EFFECT OF STERILIZATION ON ANTIOXIDANT ACTIVITY: IN THE CASE OF THAI HALAL CURRY PASTE

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ABSTRACT

Halalan and thayyiban foods are intensively important among Muslims all around the world. Besides, the demand for healthy food is escalating due to consumer health concerns. Thai cuisine usually consists of several spices and herbs meant for colouring, flavouring, enhancing taste, and having various medicinal properties. Thai red curry paste commonly contains chilli pepper, black pepper, garlic bulb, lemon grass, and turmeric as its main ingredients. In addition, the phytochemicals of particular herbs in red curry paste also have antioxidant activities. This study builds on previous research using halal red curry pasted to increase shelf-life and monitor the herbal values. The sterilization and packaging improvement could increase the shelf-life by up to 1 year. Moreover, phenolic contents, flavonoid contents and antioxidant activity (DPPH) were measured by comparison between PreS and PostS. The results show that red curry paste has a high level of phenolic, flavonoid content, and radical scavenging activity. Moreover, the health benefits of herbs and spices were increased after sterilization. This study presumed that curry paste considered a thayyiban product might be a fascinating choice for health-conscious Muslims and non-Muslims.

Keywords: *Red curry paste, Antioxidant, Total phenolic content, Total flavonoid content*

1. Introduction

Food is one of the necessities of life. According to Islamic principles, the food consumed must meet halalan and thayyiban standards. Halal means permitted and lawful, depending on Islam. Nevertheless, thayyib means healthy, nutritious, pure, and safe (Riaz &Chaudry, 2004). Global demand for halal food is growing considerably. Furthermore, food companies worldwide are increasingly joining the halal market. Recently, healthy food is becoming more and more attractive to consumers. There has been an idea of using food to prevent and perhaps control some diseases lately. Therefore, the halalan and thayyiban market is considered to be large and become an attractive and highly competitive market. Thai food is widely known for its flavour, delicacy, and health values. The local herbs, spices, and vegetables constituents of the cuisine contribute to health and also provide a unique spice taste and actual scent. Several phenolic and flavonoid compounds in local herbs have been reported to have various biological properties, such as antioxidant capacity, and play a crucial role in human health (Karakaya, 2004; Willcox et al., 2004). The essential sources of phytochemicals such as phenolic and flavonoid compounds were found in various herbs as well as the primary ingredients of Thai red curry paste, such as lemon grass (Boeira et al., 2018) and turmeric (Tanvir et al., 2017), garlic (Leelarungrayub et al., 2006), and chilli (Materska and Perucka, 2005; Wangcharoen and Morasuk, 2007).

Red curry paste has a semi-solid texture with a high moisture content, resulting in a short shelf life. The increasing popularity of halalan and thayyiban food caused the development of various products, such as developing formulas, requesting halal certification, and extending the product shelf life. The process of extending the shelf life used for red curry paste in this study was sterilization and the use of retort pouches. The packaging of the red curry paste is convenient to use and transport. The sterilization procedure for this product is

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116°C for 30 minutes. This will extend the shelf life of curry paste to 1 year and do not need to be stored at low temperatures. At the same time, sterilization to eliminate the harmful microbes and their spores might affect the phenolic and flavonoid compounds and antioxidant activity. The phytochemicals and bioactive compounds contained in foods can be lost by heat during the sterilization procedure as well as storage (Ewald et al., 1999; Kalt, 2005; Roy et al., 2007). This study aims to investigate the phenolic, flavonoid content, and antioxidant activity of the halal-certified red curry paste produced in Pattani, Thailand. Moreover, the secondary objective was to monitor the effects of heat from sterilization on antioxidant activity.

2. Materials and methods

2.1 Samples preparation

Two samples of red curry pastes including pre-sterilization (PreS) and post-sterilization (PostS) were used to investigate. Shortly, all samples (50 mg) were extracted with 1 mL of 90% methanol for TPC and TFC, with 90% ethanol for DPPH assay. The solution was mixed for 2 min and sonicated in an ultrasonic bath for 10 min at room temperature. The solution was separated by centrifugation at 13,000g for 10 min.

2.2 Total Phenolic Contents (TPC)

The extract solution was used to investigate TPC by utilizing gallic acid as a reference standard. the Folin–Ciocalteu colorimetric assay was performed (Hatami et al., 2014; Praparatana et al., 2022). In brief, 25 µl of each curry paste sample (25 mg/ml), was added into a 1.5 ml tube, followed by adding 100 µl of 10% Folin–Ciocalteu's phenol reagent and 75 µl of 10% sodium carbonate. After incubating at RT for 20 min in the dark, each solution was individually aliquoted into a 96-well plate and detected the absorbance at 735 nm with a spectrophotometer (BMG Labtech SPECTROstar Nano Microplate Reader). The results were performed in triplicate and noted as the mg of gallic acid equivalent/mg extract (mg GAE/mg extract).

2.3 Total Flavonoid Contents (TFC)

The total flavonoid contents of Thai red curry paste extracts were measured utilizing quercetin as a reference standard through an aluminium chloride colorimetric assay (Jia et al., 1998). Shortly, 0.6 ml of each sample, at a concentration of 12.5 mg/ml, in methanol, was separately added into a 5 ml tube, containing 1.120 ml of distilled water, 40 µl of 1M Potassium acetate, and 40 µl of 10% (w/v) Aluminium chloride. Then, the solution was incubated at RT in the dark for 30 min. After that, the TFC observed the absorbance at 510 nm by a spectrophotometer (BMG Labtech SPECTROstar Nano Microplate Reader). The results were done in triplicate and expressed as the mg of quercetin equivalent/mg extract (mg QE/mg extract).

2.4 DPPH (radical scavenging activity assay)

The radical scavenging activity of the ethanol extract from red curry paste samples on the DPPH radical was investigated following the preceding study by Choi et al. (2006). Briefly, 0.1 ml of sample and quercetin standard in various dilutions (1.865, 3.125, 6.25, 12.5, 25 mg/ml) were individually pipetted into 96 well plates. Then, 0.1 ml of 200 µM of DPPH solution was added. The mixture was left to stand for 30 min in the dark. The absorbance was measured at 517 nm with a UV-visible spectrophotometer (BMG Labtech SPECTROstar Nano Microplate Reader). The treatment was repeated 3 times for each concentration and averaged the absorbance values. The antioxidant activity, based on EC50

(Concentration of substances that can cause a 50 percent reduction in DPPH concentration, µg/ml) of the extract, was calculated from the concentration curve of the samples and the standard absorbance. The EC50 values of samples were compared with the EC50 of the standard.

2.5 Statistical analysis

The statistic was analysed using SPSS 15.0 program. All data were shown as mean ± standard deviation and conducted in triplicate. The significant difference between TPC and TFC was investigated by t-test, at $p < 0.05$. At the same time, the significant difference in radical scavenging activity was established by one-way analysis of variance (one-way ANOVA), at $p < 0.05$.

3. Results and Discussion

3.1 Results

3.1.1 Total Phenolic Contents (TPC)

The main ingredient of red curry paste is several herbs and spices, which is rich in phytochemical such as phenolic and flavonoid compounds. The TPC of three samples was estimated by performing colorimetric assays. TPC was calculated from the calibration curve of gallic acid: $y = 0.0037x + 0.0121$, $R2 = 0.9996$, defined as the milligram of gallic acid equivalent per mg sample (mg GAE/mg). The results showed that the TCP of PreS and PostS were 170.49±4.20 and 191.11±2.04 mg GAE/mg extract, respectively. Besides, the TPC of PostS was significantly higher than PreS (Table 1).

Table 1: Total phenolic content (TPC) and total flavonoid content (TFC) of red curry paste. Data are shown as mean ± SD (n = 3), (*p < 0.05).

Samples	TPC (mg GAE/g)	TFC (mg QE/g)
PreS	170.49±4.20*	0.99±0.16*
PostS	191.11±2.04*	2.19±0.35*

3.1.2 Total Flavonoid Contents (TFC)

Flavonoids belong to a large group of natural substances (Vinayagam and Xu, 2015; Ong and Khoo, 2000). TFC value was obtained from the calibration curve of quercetin; $y = 0.001x + 0.0046$, $R2 = 0.991$, defined as the milligram of quercetin equivalent per mg sample (mg QE/mg). The results demonstrated that the TFC of PreS and PostS were 0.99±0.16 and 2.19±0.35 mg QE/mg extract. Moreover, the TFC of PostS was significantly greater than PreS (Table 1).

3.1.3 DPPH (free radical scavenging activity)

Ethanol extracts from paste samples were used to determine the DPPH, which measures light absorption. The results showed that PreS and PostS had EC50=6.144. µg/ml and 5.355 µg/ml, respectively. The EC50 of the two samples were significantly higher than the EC50 of the standard (4.572 µg/ml). The results show that the Thai red curry paste extracted has high antioxidant activity. Additionally, the EC50 of PostS was significantly lower than PreS. The results evaluated that the free radical scavenging activity of PostS was higher than PreS (Table 2).

Table 2: Free radical scavenging activity (EC50) of DPPH. Quercetin was used as a standard. Data are presented as mean ± SD (n = 3), (*p < 0.05).

Samples	EC50 (µg/ml)
standard	4.572*
PreS	6.144*
PostS	5.355*

3.2 Discussion

Red curry paste is produced from a combination of herbs and spices, such as dried chilli pepper, black pepper, garlic bulb, lemon grass, and turmeric. The individual ingredient has a positive biological property because it contains vitamins and phytochemicals. Chilli peppers contain several compounds that play an antioxidant role, such as flavonoids, phenolic compounds, and capsaicinoids (Materska and Perucka et al., 2005). Garlic contains flavonoid compounds that affect antioxidant activity, such as quercetin (Nuutila et al., 2003). Lemongrass is a promising source of bioactive compounds with high antioxidant activity (Boeira et al., 2018). Turmeric varieties are reported to be rich in natural antioxidants, as confirmed by their high values of polyphenols, flavonoids, and antioxidant activity (Tanvir et al., 2017). In this study, red curry paste extracted revealed high values of phenolic and flavonoid, as well as the property of DPPH equal to the standard. Therefore, these results considered that Halal-certified red curry paste from Pattani (Thailand) is one of the halalan and thayyiban foods, which has health benefits on various definite biological properties such as antioxidant activity. The results indicated that the TPC, TFC, and radical scavenging (DPPH) of PostS were significantly higher than PreS. The results predicted that the sterilization process might involve the values of phenolic, flavonoid, and the capacity of radical scavenging. These results were consistent with Inchuen et al. (2011), who reported that heat treatment significantly affected TPC and DPPH. They suggested that the high heat treatment might enhance the extractability of phenolic compounds by disrupting the plant cell walls, which might release more easily of phenolic compounds from the red curry paste. Choi et al. (2006) suggested that bound polyphenolic and flavonoid compounds could be liberated by heat treatment, leading to increased polyphenolic and flavonoid contents in heated Shiitake mushrooms. Moreover, Siwarungson and Lertpringkop (2017) reported that Thai curry soups were defined as functional foods with several biological properties such as Antioxidant activity, Anti-thrombosis, Fibrinolytic activity, Anti-aging, and Anti-hypertension. Tuntipopipat et al. (2011) suggested that Thai red curry paste revealed anti-inflammatory and antioxidant properties from bioactive compounds present in the spice and herb constituents. Accordingly, further studies might be needed to confirm the effect of heat on antioxidant liberation and to study more effects on health benefits. Subsequently, product development is necessary to maintain the bioactive compound and extend the product's shelf-life.

4. Conclusion

Halal-certified red curry pastes produced in Pattani (Thailand) revealed a positive biological role in antioxidant activity, as expressed by a high value of phenolic, flavonoid, and free radical scavenging (DPPH). Incidentally, the effect of sterilization involved increasing phenolic, flavonoid contents, and radical scavenging activity. From the data presented here, it could be presumed that halal-certified red curry pastes meet a healthy food trend. It is classified as a halalan and thayyiban food suitable for Muslim and non-Muslim health lovers. Finally, red curry pastes may need further investigation on their health benefits and extended product shelf-life.

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