The influence of various cooking methods on the antioxidant compounds of local carrot variety (Daucus carota) cultivated in Jaffna District

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Abstract - Carrot (Daucus carota) is a rich source of antioxidants, mainly, β-carotene and phenolics, which are necessary to maintain eye health and prevent some types of cancers. Aim of this study was to evaluate the effect of cooking methods such as boiling, stir-frying and microwave cooking on the antioxidant potential of local carrot variety. The influence of different cooking methods on the total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) and antioxidant activity (2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity expressed as IC50 value, which is inversely proportional to antioxidant activity) of carrot was evaluated. Boiling did not have significant effect on TPC and TFC, while microwave cooking increased the TFC significantly. All cooking methods caused significant changes in the TPC except boiling. Stir-fried carrot showed significantly lower TPC, TFC and TAC than carrot cooked by other two methods except for TAC of boiled carrot. Stir-frying did not change the DPPH radical scavenging activity, while microwave cooking and boiling reduced DPPH radical scavenging activity. Microwave cooking found to be the better method than other two methods to preserve or enhance the antioxidant compounds of carrot.
Keywords - antioxidants, carrot, flavonoid content, phenolic content, radical scavenging activity.

I. INTRODUCTION

Free radicals are formed in the body cells during normal cellular function. However, excess amount of free radicals formed from endogenous or exogenous sources may lead to several human ailments [1]. Antioxidant is a substance which protects against the damage caused by free radicals [2]. Carrot (Daucus carota), belonging to family Umbelliferae, is one of the most important sources of dietary carotenoids, especially, β-carotene. Among the phenolics found in carrot, caffeic acid is the predominant. These antioxidants play vital role in maintaining human health. Carotenoids in particular, are known to protect against age related macular degeneration, cataract and some types of cancers [3].

Carrot is consumed either as fresh or after cooking. Cooking has influence on the antioxidant content of the carrot. Several studies suggested that thermal processing may increase or decrease the antioxidant potential of vegetables [4]. Understanding the effect of domestic cooking on antioxidant potential of commonly consumed vegetables is useful for selecting suitable cooking methods for retaining or enhancing activity of antioxidants. The present study was carried out to study the influence of cooking methods such as boiling, microwave cooking and stir-frying on total phenolic and flavonoid contents, antioxidant capacity and DPPH radical scavenging activity of carrot.

II. MATERIALS AND METHODS

Materials

Fresh carrot (local variety) was purchased from local market located at Thirunelvely, Jaffna on the same day of harvest. All chemicals used in the study were of analytical grade.

Sample preparation

The outer peel of the carrot was removed and the flesh was cut into small pieces (approximately 1cm) and subjected to different cooking treatments such as boiling in boiling water (100 °C), microwave cooking (560 W) and stir-frying in hot vegetable oil (230 °C) for 7, 4 and 10 minutes, respectively. The cooking time was determined based on the palatability of cooked carrot. The samples were then drained and cooled before extraction of antioxidants.

Extraction of Sample

Ethanol (70% v/v) was used to extract antioxidants from samples at the ratio of 5:1 (ethanol: sample). Sample and solvent was added in a conical flask, stoppered and shaken for 2 hours at 200 rpm using mechanical shaker (Stuart, UK) at ambient temperature. After extraction, the solvent was evaporated using rotary evaporator (Stuart, UK) to get dry extract. Dry extract stored in refrigerator at -18 ºC until analysis within two days. Before analysis, the dry extract was mixed with 70% (v/v) ethanol to get a concentration of 1 mg/mL.

Determination of total phenolic content (TPC)

TPC was determined according to the method described by [5] with minor modifications. 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)) and 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 (Thermo-Scientific) against reagent blank. The reagent blank was prepared by taking 0.3 mL of 70% ethanol instead of vegetable extract. The TPC was calculated using gallic acid as standard. The TPC was expressed as gallic acid equivalents in milligrams per gram of dry matter (mg GAE/g).

Determination of total flavonoid content (TFC)

TFC was determined according to the method explained by [6] with minor modifications. 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% NaNO2 was added. After 6 min, 0.3 mL of a 10% AlCl3.6H2O 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 UV–Vis spectrophotometer (Thermo-Scientific) against the reagent blank which was prepared by taking 0.5 mL of 70% ethanol instead of extract. The TFC was calculated using catechin standard. The TFC was expressed as catechin equivalents in milligrams per gram of dry matter (mg CE/g).

Determination of Antioxidant capacity by phosphomolybdenum method

Antioxidant capacity was determined according to [7]. 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 4 mM ammonium molybdate) was added and mixture was vortexed. The tubes were capped and incubated in a water bath at 95 °C for 90 min. The contents were cooled to room temperature and the absorbance was measured at 695 nm using a UV–Vis spectrophotometer (Thermo-Scientific) 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 ascorbic acid standard. The total antioxidant capacity was expressed as ascorbic acid equivalents in milligrams per gram of dry matter (mg AAE/g).

Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The method described by [8] was used with slight modification. The extract was taken in series of labelled test tubes (0.025-2 mL). Then, ethanol was added to make up same amount of volume. 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 using UV–Vis spectrophotometer (Thermo-Scientific). Negative control and blank were also prepared. Ascorbic acid was used as the reference antioxidant. The IC50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using inhibition curve.

III. STATISTICAL ANALYSIS

All experiments were carried out in triplicates and results were analyzed using one way analysis of variance (ANOVA) by SAS (V9.1). Duncan's new multiple range test was used to determine significant differences at 0.05 significant levels.

IV. RESULTS AND DISCUSSION

The total phenolic and flavonoid contents, antioxidant capacity and antioxidant activity of fresh and cooked carrots are shown in Table 1.

Boiling did not have significant effect on TPC and TFC, while microwave cooking increased the TFC significantly. All cooking methods caused significant changes in the TPC except boiling. Microwave cooking and stir-frying reduced TPC significantly. Stir-fried carrot had significantly lowest TPC. These results agree with the results obtained by [9]. Microwave cooked carrot possessed highest TFC followed by fresh and boiled carrot, while; stir-fried carrot had significantly lowest TFC. The results of phosphomolybdenum assay revealed that the antioxidant capacity of microwave cooked carrot was highest. Boiled and stir-fried carrot showed significantly lower antioxidant capacity than fresh and microwave cooked carrot. There was no significant difference between the antioxidant capacity of boiled and stir-fried carrot. The IC50 values of the extracts ranged from 0.43±0.02 to 0.68±0.01 mg/mL. Within the cooking methods, the low IC50 value, thus higher DPPH radical scavenging activity, was recorded in stir-fried followed by microwave cooked carrot. The high IC50 value, thus, least DPPH radical scavenging activity was recorded in boiled carrot.

Table 1: Total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity and antioxidant capacity of fresh and cooked carrots

Cooking method	TPC (mg GAE/g dry matter)	TFC (mg CE/g dry matter (DM))	Antioxidant capacity (mg AAE/g dry matter)	Antioxidant activity (IC_{50} value mg/ml)
Fresh	15.25 ± 0.90^{a}	2.67 ± 0.04^{b}	98.35 ± 5.58^{b}	0.43 ± 0.02^{c}
Boiled	15.14 ± 0.69^{a}	2.59 ± 0.05^{b}	58.28 ± 0.92^{c}	0.68 ± 0.01^{a}
Microwave cooked	10.35 ± 0.75^{b}	2.88 ± 0.05^{a}	109.44 ± 7.03^{a}	0.52 ± 0.01^{b}
Stir-fried	5.47 ± 0.89^{c}	1.26 ± 0.22^{c}	60.46 ± 2.63^{c}	0.44 ± 0.05^{c}

Data 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.

Carrots contain mainly hydroxycinnamic acids and derivatives. Among them chlorogenic acid is a major hydroxycinnamic acid. Phenolic content in different tissues decreased from peel, phloem to xylem [10]. Flavonoids, phenolics and vitamin C are water soluble compounds and heat liable compounds [11]. Thus, reduction of TPC occurred during cooking could be attributed to loss or leaching of phenolics and flavonoids. Carotenoids are relatively heat resistant antioxidants. Heat treatment may enhance the availability of β carotene of carrot due to isomerization from an all trans to a cis conformation [12] and releases the bound antioxidants. This could be the reason for increased antioxidant capacity of microwave cooked carrot while loss these compounds due to leaching into water during boiling may be the reason for lower values of boiled carrot than microwave cooked carrot. Further compounds may be degraded due to longer process and high temperature during stir-frying compared to microwave cooking.

V. CONCLUSION

Results of this study revealed that different cooking methods can cause changes in the antioxidant potential of carrot. Among the cooking methods, microwave cooking found to be the most suitable cooking method to get higher amount of TFC and antioxidant capacity than other cooking methods.

Stir-frying of carrot caused higher loss of TPC, TFC and antioxidant capacity than other cooking methods. In conclusion, microwave cooking found to be the most suitable cooking method to preserve or enhance the antioxidant potential of carrot.

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