

Effect of Different Cooking Methods on the Antioxidant properties of Bitter gourd (*Mormodica charantia*) Cultivated in Jaffna District

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ABSTRACT

Purpose: Bitter gourd (*Mormodica charantia*) is a widely cultivated vegetable crop in Jaffna. It is well-known to possess medicinal properties, mainly because of its antioxidant properties. Changes in the antioxidant properties of vegetables during different cooking methods have an influence on dietary nutrition. Therefore, this study is aimed at determining the effect of three cooking methods on the antioxidant properties of bitter gourd.

Research Method: Fresh bitter gourds were cut into small pieces and subjected to three different cooking methods (boiling, microwave cooking and stir-frying). The conditions of the cooking methods were; boiling at 100°C for 14 min, microwave cooking at 560W for 4 min and stir-frying at 230°C for 15 min. Ethanol (70 %, v/v) was used to extract the antioxidants from fresh and cooked samples and antioxidant properties were determined as total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity and DPPH radical scavenging activity. Results were analyzed using one-way analysis of variance using Statistical Analysis System (SAS 9.1).

Findings: TPC, TFC, antioxidant capacity and DPPH radical scavenging activity (IC_{50} value) of fresh bitter gourd were 27.47±1.52 mg gallic acid equivalent/g dry matter, 19.36±2.01 mg catechin equivalent/g dry matter, 103.55±3.60 mg ascorbic acid equivalent/g dry matter and 0.210±0.008 mg/mL respectively. Compared to the antioxidant properties of fresh bitter gourd, boiling has significantly ($p < 0.05$) increased TPC (by 48.63%) and TFC (by 42.77%) while, antioxidant capacity was reduced (by 17.84%). However, TPC, TFC, and antioxidant capacity of bitter gourd were reduced by 38.70%, 40.13% and 23.55%, respectively, after microwave cooking and 77.94%, 75.88% and 67.77%, respectively, after stir-frying. Thus, it can be concluded that boiling found to be the better method than other two methods to retain antioxidant properties of the bitter gourd.

Originality/ Value: This study could be useful to create the awareness among the people on different cooking methods to retain as much of the antioxidant as possible in order to get benefited by the consumption of bitter gourd.

Keywords: Bitter gourd, Flavonoid content, Phenolic content

INTRODUCTION

Fruit and vegetables are rich sources of bioactive compound (Kaur and Kapoor, 2002) mainly as phenolic and polyphenolic compounds. These compounds possess antioxidant activities, thus protecting cell components such as proteins, DNA and lipids from oxidative damages caused by free radicals by delaying or preventing the oxidation (Isabelle *et al.*, 2010; Mehta

and Gowder, 2015). Polyphenols are the most effective antioxidants found in fruit and vegetables. They are found in almost all parts of plant (Pandey and Rizvi, 2009).

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Bitter gourd (*Mormodica charantia*) belongs to *Cucurbitaceae* family. The major biological components of bitter gourd include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids. Further, bitter gourd is a source of vitamins C and A (Rezaeizadeh *et al.*, 2011) and other health promoting substances such as charantin and vicine (hypoglycaemic), momorcharin and momordicoside A and B (tumor growth inhibitors) and phenolic compounds like gallic acid, epicatechin, chlorogenic acid *etc.* and carotenoids (Behera *et al.*, 2007).

In Sri Lanka, vegetables are cooked before eating to make them palatable. Boiling and stir-frying are the most common cooking methods since long time ago. Nowadays, microwave cooking is also gaining popularity as a common cooking method. Different cooking methods have an influence on the antioxidant properties of vegetables and the extent of the effect depend on various factors including the cooking conditions. Bitter gourd is one of the vegetables consumed extensively by people in Sri Lanka. Since bitter gourd is well known to possess good antioxidant properties, it is necessary to understand the effect of different cooking methods on the antioxidant properties of this vegetable. Therefore, this study is aimed to determine the effect of three cooking methods on the antioxidant properties of a local variety of bitter gourd (Thirunelvely white).

MATERIALS AND METHODS

Plant materials

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

Chemicals

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

Sample preparation

Bitter gourds were cleaned using running tap water and cut into small pieces (approximately 1×1×1cm) and subjected to different cooking treatments such as boiling, microwave cooking and stir-frying. Duration of cooking for each method was determined using preliminary experiment until the pieces become palatable.

Cooking by boiling:

Sliced bitter gourd (35 g) was added to a beaker containing boiling water (50 mL) without a lid and cooked for 14 min and excess amount of water was drained off.

Cooking by microwave cooking:

Sliced bitter gourd (35 g) was added to the container containing water (50 mL) with lid and kept in a microwave oven at medium power (560W) for 4 min. Excess amount of water was drained off.

Cooking by 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, sliced bitter gourd (35 g) was added and stirred for 15 min.

Estimation of moisture content

Moisture content of the samples was determined gravimetrically according to AOAC (2000) method. 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.

Percentage of dry matter = 100 - moisture percentage

Extraction of Sample

Ethanol (70% v/v) was used to extract antioxidants from samples. The sample was added into 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–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 (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, UK) 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% 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 (Genie). The absorbance was measured immediately at 510 nm using a UV–Vis spectrophotometer (Thermo-scientific, UK) 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 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 (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, UK) 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 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, UK). 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 and the values are presented as mean \pm 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 SAS (V9.1). 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 bitter gourd.

RESULTS AND DISCUSSION

Antioxidants are major compounds that protect the human health by retarding or inhibiting the oxidation process via scavenging free radical produced during many environmental and

endogenous sources. The moisture content of fresh bitter gourd was $92.50 \pm 0.99\%$. The Table 01 shows the TPC, TFC, antioxidant capacity and antioxidant activity of fresh and cooked bitter gourd.

The results revealed that boiled bitter gourd showed significantly ($p < 0.05$) highest TPC followed by fresh and microwave cooked bitter gourd (Table 01). Stir-fried bitter gourd had the significantly ($p < 0.05$) lowest TPC. TPC of bitter gourd was increased by 48.63% after boiling and reduced by 38.70% after microwave cooking and 77.94% after stir-frying when compared to fresh bitter gourd. The result of TPC of bitter gourd is in line with the values reported by Tan *et al.* (2013) (1415.69 mg GAE/100g dry matter for oven dried bitter gourd), Zhang *et al.* (2009) who found 16.01 ± 1.09 mg GAE /g dry matter for fresh bitter melon leaf and Hamissou *et al.* (2013) who evaluated 13.28 ± 1.71 mg GAE/g fresh weights of fresh bitter gourd.

Boiled bitter gourd showed the highest TFC followed by fresh and microwave cooked bitter gourd. Stir-fried bitter gourd contained the significantly ($p < 0.05$) lowest TFC. There was a 42.77% of TFC increased in boiled bitter gourd. Losses of TFC in microwave cooked and stir-fried bitter gourd were 40.13 and 75.88% respectively.

Fresh bitter gourd was 103.55 ± 3.60 mg AAE/g dry matter which had the significantly highest antioxidant capacity followed by boiled bitter gourd.

Table 01: TPC, TFC, antioxidant capacity and DPPH radical scavenging activity of fresh and cooked bitter gourd.

Cooking method	TPC (mg GAE/g dry matter)	TFC (mg CE/g dry matter)	Antioxidant capacity (mg AAE/g dry matter)	Antioxidant activity (IC_{50} value mg/mL)
Fresh	27.47 ± 1.52^b	19.36 ± 2.01^b	103.55 ± 3.60^a	0.210 ± 0.008^b
Boiled	40.83 ± 1.91^a	27.64 ± 1.18^a	85.08 ± 3.23^b	0.508 ± 0.017^a
Microwave cooked	16.84 ± 1.18^c	11.59 ± 0.91^c	79.16 ± 1.52^c	0.140 ± 0.008^c
Stir-fried	6.06 ± 0.74^d	4.67 ± 0.87^d	33.37 ± 2.17^d	0.110 ± 0.012^d

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.

Microwave cooked and stir-fried bitter gourd showed significantly lower antioxidant capacity than others. There was 17.84%, 23.55% and 67.77% losses in boiled, microwave cooked and stir-fried bitter gourd respectively.

The IC₅₀ values of the extracts ranged from 0.110±0.012 to 0.508±0.017 mg/mL (Table 01). The highest DPPH radical scavenging activity thus the lowest IC₅₀ value was recorded in stir-fried bitter gourd followed by microwave cooked and fresh bitter gourd. The least DPPH radical scavenging activity thus the highest IC₅₀ value was recorded in boiled bitter gourd. This value was in agreement with the studies done by Wu and Ng (2008) who reported that ethanolic extracts from fresh bitter gourd showed DPPH scavenging activities (IC₅₀) of 156.89 µg/mL and Lu *et al.* (2012) 521 ±6µg/mL.

Boiling of bitter gourd had the highest total phenolic and flavonoid content. This may be due to the phenolic compounds in *M. charantia* were more heat stable (Choo *et al.*, 2014), The increase in the total phenolic compounds in cooked samples may be because of the intense breakdown of cell walls and release of phenolic compounds caught in the fiber of vegetables (Adefegha and Oboh, 2011). However, microwave cooked and stir-fried bitter gourd caused loss of total phenolic and flavonoid content due to cooking temperature and time (Subramaniam *et al.*, 2017). Frying caused the largest loss of antioxidants and antioxidant activity (Aminah and Permatasari, 2013).

Bitter gourd fruit contains vitamin C, vitamin E, carotenes, , chlorogenic acid, vanilic acid, gallic acid, tannic acid, (+)-catechin, caffeic acid, p-coumaric acid, benzoic acid (Hasan and Khatoon, 2012). These compounds were reported to contribute to the total antioxidant

activities of bitter gourd fruit that are associated with the protection from degenerative diseases of aging, including diabetes, cardiovascular diseases, hypertension, and cancer (Tan *et al.*, 2013).

Antioxidant properties can be altered and disturbed by processing of fruit and vegetable (Shi and Maguer 2000). Cooking is one of the processing methods of vegetables which softens the cell walls, increases the extrication of antioxidants, thus, and modifies the bioavailability of bioactive compounds of vegetables (Monreal *et al.*, 2009). Because of this, antioxidant compound of vegetables can vary when vegetables are subjected to different cooking methods such as frying, boiling, steaming and microwaving (Sultana *et al.*, 2008). The end effect of cooking on this compound depends on the temperature and localization of the structures in the vegetables, cutting and chopping process (Makris and Rossiter, 2001), processing factors, chemical nature of the specific compound (Palermo *et al.*, 2014), the heat liability and solubility of the structure (Prasad *et al.*, 1996; Pedraza-Chaverri *et al.*, 2006); bioavailability of the antioxidant compounds (Sultana *et al.*, 2008).

Correlations for total phenolic content and total flavonoid content (01), (02) total phenolic content and antioxidant capacity, (03) total flavonoid content and antioxidant capacity of bitter gourd

The results of this study showed that there is a positive strong correlation between total phenolic content and total flavonoid content ($r = 0.99$) (Figure 01), total phenolic content and antioxidant capacity ($r = 0.73$) (Figure 02), total flavonoid content and antioxidant capacity of bitter gourd ($r = 0.75$) (Figure 03).

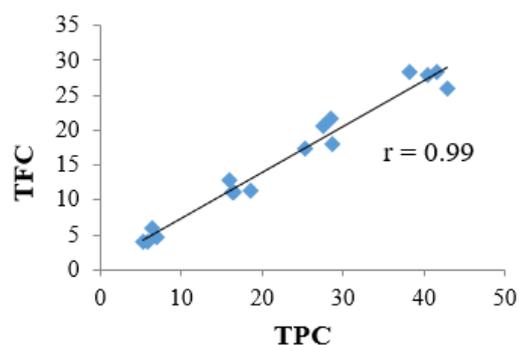


Figure 01: Correlation between total phenolic content (TPC) and total flavonoid content (TFC) of fresh and all cooked samples of bitter gourd.

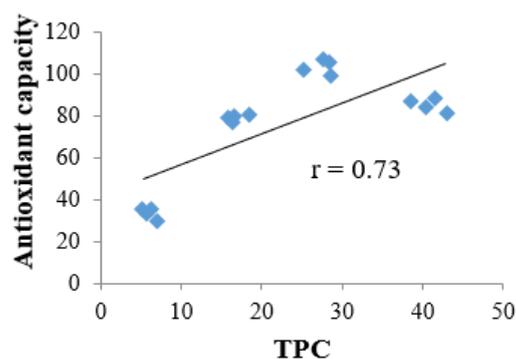


Figure 02: Correlation between total phenolic content (TPC) and antioxidant capacity of fresh and all cooked samples of bitter gourd.

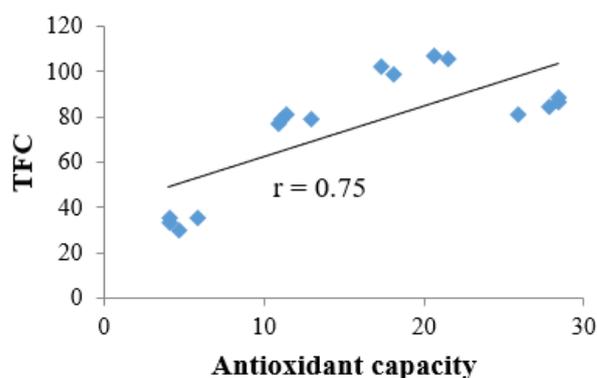


Figure 03: Correlation between total flavonoid content (TFC) and antioxidant capacity of fresh and all cooked samples of bitter gourd.

CONCLUSION

The present study has reported that boiling, microwave cooking and stir-frying causes significant changes in the antioxidant properties of bitter gourd. However, among the three methods evaluated, boiling of bitter gourd found to be the better method than the other methods studied to preserve as much of the antioxidant present in the fresh vegetable. In the correlation analysis, there was a strong positive significant ($p < 0.05$) correlation among total phenolic content, antioxidant capacity and total flavonoid content.

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Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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