## **RESEARCH ARTICLE**

# Effect of different cooking methods on antioxidant properties of Tomato (*Lycopersicon* esculentum)

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Abstract: Tomato (Lycopersicon esculentum) is one of the rich sources of antioxidants, mainly, lycopene which is known to be associated with decreased risks of chronic diseases. However, cooking influences the antioxidant properties of vegetables. Therefore, this study aimed to determine the effect of three cooking methods on the antioxidant properties of tomato. The conditions of the cooking methods of tomato were boiling at 100 °C for 6 min, microwave cooking at 560W for 40 sec and stir-frying at 230 °C for 4.5 min. Ethanol (70 %, v/v) was used to extract the antioxidant properties (such as phenolics, flavonoid and other antioxidant compounds) of tomato. These antioxidant properties were determined by using total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity and DPPH radical scavenging activity analysis. Results indicated that cooking has significant influences on the antioxidant properties of tomato. Boiling did not have significant changes in the TPC, however microwave cooking and stir-frying caused significant losses compared to fresh tomato. Stir-fried tomato had significantly the lowest TPC. All three cooking methods caused significant losses in TFC, however the losses are less significant during boiling compared to microwave cooking and stir-frying. Significantly higher antioxidant capacity was observed in microwave cooked tomato than fresh and other cooked tomatoes. The highest antioxidant activity was observed in microwave cooked tomato followed by stir-fried and fresh tomatoes.

*Keywords:* Antioxidants, Flavonoid content, Phenolic content, Tomato.

## INTRODUCTION

In the past few decades, there has been increasing interest in the use of dietary antioxidants from plant sources in order to reduce the risk of developing degenerative diseases such as cancer, cardiovascular disease and immune dysfunction (Chun *et al.*, 2005). Antioxidants play a crucial role in maintaining human health by inhibiting or delaying free radical damage in the body. Antioxidants can be defined as any molecule capable of stabilizing or deactivating free radicals before they attack cells (Rahman, 2007). Free radicals carry an unpaired electron that is looking to pair up with another. Due to this, free radicals can cause oxidative damage to cell components such as proteins, DNA and lipids which leads to increase the risk of degenerative diseases (Rezaeizadeh *et al.*, 2011). However, an antioxidant encounters a free radical by freely gives up an electron of its own which satisfies the free radical and inhibits the cellular damage (Huy *et al.*, 2008).

Antioxidants are abundant in vegetables and fruits (Sen and Chakraborty, 2011). Tomato (Lycopersicon esculentum) (Family: Solanaceae) is an extensively consumed vegetable worldwide and it is a rich source of lycopene, beta carotene, vitamin C, vitamin E and flavonoids (Willcoxe et al., 2003). Lycopene is the principal pigment of the carotenoids naturally found in tomatoes accounting for more than 80% of the total tomato carotenoids in fully red-ripe tomato (Singh and Goyal, 2008; Kamiloglu et al., 2013). Lycopene is found to be the most efficient singlet oxygen quencher with a capacity of more than twice of  $\beta$ -carotene (Shi *et al.*, 2008). Lycopene is known to protect from several diseases, especially beneficial against prostate cancer (Sen and Chakraborty, 2011). Lycopene has also been hypothesized to prevent carcinogenesis and atherogenesis by protecting critical cellular biomolecules, including lipids, lipoproteins, proteins and DNA (Agarwal and Rao, 2000).

Although some prefer consuming tomatoes as fresh, others eat tomato after processing. Antioxidant properties of tomato are influenced by cooking methods (Capanoglu *et al.*, 2010). Cooking softens the cell walls, facilitates the extraction of antioxidants, thus, modifies the bioavailability of bioactive compounds of vegetables (Monreal *et al.*, 2009). The ultimate effect of cooking on antioxidant contents depends on the processing parameters, the structure of food matrix and the chemical nature of the specific compound (Palermo *et al.*, 2014).

The present study was carried out to evaluate the effect of different cooking methods such as boiling, microwave cooking and stir-frying on total phenolics and flavoniod contents, antioxidant capacity and DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity of a local variety of tomato (*Thilina*) available in Jaffna district Sri Lanka.



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## MATERIALS AND METHODS

## **Raw materials**

Fresh ripen tomatoes (*Thilina* variety) were purchased from local farms in Jaffna soon after harvest.

## Chemicals

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

Tomatoes were cleaned using running tap water and cut into small pieces (1cm) and subjected to different cooking treatments such as boiling, microwave cooking and stirfrying. For boiling, tomato (35 g) was added to a beaker containing boiling water (50 mL) without a lid and cooked for 6 min and excess amount of water was drained off. For microwave cooking, tomato (35 g) was added to the container containing water (50 mL) with a lid and kept in a microwave oven at medium power (560W) for 0.6 min (40 sec). Excess amount of water was drained off. For stirfrying, 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. After that, tomato (35 g) was added and stirred for 4.5min. The cooking times were determined based on preliminary experiments to get the tomato tender.

## Estimation of moisture content

Sample (10 g) was weighed in a moisture can with a known weight. 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 following equation.

## Percentage of dry matter = 100 - moisture percentage

## **Extraction of Sample**

Ethanol (70% v/v) was used to extract antioxidants from samples. Sample was added in to a 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°C

until analysis within two days. Dry extract was mixed with solvent 70% (v/v) ethanol to get vegetable 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)

Prepared 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 (Genie). 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 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 in milligrams per gram of dry matter (Ismail *et al.*, 2004).

## Determination of total flavonoid content (TFC)

Tomato 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<sub>2</sub> was added. After 6 min, 0.3 mL of a 10% AlCl<sub>3</sub>.6H<sub>2</sub>O 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 (Genie). The absorbance was measured immediately at 510 nm using a UV–Vis spectrophotometer (Thermo-scientific) 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 in milligrams per gram of dry matter (Dewanto *et al.*, 2002a).

## Determination of Antioxidant capacity by phosphomolybdenum method

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 4 mM ammonium molybdate) was added and mixture was votexed (Genie). 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 calibration curve of ascorbic acid. The total antioxidant capacity was expressed as Ascorbic acid equivalents in milligrams per gram of dry matter (Girgin and Nehir, 2015).

## Determination of DPPH radical scavenging activity

The method described by Shekhar and Anju (2014) was used with slight modification. 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 (Thermo-scientific). 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 calibration curve.

#### Statistical analysis

All experiments were carried out in triplicates. The data were presented as the mean  $\pm$  standard deviation of the mean (using MS excel 2013). Results were analyzed using one way analysis of variance by Statistical Analysis System (SAS), version 9.1.3. Duncan's Multiple Range Test was used to compare the treatment means at p<0.05.

#### **RESULTS AND DISCUSSION**

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). Cooking of vegetables has mixed effects on antioxidants of cooked foods. The present study has determined the effect of three different cooking methods such as boiling, microwave cooking and stir-frying on the antioxidant properties of tomato. Figure 1 shows the TPC of fresh and cooked tomato. Boiling did not have significant changes in the TPC, however microwave cooking and stir-frying caused significant losses (16.92 and 68.07%, respectively) compared to fresh tomato. Stir-fried tomato had significantly lowest TPC. The result of TPC of tomato is in line with the values reported by Luthria et al. (2006), Natella et al. (2010) and Borguini et al. (2013). Reduction of TPC during cooking could be attributed to

loss or leaching of phenolics and flavonoids. Higher TPC in boiled tomato than microwave cooked tomato could be attributed to the release of more bound phenolic acid from vacuoles by boiling than microwave cooking (Dewanto *et al.*, 2002a). Because of heat instability of phenolics, the high temperature during stir-frying could have caused loss of phenolics, thus, stir-fried tomato had lowest TPC.

Figure 2 shows the TFC of fresh and cooked tomato. All three cooking methods caused significant losses in TFC, however the losses are less significant during boiling compared to microwave cooking and stir-frying. Losses of TFC in boiled, microwave cooked and stir-fried tomato were 13.93, 31.01 and 48.23%, respectively compared to fresh tomato. Cooking of tomato decreased the TFC. Microwave cooking of tomato causes loss of quercetin (Natella *et al.*, 2010). Also, at high temperatures, the structure of anthocyanin is opened to form chalcone, which is degraded further to brown products (Nayak *et al.*, 2015).

3 shows the antioxidant capacity Figure of fresh and cooked tomato. Based on the results of phosphomolybdenum assay, significantly higher antioxidant capacity was observed in microwave cooked tomato than fresh and other cooked tomatoes. Because of this, microwave cooked tomato was gained 70.74% of antioxidant capacity compared to fresh tomato. Boiled tomato showed significantly lowest antioxidant capacity. There was a 27.25% loss in antioxidant capacity of boiled tomato. There was no significant difference between fresh and stir-fried tomato.

Table 1 shows the DPPH radical scavenging activity of fresh and cooked tomato. The  $IC_{50}$  values of the extracts ranged from  $0.150\pm0.001$  to  $0.215\pm0.002$  mg/mL. The highest DPPH radical scavenging activity was recorded in microwave cooked tomato followed by stir-fried tomato and fresh tomato. The least DPPH radical scavenging activity was recorded in boiled tomato. The  $IC_{50}$  value of ascorbic acid was observed to be very low (1.14 µg/ mL)



Figure 1: Total phenolic content of fresh and cooked tomato. Data are presented as mean  $\pm$  standard error. Mean values with different superscripts are significantly different at p< 0.05 by analysis of variance followed by Duncan's multiple range test.



**Figure 2:** Total flavonoid content of fresh and cooked tomato. Data are presented as mean  $\pm$  standard error. Mean values with different superscripts are significantly different at p< 0.05 by analysis of variance followed by Duncan's multiple range test.



Figure 3: Antioxidant capacity of fresh and cooked tomato. Data are presented as mean  $\pm$  standard error. Mean values with different superscripts are significantly different at p< 0.05 by analysis of variance followed by Duncan's multiple range test.

Table 1: DPPH radical scavenging activity of fresh and cooked tomato.

Cooking methods	Antioxidant activity
	IC <sub>50</sub> value mg/mL
Fresh	0.196±0.003 <sup>b</sup>
Boiling	0.215±0.002ª
Microwave cooking	$0.150{\pm}0.001^{d}$
Stir-frying	0.192±0.003°

Data are presented as mean  $\pm$  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.

indicating significantly higher DPPH radical scavenging activity compared to fresh and different cooked tomatoes. Differences in scavenging activities might be due to the presence of different total phenolics and other antioxidants (Somawathi *et al.*, 2014).

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).

Phenolics, vitamin C and flavonoid compounds occur mainly in skin than pulp and seed of tomato in the hydrophilic form (Toor and Savage, 2005). Homogenization and thermal treatment can disrupt the cellular matrix (Sahlin et al., 2004) and cell wall releasing the bound antioxidants and the oxidative and hydrolytic enzymes (Dewanto et al., 2002a). Flavonoids, phenolics and vitamin C and vitamin E are the heat liable antioxidants while lycopene and carotenoids are heat resistant antioxidants. Heat treatment enhances the availability of lycopene and  $\beta$  carotene of tomato due to isomerization from an all trans to a cis conformation (Willcoxe et al., 2003). 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 minutes 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.

Time duration also has influence on antioxidants. Some studies have reported that, although a decrease in the antioxidant potential is found for short heat treatments, a recovery of these properties can occur during prolonged heat treatment. Reductions in the initial cooking can be attributed to thermal degradation of naturally occurring antioxidants and formation of early Maillard reaction products (Nayak et al., 2015). For example, Jiratanan and Liu (2004) reported that 12% reduction in the phenolic content of beets was observed during initial application of heat (15-30 minutes), but further processing increased its content equivalent to that of unprocessed beets. In the present study, duration of microwave cooking was shorter than that of boiling. This could be also a reason for the lower values of TPC and TFC of microwave cooked tomato than boiled tomato. In addition, microwave cooking of vegetables do not follow a specific trend on the phenolic antioxidants (Nayak et al., 2015). Microwave cooked

tomato had highest total antioxidant capacity than fresh tomato. Microwave cooking tomato does not stimulate the release of ascorbic acid or other antioxidants from cooked tissue and increase the availability of lycopene and  $\beta$ -carotene (Monreal *et al.*, 2009). This could be the reason for increased antioxidant capacity of microwave cooked tomato. 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).

#### CONCLUSIONS

The present study has reported that boiling, microwave cooking and stir-frying causes significant changes in the antioxidant properties of tomato. However, among the three methods evaluated, boiling and microwave cooking were better methods than the stir-frying to maintain antioxidant properties in tomato.

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