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Combined effect of carbohydrate and thermal processing on antioxidant activity of galangal coconut-milk paste extract, Tom-Kha

[1]Pengseng, N., [1,*]Siripongvutikorn, S., [1]Usawakesmanee, W. and [1]Wattanachant, S.

[1]*Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Songkhla, 90112, Thailand*

Abstract: Galangal coconut-milk soup or Tom-Kha is the sixth order of top ten Thai foods. The ingredients of Tom-Kha paste have been addressed *in vitro* system as natural antimicrobe and antioxidant. The effect of carbohydrate (glucose, sucrose and inulin) and thermal processing on antioxidant activities of gallic acid, *p*-hydroxycinnamic acid and Tom-Kha paste extract were determined to verify the antioxidant activities in food system. The results showed that antioxidant activity of gallic acid was decreased while that of Tom-kha paste extract was not changed by heat treatment. However, antioxidant activity of *p*-hydroxycinnamic acid was increased after heating. In term of mixture system, after heat treatment, glucose did not cause any negative effect on antioxidant activities of gallic acid, *p*-hydroxycinnamic acid and Tom-Kha paste extract, whereas sucrose caused a decrease on antioxidant activity of gallic acid. However, antioxidant activities of Tom-Kha paste extract and *p*-hydroxycinnamic acid were not reduced by the addition of carbohydrate.

Keywords: Galangal coconut-milk soup, Tom-Kha, antioxidant, thermal processing, carbohydrate

Introduction

The sixth most-order of top ten Thai foods is galangal coconut-milk soup or Tom-Kha due to its mild taste, sweetness and good flavor (Office of the National Culture Commission, 1999). Coconut-milk and some herbs and spices such as galangal rhizome, lemon grass, kaffir lime leaves and chili are major ingredients of soup. Numerous studies indicated that these herbs and spices have been reported as natural antimicrobe (Siripongvutikorn *et al*., 2008, 2005; Vuddhakul *et al*., 2007) and antioxidant (Seah *et al*., 2010; Ayusuk *et al*., 2009; Siripongvutikorn *et al*., 2009; Juntachote *et al*., 2007; Tachakittirungrod *et al*., 2007; Nakahara *et al*., 2002). Galangal rhizome is primarily used as a flavoring especially in the preparation of Thai curry paste and Thai soup. Two phenolic compounds, [di-(*p*-hydroxy-cis-styryl)] methane and *p*-hydroxycinnamaldehyde were isolated from chloroform galangal rhizome extract (Barik *et al*., 1987). Additionally, 1'S-1' acetoxychavical acetate derived from the rhizome was reported to possess antitumor, pungency, anti-inflammatory, antifungal, gastroprotective and xanthine oxidase inhibitory activities (Matsuda *et al*., 2003; Yang and Eilerman, 1999; Nakamura *et al*., 1998; Kondo *et al*., 1993) while *p*-hydroxycinnamaldehyde was act as osteoarthritis (Phitak *et al*., 2009). The galangal extract was applied in food system for controlling lipid oxidation (Juntachote *et al*., 2006; Cheah and Abu Hasim, 2000). Moreover, antioxidant activity of lemon grass was reported (Cheel *et al*., 2005; Middleton *et al*., 2000). Cookings in Southeast Asia commonly use kaffir lime leaves as an aromatic and astringent flavor and it could strongly inhibit the tumor-promoting activity of 12-O-tetradecanoyl-phorbol-13-acetate *in vitro* and *in vivo* (Murakami *et al*., 1995). A hot, spicy and pungent taste of food is found in chilies due to the capsaicin component (Li-E *et al*., 2008). Many studies revealed the substantial antioxidant, antigenotoxic and anticarcinogenic effects of chili extracts and capsaicin (Prasad *et al*., 2004).

The ratio of ingredients used in recipe may differ from home to home or region to region (Siripongvutikorn *et al*., 2008) that may also affect antioxidant activity. Moreover, in cooking process, food component such as protein, carbohydrate and lipid may alter antioxidant activity (Rohn *et al*., 2004; Art *et al*., 2002).

Carbohydrate is a minor ingredient of Tom-Kha soup used as sweetener may interact with bioactive compounds and reduce antioxidant activity. Inulin is a kind of carbohydrate that may obtain from the ingredients of Tom-Kha soup. It is generally well known as prebiotic and widely found in plants, including leeks, onions, garlic, asparagus, Jerusalem artichockes and chicory (Franck, 2002; Niness, 1999; Van Loo *et al*., 1995). Therefore, inulin is classified as active food ingredients for 'functional foods' (Van Loo *et al*., 1999).

In general, thermal processing decreased activity

*Corresponding author.
Email: *sunisa.s@psu.ac.th*
Tel: +66 7428 6344; Fax: +66 7455 8866

of natural bioactive compounds (Siddhuraju and Becker, 2007). However, recent studies showed that thermally processed foods, especially fruits and vegetables, have higher biological activities due to their various chemical changes during heat treatment (Seah *et al*., 2010; Ayusuk *et al*., 2009; Choi *et al*., 2006; Kim *et al*., 2006).

Since, there is limited information concerning food system as an effect of carbohydrate on the antioxidant activity of phenolic compounds. Therefore, the aim of this research was to evaluate the combined effect of carbohydrate and thermal processing on antioxidant activities of phenolic compounds and 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 red chili (*Capsicum frutescens* Linn.) were purchased from fresh market in Hat-Yai city, Songkhla, Thailand. Samples were sorted, trimmed, washed and drained on a sieve for 2 min before cut into small pieces. Tom-Kha paste was prepared by weighing all of cleaned and cut herbs/spices as recipe of Ayusuk *et al*. (2009) before brought to blend until it became a fine paste as 60-20 mesh.

Chemical and reagents

Absolute ethanol was obtained from Merck (Darmstadt, Germany). 2,2'-Azino-bis(3-ethylbenz-thiazoline-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 persulfate were procured from Fluka Chemical Co. (Buchs, Swizerland).

Extraction procedure of Tom-Kha paste extract

One gram of the Tom-Kha paste was soaked in 75% ethanol 10 ml at ambient temperature for 4 days as previous work (Ayusuk *et al*., 2009; Jantachote *et al*., 2006). The sample was 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 final volume approximately 2 ml. The sample was kept in a dark glass bottle and stored at -20°C until used. Gallic acid and *p*-hydroxycinnamic acid, test antioxidants, were separately dissolved in 75% ethanol and diluted to obtain the final concentration as 50 µM and 2,000 µM, respectively.

Preparation of carbohydrate solution

Glucose (Analytical grade, Ajax), sucrose (Analytical grade, Ajax) and inulin (Analytical grade, Sigma) were dissolved in distilled water then diluted to the final concentration of 5%.

Heat treatment

Carbohydrate (Glucose, sucrose and inulin) solution was mixed with each antioxidant (gallic acid, *p*-hydroxycinnamic acid and Tom-Kha paste extract) as the 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 antioxidant activities

DPPH scavenging activity

DPPH scavenging activity was determined by DPPH assay as described by Yen and Hsieh (1997) with some modifications. Briefly, a 100 µl of each sample was mixed with 100 µl of 0.3 mM DPPH dissolved in 75% 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 microplate reader (Power wave X, Biotek, USA). Trolox was used as antioxidant standard and results were reported as µmole Trolox equivalent (TE) per 1 g dried weight 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. $ABTS^{\bullet+}$ 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 $ABTS^{\bullet+}$ was formed at the end of this period. Then the solution was diluted with 50 ml of 75% ethanol before subjected to measure an absorbance of 1.1 ± 0.02 units at 734 nm using the microplate reader (Power wave X, Biotek, USA). Sample (15 µl) was allowed to react with 285 µl of the $ABTS^{\bullet+}$ solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the microplate reader (Power wave X, Biotek, USA). Results are expressed in µmole Trolox equivalent (TE) per 1 g dried weight of sample.

FRAP antioxidant activity

The FRAP assay was done according to Benzie

and Strain (1996) with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-Tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3.6H_2O$ solution and then warmed at 37°C before used. Sample (15 µl) was allowed to react with 285 µl of the FRAP solution for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. Results are expressed in µmole Trolox equivalent (TE) per 1 g dried weight of sample.

Statistical analyses

Data were subjected to Analysis of Variance (ANOVA) and mean comparisons were performed using the Duncan's new multiple range test (DMRT). 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 carbohydrate, antioxidants and Tom-Kha paste extract

Effect of thermal processing by heating at 121°C for 15 min, on antioxidant activities of individual carbohydrate, antioxidants and Tom-Kha paste extracts were determined according to their proton donor and electron transfer abilities as measured by DPPH, ABTS and FRAP assays (Table 1, 2 and 3). This result showed that DPPH scavenging activity of glucose and inulin were increased after heating while heat treatment did not affect that of sucrose (Table 1). In term of ABTS scavenging activity, glucose and sucrose were not significantly changed after heating ($p \geq 0.05$). While, there was no antioxidant activity of glucose and sucrose for both before and after heat treatment in FRAP assay (Table 3). However, heating process enhanced antioxidant activity of inulin in ABTS and FRAP assays (Table 2 and 3). These results might be indicated that inulin has electron transfer ability more than glucose and sucrose in aqueous solution. It implied that amount and type of monosaccharide composition lead to different antioxidant activities. Wijewickreme *et al*. (1999) and Segone *et al*. (1983) reported that glucose acted as a hydroxyl radical scavenger in biological model systems. Moreover, Peinado *et al*. (2010) found that sugars (glucose and fructose) could also display interesting antioxidant activities. From this present study, the data showed that antioxidant activity of gallic acid (benzoic acid derivative) was decreased after heating while those of *p*-hydroxycinnamic acid (cinnamic acid derivative) and Tom-Kha paste extract appeared to be heat stable. This may support heating stability of Tom-Kha paste as reported by Ayusuk *et al*. (2009). Similar to Juntachote *et al*. (2005) who reported that antioxidant activity of ethanolic extract of galangal was heat stable, even heating at 80°C for 60 min. Rehman *et al*. (2003) also found that ginger extract heated at 185°C for 120 min showed a good thermal stability and exhibited 85.2% inhibition of peroxidation of linoleic acid. Similarly, Shobana and Naidu (2002) reported that boiled (100°C for 30 min) garlic, ginger, cloves, cinnamon and pepper extracts did not only retain the antioxidant activity in lipid peroxidation assay but also showed significantly higher antioxidant activity, indicating some released bound antioxidants during heating were heat resistant and could be responsible for the higher antioxidant activity. Xu *et al*. (2007) reported that the antioxidant activity of the citrus peel extract increased with heating time and temperature in ABTS and FRAP assays. Moreover, Choi *et al*. (2006) reported that heated Shiitake mushroom at 121°C for 30 min had the antioxidant activity more than 2.0-fold and 2.2-fold compared to that of the raw sample, respectively. On the other hand, Klimczak *et*

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

Sample	DPPH scavenging activity (µmole TE/g dw)	
	Non-heated	Heated
Glucose 5%	0.04±0.02[b]	0.18±0.01[a]
Sucrose 5%	0.12±0.02[a]	0.14±0.01[a]
Inulin 5%	0.74±0.02[b]	1.28±0.01[a]
Gallic acid 50 µM	26,268.14±337.56[a]	22,214.20±406.07[a]
p-Hydroxycinnamic acid 2,000 µM	27.62±3.79[b]	39.53±2.64[a]
Tom-Kha paste extract 0.20-0.25% solid content	20.07±0.43[a]	20.67±0.53[a]

[a-b] Means within a row with different letters were significantly different ($p<0.05$).

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

Sample	ABTS scavenging activity (µmole TE/g dw)	
	Non-heated	Heated
Glucose 5%	1.00±0.05[a]	0.98±0.04[a]
Sucrose 5%	0.69±0.08[a]	0.75±0.03[a]
Inulin 5%	1.98±0.15[b]	4.52±0.07[a]
Gallic acid 50 µM	33,822.28±1,162.82[a]	22,427.61±1,641.15[b]
p-Hydroxycinnamic acid 2,000 µM	4,084.51±77.33[a]	4,050.14±58.71[a]
Tom-Kha paste extract 0.20-0.25% solid content	91.92±0.33[a]	92.06±0.65[a]

[a-b] Means within a row with different letters were significantly different ($p<0.05$).

Table 3. Effect of thermal processing on antioxidant activities of carbohydrate, test antioxidants and Tom-Kha paste extract determined by FRAP assay

Sample	FRAP antioxidant activity (µmole TE/g dw)	
	Non-heat	Heated
Glucose 5%	ND	ND
Sucrose 5%	ND	ND
Inulin 5%	0.19±0.05[b]	1.19±0.03[a]
Gallic acid 50 µM	14,911.04±935.22[a]	13,980.33±103.91[a]
p-Hydroxycinnamic acid 2,000 µM	907.90±24.48[a]	896.48±31.93[a]
Tom-Kha paste extract 0.20-0.25% solid content	32.40±1.06[a]	32.52±1.14[a]

[a-b] Means within a row with different letters were significantly different ($p<0.05$).

al. (2007) found that increasing of temperature led to a reduction of antioxidant activity of orange juices determined by DPPH and ABTS assays. This was due to a degradation of ascorbic acid which was very sensitive to oxygen, metal ions and heat (Gliguem and Birlouez-Aragon, 2005).

The DPPH and ABTS radical scavenging activities of gallic acid were decreased after heating (Table 1 and 2). It pointed out that gallic acid was not heat tolerant. This result was corresponding to that of 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. The decrease in the DPPH scavenging activity of gallic acid might have resulted in the destruction of phenolic hydroxyl groups due to heat treatments (Yen and Hung, 2000). However, thermal processing did not change reducing ability of gallic acid, *p*-hydroxycinnamic acid and Tom-Kha paste extract determined by FRAP assay. The FRAP assay determines the electron transfer ability of antioxidants (Prior *et al*., 2005), thus, it indicated that these sample's structures were heat stable. Additionally, this present study might point out that thermal processing had more affected on benzoic acid derivatives (gallic acid) than cinnamic acid derivatives (*p*-hydroxycinnamic acid) (Canas *et al*., 2004) structures were related to antioxidant activities. Generally, cinnamic acid derivatives have been resonance stabilization more than benzoic acid derivatives (Shahidi and Naczk, 2004). However, there was no change in antioxidant properties of Tom-Kha paste extract. This might be due to a balance of an increase and a decrease of some antioxidant compounds derived from each ingredient used in the paste.

inulin samples. Therefore, the antioxidants could show their activities even their values were still very low compared to those of antioxidants alone.

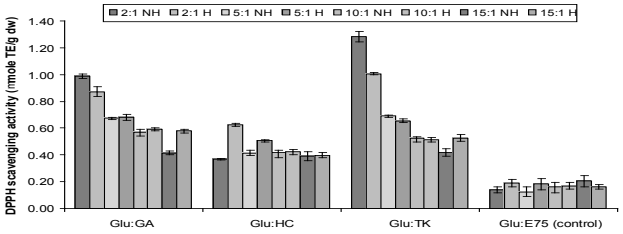

Figure 1. Combined effect of glucose and thermal processing on DPPH scavenging activity of antioxidants. The ratios of glucose: antioxidant = 2:1, 5:1, 10:1 and 15:1. Glu = glucose, GA = gallic acid, HC = *p*-hydroxycinnamic acid, TK = Tom-Kha paste extract, E75 = 75 % ethanol, NH = no heat treatment, H = heat treatment

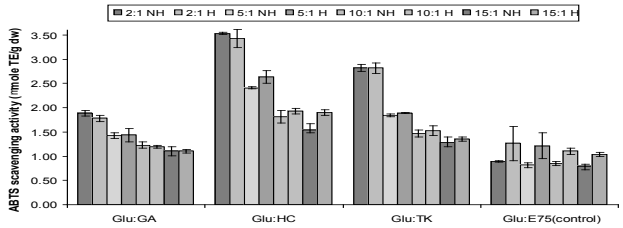

Figure 2. Combined effect of glucose and thermal processing on ABTS scavenging activity of antioxidants. The ratios of glucose: antioxidant = 2:1, 5:1, 10:1 and 15:1. Glu = glucose, GA = gallic acid, HC = *p*-hydroxycinnamic acid, TK = Tom-Kha paste extract, E75 = 75 % ethanol, NH = no heat treatment, H = heat treatment

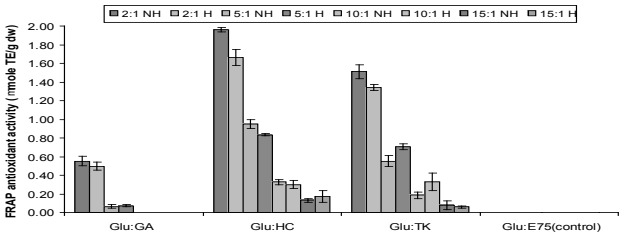

Figure 3. Combined effect of glucose and thermal processing on FRAP antioxidant activity of antioxidants. The ratios of glucose: antioxidant = 2:1, 5:1, 10:1 and 15:1. Glu = glucose, GA = gallic acid, HC = *p*-hydroxycinnamic acid, TK = Tom-Kha paste extract, E75 = 75 % ethanol, NH = no heat treatment, H = heat treatment

Combined effect of carbohydrate and thermal processing on antioxidant activities of antioxidants and Tom-Kha paste extract

In order to elucidate the antioxidant activities of model systems, three antioxidant assays (DPPH, ABTS and FRAP) were still carried out. Result showed that, combined of glucose and thermal processing did not affected antioxidant activities of gallic acid, *p*-hydroxycinnamic acid and Tom-Kha paste extract (Figure 1-3). Comparing data from Table 1-3 with histograms from Figure 1-3 indicated that antioxidant activities of all antioxidants were reduced by any carbohydrate type's addition. It meant that carbohydrate had adverse effect to all antioxidants. However, decreasing of antioxidant activities may be due to dilution and interaction effects. Moreover, it was found that heat treatment might diminish the effect of carbohydrate, particularly in glucose and

Previous results showed that gallic acid was heat labile, however, in mixture systems; at higher glucose ratio (5:1 to 15:1) seemed to prevent degradation of gallic acid from heat treatment. Sucrose decreased antioxidant activity of gallic acid while increased DPPH scavenging activity, ABTS scavenging activity and FRAP antioxidant activities of Tom-Kha paste extract after heat treatment. However, antioxidant activities of *p*-hydroxycinnamic acid were not affected by addition of sucrose and heating process (Figure 4-6). There were some scientific data addressed that sugars and polyhydric alcohols provide antioxidant activity in various systems (Ponginebbi *et al*., 1999; Regoli and Winston, 1999). However, some researchers reported that reducing sugars might act as prooxidants in food emulsions (Yamauchi *et al*., 1984; 1982). Moreover, Yamauchi *et al*. (1982) reported that carbonyl group of sugar

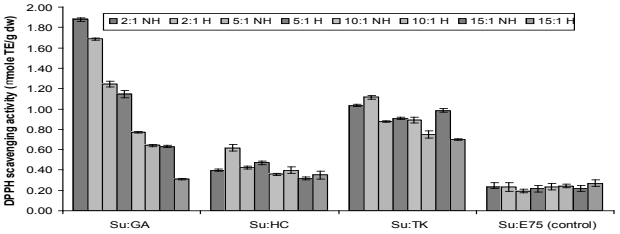

Figure 4. Combined effect of sucrose and thermal processing on DPPH scavenging activity of antioxidants. The ratios of sucrose: antioxidant = 2:1, 5:1, 10:1 and 15:1. Su = sucrose, GA = gallic acid, HC = *p*-hydroxycinnamic acid, TK = Tom-Kha paste extract, E75 = 75 % ethanol, NH = no heat treatment, H = heat treatment

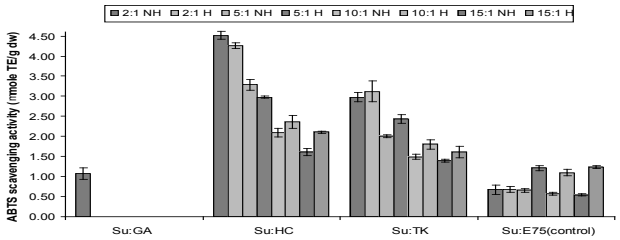

Figure 5. Combined effect of sucrose and thermal processing on ABTS scavenging activity of antioxidants. The ratios of sucrose: antioxidant = 2:1, 5:1, 10:1 and 15:1. Su = sucrose, GA = gallic acid, HC = *p*-hydroxycinnamic acid, TK = Tom-Kha paste extract, E75 = 75 % ethanol, NH = no heat treatment, H = heat treatment

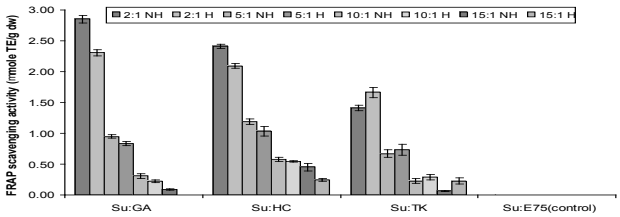

Figure 6. Combined effect of sucrose and thermal processing on FRAP antioxidant activity of antioxidants. The ratios of sucrose: antioxidant = 2:1, 5:1, 10:1 and 15:1. Su = sucrose, GA = gallic acid, HC = *p*-hydroxycinnamic acid, TK = Tom-Kha paste extract, E75 = 75 % ethanol, NH = no heat treatment, H = heat treatment

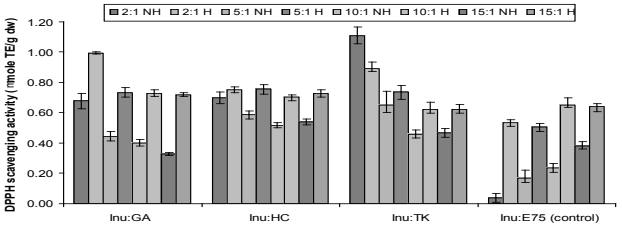

Figure 7. Combined effect of inulin and thermal processing on DPPH scavenging activity of antioxidants. The ratios of inulin: antioxidant = 2:1, 5:1, 10:1 and 15:1. Inu = inulin, GA = gallic acid, HC = *p*-hydroxycinnamic acid, TK = Tom-Kha paste extract, E75 = 75 % ethanol, NH = no heat treatment, H = heat treatment

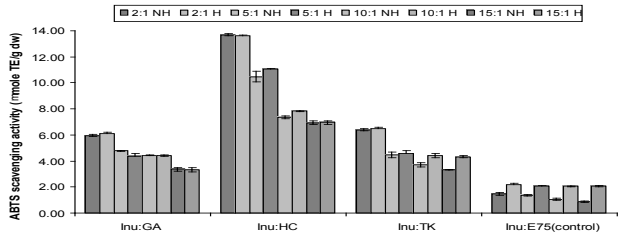

Figure 8. Combined effect of inulin and thermal processing on ABTS scavenging activity of antioxidants. The ratios of inulin: antioxidant = 2:1, 5:1, 10:1 and 15:1. Inu = inulin, GA = gallic acid, HC = *p*-hydroxycinnamic acid, TK = Tom-Kha paste extract, E75 = 75 % ethanol, NH = no heat treatment, H = heat treatment

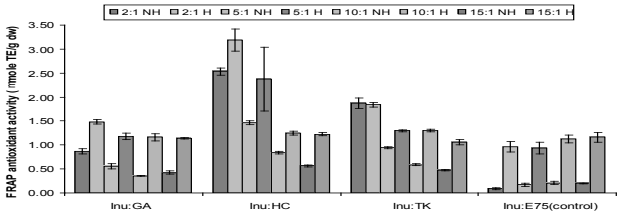

Figure 9. Combined effect of inulin and thermal processing on FRAP antioxidant activity of antioxidants. The ratios of inulin: antioxidant = 2:1, 5:1, 10:1 and 15:1. Inu = inulin, GA = gallic acid, HC = *p*-hydroxycinnamic acid, TK = Tom-Kha paste extract, E75 = 75 % ethanol, NH = no heat treatment, H = heat treatment

accelerated lipid peroxidation and the hydroxyl group of sugars and sugar analogs inhibited the oxidation. Inulin increased antioxidant activities of gallic acid, *p*-hydroxycinnamic acid and Tom-Kha paste extract (Figure 7-9). It indicated that inulin might prevent destruction of phenolic compound from heating. However, increasing of carbohydrate or decreasing of phenolic compounds in the mixture solution led to a decrease of antioxidant activity. Therefore, it could be assumed that high content of carbohydrate may interact with phenolic compounds as π-bond, hydrogen and/or glycosidic bond at hydroxyl group of carbohydrate and carboxyl group of phenolics. Another reason of reducing antioxidant activities in carbohydrate system might be due to masking effect.

Conclusion

Antioxidant activity of each carbohydrate was extremely low compared to that of each antioxidant. Thermal processing reduced antioxidant activities of gallic acid but not Tom-Kha paste extract and *p*-hydroxycinnamic acid. Heating at 121°C for 15 min did not cause any negative effect on antioxidant activities of glucose and sucrose while it could enhance antioxidant activities of inulin. In mixture model systems, antioxidant activities of gallic acid, Tom-Kha paste extract and *p*-hydroxycinnamic acid were not reduced after addition of glucose and inulin as well as heated process. On the other hand, sucrose led to decrease on antioxidant activities of gallic acid. Interesting, addition of inulin and heating did not reduce antioxidant activities of gallic acid, *p*-hydroxycinnamic acid and Tom-Kha. Therefore, using inulin in Tom-Kha soup may function as antioxidants and prevent active compounds from thermal degradation. However, antioxidant activities of Tom-Kha extract *in vivo* system should be further investigated to confirm bioavailability of this soup.

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