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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.

Table 1
Total Phenol Content, FRAP and RPA of different garlic extracts.

	TPC (mg GAE/100 g)	FRAP (mg TE/100 g)	RPA (mg TE/100 g)
ME Raw	67.52^{b}±7.8	57.64^{b}±6.2	114.76^{b}±18.9
ME Cooked	35.39^{a}±4.7	44.88^{a}±4.9	62.86^{a}±8.5
DE R0	111.44^{c}±23.6	99.26^{c}±6.6	109.65^{c}±9.7
DE Raw	334.58^{e}±32.5	129.59^{e}±8.4	308.48^{e}±28.3
DE Cooked	294.92^{d}±15.6	107.82^{d}±11.9	258.77^{d}±19.0
F Value	196.35*	165.20*	110.25*

Values are mean ± S D of four observations where ME = Methanolic Extract, DE = Digested Extract, R0 = Raw Control, GAE = Gallic Acid Equivalent, TE = Trolox Equivalent. *Within column, values with the different following superscript letter differ significantly from each other ($p \leq 0.05$).

Table 2
Radical Scavenging Ability of different garlic extracts.

	DPPHRSA (mg TE/100 g)	ABTSRSA (mg TE/100 g)
ME Raw	78.99^{d}±5.6	39.91^{c}±4.7
ME Cooked	71.32^{b}±8.2	32.48^{b}±7.4
DE R0	62.89^{a}±3.9	24.52^{a}±3.8
DE Raw	83.99^{e}±9.2	47.22^{d}±3.9
DE Cooked	74.09^{c}±4.1	39.39^{c}±1.6
F Value	0.164*	0.239*

Values are mean ± S D of four observations where ME = Methanolic Extract, DE = Digested Extract, R0 = Raw Control, GAE = Gallic Acid Equivalent, TE = Trolox Equivalent. *Within column, values with the different following superscript letter differ significantly from each other ($p \leq 0.05$).

The Ferric Reducing Antioxidant Power of different garlic extracts is depicted in Table 1. Raw chemically extracted garlic had 57.64 mg TE/100 g of FRAP. Gorinstein et al, (2009)[17] studied the FRAP of raw garlic with the help of acidic aqueous methanol and found 10.80 μM TE/g of antioxidant activity i.e. 270.31 mg TE/100 g. Raw chemically extracted garlic had almost 30% higher FRAP than cooked chemically extracted garlic which had 44.88 mg TE/100 g FRAP. The enzymatically extracted garlic had significantly higher (55%) FRAP than the chemically extracted raw garlic. Also, on cooking 20% lower FRAP was observed in the enzymatically extracted garlic than the raw. The FRAP of garlic shares a positive and strong relationship with TPC (Fig. 1).

The Reducing Power of the enzymatically extracted raw garlic was the highest (i.e. 308.4 mg TE/100 g) among all the garlic extracts. The chemically extracted raw garlic and the cooked enzymatic extract had respectively 62% and 19% lower Reducing Power than the raw enzymatic extract. Also, the chemically extracted cooked garlic had 82% lower Reducing Power than the raw chemical extract which had 114.7 mg TE/100 g of Reducing Power. The control physiological extract of raw garlic had 109.6 mg TE/100 g of Reducing Power which was lower than both chemical and enzymatic extracts of raw garlic.

Radical Scavenging Ability of different garlic extracts is shown in Table 2. Though DPPHRSA follows similar pattern as FRAP i.e. enzymatically extracted garlic has higher antioxidant capacity than the chemical extract, the difference between the two as well as raw and cooked garlic is not significant. Chemically extracted raw garlic had 78.99 mg TE/100 g of DPPHRSA whereas enzymatically extracted raw garlic had merely 6% higher DPPHRSA than the chemical extract.

ABTS Radical Scavenging Ability of enzymatic extract of raw garlic was higher (47.2 mg TE/100 g) than the chemical extract which had 39.9 mg TE/100 g of antioxidant capacity. Gorinstein et al, (2009)[17] studied the ABTSRSA of raw garlic with the help of acidic aqueous methanol and found 37.02 μM TE/g of antioxidant activity i.e. 926.5 mg TE/100 g. Cooked chemical extract suffered a loss of 22.8% in antioxidant capacity than the raw. Similarly, the enzymatically extracted cooked garlic had 19.8% lower ABTSRSA than the raw enzymatic extract which had 47.2 mg TE/100 g of ABTSRSA whereas the raw control had 24.5 mg TE/100 g of ABTSRSA.

Fig. 1 depicts strong and positive correlation between Total Phenolic Content and antioxidant capacity measured by ABTSRSA, FRAP and RPA. This shows that the antioxidant capacity is dependent on the phenolic content of garlic. The *in vitro* digestive extracts showed considerably higher antioxidant capacity than the chemical extracts in all the parameters.

4. Discussion

All antioxidant activity determinant parameters show almost similar trend in antioxidant activity of different extracts of garlic. In vitro gastrointestinal digestion gives extracts with much higher antioxidant potential than the conventional procedure. This suggests release of phenolic compounds during digestion and that the implication of antioxidant values by extraction using organic solvents may not prove true. When phenolic compounds are exposed to *in vitro* digestion, various enzymes transform them into different structural forms. These structures possess different chemical properties and functions[18] Phenolic compounds are present in

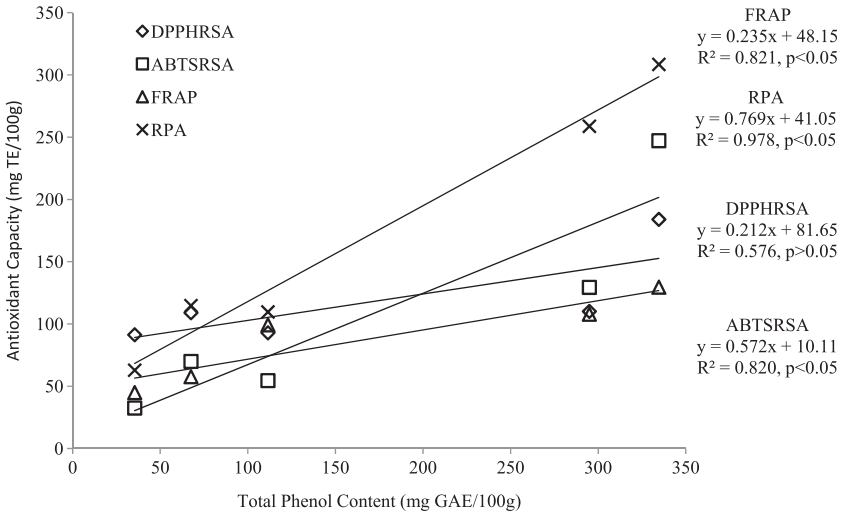

Fig. 1. Relationship between Total Phenol Content and Total antioxidant capacity of different garlic extracts. GAE = Gallic Acid Equivalent, TE = Trolox Equivalent.

bound form with proteins and other biomolecules and are gradually released during the hydrolysis process in the digestive system.[19] The amount of nutrients and phytochemicals absorbed during digestion is governed by the physical properties of the food matrix which affects the efficiency of physical, enzymatic and chemical digest. Many studies have reported antioxidant potential of different food stuffs after gastrointestinal digestion.[20–22] Jimenez and Saura-Calixto, (2005)[23] have stated that the antioxidant capacity of foods may be underestimated in the literature because the extraction solvents usually used do not allow a complete release of antioxidant compounds and additionally nonextractable polyphenols with a high antioxidant capacity are ignored. Similarly, this experiment clearly shows the difference between the conventional extraction and gastrointestinal digestion.

In the DPPHRSA, the difference between the raw and cooked garlic samples in both chemical and physiological extracts was lower than other antioxidant determinant parameters (i.e. 10% and 13% respectively). The composition of phenolic compounds as well as other antioxidant compounds may be responsible for the difference. Also, the solvent used for extraction and processing conditions add to the factors affecting the antioxidant potential of the food. According to Ryan and Prescott (2010),[18] when phenolic compounds are exposed to *in vitro* digestion, they are transformed into different structural forms and possess different chemical properties and functions. These different properties and functions may give different antioxidant activity results as evaluated by different methods. So, evaluation of antioxidant capacity measurement by more than one method is recommended by many authors.[24–26]

On the other hand, cooking leads to considerable loss of phenolic compounds. Both chemical as well as enzymatic extracts of cooked garlic had lower antioxidant potential than their counterparts. This shows depletion of phenolic compounds due to heat. Studies that support decrease in antioxidant activity after cooking have been found.[27–29] Gorinstein et al, (2008)[30] evaluated different bioactive compounds from garlic before and after various heat treatments and confirmed heat destruction of phenolic compounds. They observed the differences in the protein profile during processing. The protein profile is dependent on the time temperature combinations which in turn are responsible for different physical properties like texture, color, matrix softening, and increased extractability. These physical changes are the possible reasons why bioactive compounds, antioxidant activities, and proteins in garlic changed after cooking. Heat processing promotes polymerization of phenolic compounds to form brown-colored macromolecules[18] which may also be responsible for the drop in antioxidant activity on cooking. The release of phenolic compounds during digestion and the loss of antioxidant compounds due to heat are clearly indicated in the results. Most studies evaluating antioxidant potential do not compare raw and cooked forms of food whereas they are not consumed raw. Thus, by evaluating conventional and gastrointestinal extracts of both raw and cooked samples, this study gives complete results of garlic antioxidant potential. Difference between raw and cooked form as well as conventional and gastrointestinal extraction can be clearly seen.

5. Conclusion

Results of this study thus prove that mere extraction by organic solvents may not be sufficient for the determination of antioxidant capacity. Also, the quantity and quality of antioxidant compounds extracted by organic solvents may not reflect their bioavailability. Such conventional extraction procedures may prove misleading for assessment of the antioxidant potential of foods. By extracting the vegetables with both methods and estimating their antioxidant potential and compounds with different methods, we can conclude that conventional method of extraction using organic solvents does not imply the actual physiological conditions.

Conflicts of interest

All authors have none to declare.

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