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Influence of Different Cooking Methods on the Antioxidant Activity of Local Pumpkin Variety *(Cucurbita maxima)* Cultivated in Jaffna District

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Abstract: Antioxidants play major role in maintaining good health. Pumpkin (*Cucurbita maxima*) is one of the richest sources of β-carotene, a powerful antioxidant. However, the antioxidant activity of vegetables is influenced by the cooking methods. Therefore, this study aimed to determine the effect of three cooking methods on the antioxidant properties of pumpkin. The antioxidant properties such as total flavonoid content (TFC), total phenolic content (TPC), antioxidant capacity and antioxidant activity [2, 2-Diphenyl-1-picrylhydrazyl (DPPH), radical scavenging activity expressed as IC50 value] were determined. Fresh pumpkin was cut into small pieces and subjected to different cooking methods (boiling, microwave cooking and stir-frying) until pumpkin became tender and palatable. Ethanol (70 %, v/v) was used as the solvent to extract antioxidants. Higher loss of TPC was observed during stir-frying than microwave cooking and boiling. The highest TFC obtained from boiled sample compared to microwave cooked and stir-fried samples. Microwave cooking minimally destroyed antioxidant capacity than stir-frying and boiling compared to fresh pumpkin. Fresh pumpkin showed significantly lower IC50 than microwave cooked and stirs fried pumpkin. Boiled pumpkin showed the lowest DPPH radical scavenging activity. From this study, it can be concluded that, even though all three cooking methods studied have significant effect on antioxidant activity of pumpkin, microwave cooking has less effects on antioxidant activity than boiling and stir-frying.

Keywords: Cooking, free radical scavenging activity, pumpkin, total flavonoid content, total phenolic content

Introduction

Antioxidants can be defined as molecules capable of stabilizing or deactivating free radicals before they attack cells (Rahman, 2007). Free radicals are derived from normal metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and

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industrial chemicals (Patil *et al*., 2010). Free radicals induce oxidative stress leading to a number of human cardiovascular, neurologic and other disorders (Sen and Chakraborty, 2011). Thus, consumption of diet rich in antioxidants is associated with reduced risk of cardiovascular diseases, various forms of cancer and other degenerative diseases (Raupp *et al.*, 2011).

Vegetables are one of the richest sources of antioxidants, thus, their consumption has been associated with protection against several non-communicable diseases (Alvarez-Parrilla *et al*., 2012). Pumpkin (*Cucurbita maxima*) belongs to Cucurbitaceae family is extensively cultivated and consumed by Sri Lankans. Pumpkin is a rich source of carotenoids, especially, beta-carotene, which is a precursor of vitamin A. Thus pumpkins are considered as a source of pro-vitamin A to human diet (Dias, 2012; Priori *et al*., 2017). Epidemiological studies suggest that carotenoids are associated with enhancement of the immune response and reduction of the risk of developing degenerative diseases such as cancer, cardiovascular diseases, atherosclerosis, cataracts, and age-related macular degeneration (Azizah *et al*., 2009; Muzzaffar *et al.*, 2016). In addition it is rich in vitamin C and tocopherols (Kulaitienė *et al*., 2014).

There are many factors that affect the antioxidant activity of plants such as genotype, environmental conditions, storage and processing conditions (Raupp *et al*., 2011). Pumpkins are cooked by different methods to make it palatable. However, cooking of vegetables causes several changes in antioxidant activity. The aim of this study was to determine the effect of some processing methods on the antioxidant properties of pumpkin.

Materials and Methods

Sample collection

Fresh pumpkins (local variety) were purchased from local farms located at Thirunelvely, Jaffna on the same day of harvest.

Materials

All chemicals used in the study were purchased from Sigma Aldrich Co., St, Louis, USA. All chemicals and solvents used in the study were of analytical grade.

Sample preparation

The flesh of the pumpkin was taken and cut into small pieces (approximately 1cm). Then, that was subjected to different cooking treatments such as boiling, microwave cooking and stir-frying. For boiling sliced pumpkin (35g) was added to the container containing boiling water (50 mL) without closing with a lid and cooked for 5min. Excess water was drained off. For microwave cooking, sliced pumpkin (35 g) was added to the beaker containing water (50 mL) without closing lid and cooked in a microwave oven at medium power (560W) for 2 min and excess amount of water was drained off after cooking. For stir-frying, vegetable oil (7 mL) was heated in an unclosed stainless steel vessel (by using gas cooker at moderate blue flame) until oil reach the boiling point. Then, pumpkin (35 g) was added and stirred for 7 min.

Estimation of moisture content

Sample (10 g) was weighed in previously weighed moisture can. The moisture can was placed in an oven (Memmert, Germany) at 105 °C without lid until constant weight was obtained. After drying, the lid was replaced and moisture can was transferred into a desiccator to cool to room temperature. The weight of moisture can with sample was taken. The moisture content was calculated on wet weight basis.

Estimation of dry matter content

After estimation of moisture content, dry matter content was calculated by subtracting the moisture percentage from 100.

Extraction of Sample

Ethanol (70%, v/v) was used to extract antioxidants from samples. Sample was added in to clean dry conical flask and the solvent was added at the ratio of 5:1 [ethanol (v): sample (w)], covered with aluminum foil and stoppered and shaken at 200 rpm in a mechanical shaker for 2 hours. After 2 hours, the solvent was separated from the residue and collected in a weighed round bottom flask. Same amount of solvent was added to the residue and extracted again at the same conditions for 30 min twice and the solvents were collected at the same flask. After extraction, the solvent was evaporated using rotary evaporator (Stuart, UK) to get dry extract. Dry extract was stored in refrigerator at -18 ºC until analysis within two days. Dry extract was mixed with solvent to get extract at a concentration of 1 mg/mL to be used for further analysis to determine the antioxidant properties.

Determination of total phenolic content (TPC)

Vegetable extract (0.3 mL) was transferred into a test tube and 2.25 mL of Folin – Ciocalteau reagent (previously diluted 10-fold with distilled water) was added. The mixture was allowed to stand at room temperature for 5 min. Sodium carbonate (2.25 mL of 6%, w/v) was added to the mixture. Then, 8 mL ethanol was added and vortexed. After standing at room temperature for 30 min in dark, the absorbance was read at 725 nm using a UV–Vis spectrophotometer against the reagent blank. The reagent blank was prepared by taking 0.3 mL of 70% ethanol instead of vegetable extract. The TPC was calculated using calibration curve of gallic acid. The TPC was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry matter (Ismail *et al*., 2004).

Determination of total flavonoid content (TFC)

Vegetable extract (0.5 mL) was transferred into a test tube and 2.5 mL of distilled water was added. Then, 0.15 mL of 5% $NaNO_2$ was added. After 6 min, 0.3 mL of a 10% $AlCl_3.6H_2O$ solution was added and allowed to stand for another 5 min before 1 ml of 1 M NaOH was added. Then, this mixture was vortexed. The absorbance was measured immediately at 510 nm using a UV–Vis spectrophotometer against the reagent blank. The reagent blank was prepared by taking 0.5 mL of 70% ethanol instead of vegetable extract. The TFC was calculated using calibration curve of catechin. The TFC was expressed as catechin equivalents (CE) in milligrams per gram of dry matter (Dewanto *et al*., 2002a).

Determination of Antioxidant capacity

Vegetable extract (0.2 mL) was transferred into a screw capped test tube. Then, ethanol (0.2 mL) was added and 4 mL of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate) was added and mixture was vortexed. The tubes were capped and incubated in a water bath at 95 ºC for 90 min. The contents were cooled to room temperature and the absorbance was measured at 695 nm using a UV–Vis spectrophotometer against blank which contained 4 mL of reagent solution and appropriate volumes of the same solvent that was used for the test. The total antioxidant capacity was calculated using calibration curve of ascorbic acid. The total antioxidant capacity was expressed as Ascorbic acid equivalents (AAE) in milligrams per gram of dry matter (Girgin and Nehir, 2015).

Determination of DPPH radical scavenging activity

The extract was taken in series of labeled test tubes (0.025-2 mL). Then, ethanol was added to make up same amount of volume. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) (1 mL) solution was added to these test tubes. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min in dark room. Then, absorbance was measured at 517 nm by using UV–Vis spectrophotometer. Negative control and blank were also done. Ascorbic acid was used as the reference antioxidant. The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using inhibition curve (Shekhar and Anju, 2014).

Statistical analysis

All experiments were carried out in triplicates and the values are presented as mean ± standard deviation. All data were recorded in Microsoft Excel 2007 and calculations were done using the same. Results were analyzed using one way analysis of variance (ANOVA) with significant differences between means determined at $p< 0.05$, and mean separation was carried out using Duncan's multiple range tests using Statistical Package for the Social Science (SPSS) version 20. Correlation and Co-efficient was carried out to test relationship between antioxidant capacity and total phenolic content; total phenolic and total flavonoid content; total flavonoid content and antioxidant capacity of pumpkin.

Results and Discussion

Table 1 shows the TPC, TFC, antioxidant capacity and antioxidant activity of fresh and cooked pumpkins. From the table, it is obvious that all three cooking methods caused significant changes in the antioxidant properties. Boiling did not have influence on the TPC, however microwave cooking and stir-frying caused significant losses (10.89 and 37.36%, respectively). Stir-frying caused significantly higher losses than microwave cooking. The pattern of change in TPC of pumpkin during cooking is comparable with the values reported by Baljeet *et al*. (2016).

Boiling has increased (70.33%) the TFC significantly. In contrast, other two cooking methods caused significant losses in TFC. Losses of TFC in microwave cooked and stir-fried pumpkin were 31.83 and 9.33%,

Table 1: TPC, TFC, antioxidant capacity and DPPH radical scavenging activity of fresh and pumpkin cooked by different methods

Cooking method	TPC (mg GAE/g DM)	TFC (mg CE/g DM)	Antioxidant capacity (mg AAE/g DM)	Antioxidant activity (IC50 value mg/ml)
Fresh	9.18 ± 0.05^{a}	3.00 ± 0.03^{b}	64.02 ± 3.2^{a}	0.54 ± 0.02^{c}
Boiled	8.58 ± 0.04^{ab}	5.11 ± 0.91^{a}	26.50 ± 4.58^{d}	0.77 ± 0.02^{a}
Microwave cooked	8.18 ± 0.11^{b}	2.05 ± 0.03^{c}	53.54 ± 8.02^{b}	0.65 ± 0.01^{b}
Stir-fried	5.75 ± 0.82^{c}	2.72 ± 0.32^{bc}	43.21 ± 2.90^{c}	0.66 ± 0.02^{b}

Values are presented as mean ± standard deviation. Mean values with different superscripts in the same column are significantly different at $p < 0.05$ by analysis of variance followed by Duncan's multiple range test.

respectively. Microwave cooked pumpkin contained significantly lowest TFC. All three cooking methods reduced the antioxidant capacity of the pumpkin. There was a 58.61, 16.37 and 32.51% losses in the antioxidant capacity of boiled, microwave cooked and stir-fried pumpkin, respectively, compared to fresh pumpkin. The IC50 values of the extracts ranged from 0.54±0.02 to 0.77±0.02 mg/mL. The highest antioxidant activity (expressed as IC50 value) was recorded in fresh pumpkin, while, the least antioxidant activity was recorded in boiled pumpkin.

The plant foods are rich source of bioactive compounds. However, most of the plant foods need to be processed before consumption for better digestion and metabolism in the human digestive system. Processing of foods mainly involve heating with different energy transfer media such as water, air, oil, and electromagnetic waves (Nayak *et al*., 2015). Recent researches show that that food processing operations have positive effects that improve the quality and health benefits of foods. Some food processing methods may improve the antioxidant activities because of the increased release of bound phenolic compounds in the food matrices, whereas, some methods may decrease the antioxidant activities because temperature increase during cooking may result in destruction of phenolic antioxidants such as phenolic acids and anthocyanins (Dewanto *et al.,* 2002a, 2002b; Nayak *et al*., 2015). Chuah *et al.* (2005) has reported that cooking in boiling water decreases the radical scavenging activity of peppers, whereas microwave heating without water increases the activity. Processed tomato and sweet corn exhibited higher antioxidant activities than unprocessed ones (Dewanto *et al*., 2002a, 2002b).

Cooking of vegetables has mixed effects on phenolic antioxidants of cooked foods (Nayak *et al.*, 2015). When vegetables are subjected to various cooking processes such as boiling, stir-frying and microwave cooking, the TPC, antioxidant capacity and activity seemed to vary. This may be due to the structure of the antioxidant compounds, cooking parameters, bioavailability of the phenolic compounds

and temperature (Subramaniam *et al*., 2017) and localization of the structures in the vegetables. Phenolic contents (free and bound forms) and antioxidant activity during processing also depend on the type of fruit or vegetable (Nayak *et al*., 2015). For example, Jiratanan and Liu (2004) reported that heat treatment of table beets at 105–125 °C for 15 - 45 mins either retain or increase total phenolic content, total flavonoids, and total antioxidant activity, however, processing of green beans at similar processing conditions caused reductions in the antioxidant activity, phenolic contents, and total flavonoids. The reduction of TPC, antioxidant capacity and activity could be ascribed to the breakdown of some heat sensitive phenolic compounds; leaching of water soluble compounds; temperature induced chemical oxidation and release of oxidative and hydrolytic enzymes that could destroy the antioxidant compounds in vegetables. Boiling increased TFC which could be attributed to increased level of free flavonols by boiling (Baljeet *et al*., 2016). Less loss of antioxidant capacity during microwave cooking than boiling and stir-frying could be due to less leaching and oxidation of antioxidant compounds. In over all, high temperature processing may have detrimental effects on the phenolics and flavonoids leading to reductions in the antioxidant activities of processed fruits and vegetables. However, in some processing, antioxidant activity may increases due to intrinsic properties of the food matrix (Nayak *et al*., 2015).

Quantitative analysis was also used for carrying out the correlation between phenolic content and antioxidant capacity, total phenolic content and total flavonoid content in pumpkin. The results of this study exhibited that there is a positive weak correlation between total phenolic content and total flavonoid content (r = 0.27) (Figure 1) and between total phenolic content and antioxidant capacity (r = 0.23) (Figure 2).

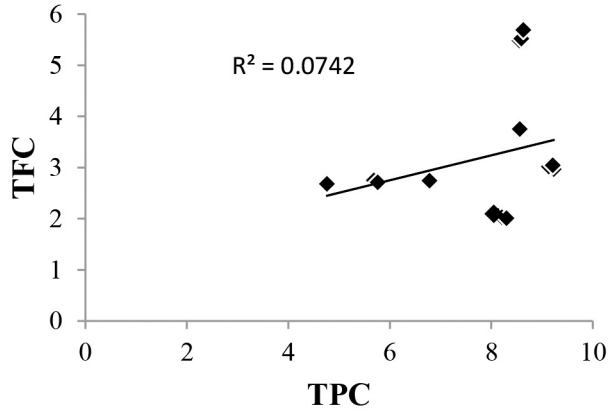

Figure 1: Correlation between total phenolic content and total flavonoid content (TFC) of fresh and all cooked samples of pumpkin.

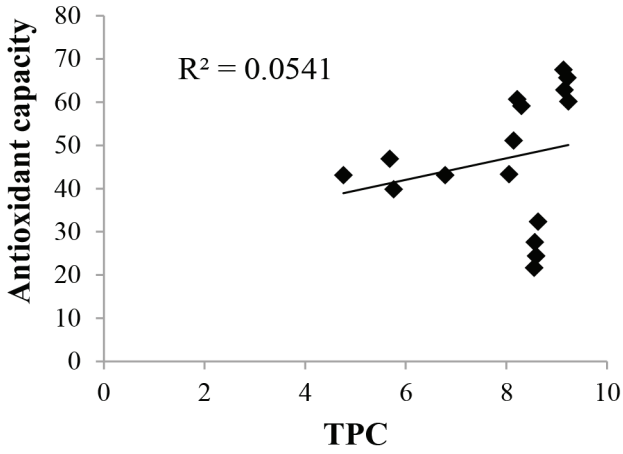

Figure 2: Correlation between total phenolic content (TPC) and antioxidant capacity of fresh and all cooked samples of pumpkin

Conclusion

The effect of three different cooking methods such as boiling, microwave cooking and stir- frying on the antioxidant activities of pumpkin was evaluated. Among different cooking methods, boiling and stir-frying caused higher losses of antioxidants than microwave cooking. Therefore, microwave cooking can be recommended to be used to cook pumpkin to preserve as much of the antioxidant in order to get the benefits of antioxidants. Further studies are needed to determine the localization of the antioxidants in the pumpkin in order to understand the reason for the changes in the antioxidant activities during various processing.

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