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Total phenolic content and antioxidant capacities of instant mix spices cooking pastes

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Abstract

Available studies to determine the total phenolic content and antioxidant capacities of cooking pastes available in Malaysia were currently limited. This study aimed to evaluate the total phenolic content (TPC) and antioxidant capacity of seven types of mix spices cooking paste in raw and cooked condition (N=33). Samples were selected based on market availability. TPC was determined by Folin-Ciocalteu method and the antioxidant capacities were evaluated by DPPH free radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and ABTS free radical scavenging assay. Results were presented in dry weight (DW). TPC in the paste samples ranged from 246.25 to 370.57 mg GAE/100 g whereas antioxidant capacities ranged from 728.54 to 1267.66 µmol TE/100 g for DPPH assay, 1247.15 to 1886.89 µmol TE/100 g for FRAP assay and 833.19 to 1589.40 µmol TE/100 g for ABTS assay. Chicken, fish and vegetarian curries were top three samples with the highest TPC and antioxidant capacities values. Cooking process had caused increment in TPC and antioxidant capacities of all paste samples, with Rendang paste showed the greatest increase in TPC (21.48%) and antioxidant capacities (24.26%-49.66%) after cooking. Linear relationships were observed between TPC and DPPH antioxidant capacity (r=0.545), FRAP antioxidant capacity (r=0.840) and ABTS antioxidant capacity (r=0.623). A positive relationship between TPC and antioxidant capacities indicated that polyphenol is one of the sources of antioxidants in mix spices cooking paste. Further investigations on the active compounds in the cooking pastes are needed to determine the bioavailability and effect of these compounds in human.

Introduction

Spices are plant products which include bark, leaves, flowers, roots, seeds or whole plants. They are generally added to food and contribute to the flavour, taste and colour of a dish. These characteristics are associated with the phenolic compounds contained in the spices (Muchuweti *et al*., 2007). In addition, spices were reported as top five foods with the highest phytochemical content in the form of polyphenols (Perez-Jimenez *et al*., 2010). Spices have been widely used since long time ago for culinary, health benefits as well as preservatives, especially in Mediterranean countries and Asia. In India, spices have been used as traditional medicine, for example, turmeric for jaundice, basil to protect heart and ginger for relieving nausea and indigestion (Tapsell *et al*., 2006). It has been estimated that an adult in India consumed as much as 4 g of turmeric daily and 50 g of garlic in a week (Tapsell *et al*., 2006). In addition to their health benefits, herbs and spices can also be used in cooking to replace ingredients such as salt, sugar and saturated fats. Several countries have recommended the usage of herbs and spices as alternatives to salt for seasoning foods, thus, help to reduce sodium intake (US Department of Agriculture, 2010; National Heatlh and Medical Research Council, 2013).

Mix spices cooking paste is a type of ready-to-cook food containing combination of various spices and condiments that were blended and thermally processed to enhance their shelf life. As several metabolic diseases are associated with oxidative processes in the human body, the antioxidant properties of spices have attracted considerable attention among researchers and consumers. Numerous studies have documented the polyphenol content and antioxidant capacities of different spices (Zheng and Wang, 2001; Pellegrini *et al.*, 2006; Carlsen *et al*., 2010). Chili pepper, one of the commonly found ingredients in mix spices cooking paste has been reported to contain substantial amount of polyphenols (luteolin and quercetin) as well as antioxidants such as carotenoids and ascorbic acids (Howard *et al.,* 2000). Similarly, others common ingredients in the mix spices cooking paste such as onion, galangal, lemon grass and ginger has been shown to be important sources of polyphenols and antioxidants (Tangkanakul *et al*., 2009).

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Study on polyphenol content in mix spices cooking paste and sauces especially after thermal processing are still limited. However, some processing method such as heating or sterilization may influence the polyphenols and antioxidants content in the cooking paste. A number of studies show different findings on the effect of processing on the polyphenols and antioxidants content of food samples. Earlier study on Thai red curry paste has reported an increase of both parameters after the introduction of heat to samples tested (Inchuen *et al.*, 2011). Another study on several Asian foods had suggested retention of natural antioxidants after heat treatment (Tangkanakul *et al*., 2009). In contrast, a study on Thai hot curry paste has reported that thermal processing led to reduction in flavonoid and TPC of the paste samples (Settharaksa *et al*., 2012). Reduction in antioxidant capacity after cooking was also reported in a study conducted on marinating sauces (Thomas *et al.*, 2010).

To the best of our knowledge, literature on polyphenols and antioxidants content in instant mix spices cooking paste sold in Malaysia, as well as the effect of cooking on these parameters has been scarcely reported. Therefore, this study aimed to determine the total phenolic content (TPC) and antioxidant capacities of seven types of instant mix spices cooking paste in Malaysia. The effect of cooking on these two variables was also assessed. The method used in this research include Folin-Ciocalteau method, DPPH radical scavenging capacity, ferric-reducing antioxidant (FRAP) and ABTS radical scavenging capacity. The correlation between TPC and the antioxidant capacity assays were investigated.

Materials and Methods

Chemicals and reagents

Gallic acid, Folin-Ciocalteu reagent, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox), 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 4,6-Tris(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride hexahydrate were purchased from Sigma Aldrich, USA. Ethanol, sodium acetate, acetic acid and hydrochloric acid were of analytical quality grade.

Mix spices cooking paste samples

A total of seven types of mix spices cooking pastes were selected for this study, which includes chicken curry (n=7), fish curry (n=5), vegetarian curry (n=4), rendang (n=6), asam pedas (n=6), tom yam (n=4) and masak merah (n=1). All samples were ready-to-cook paste which can be used to prepare main dishes. Rendang is a spicy dish normally cooked with meat. Asam pedas consist mainly chilly and served with seafood or fish. Tom yam is a spicy and sour soup originated from Thailand and widely cooked in Malaysia. Masak merah is a popular chicken dish cooked in spicy tomato sauce. The samples were purchased from hypermarkets in Selangor, Malaysia. Samples were selected based on the market availability which were identified through market surveillance. Each sample was obtained from two different hypermarkets and was homogenized.

Sample preparation

The homogenized paste samples were divided into two portions, one portion was used for analysis of raw paste samples and the second portion was cooked under low heat (85-95ºC) with a wok in 200 ml water for 20 minutes, which was then allowed to cool to room temperature. This sample was used for analysis of cooked paste samples.

Sample extraction

Extraction was carried out according to method by Velioglu *et al.* (1998). Paste sample (2 g) was added with 50 ml of 70% ethanol and was incubated in water bath shaker (BS-06/11/21/31, Jeio Tech Co., Ltd., Korea) at 150 rpm, 70°C for 2 hours. The mixture was then filtered using Whatman No.1 filter paper. The filtrate, which is the sample extract, was stored at -33°C until analysis.

Determination of moisture content

Moisture contents of both raw and cooked paste samples were identified prior to the analysis to enable expression of results in dry weight (DW). Moisture content was determined by oven-drying method at 105°C. Wet samples were dried to a constant weight at 105°C temperature by using oven and were transferred to desiccators to cool. The moisture content was determined by a differential weighing before and after drying using calculation as follow:

$$\text{Moisture content (\%)} = \frac{\text{weight of wet sample (g)} - \text{weight of dried sample (g)} \times 100\%}{\text{weight of wet sample (g)}}$$

The moisture content was then used to enable expression of results (total polyphenol and antioxidant content) in dry weight (DW) using the following formula:

$$\text{TPC/ Antioxidant content in DW} = \frac{\text{TPC/ antioxidant content in fresh weight} \times 100\%}{100\% - \text{moisture content (\%)}}$$

Determination of total phenolic content

Total phenolic content (TPC) of the mix spices cooking paste extracts was determined using the Folin-Ciocalteu method as described by Wong *et al.* (2006). The extracts (100 µl) were added to 2.5 ml of Folin-Ciocalteu reagent (x10 dilution) and the mixture was allowed to react for 5 minutes before 2.5 ml of 7% sodium carbonate solution was added into the mixture. The mixture was allowed to stand at room temperature for 1 hour. The absorbance was measured at 765 nm with UV-visible spectrophotometer (Secomam Prim, France) using distilled water as blank. The results were expressed as mg gallic acid equivalents per 100 gram DW. Each analysis was conducted in three replicates.

Determination of DPPH radical scavenging capacity

The DPPH radical scavenging capacity of the extracts was measured according to the method described by Hung and Duy (2012). Sample extract (100 µl) was added with 3.9 ml of 0.1 mM ethanolic DPPH solution. The mixture was left to stand under subdued light for 30 minutes. Absorbance was measured at 525 nm with the UV-visible spectrophotometer (Secomam Prim, France) using ethanol (70%) as blank. The results were expressed as mg Trolox equivalents per 100 gram DW. Each analysis was conducted in three replicates.

Determination of ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power of the extracts was measured according to the method of Maizura *et al.* (2010). FRAP reagent was prepared by mixing 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ and 300 mM sodium acetate buffer (pH 3.6) in the ratio of 1:1:10. Sample extract (150 µl) was added to 4.5 ml of FRAP reagent and the mixture was incubated in 37ºC for 30 minutes. Absorbance was measured at 593 nm with the UV-visible spectrophotometer (Secomam Prim, France) using ethanol (70%) as blank. The results were expressed as mg Trolox equivalents per 100 gram DW. Each analysis was conducted in three replicates.

Determination of ABTS radical scavenging capacity

ABTS radical scavenging capacity was determined according to the method as described by Lu *et al*. (2011). $ABTS^+$ cation was produced through reaction between 7 mM ABTS stock solution with 2.45 mM potassium persulfate, and the mixture was stored in the dark for at least 12 hours before use. Prior to the analysis, $ABTS^+$ solution was diluted with 70% ethanol to get an absorbance of 0.700 ± 0.02 at 734 nm. Sample extract (100 µl) was mixed with 3.9 ml of the diluted $ABTS^+$ solution and was allowed to mix for 6 minutes. Absorbance was measured at 734 nm with the UV-visible spectrophotometer (Secomam Prim, France) using ethanol (70%) as blank. The results were expressed as mg Trolox equivalents per 100 gram DW. Each analysis was conducted in three replicates.

Statistical analysis

Statistical analysis of data was carried out using SPSS version 20.0. One way ANOVA was used to compare TPC and antioxidant capacities between different types of mix spices paste while Games-Howell post hoc test was used as follow up analysis. Paired-samples T test was used to identified the effect of heating on TPC and antioxidant capacities of the mix spices paste. Relationship between TPC and antioxidant capacities was determined using Pearson's correlation. The significant value was specified at $p<0.05$.

Results

Ingredients in paste samples

Analysis of ingredients based on food labelling showed that there were variations in the ingredients of paste samples between different brands. The main ingredients which indicated in the sequence of list of ingredients were also varied by brands. Generally, the main ingredients of all paste samples were onion, chilli, ginger garlic, turmeric, galangal, lemongrass and spices.

Total phenolic content and antioxidant capacities of mix spices cooking paste

Total phenolic content of mix spices cooking pastes are shown in Table 1. Differences in TPC value between raw and cooked samples range from 2.54 to 21.48%. TPC of the paste samples ranged from 246.25 to 370.57 mg GAE/100 g DW. Chicken and fish curry paste exhibited comparable high TPC with the value of 352.58 and 370.57 mg GAE/100 g DW respectively. Conversely, lower TPC were shown by rendang, asam pedas, tom yam and masak merah paste samples.

Antioxidant capacities of mix spices cooking pastes are shown in Table 2. Results from DPPH assay showed that the antioxidant capacity of the paste samples ranged from 728.54 to 1267.66 µmol TE/100 g DW. Amongst the paste samples, chicken curry, fish curry, vegetarian curry and masak merah showed comparable high value of 1125.98, 1267.66, 1149.45 and 1074.13 µmol TE/100 g DW respectively. On

Table 1. Total phenolic content (mg GAE/100 g) in raw and cooked paste samples

Paste samples	TPC (mg GAE/100 g)		D (%)
	Raw	Cooked	
Chicken Curry	352.58 ± 49.77[a]	386.36 ± 73.40*	9.58
Fish Curry	370.57 ± 69.74[a]	379.98 ± 72.81	2.54
Vegetarian Curry	311.03 ± 33.86[ab]	325.08 ± 36.21	4.52
Rendang	258.51 ± 81.60[b]	314.05 ± 120.01**	21.48
Asam Pedas	292.30 ± 42.99[b]	314.70 ± 72.23	7.66
Tom Yam	246.25 ± 75.61[b]	257.38 ± 94.42	4.52
Masak Merah	251.27 ± 18.31[b]	302.23 ± 13.17*	20.28

Different letters in the same column showed significant difference ($p<0.05$)
Significant differences between raw and cooked samples at $^{*}p<0.05$, $^{**}p<0.01$ with Paired-Samples T Test
D= Percentage difference (%) between raw and cooked samples

the other hand, the lowest DPPH radical scavenging capacity was demonstrated by tom yam paste with 728.54 µmol TE/100 g DW.

As for the FRAP assay, the antioxidant capacity of paste samples ranged from 1247.15 to 1886.89 µmol TE/100 g DW. Chicken curry paste and fish curry paste exhibited higher antioxidant capacity of 1886.89 and 1829.42 µmol TE/100 g DW respectively. In contrast, tom yam paste showed the lowest antioxidant capacity of 1247.15 µmol TE/100 g DW. Comparable low antioxidant capacity were also documented for rendang and asam pedas paste with 1286.63 and 1247.15 µmol TE/100 g DW respectively.

In terms of ABTS assay, the antioxidant capacity ranged from 833.19 to 1589.40 µmol TE/100 g DW. Comparable high antioxidant capacity were shown by chicken curry paste and fish curry paste with 1589.40 and 1464.66 µmol TE/100 g DW respectively. In contrast, asam pedas and tom yam paste showed the lowest antioxidant capacity.

Effect of cooking on the total phenolic content and antioxidant capacities of mix spices cooking paste

Comparison of the TPC and antioxidant capacities in raw and cooked paste is shown in Table 1 and Table 2. In general, TPC value of all paste samples has increased after the cooking process. However, only chicken curry, rendang and masak merah paste samples have showed significant increase ($p<0.05$) in TPC following cooking process. Among all paste samples, rendang paste exhibited the greatest increase in TPC after cooking, with an increment of 21.48%, followed by masak merah paste (20.28%) and chicken curry paste (9.58%). Similarly, all paste samples showed significant ($p<0.05$) increase in all three antioxidant capacities following cooking process. Among the samples, rendang paste showed the greatest increment in antioxidant capacities after cooking, with percentage difference of 49.36%, 24.26% and 49.66% for DPPH, FRAP and ABTS antioxidant capacity respectively.

Correlation between total phenolic content and antioxidant capacities

There are positive and significant relationships between TPC and antioxidant capacities of samples tested ($p<0.001$) (Table 3). Moderate relationships were found between TPC and DPPH ($r=0.545$) as well as TPC and ABTS ($r=0.623$). In contrast, a good relationship were observed between TPC and FRAP ($r=0.840$).

Discussion

Seven types of mix spices cooking pastes were analysed in this study, namely chicken curry, fish curry, vegetarian curry, rendang, tom yam, asam pedas and masak merah paste. Chicken, fish and vegetarian curries were top three samples with the highest TPC and antioxidant capacities values. The main ingredients in these pastes were onion and chilli pepper. Onions are rich in flavonoids, specifically flavonols which contributed to 68% of TPC in the onion bulb (Lachman *et al*., 2003). According to Phenol-Explorer®, one of the comprehensive database on polyphenol content in foods, the TPC of chilli and onion are 326.39 mg GAE/100 g and 102.83 mg/100 g fresh weight respectively (Neveu *et al*., 2010). In addition, luteolin and quercetin were identified as the two main polyphenol compounds found in chilli pepper (Howard *et al*., 2000). Previous study on Thai red curry paste has reported to contain TPC of 255 mg GAE/ 100 g DW, which was lower as compared to our studied curry paste samples (chicken curry, fish curry and vegetarian curry) (Inchuen *et al*., 2011).

Table 2. Antioxidant capacities (μmol TE/100 g) in raw and cooked paste samples

Paste samples	DPPH (μmol TE/100 g)		D (%)	FRAP (μmol TE/100 g)		D (%)	ABTS (μmol TE/100 g)		D (%)
	Raw	Cooked		Raw	Cooked		Raw	Cooked	
Chicken Curry	1125.98 ± 237.11^{a}	1474.12 ± 472.88***	30.92	1886.89 ± 352.78^{a}	2202.78 ± 546.13***	16.74	1589.40 ± 381.17^{a}	2227.40 ± 791.83***	40.14
Fish Curry	1267.66 ± 235.12^{a}	1612.19 ± 517.60***	27.18	1829.42 ± 146.82^{a}	2021.13 ± 99.87***	10.48	1464.66 ± 142.00^{a}	2027.63 ± 199.10***	38.44
Vegetarian Curry	1149.45 ± 262.72^{a}	1603.47 ± 540.58**	39.50	1525.24 ± 137.76^{b}	1731.15 ± 105.17**	13.50	1380.10 ± 253.21^{ab}	1899.52 ± 257.51***	37.64
Rendang	834.04 ± 213.02^{bc}	1245.72 ± 344.00***	49.36	1286.63 ± 361.77^{bc}	1598.81 ± 481.26***	24.26	1076.74 ± 301.67^{bc}	1611.41 ± 390.24***	49.66
Asam Pedas	1036.06 ± 214.72^{ab}	1340.04 ± 314.66***	29.34	1379.57 ± 188.54^{bc}	1587.29 ± 244.41**	15.06	883.21 ± 213.72^{c}	1125.82 ± 252.76***	27.47
Tom Yam	728.54 ± 231.62^{c}	952.01 ± 174.97***	30.68	1247.15 ± 344.00^{bc}	1387.33 ± 390.26***	11.24	833.19 ± 324.99^{c}	1014.09 ± 341.61**	21.71
Masak Merah	1074.13 ± 17.03^{a}	1313.83 ± 37.79**	22.31	1371.93 ± 12.20^{c}	1548.50 ± 32.51**	12.87	1298.28 ± 29.47^{b}	1674.53 ± 24.62**	28.98

Different letters in the same column showed significant difference ($p<0.05$)
Significant differences between raw and cooked samples at $^{**}p<0.01$ and $^{***}p<0.001$ with Paired-Samples T Test
D= Percentage difference (%) between raw and cooked samples

The difference may be due to the different ingredients composition and processing methods. In addition, the polyphenol content in fruits and vegetables may vary with different pre- and post-harvest processing operations (Tiwari and Cummins, 2013).

All three methods used to determine antioxidant capacity used in this research measure the antioxidant capacity of food samples according to different mechanism (Gülçin, 2012). Since antioxidant capacity of a food is contributed by different antioxidants with different mechanisms of actions, it was suggested that at least two antioxidant assay should be used to provide comprehensive information on the antioxidant capacity of the food samples (Pérez-Jiménez *et al.*, 2008). In general, antioxidant capacities was highest in fish curry paste (DPPH assay) and chicken curry paste (FRAP assay and ABTS assay).

The relationship between TPC and antioxidant capacities were confirmed by positive correlation between TPC and antioxidant capacities which demonstrated that polyphenols in the cooking paste are major contributor of antioxidant capacities in these pastes. This results was in agreement with several other studies that found positive relationship between TPC and antioxidant capacity in Thai red curry paste (Inchuen *et al.*, 2011), marinating sauces (Thomas *et al.*, 2010) and Thai sauces (Tangkanakul *et al.*, 2012). Polyphenols are the compounds which responsible for the antioxidant activities of plant foods (Scalbert *et al.*, 2005). Other compounds such as ascorbic acid, tocopherols and carotenoids may also contribute to the antioxidant capacities of foods (Gülçin, 2012). Furthermore, the antioxidant capacity of food samples not only depends on the antioxidant level, but also synergy occurring between the antioxidant compounds and other plant constituents (Zheng and Wang, 2001). For example, several compounds including the carotenoids, flavonoids, ascorbic acid, tocopherol and capsaicinoids contained in chilli was suggested as the contributor for its antioxidant capacity (Ayusuk *et al.*, 2009). In addition, onion was reported to possess high antioxidant capacities due to the present of flavonoid and sulphur compounds (Griffiths *et al.*, 2002). A local study has validated the positive association between TPC and antioxidant activities several ginger varieties (Ghasemzadeh *et al.*, 2010). Likewise, allicin compound present in garlic contributed to the antioxidant activity of this herb (Rahman *et al.*, 2012).

Since the cooking paste are generally consumed after cooking, it is important to determine the changes in polyphenol and antioxidant contents following cooking process. Previous studies have reported that cooking effect on TPC and antioxidant capacity of food samples may be negative, positive

Table 3. Pearson's correlation coefficient of total phenolic content and antioxidant capacity in paste samples

Parameters	Pearson's correlation coefficient , r
DPPH antioxidant capacity	0.545*
FRAP antioxidant capacity	0.840*
ABTS antioxidant capacity	0.623*

Significant correlation at *p<0.001 with Pearson's Correlation

or none at all (Ayusuk *et al*., 2009). In our study, it was observed that cooking process causes significant ($p<0.05$) increase in TPC of the three paste samples whereas all antioxidant capacities in all paste samples demonstrated significant ($p<0.05$) increase following cooking process. The increase in TPC and antioxidant capacities of these pastes may be due to presence of onion as main ingredient. Woo *et al.* (2007) has reported that total phenolic content, flavonoid content and antioxidant capacity of onion were increased with increase in temperature and heating duration. According to Rodrigues *et al*. (2009), flavonoid is a polyphenol compound found in onion which is stable towards heat degradation. Similar results were reported by Inchuen *et al.* (2011) on Thai red curry paste samples, which showed significant increase in TPC and antioxidant capacities (DPPH and FRAP) after heat treatment. The author suggested that the increase in total phenolic content following heating may be due to increased extractability of polyphenol compounds due to cell wall disruption by heat, which enable the release of certain polyphenol compounds from the raw food samples.

The increase in antioxidant capacities indicated that the formation of new antioxidant compounds after cooking is greater than the degradation of antioxidant compounds by heat. Increase in antioxidant capacities after heat treatment can be explained by the destruction of cell walls and subcellular compartments which liberate antioxidant compounds, production of new antioxidants by thermal reaction and deactivation of certain oxidative enzymes by heat (Yamaguchi *et al*., 2001). Likewise, Tomaino *et al.* (2005) suggested that thermal oxidative degradation of certain antioxidants may be prevented by other antioxidant present in the food sample.

Conclusion

Findings in this study showed that all mix spices cooking paste samples contained substantial amount of polyphenols and antioxidants. Cooking process retained or recovered polyphenol and antioxidant contents in the cooking paste samples, indicating that mix spices cooking paste may be one of the source of polyphenols and antioxidants in Malaysian diet. A positive relationship between total phenolic content and antioxidant capacities indicated that polyphenol is one of the sources of antioxidants in mix spices cooking paste. Further investigations on the active compounds in the cooking paste are needed to determine the bioavailability after consumption and the effect of these compounds for human health.

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