Effect of drying and cooking methods on antioxidant properties of bitter gourd (*Momordica charantia*)

[Kesan pengeringan dan kaedah memasak ke atas ciri-ciri antipengoksidaan peria katak (*Momordica charantia*)]

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Keywords: bitter gourd, phenolic compound, DPPH, FRAP, cooking methods

Abstract

The effect of various cooking methods (stir frying, deep frying, boiling, steaming and microwaving) and oven-drying at three different temperatures (40, 50 and 60 °C) on antioxidant properties of bitter gourd were evaluated. Total phenolic content (TPC) was measured using Folin-Ciocalteu method while the antioxidant activity was evaluated using methanol solution of DPPH and ferric reducing potential assay using FRAP reagents. All analyses were conducted using the microplate reader spectrophotometer. The results indicated that deep frying had the highest TPC at 98.18 mg/100 g GAE, followed by microwave cooking (25.63 mg/100 g GAE). The TPC for deep-fried samples was significantly different (p <0.05) from the other cooking methods. However, microwave cooked samples have significantly (p <0.05) higher percentage of DPPH radical scavenging activity (88.54%) and FRAP (65.85 µmol/g FE) compared to oven-dried, boiling or deep frying. For oven-drying, bitter gourd dried at 40 °C retained the highest antioxidant activities compared to samples dried at 50 or 60 °C. Thus, the best drying temperature to retain antioxidant properties in bitter gourd is at 40 °C while the best cooking method is either microwave or deep fried.

Introduction

Consumption of fruits and vegetables has been associated with the prevention of chronic diseases such as cancer and cardiovascular disease (Amin and Lee 2005). Bitter gourd (*Momordica charantia*), also known as bitter melon is one of the most popular vegetables in Asia. Various methods are used to prepare the vegetable such as stir-fried, deep-fat fried, boiled, pickled, juiced and dried (Basch et al. 2003; Myojin et al. 2008). Decoction of the fruits, served as a drink, was used to prevent stomach ache, toothache, liver diseases, diabetes, hypertension and cancer and believed to be more effective in treating diabetes than the commonly eaten bitter gourd dishes (Wu and Ng 2007).

In the development of processing technologies that can optimize antioxidant retention, it is important to understand the chemical properties of the antioxidants, the enzymes that affect their content and the partitioning of antioxidants in the plant tissues (Kalt 2005). Extraction, quantification and antioxidant activities of phenolics from pericarp and seeds of bitter gourd based on maturity (Horax

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et al. 2010), influence of ripening stages on physicochemical characteristics and antioxidant properties of bitter gourd (Aminah and Anna Permatasari 2011) have been studied.

Processing can alter and often damage fruit and vegetable antioxidants. Maceration, heating and various preparation steps can result in oxidation, thermal degradation, leaching and other events that lead to lower levels of antioxidants in processed food (Shi and le Maguer 2000). Most research focussed only on the antioxidant properties of fresh tissues of vegetables (Shi and le Maguer 2000). The effect of thermal treatments on the antioxidant properties of cooked vegetables has seldom been reported. Variation in cooking treatments can profoundly affect both the texture and the nutritional value of vegetables. Lin and Chang (2005) reported that most vegetables precooked at a moderate temperature of 50 – 80 °C for a suitable period of time and subsequently cooked in boiling water showed greater firmness than those cooked directly without precooking. However, in the case of carotenoids, processing can lead to a dissociation of antioxidants from plant matrix components, an increase in carotenoid antioxidants and improved digestive absorption (Shi and le Maguer 2000). Thus the objective of this study was to determine the effect of processing and cooking methods on total phenolic content (TPC), antioxidant activity (DPPH) and antioxidant capacity (FRAP) of bitter gourd (Momordica charantia). Antioxidant activity represents the ability to inhibit the process of oxidation which usually involves a set of different reactions. It deals with the kinetics of a scavenging reaction between an antioxidant and a free radical (Resat et al. 2013). Meanwhile, antioxidant capacity measures the antioxidant power or efficiency of the extract to reduce a ferric-2,4,6-tri-2-pyridyl-s-triazine complex (Fe³⁺ TPTZ) to the ferrous form Fe²⁺ (Mohd Shukri et al. 2011).

**Materials and methods**

**Raw materials and chemicals**

Bitter gourd was obtained from the wet market in Kajang, Selangor. Total phenolics standard, gallic acid and 2,2-diphenyl-1-picrylhydrazil (DPPH) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, hexane, Folin-Ciocalteu reagent, sodium carbonate, ferric chloride, ferrous chloride and phosphate buffer were purchased from E. Merck (Darmstadt, Germany).

**Sample preparation**

The vegetables were dried and cooked in the Food Preparation laboratory, School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia. Fruit samples were cleaned and washed with tap water. After removing the non-edible portion, the fruits were cut into small cubes approximately 0.5 cm.

**Boiling**  Approximately 200 g of bitter gourd cubes were boiled in 500 ml boiling water in a covered cooking pot for 10 min.

**Steaming**  The bitter gourd cubes (200 g) were steamed for 20 min in a stainless steel vessel (500 ml). The temperature of the boiling water was 100 °C.

**Microwaving**  The bitter gourd cubes (200 g) were arranged singly in a shallow microwavable glass dish covered with a glass lid and cooked in the microwave oven (Sharp, R 9538A type Grill and Convection oven) for 10 min. The frequency and output power of the microwave oven were 2450MHz and 900 W (IEC 705) respectively. The outside dimensions of the oven were 627 m (W) x 381 mm (H) x 492 mm (D) and the cavity dimensions were 410 mm (W) x 245 mm (H) x 410 mm (D). The total capacity of the oven was 41 litres. The cooking uniformity was attained with a turntable (390 mm diameter tray system).
Stir frying For stir frying the bitter gourd were thinly sliced. A small amount of palm oil (2 – 3 ml) was placed in a non-stick frying pan (diameter 20 cm) and heated at ‘high’ on the gas stove for 10 min. The sliced bitter gourds (200 g) were then placed in the pan, the heating was reduced to ‘medium’ and the vegetable was continuously stirred for 10 min.

Deep frying About 300 ml palm oil was placed in a deep frying pan and heated (100 °C) on the gas stove for 10 min. The bitter gourd cubes (200 g) were then fried in the oil and the heating was reduced to ‘medium’. Bitter gourds were continuously fried for 10 min.

Oven drying Bitter gourd cubes (200 g) were placed in a tray and dried in the oven dryer (Memmert Schwabach, Germany) at 40, 50 and 60 °C for 24 h.

Sample extracts for antioxidant assay The bitter gourd extracts were prepared following the method described by Connor et al. (2002). About 0.5 g of bitter gourd sample was ground using a Waring blender (Waring commercial blender 314 Ella T Grasso Ave, Torrington, CT 06790) at low speed for 2 min and then mixed vigorously with 3 ml of 80% methanol and centrifuged (Hermle Gmbh, Germany) for 15 min at 3,000 rpm. The supernatant was collected in a 10 ml volumetric flask. The residue was treated again twice with 3 ml 80% methanol and centrifuged for 15 min. The supernatants were collected and standardised to a final volume of 10 ml.

Determination of total phenolic content The total phenolic content (TPC) was determined by the Folin-Ciocalteu method. The TPC was assayed colorimetrically by the procedure of Singleton and Rossi (1965) with slight modification. An aliquot (200 µl) of bitter gourd extract was mixed with 7.5% sodium carbonate (800 µl) and held for 4 – 8 min. One ml of phenol reagent solution (1:10, Folin-Ciocalteu’s reagent: water) was added to the aliquot and then shaken vigorously. The absorbance was measured at 765 nm using a microplate reader spectrophotometer (Molecular Devices, VERSAmax tunable, California, U.S.A) after 2 h held at room temperature. A mixture of water and reagent was used as a blank. The TPC was expressed as mg gallic acid equivalent (GAE)/100 g fresh weight.

DPPH radical scavenging activity The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-coloured methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The antioxidant activity of the extracts, based on its scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Akowuah et al. (2005). An aliquot of 200 µl aqueous extract of bitter gourd was added to 2 ml of 0.001 M DPPH in methanol. Absorbance at 517 nm was determined after 1 h using the microplate reader spectrophotometer (Molecular Devices, VERSAmax tunable, California, U.S.A). The percent inhibition of activity was calculated as [(Ao – Ae)/Ao] × 100 (Ao = absorbance without extract; Ae = absorbance with extract).

Ferric reducing antioxidant potential (FRAP) assay The FRAP assay was performed as described by Benzie and Strain (1996). The bitter gourd extract was first diluted with deionized water to fit within the linearity range. The working FRAP reagent was prepared by mixing 10 volume of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ in 40 mM HCl and 1 volume of 20 mM FeCl₃.6H₂O. Three millilitres of the working FRAP reagent were warmed to 37 °C. Then 100 µl of sample and 300 µl deionized water were added to the FRAP reagent. Absorbance was taken at 593 nm against the reagent blank after 30 min using
the microplate reader spectrophotometer mentioned previously. The FRAP value was calculated and expressed as millimoles of Fe$^{2+}$ equivalent (FE) per 100 g of sample using the calibration curve of Fe$^{2+}$. Linearity range of the calibration curve was 0.1 to 1.0 nM ($r = 0.99$).

**Statistical analysis**
The experiment was conducted in triplicate. All data were analysed using Analysis of Variance (ANOVA) by means of the GLM proc-SAS software (6.12 version). Significant differences between treatments were determined using Duncan Multiple Range Test (DMRT) at 95% level of significance.

**Results and discussion**

**Total phenolic content**
The effects of cooking and drying on antioxidant properties of bitter gourd are shown in Table 1. The TPC of deep fried bitter gourd was the highest at 90.18 mg/100 g GAE, while samples oven-dried at 40 °C contained 70.25 mg/100 g GAE. The TPC for samples which were microwaved, stir fried and oven-dried at 60 °C were not significantly different from each other ($p > 0.05$) at 25.63, 23.60 and 27.00 mg/100 g GAE respectively. However, stir fried samples had significantly ($p < 0.05$) lower TPC (23.60 mg/100 g GAE) compared to deep fried bitter gourd (90.18 mg/100 g GAE). This result was similar to that reported by Soupas et al.(2007) who indicated that the large surface to volume ratio of frying medium induces oxidation.

When vegetables are subjected to various cooking processes such as boiling, steaming and microwaving, the TPC seemed to vary. This may be due to the structure of the bioactive compounds, the cooking methods used, the bioavailability of the phenolic compounds (Sultana et al. 2008); the temperature and localisation of the structures in the vegetables and the process of cutting and chopping (Makris and Rossiter 2001); the stability of the structure to heat (Prasad et al. 1996; Pedraza-Chaverri et al. 2006); and the synergic activity of the structures and the reaction system assayed.

According to Sikora et al. (2008), the degree of polyphenol degradation depends very much on the processing time and the size of the vegetables. Thermal processing may release more bound phenolic acids from the breakdown of cellular constituents, although disruption of cell walls also release the oxidative and hydrolytic enzymes that can destroy the antioxidant in fruit and vegetables (Chism and Haard 1996). According to Miller et al. (2005), processed fruit and vegetables are expected to have a lower health protecting capacity than fresh ones.

However, although total phenolics are usually stored in fruit pectin or cellulose network, they can be released during thermal processing due to several factors such as drying method, type of extraction solvent, antioxidant assays used as well as interactions of several antioxidant reactions (Manzocco et al. 2001; Que et al. 2008). Individual phenolics may sometimes increase because heat can break supramolecular structures, releasing the bound phenolic which react better with the Folin-Ciocalteau reagent (Bunea et al. 2008). The last report by Capecka et al. (2005) explained that the total phenolic content obtained after drying process may be higher or lower based on the type of phenolic compounds present and their location in the cell of fruit.

Frying caused the largest loss of antioxidants and antioxidant activity. However, not all processing (drying and cooking) imposed on fruit or vegetables caused losses of antioxidant. The total phenolic content in oven-dried samples at 40 °C was high (70.25 mg/100 g GAE). Drying temperatures significantly affected the polyphenol content, with different effects according to the class of polyphenols (Del Caro et al. 2004). However, Ewald et al. (1999) reported that boiling, microwave cooking, frying or further warm holding
did not affect the levels of polyphenol, quercetin and kaempferol in onions, green beans and peas. On the other hand, losses of polyphenol upon boiling or blanching have been reported in selected cruciferous vegetables (Sikora et al. 2008), broccoli (Zhang and Hamauzu 2004), kale, spinach, cabbage, swamp cabbage and shallots (Ismail et al. 2004) probably due to the dissolution of polyphenols into the boiling water.

In contrast, Turkmen et al. (2005) recorded that cooking resulted in an increase in the phenol content in vegetables. During thermal processing, the degree of isomerisation of naturally occurring trans-isomer which is linked, is directly correlated to the degree of thermal treatment (Shi and Le Maguer 2000). Conversely, antioxidant levels were reported to decrease after aqua thermal treatment of broccoli (Zhang and Hamauzu 2004) and selected cruciferous vegetables (Sikora et al. 2008).

**DPPH radical scavenging activity**

Steamed and microwaved bitter gourd samples have high DPPH radical scavenging activity at 89.6% and 88.5% respectively (*Table 1*). These results were significantly higher (*p* <0.05) than the other treated samples. The value for boiled sample was 83.7% inhibition. This result was in contrast with those reported by Jimenez-Monreal et al. (2009). Their result showed that boiling produced low DPPH radical scavenging activity in most vegetables. The radical scavenging activity of stir fried bitter gourd cubes (44.2%) was significantly lower (*p* <0.05) than the deep fried sample(54.9%). Reduction in radical scavenging activity may be due to the dilution of antioxidant properties in oil during frying.

However, the radical scavenging activity of deep fried (54.9%) and oven dried bitter gourd at 50 °C (56.8%) were similar and showed no significant difference (*p* >0.05). Jimenez-Monreal (2009) reported that vegetables that kept their antioxidant capacity were artichoke, asparagus, garlic, onion and spinach. Vegetables that significantly (*p* <0.05) increased their scavenging capacity were eggplant, maize, pepper and swiss chard. Murakami et al. (2004) reported that the radical scavenging activity was more stable in foods during cooking and processing than the original polyphenolic compounds. On the other hand, Hatano et al. (1989) and Kimura et al. (1985) reported that the scavenging action of plant constituents has been found to be related among polyphenolic compounds, caffeic derivatives and flavonoids. Puupponen-Pimia et al. (2003) reported that the DPPH index of cauliflower decreased by 23% during blanching in water but increased by 9% in cabbage. The lowest

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**Table 1. Antioxidant properties of bitter gourd (*Momordica charantia*) as affected by various cooking methods and drying temperatures**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TPC (mg/100 g GAE)</th>
<th>DPPH (% inhibition)</th>
<th>FRAP (µmol/100 g FE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiling</td>
<td>12.00 ± 8.7f</td>
<td>83.68 ± 0.33b</td>
<td>47.65 ± 0.55c</td>
</tr>
<tr>
<td>Steaming</td>
<td>16.23 ± 8.8e</td>
<td>89.58 ± 0.91a</td>
<td>51.33 ± 0.58bc</td>
</tr>
<tr>
<td>Microwave</td>
<td>25.63 ± 1.8d</td>
<td>88.54 ± 0.48a</td>
<td>65.85 ± 4.13a</td>
</tr>
<tr>
<td>Deep frying</td>
<td>90.18 ± 7.5a</td>
<td>54.90 ± 0.40a</td>
<td>66.22 ± 5.00a</td>
</tr>
<tr>
<td>Stir frying</td>
<td>23.60 ± 3.7d</td>
<td>44.24 ± 4.00f</td>
<td>57.33 ± 0.51b</td>
</tr>
<tr>
<td>Oven drying 40 °C</td>
<td>70.25 ± 4.6b</td>
<td>66.47 ± 2.42c</td>
<td>68.27 ± 5.78a</td>
</tr>
<tr>
<td>Oven drying 50 °C</td>
<td>53.90 ± 5.7c</td>
<td>56.82 ± 0.80e</td>
<td>37.00 ± 3.36d</td>
</tr>
<tr>
<td>Oven drying 60 °C</td>
<td>27.00 ± 1.1d</td>
<td>61.73 ± 2.00d</td>
<td>27.23 ± 1.17e</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD (for each fruit n = 3). The same letter in the same column indicates no significant difference (*p* <0.05)
radical scavenging activity value (44.2%) was observed in stir-fried. According to Sathiskumar et al. (2005), methanol extracts of microwaved samples showed significant increase in DPPH scavenging activity compared to extracts from distilled water and distilled boiling water. Boiling of several vegetables could attribute to the suppression of oxidation by antioxidants due to thermal inactivation of oxidative enzymes (Yamaguchi et al. 2001). In addition, the boiling process may destruct the cell wall and sub cellular compartments thus releasing the potent radical scavenging antioxidants. Turkmen et al. (2005) reported that boiling, microwave cooking and steaming induced significant increases in total antioxidant activity of pepper, green beans, broccoli and spinach.

**Ferric reducing antioxidant potential (FRAP) assay**

Microwave cooking, deep frying and oven drying at 40 °C showed higher FRAP values, (65.85, 66.22 and 68.27 µmol/g FE fresh weight respectively) while oven dried at 60 °C contained the lowest FRAP value at 27.23 µmol/g FE fresh weight. Boiled bitter gourd cubes contained moderate FRAP value at 47.65 µmol/g FE fresh weight. The results showed that processing of fruit did not cause a drastic reduction in nutritional values and therefore, may create a new image for processed fruits. However, Andlaur et al. (2003) also reported that the FRAP values of some vegetables such as pea, spinach, cauliflower and cabbage were higher when exposed to water.

**Conclusion**

Different cooking methods and drying temperatures have significant (p <0.05) effect on antioxidant properties of bitter gourd. Samples dried at lower temperature (40 °C) retained the highest total phenolic content, DPPH scavenging activity and FRAP values compared to those dried at 50 or 60 °C. Likewise, cooking methods affect the antioxidant properties of bitter gourd. Steaming and microwaving had significantly higher (p <0.05) DPPH scavenging activities while deep frying produced bitter gourd with highest total phenolic contents and FRAP values.

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**References**


Antioxidant properties of bitter gourd (*Momordica charantia*)


Abstrak

Kesan pelbagai kaedah memasak (menumis, menggoreng jeluk, merebus, mengukus dan menggunakan mikrogelombang) dan pengeringan ketuhar (40, 50 dan 60 °C) terhadap ciri-ciri antipengoksidaan peria katak telah dinilai. Kandungan jumlah fenol (TPC) diukur menggunakan kaedah Folin-Ciocalteu sementara aktiviti antipengoksidaan telah dinilai menggunakan radikal bebas DPPH dalam larutan metanol dan assai penurunan potensi ferik menggunakan reagen FRAP. Semua analisis dilakukan dengan spektrofotometer pembaca mikroplat. Keputusan menunjukkan menggoreng jeluk mempunyai TPC paling tinggi pada tahap 98.2 mg/100 g GAE, diikuti dengan memasak menggunakan mikrogelombang (25.6 mg/100 g GAE). Nilai TPC sampel penggorengan jeluk didapati berbeza dengan signifikan (*p* <0.05) daripada kaedah memasak yang lain. Walau bagaimanapun, sampel yang dimasak menggunakan mikrogelombang mempunyai aktiviti menghapus sisa radikal bebas DPPH (88.6%) dan nilai FRAP (65.8 µmol/g FE) yang tinggi dengan signifikan (*p* <0.05) berbanding dengan sampel yang dikering, direbus atau digoreng jeluk. Bagi kaedah pengeringan ketuhar, sampel peria katak yang dikering pada suhu 40 °C mempunyai aktiviti antioksidan lebih tinggi berbanding dengan sampel yang dikering pada suhu 50 atau 60 °C. Oleh itu suhu pengeringan paling baik untuk mengenaaktiviti antioksidan ialah 40 °C manakala kaedah memasak paling baik adalah dengan menggunakan mikrogelombang atau menggoreng jeluk.

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