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Influence of Heat Treatment on Antioxidant Capacity and Color of Thai Red Curry Paste

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ABSTRACT

Thai red curry paste contains several herbs and spices well known for their health benefits. The objective of this research was to study the effects of heat treatment on the antioxidant capacity (total phenolic content (TPC) and its antioxidant activity) and colors of Thai red curry paste. The paste was subjected to heat treatment at different temperatures (60, 75, 90, 105 and 120°C) and for different times (0, 10, 20, 30, 40, 50 and 60 min). The TPC was determined by the Folin-Ciocalteu method and its antioxidant activity was elucidated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidative power (FRAP) assays and expressed as Trolox equivalent antioxidant capacity (TEAC). Thai red curry paste color was expressed as Hunter *L, a, b* values. Results showed that heat treatment had a significant ($P < 0.05$) effect on the TPC, antioxidant activity and color. The samples treated at 120°C had higher TPC and antioxidant activity than the untreated samples. The color parameter *L* values of red curry paste increased when the sample was heated higher than 60°C, while color parameters *a* and *b* were not different for most of the heated samples.

Keywords: Thai red curry paste, phenolic compounds, antioxidant, color, heating

INTRODUCTION

Thai cuisine is well known for its delicacy, but is also known to have physiological health benefits due to its ingredients of local vegetables, herbs and spices. Thai red curry paste is one of the most famous kinds of curry paste used to enhance several spicy Thai dishes. It is prepared from dried red chili, garlic, shallots, lemon grass, kaffir lime, galangal, coriander seeds, cumin, cardamon and additives, such as salt and sugar, all blended together to obtain a homogeneous orange-red paste. It provides the color, spice taste and authentic fragrance of certain dishes.

With respect to potential health benefits, phenolic compounds in herbs and spices have been found to make a major contribution to human health and multiple positive biological effects, such as antioxidant activity, antimutagenic and/or

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anticarcinogenic activity and anti-inflammatory action (Surh, 2002; Karakaya, 2004; Willcox *et al*., 2004). It has been reported that good sources of phenolic compounds and antioxidant activity can be obtained from the major ingredients of products, such as chili (Materska and Perucka, 2005; Deepa *et al*., 2007), garlic (Leelarungrayub *et al*., 2006), shallots (Fattorusso *et al*., 2002), lemon grass, galangal root (Ly *et al*., 2003; Juntachote *et al*., 2006) and coriander seeds (Wangensteen *et al*., 2004).

Fresh Thai red curry paste in a semi solid form has a short shelf life due to its high moisture content (more than 40%). The growing popularity of Thai food around the world has created the need to preserve this product. The common commercial method used to extend shelf life is thermal processing in either a can or retortable pouch. This packaging is convenient to use and facilitates easy delivery, which is especially beneficial for the export market. It is classified as a low acid canned food due to its pH of 5-6 and a_w greater than 0.85 (USFDA, 2007). For commercial sterilization, high heat treatment using a temperature greater than 100°C is required for product safety, to destroy the harmful bacteria and their spores. Nowadays, heat conditions for commercial sterilization of this product are 116-120°C to target F_0 in 6-12 min.

It is well known that heat can destroy the quality of food attributes, such as color, texture, nutrients and substances beneficial to health. The natural antioxidants contained in foods can be lost to a significant degree during processing using sterilization, pasteurization, dehydration or home handling, as well as during storage, because most bioactive compounds are relatively unstable when subjected to heat (Ewald *et al*., 1999; Hunter and Fletcher, 2002; Kalt, 2005; Roy *et al*., 2007). However, recent studies have shown that heat processing does not necessarily result in a loss of quality and health properties. In some cases, heat treatment produces no change or has an improved effect on the content and activity of naturally occurring antioxidants. For instance, it has been found that the antioxidant activity of holy basil and galangal extracts remained stable even with heating (Juntachote and Berghofer, 2005). It has also been found that heat treatment enhanced the antioxidant activity of blood-orange juice, Shiitake mushrooms and grape seed (Scalzo *et al*., 2004; Choi *et al*., 2006; Kim *et al*., 2006). Moreover, novel compounds having antioxidant activity, such as Millard's reaction products, can be formed as a result of heat treatment (Nicoli *et al*., 1997; Turkmen *et al*., 2006).

Beside the health benefits, color is an important attribute which enhances the quality of Thai red curry paste. Maintaining the natural color in thermally processed paste has been a major challenge. The orange-red color of the paste has been related to the carotenoid pigments (mainly capsanthin and capsorubin) occurring exclusively in red chilli. The red pigments are accompanied by other xanthophylls and carotenes, such as zeaxanthin, β-cryptoxanthin, violoxanthin, antheraxanthin and β-carotene. The carotenoid pattern and the pigment concentrations vary within a wide range depending on cultivars and the ripening stage (Matsufuji *et al*., 1998; Márkus *et al*., 1999). During processing, not only does the non-enzymatic browning reaction contribute to the formation of brown pigment, but pigment destruction has also been found. It has been reported that the pigment degradation of visual color (represented by the Hunter L^*a^*b values) and ΔE of red chilli puree both follow a first-order kinetic reaction during thermal processing and that the dependence of the rate constants for both Hunter L^*a^*b values and ΔE on temperature follows the Arrhenius relationship (Ahmed *et al*., 2002).

The challenge facing red curry paste producers is to produce a product which has health benefits, is visually appealing and has a safe, long shelf life. In order to optimize thermal processing

to improve the canned and pouched Thai red curry paste product, it is essential to acquire data on the correlation between temperature and heating time and on changes in target substances during heating. The objective of this study was to evaluate the effect of heat treatment on the changes in the total phenolic content (TPC), antioxidant activity and color of Thai red curry paste.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu phenol reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tripyridyl-S-triazine), gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), sodium carbonate and ferric chloride were sourced from Sigma-Aldrich Chemical Co., USA. Other common reagents were of analytical grade.

Raw materials

The Thai red curry paste used was produced by Namprick Maesri Ltd., Partnership (245 Petkasem Road, Nakornpathum, Thailand). The ingredients of this product are dried red chili (35%), garlic (23%), shallots (20%), salt (7%), lemongrass (6%), sugar (3%), kaffir lime (2%), galangal (1%) and spice (1%; coriander seed, cumin and cardamom). The moisture content, a_w and pH values of Thai red curry paste were 69.88% (dry basis), 0.883 and 5.16, respectively. About 15 g of Thai red curry paste was packed in a retort pouch (PETsioX12/DL/Ny15/DL/cpp70μ) size 150 × 190 mm^2 and the thickness of samples was 0.7 mm. Packed samples were stored at -60°C until used in the experiments.

Heat treatments

The samples were thawed to room temperature and then covered with two sheets of stainless steel size 16 × 16 cm^2, with thickness 2 mm, after which they were heated in an oil bath at five different temperatures (60, 75, 90, 105 and 120°C) for seven different holding times (0, 10, 20, 30, 40, 50 and 60 min), with all treatments repeated in triplicate. The heated samples were cooled rapidly to room temperature and stored at -60°C until analysis.

Phenolic extract preparation

One gram samples (dry basis) were homogenized with 20 mL of 80% ethanol for 10 min at room temperature and adjusted to 25 mL with 80% ethanol and then centrifuged with a benchtop centrifuge (Allegra X-12R, Beckman Coulter, Inc. USA) at 8,000 rpm, 4°C for 10 min. After centrifugation, the supernatant was separated from the residue and stored at -4°C until analysis.

Total phenolic contents determination

The TPC of each Thai red curry paste sample was determined using the Folin-Ciocalteu method, described by Choi *et al*. (2006), with some modifications.

Paste extracts of 0.5 mL were added to test tubes followed by 9.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent and 2 mL of 10% sodium carbonate solution. The contents of the test tubes were mixed thoroughly. After standing for 1 h at room temperature, the absorbance was measured at 730 nm with a UV-visible spectrophotometer (SHIMADZU 1700, Japan). The results were expressed as milligram gallic acid equivalent/gram dry matter.

DPPH radical scavenging activity assay

The scavenging activity of the red curry paste extracts on the DPPH radical was measured according to the method described by Choi *et al*. (2006), with some modifications. Red curry paste extract (0.4 mL) was mixed with 5 ml of 40% ethanol solution and 0.6 mL of 0.8 $mmolL^{-1}$ of DPPH solution. The mixture was shaken vigorously and left to stand for 30 min under subdued light. The absorbance was measured at

517 nm with a UV-visible spectrophotometer. The antioxidant capacity, based on the DPPH radical scavenging activity of the extract, was expressed as milligram Trolox equivalen/gram dry matter.

Ferric reducing antioxidative power (FRAP) assay

The FRAP of the Thai red curry paste extracts was measured according to the method of Wong *et al*. (2006), with some modifications.

FRAP reagent consists of 10 mmolL^{-1} 2,4,6-tripyridyl-S-triazine(TPTZ) (Sigma-Aldrich Chemical Co., USA.) in 40 mmolL^{-1} HCl, 20 mmolL^{-1} $FeCl_3.6H_2O$, 0.1m molL^{-1} sodium acetate buffer, pH 3.6 in the ratio of 1:1:10. Red curry paste extracts (0.1 mL) were added to 3 mL of FRAP reagent and mixed thoroughly. After standing for 8 min at room temperature, the absorbance was measured at 593 nm with a UV-visible spectrophotometer. The results were expressed as milligram Trolox equivalent/gram dry matter.

Color measurements

Visual color was measured using a Hunter colorimeter (TRI-STIMULUS model Color J.C.801, Japan) based on three color co-ordinates, namely *L* (brightness), *a* (green-red) and *b* (blue-yellow). The instrument (45°/0° geometry, D25 optical sensor, 10° observer) was calibrated against a standard white tile ($L = 90.55$, $a = -0.71$, $b = 0.39$). A glass cell containing the red curry paste was placed above the light source and post-processing *L*, *a*, and *b* values were recorded.

Experimental design and data analysis

A completely randomized design (CRD) was used to determine the effect of heat treatments on the TPC, antioxidant activity and color of the red curry paste. The results were reported as means±standard deviation (SD). The results were analyzed with one-way analysis of variance and correlations tested with the SPSS computer software, version 11 with a significance level of ($P < 0.05$). Tukey's HSD (honestly significant differences) test was used for discrimination among means. Heat treatments were used as the independent variables, while the TPC, antioxidant activity and color values were the dependent variables or responses. Pearson's correlation coefficient was used to analyze any correlation between the variables.

RESULTS AND DISCUSSION

Effect of heat treatment on total phenolic content (TPC)

The ingredients of Thai red curry paste are a mixture of herbs and spices, which are good sources of phenolic compounds. Therefore, it is important to consider the effect of heat treatment on the TPC of the red curry paste extracts. The effects of heat treatment on the TPC are shown in Figure 1. Heat treatment had a significant ($P < 0.05$) effect on TPC. The unheated sample contained 2.55 mg of gallic acid equivalent/gram dry matter. After heat treatment there was 2.59-3.02 mg of gallic acid equivalent/gram dry sample. When the pastes were heated at temperatures of 60-105°C, the TPC was not significantly different from unheated samples. There was a significant ($P < 0.05$) difference when the pastes were heated at 120°C for 30, 40, 50 and 60 min, resulting in increased TPC values by 13.78, 18.31, 19.60 and 20.15%, respectively, compared with the unheated sample. This might be attributed to the increased extractability of phenolic compounds due to the disruption of plant cell walls during high heat treatment, which might cause phenolic compounds to be released more easily than in raw materials and with lower heat treatment. These results were consistent with those of Scalzo *et al*. (2004), who reported that thermal treatment generally induced an increase in the main phenolic substances of orange juice, such as anthocyanins and total cinnamates, and those of Choi *et al*. (2006), who

reported that heat treatment of Shiitake mushrooms increased the overall content of free polyphenolic and flavonoid compounds. They suggested that bound polyphenolic and flavonoid compounds could be liberated by heat treatment. Kim *et al*. (2006) also reported an increase in the TPC in heated grape seed due to the liberated phenolic compounds.

Effect of heat treatment on antioxidant activity, DPPH radical scavenging and FRAP assays

The effects of heat treatment on the antioxidant activity of Thai red curry paste determined using the DPPH radical scavenging and FRAP assays are shown in Figures 2 and 3, respectively.

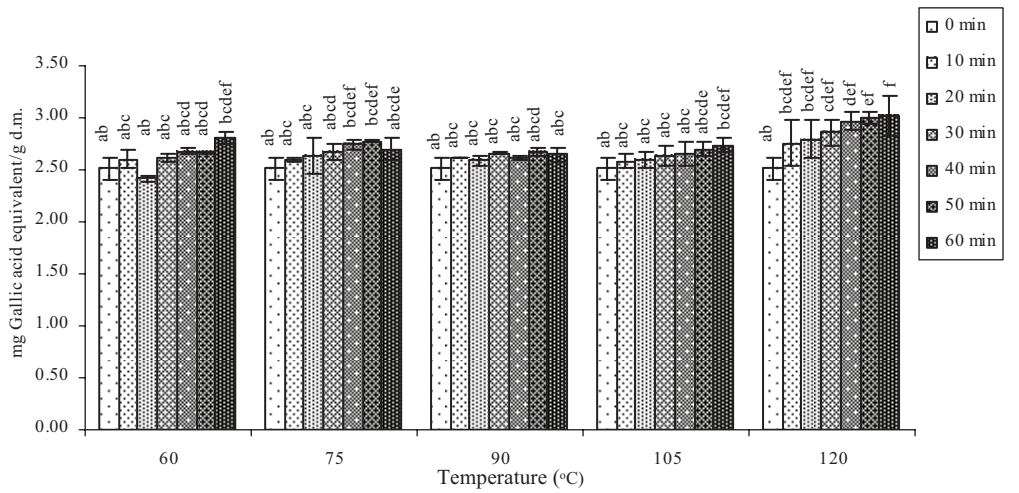

Figure 1 Mean effect of heat treatment on TPC of Thai red curry paste (n=3). ($\perp$ $\top$ represent the respective lower and upper standard deviation from the mean value.) $^{a\text{-}f}$ = Different letters above the bars indicate a significant ($P < 0.05$) difference.

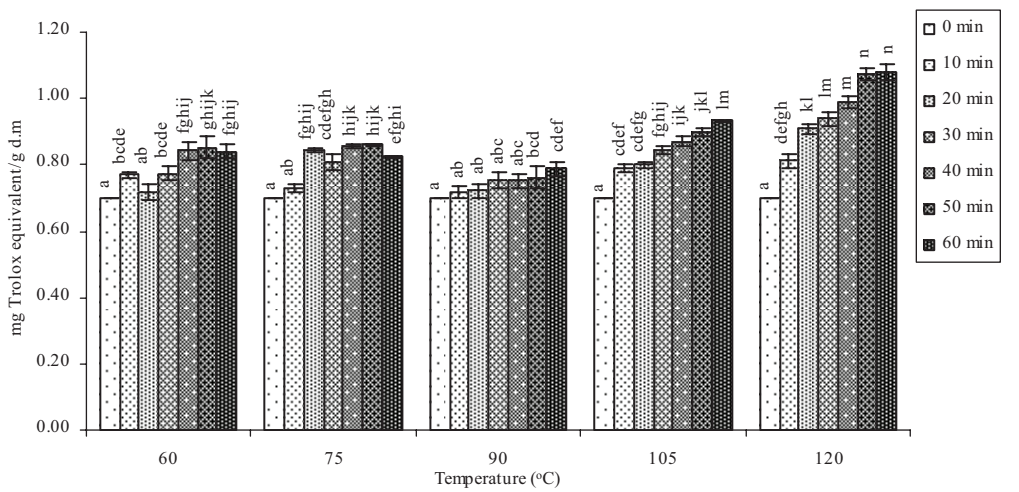

Figure 2 Mean effect of heat treatment on $TEAC_{DPPH}$ of Thai red curry paste (n=3). ($\perp$ $\top$ represent the respective lower and upper standard deviation from the mean value.) $^{a\text{-}n}$ = Different letters above the bars indicate a significant ($P < 0.05$) difference.

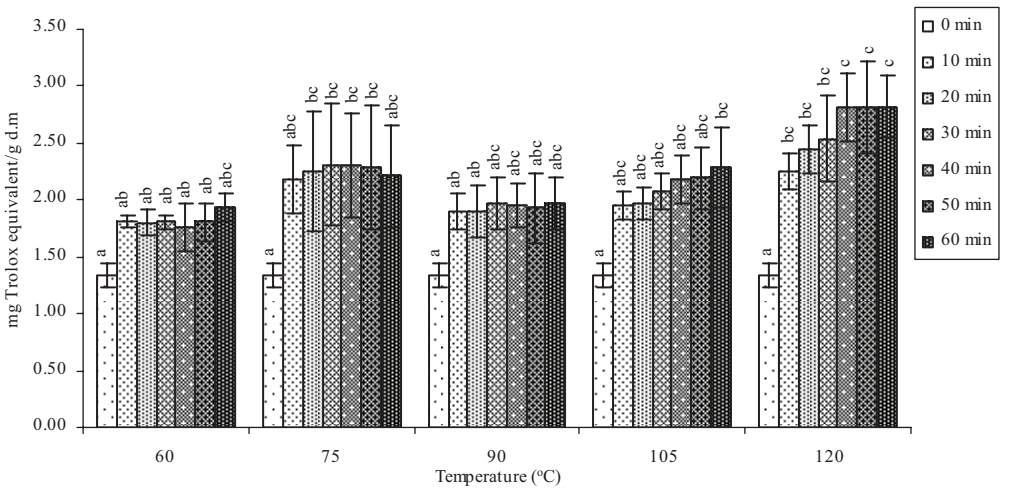

Figure 3 Mean effect of heat treatment on $TEAC_{FRAP}$ of Thai red curry paste (n=3). ($\perp\ \top$ represent the respective lower and upper standard deviation from the mean value.) $^{a\text{-}c}$ = Different letters above the bars indicate a significant ($P < 0.05$) difference.

Heat treatment had a significant ($P < 0.05$) effect on radical scavenging and ferric ion reducing activities. $TEAC_{DPPH}$ and $TEAC_{FRAP}$ values of unheated samples were 0.68 and 1.33 mg Trolox equivalent/gram dry matter, respectively, while after heat treatment they were 0.72-1.08 and 1.76-2.82 mg Trolox equivalent/gram dry matter, respectively. Most heated samples had a significant ($P < 0.05$) increase in the radical scavenging activities, except for the paste samples heated at 60°C for 10-20 min, 75°C for 10 min, and 90°C for 10-50 min. However, there was a significant ($P < 0.05$) increase in the ferric reducing activities when the pastes were heated at 105°C for 60 min and 120°C for 30, 40, 50 and 60 min compared with the unheated samples. The $TEAC_{FRAP}$ values were consistently higher than those obtained for $TEAC_{DPPH}$. Similar results were reported by Wong *et al*. (2006), who found that the DPPH radical scavenging activities of 25 edible tropical plants, expressed as Trolox equivalent, were lower than their corresponding ferric reducing activities. They suggested that the lower $TEAC_{DPPH}$ values of plant extracts could have been due to the presence of antioxidant compounds not reactive towards DPPH. The antioxidant compounds, such as polyphenols, may be more efficient reducing agents for ferric ions, but some may not scavenge DPPH radicals as efficiently, due to steric hindrance.

Phenolic compounds have been reported to be responsible for the antioxidant activities of botanical extracts. Both DPPH radical scavenging and FRAP assays have been used to measure antioxidant activity and the results of DPPH radical scavenging and FRAP assays should be correlated with those of TPC. As seen in Figures1-3, the trend of the effect of heat treatment on antioxidant activity evaluated using FRAP assay was similar to the effect on TPC. The effect of heat treatment on TPC, DPPH radical scavenging activity and FRAP values was shown clearly when pastes were heated at 120°C and the effect of heating time evaluated using DPPH radical scavenging assay was also shown clearly when samples were heated at 120°C. The results indicated that the level of TPC may contribute to the antioxidant activity of Thai red curry paste. This result was confirmed (Table 1) with a strong correlation between the values of TPC, $TEAC_{DPPH}$ and $TEAC_{FRAP}$ (P <

Table 1 Correlation coefficients for relationships between TPC and antioxidant activity.

	TPC	$TEAC_{DPPH}$	$TEAC_{FRAP}$
TPC	-	0.830**	0.830**
$TEAC_{DPPH}$	0.830**	-	0.797**
$TEAC_{FRAP}$	0.830**	0.797**	-

** = Correlation is significant at the 0.01 level (2-tailed) (n=105).

0.01). Velioglu *et al*. (1998) indicated there was a positive relationship between TPC and the antioxidant activity of plant material, such as flaxseed products and cereal products, but the relationship between phenolics and antioxidant activity for anthocyanin-rich materials and for medical plants was not significant.

Zheng and Wang (2001) suggested that not only the level of antioxidants, but also a synergy occurring between them and the other plant constituents, might influence the level of antioxidant ability of plant extracts. Tomaino *et al*. (2005) had a similar suggestion concerning natural antioxidants, such as essential oils extracted from basil, cinnamon, clove, nutmeg, oregano and thyme, which exhibited ability to protect α-tocopherol (contained in virgin olive oil) against thermal oxidative degradation.

Another reason for the improved antioxidant activity of Thai red curry paste could be the formation of novel compounds having antioxidant activity during the heat treatment or thermal processing. In the present study, non-enzymatic browning reaction products might have been formed during the heat treatment resulting in antioxidant activity (Nicoli *et al*., 1999; Choi *et al*., 2006).

In the present study, a strong correlation was found between the values of $TEAC_{DPPH}$ and $TEAC_{FRAP}$, which indicated that the compounds present in the aqueous extracts capable of reducing DPPH radicals were also able to reduce ferric ions. A similar result was found by Wong *et al*. (2006); they suggested that the reducing activity of phenolic compounds seemed to be an important factor dictating the free radical scavenging capacity of these compounds.

Effect of heat treatments on color

Besides bioactive compounds and their antioxidant activity, color is a predominant parameter for Thai red curry paste. The color parameter *L*, *a*, and *b* values of fresh samples were 29.14, 35.94 and 48.74, respectively, which indicated that the major color of red curry paste is a mixture of yellow and red, due to the presence of carotenoid from the red chili (Ahmed *et al*., 2002). Heat treatments had a significant ($P < 0.05$) effect on the color parameters *L*, *a*, and *b* values and the effects of heat treatment are shown in Figures 4, 5 and 6, respectively.

These figures show that the variation found in the color parameter *L*, *a*, and *b*, values of the paste samples was not significantly large enough to show differences in the color. However, some color parameter changes due to the effect of heat treatment were found. Heat treatment had a significant ($P < 0.05$) effect on *L*, with the color parameter *L* values of samples heated to temperatures higher than 60°C being higher than in unheated samples, while values for color parameters *a* and *b*, most of heated samples were not different. It has been reported by Ahmed *et al*. (2002) that any change in values for color parameters *a* and *b* was associated with a simultaneous change in the value of color parameter *L*. As shown in Table 2, the relationship between *L* and *a* was negative, while the relationship between parameters *L* and *b* was not significant.

Changes in the *L*, *a* and *b* values could have resulted from the effect of both pigment

destruction and non-enzymatic browning. From the results, the increase in the *L* values of heat-treated samples might have been due to the destruction of carotenoid during heating. It has been reported that the pigment degradation of visual color (represented by the *L*a*b* values of red chili puree) during thermal processing followed the first-order kinetic reaction and the dependence on temperature of the rate constants for *L*a*b* follows the Arrhenius relationship (Ahmed *et al*., 2002). The pigment degradation contributed to fading of the product color so, the *L* value increased.

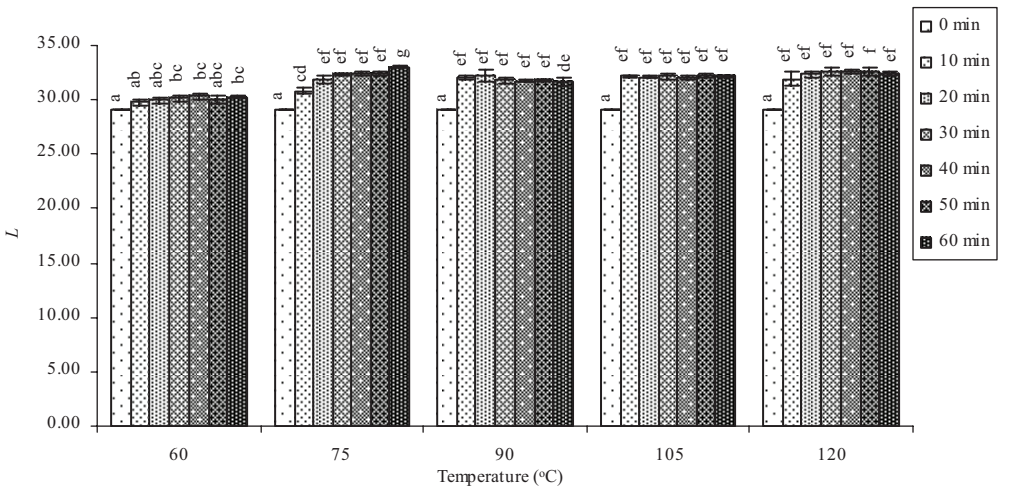

Figure 4 Effect of heat treatment on color parameter *L* of Thai red curry paste (n=3). (⊥ ┬ represent the respective lower and upper standard deviation from the mean value.) [a-f] = Different letters above the bars indicate a significant ($P < 0.05$) difference.

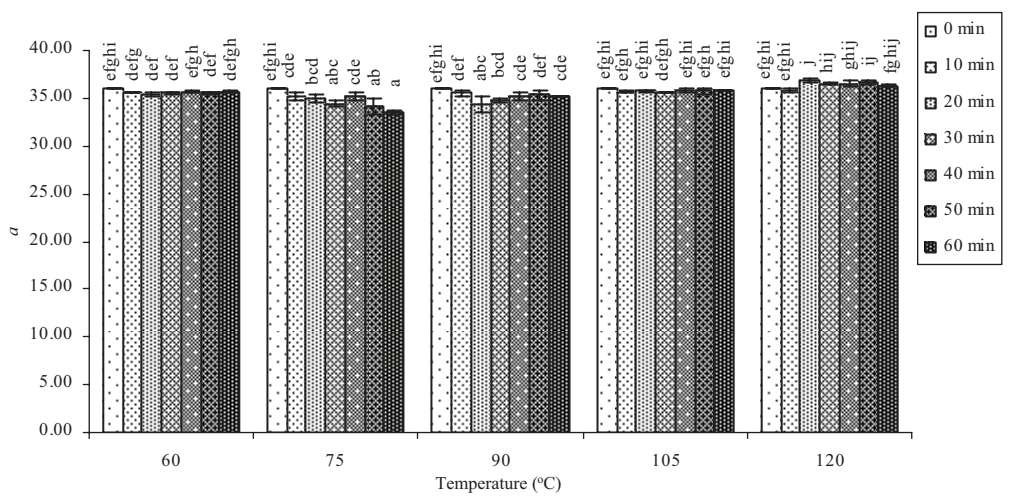

Figure 5 Effect of heat treatment on color parameter *a* of Thai red curry paste (n=3). (⊥ ┬ represent the respective lower and upper standard deviation from the mean value.) [a-j] = Different letters above the bars indicate a significant ($P < 0.05$) difference.

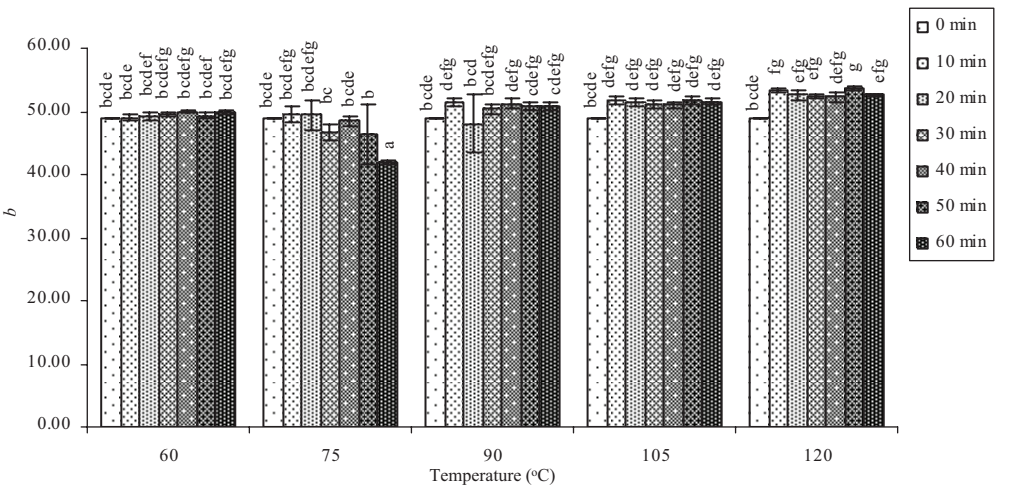

Figure 6 Effect of heat treatment on color parameter *b* of Thai red curry paste (n=3). (⊥ ⊤ represent the respective lower and upper standard deviation from the mean value.) $^{a-g}$ = Different letters above the bars indicate a significant ($P < 0.05$) difference.

Table 2 Correlation coefficients for relationships between color parameters.

	L	*a*	*b*
L	-	-0.252**	0.089
a	-0.252**	-	0.778**
b	0.089	0.778**	-

** = Correlation is significant at the 0.01 level (2-tailed) (n=105).

CONCLUSION

The heat treatments had a significant effect on the TPC, antioxidant activity and color. The study showed that heat treatment at 120°C enhanced the antioxidant activity of Thai red curry paste. Heat-treated curry paste at temperatures higher than 60°C had higher *L* values than in unheated samples. However, this research was limited in its scope, as it was conducted under laboratory conditions. Therefore, further study is necessary to validate the results in an industrial context.

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