

Research Article

Effect of thermal processing and protein nutrients on antioxidant activity of Tom-kha paste extract

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Abstract

Tom-kha or galangal coconut soup is the sixth order of top ten Thai foods. An ingredient of Tom-kha paste has been addressed as natural antimicrobial and antioxidant activities. In order to verify the antioxidant activities in food system, the effect of thermal processing and protein, beef extract on antioxidant activities of ascorbic acid, gallic acid, Trolox, ρ -hydroxycinnamic acid and Tom-kha paste extract were determined. It was found that antioxidant activities of ascorbic acid were decreased while gallic acid and Tom-kha paste extract were not changed by heat treatment. Heating at 121°C for 15 min, however, antioxidant activities of ρ -hydroxycinnamic acid were increased. After heat treatment, mixture of beef extract-Tom-kha paste extract, beef extract-ascorbic acid and beef extract-Trolox pronounced more antioxidant activity in term of DPPH assay. However, mixture of beef extract-gallic acid and beef extract- ρ -hydroxycinnamic acid had no change in DPPH activity after heating. Moreover, there was not change in ABTS assay in any mixtures after thermal process. FRAP activity, the mixture of beef extract-gallic acid, beef extract-ascorbic acid and beef extract-Trolox was decreased while beef extract-Tom-kha and beef extract- ρ -hydroxycinnamic acid were increased by heating 121°C for 15 min. To sum up, thermal processing and protein nutrient did not reduce antioxidant activity of the Tom-kha paste extract. Therefore, in consuming Tom-kha soup may promote healthy benefit.

Keywords: Protein, Beef extract, Tom-kha, Antioxidant, Thermal process

Introduction

Tom-kha or galangal coconut soup ranks sixth amongst the top ten Thai cuisines due to its mild taste, sweet and sour flavour [1]. Ingredients of tom-kha includes herbs and spices such as galangal rhizome, lemon grass, kaffir lime leaves and chili, and these have been reported as natural antimicrobials [2, 3] with antioxidant activity [4, 5, 6, 7]. Since most herbs and spices

contain many phenolic compounds these possess antioxidant and therapeutic activity. In addition, galangal rhizome is composed of 1'S-1' acetoxycinnamic acetate which was reported as antitumor [8] and anti-inflammatory [9] while *p*-hydroxycinnamaldehyde acts as osteoarthritis [10]. However, during the cooking process, food components such as protein, carbohydrate and lipids may alter antioxidant activity. For example, the interaction between protein and phenolic compounds leads to changes in the functional properties of protein [11] and antioxidant activity of phenolic compounds [12].

It is generally well known that there can be significant losses of natural compounds during thermal processing due to the fact that most of the bioactive compounds are relatively heat labile. Therefore, heat processed food is considered to have a lower health promoting capacity than the corresponding fresh one. However, recent studies have shown that thermally processed food, especially fruit and vegetables, have higher biological activities due to their various chemical changes during heat treatment [13]. There is limited information concerning the antioxidant activity of phenolic compounds in the food component system. For this reason, the effect of protein content on antioxidant activities of tom-kha paste extract before and after heating was studied.

Material and Methods

Materials

Fresh galangal rhizomes (*Alpinia galanga* Swart.), lemon grass (*Cymbopogon citratus* Stapf.), kaffir lime leaves (*Citrus hystrix* DC4.) and red bird chili (*Capsicum frutescens* Linn.) were purchased from a fresh market in Haad Yai city, Thailand. Samples were sorted, trimmed, washed, drained for 2 minutes, cut into small pieces and blended until forming a fine paste (60-20 mesh). Tom-kha paste was made in accordance with the recipe of Ayusuk *et al.* [14] by weighing all of the cleaned and cut herbs/spices before blending.

Chemical and reagents

Absolute ethanol was obtained from Merck (Darmstadt, Germany). 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride hexahydrate and potassium persulphate were procured from Fluka Chemical Co. (Buchs, Switzerland).

Methods

Sample preparation

Extraction procedure

One gram of the fresh sample was soaked in 75% ethanol 10 ml at ambient temperature for 3-5 days, depended on the composition of each sample [14, 15]. The samples were filtered through cheesecloth followed by filter paper (Whatman No. 1). The filtrate was pooled and dried by a rotary evaporator (Buchi rotavapor, Switzerland) at 40-45°C to obtain a final volume of approximately 2 ml. The sample was kept in a dark glass bottle and stored at -20°C until used. Gallic acid, Trolox, *p*-hydroxycinnamic acid and ascorbic acid were separately dissolved in 75% ethanol and diluted to obtain the final concentration as 50 µM, 250 µM, 2000 µM and 50 µM, respectively.

Beef extract preparation

Beef extract powder was dissolved in KCl phosphate buffer (pH 7.5 *I*=0.5) then diluted until the concentration was 5% protein content.

Heat treatment

Beef extract solution was mixed with each antioxidant (gallic acid, Trolox, ρ -hydroxycinnamic acid, ascorbic acid and tom-kha paste extract) at ratios of 2:1, 5:1, 10:1, and 15:1 (v/v). The samples were well mixed and heated at 121°C for 15 min using autoclave.

Determination of total phenolic compound content

The amount of total phenolic compounds in spices/herbs and tom-kha paste extract was determined as per the procedure of Chan *et al.* [16], with some modification. Gallic acid, Trolox, ρ -hydroxycinnamic acid and ascorbic acid were used as antioxidant standard and results were reported as g standard per 100 g sample of dry weight (dw).

Determination of antioxidant activity

DPPH radical scavenging activity was determined by DPPH assay as described by Yen and Hsieh [17], with some modifications. For the ABTS assay, the procedure followed the method of Re *et al.* [18], with some modifications, while the FRAP assay was done according to Benzie and Strain [19] with some modification.

Statistical analyses

Data were subjected to Analysis of Variance (ANOVA) and mean comparisons were performed using the Duncan's new multiple range test. Statistical analyses were carried out using the SPSS statistical software version 6.

Results and Discussion

Total phenolic compound content of spices and tom-kha paste extract

Total phenolic compound content of each spice/herb and tom-kha paste extract was expressed as g gallic acid equivalent (GE), g ascorbic acid equivalent (AE), g ρ -hydroxycinnamic acid equivalent (HCE) and g Trolox equivalent (TE)/100 g (Table 1). The results showed that total phenolic compound content in galangal was highest when reported as 2.78±0.03 g GE/100 g, 0.30±0.00 g AE/100 g, 3.10±0.06 g HCE/100 g and 10.90±0.12 g TE/100 g. In addition, kaffir lime leaves and tom-kha paste extract were ranked second, possessing higher antioxidant properties when compared with GE, AE and TE. In terms of HCE determination, chili was higher than tom-kha paste extract, while lemon grass had the lowest antioxidant activity. From these results it was found that bioactive compounds in all extracts had a hydrophobic form higher than a hydrophilic form. Siripongvutikorn *et al.* [4] reported that the total phenolic compound content from methanolic spice extracts used in tom-yum paste ranged from 0.19 to 3.16 g GE/100 g dw. In addition, Tangkanakul *et al.* [20] reported that total phenolic compound content from methanolic extracts of galangal was highest (2.77±0.04 g GE/100 g dw), followed by kaffir lime leaves (1.65±0.06 g GE/100 g dw), lemongrass (0.93±0.03 g GE/100 g dw) and bird chili (0.13±0.01 g GE/100 g dw), respectively. Thus the total phenolic compound content of herbs/spices reported by Tangkanakul *et al.* [20] was similar to the values obtained in this present study. Generally, however, the differences in herb preparation, freshness, physiological characteristics, variety and the solvent used might cause differences in total phenolic and antioxidant activity.

Table 1. Total phenolic compound content of spices and tom-kha paste extracts.

Sample	Total phenolics			
	g GE/100 g dw	g AE/100 g dry	g HCE/100 g dw	g TE/100 g dw
Galangal	2.78±0.03 ^a	0.30±0.00 ^a	3.10±0.06 ^a	10.90±0.12 ^a
Lemon grass	0.45±0.00 ^c	0.05±0.00 ^c	0.29±0.01 ^c	1.72±0.02 ^c
Kaffir lime leave	1.39±0.01 ^b	0.15±0.00 ^b	2.25±0.01 ^b	5.59±0.02 ^b
Chili	0.87±0.02 ^d	0.09±0.00 ^d	1.03±0.04 ^c	3.41±0.08 ^d
Tom-kha paste	1.02±0.01 ^c	0.11±0.00 ^c	0.65±0.03 ^d	3.89±0.05 ^c

^{a-c} Means within a column with different letters are significantly different (p<0.05)

Antioxidant activity of spices and tom-kha paste extracts

The antioxidant activity of the spices and tom-kha extracts was determined according to their proton and electron donor as measured by DPPH, ABTS and FRAP. Galangal rhizome showed the highest antioxidant activity in all assays (Tables 2, 3 and 4). In addition, tom-kha paste extract exhibited the second highest antioxidant properties determined by DPPH and ABTS assay. In terms of FRAP activity, kaffir lime leaves was higher than tom-kha paste extract. Lemon grass had the lowest antioxidant activity in all assays.

Several researchers reported a good correlation between the antioxidant activity of the ethanolic extract and its total phenolic compound content. This was similar to this present result which showed that the highest total phenolic compound content and highest antioxidant activity was found in galangal rhizome extract, while the lowest phenolic compound content and lowest antioxidant activity was found in the lemon grass extract. This result was in accordance with Siripongvutikorn *et al.* [4] who reported that the highest total phenolic compound content in the galangal rhizome extract and lowest phenolic compound content in garcinia well related to its antioxidant activity. In addition, the findings of Tangkanakul *et al.* [20] showed that galangal rhizome was highest in DPPH activity (1,261.00±27.24 mg AE/100 g dw) compared with bird chili (130.36±2.76 mg AE/100 g dw). On the other hand, Wangcharoen and Morasuk [21] reported that red bird chili was high in total phenolic content and ABTS activity (2,369.29±321.76 mg AE/100 g dw). Many researchers reported that other phytochemicals such as ascorbic acid, tocopherol and pigment also contribute to total antioxidant activity. However, content of bioactive compounds in vegetables greatly varies in amount, depending on spices, variety, time of the year and degree of ripeness [22].

Table 2. DPPH activity of spices and tom-kha paste extracts.

Sample	DPPH			
	µmole GE/g dw	µmole AE/g dw	µmole HCE/g dw	µmole TE/g dw
Galangal rhizome	32.14±0.12 ^a	135.19±0.50 ^a	35,217.77±152.69 ^a	96.53±0.35 ^a
Lemon grass	4.75±0.19 ^c	21.92±0.74 ^d	3,769.66±227.89 ^c	15.96±0.53 ^d
Kaffir lime leave	5.09±0.20 ^d	21.92±0.80 ^d	5,204.60±243.49 ^d	15.73±0.56 ^d
Chili	6.02±0.27 ^c	25.91±1.11 ^c	6,169.52±338.40 ^c	18.59±0.78 ^c
Tom-kha paste	10.11±0.29 ^b	43.86±1.16 ^b	10,073.59±355.22 ^b	31.53±0.82 ^b

^{a-c} Means within a column with different letters are significantly different (p<0.05)

Table 3. ABTS activity of spices and tom-kha paste extracts.

Sample	ABTS			
	µmole GE/g dw	µmole AE/g dw	µmole HCE/g dw	µmole TE/g dw
Galangal rhizome	43.98±0.15 ^a	20.34±0.07 ^a	14,061.06±49.48 ^a	196.73±0.71 ^a
Lemon grass	7.53±0.03 ^c	3.47±0.01 ^c	12,846.16±17.07 ^b	31.50±0.13 ^d
Kaffir lime leave	11.63±0.01 ^c	5.38±0.00 ^c	3,162.81±4.21 ^c	52.17±0.07 ^c
Chili	11.08±0.03 ^d	5.13±0.02 ^d	4,531.76±12.04 ^d	49.34±0.15 ^c
Tom-kha paste	15.26±0.03 ^b	7.05±0.01 ^b	11,042.54±14.69 ^c	66.87±0.15 ^b

^{a-c} Means within a column with different letters are significantly different (p<0.05)

Table 4. FRAP activity of spices and tom-kha paste extracts.

Sample	FRAP			
	µmole GE/g dw	µmole AE/g dw	µmole HCE/g dw	µmole TE/g dw
Galangal rhizome	32.49±0.58 ^a	12.28±0.15 ^a	896.42±14.47 ^a	117.263±1.69 ^a
Lemon grass	5.08±0.27 ^d	2.92±0.08 ^c	167.28±6.73 ^c	23.40±1.19 ^c
Kaffir lime leave	17.32±0.14 ^b	5.65±0.04 ^b	453.44±3.48 ^b	57.95±0.43 ^b
Chili	11.41±0.19 ^c	4.11±0.05 ^d	309.20±4.70 ^d	40.14±0.59 ^d
Tom-kha paste	11.96±0.96 ^c	5.07±0.27 ^c	344.71±24.12 ^c	45.93±3.02 ^c

^{a-c} Means within a column with different letters are significantly different (p<0.05)

Effect of protein and beef extract on antioxidant activity of antioxidants and tom-kha extract after thermal processing

The effect of heating on antioxidant activities of individual samples of beef, gallic acid, tom-kha paste extract, ascorbic acid, Trolox, ρ -hydroxycinnamic acid and KCl phosphate buffer are shown in Table 5. DPPH activity of beef extract and tom-kha paste extract was not changed by heating. Interestingly, ρ -hydroxycinnamic acid increased after heating. This may support heating stability of tom-kha paste extract as reported by Ayusuk *et al.* [14]. However, gallic acid Trolox and ascorbic acid decreased after heat treatment. In terms of ABTS activity, beef extract, tom-kha paste extract and ρ -hydroxycinnamic acid were not changed, while gallic acid, Trolox and ascorbic acid decreased after heating. Gallic acid, tom-kha paste extract, Trolox and ρ -hydroxycinnamic acid were not changed, while beef extract increased after heating when determined as FRAP activity. Not surprisingly, ascorbic acid was not detected after heat treatment. Shobana and Naidu [23] showed that boiling garlic, ginger, cloves, cinnamon and pepper extracts at 100°C for 30 min did not only retain the antioxidant activity as lipid peroxidation assay but also showed significantly higher antioxidant activity, indicating some spice constituents were resistant to thermal processes. The possible release of bound antioxidant principles during heat treatment could be responsible for the higher antioxidant activity compared to fresh spice extract. Moreover, Choi *et al.* [24] reported that the DPPH and ABTS activities of Shiitake mushroom heated at 121°C for 30 min were increased by 2.0-fold and 2.2-fold compared to the raw sample, respectively. Klimczak *et al.* [25] reported that increase of temperature and storage time caused a decrease in vitamin C content. Additionally, an increase in temperature lead to a decrease in DPPH and ABTS activity for orange juice. It is generally known that vitamin C is particularly prone to degradation because of its high susceptibility to oxidation in the presence of oxygen and metal ions and its heat sensitivity [26].

After heat treatment, mixtures of beef extract-tom-kha paste extract, beef extract-ascorbic acid and beef extract-Trolox pronounced more antioxidant activity in terms of DPPH assay (Table 5). This may due to the fact that most of the compounds in this experiment were relatively heat stable. Or it could be that the heating process may enhance the antioxidant activity due to the formation of Maillard reaction products [27]. The mixture of beef extract-gallic acid and beef extract- ρ -hydroxycinnamic acid had no change in DPPH activity after heating (Table 5). In addition, there was no change in ABTS assay in any mixtures after thermal processing (Table 6). For FRAP activity, the mixture of beef extract-gallic acid, beef extract-ascorbic acid and beef extract-Trolox decreased, while the beef extract-tom-kha and beef extract- ρ -hydroxycinnamic acid were increased by heating 121°C for 15 min (Table 7). The effect of ratios of mixtures was not significant different ($p \geq 0.05$) on the DPPH and ABTS activities while the ratios caused an increase in FRAP activity. Arts *et al.* [28] reported that antioxidant activity of flavonoids determined as Trolox equivalent antioxidant capacity, TEAC decreased after mixing with proteins (α -, β -, and κ -casein) as a masking mechanism, as proline groups have a strong affinity for the hydroxyl groups of flavonoids. Moreover, Rohn *et al.* [29] found that antioxidant activity as TEAC of quercetin decreased after mixing with bovine serum albumin, BSA. However, Almajano and Gordon [30] reported that BSA has a synergistic effect on the ABTS activity of antioxidants (caffeic acid, epigallocatechin gallate (EGCG) and Trolox) but not for tertiary butylhydroquinone, TBHQ.

Table 5. Effect of protein and beef extract on DPPH activity of antioxidants and tom-kha paste extract.

Sample	DPPH ($\mu\text{mole TE/g dry weight}$)			
	Non-heat		Heat	
Beef 5%	16.50 \pm 0.90 ^A		15.17 \pm 0.83 ^A	
Gallic acid 50 μM	26,185.71 \pm 1,500.14 ^A		22,023.67 \pm 337.56 ^B	
Tom-kha paste extract (0.20-0.25% solid content)	21.27 \pm 0.29 ^A		21.80 \pm 0.24 ^A	
Ascorbic acid 50 μM	6,107.69 \pm 387.56 ^A		ND ^B	
Trolox 250 μM	2,420.92 \pm 48.98 ^A		1,592.45 \pm 65.75 ^B	
ρ -Hydroxycinnamic acid (2,000 μM)	28.67 \pm 2.10 ^B		42.33 \pm 3.15 ^A	
KCl phosphate buffer	0.02 \pm 0.00 ^A		0.02 \pm 0.00 ^A	
Beef 5% + Gallic acid 50 μM	based on beef	based on antioxidant	based on beef	based on antioxidant
2:1	10.98 \pm 1.72 ^{a,A}	64,565.65 \pm 10,137.54 ^{a,X}	13.24 \pm 1.61 ^{a,A}	77,808.50 \pm 9,466.90 ^{a,X}
5:1	9.83 \pm 2.91 ^{a,A}	57,809.09 \pm 17,084.34 ^{a,X}	13.10 \pm 2.74 ^{a,A}	76,997.71 \pm 16,100.46 ^{a,X}
10:1	11.12 \pm 1.02 ^{a,A}	65,376.43 \pm 5,976.40 ^{a,X}	13.24 \pm 0.89 ^{a,A}	77,808.50 \pm 5,212.63 ^{a,X}
15:1	11.72 \pm 1.34 ^{a,B}	68,889.84 \pm 7,874.79 ^{a,Y}	14.89 \pm 0.68 ^{a,A}	87,537.94 \pm 3,999.52 ^{a,X}
Beef 5% + Tom-kha paste				
2:1	28.18 \pm 2.49 ^{a,B}	196.23 \pm 17.37 ^{a,Y}	36.91 \pm 2.20 ^{a,A}	257.07 \pm 15.32 ^{a,X}
5:1	29.92 \pm 0.13 ^{a,B}	208.40 \pm 10.58 ^{a,Y}	36.73 \pm 1.59 ^{a,A}	255.79 \pm 11.08 ^{a,X}
10:1	31.12 \pm 1.60 ^{a,B}	216.73 \pm 11.13 ^{a,Y}	36.27 \pm 1.31 ^{a,A}	252.59 \pm 9.13 ^{a,X}
15:1	28.59 \pm 1.23 ^{a,B}	199.12 \pm 8.54 ^{a,Y}	37.05 \pm 0.94 ^{a,A}	258.03 \pm 6.54 ^{a,X}
Beef 5% + Ascorbic acid 50 μM				
2:1	2.11 \pm 0.50 ^{a,B}	11,981.75 \pm 2,823.58 ^{a,Y}	6.11 \pm 0.90 ^{a,A}	34,692.24 \pm 5,135.27 ^{a,X}
5:1	2.39 \pm 0.50 ^{a,B}	13,547.99 \pm 2,823.58 ^{a,Y}	6.11 \pm 0.77 ^{a,A}	34,692.24 \pm 4,360.23 ^{a,X}
10:1	1.56 \pm 0.50 ^{a,B}	8,849.26 \pm 2,823.58 ^{a,Y}	6.16 \pm 0.76 ^{a,A}	34,953.28 \pm 4,313.09 ^{a,X}
15:1	2.11 \pm 0.73 ^{a,B}	11,981.75 \pm 4,143.88 ^{a,Y}	6.75 \pm 0.76 ^{a,A}	38,346.81 \pm 4,313.09 ^{a,X}
Beef 5% + Trolox 250 μM				
2:1	2.25 \pm 0.90 ^{a,B}	1,796.54 \pm 722.74 ^{a,Y}	5.70 \pm 0.97 ^{a,A}	4,551.96 \pm 771.52 ^{a,X}
5:1	1.97 \pm 0.73 ^{a,B}	1,576.10 \pm 583.21 ^{a,Y}	6.57 \pm 0.92 ^{a,A}	5,250.00 \pm 733.86 ^{a,X}
10:1	2.89 \pm 0.71 ^{a,B}	2,310.88 \pm 565.59 ^{a,Y}	6.85 \pm 1.12 ^{a,A}	5,470.44 \pm 897.67 ^{a,X}
15:1	3.35 \pm 1.18 ^{a,B}	2,678.27 \pm 941.69 ^{a,Y}	6.29 \pm 0.44 ^{a,A}	5,029.57 \pm 354.30 ^{a,X}
Beef 5% + ρ -Hydroxycinnamic acid 2,000 μM				
2:1	32.55 \pm 1.54 ^{a,B}	4,956.62 \pm 234.22 ^{a,Y}	39.31 \pm 2.11 ^{a,A}	5,985.89 \pm 321.55 ^{a,X}
5:1	32.23 \pm 3.37 ^{a,A}	4,907.60 \pm 513.38 ^{a,Y}	36.82 \pm 1.45 ^{a,A}	5,607.79 \pm 220.644 ^{a,X}
10:1	32.36 \pm 1.80 ^{a,B}	4,928.61 \pm 274.15 ^{a,Y}	37.97 \pm 2.95 ^{a,A}	5,782.84 \pm 449.05 ^{a,X}
15:1	33.37 \pm 0.42 ^{a,A}	5,082.65 \pm 64.17 ^{a,X}	35.90 \pm 2.53 ^{a,A}	5,467.75 \pm 385.61 ^{a,X}

ND: Not detected

^a Means within a column and the same condition with different letters are significantly different ($p < 0.05$),^{A-B, X-Y} Means within a row and the same condition with different letters are significantly different ($p < 0.05$)

Table 6. Effect of protein and beef extract on ABTS activity of antioxidants and tom-kha paste extract.

Sample	ABTS (µmole TE/g dry weight)			
	Non-heat		Heat	
Beef 5%	1,300.51±53.65 ^A		1,247.18±41.29 ^A	
Gallic acid 50 µM	34,786.91±1,435.59 ^A		24,899.47±1,992.28 ^B	
Tom-kha extract (0.20-0.25% solid content)	98.42±3.37 ^A		95.85±0.93 ^A	
Ascorbic acid 50 µM	16,770.83±1,942.30 ^A		ND ^B	
Trolox 250 µM	4,868±163.71 ^A		3,597.88±455.36 ^B	
ρ-Hydroxycinnamic acid 2,000 µM	3,851.78±48.09 ^A		3,890.83±44.70 ^A	
KCl phosphate buffer	0.30±0.01 ^A		0.30±0.01 ^A	
Beef 5% + Gallic acid 50 µM	based on beef	based on antioxidant	based on beef	based on antioxidant
2:1	1,129.85±279.04 ^{a,A}	6,641,465.75±164024.97 ^{a,X}	1,117.54±32.50 ^{a,A}	6,569,118.63±191,047.55 ^{a,X}
5:1	1,078.15±57.06 ^{a,A}	6,337,607.84±335407.79 ^{a,X}	1,145.44±53.25 ^{a,A}	6,733,105.44±313,021.51 ^{a,X}
10:1	1,088.82±40.67 ^{a,A}	6,400,308.68±239074.13 ^{a,X}	1,159.38±69.68 ^{a,A}	6,815,098.84±409,598.03 ^{a,X}
15:1	1,115.08±49.86 ^{a,B}	6,554,649.21±239102.59 ^{a,Y}	1,206.97±17.23 ^{a,A}	7,094,841.05±101,285.97 ^{a,X}
Beef 5% + Tom-kha				
2:1	1,217.64±55.39 ^{a,A}	8,479.39±385.72 ^{a,X}	1,142.15±54.23 ^{a,A}	7,953.72±377.64 ^{a,X}
5:1	1,176.62±385.72 ^{a,A}	8,193.70±240.80 ^{a,X}	1,206.15±18.48 ^{a,A}	8,399.40±128.66 ^{a,X}
10:1	1,188.10±50.53 ^{a,A}	8,273.69±351.86 ^{a,X}	1,190.56±18.48 ^{a,A}	8,290.84±128.66 ^{a,X}
15:1	1,197.95±22.34 ^{a,A}	8,342.26±155.54 ^{a,X}	1,114.26±73.08 ^{a,A}	1,159.45±508.89 ^{a,X}
Beef 5% + Ascorbic acid 50 µm				
2:1	1,073.23±37.28 ^{b,A}	6,093,401.29±211,644.45 ^{a,X}	1,119.18±22.87 ^{b,A}	6,354,280.86±129,856.16 ^{b,X}
5:1	1,216.82±77.37 ^{a,A}	6,908,649.93±439,290.45 ^{a,X}	1,216.82±42.17 ^{a,A}	6,908,649.93±242,469.18 ^{a,X}
10:1	1,217.64±24.78 ^{a,A}	6,913,308.50±140,685.54 ^{a,X}	1,233.23±17.23 ^{a,A}	7,001,821.21±166,734.93 ^{a,X}
15:1	1,269.33±21.22 ^{a,A}	7,206,798.01±120,493.91 ^{a,X}	1,202.05±17.23 ^{a,B}	6,824,795.79±97,829.84 ^{a,Y}
Beef 5% + Trolox 250 µm				
2:1	1,165.95±17.46 ^{a,A}	931,678.23±13,954.71 ^{a,X}	1,083.08±53.72 ^{a,A}	865,457.61±42,928.79 ^{a,X}
5:1	1,184.82±37.17 ^{a,A}	946,758.17±29,700.28 ^{a,X}	1,115.08±80.08 ^{a,A}	891,027.95±63,988.86 ^{a,X}
10:1	1,194.67±46.77 ^{a,A}	954,625.97±37,372.03 ^{a,X}	1,167.59±26.05 ^{a,A}	932,989.53±20,816.23 ^{a,X}
15:1	1,205.33±43.01 ^{a,A}	963,149.41±34,370.06 ^{a,X}	1,184.00±25.86 ^{a,A}	946,102.52±20,660.77 ^{a,X}
Beef 5% + ρ-Hydroxycinnamic acid 2,000 µM				
2:1	1,082.26±65.70 ^{b,A}	164,817.31±10,005.09 ^{b,X}	1,052.72±52.58 ^{b,A}	160,318.89±8,007.93 ^{b,X}
5:1	1,024.00±63.49 ^{b,A}	155,945.42±9,669.39 ^{b,X}	1,109.33±16.76 ^{b,A}	168,940.87±2,551.68 ^{b,X}
10:1	1,225.03±53.04 ^{a,A}	186,559.71±8,077.82 ^{a,X}	1,229.13±39.61 ^{a,A}	187,184.49±6,032.94 ^{a,X}
15:1	1,284.92±9.95 ^{a,A}	195,681.51±1,515.01 ^{a,X}	1,251.28±19.69 ^{a,A}	190,558.30±2,998.95 ^{a,X}

ND: not detected

^{a-b} Means within a column and the same condition with different letters are significantly different (p<0.05),

^{A-B, X-Y} Means within a row and the same condition with different letters are significantly different (p<0.05)

Table 7. Effect of protein and beef extract on FRAP activity of antioxidants and tom-kha paste extract.

Sample	FRAP ($\mu\text{mole TE/g dry weight}$)			
	Non-heat		Heat	
Beef 5%	12.65 \pm 0.41 ^B		19.24 \pm 0.56 ^A	
Gallic acid 50 μM	14,323.22 \pm 2390.00 ^A		12,265.85 \pm 2493.91 ^A	
Tom-kha extract (0.20-0.25% solid content)	34.09 \pm 2.74 ^A		39.37 \pm 2.75 ^A	
Ascorbic acid 50 μM	1438.33 \pm 573.65 ^A		ND ^B	
Trolox 250 μM	4077.94 \pm 678.04 ^A		3638.45 \pm 141.25 ^A	
ρ -Hydroxycinnamic acid 2,000 μM	893.94 \pm 47.55 ^A		841.91 \pm 70.75 ^A	
KCl phosphate buffer	0.00 ^A		0.00 ^A	
Beef 5% + Gallic acid 50 μM	based on beef	based on antioxidant	based on beef	based on antioxidant
2:1	13.67 \pm 1.20 ^{c,A}	80,355.04 \pm 2,805.65 ^{c,X}	6.90 \pm 0.33 ^{b,B}	40,530.21 \pm 1,662.61 ^{b,Y}
5:1	15.35 \pm 0.26 ^{b,A}	90,250.02 \pm 1,529.56 ^{b,X}	8.05 \pm 0.63 ^{a,B}	47,290.15 \pm 3,726.41 ^{a,Y}
10:1	16.25 \pm 0.18 ^{ab,A}	95,491.42 \pm 1,039.13 ^{ab,X}	8.91 \pm 0.61 ^{a,B}	52,384.59 \pm 2,597.82 ^{a,Y}
15:1	17.30 \pm 0.49 ^{a,A}	101,663.53 \pm 1,870.43 ^{a,X}	8.56 \pm 0.28 ^{a,B}	50,327.22 \pm 2,286.08 ^{a,Y}
Beef 5% + Tom-kha				
2:1	7.40 \pm 0.63 ^{d,B}	51.50 \pm 5.66 ^{d,Y}	13.59 \pm 0.55 ^{b,A}	94.61 \pm 5.29 ^{b,X}
5:1	9.20 \pm 0.39 ^{c,B}	64.03 \pm 2.74 ^{c,Y}	12.39 \pm 1.16 ^{b,A}	86.26 \pm 8.10 ^{b,X}
10:1	10.69 \pm 0.77 ^{b,B}	74.42 \pm 7.14 ^{b,Y}	17.65 \pm 0.62 ^{a,A}	122.93 \pm 1.60 ^{a,X}
15:1	12.39 \pm 0.48 ^{a,B}	86.26 \pm 2.09 ^{a,Y}	17.18 \pm 0.58 ^{a,A}	119.63 \pm 1.11 ^{a,X}
Beef 5% + Ascorbic acid 50 μM				
2:1	11.80 \pm 0.39 ^{b,A}	66,967.58 \pm 3,111.38 ^{b,X}	7.01 \pm 0.42 ^{b,B}	39,809.61 \pm 2,308.44 ^{b,Y}
5:1	15.81 \pm 1.38 ^{a,A}	89,772.71 \pm 7,849.63 ^{a,X}	7.90 \pm 0.77 ^{b,B}	44,872.16 \pm 4,398.63 ^{b,Y}
10:1	15.35 \pm 2.53 ^{a,A}	87,170.46 \pm 19,571.60 ^{a,X}	8.07 \pm 0.25 ^{b,B}	45,818.43 \pm 2,007.34 ^{b,Y}
15:1	17.17 \pm 0.35 ^{a,A}	97,484.81 \pm 2,609.55 ^{a,X}	9.65 \pm 0.60 ^{a,B}	54,760.69 \pm 2,709.91 ^{a,Y}
Beef 5% + Trolox 250 μM				
2:1	12.94 \pm 0.64 ^{c,A}	10,337.34 \pm 706.29 ^{c,X}	5.85 \pm 0.15 ^{c,B}	4,677.24 \pm 113.01 ^{c,Y}
5:1	14.58 \pm 0.87 ^{b,A}	11,649.15 \pm 697.86 ^{b,X}	8.37 \pm 0.20 ^{b,B}	6,688.24 \pm 159.81 ^{b,Y}
10:1	16.61 \pm 0.42 ^{a,A}	13,273.94 \pm 395.52 ^{a,X}	8.65 \pm 0.22 ^{b,B}	6,914.65 \pm 240.14 ^{b,Y}
15:1	17.65 \pm 0.42 ^{a,A}	14,099.64 \pm 169.51 ^{a,X}	9.22 \pm 0.36 ^{a,B}	7,367.45 \pm 282.51 ^{a,Y}
Beef 5% + ρ -Hydroxycinnamic acid 2,000 μM				
2:1	7.77 \pm 0.39 ^{b,B}	1,183.30 \pm 10.77 ^{b,Y}	11.96 \pm 0.51 ^{d,A}	1,821.65 \pm 110.38 ^{d,X}
5:1	9.29 \pm 0.53 ^{a,B}	1,414.27 \pm 80.07 ^{a,Y}	15.35 \pm 0.63 ^{c,A}	2,338.17 \pm 96.64 ^{c,X}
10:1	9.25 \pm 0.93 ^{a,B}	1,409.19 \pm 166.91 ^{a,Y}	16.34 \pm 0.56 ^{b,A}	2,487.92 \pm 78.07 ^{b,X}
15:1	9.57 \pm 0.66 ^{a,B}	1,457.42 \pm 139.99 ^{a,Y}	17.41 \pm 0.25 ^{a,A}	2,651.63 \pm 32.31 ^{a,X}

ND: not detected

^{a-d} Mean within a column and the same condition with different letters are significantly different ($p < 0.05$),^{A-B,X-Y} Mean within a row and the same condition with different letters are significantly different ($p < 0.05$)

Conclusion

Even though thermal processes reduced the activity of some antioxidants, the stability of tom-kha paste extract was still evident and this may be due to ρ -hydroxycinnamic acid being heat stable. Protein nutrients, in beef extract form, did not have any negative effects on antioxidant activities of tom-kha paste extract even after heating at 121°C. It may be concluded from this research that tom-kha, when served as a heated soup, still retains many properties beneficial to health and this can in fact be enhanced by the addition of protein without any loss of antioxidants.

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