The Effects of Boiling, Stir-Frying and Microwave Cooking on the Antioxidant Potential of Local Brinjal Variety (*Solanum melongena*) Available in Jaffna District

Thanuja Sivanathan, Subajiny Sivakanthan, Seevaratnam Vasantharuba

Department of Agricultural Chemistry, Faculty of Agriculture, University of Jaffna, Sri Lanka
Corresponding Author Email: thanuja.sivanathan@yahoo.com

Abstract: Brinjal (*Solanum melongina*) belonging to Solanaceae family is a rich source phytochemicals such as phenolic compounds (caffeic and chlorogenic acid) and flavonoids (nasunin). These phytochemicals are antioxidants, thus provide myriad of health benefits. However the antioxidant potential of vegetables is influenced by cooking. This study investigated the effects of different cooking methods on the antioxidant properties of a local brinjal variety. Fresh brinjal was cut in to small pieces and subjected to different cooking methods (boiling at 100°C for 5 min, microwave cooking at 560W for 2 min and stir-frying at 230°C for 10 min). Ethanol (70 %, v/v) was used as the solvent to extract the antioxidants. The highest total phenolic content (14.89 ± 1.84 mg gallic acid equivalent /g dry matter), total flavonoid content (13.15 ± 0.83 mg catechin equivalent/g dry matter) and total antioxidant capacity (65.0 ± 1.96 mg ascorbic acid equivalent/g dry matter) were observed in microwave cooked brinjal compared to boiled, stir-fried and fresh brinjal. Microwave cooked and stir-fried brinjal showed higher DPPH radical scavenging activity than fresh and boiled brinjal. In addition, there was a strong positive correlation ($r = 0.90$) between total phenolic content and total antioxidant capacity in raw and cooked brinjal. These results revealed that the cooking of brinjal changed the antioxidant properties and microwave cooking of brinjal is the better method than other two methods to preserve or enhance the antioxidant properties while boiling and stir-frying reduce the antioxidant potential of brinjal.

Keywords: Antioxidants, Brinjal, Flavonoid content, Phenolic content

Introduction

Nowadays, there has been much interest in the potential use of dietary antioxidants from plant foods to reduce the risk of degenerative diseases such as cancer, cardiovascular disease and immune dysfunction (Chun *et al.*, 2005). Free radicals cause oxidative damage to cell components such as proteins, DNA and lipids which leads to increase the risk of degenerative diseases (Lobo *et al.*, 2010). Antioxidants are the natural or synthetic compounds which protects against free radical damage by giving up an electron to free radical, thus, critical for maintaining good health and wellbeing.

Polyphenols are the most powerful natural antioxidants present in fruits and vegetables (Pandey and Rizvi, 2009). Brinjal (*Solanum melongina*), also known as egg plant is one of the commonly cultivated crops in the Jaffna district. The peel of brinjal has high level of nasunin which belongs to the flavonoids, while, pulp is rich in chlorogenic acid which belongs to the phenolic acid (Helyes *et al.*, 2015).

Brinjal is consumed after cooking by different methods such as boiling, deep frying, stir- frying and microwaving (Fabbri and Crosby, 2016). Cooking increases the palatability and digestibility of brinjal, while, it may cause alterations in the antioxidants of brinjal by thermal degradation, leaching or oxidation (Miglio *et al.*, 2008).

The studies on the effect of different domestic cooking methods on the antioxidant properties of local brinjal variety available in Jaffna district are scanty and very little information is available in the literature regarding the comparison between the antioxidant properties of fresh and cooked local brinjal variety (Thinnavelly purple). In this backdrop, the present study was carried out to study the effect of different cooking methods such as boiling, microwave cooking and stir-frying on total phenolics and flavonoid content, antioxidant capacity and DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity of brinjal. The information obtained from this study will be useful to create the awareness among the people regarding different cooking methods to retain as much of the antioxidant as possible in order to get benefited by the consumption of brinjal.

Materials and methods

Raw materials and chemicals

Fresh brinjals (local Thinnavelly purple variety) were purchased from local market located at Thirunelvely, Jaffna on the same day of harvest. Ethanol was purchased from VWR International S.A.S. Sodium Carbonate, Folin-Ciocalteu reagent and sodium nitrite were purchased from Himedia Laboratories Pvt. Ltd. Gallic acid standard, ascorbic acid standard, sodium hydroxide, (+)-catechin hydrate, sulfuric acid, sodium phosphate and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich Co., St, Louis, USA. Ammonium molybdate and aluminium chloride anhydrous were purchased from Research-Lab Fine Chem Industries. All chemicals and solvents used in the study were of analytical grade.

Sample preparation

The flesh of the brinjal was separated, cut into small pieces (approximately 1cm) and subjected to different cooking treatments such as;

1. Boiling:

Brinjal (35 g) was added to a beaker containing boiling water (70mL) without a lid and cooked for 5min. Excess amount of water was drained off.

2. Microwave cooking:

Brinjal (35 g) was added to the beaker containing water (2 mL) with lid. The beaker with vegetable was kept in a microwave oven at medium power (560W) for 2 min. Excess amount of water was drained off.

3. Stir-Frying:

Vegetable oil (20 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, brinjal (100 g) was added and stirred for 10 min.

Extraction of Sample

Ethanol (70% v/v) was used to extract antioxidants from samples at the ratio of 5:1 (ethanol: sample). After extraction, the solvent was evaporated using rotatory evaporator 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.

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 following equation.

$$\text{Percentage of dry matter} = 100 - \text{moisture percentage}$$

Determination of total phenolic content (TPC)

Vegetable extract (0.3 mL) was transferred into a test tube and 2.25 mL of Folin – Ciocalteu 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 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% 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 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 in milligrams per gram of dry matter (Dewanto *et al.*, 2002).

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 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 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. Negative control and blank were also done. 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.

Statistical analysis

All experiments were carried out in triplicates and results were analyzed using one way analysis of variance by SPSS (ver.20). Pearson's correlation was carried out to test relationship among antioxidant capacity, TFC and TPC of brinjal.

Results and discussion

Brinjal was selected to evaluate the antioxidant activity in terms of total phenolic (TPC), total flavonoid contents (TFC), antioxidant capacity and DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging activity. The total phenolic content and total flavonoid content were expressed as Gallic acid equivalents (GAE) and Catechin equivalents (CE), respectively. The antioxidant capacity was expressed as Ascorbic acid equivalents (AAE). DPPH radical scavenging activity was expressed by inhibition concentration at 50% (IC₅₀ value).

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

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	11.44±1.13 ^b	7.11±0.51 ^b	32.52±2.47 ^b	0.308±0.013 ^a
Boiled	7.39±0.38 ^c	6.05±0.73 ^b	25.84±3.30 ^c	0.156±0.002 ^b
Microwave cooked	14.89±1.84 ^a	13.15±0.83 ^a	65.00±1.96 ^a	0.043±0.003 ^c
Stir-fried	6.69±0.11 ^c	3.20±0.70 ^c	24.29±1.30 ^c	0.040±0.004 ^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.

The total phenolic and flavonoid contents and antioxidant capacity and antioxidant activity of fresh and cooked brinjal are shown in Table 1. All three cooking methods caused significant changes in the TPC content. Microwave cooking significantly increased the TPC while boiling and stir-frying decreased the TPC compared to fresh brinjal. There was a no significance (p> 0.05) difference between the TPC of boiled and stir-fried brinjal. Chumyam *et al.* (2013) demonstrated that both boiling and microwave cooking increased the TPC of brinjal significantly compared to raw fruits. The results of the present study agree with this result for microwave cooking. Further, finding of Saikia and Mahanta (2013) also demonstrated that microwave cooking increased TPC of brinjal compared to fresh. Microwave cooked brinjal possessed highest TFC followed by fresh and boiled brinjal, while, stir-fried brinjal had significantly lowest (p< 0.05) TFC. Saikia and Mahanta (2013) also observed that microwave cooking of brinjal increased TFC compared to raw brinjal. The results of phosphomolybdenum assay revealed that the antioxidant capacity of microwave cooked brinjal was highest. Boiled and stir-fried brinjal showed significantly (p< 0.05) lower antioxidant capacity than fresh and microwave cooked brinjal. There was no significant (p> 0.05) differences between the antioxidant capacity of boiled and stir-fried brinjal. The IC₅₀ values of the extracts ranged from 0.040±0.004 to 0.308±0.013 mg/mL. The low IC₅₀ value thus higher DPPH radical scavenging activity was recorded in stir-fried and microwave cooked brinjal followed by boiled brinjal. The high IC₅₀ value, thus, least DPPH radical scavenging activity was recorded in fresh brinjal.

The highest total phenolic, flavonoid and antioxidant capacity of microwave cooked brinjal could be attributed to the retention of phenolic compounds such as phenolic acid, chlorogenic acid and anthocyanin and other antioxidant compounds vitamin C, cysteine and glutathione during microwave cooking than other cooking methods as these compounds are water soluble and have low chemical stability (Chumyam *et al.*, 2013; Scalzo *et al.*, 2016). Due to this, these compounds may be leached into water during boiling and compounds are degraded due to longer process and high temperature during stir-frying than microwave cooking.

Correlation among the total phenolic content, total flavonoid content and antioxidant capacity of brinjal

Several studies were carried out concerning the relationship between total phenolic content and antioxidant capacity of vegetables (Kaur and Kapoor, 2002; Isabelle *et al.* 2010; Rezaeizadeh *et al.*, 2011; Xiang *et al.* 2011). Ismail *et al.* (2004) and Kahkonen *et al.* (1999) did not find any correlation between total phenolic content and antioxidant capacity of vegetables. According to them different phenolic compounds have different responses in Folin-Ciocalteu method.

However, the results of this study showed that there is a positive significant ($p < 0.05$) strong correlation between total phenolic content and antioxidant capacity ($r = 0.90$) (Figure 1) and between total phenolic content and total flavonoid content ($r = 0.91$) (Figure 2).

Figure 1: Correlation between total phenolic content (TPC) and antioxidant capacity of fresh and all cooked samples of brinjal

Figure 2: Correlation between total phenolic content and total flavonoid content (TFC) of fresh and all cooked samples of brinjal

Conclusion

This study found that different cooking methods can cause changes in the antioxidant properties of brinjal. Among the cooking methods, microwave cooking found to be the most suitable cooking method to get higher amount of total phenolic content, total flavonoid content and antioxidant capacity than other cooking methods. Stir-frying of brinjal caused higher loss of total phenolic, total flavonoid contents and antioxidant capacity than other cooking methods. Microwave cooked and stir-fried brinjal had high DPPH radical scavenging activity, while, boiled brinjal showed less DPPH radical scavenging activity. Therefore, microwave cooking found to be the most suitable cooking method to preserve or improve the antioxidant content of cooked vegetables. Findings of this study may help people on the choice of cooking method to improve and avoid the loss of antioxidants.

Acknowledgements

The financial assistance provided by the University of Jaffna, Sri Lanka (2016) is highly acknowledged.

References

- Chumyam, A., Whangchai, K., Jungklang, J., Faiyue, B.B., Saengnil, K., 2013, Effects of heat treatments on antioxidant capacity and total phenolic content of four cultivars of purple skin eggplants. *Science Asia*. 39(3), 246-251.
- Chun, O.K., Kim, D., Smith, N., Schroeder, D., Han, J.T., Chang Yong Lee, C.Y, 2005, Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture*, 85(10), 1715-1724.
- Dewanto, V., Wu, X., Adom, K.K., Liu, R.R.H, 2002, Thermal processing enhances the nutritional value of Tomatoes by increasing total antioxidant activity. *Journal of agricultural and food chemistry*, 50(10),3010-3014.
- Fabbrin, A.D.T., Crosby, G.A., 2016, A review of the impact of preparation and cooking on the nutritional quality of vegetables and legumes. *International Journal of Gastronomy and Food Science*, 3, 2-11.
- Girgin, N.F., Nehir, S, 2015, Effects of cooking on in vitro sinigrin bioaccessibility, total phenols, antioxidant and antimutagenic activity of cauliflower (*Brassica oleraceae* L. var. *Botrytis*). *Journal of Food Composition and Analysis*, 37, 119-127.
- Helyes, L., Nagy, Z.S., Daood, H., Pék, Z., Lugasi, A, 2015, The simultaneous effect of heat stress and water supply on total polyphenol content of eggplant. *Applied ecology and environmental research*, 13(2), 583-595.
- Isabelle, M., Lee, B.L., Lim, M.T., Koh, W.P., Huang, D., Ong, C.N, 2010, Antioxidant activity and profiles of common vegetables in Singapore. *Food Chemistry*. 120(4), 993-1003.
- Ismail, A., Marjan, Z.M., Foong, C.W, 2004, Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry*, 87(4), 581-586.
- Kahkonen, M.P., Hopia, A.T., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., et al. 1999, Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*, 47(10), 3954-3962.
- Kaur, C., Kapoor, H.C, 2002, Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology*, 37(2), 153-161.
- Lobo, V., Patil, A., Phatak, A., Chandra, N, 2010, Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*, 4(8), 118-126.
- Miglio, C., Chiavaro, E., Visconti, A., Fogliano, V., Pellegrini, N, 2008, Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *Journal of agricultural and food chemistry*, 56(1),139-147.
- Pandey, K.B. Rizvi, S.I, 2009, Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*, 2(5), 270-278.

- Rezaeizadeh, A., Zuki, A.B.Z., Abdollahi, M., Goh, Y.M., Noordin, M.M., Hamid, M., Azmi, T.I., 2011, Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia*. *African Journal of Biotechnology*, 10(24), 4932-4940.
- Saikia, S., Mahanta, C.L., 2013, Effect of steaming, boiling and microwave cooking on the total phenolics, flavonoids and antioxidant properties of different vegetables of assam, India. *International journal of food and nutritional sciences*, 2(3), 2320 –7876.
- Scalzo, R.L., Fibiani, M., Francese, G., Alessandro, A.D., Rotino, G.L., Pellegrino Conte, P., Mennella, G., 2016, Cooking influence on physico-chemical fruit characteristics of eggplant (*Solanum melongena* L.). *Food Chemistry*. 194, 835–842.
- Shekhar, T.C., Anju, G., 2014, Antioxidant activity by DPPH Radical Scavenging Method of *Ageratum conyzoides* Linn. Leaves. *American Journal of Ethno medicine*, 1(4): 244-249.
- Xiang, Z., Chai, J.W., kuppusamy, U.R., 2011, Customized cooking method improves total antioxidant activity in selected vegetables. *International Journal of Food Sciences and Nutrition*, 62(2), 158-163.