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Effect of heating on peroxynitrite scavenging capacity of garlic

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Abstract

The ability to scavenge peroxynitrite ($ONOO^-$) was studied in the following aqueous garlic extracts: (a) unheated extract of garlic powder (GP), (b) heated extract of garlic powder (HGP), (c) unheated extract of raw garlic (RG), (d) heated extract of raw garlic (HRG), (e) extract of boiled garlic cloves (BG), (f) extract of microwave-treated garlic cloves (MG), and (g) extract of pickled garlic (PG). All the extracts scavenged $ONOO^-$ in a concentration-dependent way. IC_{50} (mg/mL) values for each extract were 0.30 ± 0.02 and 0.35 ± 0.04 for GP and HGP, respectively; and 0.84 ± 0.08, 0.59 ± 0.04, 0.76 ± 0.09, 1.71 ± 0.19, and 1.45 ± 0.07 for RG, HRG, BG, MG, and PG, respectively. The $ONOO^-$ scavenging capacity (IC_{50} values) was not decreased in HGP (vs. GP, $p > 0.05$) and in HRG and BG ($p > 0.05$ vs. RG). In contrast, the $ONOO^-$ scavenging capacity decreased in MG and PG (vs. RG, $p < 0.001$). The IC_{50} values for the reference compounds nordihydroguiaretic acid and penicillamine were 1.1 and 4.5 μg/mL. The heating before or after garlic cutting was unable to eliminate the capacity of the extracts to scavenge $ONOO^-$; this capacity was significantly decreased in PG and MG.

Keywords: Peroxynitrite; Scavenging; Garlic; Dihydrorhodamine 123; Nordihydroguaiaretic acid; DL-penicillamine

1. Introduction

Garlic (*Allium sativum*) has been cultivated since ancient times and used as a spice and condiment for many centuries (Block, 1985). Ninety-five percent of the sulfur in intact garlic cloves is found in two classes of compounds in similar abundance: the *S*-alkylcysteine sulfoxides and the γ-glutamyl-*S*-alkylcysteines (Lawson, 1998). The most abundant sulfur compound in garlic is alliin (*S*-allylcysteine sulfoxide), which is present at 10 mg/g in fresh garlic or 30 mg/g dry weight (Lawson, 1998). When garlic cloves are cut, crushed, or chopped (or when the powder of dried cloves becomes wet in a non-acid solution), the cysteine sulfoxides, which are odorless, are very rapidly converted to a new class of compounds, the thiosulfinates, which are responsible for the odor of freshly chopped garlic. The formation of thiosulfinates takes place when the cysteine sulfoxides, which are located only in the clove mesophyll storage cells, come in contact with the enzyme allinase or alliin lyase (E.C. 4.4.1.4), which is located only in the vascular bundle sheath cells. Allinase is active at pH 4–5.8, but is immediately inhibited at acidic pH values below 3.5 or by cooking. Furthermore, microwave heating destroys allinase activity in 1 min (Song and Milner, 1999). Due to the abundance of alliin, the main thiosulfinate formed upon crushing garlic is allicin (Lawson, 1998). The half-life of allicin at room temperature is 2–16 h; however, in crushed garlic (or in garlic juice) is 2.4 days (Lawson, 1998). During the

Abbreviations: BG, Extract of boiled garlic cloves; DHR 123, Dihydrorhodamine 123; DMSO, Dimethyl sulfoxide; DTPA, Diethylenetriaminepenta acetic acid; GP, Unheated extract of garlic powder; HGP, Heated extract of garlic powder; HRG, Heated extract of raw garlic; MG, Extract of microwave-treated garlic cloves; NDGA, Nordihydroguaiaretic acid; $O_2^{\cdot-}$, Superoxide anion; $OH^{\cdot}$, Hydroxyl radicals; $ONOO^-$, Peroxynitrite; PG, Extract of pickled garlic; RG, Unheated extract of raw garlic; SAC, *S*-allylcysteine.

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past years, there has been a growing awareness of the potential medicinal uses of garlic (Banerjee et al., 2002b; Pedraza-Chaverri et al., 1998). The antioxidant properties of garlic are well documented (Banerjee et al., 2002a,b; Gorinstein et al., 2006; Chung, 2006; Rahman, 2003; Pedraza-Chaverri et al., 2000, 2001; Sener et al., 2005; Gedik et al., 2005; Kourounakis and Rekka, 1991; Prasad et al., 1995; Ide et al., 1996). Diet with 2% of garlic powder decreased the ischemia and reperfusion-induced arrhythmias (Rietz et al., 1993) and the acute renal failure and oxidative stress induced by gentamicin (Pedraza-Chaverri et al., 2000). Garlic feeding also decreased lipid peroxidation and prevented the decrease in glutathione peroxidase activity in red blood cells of mice treated with adriamycin (Thabrew et al., 2000). In addition, chronic administration of raw garlic homogenate protects the heart against oxidative damage induced by ischemia and reperfusion (Mukherjee et al., 2002). Furthermore, it has been found that an aqueous extract of raw garlic scavenges hydroxyl radicals ($OH^{\bullet}$) (Prasad et al., 1996; Kim et al., 2001) and superoxide anion ($O_2^{\bullet-}$) (Kim et al., 2001), inhibits lipid peroxidation (Prasad et al., 1996; Yin and Cheng, 1998), Cu^{2+}-induced lipoprotein oxidation (Pedraza-Chaverri et al., 2004b), and the formation of lipid hydroperoxides (Prasad et al., 1996; Kim et al., 2001). Furthermore, an aqueous extract of garlic powder is also able to inhibit lipoprotein oxidation (Pedraza-Chaverri et al., 2004b) and to scavenge $OH^{\bullet}$ (Lewin and Popov, 1994) and $O_2^{\bullet-}$ (Torok et al., 1994). There is some evidence that garlic prevents nitrosative damage. It has been found that aged garlic extract (Maldonado et al., 2003) and *S*-allylmercaptocysteine (Pedraza-Chaverri et al., 2004a), an organosulfur compound found in garlic; prevent the gentamicin-induced increase in renal 3-nitrotyrosine immunostaining, an index of nitrosative stress. In addition Morihara et al. (2005) found that aged garlic extract and *S*-allylcysteine (SAC), a garlic compound with antioxidant properties, were able to prevent the $ONOO^-$ induced hemolysis. However, to our knowledge, there are no studies characterizing the potential $ONOO^-$ scavenging properties of garlic and the effect of heating on this ability. In the present paper we studied if the ability of aqueous garlic extracts to scavenge $ONOO^-$, assessed as IC_{50} values, is altered in the aqueous preparations listed in Table 1. When the thermo-labile enzyme allinase is inhibited before garlic cutting in boiled garlic (BG), microwave-treated garlic cloves (MG) and pickled garlic (PG), allicin is not formed and allin is found in high concentrations. In contrast, when garlic cloves are crushed (RG) or when garlic powder becomes wet (GP), allicin is rapidly formed by the action of allinase on allin. The heating of extract of garlic powder (HGP) or raw garlic (HRG) may decrease the concentration of heat labile compounds.

Table 1
Garlic aqueous preparations used in this study

BG	Extract of boiled garlic cloves
MG	Extract of microwave-treated garlic cloves
PG	Extract of pickled garlic
HGP	Heated extract of garlic powder
HRG	Heated extract of raw garlic
RG	Unheated raw garlic
GP	Unheated garlic powder

GP was the control of HGP and RG was the control of HRG, BG, MG, and PG.

2. Materials and methods

2.1. Reagents

Bulbs of garlic were obtained in a local market. Garlic powder was obtained from McCormick (Mexico City, Mexico). Dihydrorhodamine 123 (DHR 123) was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Diethylenetriaminepenta acetic acid (DTPA), potassium nitrite, manganese dioxide, DL-penicillamine, dimethyl sulfoxide (DMSO) and nordihydroguaiaretic acid (NDGA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade and were commercially available.

2.2. Preparation of aqueous extracts of garlic

2.2.1. Extract of garlic powder (GP)

Garlic powder was weighed (0.3 g), dissolved, and stirred with 3 mL of distilled water for 10 min. This solution was centrifuged at $20{,}124 \times g$ for 10 min at 4 °C. The supernatant was recovered and used at the indicated final concentrations.

2.2.2. Heated extract of garlic powder (HGP)

The procedure was similar to the previous one except that the mixture was boiled for 30 min before the centrifugation step. The amount of water evaporated was replaced at the end of the heating. The supernatant was recovered and used at the indicated final concentrations.

2.2.3. Extract of raw garlic (RG)

Garlic cloves were peeled off, weighed, chopped, and homogenized with distilled water in a Polytron (Model PT2000, Brinkmann, Switzerland). This homogenate was centrifuged at $20{,}124 \times g$ for 10 min at 4 °C. The supernatant was recovered and used at the indicated final concentrations.

2.2.4. Heated extract of raw garlic (HRG)

The procedure was similar to the previous one except that the homogenate was boiled for 30 min before the centrifugation step. The amount of water evaporated was replaced at the end of the heating. The supernatant was recovered and used at the indicated final concentration.

2.2.5. Extract of boiled garlic cloves (BG)

Unpeeled garlic cloves were boiled in water for 30 min. After this time, garlic cloves were peeled off and the aqueous extract was prepared as described before (extract of raw garlic). The supernatant was recovered and used at the indicated final concentrations.

2.2.6. Extract of microwave-treated garlic cloves (MG)

Unpeeled garlic cloves were submitted to microwave heating for 30 s (1100 W). After this time, garlic cloves were peeled off and the aqueous extract was prepared as described before (extract of raw garlic). When allinase is inactivated by heating, the cascade of thiosulfinate formation is blocked from alliin, and allicin and its derivates can not be formed. It has been shown that as little as 60 s of microwave heating (600 W) can totally destroy allinase enzyme activity, whereas microwave heating for 30 s (600 W) inhibits 90% of allinase activity compared with unheated garlic.