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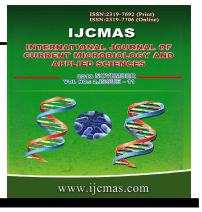

Original Research Article

Efficacy of heat treatment on the *in vitro* antioxidant activity of selected spices

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A B S T R A C T

The effect of cooking on total phenolic content, total flavonoid content and total antioxidant capacity of spices were investigated. In the present study spices selected for antioxidant analysis were Black pepper (*Piper nigrum*), cloves (*Syzygium aromaticum*), and cinnamon (*Cinnamomum verum*) in three different treatments like fresh, heat treatment for 1 hour and 2 hour. These treated spices were taken as experimental group and fresh spices without any heat treatment were taken as control group for further analysis. After heat treatment, the bioactive compounds were extracted to determine the antioxidant activity, total phenol and total flavonoids content were measured and analysed.

Keywords

Total Phenolic Content; Total Antioxidant Capacity.

Introduction

Spices are vital culinary addendums enhancing organoleptic characteristics of food. Spice powders and spice blends are an integral part of Indian cuisine. (Narayanan, 2006). Spice is a dried seed, fruit, root, bark or vegetative substance used in nutritionally insignificant quantities as a food additive for the purpose of flavouring and sometimes as preservative by killing or preventing the growth of bacteria. (Adamson, 2004).

Though spices are mainly used to increase food palatability, they do have medicinal and preservation effects due to the presence of a number of phytochemical and antimicrobial compounds. (Sen *et al*., 2008).

Spices are abundant sources of polyphenolic compounds that have strong antioxidant capacities. Consumption of spices has been implicated in prevention of cardiovascular diseases, carcinogenesis, inflammation and atherosclerosis. (Hussain, 2006) Phenolic compounds are the major bioactive compounds found in spices. Spices that have antioxidant property can function as antimutagens. Since mutagenesis has a direct bearing on cancer initiation, antimutagenic spices can probably be anticarcinogenic too. (Kawamori *et al*., 2003) Flavonoids are phytonutrients in plant-based food products that often contribute to the color of the foods. They provide antioxidant activity which may play a significant role

in cardiovascular health and may help to prevent against diseases such as cancer caused by free-radical damage. They may also provide benefit in the prevention of other chronic conditions such as osteoporosis and diabetes. (Sherakat, 2009).

Spices are usually consumed after thermal cooking. Therefore antioxidant activity of spices may be affected by thermal cooking. Only scarce information was available on the effect of heat treatment on the *in vitro* antioxidative activities of spices (Ademoyegun, 2010).

The objectives of this study were (1)To investigate the *in vitro* antioxidant capacities for five spices. (2) To analyse antioxidant activity by using different assays. (3) To ascertain the total flavonoid content and total phenolic content from the spice extracts in correlation with the antioxidant activities

Materials and Methods

Selection of spices

Fresh and clean spices were procured from Tamilnadu Agriculture University, Coimbatore for the study. The spices selected were Black pepper (*Piper nigrum*), Cloves (*Syzygium aromaticum*) and Cinnamon (*Cinnamomum verum).*

Heat Treatment

Each spices Black Pepper, cloves and cinnamon was pounded with mortar and pestle to have a thoroughly mined and fine powder spices. Each selected spice (1 g) was put in a light – capped test tube which was placed in a boiling water bath and heated at 100^0c for 1hour and 2 hour to prevent oxidation and loss of active components by evaporation. These treated spices were taken as experimental group and fresh spices without any heat treatment are taken as control group for further analysis.

Extraction of bioactive compounds from spices

After heat treatment, the tube was allowed to cool, and then the bioactive compounds were extracted with 20 ml of methanol by shaking for 20 min and centrifuging at 2,000 rpm for 20 min. The supernatant was used to determine the antioxidant activity, total phenol and total flavanoid content were measured using this extract solution. Three measurements were performed for each spice sample, and the results were expressed as the mean value± SD.

Analysis procedure

Determination of total phenolic content of spices

Total soluble phenolic content of spice extract were determined by spectrophotometric method (SINGLETON *et al*., 1999). Gallic acid is used as the standard which represents the phenolic compound in the sample extract. 10mg of Gallic acid monohydrate was dissolved in 100mL of methanol to give a concentration of 100μg/mL. Aliquots of 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5mL from the standard solution were taken in 6 different 10mL volumetric flask. To each flask 2.5mL of 1N Folin- Ciocalteu reagent and 2mL of 20% sodium carbonate were added. The mixture was allowed to stand for 15 minutes and the volume was made up to mark with water to get a concentration ranging from 2.5-25μg/mL. The absorbance of the resulting solutions

was measured at 765nm against reagent blank. A standard calibration curve of was prepared by plotting absorbance Vs concentration and it was found to be linear over this concentration range.

Determination of the total flavonoid content of spices

Total flavanoid content of spice extract was determined by described by spectrophotometric method (QUETTIER *et al*., 2000). Rutin is used as the standard for estimation of total flavonoids in the prepared extract. 10mg of rutin was dissolved in 10mL of methanol to get 1000µg/mL solution. Aliquots of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0mL from the above stock solution were taken in 6 different 10mL volumetric flask. To each flask 1.5mL of methanol, 0.1mL of 10% aluminum chloride, 0.1mL of 1M potassium acetate and 2.8 mL of distilled water was added. The reaction mixture was kept aside at room temperature for 30 min and the volume was made up with water. The absorbance of the resulting solutions was measured at 415nm against reagent blank. The calibration curve was prepared by plotting absorbance Vs concentration and it was found to be linear over this concentration range of 10-100µg/mL.

Determination of total antioxidant capacity of spices

The antioxidant activity of the spice extracts were evaluated by standard method (TEKAO *et al*., 1994). The principle of this method is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 5 mL of a (10 mmol/L) TPTZ (2, 4 ,6- tripyridyl- s-triazine) solution in 40 mmol/L HCL plus 5 mL of FeCl3 (20 mmol/L) and 50 mL of acetate buffer, (0.3 mol/L, pH=3.6) and was prepared freshly and warmed at 37ºC . Aliquots of 100 µL sample were mixed with 3 mL FRAP reagent and the absorbance of reaction mixture at 593 nm was measured\ spectrophotometrically after incubation at 37ºC for 10 min. For construction of calibration curve five concentrations of FeSO4 7H2O (1000, 750, 500, 250, 125 µmol/L) were used and the absorbencies were measured as sample solution. The values were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L FeSO4.

Statistical analysis

All data were recorded as means ± SD and analyzed by SAS (2003). One – way analysis of variance (ANOVA) were carried out to test any significant differences between raw and cooked spices.

Results and Discussion

Determination of total phenolic content of spices

Data on total phenolic in heat treatment spices are very limited. Khatun *et al.*, (2006) reported that cloves and cinnamon contain 8.3 mg GAE/g and 5.8 mg GAE/g uncooked and retained 75% and 172.41% for 1 hour respectively. The difference may have been due to the differences in the extraction method, solvent used and cooking method. The fresh spices contained 0.07 - 95.0 mg gallicacid equivalents / g of dry power and the ranking were clove > cinnamon > pepper.

Table.1 Total phenolic content of spices

SPICES	Total Phenol mg gallicacid equivalents / g of dry power		
	Fresh	**1 Hour**	**2 Hour**
Cinnamon (G1)	4.9 ± 0.7a*	4.8 ± 1.23a*	4.3 ± 1.5a*
Clove (G2)	9.5 ± 1.2b*	10.5 ± 1.37b*	11.0 ± 0.9b*
Pepper (G3)	0.07 ± 0.03c*	0.10 ± 0.06c*	0.11 ± 0.02c*
CD (p<0.05)	0.74	0.82	0.95

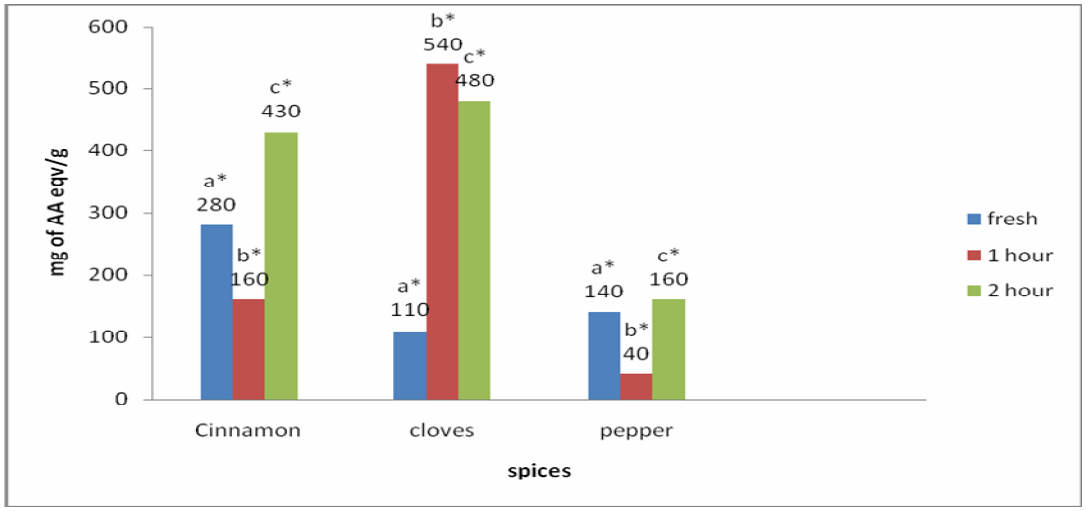
Values are mean ± SD of five samples in each group
Groups compared: a – G1 vs G2; b – G2 vs G3; c – G3 vs G1
Significance : *- Significant at 5% level (p<0.05); ns – Not significant

Table.2 Total flavonoid content of spices

Species	Total flavonoids (mg catechin eqv/g of w)		
	Fresh	**1 Hour**	**2 Hour**
Cinnamon (G1)	36 ± 1.2a*	42 ± 1.3a*	51 ± 1.7a^{ns}
Clove (G2)	32 ± 2.4b*	32 ± 1.7b^{ns}	52 ± 3.1b^{ns}
Pepper (G3)	30 ± 1.9c*	31 ± 2.2c*	50 ± 2.0c^{ns}
CD (p<0.05)	**1.86**	**2.05**	**2.73**

Values are mean ± SD of five samples in each group
Groups compared: a – G1 vs G2; b – G2 vs G3; c – G3 vs G1
Significance : *- Significant at 5% level (p<0.05); ns – Not significant

Figure.1 Total antioxidant content of spices



Groups compared: a – G1 vs G2; b – G2 vs G3; c – G3 vs G1
Significance : *- Significant at 5% level (p<0.05); ns – Not significant

In table VI, after cooking procedures for 1 hour, the total phenolic content of cinnamon was significantly ($p<0.05$) reduced and the reduction was the same for 2 hours cooking. Conversely, of pepper and clove, total phenolic content was significantly ($p<0.05$) increased to various extents for 1 hour. Heat treatment and increment was the same for 2 hours for pepper and clove.

Data on total phenolic in heat treatment spices are very limited. Khatun *et al*, (2006) reported that cloves and cinnamon contain 8.3 mg GAE/g and 5.8 mg GAE/g uncooked and retained 75% and 172.41% for 1 hour respectively. The difference may have been due to the differences in the extraction method, solvent used and cooking method. The total phenolic content of selected spices is presented in Table 1.

Determination of the total flavonoid content of spices

The total flavonoids range from 0.81-36mg catechin equivalent/g of weight with the ranking cinnamon > clove > pepper. The effect of heated spices were significant ($p<0.05$) for pepper, clove and cinnamon. Cinnamon, clove, pepper shows an increase in total flavonoid at 1 hour and also when further heated for 2 hour. It was reported that heat treatment increased the level of free flavonoid Stewart *et al.* (2000). For cloves and pepper the effect of heat on the total flavonoid content has no significant ($p<0.05$), which show that the flavonoid content is relatively stable under thermal heat. The total flavonoid content of some spices is presented in **Table 2.**

Determination of total antioxidant capacity of spices

The antioxidant activity of spices are in the order Cinnamon > Pepper > Cloves. The total antioxidant activity of spices ranged from 540 – 110 equivalent to ascorbic acid mg/g extract for fresh spices and for heated spice range from 720-160 equivalent to ascorbic acid mg/g of spice extract. The total antioxidant capacity of spices is presented in Figure 1. The relatively stable organic radical, DPPH, has been widely used in the determination of antioxidant activity of single compounds, as well as of different plant extracts (Katalinic et al., 2006).Umbelliferae include many common spice plants, e.g. cumin, coriander and various kinds of peppers. Some researchers have previously reported that spices of this family exhibited a moderate antioxidant effect of 169 equivalents to ascorbic acid mg/g (Shan, Cai, Sun, & Corke, 2005).

Total antioxidant activity of cloves significantly ($p<0.05$) increased after 1 hour of cooking when compared to fresh, but total antioxidant activity of pepper and cinnamon decreased during 1 hour and significantly increased during 2 hour of cooking

Conclusion

The fresh spices contained 0.07 - 95.0 mg gallicacid equivalents / g of dry power and the ranking were clove > cinnamon > pepper. The total flavonoids range from 0.81- 36mg catechin equivalent/g of weight with the ranking cinnamon > clove > pepper. The antioxidant activity of spices are in the order cinnamon > pepper > cloves. The total antioxidant activity ranged from 540 – 110 equivalent to ascorbic acid mg/g extract for fresh spices and for heated spice range from 720-160 equivalent to ascorbic acid mg/g of spice extract.

From the results of this study, it is clear that that spices have strong antioxidant activities in a methanol extract solution. In conclusion, the results illustrated that the health benefits from plant sources remained in the products after thermal process that it, heat do not denatured the antioxidant activities in all the selected spices studied. Spices are expected to be a valuable food constituent for promoting good health in our daily lives.

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