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Original article

Antioxidant activity of garlic using conventional extraction and *in vitro* gastrointestinal digestion

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ABSTRACT

Introduction: Garlic is well known for its health protective abilities. Many studies have also proven garlic as an oxidative stress fighter with unique antioxidant potential. These studies have extracted raw garlic in conventional manner i.e. using organic solvents. Such antioxidant capacities cannot be well implicated for health purposes.

Methods: This study deals with measurement of antioxidant capacity of raw as well as cooked garlic extracted by chemical as well as physiological method (*in vitro* gastrointestinal digestion). The Total antioxidant capacity was measured by methods like DPPH Radical Scavenging Ability, ABTS Radical Scavenging Ability, Ferric Reducing Antioxidant Power and Reducing Power Assay. Total Phenol was also evaluated.

Results: Results show a wide difference between the antioxidant capacity of conventional and physiological extracts. The *in vitro* digested extracts of raw garlic show highest antioxidant capacity of all raw and cooked garlic extracts. Loss of phenolic compounds and antioxidant potential on cooking can also be clearly observed in both chemical and physiological extracts.

Conclusion: It can be thus concluded that the physiological method of antioxidant extraction is more applicable and reliable than the conventional chemical extraction methods that do not resemble the biological behavior of antioxidants.

1. Introduction

Excess production of oxygen radical species such as hydrogen peroxide, superoxide anion radical, and the hydroxyl radical are thought to cause damage in cells.[1] The oxidative damage to cells is one of the factors causing many diseases, including atherosclerosis, diabetes and cancer.[2] Garlic (*Allium sativum*) has been considered to be one of the best disease-preventive foods. Dietary foods contain a wide variety of free radical scavenging antioxidants.[3] Garlic is composed mainly of fructose-containing carbohydrates and sulfur compounds. According to Banerjee et al, (2002),[4] garlic possesses antiproliferative properties. A number of investigations have reported that garlic extract has a wide range of health benefits, e.g., against cancer and cardiovascular disorders[5] and as an antioxidant.[6,7] Numerous studies have been found reporting the antioxidant compounds in garlic as well as the antioxidant capacity of garlic. These studies extract raw garlic which is seldom consumed so. Food processing steps such as dehulling, peeling, thermal processing, mashing, etc. contribute to degradation and loss of phenolic compounds.[8,9] Also, we know that phenolic compounds mainly exist as glycosides linked to various sugar moieties or as other complexes linked to organic acids, amines, lipids, carbohydrates, and other phenols. Cooking sets the phenolic compounds free from these linkages to make them more bioaccessible. Moreover, garlic is extracted in organic solvents or their mixtures. The enzymatic treatments hydrolyze starch and protein, which may favor the release of polyphenols. The biological properties of antioxidants depends on the release of phenolic compounds from the food matrix during the digestion process (bioaccessibility) and may differ quantitatively and qualitatively from those produced by the chemical extraction employed in most studies.[10] Thus this study deals with analysis of both conventional as well as physiological extracts of raw and cooked garlic.

Abbreviations: ABTS, 2,2 azinobis (3–ethylbenzothiazoline-6-sulfonic acid) diammonium salt; ABTSRSA, ABTS Radical Scavenging Ability; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; DPPHRSA, DPPH Radical Scavenging Ability; FRAP, Ferric Reducing Antioxidant Power Assay; RPA, Reducing Power Assay; TEAC, Trolox Equivalent Antioxidant Capacity; TPTZ, 2,4,6–tris (2-pyridyl)–s–triazine; Trolox, 6-hydroxy-578-tetra methyl-chromane-2 carboxylic acid.

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2. Materials and methods

2.1. Chemicals

Pepsin (P-7000), Pancreatin (P-1750), Lipase (L-3126), Bile Extract Porcine (B-8631), α-Amylase (A-3176), Amyloglucosidase (A-7095), ABTS (A-1888), DPPH (D-9132), Catechin (C-1251), Vanillin (V-2375), Rutin (R-5143), Gallic acid (G-7384) and TPTZ (T-1253) were purchased from Sigma Aldrich-Germany and Trolox–56510 was purchased from Fluka.

2.2. Sample preparation

Experiment was done in two duplicate batches with two separate purchases in the same season. Garlic was purchased from the local market, peeled and finely pound. For cooking, 50 g of peeled garlic was pressure cooked without direct addition of water for 10 min. This cooked sample was cooled and pound like the raw sample. Further, it was extracted along with raw sample as stated below.

2.3. Chemical extraction

900 mg of raw and cooked garlic sample was extracted twice in 80% acidic methanol (pH set 2.0 with 1 N HCl) by shaking at room temperature for 45 min. Supernatants were filtered and centrifuged and volume was made up to 30 ml with the solvent. All samples were transferred to Eppendorf tubes and stored at −20 °C for antioxidant determination.

2.4. Extraction by 'in vitro gastrointestinal digestion'

900 mg of raw and cooked garlic sample was used for *in vitro* gastrointestinal digestion. The digestive enzymatic extraction was carried out by using the *in vitro* procedure previously described by Serrano et al, (2007).[10] Samples were successively incubated with digestive enzymes to simulate digestion in the small intestine. A control of sample was also incubated similarly with buffers without addition of enzymes.

Sample was incubated with pepsin (0.6 ml of a 300 mg/ml solution in a buffer of 0.2 M HCl–KCl, pH 1.5, 40 °C, 1 h), pancreatin (3 ml of a 5 mg/ml solution in 0.1 M phosphate buffer, pH 7.5, 37 °C, 6 h), lipase (6 ml of a 7 mg/ml solution in 0.1 M phosphate buffer, pH 7.5, 37 °C, 6 h), bile extract porcine (6 ml of a 17.5 mg/ml solution in 0.1 M phosphate buffer, pH 7.5, 37 °C, 6 h) and α-amylase (3 ml of a 120 mg/ml solution in 0.1 M tris-maleate buffer, pH 6.9, 37 °C, 16 h).

Then, the samples were centrifuged (15 min, 6000 rpm) and supernatants were collected. Residues were washed twice with 5 ml of distilled water, and all supernatants were combined. Each supernatant was incubated with 300 μl of amyloglucosidase for 45 min at 60 °C. Volume of all samples was made up to 30 ml. All samples were transferred to Eppendorf tubes and stored at −20 °C for antioxidant determination.

Both chemical and digestive extracts (control and enzymatic) were used to determine the antioxidant capacity.

2.5. Total Phenol estimation

Folin–Ciocalteu method[11] was used to determine the total phenol content of the chemical and physiological extracts. Different aliquots of known concentration of gallic acid were taken as standard.

2.6. Ferric Reducing Antioxidant Power

Total antioxidant capacity of the chemical and physiological extracts for FRAP was determined by using the method of Benzie and Strain (1999).[12] Different aliquots of Trolox were treated as standard and results were expressed in terms of TEAC (mg of Trolox Equivalent/100 g).

2.7. Reducing Power Assay

This assay was performed as suggested by Oyaizu (1986).[13] Different aliquots of Trolox were treated as standard and results were expressed in terms of TEAC (mg of Trolox Equivalent/100 g).

2.8. DPPH Radical Scavenging Ability

The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical, was determined by the method described by Brand-Williams et al, (1995).[14] The percent inhibition and IC 50 was calculated and results were expressed in terms of TEAC (mg of Trolox Equivalent/100 g).

2.9. ABTS Radical Scavenging Ability

The radical scavenging ability of was determined using the modified ABTS radical decolorization assay.[15] The percent inhibition was calculated and results were expressed in terms of TEAC (mg Trolox Equivalent/100 g).

2.10. Statistical analysis

Differences between variables were tested for significance by using a one-way analysis of variance, DUNCAN using the level significance of $p \leq 0.05$ by SPSS.

3. Results

Many studies measuring the antioxidant capacity of garlic have been found. Usually, different organic solvents and their mixtures are used for extraction of antioxidant compounds. This extraction method does not imply to the physiological absorption. Thus in this study, the bioavailable antioxidant capacity of garlic is measured by simulation of gastrointestinal conditions. In physiological extraction, raw and cooked garlic were digested *in vitro* with enzymatic treatments. A control of sample was also incubated similarly with buffers without addition of enzymes. This can clearly show the difference between antioxidant capacity of chemical and physiological extracts. Control shows the degree of activity of enzymes. Also, comparison between raw and cooked can be made.

Table 1 shows the Total Phenolic Content of different garlic extracts. The Total Phenolic Content of chemically extracted raw garlic was 67.5 mg GAE/100 g. Bozin et al, (2008)[6] extracted garlic in 80% methanol and found that Total Phenol Content of garlic was 50 mg GAE/100 g. The chemically extracted cooked garlic suffered a loss of 90% in phenolic content. Park et al, 2009[16] also found similar results on heating of garlic. However, the extraction of garlic in physiological conditions helps better extraction of phenolic compounds. The control raw garlic extract had 111.44 mg GAE/100 g of TPC whereas enzymatically extracted raw garlic had 334.58 mg GAE/100 g of TPC which was around 80% more than the chemically extracted raw garlic. Also, the enzymatically extracted cooked garlic showed better TPC than the chemical counterpart. Only 13–14% loss of TPC can be observed on cooking in enzymatic extracts.