

Effect of Heat Treatment on Antioxidant Properties of Tom-Kha Paste and Herbs/Spices Used in Tom-Kha Paste

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ABSTRACT

The research studied tom-kha paste for the effects of heat treatment (100°C, 10, 20 and 30 min) on the total phenolic content by Folin-Ciocalteu test and the antioxidant activities of the paste and its ingredients, including galangal rhizome, lemon grass, chili and kaffir lime leaves were investigated by three different methods: DPPH radical scavenging assay, ABTS radical cation decolorization assay and ferric ion reducing/ antioxidant power (FRAP) assay. The results showed the total phenolic content of chili and tom-kha paste extracts decreased as a result of heating at 100°C for 30 min, while the total phenolic content in other samples did not change. The FRAP value of galangal extract increased, while the ABTS and FRAP values of lemon grass extracts decreased after heating. The DPPH and FRAP values of kaffir lime leaf extract increased following heating. The DPPH, ABTS and FRAP values of chili extract decreased after heat treatment. For tom-kha paste, the DPPH and ABTS values decreased, but the FRAP values increased following heat treatment

Key words: Tom-kha, antioxidant, heat, galangal, lemon grass, kaffir lime leaves, chili

INTRODUCTION

Tom-kha (coconut milk soup with Siamese galangal), is the sixth most-ordered Thai dish among consumers, both in Thailand and worldwide, because of its mild taste, sweetness and flavor (Office of the National Culture Commission, 1999). The soup usually consists of coconut milk, galangal rhizome, lemon grass, chili and kaffir lime leaves. However, the ratio of spices/ ingredients used may differ from home to home or region to region (Siripongvutikorn *et al.*, 2008). There is growing evidence that several herbs and spices ordinarily used in Thai cuisine promote

antioxidant activity and have health benefits. Galangal rhizome has been reported as having antitumorous (Matsuda *et al.*, 2003; Zaeoung *et al.*, 2005), antibacterial (Vuddhakul *et al.*, 2007), antifungal (Ficker *et al.*, 2003) and antioxidant (Juntachote and Berghofer, 2005) activity. In addition, galangal extract showed antioxidant activity in a model system (Cheah and Abu-Hasim, 2000) and in pork (Juntachote *et al.*, 2006). Lemon grass leaves contain essential oil up to $1 \pm 5\%$ (dry weight) with mainly citral compounds (Lewinsohn *et al.* 1998). The ethanol extract of lemon grass exhibited anti-mutagenic activity (Vinitketkumnueen *et al.*, 1994). Kaffir lime leaves

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are utilized for various medical and culinary proposes in Southeast Asia. Siripongvutikorn *et al.* (2005) reported that kaffir lime leaves were a good source of β -carotene in tom-yum paste at levels of $173.60 \pm 61.45 \mu\text{g/g}$ sample. Chilies are used in cooking to provide a hot, spicy and pungent taste, due to the capsaicin component (Li-E *et al.*, 2008). Many studies have revealed the substantial antioxidant, antigenotoxic and anticarcinogenic effects of chili extracts and capsaicin (Prasad *et al.*, 2004).

It is generally known that some natural compounds could be substantially lost during thermal processing (Azevedo-Meleiro and Rodriguez, 2005). Therefore to address this, the objective of this research was to determine the effect of heat on the total phenolic content and antioxidation activities of tom-kla paste and the herbs/spices used in the paste.

MATERIALS AND METHODS

Materials

Fresh galangal rhizomes (*Alpinia galanga* Swart.), lemon grass (*Cymbopogon citratus* Stapf.), kaf fir lime leaves (*Citrus hystrix* DC4.) and red bird chili (*Capsicum frutescens* Linn.) were purchased from a fresh market in Hat Yai city, Songkhla province, Thailand. Samples were cleaned, washed with water, cut into small pieces and ground in a blender. Tom-kha paste was made by weighing the small pieces of galangal rhizomes, lemon grass, kaffir lime leaves and red bird chili according to the recipe and grinding with the blender until a fine paste was produced.

Chemical

Ethanol was obtained from Merck (Darmstadt, Germany). 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2-diphenyl-1-picryl hydrazyl (DPPH) were

purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2,4,6-Tripyridyl- s-triazine (TPTZ), ferric chloride hexahydrate and potassium persulfate were procured from Fluka Chemical Co., (Buchs, Switzerland).

Extraction procedure

One gram of the fresh sample was soaked in 75% ethanol (10 ml) at ambient temperature for 5 d, according to the method of Li-E *et al.* (2008). The samples were filtered through cheesecloth, followed by filter paper (Whatman No. 1). The supernatant was collected, while the residue was re-extracted as described above. The filtrate was pooled and evaporated by a rotary evaporator (Buchi rotavapor, Buchi, Switzerland) at 40-45°C until solid content was approximately 0.09-0.15%/g dw (dry weight). The sample was kept in a dark glass bottle and stored at -20°C until used.

Effect of heating on total phenolic content and antioxidant activity.

Samples of 5 ml of crude ethanolic extract with a solid content between 0.09- 0.15 dw (dry weight)/g was added into a test tube and heated in an oil bath at 100°C for 10, 20 and 30 min. Samples were then cooled to room temperature before being evaluated for the total phenolic content and antioxidant activities by DPPH, ABTS and FRAP assays.

Determination of total phenolic content

The amount of total phenolics in the extracts was determined according to the Folin-Ciocalteu procedure, as described by Kahkonen *et al.* (1999), with some modification.

Determination of antioxidant activity

DPPH radical scavenging activity was determined by DPPH assay, as described by Wu *et al.* (2003). The ABTS assay procedure followed the method of Arnao *et al.* (2001) and the FRAP

assay was carried out according to Benzie and Strain (1996), with some modification.

Statistical analyses

Data were subjected to analysis of variance (ANOVA) and mean comparisons were performed using Duncan's new multiple range test. Statistical analyses were carried out using SPSS statistical software, version 7.

RESULTS AND DISCUSSION

Total phenolic content and antioxidant activity in tom-kha paste and its ingredients

The total phenolic content and antioxidant activity of tom-kha paste and the spices and herbs used in tom-kha paste are shown in Table 1. The results showed that the total phenolic content in chili and galangal were highest at 1.87 ± 0.20 and 1.80 ± 0.03 g gallic acid equivalent (GAE)/100 g dw, respectively, followed by kaffir lime leaves, tom-kha paste and lemon grass at 1.21 ± 0.15 , 1.18 ± 0.07 and 0.46 ± 0.06 g GAE/ 100 g dw, respectively.

Galangal rhizome showed the highest antioxidant activity measured by DPPH and FRAP assay with a value of 60.75 ± 1.61 and 104.38 ± 3.85 μ mole TE/ g dw, respectively. However, red bird chili showed the highest antioxidant activity by ABTS assay with a value of 223.32 ± 5.55 μ mole TE/ g dw. This result was similar to that reported by Wangcharoen and Morasuk (2007), who found that red and green bird chili and red

holy basil showed the highest ABTS activity followed by red chili spur pepper and white holy basil, green chili spur pepper, garlic and pumpkin, respectively. The antioxidant activity of chili extracts has been reported to be due to the existence of compounds, such as carotenoids (Siripongvuttikorn *et al.*, 2005), flavonoids (Miean and Mohamed, 2001), ascorbic acid and tocopherol (Daood *et al.*, 1996; Ching and Mohamed, 2001) and capsaicinoids (Materska *et al.*, 2003).

Though the total phenolic content in kaffir lime leaves and tom-kha paste was not significantly different ($p \geq 0.05$), DPPH and ABTS activity in tom-kha paste were higher than in kaffir lime leaves. This suggested that the total phenolic content may not be a good indicator of antioxidant activity. Chu *et al.* (2000) reported that crown daisy, with a low phenolic content in terms of total flavonoids (1.12 ± 0.03 mg/kg), had moderate antioxidant activity equal to $92.18 \pm 0.23\%$, $91.90 \pm 0.10\%$, $72.101.50\%$ and 79.03 ± 0.47 determined by DPPH scavenging activity, superoxide scavenging activity, hydroxyl radical scavenging activity and lipid peroxidation inhibition, respectively. Many researchers observed that other phytochemicals, such as ascorbic acid, tocopherol and pigment, also contributed to total antioxidant activity. While phenolic compounds undergo a complex redox reaction with the phosphotungstic and phosphomolybdic acids present in the reagent, the assay has been shown to be specific not to just polyphenols, but to any other substance that

Table 1 Total phenolic content and antioxidant activity of tom-kha paste and its ingredients.

SampleIngredient	Total phenolics (g GAE/ 100 g dw.)	DPPH	ABTS μ mole TE/ g dw.	FRAP
Galangal	1.80 ± 0.03^a	60.75 ± 1.61^a	167.48 ± 5.19^b	104.38 ± 3.85^a
Lemon grass	0.46 ± 0.06^c	28.08 ± 1.11^c	35.47 ± 3.67^e	39.37 ± 1.36^c
Kaffir lime leaves	1.21 ± 0.15^b	17.65 ± 1.18^d	73.75 ± 1.49^d	37.22 ± 0.09^c
chili	1.87 ± 0.20^a	55.75 ± 4.89^b	223.32 ± 5.55^a	56.38 ± 3.01^b
Tom-kha	1.18 ± 0.07^b	26.14 ± 0.08^c	97.00 ± 4.55^c	33.00 ± 2.62^c

^{a-c} = means within a column with different letters are significantly different ($p < 0.5$).

could be oxidized by the Folin reagent, Various researchers have reported the poor specificity of the assay (Singleton *et al.*, 1999). In addition, phenolic compounds, depending on the number of phenolic groups they have, respond differently to the Folin–Ciocalteu reagent (Singleton *et al.*, 1999). The differences in antioxidant activity could be explained by the different mechanisms of the analytical methods. ABTS and DPPH assays are based on reduction of the ABTS radical cation (ABTS^{•+}) (Re *et al.*, 1999) and the DPPH radical (DPPH[•]) (Roginsky and Lissi, 2005). The important difference between ABTS and DPPH is that ABTS^{•+} can be dissolved in aqueous and organic media, in which the antioxidant activity can be measured, due to the hydrophilic and lipophilic nature of the compounds in samples. In contrast, DPPH[•] can only be dissolved in organic media, especially in ethanol, this being an important limitation when interpreting the role of hydrophilic antioxidants. That may be the reason why the ABTS value was higher than the DPPH value in all sample extracts (Wojdylo *et al.*, 2007). The FRAP assay measures the ability to reduce ferric tripyridyltriazine (Fe³⁺-TPTZ) in samples to the ferrous form (Fe²⁺-TPTZ) (Wojdylo *et al.*, 2007).

Effect of heat treatment on total phenolic content of tom-kha paste and its ingredients.

The effect of heating at 100°C for 10, 20 and 30 min on the total phenolic content of the paste and its ingredients is shown in Figure 1. From the results, the total phenolic content of galangal increased by heating for 10 min and slightly decreased as time increased. It was found that the heating time did not cause a significant change ($p \geq 0.05$) in the total phenolic content in lemon grass and kaffir lime leaves, while the total phenolic contents of chili and tom-kha paste extracts significantly decreased ($p < 0.05$) when the heating time increased. Kim *et al.* (2006) concluded that the total phenolic contents in whole grape seed extract (WGSE) and powdered grape seed extract (PGSE) were significantly increased by heat treatment, however, heating at 200°C decreased the total phenolic content of WGSE and PGSE significantly ($p < 0.05$). On the other hand, Xu *et al.* (2007) reported that the total amount of phenolic acids in huyou peel extract decreased after heat treatment, which indicated that some phenolic acids probably were destroyed by heat treatment and converted insoluble phenolic compounds to soluble phenolics. This indicated that the phenolic compounds of plants may be

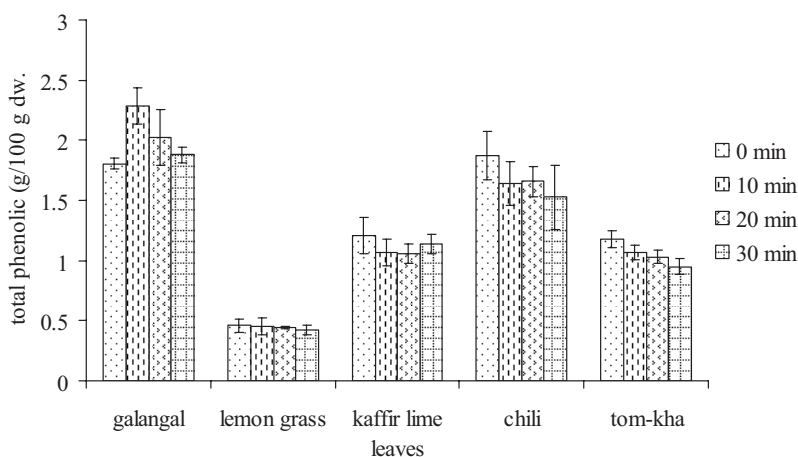


Figure 1 Effect of heat treatment on the total phenolic content of tom-kha paste and its ingredients.

present in different bound forms depending on the species. Therefore, the effective processing step to liberate antioxidant compounds from different plant species may not be similar (Kim *et al.*, 2006).

Effect of heat treatment on antioxidant activity of Tom-kha paste and its ingredients.

The effect of heat treatment on antioxidant activity of tom-kha paste and its ingredients is presented in Figure 2-4. The DPPH and ABTS values of galangal rhizome did not

change during heating at 100°C for 10 to 30 min. However, the FRAP value of the galangal extract was significantly increased by heating at 100°C for 20 min and significantly decreased ($p < 0.05$) when time increased to 30 min. Juntachote and Berghofer (2005) reported that the ethanolic extracts of galangal showed good heat stability (80°C, 1 h). ABTS and FRAP assays of the lemon grass extract slightly decreased after heating for any time, while DPPH did not change. Heat treatment could increase DPPH and FRAP

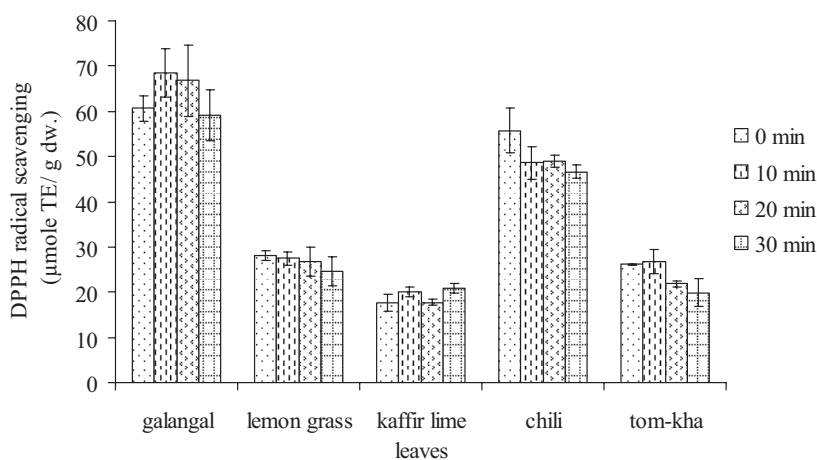


Figure 2 Effect of heat treatment on the DPPH radical scavenging activity of tom-kha paste and its ingredients.

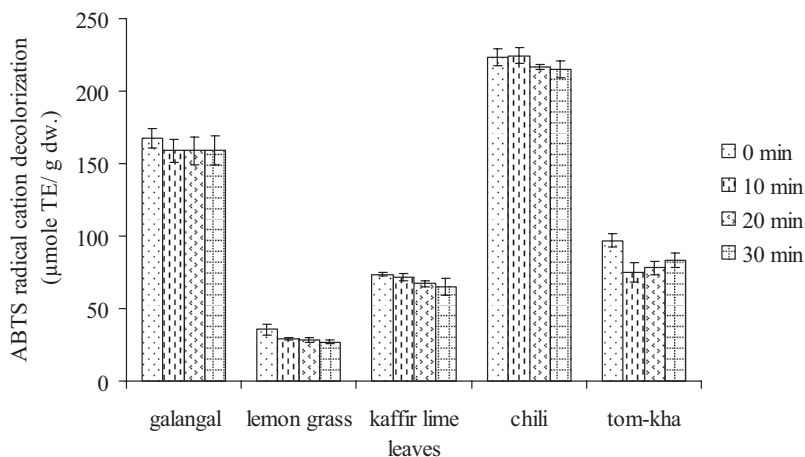


Figure 3 Effect of heat treatment on the ABTS radical cation decolorization of tom-kha paste and its ingredients.

($p < 0.05$), while decreasing ABTS assay values of kaffir lime leaf extract. Berhow *et al.* (1996) reported that flavanone glycosides (e.g., narirutin-4'-glucoside, edocitrin, neoeriocitrin, narirutin, hesperidin, neohesperidin and lidymin) and flavone/ol glycosides (e.g., rutin) were found in kaffir lime leaves. Xu *et al.* (2007) reported that heat treatment (90°C, 30 min) decreased narirutin, naringin, hesperidin, and neohesperidin. An increase in the DPPH and FRAP values of kaffir lime leaf extract was due to the breakdown of flavonoid glycosides to free flavonoid. It has often been reported that glycosylation reduces flavonoid activity (Figueirinha *et al.*, 2008). In chili extracts DPPH, ABTS and FRAP significantly decreased ($p < 0.05$) after heat treatment due to the loss of ascorbic acid and some phenolic compounds. Ching and Mohamed (2001) reported that chilies were rich in vitamins, especially pro-vitamin A and C. The loss of ascorbic acid was expected, as thermal treatment was known to accelerate the oxidation of ascorbic acid to dehydroascorbic acid, followed by hydrolysis, 3-diketogulonic acid and eventually polymerization to other nutritionally inactive components. Klimczak *et al.* (2007) reported that each increase of temperature by 10°C caused a distinct decrease in the concentration of ascorbic acid, and the decrease

of vitamin C content upon storage was reflected by the decrease in the antioxidant capacity of orange juices. Miean and Hohamed (2001) reported that bird chili had a total flavonoids content of 1,663.0 mg/kg including myricetin (236.0 ± 0.03 mg/kg dw), quercetin (392.0 ± 0.02 mg/kg dw), luteolin (1035.0 ± 0.03 mg/kg dw). Crozie *et al.* (1997) reported that cooking (microwaving, boiling and frying) lowered the quercetin content of tomatoes and onions. Tom-kha paste is composed of galangal rhizome, lemon grass, chili and kaffir lime leaves in the ratio of 16.67: 18.89: 3.29: 1. Therefore, the changes in the antioxidant activity agreed with the changes found in the main ingredients of lemon grass and galangal after heating.

CONCLUSION

Tom-kha paste and its ingredients possessed antioxidant activities, particularly galangal rhizome. However, a long heating time could reduce the total phenolic content and antioxidant activities. Therefore, to maintain the antioxidant activities, the optimum heating time at cooking temperature for tom-kha paste was very important.

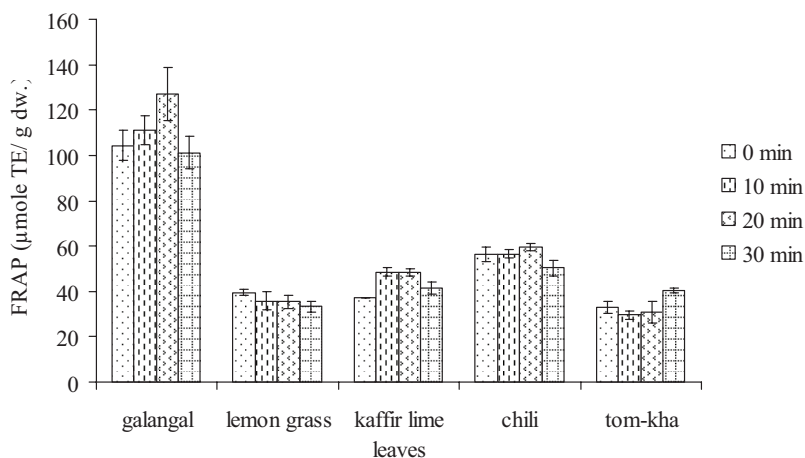


Figure 4 Effect of heat treatment on the FRAP of tom-kha paste and its ingredients.

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