

Influence of Thermal Sterilization on Antioxidant Capacity and Total Phenolics of Spicy Thai Foods

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ABSTRACT

Two different styles of spicy Thai food were developed. The first group were sauces for ready-to-cook products—‘Mee Kathi’ sauce (MeKa), ‘Phat Mee Paktai’ sauce (PMeP), ‘Phat Khimao’ sauce (PKhi), ‘Kaeng Khua’ sauce (KK), ‘Om Isan’ sauce (OmIs), ‘Chu Chi’ sauce (CC) and ‘Homok’ sauce (HoM). The second group was ready-to-eat foods—‘Khao Soi Nuea’ (KSoi), ‘Mu Phat Phrik Khing’ (PPK) and ‘Khua Kling Mu’ (KKM). These foods and sauces were each packed in a stand-up type aluminum pouch and were heated at 120 °C for an appropriate time of 15, 25 or 30 min. The antioxidant capacity of the methanol extracts obtained from the sterilized food samples ranged from 5.77 to 42.11 mg vitamin C equivalent per 100 g and the total phenolic content of these foods ranged from 27.73 to 145.93 mg gallic acid equivalent per 100 g. Five selected foods, MeKa, KSoi, PKhi, PPK and KKM, were studied for the influence of heat on natural antioxidants to scavenge the 1, 1-diphenyl-2-picrylhydrazyl radical and for changes of the phenolic content. The results showed that the antioxidant capacity in KSoi, KKM, MeKa and PPK was enhanced by amounts of 3–113% and the phenolic content was increased in all products, by amounts of 4–52%.

Keywords: Thai foods, total phenolics, DPPH, scavenging capacity, processed foods

INTRODUCTION

Thai food is one of the popular ethnic restaurant cuisines consumed worldwide due to its harmonious blend of flavors, aromas and health benefits. Numerous herbs and spices, usually used to enhance and complement the flavors of a wide variety dishes, are an excellent source of phenolic compounds which have been reported to possess high antioxidant activity (Murakami *et al.*, 1995). Culinary herbs and spices such as lemon grass, ginger, galangal, sweet basil and holy basil are widely used in Thai cooking and have shown antioxidant activity (Javanmardi *et*

al., 2003; Jayasinghe *et al.*, 2003; Surveswaran *et al.*, 2007).

The health benefits and the rising need for convenience foods are two major awareness factors of consumers as they eat alone more often and spend less time preparing food. Ready-to-eat meals are an ideal concept to satisfy the busy lifestyle many people have nowadays. In Thailand, there are four styles of ethnic food grouped by geography and ethnology with the demand for ethnic foods increasing. This information has inspired the idea of developing processed local foods. However, thermal treatment has been observed to affect the antioxidant activity and

the phenolic content of vegetables (Gazzani *et al.*, 1998; Ismail *et al.*, 2004; Turkmen *et al.*, 2005; Roy *et al.*, 2007). Jiratanan and Liu (2004) reported thermal processing may enhance, reduce or cause no change in the total antioxidant activity. Other research indicated that processing caused no change to the antioxidant potential of fruit and vegetables or enhanced it due to the improvement of the antioxidant properties of naturally occurring compounds or the formation of novel compounds such as Maillard reaction products having antioxidant activity (Nicoli *et al.*, 1997; Manzocco *et al.*, 2001). Dewanto *et al.* (2002a, b) reported that thermal processing increased the antioxidant activity of tomatoes and sweet corn.

Thai foods, in ready-to-eat or ready-to-cook form were developed in a pilot plant as part of the current study, to make new food prototypes and develop the transfer technique to the food factory. In view of the considerable advancement in technological processes, the adoption of modified recipes and the development of several new products to provide variety in the diet, it is necessary to evaluate their antioxidant activity.

To date, little information is available on the effects of thermal processing on the functionality of heat-treated foods. Therefore, the objective of this research was to investigate the effects of thermal processing on the total phenolic content and the antioxidant activity of heat-treated foods.

MATERIALS AND METHODS

Materials

The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteu reagent was obtained from Merck (Darmstadt, Germany). Ascorbic acid was purchased from Fisher Scientific UK Ltd., Loughborough UK. All other chemicals used were of analytical grade. Herbs and spices were purchased from local

markets in Bangkok, Thailand.

Preparation of foods

The selected Thai foods in the present study were 'Mee Kathi' (stir fried rice noodle with coconut milk), 'Phat Mee Paktai' (stir fried noodle with spicy coconut milk), 'Phat Khimao' (spicy fried noodles with holy basil), 'Kaeng Khua' (meat in spicy coconut milk with dried fish), 'Om Isan' (mix vegetables in fermented fish spicy soup), 'Homok' (steamed curried fish), 'Chu Chi' (fish or shrimp in thick red curry), 'Khao Soi Nuea' (wheat noodles with beef curry), 'Mu Phat Phrik Khing' (spicy stir fried pork) and 'Khua Kling Mu' (stir fried pork with turmeric). They were developed into two different styles. The first group was as a sauce for ready-to-cook products—Mee Kathi sauce (MeKa), Phat Mee Paktai sauce (PMeP), Phat Khimao sauce (PKhi). Kaeng Khua sauce (KK), Om Isan sauce (OmIs), Chu Chi sauce (CC) and Homok sauce (HoM). The second group was as ready-to-eat food—Khao Soi Nuea (KSoi), Mu Phat Phrik Khing (PPK) and Khua Kling Mu (KKM). The ingredients of each product are given in Table 1.

Both the sauces and ready-to-eat food were packed separately in stand-up laminated aluminum pouches (PET12/NY15/AL 9/PP80, 120 × 180 × 35 mm), sealed with HENKOVAC, and sterilized by a hot water spray retort (Model RCS-60 SPXTG; Hisaka Works Ltd.; Osaka, Japan) at 120 °C but with different duration times: sauces of MeKa, PMeP, PKhi, KK, CC and HoM were sterilized for 25 min; OmIs sauce was processed for 15 min; and KSoi, PPK and KKM were heated for 30 min.

The effects of thermal processing on the antioxidant capacity and total phenolic content were studied on five selected products—namely, MeKa, KSoi, PKhi, PPK and KKM. Each product was sampled before and after the sterilization process. Samples from the 10 sterilized products: MeKa, PMeP, PKhi, KK, OmIs, HoM, CC, KSoi,

Table 1 List of ingredients in sterilized food products. (Proportions of major ingredients calculated based on weight).

Food type	Major ingredients (%)	Herbs and spices (%)
<i>Ready-to-cook foods</i>		
Mee Kathi sauce (MeKa)	dried chili (0.1), shallot (7.2), coriander root (0.7), pepper (0.2), tamarind juice (5.7), fermented soybean (7.2), pork (7.2), prawn (10.7), tofu (7.2), palm sugar (10.0), salt (0.9), coconut milk (25.0), water (17.9)	13.9
Phat Khimao sauce (PKhi)	bird chili (1.9), chili (3.8), garlic (9.4), pepper (0.1), holy basil (6.6), vegetable oil (9.4), fish sauce (3.8), soy sauce (4.7), sugar (3.8), water (56.6)	21.8
Phat Mee Paktai sauce (PMeP)	dried chili (1.4), dried bird chili (0.2), shallot (9.3), shrimp paste (1.2), palm sugar (14.0), fish sauce (3.5), salt (0.7), coconut milk (46.5), water (23.3)	10.9
Kaeng Khua sauce (KK)	dried chili (0.9), garlic (2.3), shallot (3.5), lemon grass (1.4), galangal (0.5), kaffir lime peel (0.2), kaffir lime leaves (0.2), salt (0.6), shrimp paste (0.6), roasted dried fish (0.8), fish sauce (3.5), palm sugar (1.4), coconut milk (57.7), water (26.5)	9.0
Om Isan sauce (OmIs)	bird chili (0.5), garlic (0.7), shallot (4.8), lemon grass (2.1), fermented fish (4.3), fish sauce (1.1), roasted glutinous rice (1.4), stock soup (85.1)	8.1
Homok sauce (HoM)	dried chili (2.0), dried bird chili (0.3), garlic (6.6), shallot (4.9), lemon grass (2.0), galangal (0.7), coriander root (0.8), fingerroot (2.5), pepper (0.2), kaffir lime peel (0.5), kaffir lime leaves (0.3), shrimp paste (1.6), fish sauce (6.6), palm sugar (2.5), coconut milk (67.9), salt (0.7)	20.8
Chu Chi sauce (CC)	dried chili (0.9), dried bird chili (0.2), garlic (2.8), shallot (3.2), lemon grass (0.9), galangal (0.4), coriander root (0.5), kaffir lime peel (0.2), kaffir lime leaves (0.4), shrimp paste (0.7), salt (0.4), pepper (0.1), palm sugar (1.8), fish sauce (2.3), coconut milk (50.5), water (34.7)	9.6

Food type	Major ingredients (%)	Herbs and spices (%)
<i>Ready-to-eat foods</i>		
Khao Soi Nuea (KSoi)	dried chili (0.4), dried bird chili (0.1), garlic (0.9), shallot (2.0), coriander root (0.4), ginger (0.7), coriander seeds (0.1), cumin (0.1), curry powder (0.2), beef (21.9), sugar (1.1), soy sauce (1.1), fish sauce (0.4), salt (0.5), coconut milk (17.5), water (52.5)	4.9
Mu phat phrik khing (PPK)	dried chili (1.9), dried bird chili (0.6), garlic (4.6), shallot (5.7), lemon grass (1.7), galangal (0.7), kaffir lime peel (0.5), kaffir lime leaves (0.5), salt (0.6), shrimp paste (0.6), pork (42.9), vegetable oil (5.7), palm sugar (4.0), fish sauce (1.4), water (28.6)	16.2
Khua kling mu (KKM),	bird chili (2.5), dried chili (1.0), garlic (3.2), shallot (3.2), lemon grass (2.5), galangal (1.0), turmeric (1.3), kaffir lime leaves (0.9), white pepper (0.3), black pepper (0.3), pork (63.0), salt (0.6), shrimp paste (2.5), vegetable oil (3.2), fish sauce (1.3), sugar (0.8), water (12.4)	16.3

PPK, KKM and the non-sterilized samples of five products: MeKa, KSoi, PKhi, PPK and KKM were homogenized (in three replicates) and kept at -20 °C for further analysis.

Sample extraction

Each homogenized sample was extracted in 100% methanol at room temperature. The extracting ratio of the sample to methanol was 1:5 (weight by volume). The supernatants were stored in capped bottles and kept at -20 °C until further use to determine the antioxidant capacity, total phenolic content and high-performance liquid chromatography (HPLC) analysis.

Antioxidant capacity assay

DPPH scavenging activity was determined according to a method of Ohnishi *et al.* (1994). The percentage of scavenging activity was calculated

from Equation 1:

$$(1 - X / C) * 100 \quad (1)$$

where X = the absorbance of the sample extract and C = the absorbance of methanol. Vitamin C (ascorbic acid) was used as the standard, and the antioxidant capacity was expressed as milligrams of vitamin C equivalent (VCE) per 100 g fresh weight.

Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu reagent, adapted from Singleton and Rossi (1965). Gallic acid was used as the standard. The results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g fresh weight.

HPLC analysis

An HPLC system comprising a vacuum

degasser, quaternary pump, autosampler, thermostated column compartment, and UV detector was used. A 100 mm x 4.6 mm inside diameter TSKgel Super-ODS (Tosoh Co.; Tokyo, Japan) was maintained at 40 °C. The analytical condition was monitored through a linear gradient using 0.5% formic acid and acetonitrile from 10% to 70% for 30 min, eluted at a flow rate of 1 mL min⁻¹ and detected at 280 nm.

RESULTS AND DISCUSSION

Antioxidant capacity and total phenolic content of sterilized food products

The plant content in the studied foods ranged from 4.9% in KSoi to 21.8% in PKhi (Table 1). Influences on the antioxidant capacity and total phenolic content came only from the fresh herbs and dried spices, unlike previous studies that contained vegetables which could play an important role (Tangkanakul *et al.*, 2006, 2009, 2011).

Methanol extracts prepared from the sterilized food were determined for their antioxidant capacity and total phenolic content

as shown in Table 2. These foods exhibited antioxidant capacity ranging from 5.77 to 42.11 mg VCE per 100 g. PKhi and PPK exhibited comparable high antioxidant capacity with 42.11 and 40.16 mg VCE per 100 g, respectively, while OmIs had the lowest capacity with 5.77 mg VCE per 100 g food.

Correlation between the plant content and antioxidant capacity was relatively low ($R^2 = 0.650$). The foods that caused such low correlation were HoM and KSoi. HoM exhibited lower antioxidant capacity than expected. Various herbs used in HoM were similar to CC, except for fingerroot which presented only in HoM. Data from the individual herb analyses on antioxidant capacity showed that one hundred grams of fresh fingerroots provides 0.06 g VCE (Tangkanakul *et al.*, 2011) which suggests that fingerroot did not give an antioxidant capacity benefit to HoM. For KSoi, the antioxidant capacity was greater than expected which may have been derived from the added dried spices, cumin and curry powder as the antioxidant capacities of cumin and curry powder were 302 and 236 mg VCE per 100 g, respectively (Tangkanakul *et al.*, 2009).

Table 2 Antioxidant capacity and total phenolic content (mean \pm SD) in selected sterilized foods (n = 3).

Food	Antioxidant capacity (mg VCE per 100 g food)	Total phenolic content (mg GAE per 100 g food)
MeKa	20.11 \pm 0.69	108.58 \pm 0.21
PKhi	42.11 \pm 1.29	80.52 \pm 2.47
PMeP	28.11 \pm 0.17	95.78 \pm 0.62
HoM	28.20 \pm 0.13	105.67 \pm 2.26
CC	17.39 \pm 0.28	72.97 \pm 0.69
KK	13.31 \pm 0.80	55.23 \pm 0.33
OmIs	5.77 \pm 0.16	27.73 \pm 0.41
KSoi	15.43 \pm 1.32	60.47 \pm 2.19
PPK	40.16 \pm 1.47	145.93 \pm 1.08
KKM	31.38 \pm 0.69	131.10 \pm 0.41

MeKa = Mee Kathi sauce; PMeP = Phat Mee Paktai sauce; PKhi = Phat Khimao sauce; KK = Kaeng Khua sauce; OmIs = Om Isan sauce; CC = Chu Chi sauce; HoM = Homok sauce; KSoi = Khao Soi Nuea; PPK = Mu Phat Phrik Khing; KKM = Khua Kling Mu.

VCE = Vitamin C equivalent; GAE = Gallic acid equivalent.

The total phenolic content of all products was in the range 27.73–145.93 mg GAE per 100 g food (Table 2). Four kinds of foods—PPK, KKM, MeKa and HoM—showed high total phenolic content with values greater than 105 GAE per 100 g food. The phenolic contents of PPK, KKM, MeKa and HoM were 1.3–1.8 times higher than PKhi. Nevertheless, PKhi showed the highest antioxidant capacity with 42.11 mg VCE per 100 g food, even though its total phenolic content was not as high as in the other four foods. This may have resulted from the presence of compounds with antioxidant activity that were not phenolic compounds or the presence of phenolic antioxidants having strong radical scavenging activity. When a correlation between the antioxidant capacity and the phenolic content of the ten tested foods was considered, a low level of linear correlation ($R^2 = 0.581$) was found which increased to 0.905 when the PKhi data were excluded. Thus it was probable that PKhi contained phenolic antioxidants having strong radical scavenging activity.

The major herb ingredients in PKhi were garlic and holy basil (Table 1). One hundred grams each of fresh garlic and holy basil leaves provides total phenolics of 0.08 and 0.55 g GAE, respectively, with antioxidant capacity as measured by 0.01 and 0.25 g VCE, respectively (Tangkanakul *et al.*, 2011). This demonstrated that the substances in the holy basil rather than in the garlic play an important role as strong antioxidants. The phenolic compounds found in holy basil are isothymusin, ursolic acid, carnosic acid, eugenol, sinapic acid and rosmarinic acid (Kelm *et al.*, 2000; Hakkim *et al.*, 2007). Chen and Ho (1997) examined the scavenging effects of several antioxidants on the DPPH radical and revealed that rosmarinic acid possessed the best ability when compared with caffeic acid, chlorogenic acid, α -tocopherol and ferulic acid.

Influence of thermal processing on antioxidant capacity and total phenolic content

MeKa, KSoi, PKhi, PPK and KKM

were studied to compare the antioxidant capacity and total phenolic content of products before and after sterilization. The antioxidant capacity of MeKa, PPK and KKM were significantly ($P < 0.05$) increased after sterilization (Figure 1). The highest level of increase was observed in MeKa (113%) followed by PPK (102%) and KKM (35%) with there being no difference in KSoi and PKhi. The greatest increased antioxidant capacity as shown with MeKa could have been due to the heat stability of the bioactive compound in shallot which made up 7.2% of the ingredients in this sauce. According to Shahidi and Wanasundara (1992), shallots contain a high flavonoid content which may have contributed to their high antioxidant activity, although cooking shallots for one minute in boiling water did not produce a difference in the antioxidant activity (Ismail *et al.*, 2004). Gazzani *et al.* (1998) demonstrated that thermal treatment increased the antioxidant activity of vegetables which became greater with increasing time.

Similar positive heat effects were found in processed tomatoes and sweet corn, where thermal processing could lead to the formation of novel compounds with antioxidant activity or the release of bound phenolic compounds (Dewanto *et al.*, 2002a, b). Turkmen *et al.* (2005) reported that different methods of cooking either increased the antioxidant activity or it remained unchanged depending on the type of vegetables. Yamaguchi *et al.* (2001) reported that after boiling, the antioxidant activity of the cooked tissue and the cooking water of broccoli, cabbage and pumpkin decreased, but the total activity increased by 112 to 163%. The authors suggested the increase in the antioxidant activity of the vegetables after boiling was possibly due to the thermal destruction of the cell walls and subcellular compartments which liberated a great amount of antioxidant compounds or there was potent production of antioxidants by the thermal chemical reaction or there was inactivation of oxidative enzymes by heating.

The total phenolic contents of the five tested foods before and after sterilization ranged

from 58.33 to 116.71 mg GAE per 100g food and 60.47 to 145.93 mg GAE per 100g, respectively. The results showed that sterilization increased the total phenolic content of all tested samples (Figure 2) although a significant ($P < 0.05$) increase

was observed only in MeKa and PPK. This was similar to the study by Jiratanan and Liu (2004) which indicated that the total phenolic content of processed beets at 125 °C increased by 14% compared with the control.

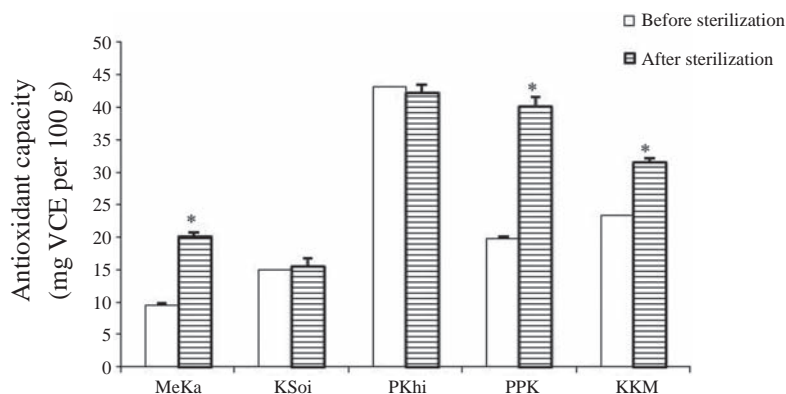


Figure 1 Antioxidant capacity of five tested food extracts. Asterisk (*) indicates a significant difference at $P < 0.05$ between before and after sterilization for a product. The vertical bar (\top) above each column represents the standard deviation.

MeKa = Mee Kathi sauce; KSoi = Khao Soi Nuea; PKhi = Phat Khimao sauce; PPK = Mu Phat Phrik Khing; KKM = Khua Kling Mu.

VCE = Vitamin C equivalent.

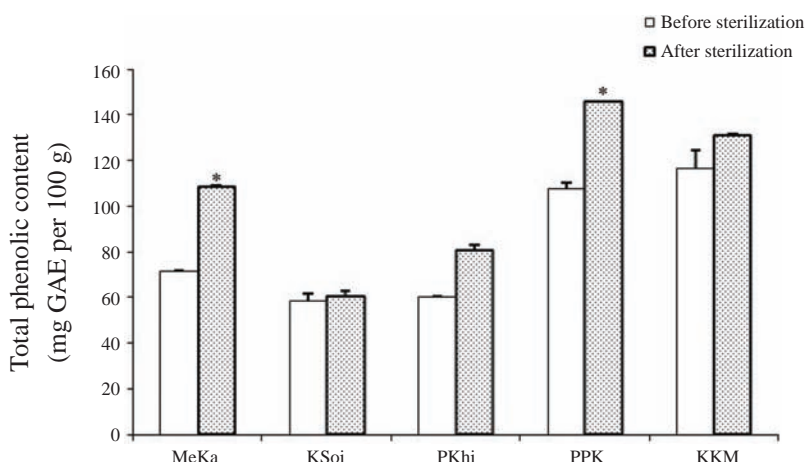


Figure 2 Total phenolic content of five tested foods extracts. Asterisk (*) indicates a significant difference at the level $P < 0.05$ between before and after sterilization. The vertical bar (\top) above each column represents the standard deviation.

MeKa = Mee Kathi sauce; KSoi = Khao Soi Nuea; PKhi = Phat Khimao sauce; PPK = Mu Phat Phrik Khing; KKM = Khua Kling Mu.

GAE = Gallic acid equivalent.

HPLC analysis was employed to study the pattern change of substances after heat processing. Similar chromatograms of the food before and after sterilization were detected in Ksoi, PKhi and KKM (data not shown) which agreed with the results for the total phenolic content. The analysis also demonstrated changes in the chemical composition of the methanol extracts of MeKa and PPK after sterilization treatment (Figure 3). Greater amounts of high polarity compounds together with the minimizing of low polarity compounds were detected in both products. These compounds could be generated by condensation or by a hydrolysis reaction in the low polarity compounds during sterilization similar to a report of Manzocco *et al.* (2001) who studied pasteurized tea.

CONCLUSION

The findings in this study indicated that each type of food product had a different antioxidant activity and total phenolic content which were contributed by the different ingredients of herbs and spices. Thermal treatment probably generated an increased amount of newly active substances with antioxidant activity, especially from the fresh plant ingredients. The thermal-processed pouched foods retained their antioxidant activity and total phenolic content. This information will add to the knowledge that will impact consumer food selection and enhance their awareness of the health benefits of processed foods in the prevention of chronic disease.

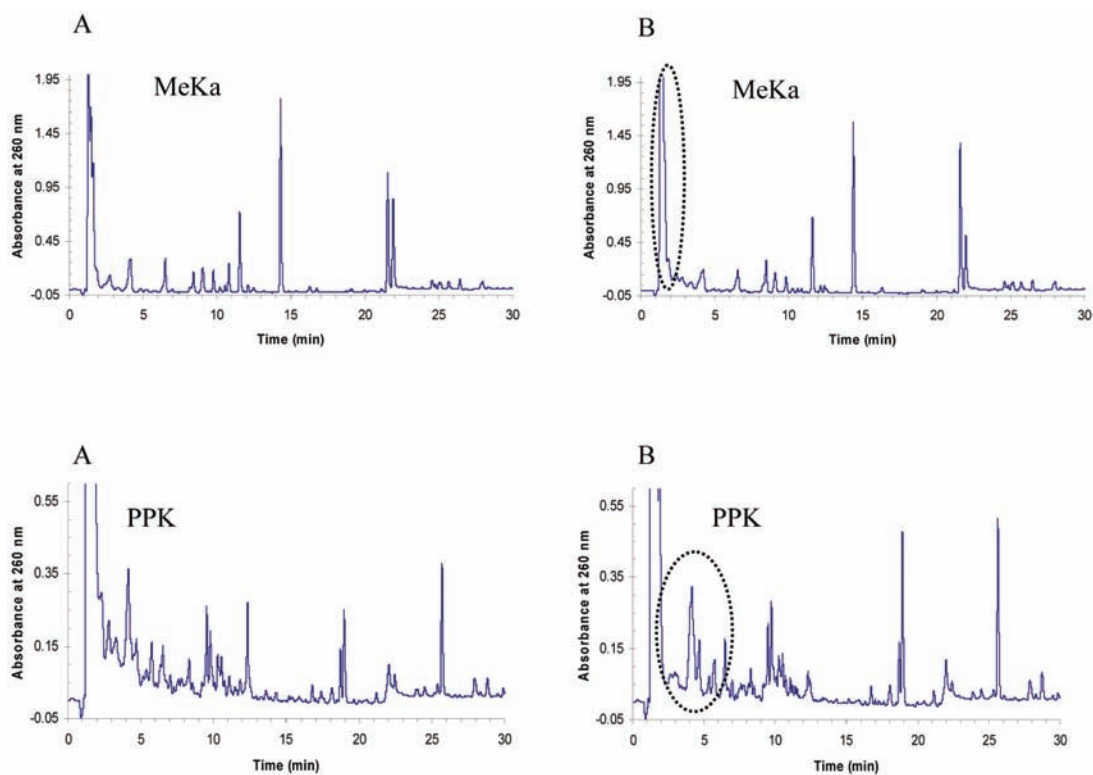


Figure 3 High-performance liquid chromatography profiles (260 nm) of methanolic extracts from MeKa and PPK: (A) before; and (B) after sterilization process. Dotted ellipse areas indicate high polarity compounds.

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