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Full Length Research Paper

The quality of garlic decreases with time

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Fruits and vegetables are the staple sources of antioxidants. The antioxidant properties may wear off with time. The present study was aimed to investigate and compare the antioxidant capacity of aged garlic (three-month old) with that of the fresh (newly harvested) garlic. In the present interventional study, an ethanol extract was obtained from fresh and aged cloves of garlic and their antioxidant capacity were measured in linoleic and β-carotene linoleate models. Phenol compounds were assayed using Folin-Ciocalteu colorimetry equivalent to Gallic acid and the flavonoid and flavonol compounds were assayed utilizing chloride aluminum colorimetry method equivalent to rutin. The allicin level was measured through spectrophotometry. Fresh garlic was more efficacious (35.63) than the three-month old garlic (10.2) in inhibiting oxidation. In the linoleic acid and β-carotene linoleate models, no significant difference was found between the fresh garlic and old garlic extracts in terms of optical density ($p>0.05$). The phenol compounds in the fresh garlic (12.61mg/g) were higher than those of the three-month old garlic (2.89 mg/g). Allicin level in the fresh garlic extract (15 µg/ml) was shown to be higher than three-month old garlic extract (8 µg/ml). The results of the present study suggest that garlic should be preferably consumed fresh as it maintains its beneficial compounds.

Key words: Antioxidant, garlic, flavonoid, phenolic compounds.

INTRODUCTION

Fruits and vegetables contain a vast range of phenol and carotenoid compounds with antioxidative properties. Studies show a strong relationship between fruit and vegetable consumption and a drop in the likelihood of cardiovascular diseases, cancer, Alzheimer, diabetes and age-related psycho-physiological problems (Liu, 2003; Shirzad et al., 2011).

An imbalance between oxidation agents and the antioxidative capacity of the body can increase the risk of cancer and cardiovascular conditions (Liu and Hotchkiss, 1995). Extensive use of chemical compounds including butylated hydroxytoluene, butylated hydroxyanisole, selenium and vitamin E which are proven antioxidants has been restricted because of their toxicity. Therefore, studies on the natural antioxidant compounds are recommended. The flavonoid compounds from certain herbal extracts function as antioxidant and have less adverse side-effects compared to synthetic chemical compounds (Ginsberg and Karmally, 2000). Garlic (*Allium sativum* from the Alilaceae family) has compound cloves with multiple bulbs which contain efficacious medicinal substances including alliin, allicin, alliinase enzyme and vitamins A, B and C (Touloupakis and Ghanotakis, 2011). Garlic has been shown to have curative properties for certain types of cancer, thanks to its antioxidant agents (Rosen et al., 2001; Xiao et al, 2003; Lu et al., 2004; Miron et al., 2003; Xiao et al., 2004).

A study conducted in 2006 indicated greater stability of polar compounds such as polyphenols during cooking and

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storage (Lanzotti, 2006). Gazzani and colleagues showed that heat had considerable effect on the antioxidative activity of garlic (Gazzani and Papetti, 1998). Since the medicinal effect of garlic is mainly attributed to its antioxidant activity and its sulfur components, this study was attempted to compare the antioxidant activity and some bioactive components of fresh and three month old garlic.

MATERIALS AND METHODS

In the present study, the antioxidant capacity and the levels of allicin, flavonoids, flavonol and phenolic compouds were measured in the hydroalcoholic extracts of fresh and three-month old garlic. The fresh garlic (harvested from Fereydoun-Shahr, Esfahan, Iran) was cleaned, crushed and left in the room temperature for half an hour before obtaining the extract through maceration method. A certain amount of the same garlic was left in room temperature for three months and extracted similarly.

Garlic ethanol extract preparation

Four hundred ml of 96% ethyl alcohol was added to 50 g of crushed garlic and left for 24 h and filtered. The extraction was repeated with the same amount of 70% ethyl alcohol and added to the previous extract. The solvent was evaporated using a Rotary evaporation until the remaining volume reached to one-fifth of the initial one. The extract then decanted three times by 50 ml of chloroform. The remaining extract was dried at 50°C in the oven and stored at -70°C until use (Baghalian et al., 2004).

Measurement of antioxidant activity in β-carotene model

To measure the antioxidant activity of the extract, 0.5 ml chloroform, 5 ml β-carotene (0.2 mg), 20 ml linoleic acid (20 mg) and 0.2 ml Tween 40 were mixed in a test tube. The test tube was then incubated at 50°C for 10 min in order to remove the solvent. The solution was diluted with distilled water and mixed with 4 ml of aliquots in the following manner.

The control solution was prepared including 0.2 ml ethanol and 0.2 ml of the extract sample with 0.15 ml ethanol and 0.05 ml turmeric extract. The optical density of the control group was recorded at t=0 and t=90 at 470 nm wavelength and similar to the standard group. The samples were incubated in a bain-marie at 50°C. The antioxidant activity was measured on the basis of the ability of the samples in preventing the washing of β-carotene. The antioxidant activity was calculated through Formula 1 below (Jayaprakasha et al., 2002).

$$AA = 100\,[1-(A_o - A_t)/(A^o_o - A^o_t)] \quad (1)$$

Where, A_o: the optical density at t = 0; A_t: optical density of the sample at t = 90; A^o_o and A^o_t: as optical density values in the control samples at t = 0 and t = 90, respectively.

Assaying antioxidative power in linoleic acid model

We mixed 2 cc of the extract solution with a concentration of 200 mg/l, 2 cc of linoleic acid 2.51% in ethanol, 4 cc of phosphate buffer 0.05 M with pH=7 and 2 cc of distilled water in a cap-on glass and transferred to an oven heated at 40°C. Optical density in the sample was measured through tiosianat method after 6 and 12 h and repeated every 12 h. To read the optical density values in the samples, 0.1 cc of emulsion with 9.7 cc ethanol 75% and 0.1 cc 0.02 M chloride from solution in chloridric acid 10% were mixed. After 3 minutes, 0.1 cc tiosianat ammonium 30% was admixed to this solution and its optical density was measured at 500 nm wavelength. This method is based on iron (II) oxidation by peroxides. The iron (III) created by tiosianat ammonium formed a red complex. The created complex had the highest optical density at 500 nm wavelength which was regarded as a measure of existing peroxide (Farhoosh et al., 2007).

Measurement of total phenolic compounds

Total phenolic compounds were assayed equivalent to gallic acid using Folin-Ciocalteu colorimetry (Singleton and Rossi, 1965). The standard solutions were prepared with concentrations of 12.5, 25, 50, 62.5, 100 and 125 ppm of gallic acid in methanol 60%. Then, 0.1 ml from each sample was transferred into a test tube and 0.5 ml Folin-Ciocalteu 10% was added as reactive agent. The solutions were left for 8 min at room temperature and then 0.4 ml sodium carbonate 7.5% was added. The tubes were maintained for 30 min at the laboratory temperature and then assayed in three intervals by a spectrophotometer (Unico uv 2010) at 765 nm wavelength. To measure the overall phenol in the extracts, 0.01-0.02 g of the extracts was solved in 60% methanol, reaching 10 ml and then, using Folin-Ciocalteu method, the overall level of phenol was measured. However, instead of using the standard solution, 0.1 ml extract solution was added. Finally, the overall phenol level was obtained from the read optical density in mg/g extract in gallic acid equivalent.

Assaying flavonoid and flavonol compounds

Chloride aluminum colorimetry and rutin method was used to assay the total flavonoids (Pourmorad et al., 2006). First, standard solutions (Rutin in methanol 60%) with concentration levels of 25, 50, 100, 250 and 500 ppm were prepared. Then 1 ml from these solutions was transferred into test tubes and mixed with 1 ml of chloride aluminum 2%. Afterwards, 6 ml potassium acetate 5% was added and the optical density level was read after 40 min at 415 nm wavelength. The concentration levels of the standard solutions were assayed in three intervals.

In order to measure the overall level of flavonoids in the extracts, 0.01-0.02 g of the extracts was dissolved in methanol 60%, reaching 10 ml. Then, using chloride aluminum colorimetry the total level of flavonoids was measured. However, instead of using the standard solution, 1 ml of the extract was added. The total flavonoid level was calculated in mg per one gram extract, equivalent to rutin.

The total flavonol was also measured using chloride aluminum colorimetry and Rutin procedure, however the optical density level reading, was obtained after 2.5 h at 440 nm wavelength (Loziene et al., 2006).

Assaying allicin

A certain amount of 2-mercaptoethanol (5 ml) was added to 1 g of 2-nitrobenzoic acid in 50 ml of 0.5 M trace and after 5 min acidified up to 1.5 pH with HCl and maintained at 4°C overnight. The formed orange crystals were rinsed with dilated HCl and dried in vacuum.One ml of the prepared extract with maximum 10 µg allicin was added to 1 ml of 1.2×10^{-4} 2-nitro5-benzoic acid in 50 mM

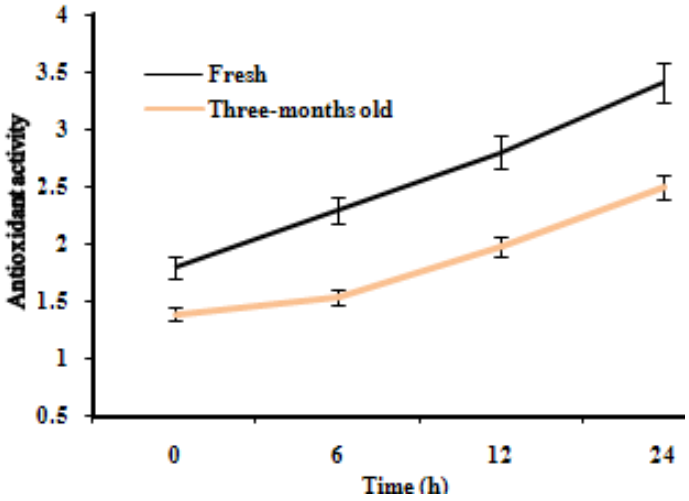

Figure 1. The mean (± standard error) antioxidant activity in fresh and three month old garlic extracts in linoleic acid model (p<0.05).

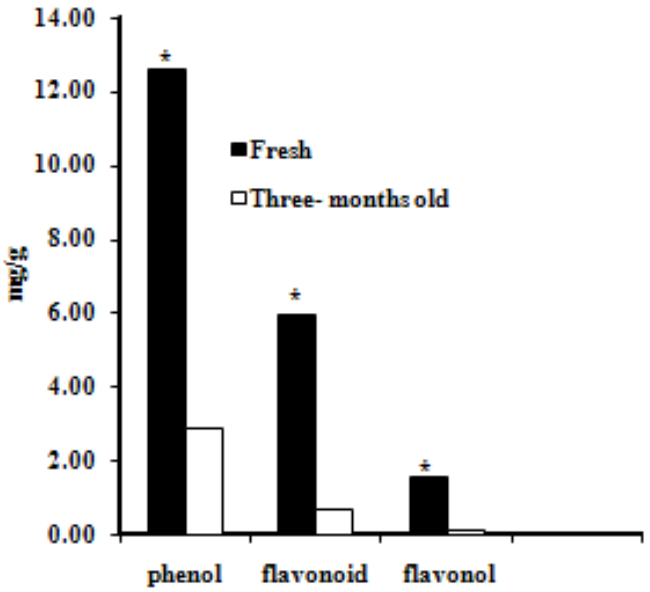

Figure 2. Total phenolic, flavonoid and flavonol compounds in fresh and three month old garlic extracts.

sodium phosphate and after 30 min the optical density level at 412 nm was measured. The results were plugged into the Formula 2 to calculate the concentration of allicin.

$$C_{allicin}(mg/ml) = \frac{\Delta A_{412} \times 162}{28300} = \frac{(A_2 - A_1)_{412nm} \times 162}{28300} \quad (2)$$

Where, A_2: Optical density level of 2-nitro-5-benzoic acid before extract added; A_1: Optical density level of 2-nitro-5-benzoic acid and the extract after 30 min.

RESULTS

The β-carotene linoleate model measurement showed that the antioxidant activity of fresh garlic extract was higher than that of three-month old garlic (P<0.05). Figure 1 shows the optical density levels at 500 nm wavelength after 6, 12 and 24 h.

At 470 nm wavelength, the optical density values of β-carotene in fresh and three-month old garlic extracts were 35.63 and 10.2 (P<0.05), respectively.

In addition, fresh garlic extract contained significantly higher levels of phenolic and flavonoid compounds compared to three month old garlic (p<0.05) however, there was not significantly difference between flavonol level in fresh and three month old garlic extracts (p>0.05) (Figure 2).

A significant difference was observed between fresh garlic and three month old garlic in terms of both phenolic and flavonoid compounds (p<0.05), but not in flavonols (p>0.05).

In addition, the allicin in the fresh garlic extract measured to be 15 µg/ml compared to 8 µg/ml in three-month old garlic.

DISCUSSION

The present study aimed to compare the antioxidant activity of the extracts from fresh and three-month old garlic. The findings show that fresh garlic extract enjoys a higher antioxidant activity than that of a three-month old one. In addition, fresh garlic contains higher levels of allicin as well as flavonoid and phenolic compounds than three-month old garlic extract. Wearing off the property or the enzymes producing such substances through the time may be the reason behind it. Cooking methods, including boiling, seems also result in the reduction of total flavonol, phenol and flavonoid compounds, allicin and the oxidative capacity in garlic (Zhan and Hamauzuy, 2004). It has been shown that a process like cooking softens the cellular walls and facilitates carotenoids extraction (Rodriguez-Amaya, 1999). Other studies also show that temperature change during cooking reduces the amount of vitamins in vegetables (Lin and Chang, 2005).

The results of this study as well as other investigations indicate that the composition of the garlic may change considerably by time or heating. Hence, the pharmacological properties of garlic may be affected by preparation methods or processes. Therefore, it is important to have understanding of the active substrate exist in particular preparation and its selective therapeutic potential for the concerned situation.

Most of investigations on garlic's components have focused on sulfur compounds and its antioxidant activity. The reasons for the interest are unusually high content of

these compounds, particularly its organosulfur components, compared to other food plants (Touloupakis and Ghanotakis, 2011). Furthermore, elimination of volatile sulfur compounds, of which allicin is the most abundant, from crushed garlic results in the removal of all or most of garlic's antibacterial antifungal antiatherosclerotic, thrombolytic and blood lipid lowering effects (Lawson et al., 1992; Plengvidhya et al., 1988; Shen-qin, 2004).

Alliin, allicin and the two main γ–glutamyl cysteines constitute the majority (about 72%) of the sulfur compounds in whole or crushed garlic. Alliin is the parent compound of allicin and allicin is the parent compound of diallyl sulfides (Wang et al., 2011). Alliin has no pharmacologic activity unless converted to allicin by a garlic enzyme – alliinase (Kaschula et al., 2010).

Garlic's medicinal effects mostly have been ascribed to allicin. Therefore, reduction of allicin and other components in three month old garlic may reduce its medicinal properties. The γ-glutamylcysteines play an important function as reserve compounds for producing additional alliin and isoalliin during wintering and sprouting, increasing the antibiotic capacity of the young plants (Lancaster and Shaw, 1991). These compounds have been found to be fairly stable when cloves are maintained at room temperature (Wang et al., 2011).

The enzyme responsible for the lysis of alliin is alliinase or alliin lyase (Kaschula et al., 2010). A unique feature of alliinase is that it is present in garlic in unusually large amounts for an enzyme, consisting of at least 10% of the total clove protein, meaning thereby that garlic cloves contain approximately equal amounts (10 mg/g fresh weight) of alliinase and alliin. This may well explain why alliin is converted so very rapidly to allicin when garlic is crushed. The stability of alliinase is also quite remarkable, since garlic powders stored for up to 5 years show little loss in ability to produce allicin. Allicin (diallyl thiosulphate) is one of the most active components of garlic and is also responsible for its typical pungent smell. Allicin does not exist in garlic until it is crushed or cut. Injury to the garlic bulbs activates the enzyme allinase which metabolizes alliin to allicin (Kaschula, 2010). Whole garlic is well known to be odor free until the cloves are cut or crushed, indicating that alliinase and the cysteine sulfoxides are stored in separate compartments. A question may rise that if they are stable and are in different compartments, what is the main reason for the reduction of allicin, tannins, flavonoids and phenolic components as well as the antioxidant activity of three month old garlic compared to fresh one. Therefore, further investigation is needed to answer this question.

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