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INFLUENCE OF BLANCHING ON ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF RAW GARLIC (*ALLIUM SATIVUM*)

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Abstract:

The present study was designed to delineate the influence of blanching on garlic, i.e. Allium sativum regarding in vitro antioxidant profile and antimicrobial activities. Common blanching methods like soaking at hot water at 80°C for 4 minutes and heating in a microwave at high temperature for 90 seconds were engaged. The antioxidant assays performed were DPPH radical decolorization assay, reducing power assay, estimation of total polyphenols, total flavonoids and ascorbic acid. Antimicrobial activities of raw and blanched garlic were adjudicated against the common food borne pathogens like Bacillus cereus, Escherichia coli, Staphylococcus aureus, Klebsiella aerogenes, Enterobacter aerogenes and Bacillus subtilis. The study indicated that blanching by normal heating markedly improved DPPH radical scavenging ability in gallic acid equivalence scale (from 215.97±15.38 µg/gm to 235.22±35.59 µg/gm) but lowered contents of total phenolics and ascorbic acid. Flavonoid content was improved (from 35.01±2.88 µg/gm to 45.67±3.14 µg/gm). Blanching using microwave deteriorated the antioxidant profile. It was also observed that blanching have declining effect on antimicrobial activity of garlic although some of the activity was still retained. The study indicated that common blanching processes alone had a moderate effect on antioxidant potential and phytochemical constituents of garlic and had no positive effect on their antimicrobial activities.

Keywords: *Antioxidant, Blanching, Edible flowers, Polyphenolics, DPPH, Anthocyanin*

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INTRODUCTION:

The classical nutrition research has taken an important turn in the last few decades where apparently pharmacologically inert food additives like spices have demonstrated profound effects in health and diseases [1] . Dietary spices are one of such nutrients that are being identified vital to maintain human health by their antioxidative, chemopreventive, antimutagenic, antiinflammatory, immune modulatory effects on cells and a wide array of beneficial effects on human health via action on gastrointestinal, cardiovascular, respiratory, metabolic, reproductive, neural and other systems [2]. Herbs and spices have long been used as food additives in different human cultures. Spices play important role both as functional food ingredients and nutritional supplements. They could enhance the taste and flavour of food. In addition, spices like garlic, ginger, cardamom, peppers and chilies have also been used for treating several disorders as they have potent medicinal properties [3].

Garlic (*Allium Sativum*) is well known for its medicinal benefits, especially in its role in preventing cancer and cardiovascular diseases [4]. It is a species of the onion family – Alliaceae. The bulb is divided into numerous fleshy section called cloves. The cloves are used for culinary consumption (raw or cooked) or for medicinal purposes. It is replete of fructose-containing carbohydrates and sulfur containing compounds, which are vital antioxidant principles [5]. The modulatory effects of garlic on hepatic and blood oxidant–antioxidant status might play a key role in preventing cancer development [6]. Garlic has been used since ancient times in India for its valuable effect on the heart and circulation [7,8,9], and it has been found that the regular use of garlic may help to prevent malaria and to raise immunity. It has been also proposed that garlic has been used to treat asthma, candidiasis, colds, diabetes and antibacterial effect against food borne pathogens [10]. Therapeutic use of garlic has been recognized for thousands of years against different micro-organisms. Even some researchers have suggested that it might have a role as a food additive to prevent food poisoning. There are also some evidences that fresh garlic, but not aged garlic, can inhibit certain bacteria like *E. coli*, antibiotic-resistant *Staphylococcus aureus*, and *Salmonella enteritidis* in the laboratory.

Usually, thermal treatments associated with cooking processes affect the levels of nutritional and antioxidant factors of the food ingredient [11,12,13]. Blanching of foods involves mild heating in water with the objective to inactivate enzymes and to partially destroy microorganisms [14]. However, the process can decrease or increase antioxidant activity of agricultural products [15]. Hence, it is a necessity to keep blanching conditions at a level just sufficient to cause inactivation of the deleterious enzymes but with minimal effect on other beneficial attributes. In view of above, the present study was designed in such a way that it resembled closely with the common blanching processes practiced in India and to ascertain the change in the antioxidant and phytochemical profile of garlic. To our knowledge, it was one of the very few studies that dealt with the change in quality of human consumable spices before and after blanching for their radical scavenging abilities, and probably the first with raw garlic, directly collected from agricultural fields. In this way, we would be able to know the appropriate blanching methods, which would retain the effectiveness of the spice. The present study reports the achievement of the aim through some common *in vitro* antioxidant and antimicrobial assays.

MATERIALS AND METHODS:

Chemicals and Reagents:

2,2′-Diphenyl-1-picryl hydrazyl (DPPH) was purchased from Himedia, India. Folin-Ciocalteau reagent, gallic acid, Ascorbic acid, potassium hexacyanoferrate and ferric chloride were obtained from Merck, India. All other reagents and chemicals used were of analytical grade procured from local sources. Deionized distilled water was used in the entire study. Garlic was purchased from a local market of Kolkata. The samples were checked for dirt or any visible damages, and were discarded if found.

Blanching and Extraction:

The samples were washed well with water before blanching. About 5 gms of samples were suspended in water with a solid-to-solvent ratio of 1:3 (w/v). Blanching for each sample was done in quadruplicate. The following are the two common methods of blanching [16].

Hot water method – The samples were put in water at 80^{o}C for 4 minutes. Then the water was drained off, the samples were dried with a hand drier and hot extracted with 60% aqueous methanol for 5 minutes. Solid-to-solvent ratio for extraction was maintained 1:10 (w/v). After extraction, the mixture was filtered through Whatman 1 and used for further studies.

Microwave method – The samples in water were heated at high power for 90 seconds in a commercial microwave oven. Then the water was drained off, the samples were dried with a hand drier and hot extracted with 60% aqueous methanol for 5 minutes. Solid-to-solvent ratio for extraction was maintained

1:10 (w/v). After extraction, the mixture was filtered through Whatman 1 and used for further studies. For the antimicrobial studies, the unblanched and blanched samples were extracted with water in ratios mentioned later in the antimicrobial assay protocol.

Antioxidant assays:

DPPH assay was performed using a previously described procedure [12]. Decolorization of DPPH radical solution by the samples was monitored by decrease in absorbance of the mixture at 517 nm in a Systronics spectrophotometer (Model – 2202). The concentration that causes a decrease in the absorbance of initial oxidants by 50% is defined as IC_{50} of the samples. Gallic acid was used as positive control and results were expressed as gallic acid equivalents (GAE). Reducing powers of the samples were determined using a previously described procedure [17]. Sample solutions were mixed with phosphate buffer (pH 7.4) and aqueous potassium ferricyanide solution. This mixture was kept at 50±2ºC in water bath for 20 minutes. After cooling, 10% (w/v) trichloroacetic acid was added and centrifuged at 3000 rpm for 5 min. The supernatant was mixed with freshly prepared ferric chloride solution (1%, w/v). The absorbance was measured at 700 nm in a Systronics spectrophotometer (Model – 2202). Gallic acid was used as positive control and comparing with its' IC_{50} and the results were expressed as gallic acid equivalents (GAE). Total phenolics content was determined using a previously described procedure [18]. Samples were mixed with Folin-Ciocalteau's solution (1:10 v/v diluted with distilled water) and allowed to stand for 28±2°C for 5 min. Then 7% (w/v) aqueous sodium carbonate solution was added and the mixture were allowed stand for another 90 min and at darkness. The absorbance of the blue colors that developed were measured at 725 nm using a Systronics spectrophotometer (Model – 2202). Gallic acid was used as standard and the total phenolic concentration in the spice extract was expressed as gallic acid equivalent (GAE). For total flavonoid estimation, aluminum chloride method was used following a published procedure [16]. Briefly, sample extractives were mixed with methanol, 10% (w/v) aqueous aluminum chloride solution, 1 M aqueous potassium acetate solution distilled water and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam UV-Vis spectrophotometer (model – Systronics 2202). Total flavonoids content were calculated using a calibration curve of quercetin as standard and the results were expressed as quercetin equivalent (QE). Determination of ascorbic acid contents was done following a published procedure with minor modifications [13]. Stock iodine solution was standardized by titrimetric method using standard (N/100) sodium thiosulphate solution followed by addition of 1% starch solution, when the solution turned blue and continue the titration until the blue color just discharged. The infusions were then titrated with the standard iodine solution. In a similar procedure, standard ascorbic acid solution was also titrated. Comparing the titers, the results were expressed as mg ascorbic acid/gm fresh sample.

Antimicrobial Assays:

The bacterial strains used in this study included *Bacillus cereus* (MTCC 1272), *Escherichia coli* (MTCC 1610), *Staphylococcus aureus* (MTCC 9542), *Klebsiella aerogenes* (MTCC 9544), *Enterobacter aerogenes* (MTCC 2822) and *Bacillus subtilis* (MTCC 1305). The strains were obtained from IMTECH, Chandigarh, India and preserved at Department of Microbiology, Ramakrishna Mission Vidyamandira, Howrah, India. The microorganisms were inoculated into 10 mL of sterile nutrient broth, and incubated at 37ºC for 16-18 hours.Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile Mueller Hinton Agar (HiMedia M173) plates. Agar wells were prepared with the help of sterilized cork borer with 10 mm diameter [19]. Using a micropipette, 100 microlitres of different concentrations of garlic extracts (viz. 25%, 50%, 75%, and 100%, w/v) were added to different wells in the plate. The plates were incubated in an upright position at 37ºC for 24 hours. The diameter of inhibition zones was measured in mm. Each experiment was done in quadruplicate sets.

Statistical Analyses:

All the assays were performed in quadruplicate and data are presented as mean ± standard deviation. The analyses were done with the software 'Prism 4.0' (GraphPad Inc., USA).

RESULTS AND DISCUSSION:

Antioxidant Assays:

It is of great importance to consider that only a small amount of vegetables is consumed in the raw state and most of them needed to be processed for safety and quality. However, many food composition data bases never consider the fact that concentrations of nutrients and their activity might change through heating effects, such as blanching [20]. It has also been observed that blanching mostly reduced effectiveness of the vegetables, although in some

cases, it improved the contents of a few antioxidative phytochemicals [16].

The results of DPPH assay indicated that radical scavenging activities of garlic was relatively higher after blanching at 80ºC (Table 1). However, upon microwave treatment, the radical scavenging effect reduced significantly compared to control (U, 0 min blanching). The improved activity during heat blanching might be due to solubilization of some antioxidant bioactives at higher temperature. Reduced activity after microwave treatment might be due to the disintegration of the extracted bioactives.

Reducing power assay indicates presence of electron donors in an extract which might serve as good antioxidant. Heat blanching showed non-significant decrease of reducing power (from 113.53±10.52 µg/gm GAE to 105.08±6.56 µg/gm GAE, Table 1). However, after microwave blanching, significant reduction of reducing power was observed, which was in accordance with results of DPPH assay.

Total phenolic contents also decresed non-significantly upon heat blanching in comparison to control (from 1000.00±72.57 µg/gm GAE to 863.75±81.48 µg/gm GAE, Table 1). However, after microwave blanching, significant reduction was observed, which again was in accordance with results of DPPH assay.

Total flavonoid content of garlic was increased upon heat blanching compared to control (from 35.01±2.88 µg/gm QE to 45.67±3.14 µg/gm QE, Table 1). This might be the reason of better radical scavenging ability of the samples in DPPH assay protocol. However, contents reduced significantly after blanching by microwave.

Ascorbic acid contents were significantly reduced in both methods of blanching. All these antioxidant assays indicated that overall antioxidant profiles of the blanched samples were deteriorated compared to fresh vegetable.

Antimicrobial Assays:

Garlic extract showed excellent antibacterial activity at all concentrations (100%, 75%, 50% and 25%, w/v) to all microorganisms of the present investigation, and the activity was a linear function of concentration (Table 2). Each organism responded differently to the garlic extract at different concentrations. At 100% concentration, *Staphylococcus aureus* was least sensitive (25.25±2.4 mm) and *Bacillus cereus* was most sensitive (32±2.2 mm). At 100%, garlic extract showed antimicrobial activity against the entire range of test organisms also. 75% and 50% concentrations of the extract also showed moderate antibacterial activity against these strains. After blanching by the two methods, although there were linear correlation with the antimicrobial activity, the activity was diminished when compared with unblanched samples at same concentrations (Table 2). This was observed for all the six test microorganisms. The antibacterial activity of garlic is reported to be due to the action of allicin or diallylthiosulphinic acid or diallyldisulphide [21]. Our results revealed differences in the sensitivity of different food borne pathogens to garlic extract, suggesting that mechanisms of resistance might be developing in these organisms. Garlic can be used as a potent inhibitor of food pathogens, however blanching might have some negative effect on its' antimicrobial activity, probably due to deactivation of the necessary bioactives.

Table 1: Antioxidant Activities of Garlic Extracts (before and after blanching). Results are expressed as mean±SD.

Assay method	Sample		
	U	B_H	B_M
DPPH assay (GAE, µg/gm)	215.97±15.38	235.22±35.59	63.43±4.62
Reducing power assay (GAE, µg/gm)	113.53±10.52	105.08±6.56	85.02±6.18
Total phenolics content (GAE, µg/gm)	1000.00±72.57	863.75±81.48	568.00±37.96
Total flavonoids content (QE, µg/gm)	35.01±2.88	45.67±3.14	25.80±3.28
Ascorbic acid content (mg/gm)	0.73±0.07	0.47±0.05	0.48±0.11

Legend: U – Unblanched, B_H – Blanched by heating at 80ºC, B_M – Blanched using microwave, GAE – Gallic acid equivalence, QE – Quercetin equivalence

Table 2: Antimicrobial Activities of Garlic Extracts (before and after blanching). Results are expressed as mean diameter of zone of inhibition (in mm)±SD.

Microorganism	Concentration	Sample		
		U	B_H	B_M
Bacillus cereus	25%	13.00±1.8	7.00±1.2	6.5±1.3
	50%	18.75±1.7	14.75±1.7	14.25±2.2
	75%	24.50±1.7	16.25±2.1	14.00±1.8
	100%	32.00±2.4	26.50±2.4	25.25±2.2
Escherichia coli	25%	9.75±1.5	5.00±1.4	6.25±1.3
	50%	15.00±1.4	7.00±1.2	8.50±1.3
	75%	19.25±2.1	11.25±1.5	13.50±1.3
	100%	28.25±1.7	19.25±2.1	19.50±1.3
Staphylococcus aureus	25%	5.75±1.00	6.00±1.4	5.75±1.00
	50%	12.00±1.8	10.00±1.8	9.25±1.3
	75%	16.50±1.3	12.75±2.2	10.25±1.5
	100%	25.25±2.2	16.50±2.1	14.50±1.3
Klebsiella aerogenes	25%	10.25±1.5	7.75±1.0	9.50±1.3
	50%	16.75±1.5	10.75±1.5	11.25±1.5
	75%	22.50±2.4	14.00±1.8	16.50±1.3
	100%	28.50±1.3	18.50±2.4	20.75±1.0
Enterobacter aerogenes	25%	9.25±1.0	7.00±0.8	9.25±1.0
	50%	17.00±1.8	8.25±1.0	11.50±1.3
	75%	21.75±1.5	11.75±2.1	11.75±2.1
	100%	27.25±1.3	18.25±1.3	20.75±1.7
Bacillus subtilis	25%	10.75±1.5	9.00±0.8	9.00±1.8
	50%	16.75±1.5	12.75±1.0	12.00±1.4
	75%	22.50±1.3	16.75±1.3	15.75±1.7
	100%	28.25±1.3	24.25±1.5	25.00±1.8

Legend: U – Unblanched, B_H – Blanched by heating at 80°C, B_M – Blanched using microwave

CONCLUSION:

The present study elaborated comparison of the antioxidant activity of garlic before and after blanching that mimic normal cooking procedure. The major conclusions arising out from the study was that significant improvement in DPPH radical scavenging activity was observed (from 215.97 µg/gm to 235.22 µg/gm) after blanching by heating in water at 80°C for 4 mins. This might be due to the increment of total flavonoid content in the extract (from 35.01 µg/gm to 45.67 µg/gm). Increment in flavonoid content might occur due to incresed solubilization of the component. Other components might be destroyed at higher temperature during blanching. That is why, antioxidant profile deteriorated after blanching in microwave. The antimicrobial activity profile of garlic was also lost after blanching by both heating and by treating in a microwave, against all the six food borne pathogens used in this study. The present study thus indicated that blanching could have a telling effect on the antioxidant and antimicrobial profile of garlic and should be avoided in case of this spice.

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