

# Effect of lipids and thermal processing on antioxidant activity of galangal seasoning, tom-kha paste extract

<sup>1</sup>Naiyana Pengseng and \*<sup>1</sup>Sunisa Siripongvutikorn

<sup>1</sup> Department of Food Technology, Prince of Songkla University, Songkhla 90112, Thailand

\*Corresponding Author: sunisa.s@psu.ac.th, sunisasiripongvutikorn@gmail.com

**Abstract** The effect of lipids on antioxidant activities of tested antioxidants and the galangal seasoning, Tom-Kha paste extract were determined. The result showed that heating caused a decrease of DPPH scavenging activity of lauric acid but did not affected on ABTS scavenging activity of both lauric acid and virgin coconut oil. However, ABTS scavenging activity of *p*-hydroxycinnamic acid and the paste extract in both lauric acid and virgin coconut oil systems increased after thermal processing. In the system of lauric acid, peroxide value (PV) of almost mixtures was increased ( $p < 0.05$ ) by heating. Thiobarbituric acid reactive substances (TBARS) of the mixtures were not significantly different ( $p < 0.05$ ) after thermal processing while *p*-anisidine value (AV) of only lauric acid-gallic was enhanced after heating. The results showed that PV of virgin coconut oil added with all tested antioxidant was not changed after heating. TBARS of virgin coconut oil added with antioxidant samples seemed to slightly increase after heating. AV of virgin coconut oil with added gallic acid and the paste extract were not changed while AV of virgin coconut oil with added *p*-hydroxycinnamic acid and Trolox seemed to decrease after heating.

**Key words:** Antioxidant, thermal processing, Tom-Kha, lauric acid, virgin coconut oil

## Introduction

Many factors, such as light, metal ions, oxygen, temperature and enzymes influence the oxidative stability of lipid-containing food (Nawar, 1996). The lipid oxidation process mainly involves the oxidation of unsaturated fatty acids or their derivatives (Wheatley, 2000). The degradation process has been generally established being a free radical mechanism, yielding primary oxidation products, which in turn degrade to yield secondary oxidation products (e.g. aldehydes, ketones, lactones, alcohols and acids) and often associated by the unwanted flavor which is broadly described as rancidity (Madhavi et al. 1996). Galangal coconut milk soup or Tom-Kha has been classified as the sixth order of top ten Thai cuisines due to its perfect combination of mild taste, sweet and sour flavor (Office of the National Culture Commission, 1999). In general, the ingredient of the soup consists of herbs and spices such as galangal rhizome, lemon grass, kaffir lime leaves and chili have been reported as natural antimicrobial agents (Nakahara et al. 2002; Siripongvutikorn et al. 2005, 2008) and antioxidants (Juntachote et al. 2007; Siripongvutikorn et al. 2009). In cooking procedure, a cup of coconut milk is recommended to add (Thai food to the world, 2005). However, the ratio or/and kind of the ingredients as well as coconut milk used in recipe may differ from home to home or region to region (Siripongvutikorn et al. 2008) that may also alter consumer preference and antioxidant activity.

To improve eating quality and safety of food products, and to extend the shelf life of the products, thermal processing is one of the most important processes widely used in the food industry (Wang & Sun, 2006). On the other hand, thermal processing can promote lipid oxidation by disrupting cell membranes and releasing prooxidants which rapidly developed in food during cooking and storage (Madhavi et al. 1996). Some studies have been showed that cooking methods had negative affected to the contents of nutrient and health-promoting compounds such as vitamin C, carotenoids and polyphenols as well as natural bioactive compounds (Siddhuraju & Becker, 2007). However, recent studies showed that thermally processed foods, especially fruits and vegetables, have higher biological activities (Choi et al. 2006; Ayusuk et al. 2009; Seah et al. 2010). Four possibilities are suggested for the increase in antioxidant activity of some vegetables after cooking: (1) the liberation of high amounts of antioxidant components due to the thermal destruction of cell walls and sub cellular compartments; (2) the production of stronger radical-scavenging antioxidants product by thermal chemical reaction; and/or (3) increasing of the oxidation capacity of antioxidants by thermal inactivation of oxidative enzymes; (4) production of new non-nutrient antioxidants or the formation of novel compounds such as Maillard reaction products with antioxidant activity (Morales & Babel, 2002). Based on basic knowledge, antioxidant of herbs/spices may be influenced by many factors and the information of combined effect of lipid and thermal processing in model food system are limited. Therefore,

the objectivity of this study was to determine the effect of lipid and thermal processing on antioxidant activity of galangal coconut milk paste extract, Tom-Kha.

## Materials and Methods

### Materials

Fresh galangal rhizomes (*Alpinia galanga* Swart.), lemon grass (*Cymbopogon citratus* Stapf.), kaffir lime leaves (*Citrus hystrix* DC4.) and chili (*Capsicum frutescens* Linn.) were purchased from fresh market in Hat-Yai city, Songkhla, Thailand.

### Chemical and reagents

6-Hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2'-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, *p*-hydroxycinnamic acid, *p*-anisidine and thiobarbituric acid (TBA) were purchased from Fluka (Sigma-Aldrich Chemie GmbH, Germany). Absolute ethanol and hydrochloric acid were purchased from Merck (Darmstadt, Germany).

### Material Preparation

Fresh samples were sorted, trimmed, washed, drained for 2 min, cut into small pieces. Tom-Kha paste was made by weighing all of cleaned and cut herbs/spices according to recipe composed of galangal, lemon grass, kaffir lime leaves and chili as 41, 47, 3 and 9 g, respectively (Ayusuk et al. 2009) before brought to blend until it became a fine paste as 60-20 mesh.

### Extraction Procedure

One g of the blended Tom-Kha paste was soaked in 75 ml/100ml ethanol for 4 days before subjected to filter 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 volume approximately 2 ml then adjust final volume as 2 ml. The sample was kept in a dark glass bottle and stored at -20°C until used.

### Preparation of Tested Antioxidants

Gallic acid, Trolox and *p*-hydroxycinnamic acid were separately dissolved in absolute ethanol and diluted to obtain the final concentration as 50 µM, 250 µM and 2,000 µM, respectively.

### Heat Treatment

Virgin coconut oil (A-tiss D-life, Thailand) and lauric acid (Kosher, Sigma-Aldrich Co., USA) represented as lipid sample were sonicated at 50°C for 60 min with sonicator (RK 100 H, Bandelin SONOREX, Germany) to initiate lipid oxidation. Sonicated samples were mixed with each antioxidant (gallic acid, *p*-hydroxycinnamic acid, Trolox and Tom-Kha paste extract) as the ratios of 2:1, 5:1, 10:1 and 15:1 (v/v) using vortex and sonicated at 50°C for 15 min. The samples were heated at 121°C for 15 min using autoclave (SS352, Tomy Seiko Co., Ltd, Japan).

### Determination of Antioxidant Activities

#### DPPH Scavenging Activity

DPPH scavenging activity was determined by DPPH assay as described by Yen & Hsieh (1997) with some modifications. Briefly, a 1.5 ml of each sample was mixed with 1.5 ml of 0.3 mM DPPH dissolved in absolute ethanol. The mixture was shaken vigorously and left at ambient temperature for 30 min in the dark. The DPPH scavenging activity was determined by measuring the absorbance at 517 nm using a UV-Visible spectrophotometer (UV-16001, Shimadzu, Kyoto, Japan). Trolox was used as antioxidant standard and results were reported as µmole Trolox equivalent (TE)/g dried weight (dw) of sample.

#### ABTS Scavenging Activity

The procedure of ABTS scavenging activity followed the method of Re et al. (1999) has been used with minor modifications. ABTS<sup>•+</sup> radical cation was produced by mixing of 7.4 mM aqueous ABTS and 2.6 mM potassium persulfate then mixture was kept in the dark area at ambient temperature for 12 h. Blue-green ABTS<sup>•+</sup> was formed at the end of this period. Then the solution was diluted with 50 ml of absolute ethanol before subjected to measure an absorbance of 1.1±0.02 units at 734 nm using the UV-Visible spectrophotometer (UV-16001, Shimadzu, Kyoto, Japan). 0.15 ml of the sample was allowed to react with 2.85 ml of the ABTS<sup>•+</sup> solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the UV-Visible spectrophotometer (UV-16001, Shimadzu, Kyoto, Japan). Results are expressed as µmole Trolox equivalent (TE)/g dw of sample.

### **Peroxide Value (PV)**

The formation of primary products of lipid oxidation (peroxides) was evaluated on an aliquot of the fat extract according to Paquot (1979). The sample (2 to 5 g) was dissolved in 25 ml chloroform-acetic acid mixture (2:3, v/v), treated with 1 ml of saturated potassium iodide (KI) solution, and kept in the dark for 5 min. The mixture was treated with 30 ml of distilled water and shaken. One ml of starch solution (1% w/v) was added as an indicator. The peroxide value was determined by titrating iodine liberated from potassium iodide with sodium thiosulphate solution. The PV was defined as the reactive oxygen content, expressed as milliequivalents of active oxygen per kg of lipid (meq of active oxygen/kg).

### **Thiobarbituric Acid Reactive Substances (TBARS)**

Thiobarbituric acid reactive substances were determined as described by Buege & Aust, 1978. One ml of sample was dispersed in 4 ml of thiobarbituric acid solution (0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl). The mixture was heated in boiling water for 10 min, followed by cooling in running tap water. The mixture was centrifuged at 3,600×g for 20 min at room temperature using a centrifuge (Hermle Z323, Hermel Labortechnik, Germany). The absorbance of the supernatant was measured at 532 nm using the UV-Visible spectrophotometer (UV-16001, Shimadzu, Kyoto, Japan). The standard curve was prepared malondialdehyde bis (dimethyl acetal) (MDA) at concentrations ranging from 0 to 10 µg/ml. TBARS value in each sample was expressed as mg MDA/kg of lipid.

### **p-Anisidine Value (AV)**

p-Anisidine value of oil was analyzed according to Paquot (1979). The sample (0.5-4.0 g) was dissolved in 25 ml of hexane and measured at 350 nm using UV-visible spectrophotometer (UV-16001, Shimadzu, Kyoto, Japan). This solution (5 ml) was mixed with 1 ml of p-anisidine reagent (2.5 g/l of p-anisidine in glacial acetic acid) for 10 min. The absorbance was read at 350 nm. The p-anisidine value was calculated by this formula: p-anisidine value =  $25 \times [(1.2 \times A_2 - A_1)/W]$ ; where  $A_1$  = the absorbance before adding p-anisidine,  $A_2$  = the absorbance at 350 nm after adding p-anisidine and W = weight of sample (g).

### **Statistical Analyses**

Data were subjected to Analysis of Variance (ANOVA) and mean comparisons were performed using the Duncan's multiple range test (DMRT). Differences between the mean scores from non-heated and heated samples were determined using the t-test. Statistical analyses were carried out using the SPSS statistical software (SPSS, Inc., Chicago, IL).

## **Results and Discussion**

### **Effect of Thermal Processing on Antioxidant Activities of Lauric Acid, Virgin Coconut Oil, Tested Antioxidants and Tom-Kha Paste Extract**

DPPH and ABTS assays are considered to be mainly method based on a proton abstraction and electron transfer reaction. The differences in the antioxidant capacity in the ABTS and DPPH assays depend mainly on the reactivity of the free radical and the solubility of the different compounds in the testing solution (Prior et al. 2005). Additionally, the ABTS method has the extra flexibility at different pH levels and ABTS is soluble in both aqueous and organic solvents thus, ABTS is useful in assessing antioxidant activity of samples in different media (Awika et al. 2003). Effect of thermal processing on antioxidant activity of lauric acid, virgin coconut oil, tested antioxidants and Tom-Kha paste extract determined by DPPH and ABTS assay were showed in Table 1 and 2, respectively. DPPH scavenging activity of lauric acid, gallic acid and Trolox were decreased while p-hydroxycinnamic acid and Tom-Kha paste extract were not changed after heating at 121°C for 15 min. In addition, ABTS scavenging activity of gallic acid and Trolox decreased after heating while lauric acid, virgin coconut oil, p-hydroxycinnamic acid and Tom-Kha paste extract were not significantly changed after heating. From this result, gallic acid and Trolox were classified as heat labile antioxidant while p-hydroxycinnamic acid and Tom-Kha paste extract were heat stable antioxidant. Decreasing of DPPH and ABTS scavenging activities of gallic acid and Trolox might be occurred by the destruction of phenolic hydroxyl groups from high temperature (Yen & Hung, 2000). This result corresponding with Chen et al. (2007) who reported that a decrease of DPPH scavenging activity of gallic acid occurred after heating at 90°C for 30 min. Arabshahi-D et al. (2007) reported that heating at 100°C for 15 min resulted in a significant

decrease ( $p < 0.05$ ) in antioxidant activity in drumstick leaves extract due to degradation of natural antioxidant and formation of novel compounds as prooxidant. Moreover, Murcia et al. (2009) found that canned vegetables more pronounced loss of antioxidant activity than frozen vegetables and fresh one. The reduction in antioxidant activity as a result of degradation of phenolic compounds in canning might be caused by the high temperatures used during processing.

The result showed that virgin coconut oil seemed to have ABTS activity as lauric acid. However, it was found that there was no DPPH activity detected in virgin coconut oil may be due to dissolved problem. Therefore, using each antioxidant activity assay would be considered otherwise fault detection may occur. Heating, 121°C for 15 min caused decreasing on DPPH activity of lauric acid but on ABTS scavenging activity of both lauric acid and virgin coconut oil. This result similarly with Marina et al. (2009b) who reported that the antioxidant activity of virgin coconut oil samples ranged from 7.78 to 29.18 mg GE/100 g oil which was higher than antioxidant activity of refined, bleached and deodorized coconut oil (RBDCO). May be due to total phenolic contents of virgin coconut oil samples (7.78-29.18 mg GE/100 g oil) were significantly higher than RBDCO (6.14 mg GE/100 g oil). However, Seneviratne et al. (2009) reported that the coconut oil extracted under hot conditions (HECO) contained more phenolic substances than the coconut oil extracted under cold conditions (CECO) and the antioxidant potential of HECO was higher than that of CECO as determined by DPPH assay, deoxyribose assay and in vivo assay of serum antioxidant capacity. Seneviratne et al. (2009) also reported that gallic acid, (-)-epigallocatechin and syringic acid were the major phenolic compounds present in VCO while gallic acid (-)-epigallocatechin, (+)-catechin, *p*-hydroxybenzoic acid, (+)-epicatechin, caffeic acid, syringic acid and ferulic acid were identified in traditional coconut oil (hot extraction). Moreover, Marina et al. (2009a) reported that the major phenolic acids in virgin coconut oil were ferulic acid and *p*-hydroxycinnamic acid. To sum up, antioxidant activity of materials may differ from type and part of used sample, extraction process, assay protocol and assay methods.

Table 1. Effect of thermal processing on DPPH scavenging activity of lipids, tested antioxidants and Tom-Kha paste extract

Samples	DPPH scavenging activity ( $\mu\text{mole TE/g dw}$ )	
	Non-heated	Heated
Lauric acid	0.03 $\pm$ 0.00 <sup>a</sup>	0.01 $\pm$ 0.00 <sup>b</sup>
Virgin coconut oil	ND	ND
Gallic acid	26,057.60 $\pm$ 101.53 <sup>a</sup>	18,786.47 $\pm$ 382.24 <sup>b</sup>
<i>p</i> -Hydroxycinnamic acid	30.82 $\pm$ 0.21 <sup>b</sup>	37.27 $\pm$ 2.93 <sup>a</sup>
Trolox	2,208.61 $\pm$ 7.19 <sup>a</sup>	2,103.26 $\pm$ 61.09 <sup>b</sup>
Tom-Kha paste extract	18.27 $\pm$ 0.09 <sup>a</sup>	18.15 $\pm$ 0.32 <sup>a</sup>

ND=not determined,<sup>a,b</sup> Means within a row and same condition with different letters were significantly different ( $p < 0.05$ ).

Table 2. Effect of thermal processing on ABTS scavenging activity of lipids, tested antioxidants and Tom-Kha paste extract

Samples	ABTS scavenging activity ( $\mu\text{mole TE/g dw}$ )	
	Non-heated	Heated
Lauric acid	0.08 $\pm$ 0.01 <sup>a</sup>	0.09 $\pm$ 0.01 <sup>a</sup>
Virgin coconut oil	0.10 $\pm$ 0.00 <sup>a</sup>	0.10 $\pm$ 0.00 <sup>a</sup>
Gallic acid	35,227.42 $\pm$ 163.09 <sup>a</sup>	19,732.48 $\pm$ 142.59 <sup>b</sup>
<i>p</i> -Hydroxycinnamic acid	3,719.95 $\pm$ 66.90 <sup>a</sup>	3,695.58 $\pm$ 338.57 <sup>a</sup>
Trolox	4,844.44 $\pm$ 3.91 <sup>a</sup>	4,073.64 $\pm$ 12.42 <sup>b</sup>
Tom-Kha paste extract	91.48 $\pm$ 2.10 <sup>a</sup>	94.48 $\pm$ 2.57 <sup>a</sup>

<sup>a,b</sup> Means within a row and same condition with different letters were significantly different ( $p < 0.05$ ).

### **Combined Effect of Lipids and Thermal Processing on Antioxidant Activities of Tested Antioxidants and Tom-Kha Paste Extract**

Combined effect of lauric acid (LA) and thermal processing on DPPH scavenging activity of gallic acid, *p*-hydroxycinnamic acid, Trolox and Tom-Kha paste extract as the ratio of 2:1, 5:1, 10:1 and 15:1 expressed in  $\mu\text{mole TE/g dw}$  of sample were showed in Figure 1 (a). From this present found that DPPH scavenging activity of all mixtures were significantly decreased ( $p < 0.05$ ) after thermal processing at 121°C for 15 min. Although the previously results showed that thermal processing did not have negative affected on DPPH scavenging activity of *p*-hydroxycinnamic acid and Tom-Kha paste extract. It implied that lauric acid reduced antioxidant

activity of all antioxidants and Tom-Kha paste extract may be due to van der waals interaction between fatty acid chains and phenolic compounds (Weber & de Bont, 1996). Moreover, thermal processing induced autoxidation of lauric acid (Madhavi et al. 1996). Thereafter thermal processing brought to the destruction of phenolic hydroxyl groups and degradation of phenolic compounds (Murcia et al. 2009). In addition, Yen et al. (2002) reported that ascorbic acid and gallic acid at lower concentrations (0.004-0.24 mM) would change to be prooxidant when testing with high concentrations of free radical or active oxygen and metal ion. It was explanation by their weak metal-chelating effects and their strong electron-donating effects (reducing ability), as well as their stimulation of oxidative effects.

ABTS scavenging activity of lauric acid and virgin coconut oil systems was showed in Figure 1. (b). The result showed that ABTS scavenging activity of the mixtures of lauric acid and antioxidants (LA: GA and LA: T) was decreased ( $p < 0.05$ ) at ratio 2:1 and 5:1 while at ratios as 10:1 and 15:1 were not changed after heating. Additionally, in systems of virgin coconut oil mixed with gallic acid and Trolox were decreased after heating. Surprising ABTS scavenging activity of *p*-hydroxycinnamic acid and Tom-Kha paste extract in both lauric acid and virgin coconut oil system increased ( $p < 0.05$ ) after thermal processing. This may be due to the solubility of polar phenolic substances such as gallic acid, *p*-hydroxybenzoic acid, caffeic acid, syringic acid and ferulic acid in non-polar coconut oil was certainly improved at high temperatures, then more phenolic substances would be more dissolved in coconut oil during the heating (Seneviratne et al. 2009). Moreover, Rice-Evans et al. (1996) addressed that *p*-hydroxycinnamic acid has relatively high antioxidant capacity because of its CH=CH-COOH group compared with gallic acid when heating was applied. Generally, cinnamic acid derivatives have been resonance stabilization more than benzoic acid derivatives (Shahidi and Naczka, 2004) due to the greater delocalization of the unpaired electron of the antioxidant radical caused by the conjugated side chain. Furthermore, the electron-withdrawing properties of the carboxyl group attached to the aromatic ring have a negative influence on the hydrogen-donating abilities of hydroxybenzoic acids (Pekkarinen et al., 1999). According to Seneviratne et al. (2009) who reported that the phenolic extracts of HECO is superior to that of CECO at all tested phenolic concentrations. This meant that the phenolic extracts of HECO display higher antioxidant capacities than those of CECO at any given total phenol concentration. This higher antioxidant capacity of the phenolic extracts of HECO should be associated with the more complex phenolic composition of HECO (Seneviratne et al., 2009).

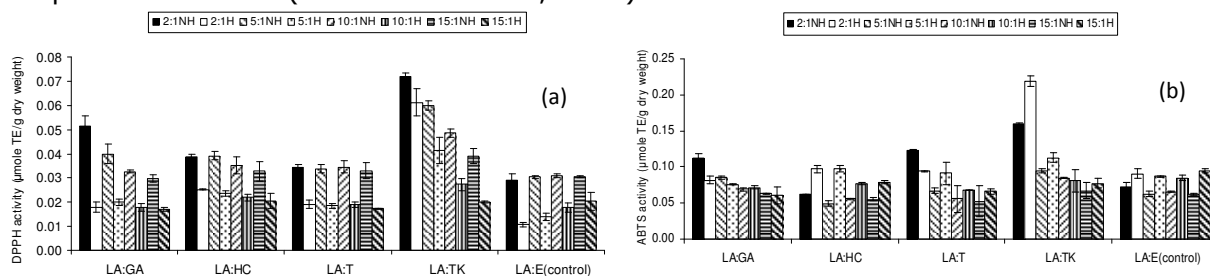


Figure 1. Combined effect of lauric acid (LA) and thermal processing on (a) DPPH scavenging activity (b) ABTS scavenging activity of gallic acid (GA), *p*-hydroxycinnamic acid (HC), Trolox (T), Tom-Kha paste extract (TK) and absolute ethanol (E). The ratios of LA: antioxidant = 2:1, 5:1, 10:1 and 15:1. NH = no heat treatment, H = heat treatment

## Conclusions

The results showed that gallic acid and Trolox were heat labile antioxidant while *p*-hydroxycinnamic acid and Tom-Kha paste extract were heat stable antioxidant. Antioxidant activities of the mixture systems of lauric acid and antioxidants and thermal processing reduced DPPH scavenging activity of all treatments. A decrease of DPPH scavenging activity of all lauric acid-antioxidant mixtures was found after heat treatment. ABTS scavenging activity of the mixtures of lauric acid-gallic acid and lauric acid-Trolox were decreased while the mixtures of lauric acid-*p*-hydroxycinnamic acid and Tom-Kha paste extract were increased after heat treatment. The used antioxidants and Tom-Kha paste extract could prevent the propagation step of oxidation of lauric acid induced by heating. Antioxidant and or prooxidant properties in lauric

acid system depended on concentration and heating conditions. Tested antioxidants and Tom-Kha paste extract could inhibit oxidation of virgin coconut oil by thermal processing. Moreover, tested antioxidants and Tom-Kha paste extract may possess antioxidant and/or prooxidant activities depended on method assay, concentration and type of tested antioxidant and lipid systems as well as heating. However, further research about antioxidant activities of Tom-Kha paste extract in vivo system to confirm bioavailability should be carried out.

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