MJoSHT
Malaysian Journal of Science, Health & Technology
[mjosht.usim.edu.my]

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Effect of Cooking Time and Temperature on Extraction Yield and Antioxidant Properties of Beef Rendang

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Article Info
Article history:
Received: 22 September 2022
Accepted: 13 June 2023
Published:12 September 2023

Academic Editor:
Sharifah Fairuz Syed Mohamad

Malaysian Journal of Science, Health & Technology

MJoSHT2023, Volume 9, Issue No. 2
eISSN: 2601-0003

https://doi.org/10.33102/mjosht.v9i2.317

Abstract*— Rendang is a popular traditional cuisine in Southeast Asia, particularly Malaysia and Indonesia. Rendang ingredients vary according to different countries and mainly consist of spices, coconut milk, and meat, either chicken or beef. Spices such as coriander, fennel, cumin, ginger, galangal, lemongrass, garlic, and onion used in rendang mostly contributed to its antioxidant properties. However, the beef rendang's antioxidant properties may be lost due to the long cooking period. This study aims to determine the effect of cooking time and temperature on the extraction yield and antioxidant properties of beef. Rendang samples prepared using lean beef and mixed with coconut milk and other spices were collected every hour up to five hours of cooking. The samples were subjected to methanol extraction for total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay using Folin-Ciocalteu and DPPH solution, respectively. The data were analysed by one-way analysis of variance (ANOVA) followed by Tukey's test. Data for extraction yield showed that rendang samples cooked for one and two hours had significantly lesser yield ($p<0.05$) than those cooked for three to five hours. For TPC analysis, rendang that was cooked for three hours had the highest total phenolic content (8.018 ± 0.911 mg GAE/g) ($p<0.05$) compared to those that were cooked for one, two, four, and five hours (4.205±0.364, 2.368±0.127, 2.746±0.080, and 1.839±0.110 mg GAE/g). For the DPPH assay, one-hour beef rendang sample had the lowest EC50 (20.278±0.733 µg/ml) ($p<0.05$) followed by those cooked for two, three, four, and five hours (39.041±1.368, 40.143±4.502, 48.338±2.115, and 58.159±3.060 µg/ml), indicating a low EC50 value which exhibited high antioxidant power. It can be concluded that antioxidant properties were significantly lost due to prolonged heating with increasing cooking time.

***Keywords*— Beef Rendang; Cooking time; Temperature; Extraction Yield; Antioxidant Properties**

I. INTRODUCTION

Rendang is one of the most well-known dishes in Southeast Asia, especially in Malaysia and Indonesia. Rendang is a very familiar dish that is usually served during Islamic festivals in these countries, which is Eid day. The origin of rendang is yet to be confirmed as a few communities claimed that rendang is from their culture. Foreign culinary traditions influenced rendang, which is related to the West Sumatra culinary

tradition and the rendang. In Malaysia, either chicken rendang or beef rendang will be found in common occasions. Beef rendang is made of beef meat, spices, coconut milk, sugar, salt, cumin, fennel, and coriander. Beef rendang would require more time to cook compared to chicken rendang. Rendang from Malaysia is usually cooked for a prolonged duration of up to 5 hours at temperatures ranging between 90^{O}C-120^{O}C. Thus, prolonged cooking time is needed for the preparation of beef rendang, and this will somehow affect the antioxidant properties in the rendang.

Compared to fresh beef, the chemical composition of beef rendang clearly gives different data for both fat and protein content. Based on a study [1], the protein content in fresh beef is higher than in beef rendang, while the fat content in beef rendang is higher than in fresh beef because coconut milk is added as one of the main ingredients. Cooking food is primarily used to inactivate diseases caused by bacteria (pathogens) and enzymes, as well as to lower the moisture content in processed meals and soften the outer tissue. In terms of colour, texture, and flavour, however, processing can result in a variety of changes of appearance, composition, nutrition, and sensory qualities in processed foods. Food processing is generally acknowledged as one of the main contributors to the destruction or alteration of natural phytochemicals, which may affect the antioxidant potential of foods [2].

Food producers use antioxidants to stabilise food lipids and avoid product consistency degradation. Antioxidants are compounds that can regulate or prevent oxidative processes that contribute to food quality degradation or the initiation and proliferation of degenerative diseases at a very low amount [3]. Other than the beef meat, other ingredients added to make beef rendang also influence the antioxidant properties. Coconut milk, for example, apart from contributing to the rich flavour of rendang, also affects the antioxidant properties of rendang [4].

Some spices could also contribute to the antioxidant effect. In the making of rendang, spices that are commonly used are ginger, garlic and sometimes turmeric. A study [5] reported that ginger has the highest antioxidant activity among these spices, followed by turmeric and garlic. Hence, the amounts of spices added would impact the antioxidant properties of the rendang. Cooking temperature had a significant impact on the phenolic compound and antioxidant activity of garlic [6]. Other than that, a study [7] analysed the effect of heating temperature on onion in terms of antioxidant properties. The ingredients of rendang have variations in all rendang dishes as different people would use different compositions of ingredients to make rendang.

Rendang is usually made by cooking for extended hours. Due to the long cooking time, the antioxidant properties of the beef rendang would be a concern. Temperature and heat processing time significantly influence the composition of the beef rendang and sensory attributes. Due to various rendang recipes, there are many types of rendang with various durations of preparation required. The variety of recipes is a concern as this might also affect the shelf life of the rendang as well as its nutritional content.

Aside from the ingredients used in the recipe, the processing time of the rendang is a major important factor. Studies [8, 19] on the physicochemical features of the food and the processing parameters have a significant impact on the antioxidant capacity of Maillard reaction products, which is a non-enzymatic reaction due to food processing. This shows that the physiochemical of rendang would be affected as well as antioxidant properties, due to prolonged heat processing. Antioxidants are needed to stop the oxidation of oils and fats because the chemicals (hydroperoxide, hydroxyl radical, and a single oxygen capacity) that are made when oil is oxidised can lead to oxidative stress in biological systems [9].

Oxidative breakdown affects meat proteins as well. Reactive nitrogen species (RNS), reactive oxygen species (ROS), by-products of oxidative reactions, and transition metal ions are all pro-oxidants found in meat [10]. Oxidation of lipids in rendang is more likely due to the high-fat content of the coconut milk and cooking oil used to cook the ingredients. Light, temperature, degree of unsaturation, pH, and the prooxidant or antioxidant's balance influence lipid oxidation [11]. Therefore, this study aims to determine the effect of prolonged processing time of rendang towards antioxidant value.

II. MATERIALS AND METHODS

A. Chemicals

Methanol (R&M, Subang, Malaysia), Folin-Ciocalteu's reagent (Sigma Aldrich, St. Louis, MO, USA), sodium carbonate (Systerm, Shah Alam, Selangor, Malaysia), 2,2-diphenyl-1-picrylhydrazyl (Sigma Aldrich, St. Louis, MO, USA), gallic acid (Sigma–Aldrich, St. Louis, MO, USA), pure ethanol (Systerm, Shah Alam, Selangor, Malaysia).

B. Sample preparation

All ingredients were bought from Pasar Borong Selangor, Seri Kembangan, Selangor, Malaysia. Lean beef (12 kg) from the shank part was cut into 3 x 5 x 5 cm pieces, boiled by gas stove for 2 h, and cooled to room temperature for 1 h. The boiled meat was shredded manually and stored overnight in a chiller (6-8 ºC). Coconut milk (10 kg), *kerisik* (400 g), blended dried chilli (1 kg), blended red onion (3 kg), blended garlic (1 kg), blended galangal (120 g), blended lemongrass (1.5 kg), tamarind slice (100 g), blended ginger (120 g), coriander seed powder (100 g), fennel powder (50 g), cumin powder (50 g), sugar (200 g), and salt (100 g) were cooked together in automatic braising pan (Salsamat, Nilma, Parma, Italy) with the shredded beef for 1 to 5 h, with 1 h interval which corresponded to T1 (gravy), T2 (wet), T3 (dry) T4 (intermediate floss), T5 (floss), respectively. Table I shows the different heating temperatures for each condition. Six replicates (n=6) of rendang samples were taken every hour for each condition (T1, T2, T3, T4 and T5). Each sample was weighed at 200 g and stored in an aluminium pouch at -20 ºC until further analysis.

C. Extraction of sample

Extraction of beef rendang was done according to Barros et al. [12] with modifications. The samples (3 g) were extracted in 100 mL of methanol (100%, v/v) at 26°C. The mixture was then shaken at 160 rpm (Scilogex, Rocky Hill, USA) for 24 h. Next, it was filtered through Whatman #4 paper before drying at 40 °C using a rotary evaporator (Heidolph Hei-VAP Silver 3, Germany) for 2 h. Finally, the extract was redissolved in methanol to obtain a concentration of 50 mg/mL and stored at 4 °C for analysis.

TABLE I . COOKING TIME AND TEMPERATURE FOR BEEF RENDANG

Condition of rendang	Time of cooking (hour)	Heating temperature (°C)
T1	1	120
T2	2	120
T3	3	110
T4	4	90
T5	5	90

D. Total phenolic content (TPC)

The total phenolic content was determined using Folin-Ciocalteu method according to Ee et al. [13]. A volume of 250 µL of 10 mg/mL from the stock extract solution was combined with 500 µL of Folin-Ciocalteu's phenol reagent and 2.5 mL of distilled water. The mixture was vortexed (VTX-300L, LMS, Tokyo, Japan) and stored at room temperature (25°C) for 5 min in the dark. The mixture was then mixed with 5 mL of 7% (w/v) of sodium carbonate solution and 4.25 mL of distilled water. Next, the mixture was vortexed for another 30 s and further incubated in the dark for 2 h. The absorbance of the mixture (225 µL) was measured at 750 nm using a microplate reader spectrophotometer (Multiskan™ FC Microplate Reader, Thermo Scientific™, MA, USA). TPC was expressed as milligrams of gallic acid equivalent (GAE) per gram (mg GAE/g) of the extract.

E. DPPH assay

DPPH free radical scavenging activity of beef rendang was assessed according to Naziruddin et al. [14] with slight modifications. To prepare 0.10 mM DPPH solution, a total of 0.02 g of DPPH powder was solubilised in 500 mL of ethanol. The 0.1 mg/mL gallic acid (GA) stock solution was made by dissolving 0.01 g of GA in 100 mL ethanol. From the stock solution, five different concentrations of GA were prepared: 0.05 mg/mL, 0.025 mg/mL, 0.0125 mg/mL, 6.25×10^{-3} mg/mL, and 3.125×10^{-3} mg/mL. A series of stock solutions from methanolic extract of beef rendang from all stages were made with concentrations of 0 µg/mL, 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL and 25 µg/mL. For the assay, 250 µL of each sample was mixed with 750 µL of ethanolic DPPH solution. Pure ethanol was used as a control. The test tube was vigorously shaken before being left to incubate for 30 min without light exposure. A microplate reader spectrophotometer (Multiskan™ FC Microplate Reader, Thermo Scientific™, MA, USA) was used to measure the absorbance of the samples, standard, and control at 517 nm. The experiment was done in triplicates. The following equation was used to determine the DPPH radical's scavenging activity:

Radical scavenging activity (RSA) % =

$$\left[1 - \frac{\text{Abs sample}}{\text{Abs control}}\right] \times 100\% \quad (1)$$

where $Abs_{control}$ denotes the absorbance of the control reaction (which contains all reagents excluding the test compound) and Abs_{sample} denotes the absorbance of the test compound. A standard curve of percentage inhibition versus extract concentrations was plotted, and half-maximal effective concentration (EC_{50}) of the extract was derived.

F. Statistical analysis

All data were analysed using the Minitab software for one-way ANOVA, followed by Tukey's test to determine the significant difference between means at the 5% level. A p-value <0.05 was regarded as significant. The Pearson correlation coefficient analysis was carried out to determine the correlation between the total phenolic content and DPPH results.

III. RESULTS AND DISCUSSION

A. Extraction yield of beef rendang

Table II shows the mean of extraction yield of the beef rendang. The average weight of the extract yield was in the range of 0.614 to 1.235 g. There was a significant difference ($p<0.05$) between samples cooked for 3 to 5 h and those cooked for 1 to 2 h. Different conditions of rendang may have different water content, as an increase in cooking time may lead to a decrease in water content. This contributes to the difference in extraction yield as the duration of extraction for each rendang condition was constant, which may influence the antioxidant properties of the beef rendang.

TABLE II. EXTRACTION YIELD OF BEEF RENDANG AT DIFFERENT COOKING TIME

Time of cooking (h)	Mean ± SD (g)
1	0.614 ± 0.069^{b}
2	0.739 ± 0.09^{b}
3	1.088 ± 0.161^{a}
4	1.064 ± 0.106^{a}
5	1.235 ± 0.030^{a}

[a-b] Values with different letters differ significantly ($p<0.05$).

Extraction is crucial in the isolation and purification of numerous bioactive components found in food. There are various techniques for determining antioxidant properties. Several extraction procedures to identify the polyphenols from food samples have been reported, from conventional to advanced techniques. Methanol was chosen as the extraction solvent for antioxidant analyses as it can provide the highest recovery of phenols in the sample [15]. The extraction yield of the sample may vary due to the composition of the sample. According to Ismail et al. [16], the low extraction yield of cantaloupe seed is most likely due to the methanol solubility to the major components like fat, carbohydrate, and protein. In general, phenolic compounds have a better yield and greater solubility in organic solvents.

B. Total phenolic content (TPC)

Total phenolic content was done by using the Folin-Ciocalteu method. The calibration curve was y = 0.0019x with R^2 = 0.9885 (Figure 1).

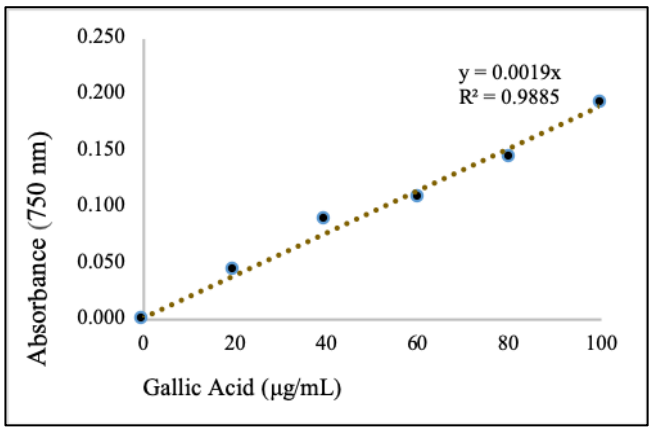

Figure 1. Standard curve for gallic acid

Table III shows the mean of total phenolic content from each sample that was tested in six replicates. The total phenolic content in all cooking time ranged from 1.839 to 8.018 mg GAE/g. Samples that were cooked for 3 h had the highest total phenolic content (8.018 mg GAE/g) ($p<0.05$) compared to those that were cooked for 1, 2, 4 and 5 h (4.205, 2.368, 2.746, and 1.839 mg GAE/g).

TABLE III. TOTAL PHENOLIC CONTENT OF BEEF RENDANG AT DIFFERENT COOKING TIMES AND TEMPERATURES

Cooking time (h)	Heating temperature (°C)	TPC (mg GAE/g)
1	120	4.205±0.364[b]
2	120	2.368±0.127[c]
3	110	8.018±0.911[a]
4	90	2.746±0.080[c]
5	90	1.839±0.110[c]

[a-c] Values with different letters differ significantly ($p<0.05$).

The reduction of phenolic compounds in rendang was due to the prolonged heat during cooking. The phenolic content fluctuated during prolonged cooking, and the results showed that TPC significantly decreased from 1 to 2 h at similar temperatures. After 3 h of cooking at 110 ºC, TPC increased significantly ($p<0.05$), then prolonged heating at 4 to 5 h at 90 ºC decreased the TPC. The phenolic compounds from the ingredients, especially herbs and spices as main sources of antioxidants in rendang, were destructed upon heating, as most of the phenolics were heat labile.

According to Sharma et al. [7], there was an increase in the amount of phenolic content in onions that were heated at 120 ºC for 30 min. However, prolonged heating after 30 min reduced the phenolics significantly. Furthermore, antioxidants may also be derived from coconut milk (fat) [4]. Heating fat causes a variety of chemical reactions, including oxidation. The chemical mechanism of thermal oxidation is nearly identical to that of autoxidation. Thermal oxidation occurs at a higher rate than autoxidation, and the unstable primary oxidation products, hydroperoxides, are swiftly degraded into secondary oxidation products such as aldehydes and ketones [17].

On the other hand, phenolics may differ according to the types of spices used as ingredients, such as garlic, which were released by the high temperature of cooking. A study by Panpatil et al. [5] found that the TPC of garlic increased as the cooking temperature was raised, most likely due to the release of bound phenolic and flavonoid compounds in the cooking water. The increase in TPC after heating may also be attributed to the inactivation of the polyphenol oxidase enzyme, which prevents polyphenolics from degrading [6]. The quantity and structure of phenoxy groups are critical factors in improving antioxidant activities. Free radical scavenging is improved with a catechol structure (phenols in positions 3′ and 4′) with an enol group in position 3 [18]. Furthermore, the presence of an enone moiety aids in the enhancement of antioxidant capabilities.

C. DPPH assay

Table IV shows the mean of the DPPH activity of rendang presented as EC_{50} values. The EC_{50} value denotes the concentration of beef rendang extract required to inhibit the initial DPPH radical concentration by 50%.

TABLE IV. DPPH ACTIVITY (EC_{50} VALUE) OF BEEF RENDANG AT DIFFERENT COOKING TIMES AND TEMPERATURES

Cooking time (h)	Heating temperature (°C)	EC_{50} value (µg/mL)
1	120	20.278±0.733[d]
2	120	39.041±1.368[c]
3	110	40.143±4.502[c]
4	90	48.338±2.115[b]
5	90	58.159±3.060[a]

[a-d] Values with different letters differ significantly ($p<0.05$).

The results showed a significant increase ($p<0.05$) of DPPH between 1 to 5 h. There was no significant effect ($p>0.05$) of cooking time between 2 to 3 h on EC_{50} values at different heating temperatures. It was also observed that prolonged

cooking would affect the bioactive compounds (e.g., phenolic components) present in the samples, thus influencing the DPPH activity of the samples.

The mean value of EC_{50} of every stage of rendang agreed well with the results of total phenolic content, whereby lower cooking time has the lowest EC_{50} values, i.e., having stronger strength of antioxidants to scavenge the DPPH radical. This can be shown at the EC_{50} value of gravy (T1), which has the highest content of antioxidant compounds. The mean value of EC_{50} shows that when rendang was cooked for 1 h, the ability to scavenge 50% of DPPH free radicals was maximum.

Generally, the data shows that an increase in cooking time for beef rendang will increase the EC_{50} value. This is because the phenolic content affects the increasing rate of antioxidant activity. Besides phenolics, other compounds such as flavonoids, which can be found majorly in spices, also contribute to the antioxidant activity [18] of the beef rendang. Other than that, Chandra et al. [20] demonstrated the link between phenolic compounds and antioxidant activity in several vegetables using the DPPH assay. This is supported by the Pearson correlation, where negative correlations and significant values were obtained between TPC and EC_{50} values for all five stages of cooking conditions (Table V). The table shows an inversely proportional relationship between the two variables, where the higher the TPC, the lower the EC_{50}.

TABLE V. PEARSON CORRELATION BETWEEN TPC AND EC_{50} VALUES OF BEEF RENDANG AT DIFFERENT STAGES

		TPC				
		T1	**T2**	**T3**	**T4**	**T5**
EC_{50}	**T1**	-0.578 .023*				
	T2		-0.573 .023*			
	T3			-0.539 .027*		
	T4				-0.277 .029*	
	T5					-0.136 .019*

*denotes significance at $p<0.05$

DPPH assay was used to determine antioxidant activity in this experiment. For instance, antioxidants are divided into primary and secondary antioxidants based on their method of action. They can act as hydrogen donors or acceptors of free radicals, inhibiting the chain reaction of oxidation and resulting in more stable radicals. Besides, antioxidant vitamins, antioxidant minerals, and phytochemicals mostly have a phenolic structure, which includes flavonoids, carotenoids, lycopene, β-carotene, catechins, diterpene and their derivatives. These components interact by various mechanisms, including metal ion binding, converting hydroperoxides to non-radical species, scavenging reactive oxygen species, absorbing UV radiation, and/or deactivating singlet oxygen [21].

Moreover, the DPPH assay assesses a compound's antioxidant capabilities by determining its ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical [22]. DPPH is a commercially accessible and very stable free radical that can take an electron or a hydrogen atom. Thus, the DPPH assay can be considered an economic, easy, and accurate method to evaluate antioxidants' radical scavenging activity (RSA). Specific chemicals or extracts can react with a stable radical, DPPH, in a methanol solution to determine their antioxidative activity. During the reaction, the decrease in DPPH absorbance at a characteristic wavelength is monitored. DPPH absorbs at 515 nm in its radical form, but this absorption is lost when it is reduced by an antioxidant (AH) or a radical species (Re).

IV. CONCLUSION

This study has shown that there was a significant effect of cooking time and temperature on extraction yield and antioxidant properties of beef rendang. The reduction of antioxidant abilities of beef rendang through TPC and DPPH analysis from 1 to 5 h indicated that prolonged cooking time had a detrimental effect on the antioxidant properties. This study has examined the effect of cooking time and temperature on the antioxidant properties of beef rendang. A further extensive study is warranted to determine the effect of prolonged cooking time and temperature on the rendang's lipid and protein co-oxidation due to the presence of spices and coconut milk as the main ingredients. This is because the prolonged cooking process is susceptible to oxidation reactions and the formation of metabolites.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

ACKNOWLEDGEMENT

The authors thank the Faculty of Food Science and Technology and the Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia, for their support and resources. This research was funded by the Ministry of Higher Education Malaysia under the Fundamental Research Grant Scheme (FRGS/1/2020/STG04/UPM/01/1).

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