

## Fatty acid profile, phenolic compounds and potential antioxidant activity of fresh and cooked Massaman curry paste

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**Submission Date:** August 1<sup>st</sup>, 2019. **Acceptance Date:** August 28<sup>th</sup>, 2019. **Publication Date:** July 31<sup>st</sup>, 2019.

**Citation:** Dat-arun P., Chakree K., Settaraksa S. Fatty acid profile, phenolic compounds and potential antioxidant activity of fresh and cooked Massaman curry paste. *Functional Foods in Health and Disease* 2019; 9(8): 543-557. DOI: <https://doi.org/10.31989/ffhd.v9i8.644>

### ABSTRACT

**Background:** The fresh herbs and spices used in Thai food are what make Thai cuisine unique. Some of these herbs and spices have been scientifically studied for health benefits. However, there is limited evidence for cooked food.

**Objective:** To determine proximate composition, fatty acid profile, phenolic and flavonoid contents and antioxidant activity of fresh Massaman curry paste (MCP) and cooked Massaman curry including beef and chicken Massaman curry (BMC and CMC, respectively).

**Methods:** The chemical composition and fatty acid profile of freeze-dried samples were analyzed. Food samples were extracted by three solvents: water, absolute methanol or ethanol. The total phenolic and flavonoid contents (TPC, TFC, respectively) of the extracts were measured spectrophotometrically and identified through high performance liquid chromatography (HPLC). The antioxidant activities of extracts were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and ferric reducing antioxidant power (FRAP) assays.

**Results:** Carbohydrate, protein, and fat were the main components of all sample studies. Chicken Massaman curry (CMC) contained highest fat and energy contents. Fatty acid profile of CMC showed high content of lauric acid (9460 mg/100 g), oleic acid (6050 mg/100 g) and palmitic acid (4220 mg/100 g). MCP showed high content of linoleic acid (213.70 mg/100 g) and palmitic acid (195.71 mg/100g). Methanol extract of MCP showed the highest content of TPC (1.076±0.013 mg gallic acid equivalent (GAE)/100g crude extract), TFC (27.10±0.31 mg CE/ 100g crude extract) and antioxidant activity by FRAP assay (0.311 ± 0.002 mg

Trolox/100g crude extract). While ethanol extract of MCP showed the highest activity of DPPH at value as  $11.81 \pm 0.06$  mg GAE/100 g crude extract, following by methanol extract of MCP ( $11.02 \pm 0.05$  mg GAE/100 g crude extract). Cooked Massaman curry including BMC and CMC showed low content of TPC and TFC and antioxidant than fresh MCP. Ferulic acid was the main phenolic acid in these samples which was the highest in methanol extract, followed by ethanol and then water extraction. After cooking process, some activities of food were altered.

**Conclusion:** The results of this study suggest that fresh Massaman curry paste and cooked Massaman curry in ready-to-eat foods could potentially show antioxidant activity. It provided that the health benefits from plants source still remained in the products after cooking process.

**Keywords:** Thai food, Massaman curry, Antioxidant activity, Fatty acid profile, Phenolic compounds

## BACKGROUND

The famous reputation of Thai food about the flavor and meticulous preparation is well known internationally for over decade. Thai dishes were valued not only the taste but also its health benefits, using the folk knowledge to create healthy and delicious food [1]. About Thai curry such as Tom-Yum, Red or Massaman curry and Green curry has gained popularity dishes [2]. Especially, Massaman curry, was recognized to be the number one most delicious food in the world, based on 50 popular food surveys by Cable News Network [3].

The major ingredients of Massaman curry paste including garlic, shallot, guajillo chili, lemon grass, galangal, coriander seed, cinnamon, kaffir lime peel and shrimp paste. Garlic and shallot have been known as a grate antibacterial [4], antidiabetic, hypocholesterolemic, antifungal, antiparasitic, antiviral activities and cancer preventive agent [5, 6]. Moreover, other ingredients are effective in several activities such as guajillo chili: antioxidants, anti-inflammatory, antidiabetic, antihypertensive, anti-mutagenic and chemo preventive properties [7]; galangal: antiallergic, antidermatophytic, antimicrobial, antiviral, anticancer, anti-inflammatory, hypolipidaemic, immune-stimulating activities, gastroprotective and cardiovascular and related effect [8]. Coriander seed or *Coriandrum sativum* L., its main bioactive is essential oil, fatty acids, tocol, sterol and carotenoids and its biological activities is antimicrobial and antioxidant [9]. Cinnamon, this spice has been found to have strong antioxidant, antibacterial, antipyretic and anti-inflammatory properties and to use for the treatment of cardiovascular diseases, chronic gastrointestinal and gynecological disorders in oriental herbal medicine [10]. For essential oil, from lemon grass, it is mainly comprised of citral which exhibited a broad antifungal property [2].

Currently there is some research about the health properties of curry paste such as yellow curry, red curry and green curry. But there is no data about the health effect of the Massaman curry which has different components from other curry and may result in different health effects. Therefore, the researchers were interested in studying the effect of bioactive compounds extracts, including the amount and types of fat composition, from fresh and cooked Massaman curry on health properties. But not only this information will be scientific evidence of the health benefits of popular Thai food, they also can be the needs of consumers who are aware of health, especially for global consumers and also are helpful in publicize

Thai healthy food which should be useful in terms of international marketing. The aims of this study was to determine the chemical composition, fatty acids profile, antioxidant activity and identify some types of bioactive in fresh and cooked Massaman curry extracts.

## MATERIALS AND METHODS

### *Raw materials*

Massaman curry paste consists of 4.88% coriander seed (*Coriandrum sativum*), 2.09% caraway (*Ocimum gratissimum* L.), 2.09% cloves (*Syzygium aromaticum* L. Merrill and Perry), 2.09% cardamom seed (*Amomum testaceum* Ridl), 3.48% cinnamon (*Cinnamomum bejolghota* Sweet), 1.39% mace (*Myristica fragrans* Houtt.), 13.94% guajillo chili (*Capsicum annuum*), 1.74% salt (sodium chloride), 2.09% white pepper (*Piper nigrum*), 6.97% galangal (*Alpinia galangal* Linn), 10.45% lemon grass (*Cymbopogon citratus*), 3.48% kaffir lime peel (*Citrus hystrix* DC), 27.87% garlic (*Allium sativum*), 13.94% shallot (*Allium ascalonicum*), and 3.48% shrimp paste was collected from local market in Hat-Yai, Songkhla, Thailand.

Generally, beef or chicken is used in the preparation of Massaman curry, so two type of Massaman curry were prepared included beef and chicken Massaman curries. These were composed of 500 g beef or chicken meat, 200 g of Massaman curry paste, 6 cups of coconut milk, 300 g of potatoes, 200 g of onion, 50 g of roasted peanuts, 10 g of roasted Siam cardamoms, 8 table spoon of tamarind juice, 100 g of palm sugar and 20 g of sodium chloride.

### *Sample preparation*

The preparation of curry was conducted by adapting the process used by Royal Thai Cuisine (2006) [11]. To prepare the Massaman curry, herbs and spices (coriander seed, caraway, cloves, cardamom seed, cinnamon stick and mace) were individually roasted in a pan over low heat until fragrant about 15 min then were finely ground with a blender machine. Chilies and salt were added followed by the addition of white pepper, galangal, lemongrass, kaffir lime peel, garlics, and shallots into a fine paste. Finally, shrimp paste and the ground spice were added properly. The curry was kept at -20 °C for further study. To make the curry, beef, chicken, and other ingredients including onion, tamarind juice, potato, and peanut were prepared. The food recipe was obtained from Thai cooking specialists. One kg of curry paste and ready-to-eat prepared food were blended (Panasonic Blender MX 151 SP, Thailand) and freeze-dried under the condition as followed; 0.055 mbar, 12 h, at -40 °C (Dura Dry, Dura Freeze Dryer, Canada). The dry food was milled to a powder at 3s (Super Blender, AIKO, China), then the powder was stored in plastic bottles at -20 °C until used.

### *Proximate composition analysis*

The proximate analysis of food was determined according to AOAC [12] procedures and included the following: ash (AOAC method 920.153); moisture (AOAC method 925.45); protein (AOAC method 981.10); fat (AOAC method 948.15). However, carbohydrate was calculated.

### *Fatty acids composition*

Fatty acids from Massaman curry paste, beef Massaman curry, and chicken Massaman curry were detected as their methyl esters (FAME's) according to Department of Medical Science

and National Bureau of Agricultural Commodity and Food Standards [13]. Separation of FAME was operated by gas chromatography (Hewlett Packard 6850A) equipped with capillary column, flexible fused silica, 25 m and 0.2-0.35 mm i.d. A flame-ionization detector was used and helium was used as a carrier gas. Results were expressed as fatty acids composition per 100 g food.

### ***Sample extraction***

The extraction was conducted by adapting the method used by Do *et al* [14]. In brief, samples were extracted by three solvents: 100% methanol, 100% ethanol and water at room temperature. Extracting ratio of sample to solvent was 1:6. The mixture was homogenized and shaken at 1000 rpm/min for 1 h. The supernatant was filtered through filter paper, and then evaporated by rotary evaporator, freeze-dried and preserved in brown bottles at -20 °C for further analysis.

### ***Determination of total phenolic content (TPC)***

Total phenolic content (TPC) was determined using Folin-Catiocalteau method adapted from Slinkard and Singleton [15]. Briefly, the food extracts were diluted with distilled water at concentrations of 0.1, 0.5, 1, 2, 3, 4, 5 mg/ml. A volume of 12.5 µl of the extract from all concentrations and standard solutions (containing 5, 10, 25, 50, 100, 150, and 200 µg/ml of gallic acid in distilled water) were individually subjected into 96-well plate, 12.5 µl of Folin Catechau reagent was added to each well and left for 6 min. Afterwards, 125 µl of Na<sub>2</sub>CO<sub>3</sub> solution (7% w/v in distilled water) and 100 µl of distilled water were added. The mixture was incubated for 90 min in the darkness and the absorbance was measured at 760 nm using the microplate reader (Bio-TeK PowerWave XS, USA). The results were expressed as mg of gallic acid equivalents (GAE) per 100 g dry weight.

### ***Determination of total flavonoid contents (TFC)***

Total flavonoid content (TFC) was measured by AlCl<sub>3</sub> assay using catechin as reference compound [16]. Briefly, 125 µl of the extract and 75 µl of 5% NaNO<sub>2</sub> were mixed in 96-well plate. The mixture solution was allowed to stand for 6 min at ambient temperature and then 150 µl of 10% AlCl<sub>3</sub> was added. After 5 min, 750 µl of NaOH (1M) was added and incubated for 5 min again. The final volume of the mixture was adjusted to 2500 µl with distilled water. The mixture was allowed to stand for 15 min and absorbance was determined at 510 nm. The TFC results were calibrated with a standard curve of Catechin and expressed as Catechin Equivalent (mg CE per 100g dry crude extract).

### **Antioxidant activities**

#### ***DPPH radical scavenging activity assay***

DPPH (2,2-Diphenyl-1-picryl hydrazyl) radical scavenging activity was measured using the modified method of Bran-Williams *et al* [17]. One hundred µl of the extraction sample and gallic acid standard solutions (2-10 µg/ml) were individually placed in a 96-well microtiter plate. Then 100 µl of DPPH solution (0.2 mM in ethanol) was added. The mixture was left at

room temperature for 30 min in darkness and the absorbance was measured at 517 nm using the microplate reader (BioTeK PowerWave XS, USA). The activity was reported as mg Catechin equivalent (mg CE per 100 g dry crude extract).

#### ***Ferric Reducing Antioxidant Power (FRAP)***

The FRAP assay was determined according to the method of Benzie and Strain [18]. The FRAP solution was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O. TPTZ solution (2.5 ml), and FeCl<sub>3</sub>. 6H<sub>2</sub>O solution (2.5 ml) were then mixed and incubated at 37°C for 30 min in a warm water bath prior to use (FRAP solution). The food extract samples were diluted with distilled water to 10, 15, 20, 25, 30, 35, 40 mg/ml. Thirty µl each of these diluted food extracts and thirty µl of Trolox standard solutions (5, 10, 15, 20, 25, 30 mg/ml) in ethanol were placed in a 96-well micro-titer plate. Then FRAP solution (270 µl) was added to both food extracts and standard wells, kept for 30 min in darkness, and assayed in triplicate. In this reaction, ferric-tripyridyltriazine (Fe<sup>3+</sup>-TPTZ) complex is reduced from ferric (yellow) to ferrous (blue) form. Development of ferrous tripyridyltriazine complex was measured by absorbance at 595 nm using a microplate reader (Microplate reader, Biotek, Power wave X, Winooski, USA). The results were expressed as mg Trolox per 100g dry crude extract.

#### ***Identification of phenolic compounds by High Performance Liquid Chromatography (HPLC)***

Phenolic acid standards were consisted of gallic acid, protocatechuic acid,  $\rho$ -coumaric acid, chlorogenic acid, vanillic acid, catechin, ferulic, rutin, cinnamic, quercetin, caffeic acid and syringic acid. The standard solutions were performed by dissolving 0.01 g of each in 10 ml distilled water. Working solution was made to 20-200 µg/ml. Massaman curry paste, beef Massaman curry and chicken Massaman curry crude extracts (0.02 g) were solubilized in 2 ml water. The standard and sample solutions were filtered through 0.2 µm Nylon-syringe filter and the mobile phase were degassed before the injection of the solutions.

#### ***HPLC condition***

HPLC analysis was carried out using an Agilent 1200 series (Agilent 1200, Waldbronn, Germany). The separation was carried out using Eclipse XDB-C18 (250 mm × 4.6 mm i.d., 5 µm, Agilent, Santa Clara, CA, USA). The mobile phase consisted of 1% formic acid in water (A) and methanol (B) at a flow rate 0.8 ml/min. The mobile phase was performed consecutively in a linear gradient as follows: 0 min (100% A); 0-10 min (90% A); 10-20 min (75% A); 20-30 min (40% A); 30-40 min (30% A). The wavelengths were detected at 254, 280 and 325 nm. The column temperature was controlled at 25°C and the injection volume was kept at 10 µl for each of the sample solutions.

#### ***Statistical analysis***

All analyses were carried out in triplicates and reported as means ± standard deviation. Data were analyzed by analysis of variance (ANOVA) and statistical significance with Duncan's multiple range test (P < 0.05) using SPSS (version 14.0).

## RESULTS AND DISCUSSION

### *Proximate analysis*

The results of proximate analysis of Massamun curry paste (MCP), beef Massaman curry (BMC) and chicken Massaman curry (CMC) are presented in Table 1. It is noticeable that the highest amount of ash, moisture and carbohydrate belonged to MCP showed values of 10.10 g/100 g, 7.91 g/100 g, and 63.32 g/100 g. While fat and energy in the crude extract were the highest in CMC which recorded values of 31.93 g/100 g and 534.57 g/100 g, respectively. However, protein content was the highest in BMC and recorded 38.84 g/100 g. Moreover, beef and chicken Massaman curry shared a similar pattern in most approximate analysis.

From the proximate analysis conducted, carbohydrate content in the samples was calculated using the formula [100%-moisture-ash-fat-protein]. Carbohydrate content was the highest in MCP (63.32%), followed by CMC (50.39%) and BMC (42.17%). Carbohydrate is also known as monosaccharides, disaccharide, and polysaccharides. However, polysaccharides are the main type of carbohydrate in this study which included digested and non-digested (dietary fiber) polysaccharide by human enzymes. The results showed that mixture of herbs and spices showed the highest content of digested and non-digested polysaccharide. While a high content of carbohydrate was found in curry paste other researchers found that some of Thai curry paste such as Thai red curry paste significantly reduces blood glucose, serum malonaldehyde in diabetic group of rats received curry paste compared to the control [19]. Moreover, when mixed curry pastes with other protein source in whole dish such as MCP and CMC, the macronutrient including protein, carbohydrate, and fats content was appropriate for human requirement [20].

**Table 1** Proximate composition of different Massaman Curry per 100 g edible portion.

Parameters (g/100 g)	MCP	BMC	CMC
Ash (g)	10.10	6.25	5.75
Moisture (g)	7.91	0.01	0.57
Protein (g)	17.94	38.08	11.32
Crude fat (g)	0.73	13.49	31.97
Carbohydrate (g)	63.32	42.17	50.39
Energy (Kcal)	327.96	442.41	534.57

MCP; Massaman curry paste, BMC; beef Massaman curry; CMC; chicken Massaman curry

### *Fatty acid compositions*

Massaman curry is the most delicious Thai food in the world, surveyed by Cable News Network [3]. They contained plenty of herbs and spices and high contents of coconut milk. Coconut milk is an important ingredient of Thai curry. It is an oil-in-water emulsion pressed from the solid endosperm of coconut [21]. Coconut milk is serves as a natural solvent during

food preparation by extracting both polar and non-polar compounds from various herbs and spices in Thai curry paste [22]. However, health conscious people are discouraged from consuming coconut milk due to its higher content of high saturated fatty acid. The amount of fatty acid composition in MCP, BMC, and CMC is presented in Table 2.

**Table 2.** Fatty acid composition of different Massaman curry by gas chromatography (GC, g/100 g)

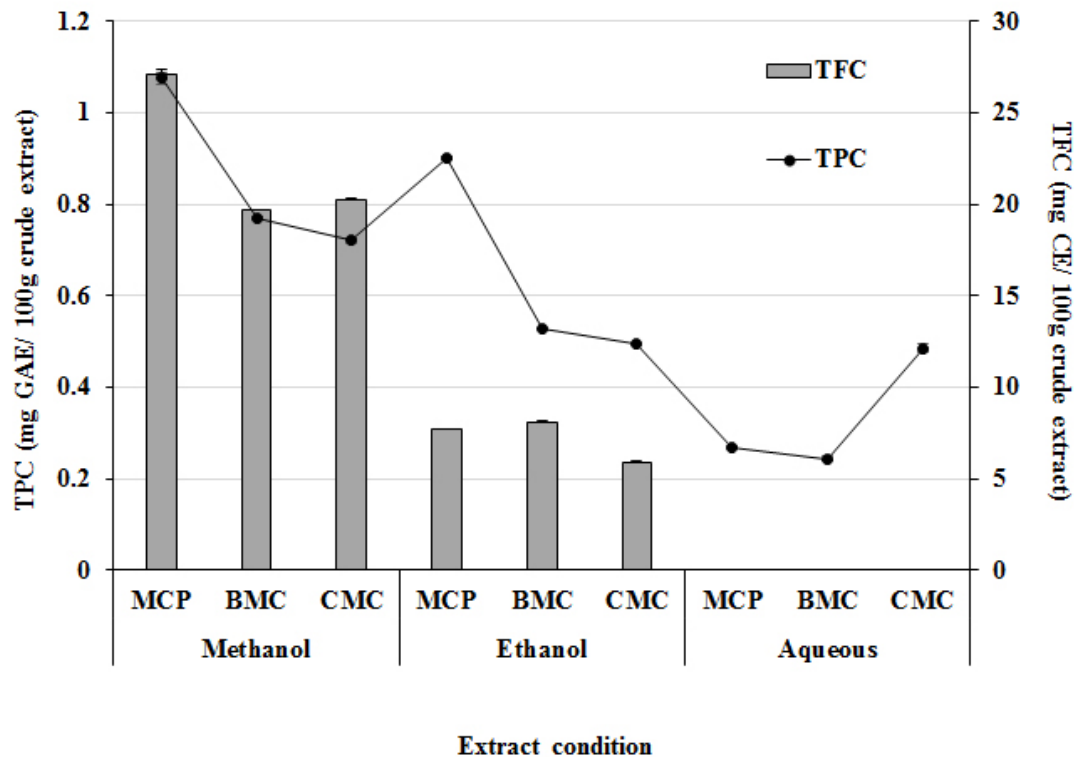
Fatty acid composition	MCP	BMC (mg/100 g)	CMC
Butyric acid (C4:0)	0.00	0.00	0.00
Caproic acid (C6:0)	0.00	30.00	70.00
Caprylic acid (C8:0)	0.00	530.00	1250.00
Capric acid (C10)	0.00	510.00	1160.00
Undecanoic acid (C11:0)	0.00	0.00	0.00
Lauric acid (C12:0)	0.00	4180.00	9460.00
Tridecanoic acid (C13:0)	0.00	0.00	0.00
Myristic acid (C14:0)	19.75	1260.00	2920.00
Myristoleic acid (C14:1)	0.00	0.00	0.00
Pentadecanoic acid (C15:0)	0.00	0.00	0.00
Cis-10-Pentadecanoic acid (C15:1n10)	0.00	0.00	0.00
Palmitic acid (C16:0)	195.71	1440.00	4220.00
Palmitoleic acid (C16:1n7)	0.00	0.00	460.00
Heptadecanoic acid (C17:0)	0.00	0.00	0.00
Cis-10- Heptadecanoic acid (C17:1n10)	0.00	0.00	0.00
Stearic acid (C18:0)	27.45	600.00	1180.00
Elaidic acid (C18:1n9t)	0.00	0.00	0.00
Oleic acid (C18:1n9c)	172.70	2250.00	6050.00
Linolelaidic acid (C18:2n6t)	0.00	0.00	0.00
Linoleic acid (C18:2n6)	213.70	1570.00	2540.00
Gamma-Linolenic acid (C18:3n6)	0.00	0.00	0.00
Alpha Linolenic acid (C18:3n3)	59.51	0.00	0.00
Arachidic acid (C20:0)	0.00	0.00	0.00
cis-11-Eicosenoic acid (C20:2n6)	0.00	0.00	0.00
cis-11,14-Eicosadienoic acid (C20:2n6)	0.00	0.00	0.00
Heneicosanoic acid (C21:0)	0.00	0.00	0.00
Cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.00	0.00	0.00
Arachidonic acid (C20:4n6)	0.00	0.00	0.00
Cis-11,14,17-Eicosatrienoic acid (C20:3n3)	0.00	0.00	0.00
Behenic acid (C22:0)	0.00	0.00	0.00
cis-5,8,11,14,17-Eicosapentaenoic acid; EPA (C20:5n3)	0.00	0.00	0.00
Erucic acid (C22:1n9)	0.00	0.00	0.00
Cis-13,16-Docosadienoic acid (C22:2)	0.00	0.00	0.00
Tricosanoic acid (C23:0)	0.00	0.00	0.00

The MCP, BMC, and CMC recorded fatty acid composition at values ranged from 19.75 to 213.70, 30.00 to 4180.00 and 1160 to 9460.00 mg/100 g, respectively. The data also revealed that palmitic acid, oleic acid, linoleic acid, stearic acid, alpha linolenic acid and

myristic acid were found in MCP, BMC and CMP, which are the major fatty acids observed in all the samples as reported in most plant. However, high content of palmitic acid was found in MCP, this type of fat was decreasing in cooked Massaman. Furthermore, oleic acid can reduce blood pressure, increase fat burning and may prevent type 2 diabetes [23]. While linoleic acid can be used to reduce blood cholesterol and lower risk of coronary heart disease [24]. Alpha-linolenic acid was found only in MCP because it is rich in vegetable oil, seeds, nuts and some vegetables which are known to be the dietary sources of alpha-linolenic acid [25]. Linoleic acid and alpha-linolenic acid are known as essential fatty acids for humans, which the human body cannot synthesize on its own [26]. Lauric acid was found only in BMC and CMC due to their content of coconut milk. Perera *et al* report that dominant saturated fatty acid in coconut milk is lauric acid and it accounts for half of the total saturated fatty acids [27]. Moreover, stearic acid was the most in CMC (1180 mg/100 g) of fatty acid in sample, same as found in *Capsicum* spp [28]. This fatty acid might be taken from *Capsicum annum*. The current results showed that the major fatty acid in MCP were linoleic, palmitic, oleic, alpha-linolenic acid, stearic, and myristic acids.

**Total phenolic and total flavonoid contents**

The results of total phenolic contents (TPC) and total flavonoid contents (TFC) of Massaman curry paste (MCP) and ready-to-eat, Massaman curry including beef Massaman Curry (BMC) and chicken Massaman Curry (CMC) extracted by different solvents (methanol, ethanol and water) are presented in Figure 1.



**Figure 1.** TPC and TFC of different Thai Massaman curry using methanol, ethanol and aqueous extraction. All experiments were performed in triplicate. GAE = gallic acid equivalent, CE= catechin equivalent, MCP = Massaman Curry Paste, BMC = Beef Massaman Curry and CMC = Chicken Massaman Curry.



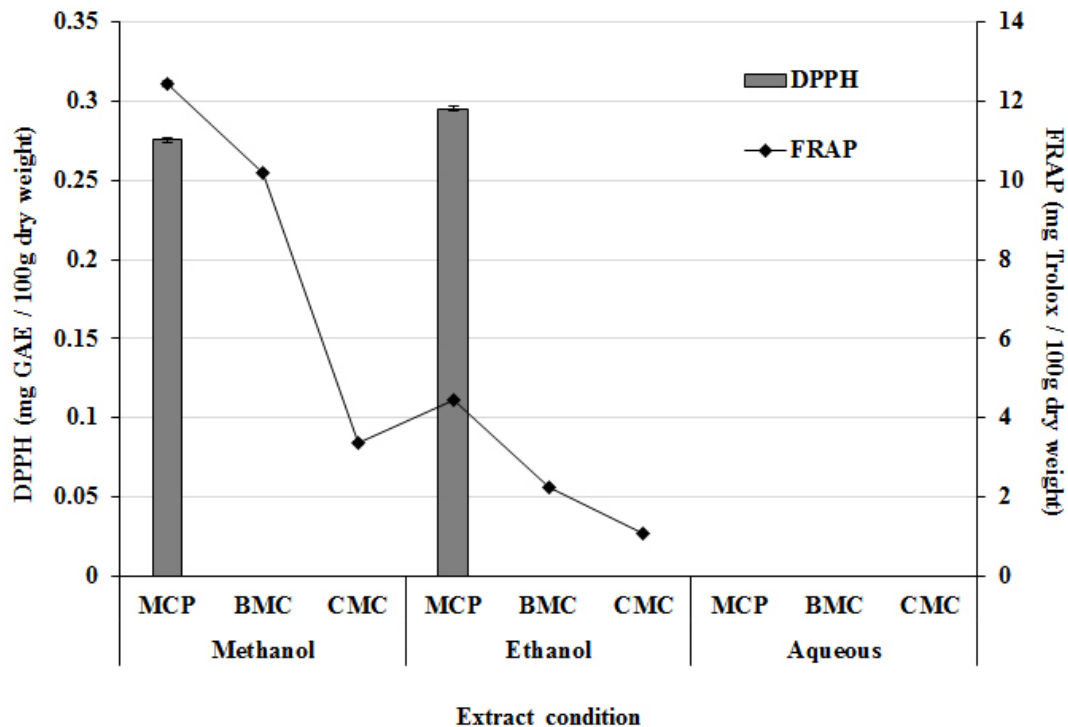
The TPC of various solvent extracts distinctly varied and ranged from  $0.244 \pm 0.005$  to  $1.076 \pm 0.013$  mg GAE/100 g crude extract. The TPC in MCP, BMC, and CMC extracted with methanol, ethanol, and water showed values ranged from  $0.721 \pm 0.005$  to  $1.076 \pm 0.0131$ ,  $0.494 \pm 0.00$  to  $0.902 \pm 0.003$  and  $0.244 \pm 0.005$  to  $0.486 \pm 0.008$  mg GAE/100 g crude extract respectively. Methanol solvent could result in the highest amount of TPC, followed by ethanol then water. According to the results of TPC for each sample, it was found that the methanol extract of MCP contained consistently the highest of TPC ( $1.076 \pm 0.013$  mg GAE/100 g crude extract), followed by those in ethanol ( $0.902 \pm 0.003$  mg GAE/100 g crude extract). For cooked samples, BMC and CMC also showed the highest TPC in methanol extracted compared with ethanol and aqueous extracted. However, it was found that the highest TPC in aqueous solutions was found in CMP, followed by MCP and BMC. Total flavonoid contents (TFC) were ranged from  $19.70 \pm 0.03$  to  $27.10 \pm 0.31$  mg GAE/100g and  $5.88 \pm 0.09$  to  $7.71 \pm 0.05$  mg GAE/100g in methanol and ethanol, respectively. The highest value of TFC was recorded in methanol extract, followed by ethanol. It was observed that TFC in the methanol extract shown to be more than two times when comparing with the ethanol extracts. Unfortunately, TFC could not detect in the aqueous extracted.

The efficiency of bioactive compounds extraction has been reported to be affected by several factors, including the chemical nature of phytochemicals, the extraction method used, the sample particle size, the solvent used, as well as the presence of interfering substances [29]. Under the same extraction temperature and time, solvent and composition of the sample are known as the most important parameters [30]. In the current work, fresh MCP and ready-to-eat Massaman curry including BMC and CMC were extracted by using methanol, ethanol and water. The effect of solvents on TPC was observed to be similar to that on TFC. The highest TPC and TFC were found in methanol, followed by those in ethanol extract, while the TPC and TFC of the water extracts is significantly less than that of other solvents ( $p < 0.05$ ). The difference observed in the phenolic content of these extracts can be attributed to the nature of the biochemical structure of the extracted solvent and the chemical properties of the phenolic compounds available [31]. It may also be caused by the possible complex formation of some phenolic compounds in the extract that are soluble in methanol and ethanol. These phenolic compounds may possess more phenol groups or have higher molecular weights than the phenolics in the water extract [30]. The results of this study are in agreement with Settharaksa *et al* [32] and Turkmen *et al* [33] who found that the content of phenolic and flavonoid varied in different solvents. These authors also reported that solvents with different polarity (ethanol and water) have played an important role on polyphenol content. This observation is also in line with those reported by Sepahpour *et al* [34] who showed that the phenolic content of turmeric extracted with 80% acetone, 80% ethanol, 80% methanol and cold water were respectively 221.7, 172.1, 90.1 and 3.8 mg GAE/g of freeze-dried crude extract. Base on the results of TPC and TFC, the best extracting solvent was methanol.

### ***Antioxidant activity***

Antioxidant activity in methanol, ethanol, and aqueous extract from MCP, BMC, and CMC as determined by DPPH radical scavenging and Ferric Reducing Antioxidant Power (FRAP) were shown in Figure 2. DPPH radical scavenging activity was found only in methanol and ethanol extraction in MCP. Ethanol extract of MCP was higher than methanol extraction and

the recorded values were  $11.81 \pm 0.06$  and  $11.02 \pm 0.05$  mg GAE/ 100 g dry crude extract, respectively. However, the radical scavenging properties in BMC and CMC using the three solvents extraction (methanol, ethanol and water) were not detected. The results of Ferric reducing antioxidant power (FRAP), one of the assays used to determine the antioxidant activity are presented in Figure 2. These results showed that FRAP value were higher in methanol extracted samples compared to ethanol extraction for all of three food samples with an undetectable FRAP activity in water extraction. This indicated that methanol extraction was more capability in extracting antioxidant in MCP, BMC, and CMC compared to ethanol and water.



**Figure 2.** Antioxidant activity by DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) of different Thai Massaman curry.

The fact, organic solvents normally exhibit good antioxidant capacity than aqueous solvent has already been reported by Array *et al* (2007) who demonstrated the antioxidant activity by methanolic and ethanolic extracts of turmeric was significantly higher than that of the aqueous extracts [31]. In this study, MCP extract observed by using a pure organic solvent (methanol and ethanol) gave stronger radical scavenging ability than that of water extract. Nevertheless, aqueous extract both of MCP and CMC undetectable of antioxidant activity using DPPH radical scavenging and FRAP assay. The TFC also not detectable when all samples were extracted by aqueous solution. This might be the nature of active compounds in that samples not be appropriated to dissolve in extraction method of this study.

Antioxidant activity is not necessarily in the same order as TPC or TFC indicating that high TPC or TFC do not always mean high antioxidant capacity and vice versa. However, the current study revealed that the highest antioxidant activity of MCP in DPPH radical scavenging and FRAP assay were highest in methanol and ethanol extracts. In addition to radical scavenging, polyphenols are also known as metal chelators. Chelation of transition

metals such as  $\text{Fe}^{2+}$  can directly reduce the rate of Fenton reaction, thus preventing oxidation caused by highly reactive hydroxyl radicals [35, 36]. Polyphenols do not act alone, it has been found that polyphenols can actually function as a co-antioxidant, and are involved in the regeneration of essential vitamins [37]. Siwarungson and Lertpringkop found that Massaman curry paste showed strongly antioxidant activity comparing with red curry, phanang curry and green curry; although it showed activity lower than green curry [38]. These authors reported that the descending ability of antioxidant properties as followed; green curry > Massaman Curry > red curry > Phanang curry.

#### *Identification of phenolic acids of Massaman curry and their products*

Results of phenolic and flavonoid compounds in MCP, BMC, and CMC by HPLC method are presented in Table 3. The main phenolic acid in these samples is ferulic acid which was the highest content in methanol extracted, followed by ethanol then water extraction. Moreover, syringic acid, chlorogenic acid and coumaric acid were also found in a high content in methanol extract of MCP. Various bioactive compounds are derived from Massaman curry paste and their products, including, vanillic acid, cinnamic acid, gallic acid, and catechin. Altogether, these results suggest that ferulic acid was high content in MCP and in cooked Massaman curry extracted.

Phenolic compounds	Methanol			Ethanol			Aqueous		
	MCP	BMC	CMC	MCP	BMC	CMC	MCP	BMC	CMC
Gallic acid	+	++	++	+	+	+	+	+	+
Protocatechuic	-	-	-	-	-	-	-	-	-
Catechin	-	++	+++	-	-	+	++	+	+
Chlorogenic acid	++	+++	+++	+	-	-	++	-	-
Caffeic	-	-	-	-	-	-	-	-	-
Syringic acid	+++	++	++	+	-	-	-	-	-
Vanillic acid	++	+	+	-	+	+	-	-	-
Coumaric	++	++	++	++	++	++	+	+	+
Ferulic	++++	+++	+++	++++	+++	+++	+	+	+
Rutin	-	-	-	-	-	-	-	-	-
cinnamic acid	++	+	+	++	++	++	++	++	++

(+) presence low values contents (less than 10 mg/100g dry crude extracts), (++) presence medium values contents (10-50 mg/100g dry crude extracts), (++++) presence moderate-high values contents (50-100 mg/100g dry crude extracts), (++++) presence high values contents (>100 mg/100g dry crude extracts), and (-) = absence. Each value was obtained by calculating the average of two experiments. Massaman Curry Paste (MCP); Beef Massaman Curry (BMC) and Chicken Massaman Curry (CMC).

Ferulic acid, a ubiquitous natural phenolic phytochemical present in seeds and leaves, both in its free and covalently conjugated form in the cell wall of several plants [39]. It is insoluble in water at room temperature but soluble in hot water, ethyl acetate, ethanol, and ethyl ether, and has been found that ethanol (60%) is suitable for the successful extraction of ferulic acid [40]. Therefore, this study found low content of ferulic acid in water extracted.

In ready-to-eat Massaman curry, coconut milk is one of the main ingredients, it rich in nutrients and phytochemical such as protein, fatty acid and phenolic compounds [41] and has exhibited antioxidant effects [42]. It is also rich in the phenolic compounds such as  $p$ -coumaric acid, ellagic acid and ferulic acid [41]. Gallic acid and coumaric acid normally are found in onion, cumin, nutmeg, fennel, and peppermint [43]. This study found that all samples contain gallic acid, coumaric acid, ferulic acid and cinnamic acid.

It is clear that each spices or herbs employed in Thai foods supplies antioxidants to some extent. However, the influence of the mixture and cooking process, especially in ready-to-eat in whole dish on antioxidant properties and phenolic contents in Asian foods was limited in the literature. There are a few reports on antioxidant activity, phenolic and flavonoids contents in the cooked foods [44]. These results showed that Thai curry paste like Massaman curry and their food products can show amount of active compound with potential antioxidant activity. Therefore, Thai food may be able to delay or prevent the onset of antioxidant health-related disease.

## CONCLUSION

Massaman is one of the most popular Thai curries. It is a mild coconut milk-based soup containing high nutritive value. MCP extract showed the highest of carbohydrate ash and moisture content, and for extract in methanol and demonstrated higher TPC than the ethanol and aqueous solution. Methanol extract is the highest antioxidant both in DPPH radical scavenging activity and FRAP assay and also gave the highest content of TFC. Moreover, various types of phenolic acid compound were detected including gallic acid, coumaric acid, and cinnamic acid. This study showed that Massamun curry paste both fresh and cooked also provide antioxidant capacity derived from various herbs and spices used as ingredients. The results suggest that the health benefits from these plants source still remained in the products after being cooked.

**List of Abbreviations:**  $AlCl_3$ , Aluminium chloride; ANOVA, Analysis of variance; AOAC, Association of Official Analytical Chemists; BMC, Beef Massaman curry; CE, Catechin equivalent; CMC, Chicken Massaman curry; DPPH, 2,2-dyphenyl-1-picrylhydrazyl;  $FeCl_3$ , Ferric Chloride; FRAP, Ferric Reducing Antioxidant Power; GAE, Gallic acid equivalent; HPLC, High performance liquid chromatography; MCP, Massaman curry paste;  $Na_2CO_3$ , Sodium carbonate; NaOH, Sodium Hydroxide;  $NaNO_2$ , Sodium nitrite; ND, Non detected; SD, Standard deviation; TFC, Total flavonoid content; TPC, total phenolic content; TPTZ, 2, 4, 6-tripyridyl-s-triazine.

**Competing Interests:** The authors declare that there is no conflict of interest.

**Author's Contributions:** All authors contributed to these studies.

**Acknowledgements and Funding:** This work was financially supported from Government Budget Grant (PHY610103S), Prince of Songkla University, Hat Yai, Songkhla, Thailand.

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