

Effects of heat treatments on antioxidant capacity and total phenolic content of four cultivars of purple skin eggplants

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ABSTRACT: We investigated the effects of three heat treatments on antioxidant capacity and total phenolic content in purple skinned eggplant fruits. Four cultivars of eggplant: ‘Muang Lek’, ‘Muang Lot Fai’, ‘Muang Kan Dam’, and ‘Muang Kan Khiao’ were subjected to boiling, steaming, and microwaving for 5, 10, and 15 min in each treatment. The results show that antioxidant capacity (DPPH and ABTS) and total phenolic content significantly increased in all eggplant cultivars with all cooking methods compared with those of raw fruits. Fruits microwaved for 10 and 15 min had the highest antioxidant capacities and total phenolic content in all cultivars. ‘Muang Lot Fai’ had the highest antioxidant capacity and total phenolic content of the four cultivars examined. A highly positive correlation for each heat treatment was found between antioxidant capacity and total phenolic content.

KEYWORDS: boiling, cooking methods, microwaving, steaming, vegetables

INTRODUCTION

Eggplant (*Solanum melongena* L.) is a common vegetable consumed throughout the world. It contains important phytonutrients such as phenolic and flavonoid compounds which have high antioxidant capacities^{1–3}. Phenolic compounds and antioxidant activity are found in most parts of the eggplant, including calyx, leaf, peel, pulp, and stem³. In the fruits, the antioxidant capacities and phenolic compounds are found in both the pulp and skin⁴. The antioxidant capacity and total phenolic content of purple skin eggplants have been reported to be higher than those of green and white skin cultivars^{5,6}.

Most vegetables are heated either by boiling, steaming or microwaving before being consumed⁷. Several studies have reported that these heating processes cause many changes in the chemical composition of vegetables. For example, the antioxidant capacity and total phenolic content of pepper (*Capsicum* spp.), green bean (*Phaseolus vulgaris*), broccoli (*Brassica oleracea*), spinach (*Spinacia oleracea*), and sweet corn (*Zea mays*) increase after boiling, steaming, or microwaving^{8,9}. Heating also increased

total phenolic content in pungent peppers (*Capsicum annuum*)¹⁰. In contrast, total phenolic content and antioxidant capacity of tomatoes (*Lycopersicon esculentum*) decrease by boiling, baking, or frying¹¹. The antioxidant capacity and total phenolic content of fresh cut broccoli decrease significantly by microwave heating¹². The antioxidant capacity of garlic (*Allium sativum*) decreases by drying and steaming¹³. Also, the phenolic content was found to decrease in non-pungent peppers (*Capsicum annuum*) by heating¹⁰.

Eggplant is one of the most common vegetables used in Thai cuisine² and is usually cooked. The objective of our study was to investigate the effects of different heat treatments including boiling, steaming, and microwaving on antioxidant capacities and total phenolic contents of four purple skin eggplant cultivars in Thailand.

MATERIALS AND METHODS

Plant materials

Four cultivars of purple skin eggplants (*Solanum melongena*) at commercial maturity, namely ‘Muang Lek’, ‘Muang Lot Fai’, ‘Muang Kan Dam’, and



Fig. 1 Four cultivars of purple skin eggplant fruit: (a) 'Muang Lek', (b) 'Muang Lot Fai', (c) 'Muang Kan Dam', (d) 'Muang Kan Khiao'.

'Muang Kan Khiao' (Fig. 1), were purchased from local markets in Chiang Mai, Thailand. The fruits were cleaned with tap water and dried with tissue paper.

Whole fruits were subjected to boiling, steaming, and microwave heating to study antioxidant capacity and total phenolic content. Six fruits were used for each treatment and raw fruits without heating were used as a control.

Boiling process

The fruits were heated in 2 l of boiling water in a commercial pot for 5, 10, and 15 min, respectively, and cooled at room temperature before testing.

Steaming process

One litre of distilled water was added to a commercial pot (10 l capacity) with a sieve, and then eggplant fruits were steamed for 5, 10, and 15 min, respectively. After steaming, the heated fruits were cooled at room temperature before testing.

Microwaving process

Eggplants were placed in a dish covered with a plastic cap and then heated in a microwave oven (Goldstar ER-762MD, 700 W) for 5, 10, and 15 min. After microwaving, the heated fruits were cooled at room temperature before testing.

Extraction of samples

Samples were extracted by the modified method of Ahmed and Osama¹⁴. Flesh and skin of each heated or raw fruit of eggplants were cut into small pieces. A sample (1 g) was then homogenized for 1 min in 25 ml of 80% methanol by using a mortar and pestle at 4 °C. The extract solution was centrifuged at 15 950g for 20 min at 4 °C (Herml model Z383K, Germany) and the supernatant was collected as a sample solution to determine antioxidant capacities and total phenolic contents.

DPPH radical scavenging capacity assay

The DPPH method was carried out according to the procedure of Mun'im et al¹⁵ with slight modifications. The principle of this assay is that the free radical DPPH[•] possesses a characteristic absorption at 517 nm, which decreases significantly on exposure to antioxidants by receiving hydrogen atoms or electrons. A lower absorbance at 517 nm indicates a higher antioxidant capacity of the extract¹⁶. The method is a standard assay in antioxidant capacity studies and offers a rapid technique for analysing the antioxidant capacity of extracts. In our study, the sample extract (100 µl) was mixed with 400 µl of 0.3 M acetate buffer pH 5.5 in a test tube. Then 2.5 ml of 0.12 mM DPPH[•] in 100% methanol was added to the tube. The mixed solution was placed in

a dark at room temperature (25 °C) for 30 min. The radical scavenging capacity was calculated from the difference in absorbance at 517 nm between a blank and the sample using a spectrophotometer (Thermo Spectroni model Helios Epsilon, USA). The blank was determined without a sample solution and 100% methanol was used as a control. A standard curve of 0–1000 µM Trolox was used to quantify the antioxidant capacity and the antioxidant capacity was expressed as µmol Trolox equivalents/100 g of fresh weight.

Determination of ABTS radical scavenging capacity

The ABTS method was carried out according to the procedure of Huang et al.¹⁷ with slight modifications. The antioxidant capacities of the sample were determined by measuring a decrease in the intensity of the blue colour of the ABTS^{•+} radical as a result of a reaction between the ABTS^{•+} radical and the antioxidant compounds in the sample¹⁸. In our assay, the ABTS^{•+} radical was generated by persulphate oxidation of ABTS. Specifically, 7 mmol of ABTS ammonium was dissolved in water and treated with 2.45 mmol of potassium persulphate and the mixture was kept at 5 °C for 16 h to give a dark blue solution. The dark blue solution was diluted with 80% ethanol until the absorbance at 734 nm was read as 0.7 using a spectrophotometer (Thermo Spectroni model Helios Epsilon, USA); this solution was called the ABTS^{•+} solution. Then, 2 ml of the ABTS^{•+} solution was mixed with 20 µl of sample. The absorbance at 734 nm was read at 10 min after mixing. The radical scavenging capacity was calculated from the difference in absorbance at 734 nm between the blank and the sample. The blank was determined without a sample solution and 80% methanol was used as a control. The antioxidant capacity was expressed as µmol Trolox equivalents/100 g of fresh weight as described above.

Determination of total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu methods according to the procedures of Singleton and Rossi¹⁹ with slight modifications. Two ml of sample extract were mixed with 10 ml of 10% Folin-Ciocalteu's phenol reagent in a flask and placed at room temperature for 8 min. Then, 8 ml of 7.5% Na₂CO₃ was added to the flask. The mixed solution was placed at room temperature for 2 h. The absorbance was measured at 765 nm using a spectrophotometer (Thermo Spectroni model Helios Epsilon, USA). A mixture of distilled water and reagents was used as a blank. A standard curve of 0–

100 µg/ml gallic acid was used to quantify the total phenolic content. The total phenolic content was expressed as mg gallic acid equivalents/100 g of fresh weight.

Statistical analysis

Experiments were repeated twice and similar results were obtained. The results of one representative experiment are shown and the data are presented as mean. Differences between treatments were considered at $p < 0.05$ by One Way ANOVA using SPSS for Windows version 15.0.

RESULTS AND DISCUSSION

As shown in Table 1, the antioxidant capacity by DPPH radical scavenging assay in eggplant fruits of all cultivars significantly ($p < 0.05$) increased when the fruits were heated with either by boiling, steaming, or microwaving compared with the control fruits (without heating). Microwaving for 5, 10, or 15 min showed higher antioxidant capacities than those of steaming and boiling methods in all cultivars (Table 1). The antioxidant capacity in fruits heated with a microwave increased and reached the highest at 10 min; then the antioxidant capacity reached a plateau (Table 1). Among four cultivars, 'Muang Lot Fai' had the highest antioxidant capacity (Table 1). The antioxidant capacity in 'Muang Lot Fai' at 10 min of microwave heating was 1.3, 1.4, and 1.2 times higher than that of 'Muang Lek', 'Muang Kan Dam', and 'Muang Kan Khiao', respectively. For steaming, it was found that the highest antioxidant capacities of cultivars 'Muang Lek' and 'Muang Kan Dam' were at 10 min of heating, whereas 'Muang Lot Fai' and 'Muang Kan Khiao' had the highest capacity at 15 min. The antioxidant capacities activated by steaming were still lower than those by microwaving (Table 1). For the boiling treatment, 'Muang Lek' and 'Muang Kan Dam' showed the highest antioxidant capacity at 10 min, whereas 'Muang Lot Fai' and 'Muang Kan Khiao' were highest at 15 min. These antioxidant levels were still lower than those by microwaving (Table 1). The results also showed that an increase in antioxidant capacities by steaming was higher than that of boiling method when the fruits were heated for 5, 10, or 15 min. It is worth mentioning that fruits cooked for 5–15 min by steaming and boiling were consumable, whereas fruits cooked by a microwave for 15 min were unpalatable according to Thai eating preferences.

The ABTS radical scavenging assay revealed that antioxidant capacities of eggplants heated by boiling, steaming or microwaving had the same trend as with

Table 1 Antioxidant capacity and total phenolic content after different heat treatments of four cultivars of eggplant fruits.

Cultivar/ heat treatment	Antioxidant capacity ($\mu\text{mol Trolox equivalent}/100 \text{ g fresh weight}$)								Total phenolic content (mg gallic acid equivalent/100 g fresh weight)			
	DPPH radical scavenging assay				ABTS radical scavenging assay							
	0 min	5 min	10 min	15 min	0 min	5 min	10 min	15 min	0 min	5 min	10 min	15 min
'Muang Lek'												
Boiling	55.2 ^d	89.3 ^c	160.1 ^a	133.7 ^b	8.8 ^d	14.9 ^c	25.7 ^a	22.8 ^b	78.3 ^d	113.0 ^c	166.2 ^a	145.6 ^b
Steaming	55.2 ^c	121.4 ^b	169.3 ^a	161.1 ^a	8.8 ^c	16.3 ^b	29.1 ^a	28.1 ^a	78.3 ^d	136.2 ^c	174.5 ^a	150.7 ^b
Microwaving	55.2 ^c	148.7 ^b	205.3 ^a	202.1 ^a	8.8 ^c	22.1 ^b	35.6 ^a	36.2 ^a	78.3 ^c	155.2 ^b	207.7 ^a	200.0 ^a
'Muang Lot Fai'												
Boiling	58.4 ^d	93.5 ^c	116.0 ^b	164.2 ^a	10.6 ^c	12.2 ^c	20.2 ^b	26.0 ^a	125.4 ^d	147.3 ^c	213.0 ^b	264.0 ^a
Steaming	58.4 ^d	120.3 ^c	152.7 ^b	202.3 ^a	10.6 ^c	15.2 ^b	27.4 ^a	30.3 ^a	125.4 ^d	201.1 ^c	248.3 ^b	334.3 ^a
Microwaving	58.4 ^c	185.6 ^b	266.3 ^a	269.1 ^a	10.6 ^c	25.3 ^b	40.5 ^a	41.1 ^a	125.4 ^c	298.9 ^b	386.4 ^a	389.4 ^a
'Muang Kan Dam'												
Boiling	44.1 ^d	63.4 ^c	121.4 ^a	95.3 ^b	10.0 ^b	13.1 ^b	19.4 ^a	17.4 ^a	98.8 ^c	108.0 ^c	174.4 ^a	140.0 ^b
Steaming	44.1 ^c	93.2 ^b	134.3 ^a	102.4 ^b	10.0 ^d	16.9 ^c	25.7 ^a	20.8 ^b	98.8 ^d	125.4 ^c	196.6 ^a	145.3 ^b
Microwaving	44.1 ^c	127.7 ^b	186.2 ^a	179.7 ^a	10.0 ^c	23.2 ^b	36.0 ^a	36.1 ^a	98.8 ^c	173.3 ^b	232.9 ^a	224.3 ^a
'Muang Kan Khiao'												
Boiling	49.2 ^c	54.2 ^c	73.7 ^b	102.2 ^a	7.1 ^c	8.7 ^{bc}	11.3 ^b	15.6 ^a	85.6 ^d	99.0 ^c	125.7 ^b	147.4 ^a
Steaming	49.2 ^d	63.0 ^c	98.1 ^b	121.3 ^a	7.1 ^c	12.4 ^b	14.5 ^b	22.2 ^a	85.6 ^d	115.3 ^c	156.8 ^b	188.3 ^a
Microwaving	49.2 ^c	114.3 ^b	229.6 ^a	233.6 ^a	7.1 ^c	20.4 ^b	32.2 ^a	33.5 ^a	85.6 ^c	165.7 ^b	286.1 ^a	285.3 ^a

Data are expressed as mean ($n = 6$). Values followed by different letters for each combination of cultivar and cooking method are significantly different ($P < 0.05$).

the DPPH radical scavenging assay. All three heating processes increased antioxidant capacities compared with raw fruits, and the fruits heated by microwaving had the highest antioxidant capacities compared with boiling and steaming (Table 1). Heating for 10 min in a microwave had the highest antioxidant capacity; after that the capacity plateaued (Table 1). 'Muang Lot Fai' had the highest antioxidant capacity (Table 1).

We found that antioxidant capacities of all eggplant cultivars determined by both DPPH and ABTS radical scavenging capacity assay significantly increased with microwaving, steaming and boiling (Table 1). These results are consistent with the work of Dewanto et al⁸, Turkmen et al⁹ and Francisco and Resurreccion²⁰ who reported that heat treatments enhanced antioxidant capacity in pepper, green bean, broccoli, spinach, sweet corn and peanut (*Arachis hypogaea*). The results also agree with Ng et al⁷ who found that boiling and microwaving significantly increased antioxidant capacities in water morning glory (*Ipomoea aquatica*) and bitter melon (*Momordica charantia*), respectively. Many antioxidant compounds in plants are mainly present as covalently bound forms with insoluble polymers²¹. It is possible that heat disrupts the cell wall and releases antioxidant compounds, leading to an increase in antioxidant capacity²¹.

It is well known that phenolic compounds are antioxidants¹⁻³ which were found in all parts of eggplant^{3,4}, and the content of phenolic compounds are changed during cooking^{8,9}. In our work, total phenolic content in eggplant cultivars significantly ($p < 0.05$) increased when the fruits were heated for 5, 10, or 15 min with either boiling, steaming, or microwaving compared with raw fruits (Table 1). Microwaving resulted in higher total phenolic content than steaming and boiling methods in all cultivars (Table 1). 'Muang Lot Fai' had the highest total phenolic content which was 1.9, 1.7, and 1.4 times higher than that of 'Muang Lek', 'Muang Kan Dam', and 'Muang Kan Khiao', respectively, at 10 min of heating. It is interesting to note that although antioxidant capacity and total phenolic content by microwaving for 10 or 15 min was not significantly different, we found that heating for 15 min destroyed the texture of fruit and gave an unpleasant shape of fruit (data not shown). For steaming, it was found that the highest total phenolic content for cultivars 'Muang Lek' and 'Muang Kan Dam' were 10 min, whereas the cultivars 'Muang Lot Fai' and 'Muang Kan Khiao' had the highest total phenolic content at 15 min (Table 1). For boiling, 'Muang Lek' and 'Muang Kan Dam' showed the highest total phenolic content when fruits were heated for 10 min, whereas 'Muang Lot Fai' and 'Muang

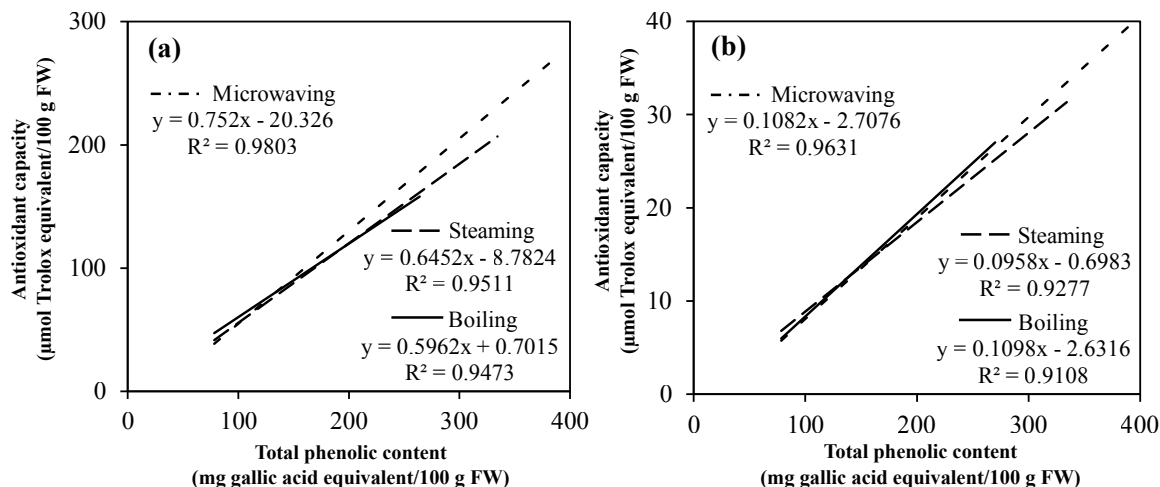


Fig. 2 Correlation between antioxidant capacity and total phenolic content of four cultivars of eggplant fruits using (a) the DPPH method or (b) the ABTS method.

Kan Khiao' had the highest at 15 min (Table 1). An increase in total phenolic content by steaming was higher than by boiling.

The result is in agreement with Dewanto et al⁸ and Turkmen et al⁹ who reported that total phenolic content of pepper, green bean, broccoli, spinach, and sweet corn increased after boiling, steaming, or microwaving. Our results also agree with the work of Ornelas-Paz et al¹⁰ who showed that total phenolic content in pungent peppers was increased by boiling and grilling. Similarly, our finding correlates with Ng et al⁷ who reported that boiling and microwaving significantly increased water-soluble phenolic content in water morning glory and bitter melon. Our results could be possibly explained that boiling, steaming and microwaving change bound phenolic compounds to be free forms and released into cytosol^{8,21}. Ju et al²² found that phenolic acids such as vanillic acid, protocatechuic acid, syringic acid, and 2,5-dihydroxyterephthalic acid in Chaga mushroom (*Inonotus obliquus*) significantly increased after steaming. These authors suggested that the liberation of phenolic compounds was enhanced by steaming.

A highly positive correlation of each heat treatment was found between antioxidant capacity by DPPH and ABTS radical scavenging assay and total phenolic content. For DPPH radical scavenging assay, microwaving had the highest correlation ($R^2 = 0.9803$), followed by steaming ($R^2 = 0.9511$), and boiling ($R^2 = 0.9473$), respectively (Fig. 2a). Similarly, microwaving had the highest correlation ($R^2 = 0.9631$), followed by steaming ($R^2 = 0.9277$), and boiling ($R^2 = 0.9108$), respectively, for ABTS

radical scavenging assay (Fig. 2b). This suggests that increasing amounts of phenolic compounds directly enhanced radical scavenging capacity²². The result is consistent with the work of Akanitapichat et al², Huang et al⁴, and Singh et al²³.

The different levels of antioxidant capacity in fruits and vegetables depends on plant cultivars and cooking methods^{2,4,7,23–25}. We found that microwaving caused the highest antioxidant capacity, followed by steaming, and boiling. The possible reason is that phenolic and antioxidant compounds activated by microwaving were retained in eggplant fruits, whereas cooking by boiling and steaming processes released them to water, as fruits heated with these two methods have direct and indirect contact with hot water, respectively. Our explanation is supported by comparing results between boiling and steaming methods in which steaming had higher total phenolic content than boiling (Table 1). In addition, heating fruits in a microwave oven increased temperature faster than other heating methods²⁵. This destroyed cell membranes of wild blueberries (*Vaccinium angustifolium*) and allowed antioxidants to be easily extracted from the fruits²⁶. Our result agrees with Chuah et al²⁴ who reported that cooking peppers by microwaving had more antioxidants than stir-frying and boiling. Microwaving for 10 min was the best method in enhancing antioxidant capacity, total phenolic content and having agreeable appearance for eating eggplant fruits, which was highest in 'Muang Lot Fai'.

In conclusion, our results showed that boiling, steaming and microwaving for 5–15 min increased total phenolic contents and antioxidant capacities in

all eggplant cultivars. 'Muang Lot Fai' had the highest antioxidant level. Microwaving for 10 min produced the highest level of total phenolic compounds and antioxidant capacity. Microwave cooking for 10 min is best to enhance the antioxidant capacity of eggplant fruits. Further studies are needed to identify and quantify phenolic composition of eggplant fruits after being heated.

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