared manually. The edible parts were weighed and divided into 4 parts: 1 is to be used raw, and the other 3 for boiling at 100°C for 20, 40, and 60 minutes, respectively. Water from boiled samples was removed by filtration. The samples were cooled, crushed, and frozen under a temperature of −20°C. After lyophilization, 500 mg of fresh and boiled garlic samples was equal to 25 mg of lyophilized garlic.

2.3. Determination of the bioactive compounds

Dietary fiber, minerals, minor elements, total polyphenols, total tocopherols, and its most abundant and active isomer α-tocopherol were determined as previously described [16-18]. The total phenols were extracted with 5 mL of 1.2 mol/L HCl in 50% methanol/water from 50-mg aliquot of deproteinated lyophilized garlic samples. The samples were cooled and diluted to 10 mL with methanol and centrifuged for 5 minutes at 4000 × *g* with a benchtop centrifuge to remove solids. The phenols were measured at 750 nm after reacting for 10 minutes, using the Folin-Cocialteu reagent that was diluted 5-fold before use, with gallic acid as standard [16-18].

2.4. Determination of the total antioxidant potentials

There are many methods for total antioxidant determination with specific limitations. Some of the antioxidant assays give different antioxidant activity trends [19]. Therefore, the antioxidant potential of raw and boiled garlic samples was determined by 2 complementary methods using 2,2′ -azino-bis (3-ethyl-benzothiazoline- 6-sulfonic acid) diamonium salt ($ABTS^{\bullet+}$) with $K_2S_2O_8$ [19] and copper (II)-neocuproine [Cu (II)-Nc] [20].

$ABTS^{\bullet+}$ radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). After addition of 990 μL of $ABTS^{\bullet+}$ solution to 10 μL of different garlic extracts (0.2 mg/mL) or trolox standards (final concentration 0-20 μM) in ethanol or phosphate-buffered saline, the absorbance was monitored exactly 1 and 6 minutes at 734 nm after the initial mixing [19].

Cupric-reducing antioxidant capacity (CUPRAC) is based on using the Cu (II)-Nc reagent as the chromogenic oxidizing agent. To the mixture of 1 mL of Cu (II), Nc, and NH_4Ac buffer solution, antioxidant sample (or standard) solution (x mL) and H_2O ([1.1 − x] mL) were added to make the final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank [20]. The percentage decrease of the absorbance was calculated and plotted as a function of sample concentration and of trolox for the standard reference data [19,20].

2.5. Animals and diets

The Animal Care Committee of Warsaw Agricultural University approved this study. 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 with 7 rats each: control, Raw, Gar20, Gar40, Gar60, Chol, Chol/Raw, Chol/Gar20, Chol/Gar40, and Chol/Gar60.

The diets of all rat groups are summarized in Table 1. As can be seen, the control group was fed basal diet (BD), which included wheat starch, casein, soybean oil, cellulose, mineral, and vitamin mixtures. To the BD of the 9 other groups, 25 mg of lyophilized raw garlic (equivalent of 500 mg raw garlic/kg body weight [Raw]), the same quantity of garlic boiled for 20, 40, and 60 minutes for Gar20, Gar40, and Gar60, respectively, 1% of cholesterol (Chol), 1% Chol and 25 mg/kg body weight of lyophilized raw garlic (Chol/Raw), 1% Chol, and the same quantity of