Enhanced Knowledge in Sciences and Technology Vol. 3 No. 1 (2023) 257-265

EKST

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/ekst
e-ISSN : 2773-6385

Effect of Heat Treatment on Phytochemical Content and Antioxidant Activity of Fresh and boiled *Capsicum Annum variety Kulai*

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DOI: https://doi.org/10.30880/ekst.2023.03.01.029
Received 15 January 2023; Accepted 11 April 2023; Available online 3 August 2023

Abstract: *Capsicum annuum L. var Kulai* belongs to the Solanaceae family or better known as red chili. Fresh Chili is a vegetable that originally has a high antioxidant content. However, the antioxidant content in chili can reduced after heat treatment i.e., boiling which uses three different temperatures which are 60, 80 and 100 ℃. Therefore, in this research the total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical scavenging activities of fresh and boiled (60,80 and 100 ℃)*C.annum L. var Kulai* were determined. The total phenolic and total flavonoid content were evaluated using Folin -ciocaltue reagent and aluminium chloride and capsaicin content was measured using High- Performance Liquid Chromatography (HPLC). The antioxidant activity was tested using 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging assay. The results showed chili heated with 60 ℃ stated the highest total phenolic content of 94.05±0.06 mg GAE/g. Chili was treated with 60 ℃ also demonstrated the highest total flavonoid content and capsaicin content (63.84±0.13 QE/g) and (150.39±1.53 µg/g) .*C.annum L. var Kulai* at 60 ℃ recorded the highest of antioxidant activity (75.45 %).In addition, fresh *C.annum L. var Kulai* reported the highest TPC ,TFC and DPPH . Thus, it can concluded the heat treatment with higher temperature can reduce phytochemical compound and antioxidant activities in *C.annum L. var Kulai.*

Keywords: Antioxidant Activity, *Capsicum Annum Variety Kulai,* Capsaicin, Flavonoid,Phytochemicals.

1.0 Introduction

Chilli pepper, also known by its scientific name, *Capsicum annum*, is consumed in large quantities across the globe and continues to experience tremendous expansion. As a result of increased demand and the costs associated with production, 62 % of Malaysia's chilli supply comes from imports from other country [1]. Chilli pepper *(Capsicum annuum L.)* is considered to be the second most popular vegetable fruit in Malaysia. The genus contains 25 different species, all of which have been successfully cultivated in various parts of the world. *C. Annuum, C. Chinense, C. Frutescence, C. Pubescence,* and

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C. Baccatum are few species that have been identified in Asia [2,3]. In addition to this, the quantity of flavonoids, and capsaicinoids that are contained in chilli is fairly considerable. Chili has been the subject of many studies, mainly to evaluate the chemical composition or antioxidant activities of various cultivars of pepper, as well as to evaluate the effect of drying methods on the chili. Chili is an essential component of Asian cuisine, and the most common methods for preparing them include boiling or stir-frying with a variety of other meals and vegetables. According to the findings of the earlier research, among the species, the *C. annuum* variation or "*Kulai*" is the one that is most frequently cultivated by Malaysian farmers because of the high marketable value it possesses.

Heating treatments, often conducted to improve the palatability and raise the edibility of vegetables [3]. The chemical compositions can change as well as the physical properties of the vegetables that through heat treatment. It was a common concept that thermally treatment can affect a reduction in phytochemical content in chilies such as flavonoid, antioxidant and capsaicin content. Due to the fact that certain phytochemical content in vegetables can lost during the heat treatment. On the other hand, it was hypothesized that heat treatment affected the antioxidant activity due to the reduction of phenolic content [4].

Previous studies have been conducted to investigate the effect of drying conditions and temperature on phytochemical compounds and antioxidant activity found in chilies of different genera such as *C. annuum, C. chinense, C. frutescence, C. pubescence* found in Malaysia and Asian countries. In addition, the most recent study only focused on the effect of different cooking on the antioxidant content of bell pepper [5]. However, there is a lack of information about the famous chili genus cultivated in Malaysia, which is *C.annum L. var Kulai*. Therefore, this study focuses on the effect of heat treatment (boiling) on fresh *C.annum L. var Kulai* and that which has been boiled using different temperatures of 60,80,100℃ for 20 minutes on the phytochemicals content and antioxidant activity.

2. Materials and Methods

2.1 Reagents and Apparatus

The chemical used in this study were aluminium chloride hexahydrate, methanol, sodium carbonate, sodium hydroxide, Folin -ciocaltue reagent (Bendosen Laboratory Chemicals, Norway). Gallic acid,quercetin and 2,2-diphenyl-1-picryhydrazyl (DPPH) (Systerm Chemical,USA).Pure capsaicin (Sigma Aldrich,USA).

The apparatus used in this study were freeze dryer (Daihan, hypercool hc3110 h, Mumbai), rotary evaporator (Butchi, rv 10 digital v-c, Germany,water bath (Memmert, wtb11, German),UV-vis Spectrophotometer (Hach, DR 5000, Canada),High Performance Liquid Chromatography (Shimadzu, LC-20AT, Japan),grinder (Retsch, GM 200, Germany),drying oven (Memmert, U40, Germany).

2.2 Sample Preparation

Fresh chilies (*C. annum L. var Kulai*) were collected from Pasar Tani Pagoh of Johor. Fresh chilies were weighed and washed under running water to remove extraneous material. The chilies that have been washed are divided into two parts where the first part, the fresh chilies were dried using a freeze dryer with the aim of preserving the phytochemicals in the fresh chilies. Second part, the fresh chilies were dried using a drying oven. The dried chilies were kept in the airtight container at room temperature (25 ±2°C) for heat treatment process (boiling).

Drying of chili:Fresh chilies that have been cut were placed on a tray to be dried with a drying oven for 72 hours at 60 ℃. During the drying process, the chilies were always flipped upside down to ensure that the chilies dry evenly [6]. The main reason to dry the chilies in an appropriate method to produce good quality of color and pungency [7].

Boiling: The dried chilies boiled at various temperatures, which were 60℃, 80℃ and 100℃ for 20 min. The 300g dried chilies was weighed and added 500ml distilled water in a stainless pot. During the boiling process, thermometer was used to monitor the temperature [8]. Heat treatment method such as boiling can inactivation or decrease in number of microorganisms in order to develop safe product such as chili paste [9].

Grinding: The boiled chilies was ground using a grinder (Retsch, GM200, Germany) constantly at a speed of 200 rpm for 180 seconds to ensure that the chili was crushed and becomes like a paste. The main purpose of chili being ground and made into a paste was to ensure that the chili paste was dry constantly and easy for extraction [10]. Fresh chilies that have been dried using a freeze dryer also ground using a grinder at a speed of 200 rpm for 180 seconds until it becomes a fine powder [11].

Drying: The chili paste that has been ground was put into a centrifuge tube up to 350 ml and covered with a lid. After that, the centrifuge tube containing the chili paste was frozen in a freezer (New Brunswio,USA) for one night using a temperature of -80 ℃. Next, the frozen chili paste was put into the freeze dryer for a drying process at 96 hours [12].

2.3 Methanolic Extraction

Firstly, 2 g chili powder (fresh chili and chili after boiled undergo freeze dry) and mixed with 20 ml of methanol in 100 ml conical flask. The conical flask was covered with aluminium foil and tight it using parafilm. Then, the mixture was incubated at 40℃ for 2 hours in shaker with a speed 150rpm.After that, the supernatant was collected using a Whatman No.1 filter paper. The supernatants were pooled together and transferred to a rotary evaporator using 40℃ temperatures to remove the methanol in sample. Lastly the evaporated extract was kept in a sealed dark glass bottle and stored at -20 ℃ for the further analysis [12].

2.4 Extraction of Capsaicin from chili

All the chili samples were used powdered and dried (fresh chilies and heat treatment chillies), then this chili sample was extracted with a slight modification that was each dried chilies was weighed as much as 5 g and placed in methanol (10 ml) in a conical flask and swirl for a few minutes. Next, transferred the sample into the centrifuge tube. The centrifuge was tightly closed and wrapped with aluminium foil around the centrifuge tube. Next, the centrifuge tube was placed in a water bath at 80 °C for 4 hours, then swirled centrifuge manually every hour. Samples were removed from the water bath and cooled to room temperature. The supernatant of each sample was filtered using a 0.45 µm nylon filter into an HPLC sample vial using a 5 mL disposable syringe. The vial was capped and stored at 5 °C in a refrigerator until analysis [13].

2.5 Preparation of Standard Solution

Gallic Acid: 10 mg of gallic acid was weighed in 10 ml of methanol to get the concentration (1mg/mL), the solution was stirred until it dissolved. Various concentration of gallic acid solutions in methanol (20,40,60, 80,100µg/ml) were prepared from the standard solution [12].

Standard dilution: 10 mg of quercetin was dissolved in 10 ml of methanol to get the concentration (1mg/ml). Various concentration of quercetin solutions in methanol (0.1,0.5,1.10, 2.5, 5.0 mg/ml) were prepared from the standard solution [12].

2.6 Determination of Total Phenolic Content (TPC)

An aliquot of 0.5 ml sample extract was mixed with 0.5 ml Folin-ciocalteu reagent, followed by addition of 10 ml of 7% Na_2CO_3 solution. The mixture was incubated for 1 h at 25 ± 2 °C in the dark and then absorbance was measured at 765 nm using a UV–vis Spectrophotometer. The amount of TPC

was expressed as milligram of gallic acid equivalents (GAE) per g of sample (mg GAE/ g DW). All the experiment performed in triplicate [12].

2.7 Determination of Total Flavonoid Content (TFC)

A 0.5 ml aliquot of sample extract was mixed with 2.25 ml of distilled water in a black covered test tube followed by addition of 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.3 ml of 10% $AlCl_3.6H_2O$ solution was added followed by addition of 1.0 ml 1 M NaOH. The mixture was mixed well by vertexing. The absorbance was measured at 510 nm by the Uv -vis spectrophotometer. Results were expressed as milligram of quercetin equivalent (QE) per g of sample (mg QE/ g DW). All the experiment performed in triplicate [15].

2.8 Scavenging activity (DPPH) assay

Firstly, 5 ml of 0.1 mM DPPH was prepared in methanol and mixed with 1ml of the extracted sample. The DPPH solution must be protected from light by covering the flask with aluminium foil. The next step is followed by incubation at 37 ℃ in in the dark for 30 minutes. The absorbance of the reaction mixture will be measured at 517nm using a UV-VIS spectrophotometer. The test will perform in triplicate and the result was average [15]. The scavenging activity was calculated as following (Eq.1):

$$\text{DPPH scavenging effects (\%)} = \frac{\text{Absorbance Control-Absorbance sample}}{\text{Abscontrol}} \times 100 \qquad \textit{Eq.1}$$

Where Absorbance control is the absorbance of DPPH with methanol, while Absorbance sample is the absorbance of DPPH with sample (fresh and boiled).

2.9 Chromatographic conditions

The HPLC analyses were carried out on a Thermo HPLC system equipped with a Finnigan Surveyor Auto Sampler Plus. A Finnigan Surveyor LC Plus quaternary pump and a surveyor photodiode array (PDA) detector was used.The chromatographic conditions were as follows used Betasil C18 column (particle size 3 µm, dimension 150 × 4.6 mm) .The column temperature was set at 60 °C and the sample temperature set at 20 °C. The sample volume of HPLC was set at 5 µL. The UV detection was used the wavelength at 222 nm. Then, the mobile phase was used binary mixture water- methanol at a 50:50 ratios with flow rate was 1.5 mL/min to detect the peak [15]. The contents (W, mg g^{-1}) of capsaicin in chili pepper was calculated as following (Eq. 2):

$$W = C.\ V/m \qquad \textit{Eq.2}$$

Where C is the concentration (mg mL^{-1}) of capsaicin calculated from the calibration curves for standard capsaicin while V is the total volume of extract and m is the sample weight in g.

2.10 Statistical Analysis

Since the test were carried out in triplicate results reported as mean ± standard deviation (SD) values. The significant differences among the means were determined with a one-way analysis of variance (ANOVA) by using IBM SPSS (version 27, Chicago,USA) at $\alpha = 0.05$

3.Results and Discussion

3.1 Effect of heat treatment (boiling) on Total Phenolic and Total Flavonoid Content

Fresh *C.annum L. var kulai* showed a higher Total Phenolic Content 95.61 mg/g±0.47 as compared to chilies that through heat treatment.Based on a previous study conducted by Hwang *et al*[16] it was found that each species of *C. annum* cultivation has a different phenol content where the results shown the fresh *capsicum* such as *Capezzolo* (*C. chinense*) and *Hierro pepper* (*C. annuum*) has a phenol content range of 23-71.4 mg/g [16]. Based on Table 1 showed the TPC in *C.annum L. var Kulai* was significantly decreased ($p<0.05$) according to the increase in temperature.TPC values of 60 ℃ (94.05 mg/g ±0.06), 80 ℃ (87.82 mg/g±0.53) and 100℃ (79.70 mg/g±0.55).The results of this study were consistent with previous studies reported by Ismail *et al* [17] that heat treatment reduces TPC in vegetables because heat has a tendency to reduce polyphenols due to phenols leaching into boiling water, as well as phenolic breakdown during processing. Another study conducted by Lin and Tang [18] also found that TPC in green chili also showed a reduction due to increased steaming, boiling and blanching temperatures. Previous study by Hwang *et al* [16] reported boiling and steaming temperatures at 40℃, 60 ℃ ,80℃ and 100 ℃ where bell pepper that was boiled and steamed at a temperature of 100 ℃ showed a TPC losses rate of 66.99 %.

Table 1: The total phenolic content and total flavonoid contents, in fresh *C. annuum L.variety Kulai* and after heat treatment (boiling)

Parameters	Heat treatments			
	Fresh	60℃	80℃	100℃
Total Phenolic Content (TPC)[1]	95.61±0.47[a]	94.05±0.06[b]	87.82±0.53[c]	79.70±0.55[d]
Total Flavonoid Content (TFC)[2]	71.57±0.09[a]	63.84±0.13[b]	56.27±0.28[c]	50.58±0.58[d]

[1] mg GA equivalent/gDW.[2] mg Quercetin equivalent/gDW. [a,b,c,d] Values with different letters in the row were significantly different($p<0.05$).

Besides that, Total Flavonoid Content (TFC) in fresh *C.annum L. var Kulai* was higher (71.57 mg/g) as compared to chillies that through heat treatment which ranged TFC (63.84-50.58 mg/g). Based on Table 1, a significant decrease in TFC ($p<0.05$) has been shown between fresh and heat-treated chillies. This study was confirmed by a previous study Lin and Tang [18] reported that thermal treatment affects the stability of the flavanol in chili where TFC in raw red bell pepper and red bell pepper subjected to heat treatment was different. Another author stated boiling at 90-100˚C directly can destroy enzyme activity and block the synthesis pathway of flavonoids [19]. Thermal instability of quercetin and kaempferol in vegetable tissue and in boiling water that will affect TFC in chili [20].

3.2 Effect of heat treatment (boiling) to Antioxidant Activity in chili

Based on Table 2, the highest percentage of scavenging activity was fresh *C.annum L.var Kulai* while the treated *C.annum L.var Kulai* with 60 ℃ recorded the higher percentage of scavenging activity. A previous study conducted by Hwang *et al* [16] demonstrated that the DPPH radical scavenging activity of *C. annuum* bought in South Korea local market was significantly reduced after heat treatment. The DPPH radical scavenging activity was reduced 42.0% of its initial capacities after boiling, followed by steaming (23.5 %) roasting (11.6 %), and stir-frying (4.6 %). Besides that, at temperatures of 80 and 100℃ it caused the inhibition percentage to decrease. Based on the previous study, reported boiling chili for 5 minutes with 80℃ reduces the radical scavenging activity to below

77 % from its initial level, while boiling time is prolonged to 30 minutes with 90 ℃, the result in reducing scavenging activity to 64 % [21].

Table 2 : Antioxidant activity(DPPH scavenging effects(%)of fresh and heat treatment (boiling) of *C.annum L var Kulai*

Parameters	Fresh Sample	Heat treatments		
		60℃	80℃	100℃
DPPH scavenging effects (%)	80.73[a]	75.45[b]	66.53[c]	44.42[d]

[a,b,c,d] Values with different letters in the row were significantly different($p<0.05$).

3.3 Effect of heat treatment (boiling) on Capsaicin Content in Chili.

The capsaicin content was measured by using the standard curve in Figure 1, where the equation was y = 9359.2x + 426201, where R^2=0.9879. Based on Figure 2 showed the chromatogram of the peak of capsaicin content in the sample. As shown in Table 3, the capsaicin content of treated chilies for three different temperatures was around 150.39-89.87 µg/g meanwhile, the capsaicin content of fresh 178.39 µg/g. Previous study by Srinivasan *et al* [22] reported moderate of heat treatment temperature of 20-65 ℃ only gives a little effect on the Jalapeño pepper which was 1.1–28.1%. Another study conducted by Alvarez *et al* [23] reported the high temperature was used in heat treatment (boiling) degraded the capsaicin content. The losses of capsaicin content during boiling was due to the capsaicin was leached into boiling water [24].

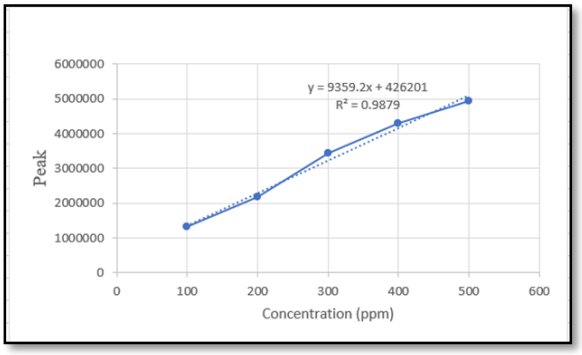

Figure 1: Standard calibration Curve of Capsaicin

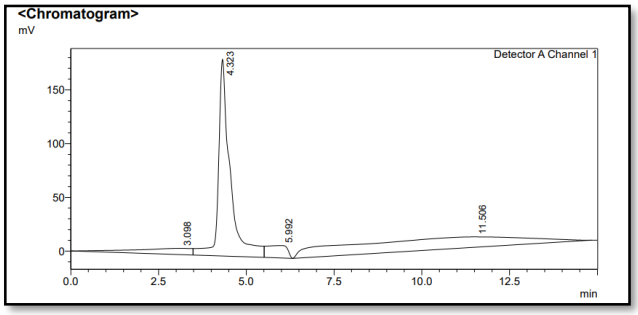

Figure 2: Chromatogram of the chilies using heat treatment (boiling) at 80 ℃

Table 3: The capsaicin content in fresh *Capsicum annum L var Kulai* and after heat treatment(boiling)

Parameters	Fresh Chili	Heat treatments		
		60℃	80℃	100℃
Capsaicin (µg/g)	178.39±0.68[a]	150.39±1.53[b]	121.13±1.235[c]	89.87±0.38[d]

[a,b,c,d] Values with different letters in the row were significantly different($p<0.05$).

4. Conclusion

In conclusion, fresh *C.annum L. var Kulai* shows the highest total phenolic, flavonoid and capsaicin content as compared to *C.annum L.var Kulai* that has through heat treatment (boiling). Different temperatures in heat treatment also play an important role in maintaining the phytochemicals in the chili. This study showed that the temperature 60℃ was suitable for maintaining the higher TPC, TFC and capsaicin content same as the fresh *C.annum L.var Kulai* . Besides that, the higher temperature of 80 and 100℃ was not suitable for heated the chili because the high temperature can lead the deterioration the phytochemical and antioxidant activity. Last but not least,the phytochemical content and the temperature was used also affect the antioxidant activity in chili.

Acknowledgement

The authors would also like to thank the Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia for providing facilities in the execution of this study.

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