Journal of Food Measurement and Characterization (2025) 19:5750–5768
https://doi.org/10.1007/s11694-025-03352-0

ORIGINAL PAPER

A comparative study of antioxidant properties and oxidative stability in beef rendang under prolonged heating and varying coconut milk percentages: a chemometric approach

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Received: 4 February 2025 / Accepted: 15 May 2025 / Published online: 7 June 2025

Abstract

This study investigated the impact of varying coconut milk percentages (0–125%) and cooking times (0–4 h) on the antioxidant properties and oxidative stability of beef rendang, a traditional Southeast Asian dish. Antioxidant activity was assessed through total phenolic content (TPC), total flavonoid content (TFC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, and Ferric reducing antioxidant power (FRAP) assays, while lipid and protein oxidation were evaluated via conjugated dienes, anisidine values, carbonyl content, and soluble protein. Creatine and creatinine levels were also monitored. Results revealed that higher coconut milk concentrations (50–125%) and moderate cooking times (2–4 h) enhanced antioxidant activity and reduced lipid and protein oxidation. However, prolonged cooking diminished antioxidant properties due to increased oxidation reactions. Chemometric analysis highlighted significant interactions between coconut milk and cooking time. This study underscores the need to optimise coconut milk levels and cooking duration to improve the quality and safety of rendang, benefiting both manufacturers and consumers.

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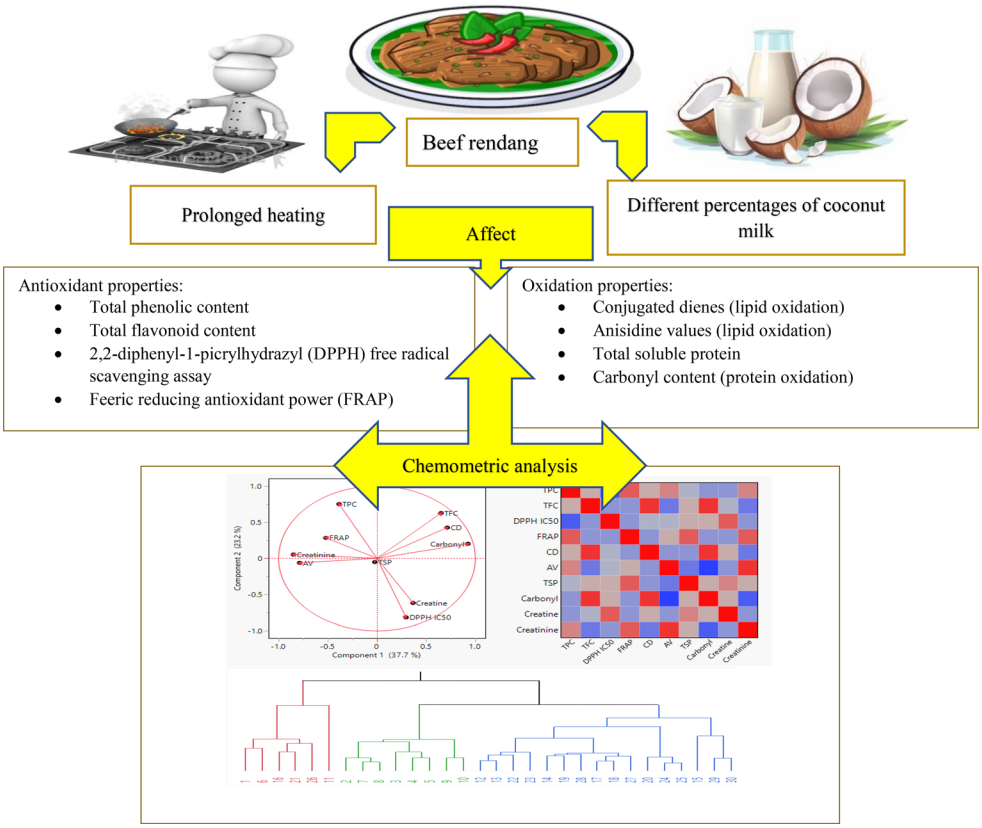
Graphical Abstract



Keywords Antioxidant · Beef Rendang · Coconut milk · Creatine · Creatinine · Oxidation · Prolonged heating

Introduction

Beef rendang, a traditional dish originating from Indonesia and a famous cuisine in Malaysia, is renowned for its rich flavours developed through a slow cooking process involving a blend of spices, coconut milk, and meat protein [1]. Various meat proteins, such as beef, chicken, and shellfish (e.g., cockles and oysters), can be used to prepare rendang. The spices responsible for enhancing the flavour and aroma of the dish include garlic, onion, ginger, galangal, fennel, cumin, lemongrass, turmeric, coriander, and tamarind. These spices exhibit antioxidant properties that contribute to the dish's overall nutritional value [2]. Compared to chicken rendang, beef rendang requires a longer cooking time. Malaysian rendang is often cooked for up to five hours at temperatures between 90 °C and 120 °C. This extended cooking period is essential for developing the dish's distinctive texture and flavour while enhancing its preservation [2]. A study found that while fresh beef has a higher protein level than beef rendang, the key ingredient in beef rendang, i.e., coconut milk, has a higher fat content than fresh beef [3].

Coconut milk significantly influences the antioxidant properties of rendang. It contains phenolic compounds and lauric acid, which contribute additional antioxidants. The percentage of coconut milk used is crucial; higher concentrations may increase antioxidant content, but also introduce more lipids, which are prone to oxidation. Additionally, coconut milk is rich in saturated fats and contains antioxidants such as tocopherols and polyphenols [4]. During the prolonged cooking process, lipid and protein oxidation occur simultaneously. This oxidation leads to the degradation of amino acids, loss of essential proteins, and the formation of protein-lipid cross-links. The heat-induced oxidative stress on beef proteins is exacerbated by lipid oxidation, as reactive oxygen species (ROS) and lipid peroxides can attack protein molecules [5]. The natural antioxidants present in the spices and coconut milk play a role in inhibiting protein oxidation, though their effectiveness depends on their stability during prolonged cooking.

Thermal processing in beef rendang not only enhances flavour and shelf life, but also inactivates bacteria and anti-nutritional components [6]. However, heating can lead to the degradation of thermolabile nutrients and the formation

of less bioactive and potentially harmful substances, such as diterpenoids, trans fatty acids, heterocyclic aromatic amines, and certain Maillard reaction products [7]. Cooking may also cause the degradation or loss of some phenolic structures, which are sensitive to heat [8]. Additionally, there are minimal reports on the interactions between phenolic chemicals and other food components, such as proteins, lipids, and sugars. By participating in the Maillard reaction at high temperatures, these compounds generate melanoidins, which lower the food's phenolic content [7]. Consequently, cooking methods have a significant impact on the bioavailability of polyphenols and the health benefits associated with them.

This study aimed to investigate the effects of different coconut milk percentages and prolonged cooking on the antioxidant properties and oxidative stability of beef rendang. By analysing antioxidant activity, lipid and protein oxidation, and chemometric interactions, this research sought to determine the optimal coconut milk concentrations and cooking durations that enhance the dish's nutritional quality and stability. The findings will provide valuable insights for consumers and food manufacturers seeking to improve the quality, safety, and shelf life of traditional beef rendang. While previous studies have investigated the antioxidant properties of rendang ingredients individually, this study is the first to systematically analyse the interactive effects of coconut milk concentration and prolonged heating on both lipid and protein oxidation using a chemometric approach. This research introduced a novel combination of two-way ANOVA and chemometric analysis (Principal Component Analysis and Hierarchical Cluster Analysis) to uncover patterns in antioxidant degradation and oxidative stability, offering deeper insights into optimising cooking conditions for improved food quality.

Methodology

Materials

All chemicals and reagents were of analytical grade and purchased from Thermo Fisher Scientific (New Hampshire, USA), Merck KGaA (Germany), R&M Chemicals (UK), and Sigma-Aldrich (St. Louis, MO, USA), unless stated otherwise. The reagents included sodium carbonate, gallic acid, methanol, Folin-Ciocalteu's reagent, pure ethanol, chloroform, iso-octane, *n*-hexane, *p*-anisidine, glacial acetic acid, bovine serum albumin (BSA) standard, phosphate-buffered saline (PBS, pH 7.4), Bradford reagent (Bio-Rad), 0.15 M potassium chloride buffer, trichloroacetic acid, sodium dodecyl sulphate (SDS) solution, dinitrophenylhydrazine (DNPH) solution, hydrochloric acid, ethyl acetate, guanidine hydrochloride, monosodium phosphate, creatine standard (C0780), creatinine standard (C4255), diethyl ether, picric acid, sodium hydroxide, diacetyl, and 1-naphthol.

Preparation of beef Rendang samples

All ingredients were bought from a wholesale market in Seri Kembangan, Selangor, Malaysia. A total of 12 kg of lean beef shank was cut into 3 × 5 × 5 cm pieces, cooked on a gas stove for two hours, and then left to cool at room temperature for one hour. The boiled flesh was manually shredded and chilled (6–8 ºC) overnight. Coconut milk (10 kg), 400 g of coconut butter (*kerisik*), 1 kg of blended dried chilli, 3 kg of blended red onion *(Allium cepa L.)*, 1 kg of blended garlic (*Allium sativum)*, 120 g of blended galangal *(Alpinia galangal)*, 1.5 kg of blended lemongrass *(Cymbopogon citratus)*, 100 g of tamarind *(Tamarindus indica L.)*, 120 g of blended ginger *(Zingiber officinale)*, 100 g of coriander *(Coriandrum sativum)* seed powder, 50 g of fennel *(Foeniculum vulgare)* powder, 50 g of cumin *(Cuminum cyminum)* powder, 200 g of sugar, and 100 g of salt were added to an automatic braising pan (Salsamat, Nilma, Parma, Italy). The rendang was prepared using six different coconut milk percentages (0%, 25%, 50%, 75%, 100%, and 125%) and cooked for five different durations (0, 1, 2, 3, and 4 h) at a maintained temperature of 120 °C. All samples were prepared in triplicate to ensure data reliability and minimise experimental variation. After cooking, all samples were lyophilised to prevent post-cooking oxidation before further analysis.

Antioxidant analysis

Extraction procedure

The extraction procedure for rendang samples was conducted according to a method described in a study, with slight modifications [9]. This extraction was performed to obtain antioxidant compounds present in the beef rendang samples, which were then analysed for their phenolic content and radical scavenging activities. Briefly, a 0.5 g rendang sample was weighed into a 50-mL centrifuge tube, followed by the addition of 12 mL of 100% (v/v) methanol. The mixture was vortexed for 1 min and sonicated for 30 min at room temperature to enhance extraction efficiency. Subsequently, the mixture was centrifuged at 5,000 rpm for 10 min at ambient temperature (22–24)°C, and the supernatant was filtered through a 0.45 µm syringe filter for further antioxidant analysis.

Total phenolic content

The concentration of phenolics in rendang extracts was measured using a modified Folin-Ciocalteau method [10]. This assay was conducted to determine the total phenolic content (TPC), as phenolic compounds are key contributors to the antioxidant capacity of the rendang samples. Gallic acid served as the standard phenolic compound. For the analysis, a methanolic extract solution with a concentration of 41.67 mg/mL was employed. 50 µL of distilled water, 100 µL of 10% (v/v) Folin-Ciocalteau reagent solution, and 50 µL of methanolic extract solution or standard gallic acid solutions (1.5625–200 µg/mL) were combined to create the reaction mixture. After 6 min, 100 µL of a 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was added and gently stirred. The reaction mixture was then incubated in the dark for 2 h, and its absorbance was determined at 765 nm against distilled water as a blank solution using the microplate reader. The TPC was calculated from known quantities of the gallic acid standard and expressed as mg GAE/g dry weight (DW).

Total flavonoid content

The total flavonoid content (TFC) was determined using a method adapted from a previous study, with minor modifications [11]. This analysis aimed to assess the flavonoid content in the rendang samples, as flavonoids are significant antioxidants that contribute to oxidative stability. Briefly, 100 µL of the sample (41.67 mg/mL) or standard quercetin solutions (ranging from 0.7813 to 100 µg/mL) was mixed with 100 µL of 2% (w/v) aluminium chloride ($AlCl_3$) ethanol solution in a 96-well plate. The mixture was incubated in the dark for 1 h at room temperature, after which the absorbance was measured at 420 nm. A calibration curve was constructed using quercetin as the standard, and the TFC was expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW).

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was conducted to evaluate the antioxidant capacity of the rendang samples by measuring their ability to neutralise free radicals. This assay assessed the efficiency of antioxidants in the sample to donate hydrogen atoms or electrons, thereby stabilising DPPH radicals, which serve as a key indicator of their potential to prevent oxidative degradation. The procedure was carried out according to a previously described method, with slight modifications [12]. A 0.10 mM (0.004%) DPPH solution was prepared by dissolving 4 mg of DPPH powder in 100 mL of 100% (v/v) aqueous methanol. A Trolox stock solution (0.4 mg/mL) was prepared by dissolving 4 mg of Trolox in 10 mL of 100% (v/v) aqueous methanol. From this stock solution, five Trolox concentrations were prepared: 400, 200, 100, 50, 25, and 12.5 µg/mL. Similarly, a series of stock solutions were prepared from the methanolic extract of beef rendang at various stages, with concentrations of 1.875, 3.75, 7.5, 15, 30, and 60 µg/mL. For the assay, 100 µL of each extract was mixed with 200 µL of the DPPH solution. After incubating the mixture in the dark for 30 min at room temperature, the absorbance was measured at 517 nm using a BIOTEK GEN5 Eon Microplate Spectrophotometer (Winooski, Vermont, USA). The DPPH free radical scavenging activity (% RSA) was calculated using the following equation (Eq. 1):

$$\text{Radicalscavengingactivity (RSA)} = \frac{\text{Absblank} - \text{Abssample}}{\text{Absblank}} \times 100 \quad (1)$$

Where Abs_{blank} denoted the absorbance of the control reaction (which contained all reagents except the rendang extract), and Abs_{sample} denoted the absorbance of the rendang extract. A standard curve of percentage inhibition versus extract concentrations was plotted, and the half-maximal inhibitory concentration (IC_{50}) of the extract was derived. Trolox was used as a positive control.

Determination of ferric reducing antioxidant power (FRAP) assay

The FRAP (ferric reducing antioxidant power) assay was conducted to evaluate the reducing power of antioxidants in the rendang samples, which serve as an important indicator of their ability to mitigate oxidative stress. This assay measured the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in the presence of antioxidants, reflecting the electron-donating capacity of the sample. The FRAP assay was performed according to a previously described method [13], with slight modifications. Briefly, the FRAP reagent was freshly prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6\ H_2O$ in a 10:1:1 (v/v/v) ratio. The mixture was incubated at 37 °C for at least 10 min. For the assay, 20 µL of the extract solution (41.67 mg/mL) and 80 µL of distilled water were added to 200 µL of the freshly prepared FRAP reagent in a 96-well plate. The solution was thoroughly mixed, incubated for 8 min at room temperature, and the absorbance was measured at 593 nm using distilled water as the control. A standard curve was constructed using ferrous sulphate heptahydrate ($FeSO_4 \cdot 7\ H_2O$) solutions with concentrations ranging from 3.125 to 400 µg/mL. The

FRAP values were expressed as milligrams of Fe (II) per gram of dry weight (mg Fe (II)/g DW).

Lipid oxidation analysis

Lipid oxidation analysis was performed to assess the oxidative stability of fats in beef rendang. Lipid oxidation contributes to rancidity, the formation of off-flavours, and the degradation of product quality. The analysis included measurements of conjugated dienes and anisidine values, which indicate the extent of primary and secondary lipid oxidation, respectively.

Lipid extraction method

The extraction process using n-hexane involved placing 40 g of freeze-dried beef rendang into a beaker with 200 mL of n-hexane. The mixture was stirred at 300 rpm for 20 h at 45 °C. To prevent solvent evaporation, the samples were covered with aluminium foil. After extraction, the hexane solution was filtered through 125 mm F1113-grade filter paper and then concentrated using a rotary evaporator at 40 °C for 20 min. The extracted oil was collected and stored at -20 °C until further analysis. The de-oiled samples (crude samples) were retained for subsequent protein co-oxidation analysis.

Conjugated dienes analysis

Conjugated dienes (CD) analysis was conducted to assess the extent of primary lipid oxidation in the rendang samples. Conjugated dienes are early-stage oxidation products formed when polyunsaturated fatty acids undergo structural changes. Measuring their concentration provides valuable insight into the initial oxidation process and the oxidative stability of the lipids in rendang. The analysis was performed according to the method recommended by the International Union of Pure and Applied Chemistry (IUPAC) [14], with minor modifications.

Approximately 0.5 g of the lipid extract was dissolved in 25 mL of iso-octane and vortexed for 1 min to ensure homogenisation. The conjugated dienes were quantified by measuring the absorbance at 234 nm, and the results were expressed as absorbance E 1% 1 cm, calculated using Eq. (2):

$$\boldsymbol{E}\,1\%\,1\,\boldsymbol{cm}\,(234) = \frac{\boldsymbol{A\lambda}}{\boldsymbol{C}\,\boldsymbol{x}\,\boldsymbol{d}} \tag{2}$$

Where Aλ is the absorbance at 234 nm; C is the concentration of the lipid solution in iso-octane (g 100 mL^{-1}), and d is the length of the cuvette.

Anisidine value (AV)

The anisidine value (AV) analysis was conducted to assess the extent of secondary lipid oxidation in the rendang samples. As lipid oxidation progresses, primary oxidation products, such as conjugated dienes, degrade into aldehydes, which contribute to off-flavours and rancidity. The p-anisidine value (p-AV) was determined according to the AOCS Official Method Cd 18–90 [15], with slight modifications. Briefly, 0.5 g of the extracted lipid sample was diluted to 25 mL with iso-octane in a volumetric flask and vortexed for homogenisation. The initial absorbance (A_1) was measured at 350 nm using a UV-visible spectrophotometer. Next, 5 mL of the lipid mixture was combined with 1 mL of 2.5 mg mL^{-1} p-anisidine in glacial acetic acid, vortexed, and incubated in the dark for 10 min. The absorbance at 350 nm (A_2) was then measured using a UV-2550 spectrophotometer (Shimadzu, Japan) against a reagent blank. The p-AV was calculated using the formula provided in Eq. 3:

$$\boldsymbol{p-AV} = \frac{25 \times (1.2\mathbf{A}2 - \mathbf{A}1)}{\boldsymbol{m}} \tag{3}$$

Where, A2 = Absorbance of the lipid solution after reaction with the p-anisidine reagent, A1 = Absorbance of the lipid solution alone, and m = Mass of the sample (g).

Protein-co oxidation analysis

Protein oxidation was assessed by measuring carbonyl content and total soluble protein. Protein oxidation contributes to changes in texture, loss of nutritional quality, and the potential formation of undesirable compounds. The carbonyl content was determined using the dinitrophenylhydrazine (DNPH) method, which quantifies protein carbonylation, a key indicator of oxidative damage.

Carbonyl value

The carbonyl value analysis was conducted to evaluate the extent of protein oxidation in the rendang samples. Protein oxidation, caused by prolonged cooking and oxidative stress, led to the formation of carbonyl compounds, indicating protein degradation and potential loss of nutritional and textural quality. The total carbonyl content was analysed using the DNPH (2,4-dinitrophenylhydrazine) method with slight modifications from a previous study [16]. Briefly, 1 g of cooked meat was homogenised with 10 mL of 0.15 M KCl buffer. Then, 100 µL of the homogenate was mixed with 1 mL of 10% trichloroacetic acid (TCA) and centrifuged at 5000 × g for 5 min. The resulting pellets were dissolved in 400 µL of 5% sodium dodecyl sulphate (SDS) solution.

Next, 0.8 mL of 0.3% (w/v) DNPH in 3 M HCl was added, and the mixture was incubated at room temperature for 30 min. After incubation, proteins were precipitated by adding 400 µL of 40% TCA, followed by centrifugation at 5000 × g for 5 min. The pellets were washed three times with an ethanol-ethyl acetate mixture (1:1, v/v) and dissolved in 1.5 mL of 6 M guanidine hydrochloride in 20 mM sodium phosphate buffer (NaH_2PO_4, pH 6.5) through overnight incubation at 4 °C. The absorbance was measured at 280 nm and 370 nm using a UV-VIS Lambda 35 spectrophotometer (PerkinElmer, Waltham, MA) at 25 °C to determine protein concentration and carbonyl content, respectively. The carbonyl content was calculated using the formula provided in Eq. 4 and expressed as nmol/mg protein.

$$\text{Carbonylcontent}\left(\frac{\text{nmol}}{\text{mg}}\right) = \frac{\text{Abs370} - \text{Abs370 (blank)}}{[22,000\text{xAbs280} - (\text{Abs370} - \text{Abs370 (blank)} \times 0.43]} \times 10^6 \quad (4)$$

Total soluble protein

The total soluble protein analysis was conducted to evaluate the extent of protein denaturation and aggregation in the rendang samples. Heat and oxidative stress can reduce protein solubility by inducing structural changes, potentially affecting the nutritional quality, texture, and functional properties of the dish. The soluble protein content was determined using Bradford's method [17] and expressed as the percentage of total soluble proteins relative to the sample weight (w/w, dry basis). De-oiled samples from Sect. 2.4.1 were extracted overnight at 150 rpm with continuous shaking in a sodium phosphate buffer (0.2 M, pH 7.9) containing 0.02% sodium azide. The mixture was then centrifuged at 14,000 × g for 30 min. The supernatant was collected, flushed with argon gas, and stored at −80 °C until analysis. Bovine serum albumin (BSA) was used as the protein standard for this analysis.

Creatinine and creatinine content

The creatine and creatinine content of the beef rendang samples were analysed to evaluate the effect of prolonged cooking and varying coconut milk percentages on muscle protein degradation. Creatine is a natural component in meat that degrades into creatinine during heat treatment, and its levels can indicate the extent of thermal processing and protein stability in the samples.

Creatine content

The creatinine content of the samples was analysed using a method adapted from a previous study [18], with slight modifications. To remove precipitated proteins, 1 g of the sample was weighed into a beaker, mixed with 20 mL of trichloroacetic acid, and homogenised for 5 min. The mixture was then filtered through filter paper. Next, 8 mL of the filtrate was collected and de-oiled with 4 mL of diethyl ether. After a 10-minute phase separation, 4 mL of the refined extract was mixed with 1.5 mL of picric acid in sodium hydroxide solution. The mixture was thoroughly mixed, heated at 40 °C for 10 min, and the creatinine content was determined spectrophotometrically at 500 nm.

Creatine content

The creatine content of the samples was determined according to a method adapted from a previous study [18], with slight modifications. Briefly, to separate precipitated proteins, 0.25 g of the sample was weighed into a beaker, mixed with 100 mL of trichloroacetic acid, and homogenized for 5 min. The mixture was then filtered through filter paper. Next, 20 mL of the filtrate was collected and de-oiled with 10 mL of diethyl ether. After a 10-minute phase separation, 4 mL of the refined extract was combined with 2 mL of 1-naphthol and 2 mL of diacetyl in sodium hydroxide solution. The mixture was thoroughly mixed, heated at 40 °C for 5 min, and the creatine content was determined spectrophotometrically at 520 nm.

Statistical analysis

All assays were performed in triplicate, and results were reported as the mean ± SEM to ensure data reliability. The differences between the mean values were assessed using two-way analysis of variance (ANOVA) to evaluate the interactive effects of coconut milk percentage and cooking time on antioxidant activity and oxidative stability. Tukey's post-hoc test was applied for multiple comparisons at a significance level of 0.05 ($p < 0.05$) to determine statistically significant differences between treatment groups. Statistical analyses were performed using Minitab 21 statistical software (Minitab Inc., State College, PA, USA).

To enhance the computational analysis, a comprehensive chemometric approach was applied using JMP® Pro software (version 17.0.0, SAS Institute Inc., Cary, NC, USA). Pearson's correlation analysis was conducted to identify relationships between antioxidant activity, lipid oxidation, and protein oxidation markers. Bartlett's test of sphericity and the Kaiser-Meyer-Olkin (KMO) test were used to validate the suitability of the data for multivariate analysis,

ensuring the robustness of the statistical approach. Principal Component Analysis (PCA) was employed to uncover dominant patterns and variability across samples, while Hierarchical Cluster Analysis (HCA) classified samples based on their antioxidant and oxidation profiles. Additionally, a factor loadings analysis was performed to determine the contribution of individual variables to overall variance. The integrated statistical and chemometric approach provided a more holistic and data-driven evaluation, offering deeper insights into antioxidant stability and oxidative interactions beyond traditional univariate methods.

Results and discussion

Antioxidants analysis

Table 1 presents the total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP) across different coconut milk concentrations and cooking durations. The data show that TPC and FRAP values peaked at 50% coconut milk concentration and a 4-hour cooking duration, indicating optimal antioxidant retention under these conditions. This aligns with previous studies suggesting that moderate fat content enhances polyphenol stability, whereas excessive fat levels may promote oxidation [2, 8].

Based on the results obtained, the highest TPC and FRAP values after heat treatment were 457 mg GAE/g and 1140 mg FE/g, respectively, in beef rendang prepared with 50% of coconut milk and cooked for 4 h. Meanwhile, the

Table 1 Antioxidant properties of beef Rendang: total phenolic content, total flavonoid content, DPPH radical scavenging activity (IC_{50}), and ferric reducing antioxidant power (FRAP) analysed using Two-Way ANOVA with interaction effects (Coconut milk × cooking Time)

Variables	Cooking time					*P*-value		
Coconut milk percentage	0 h	1 h	2 h	3 h	4 h	CM	CT	CM x CT[1]
Total phenolic content (mg GAE/ g)								
0%	277 ± 8.38^{Bc}	382 ± 5.45^{Aa}	349 ± 6.54^{Aa}	373 ± 12.2^{Aa}	373 ± 13.7^{Aa}	0.423	0.083	0.005
25%	329 ± 8.12^{ABab}	339 ± 9.41^{Aa}	339 ± 5.07^{Aa}	277 ± 14.6^{Bc}	295 ± 15.4^{ABa}			
50%	159 ± 5.28^{Ad}	320 ± 13.4^{Aa}	307 ± 8.04^{Aa}	294 ± 7.93^{Abc}	457 ± 16.6^{Aa}			
75%	331 ± 8.55^{ABab}	343 ± 7.81^{ABa}	324 ± 24.9^{ABa}	350 ± 4.9^{Aab}	288 ± 0.819^{Ba}			
100%	362 ± 14.5^{Aa}	336 ± 21.5^{Aa}	321 ± 6.15^{Aa}	302 ± 21.1^{Abc}	326 ± 9.7^{Aa}			
125%	316 ± 7.19^{Abc}	314 ± 25.3^{Aa}	355 ± 6.31^{Aa}	324 ± 9.61^{Aabc}	334 ± 8.44^{Aa}			
Total flavonoid content (mg QE/ g)								
0%	51.2 ± 1.47^{Ac}	37.1 ± 0.754^{Ba}	30.7 ± 0.067^{Ca}	30 ± 0.623^{Ca}	32.3 ± 0.691^{Ca}	< 0.001	< 0.001	< 0.001
25%	64 ± 0.835^{Ab}	27.5 ± 0.545^{Bb}	26 ± 0.485^{Ba}	17.3 ± 0.453^{Cb}	17.8 ± 1.48^{Cb}			
50%	11.1 ± 0.202^{Ad}	14.4 ± 0.642^{Ad}	13.8 ± 0.678^{Ab}	11.8 ± 0.506^{Ab}	19.1 ± 4.47^{Ab}			
75%	46.2 ± 0.509^{Ac}	21.5 ± 1.15^{Bbcd}	15.9 ± 0.327^{Cb}	17.3 ± 0.094^{Cb}	16 ± 0.557^{Cb}			
100%	45 ± 4.38^{Ac}	16.3 ± 3.73^{Bcd}	13.3 ± 0.187^{Bb}	15.7 ± 3.16^{Bb}	14.8 ± 0.499^{Bb}			
125%	73.9 ± 0.605^{Aa}	24.8 ± 1.83^{Bbc}	24.4 ± 0.676^{Ba}	17.5 ± 1.72^{Cb}	20.6 ± 0.477^{BCb}			
DPPH IC_{50} (% inhibition)								
0%	27.7 ± 0.485^{Aa}	17.8 ± 1.24^{Cd}	22.7 ± 2.24^{ABCab}	20.8 ± 1.38^{BCc}	24.6 ± 0.963^{ABb}	0.004	0.026	< 0.001
25%	23.9 ± 0.993^{Aa}	26.1 ± 0.858^{Abc}	18.9 ± 1.33^{Bb}	25.2 ± 1.08^{Abc}	21.7 ± 0.971^{ABb}			
50%	76.5 ± 29.2^{Aa}	21.8 ± 0.333^{Acd}	20.7 ± 1.25^{Aab}	18.1 ± 1.04^{Ac}	19.9 ± 1.33^{Ab}			
75%	29.4 ± 0.328^{Aa}	29.5 ± 1.13^{Aab}	29.5 ± 0.847^{Aab}	28.7 ± 0.355^{Aab}	33.1 ± 1.71^{Aa}			
100%	27.8 ± 1.62^{Aa}	33.2 ± 2.91^{Aa}	37.7 ± 9.2^{Aa}	33.8 ± 3.29^{Aa}	34.7 ± 2.65^{Aa}			
125%	20.4 ± 0.537^{Ba}	21.9 ± 0.904^{ABcd}	21.4 ± 0.312^{ABab}	22.6 ± 0.568^{ABbc}	23.4 ± 0.571^{Ab}			
Ferric reducing antioxidant power (mg FE/ g)								
0%	371 ± 12.7^{Bd}	479 ± 3.44^{Abc}	359 ± 15^{Bb}	351 ± 14.3^{Be}	353 ± 12.9^{Bb}	< 0.001	0.028	< 0.001
25%	377 ± 19.8^{Cd}	383 ± 36.8^{Cc}	408 ± 14.1^{BCb}	594 ± 11.4^{Ac}	503 ± 26.7^{ABab}			
50%	273 ± 4.11^{Be}	565 ± 4.69^{ABb}	611 ± 10.2^{ABab}	699 ± 30.4^{ABb}	1140 ± 345^{Aa}			
75%	549 ± 11.8^{Cc}	695 ± 31^{ABa}	622 ± 21.1^{BCab}	751 ± 20^{Aab}	536 ± 31.9^{Cab}			
100%	732 ± 15.5^{ABa}	769 ± 12.3^{Aa}	609 ± 138^{ABCab}	457 ± 5.13^{BCd}	436 ± 15.7^{Cb}			
125%	644 ± 9.8^{Cb}	537 ± 4.36^{Db}	753 ± 21^{ABa}	801 ± 2.81^{Aa}	704 ± 3.04^{Bab}			

Values are means ± SEM, $n = 3$ per treatment group. IC_{50} indicates half maximal inhibitory concentration

The uppercase letter denotes the significant difference ($P < 0.05$) between columns (cooking time), meanwhile the lowercase denotes the significant difference between rows (coconut milk percentages), in each analysis as analysed by two-way ANOVA and the TUKEY test. [1]CM × CT = COCONUT MILK × COOKING TIME interaction effect and are significantly ($p < 0.05$) different ($n = 3$)

lowest TPC and FRAP values, 159 mg GAE/g and 273 mg FE/g, respectively, were recorded in beef rendang with 50% of coconut milk at 0 h of cooking (Table 1). Heating causes enzymes to break down, which stops them from reducing the antioxidant levels in the dish as they normally would. This explains the liberations of antioxidants in beef rendang spices from the cellular structure due to cell wall hydrolysis, which occurs because of prolonged cooking [19]. Based on the TPC results for beef rendang, lauric acid in coconut milk, which is a highly stable fat did not deteriorate quickly at high temperatures, allowing antioxidants to rise. However, lauric acid tends to be susceptible to a few alterations if heated for an extended period, leading to the formation of trans fatty acid [20]. In general, coconut milk tends to exhibit higher TPC levels after prolonged heating, as heat facilitates the breakdown of cell walls in spices, thereby releasing previously bound phenolic compounds [21]. This process contributes to the higher TPC content observed at 4 h of cooking time across all formulations.

As for the rendang with 0% coconut milk, the source of TPC content was likely derived from spices such as coriander, lemongrass, cumin, fennel, garlic, and onion. Despite the absence of coconut milk as an antioxidant source, these spices contributed antioxidant levels, resulting in no significant difference compared to rendang formulations containing coconut milk. Hence, based on the findings of this study, it can be said that longer cooking times can increase antioxidant levels when moderate amounts of coconut milk are added. With higher coconut milk concentrations, not only were polyphenol oxidase enzymes more easily degraded, but their polyphenols were also affected. The higher the PO antioxidant level, the more susceptible it was to degradation [22]. Moreover, when a moderate amount of coconut milk was added, the tannins produced also increased. However, due to the breakdown of trapped antioxidants, they were not absorbed into the circulation, thereby reducing their ability to protect antioxidant cells [23]. Thus, when 75% coconut milk was added to the beef rendang, the higher fat content made it more difficult for the antioxidants to reach their target cells. There was a possibility that the total amount of TPC antioxidants in the food would be reduced. The interaction effect (Coconut Milk × Cooking Time) had a highly significant p-value of **0.005** (Table 1), indicating that the combination of the treatment method and cooking duration significantly affected the TPC. This suggests that while individual factors (coconut milk and cooking time) alone did not greatly impact TPC, their combined effect led to noticeable changes. For instance, certain cooking methods might preserve or enhance TPC better when applied for specific durations, while others might degrade phenolic more rapidly depending on the cooking time. Some antioxidants, however, like melanoidin, were generated during the long cooking process. This is because new antioxidants are created in the foods themselves [24].

The TPC results slightly contradicted the TFC results, as during extended period of cooking, the TFC content in beef rendang reduced significantly compared to 0 h of cooking. TFC values from Table 1 displayed significant variability across treatments, ranging from **11.1 ± 0.202** to **73.9 ± 0.605** mg QE/ g, indicating that flavonoid content is highly sensitive to the treatment conditions. Beef rendang with the highest percentage of coconut milk (125%) exhibited the highest TFC content for the 0-hour cooking time formulation. Over the cooking period, TFC significantly reduced because, when beef rendang was heated for over an hour, its water content evaporated, leading to lipid oxidation [25]. At the same time, the spices began to break down, reducing their antioxidant levels. Prolonged heating could also denature antioxidant proteins in the meat and reduce the activity of antioxidant enzymes [25]. The interaction effect between coconut milk percentages and cooking time was also significant ($p < 0.001$), implying that the effect of cooking time on TFC depends heavily on the percentage of coconut milk used. For example, a particular percentage of coconut milk (25%, 75%, and 125%) may retain high TFC when used briefly but cause substantial flavonoid loss if the cooking time is extended.

The FRAP results (Table 1) varied considerably, with values ranging from **273 ± 4.11** to **1140 ± 345** mg FE/g, showing a wide range of antioxidant potential. However, the p-values were significant across all categories, with < 0.001 for coconut milk interaction, **0.028** for cooking time interaction, and **<0.001** for the interaction between coconut milk and cooking time (highly significant). Generally, at higher temperatures and with prolonged heat application, the structure of coconut milk in beef rendang might have been altered, which could have denatured proteins and antioxidants. Such alteration may make coconut milk more oxidisable and potentially produce free radicals [26]. When coconut milk was heated to 80 °C and above, it lost the ability to maintain its protein structure, therefore reducing antioxidant properties. FRAP tests work through oxidation and reduction reactions, where ferric ions are converted into ferrous ions [26]. Spices in rendang, due to their strong antioxidant properties, can prevent or delay lipid oxidation. During the early stage of autoxidation, free radicals are produced. The antioxidants in coconut milk and spices help by either neutralising these free radicals through complex formation with metal ions (via the Fenton reaction) or by stopping them from forming in the first place [27]. As heating time increased, the protein in the mixture started to denature, leading to changes in its properties and thickness. However, the FRAP value reached its peak after 4 h of heating. This happens because antioxidants steadily reduce ferric ions (Fe^{3+}) to ferrous ions

(Fe^{2+}), a process that does not rely on enzymes. Additionally, spices and a large amount of coconut milk can slow down the denaturation of antioxidants [28]. As expected, antioxidant-rich spices such as turmeric, ginger, and garlic were used in preparing beef rendang, which requires long cooking process. During this process, these spices released their antioxidant properties into the dish [29]. When additional coconut milk was added, it contained enzymes, including proteolytic enzymes, lipase, and lipoxygenase, which can break down proteins. These proteolytic enzymes specifically target proteins that could act as pro-oxidants—molecules that can accelerate the oxidation process. Therefore, denatured proteins could have reduced pro-oxidants in the beef rendang, contributing to the FRAP content [28].

The DPPH IC_{50} results (Table 1) provided valuable insight into how different coconut milk percentages and cooking times influenced the antioxidant efficacy of the samples, specifically their capacity to scavenge free radicals. DPPH IC_{50} is a measure of the concentration needed to inhibit 50% of DPPH radicals, so lower IC_{50} values indicate stronger antioxidant activity. Although the results showed no significant difference in IC_{50} values for each formulation except for beef rendang with 50% coconut milk (0-hour cooking time), where it had the highest IC_{50} value, the highly significant interaction effect ($p < 0.001$) between coconut milk percentages and cooking time meant that the impact of cooking time on DPPH IC_{50} depended on the specific percentages of coconut milk applied. As the percentage of coconut milk in beef rendang increased, so did the level of saturated lauric acid. A study found that levels of saturated fats like lauric, myristic, and palmitic acids rose significantly in rendang spices [30]. Additionally, heating caused an increase in total trans fats in both the beef and spices. As moisture in the beef rendang decreased, antioxidants began to break down, potentially due to the formation of phenolic radicals within BHA molecules [31]. However, in this case, extended cooking time allowed for the gradual release of antioxidants from the spices used in beef rendang, such as turmeric, ginger, and garlic [29]. As these antioxidants were extracted into the dish, they enhanced its ability to scavenge free radicals. The breakdown of cellular structures in the meat and spices during prolonged heating could have made more bioactive compounds available for antioxidant activity. This included the release of phenolic compounds, which are known to contribute to antioxidant capacity. Following that, some cooking processes led to the formation of new antioxidant compounds through the Maillard reaction and other cooking-induced transformations [24]. Meanwhile, the combination of antioxidants from both the coconut milk and the spices could have had a synergistic effect, enhancing the overall antioxidant activity. This means that the presence of coconut milk may help to boost the effectiveness of the antioxidants present in the spices, further reducing the IC_{50}. There may be a threshold concentration of antioxidants needed to achieve a noticeable effect on the IC_{50}. Once this threshold is reached, additional increases in coconut milk may not result in further improvements in antioxidant capacity. The overall composition of beef rendang is complex, with various ingredients interacting in ways that can influence antioxidant activity. Increasing coconut milk may not have significantly altered the antioxidant dynamics if other factors, such as the balance of spices, meat, and cooking time, remained constant. The antioxidant activity trends observed in our study (TPC, TFC, FRAP, and DPPH) aligned with previous findings on the impact of prolonged heat on phenolic stability and lipid oxidation in meat-based dishes [2].

Oxidation analysis

Table 2 summarises lipid and protein oxidation markers, including conjugated dienes (CD), anisidine values (AV), total soluble protein (TSP), and carbonyl content. Notably, CD values increased with prolonged cooking, indicating progressive lipid peroxidation. However, at 100% coconut milk concentration, CD levels were lower than expected, potentially due to the protective role of lauric acid, which slows oxidation.

Table 2 presents a two-way ANOVA analysis of the effects of Coconut Milk (CM), cooking time (CT), and their interaction (CM × CT) on four variables associated with oxidation: CD (Conjugated Dienes), AV (Anisidine Value), TSP (Total Soluble Protein), and Carbonyl Content. All factors (CM, CT, and their interaction) were significant ($p < 0.001$), indicating that both the coconut milk percentages and cooking time significantly affected lipid oxidation and protein co-oxidation, with interactive effects observed as well. The highly significant interaction effects for each parameter ($p < 0.001$) indicate that the presence of coconut milk heavily modulated the influence of cooking time on oxidation. For example, coconut milk may help reduce oxidation when used with shorter cooking times but may lose effectiveness as cooking time increases. The combination of coconut milk percentages and specific cooking times could, therefore, be optimised to minimise oxidation.

In the early stages of lipid oxidation, non-conjugated double bonds in unsaturated fats were transformed into conjugated double bonds, producing conjugated dienes. These changes are often tracked using spectrophotometry, with characteristic absorption peaks appearing around 232–234 nm [32]. In beef rendang, the long cooking process often lasting several hours at 80–90 °C, promoted the oxidation of lipids found in both the beef and coconut milk [25, 30]. While the extended cooking time intensified flavour, it

Table 2 Lipid and protein oxidation parameters in beef Rendang: conjugated dienes, Anisidine values, total soluble protein, and carbonyl content analysed using Two-Way ANOVA with interaction effects (Coconut milk × cooking Time)

Variables	Cooking time					*P*-value		
Coconut milk percentage	0 h	1 h	2 h	3 h	4 h	CM	CT	CM x CT[1]
Conjugated dienes (E 1% 1 cm)								
0%	$0.110 \pm 5e\text{-}04^{Bab}$	0.0975 ± 0.0095^{Ca}	$0.0545 \pm 5e\text{-}04^{Dab}$	0.100 ± 0.0035^{BCa}	0.127 ± 0.001^{Aa}	< 0.001	< 0.001	< 0.001
25%	0.174 ± 0.0185^{Aa}	0.083 ± 0.009^{Bab}	$0.0675 \pm 5e\text{-}04^{Ba}$	$0.0615 \pm 5e\text{-}04^{Bb}$	$0.0585 \pm 5e\text{-}04^{Bb}$			
50%	0.103 ± 0.004^{Ac}	$0.0385 \pm 5e\text{-}04^{Cc}$	0.045 ± 0^{Cab}	0.0685 ± 0.0025^{Bb}	0.073 ± 0.002^{Bb}			
75%	0.0985 ± 0.0085^{Ac}	0.0625 ± 0.0075^{Bb}	0.0535 ± 0.0035^{BCab}	0.041 ± 0.003^{CDc}	$0.0295 \pm 5e\text{-}04^{Dc}$			
100%	0.132 ± 0.0015^{Ab}	0.033 ± 0.002^{Bc}	0.03 ± 0.001^{Bc}	0.032 ± 0.004^{Bc}	$0.0235 \pm 5e\text{-}04^{Bc}$			
125%	0.101 ± 0.001^{Ac}	0.045 ± 0.004^{Bbc}	0.064 ± 0.003^{Aba}	$0.0365 \pm 5e\text{-}04^{Bc}$	$0.0295 \pm 5e\text{-}04^{Bc}$			
Anisidine values								
0%	7.45 ± 0.48^{Ca}	9.24 ± 0.955^{Ca}	14.2 ± 0.245^{Ba}	15.8 ± 0.875^{ABa}	20.4 ± 0.69^{Aab}	< 0.001	< 0.001	< 0.001
25%	7.08 ± 0.41^{Ba}	9.1 ± 0.21^{ABa}	9.04 ± 0.535^{ABb}	11.2 ± 1.13^{ABb}	19.4 ± 0.955^{aAb}			
50%	4.25 ± 0.21^{Cbc}	7.46 ± 0.19^{BCab}	9.34 ± 0.425^{Bb}	11.9 ± 0.655^{Bb}	20.4 ± 0.215^{Aab}			
75%	2.72 ± 0.405^{Dc}	6.28 ± 0.67^{CDb}	8.44 ± 0.425^{BCb}	12.1 ± 0.26^{Bb}	20 ± 0.775^{Aa}			
100%	5.35 ± 0.565^{Cb}	6.3 ± 0.265^{Cb}	12.7 ± 0.615^{Ba}	18.9 ± 0.29^{Aa}	21.9 ± 0.215^{Aa}			
125%	6.4 ± 0.185^{Cb}	7.16 ± 0.575^{Cab}	10.2 ± 0.035^{Cb}	12.9 ± 0.745^{Bb}	21.3 ± 0.095^{Aa}			
Total soluble protein (mg protein/ml extract)								
0%	0.721 ± 0.063^{Ab}	0.678 ± 0.0125^{ABb}	0.575 ± 0.0045^{Bc}	0.67 ± 0.017^{ABc}	0.587 ± 0.0045^{Bcd}	< 0.001	< 0.001	< 0.001
25%	0.645 ± 0.0065^{Ac}	0.637 ± 0.004^{Ab}	0.663 ± 0.0085^{Ad}	0.589 ± 0.005^{Ad}	0.6 ± 0.0015^{Ad}			
50%	0.71 ± 0.002^{Cbc}	$0.788 \pm 5e\text{-}04^{ABa}$	0.786 ± 0.0105^{Aab}	0.75 ± 0.0105^{BCb}	0.684 ± 0.0035^{Cb}			
75%	0.849 ± 0.0015^{Aa}	0.79 ± 0.0095^{Ba}	0.734 ± 0.0095^{Cc}	0.746 ± 0.0115^{BCab}	0.727 ± 0.005^{Cab}			
100%	0.819 ± 0.007^{Aa}	0.818 ± 0.0065^{ABa}	0.762 ± 0.0065^{BCbc}	0.704 ± 0.001^{Cb}	0.693 ± 0.003^{Cbc}			
125%	0.815 ± 0.004^{Aa}	0.758 ± 0.0175^{Aa}	0.838 ± 0.0035^{Aa}	0.824 ± 0.0105^{Aa}	0.831 ± 0.0165^{Aa}			
Carbonyl content (nmol/mg protein)								
0%	15.6 ± 0.383^{Aa}	11.9 ± 0.138^{Ba}	8.92 ± 0.243^{Ca}	7.17 ± 0.353^{Ca}	3.76 ± 0.113^{Da}	< 0.001	< 0.001	< 0.001
25%	16.8 ± 0.126^{Aa}	11.5 ± 0.0789^{Ba}	9.07 ± 0.671^{BCa}	6.74 ± 0.702^{CDa}	3.87 ± 0.632^{Da}			
50%	14.5 ± 0.483^{Aab}	11.1 ± 0.0272^{Ba}	6.01 ± 0.326^{Cb}	4.4 ± 0.237^{Cab}	1.25 ± 0.304^{Dc}			
75%	16.5 ± 0.183^{Aa}	11.5 ± 0.343^{Ba}	7.69 ± 0.267^{Cab}	3.99 ± 0.86^{Db}	2.28 ± 0.17^{Db}			
100%	11.2 ± 0.267^{Ab}	7.79 ± 0.662^{Bb}	2.12 ± 0.655^{Cc}	1.16 ± 0.128^{Cc}	0.866 ± 0.115^{Cc}			
125%	15.9 ± 0.883^{Aa}	9.73 ± 0.136^{Bab}	8.64 ± 0.372^{Bab}	4.38 ± 0.476^{Cab}	0.982 ± 0.225^{Dc}			

Values are means ± SEM, $n = 3$ per treatment group. IC_{50} indicates half maximal inhibitory concentration

The uppercase letters denote the significant differences ($P < 0.05$) between columns (cooking time), meanwhile lowercase letters denote significant differences between rows (coconut milk percentages), in each analysis as analysed by two-way ANOVA and the TUKEY test. [1]CM × CT = COCONUT MILK × COOKING TIME interaction effect and are significantly ($p < 0.05$) different ($n = 3$)

also increased the chances of lipid oxidation, resulting in the formation of conjugated dienes[2]. From Table 2, conjugated dienes values varied widely from **0.0235 ± 5e-04** to **0.174 ± 0.0185**, indicating that lipid oxidation levels differed significantly across treatments. The highest value was observed in the sample of beef rendang with 25% coconut milk and without heating, while the lowest value was recorded in the sample with 100% coconut milk, cooked for 4 h. Research indicates that natural antioxidants can play a major role in reducing the formation of conjugated dienes, thus improving the oxidative stability of meat products [33]. For example, antioxidants from spices have been shown to effectively slow down lipid peroxidation, resulting in lower levels of conjugated dienes in the final product [32, 33]. This is particularly important for beef rendang, where high cooking temperatures and the rich lipid content from both the meat and coconut milk create ideal conditions for oxidation to occur. Regarding the highest conjugated diene value, when raw beef and coconut milk were combined without cooking, they contained higher levels of pre-existing lipid oxidation products. This could have been due to storage or exposure to oxygen before the experiment, leading to initial lipid oxidation. Without the cooking process to alter lipid structure, these fats showed a higher baseline level of conjugated dienes. The antioxidants in coconut milk, such as phenolic compounds, may not have been fully effective at inhibiting oxidation in raw mixtures, especially since they might have required some heat activation to function optimally. However, the significant interaction effect ($p < 0.001$) between percentages of coconut milk and cooking time suggests that the presence of coconut milk influences the impact of cooking time on lipid oxidation. This interaction

may mean that while coconut milk can protect against lipid oxidation under shorter cooking times, its protective effect diminishes with longer cooking durations.

The anisidine value reflects secondary oxidation products, especially aldehydes, which form after initial peroxide formation during lipid oxidation [25]. In high-fat dishes like beef rendang with coconut milk, extended cooking time can accelerate oxidation, leading to the accumulation of these secondary oxidation byproducts. Table 2 shows the anisidine values varied significantly across treatments, ranging from **2.72 ± 0.405** to **21.9 ± 0.215**. Notably, beef rendang without coconut milk exhibited higher anisidine values compared to samples containing coconut milk, indicating the coconut milk effects against secondary oxidation. Coconut milk contains antioxidants, including phenolic compounds such as catechins and tocopherols (forms of vitamin E), which scavenge free radicals and inhibit aldehyde formation [34]. These antioxidants help stabilise lipids and prevent breakdown of lipid peroxides into secondary oxidation products that contribute to higher anisidine values. Overall, anisidine values showed an increasing trend from 0-hour to 4-hour cooking time across all treatments. As cooking duration lengthens, primary lipid peroxides have more time to degrade into aldehydes, elevating anisidine values [35]. Prolonged heat exposure accelerates this conversion, especially in unsaturated fats prone to oxidative degradation. Furthermore, extended heating can deplete natural antioxidants, diminishing their protective capacity and allowing secondary oxidation to progress [36]. Following that, the significant interaction effect ($p < 0.001$) between coconut milk percentages and cooking time suggests that the amount of coconut milk modulates the influence of cooking time on anisidine values. In samples containing coconut milk, the increase in anisidine values over time appears to be moderated by its antioxidant properties, which help suppress the formation of secondary oxidation products.

Protein solubility is an indicator of protein integrity, where lower total soluble protein values suggest protein denaturation or aggregation, often caused by heat or oxidative stress [37]. During cooking or heating, proteins may unfold and aggregate, reducing their solubility and affecting their functionality. This loss of solubility alters the structural integrity of meat proteins, impacting texture and quality, particularly in food products subjected to high temperatures [38]. Table 2 shows that total soluble protein values varied from **0.575 ± 0.0045** to **0.849 ± 0.0015 mg/ml** demonstrating significant differences in protein solubility across treatments. The highly significant p-values ($p < 0.001$) for all factors suggest that coconut milk percentage, cooking time, and their interaction strongly influenced protein solubility in beef rendang. Coconut milk appears to help preserve protein solubility, mainly due to its antioxidant properties, which reduce protein oxidation. As reported by a study, coconut milk contains phenolic compounds and tocopherols (forms of vitamin E) that help stabilise protein structures by neutralising oxidative agents [39]. These antioxidants shield proteins from oxidation-induced denaturation and aggregation, helping maintain their solubility and integrity. Prolonged cooking times, however, are associated with decreased TSP levels, indicating protein unfolding and aggregation due to sustained heat. At high temperatures, protein structures lose their natural shape, forming aggregates or cross-links, which reduces their ability to dissolve in water [40]. This loss of solubility reflects a decrease in protein integrity as heat-induced modifications alter their original structure. Generally, the significant interaction effect ($p < 0.001$) indicates that coconut milk mitigates the impact of cooking time on protein solubility. For example, at shorter cooking durations, the antioxidants and protective fats in coconut milk maintain higher TSP values by minimising protein oxidation. However, as cooking time increases, the protective effect may weaken, leading to more noticeable protein denaturation.

On the other hand, the carbonyl content results revealed the level of protein oxidation in the samples, as evidenced by the development of carbonyl groups on protein side chains. This process, known as protein carbonylation, serves as a marker of oxidative damage [41]. It typically arises from extended heat exposure or oxidative stress, which degrades both the quality and functional properties of proteins [42]. Based on Table 2, carbonyl content values varied widely from **0.866 ± 0.115** to **16.8 ± 0.126 nmol/mg protein**, indicating considerable differences in the degree of protein oxidation across treatments. The two-way ANOVA analysis showed highly significant effects ($p < 0.001$) for coconut milk percentage, cooking time, and their interaction, demonstrating that each factor, both independently and in combination, significantly influenced carbonyl content in the beef rendang samples. Higher coconut milk concentrations generally corresponded with lower carbonyl content, indicating its protective role against protein oxidation. Our results on lipid oxidation (CD and AV) correlate with studies showing how saturated fats in coconut milk may contribute to oxidative stability. Coconut milk is rich in antioxidants, including phenolic compounds and tocopherols, which actively neutralise free radicals, helping to prevent oxidative damage to proteins [42]. This antioxidant action reduces the formation of carbonyl groups by blocking oxidation pathways that would otherwise degrade protein molecules [43]. Additionally, the fats and emulsifying properties of coconut milk may create a barrier around proteins, shielding them from exposure to oxygen and lowering the chance of oxidative changes [34]. This combined effect helps maintain protein structure and lower carbonyl levels when coconut

milk is present. Contrary to the typical trend where prolonged cooking leads to increased protein oxidation, in this case, carbonyl content significantly decreased with longer cooking times. This suggests that extended cooking may reduced detectable protein oxidation levels, potentially due to the breakdown of initial oxidation products or complex interactions with antioxidants in coconut milk. One possible explanation for lower measurable carbonyl levels at higher temperatures is that some carbonyl groups may break down or react further over time. Extended heat exposure could also facilitate secondary reactions that further alter oxidation byproducts, ultimately reducing detectable carbonyl content. Therefore, the synergistic interaction between coconut milk and cooking time likely contributes to the significant reduction in carbonyl content observed, with coconut milk's antioxidants potentially working in tandem with extended cooking to reduce protein oxidation more effectively than either factor alone.

For home cooks and culinary professionals, these findings offer practical guidance on balancing traditional cooking techniques with the preservation of optimal food quality. Many traditional cooking methods rely on prolonged heating, which can lead to excessive oxidation and nutrient degradation. Adjusting coconut milk concentration to an optimal range of 50–75% and limiting cooking time to 2–3 h can help manufacturers enhance product stability and shelf life by reducing lipid oxidation and maintaining antioxidant content. This is particularly beneficial for food companies producing packaged, frozen, or canned rendang, where maintaining quality over time is essential for consumer satisfaction and safety. Additionally, the results provide valuable insights for developing functional food formulations that prioritise antioxidant retention while minimising oxidation-related deterioration.

Creatine and creatinine content

Table 3 presents the conversion of creatine to creatinine under varying cooking conditions. As expected, creatine levels decreased significantly with prolonged heating, accompanied by a corresponding rise in creatinine formation. The highest creatine retention was observed in samples with 100% coconut milk content and shorter cooking durations, indicating that the antioxidant properties of coconut milk may help slow creatine degradation. The highly significant interaction effect ($p<0.001$) between coconut milk percentage and cooking time suggests that both factors played a crucial role in determining the rate of creatine breakdown.

Creatine is a naturally occurring compound in muscle tissue that can convert to creatinine, especially during heat exposure, through non-enzymatic processes [44]. Monitoring these two compounds provides insights into how cooking and coconut milk impact the stability and transformation of creatine in foods like beef rendang. Based on the results in Table 3, creatine levels decreased significantly with prolonged cooking times, ranging from **41.1 ± 0.626** mg/g at 0 h (without coconut milk) to as low as **1.08 ± 0.0914** mg/g after extended cooking (4 h) in certain treatments. Meanwhile, creatinine levels generally increased with cooking time, with values ranging from **8.54 ± 1.55** mg/g at shorter cooking times to **41.7 ± 0.195** mg/g after extended cooking (4 h) with high coconut milk levels. The p-values for creatine and creatinine content were also highly significant ($p<0.001$) across all factors—coconut milk percentage, cooking time,

Table 3 Creatine and creatinine content in prolonged cooking beef Rendang with different percentages of coconut milk using Two-Way ANOVA

Variables	Cooking time					*P*-value		
Coconut milk percentage	0 h	1 h	2 h	3 h	4 h	CM	CT	CM x CT[1]
Creatine content (mg/g)								
0%	34.6 ± 1.81^{Aab}	11.5 ± 1.20^{CDc}	19.3 ± 0.242^{Bc}	14.7 ± 1.06^{BCb}	8.27 ± 1.13^{Dbc}	< 0.001	< 0.001	< 0.001
25%	12.5 ± 0.213^{Acd}	8.9 ± 0.182^{Cc}	10.2 ± 0.345^{Be}	1.08 ± 0.0914^{Ee}	4.18 ± 0.158^{Dd}			
50%	41.1 ± 0.626^{Aa}	33.5 ± 0.439^{Aa}	30.0 ± 0.584^{ABb}	15.7 ± 0.529^{ABb}	6.57 ± 0.284^{Bcd}			
75%	18.8 ± 0.399^{Abc}	16.6 ± 1.1^{ABb}	15.8 ± 0.172^{Bd}	8.1 ± 0.0839^{Cc}	10.5 ± 0.268^{Cb}			
100%	37.8 ± 0.623^{Aa}	33.6 ± 1.79^{Aba}	34.9 ± 1.21^{Aa}	29.2 ± 0.931^{Ba}	21.5 ± 1.05^{Ca}			
125%	2.25 ± 0.258^{Bd}	2.17 ± 0.0629^{Bd}	5.25 ± 0.221^{Af}	4.58 ± 0.151^{Ad}	4.43 ± 0.454^{Ad}			
Creatinine content (mg/g)								
0%	8.54 ± 1.55^{Dcd}	14.0 ± 0.197^{Cc}	21.7 ± 0.114^{Babc}	24.9 ± 0.29^{ABb}	27.5 ± 0.452^{Ac}	< 0.001	< 0.001	< 0.001
25%	6.68 ± 0.511^{Cd}	8.99 ± 0.177^{BCd}	14.2 ± 3.17^{ABc}	13.7 ± 0.46^{ABd}	18.6 ± 0.463^{Ad}			
50%	12.6 ± 0.248^{Cbc}	17.3 ± 1.18^{BCb}	23.4 ± 2.36^{Bab}	25.2 ± 0.545^{Bb}	34.9 ± 3.17^{Ab}			
75%	13.6 ± 0.321^{Cab}	19.2 ± 0.329^{ABb}	18.3 ± 0.463^{Bbc}	18.4 ± 0.248^{Bc}	20.6 ± 0.147^{Ad}			
100%	8.54 ± 1.55^{Dcd}	14 ± 0.197^{Cc}	21.7 ± 0.114^{Babc}	24.9 ± 0.29^{ABb}	27.5 ± 0.452^{Ac}			
125%	17.7 ± 0.108^{Ea}	22.6 ± 0.182^{Da}	26.7 ± 0.134^{Ca}	39.2 ± 0.449^{Ba}	41.7 ± 0.195^{Aa}			

Values are means ± SEM, $n=3$ per treatment group. IC_{50} indicates half maximal inhibitory concentration

The uppercase letters denote significant differences ($P<0.05$) between columns (cooking time), meanwhile the lowercases denote significant differences between rows (coconut milk percentages), in each analysis as analysed by two-way ANOVA and the TUKEY test. [1]CM × CT = COCONUT MILK × COOKING TIME interaction effect and are significantly ($p<0.05$) different ($n=3$)

and their interaction effect—indicating that both the amount of coconut milk and cooking duration, along with their interaction, strongly influence creatine degradation and creatinine formation. The results in Table 3 further confirm that prolonged cooking significantly reduces creatine content, with a corresponding increase in creatinine levels. This suggests that beef rendang cooked for extended durations may have reduced nutritional value as a dietary creatine source. For individuals who rely on natural dietary sources of creatine, such as athletes, bodybuilders, and older adults at risk of sarcopenia (muscle loss), consuming beef rendang prepared with shorter cooking durations (2–3 h) may help better preserve creatine content. These findings emphasise the importance of optimising cooking time to retain nutritional benefits.

Creatine levels decreased noticeably with extended cooking time, which is expected as heat drives the breakdown of creatine into creatinine [45]. This breakdown process intensified with prolonged heat exposure, reflecting creatine's sensitivity to high temperatures. As cooking continued, creatinine levels rose in parallel with creatine depletion, highlighting the conversion process [46], a common reaction in meat dishes like beef rendang. Therefore, longer cooking times result in lower creatine content while promoting creatinine formation due to heat-driven transformations. Higher coconut milk concentrations seem to help preserve creatine levels, particularly during shorter cooking times. The antioxidants in coconut milk likely slow down creatine degradation by mitigating oxidative stress, thereby delaying its conversion into creatinine [47]. However, as cooking time increases, the protective effects of these antioxidants weaken, possibly due to prolonged heat exposure overwhelming their ability to counteract oxidation. In treatments with higher coconut milk content, creatinine levels still rise over time, suggesting that while coconut milk initially shields creatine, its efficacy diminishes with extended cooking. Eventually, the heat-driven transformation into creatinine becomes unavoidable, and certain compounds in coconut milk may even participate in reactions that accelerate this process as cooking continues [46, 48]. Creatinine is a metabolic waste product excreted through the kidneys. Table 3 indicates that longer cooking durations lead to increased creatinine formation, which may have implications for individuals with chronic kidney disease (CKD) or impaired kidney function. While healthy individuals can efficiently excrete creatinine, those with preexisting kidney conditions may need to regulate their intake of creatinine-rich foods to reduce the renal excretory burden. Therefore, moderating cooking duration could be a practical strategy for individuals managing kidney health, helping to minimise excessive creatinine intake from overcooked meat.

While creatine is not a primary focus in culinary choices for rendang, its inclusion in the analysis serves to illustrate the extent of nutrient transformations during slow cooking. Results show that creatine levels declined markedly with prolonged heating, whereas creatinine formation increased accordingly. This transformation reflects the sensitivity of certain meat-derived compounds to thermal processing. The findings highlight the broader implications of heat-induced nutrient changes in traditional dishes such as rendang and may inform cooking practices that aim to balance culinary authenticity with nutritional retention.

Chemometric analysis

The Pearson correlation results (Table 4; Fig. 1) highlight important relationships between antioxidant compounds, lipid and protein oxidation markers, and creatine stability in beef rendang. These correlations offer deeper insights into how antioxidant levels influence lipid oxidation and protein stability. By integrating multivariate analysis, the study underscores the importance of optimising ingredient proportions and cooking time in enhancing oxidative stability in beef rendang.

Key findings underscore the protective role of TPC in enhancing antioxidant capacity, the strong association of TFC with both lipid and protein oxidation, and the inverse relationship between carbonyl content and AV. These

Table 4 Pearson correlation between antioxidant properties and oxidation products in beef Rendang

	TPC	TFC	DPPH IC_{50}	FRAP	CD	AV	TSP	Carbonyl	Creatine	Creatinine
TPC	**1.0000**	0.1708	-0.6711*	0.4631*	0.0709	0.2630	-0.0201	-0.2215	-0.3480	0.2960
TFC	0.1708	**1.0000**	-0.2610	-0.2039	0.7463*	-0.4609*	0.0235	0.6911*	-0.1257	-0.4188*
DPPH IC_{50}	-0.6711*	-0.2610	**1.0000**	-0.2886	-0.0061	-0.1364	0.0450	0.1037	0.5499*	-0.2143
FRAP	0.4631*	-0.2039	-0.2886	**1.0000**	-0.3185	0.0504	0.5213*	-0.3395	-0.2063	0.4405*
CD	0.0709	0.7463*	-0.0061	-0.3185	**1.0000**	-0.3829*	-0.2358	0.6589*	0.0294	-0.5065*
AV	0.2630	-0.4609*	-0.1364	0.0504	-0.3829*	**1.0000**	-0.4005*	-0.8603*	-0.2665	0.6503*
TSP	-0.0201	0.0235	0.0450	0.5213*	-0.2358	-0.4005*	**1.0000**	0.1182	0.2210	0.1926
Carbonyl	-0.2215	0.6911*	0.1037	-0.3395	0.6589*	-0.8603*	0.1182	**1.0000**	0.1816	-0.7186*
Creatine	-0.3480	-0.1257	0.5499*	-0.2063	0.0294	-0.2665	0.2210	0.1816	**1.0000**	-0.3546
Creatinine	0.2960	-0.4188*	-0.2143	0.4405*	-0.5065*	0.6503*	0.1926	-0.7186*	-0.3546	**1.0000**

* the strength of the linear relationship, with values (r) closer to ± 1 indicating stronger relationships. Correlation is significant at $p < 0.05$

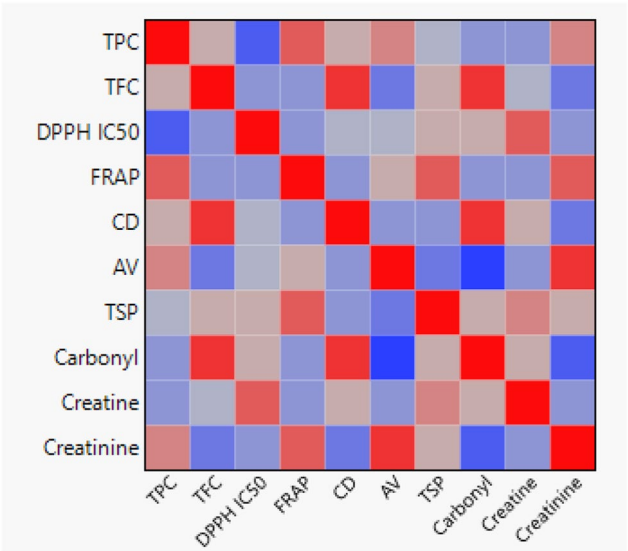


Fig. 1 Pearson's correlation coefficients (r) analysis between antioxidant properties, oxidation, creatine, and creatinine content of beef. Red = positive correlation, blue = negative correlations. Intensity of the colour represents the magnitude of the correlations (Colour figure online)

correlations provide valuable insights into optimising ingredient levels and cooking conditions to enhance antioxidant stability and minimise oxidative damage in beef rendang. Results demonstrate that TPC exhibited a significant negative correlation with DPPH IC_{50} (r=-0.6711), suggesting that higher TPC levels correspond to stronger antioxidant capacity (lower IC_{50} values). However, TPC showed a positive correlation with FRAP (r=0.4631), indicating that higher TPC is linked to greater reducing power.

On the other hand, TFC exhibited a strong positive correlation with conjugated dienes (r=0.7463) and carbonyl content (r=0.6911), suggesting that higher flavonoid levels may correspond with the initial stages of lipid oxidation and protein oxidation. Flavonoids, while generally antioxidants, can sometimes act as pro-oxidants, especially when exposed to metal ions or when generating reactive oxygen species (ROS) as part of their antioxidant activity. In a complex food matrix like beef rendang, this pro-oxidant effect may promote the formation of conjugated dienes, leading to elevated conjugated diene (CD) levels despite high total flavonoid content (TFC). Additionally, intense cooking heat can alter flavonoid structure, reducing their ability to fully prevent primary lipid oxidation. The ROS generated can attack protein side chains, leading to carbonyl formation and an increase in carbonyl content. Although flavonoids initially protect proteins, they may degrade over time, potentially adding to an oxidative environment under prolonged heat exposure. In contrast, TFC showed a significant negative correlation with anisidine value (r=-0.4609), suggesting that higher flavonoid levels may inhibit secondary lipid oxidation. While flavonoids may not completely stop primary oxidation (as reflected in increased CD levels), they can effectively suppress secondary lipid oxidation stages. Anisidine value (AV) measures aldehyde compounds, such as 2-alkenals, which arise as secondary oxidation products following the initial creation of conjugated dienes. Flavonoids can neutralise aldehydes or stabilise lipid radicals, potentially preventing these secondary oxidation products even when primary oxidation has already occurred.

In addition, conjugated dienes showed a positive correlation with carbonyl content (r=0.6589), suggesting that primary lipid oxidation products may lead to protein oxidation. However, the negative correlation (r= -0.3829) of CD and anisidine values (AV) indicates that as primary lipid oxidation (CD) increases, secondary lipid oxidation (AV) may be inversely affected, possibly due to antioxidant effects from coconut milk in beef rendang. The increase in CD alongside a decrease in AV likely reflects a scenario where antioxidants or cooking conditions permit the formation of conjugated dienes during primary oxidation but inhibit the progression to secondary oxidation, thus keeping aldehyde formation (AV) low. This indicates that antioxidants may be stabilising primary lipid oxidation products or selectively scavenging secondary oxidation products, preventing a full oxidation cascade from taking place.

As for creatine, its positive correlation with DPPH IC_{50} (r=0.5499) suggests that as higher antioxidant capacity is associated with greater creatine retention. Conversely, creatinine showed an inverse correlation with TFC (r=-0.4188) but a strong positive correlation with FRAP (r=0.4405), indicating that flavonoids may play a role in reducing creatine's conversion to creatinine. Nevertheless, as reducing power increases, creatine degradation into creatinine might also rise, possibly due to oxidative stress conditions or prolonged cooking. Other than that, creatinine exhibited a strong correlation with AV (r=0.6503) and a strong inverse correlation with carbonyl content (r=-0.7186), indicating that higher AV values correspond with increased creatinine levels, possibly due to prolonged cooking and protein breakdown. Meanwhile, greater protein oxidation (higher carbonyl content) appears to be linked to reduced creatine conversion.

Principal Component Analysis (PCA) was used to better understand how various oxidation parameters and antioxidant properties are interrelated, revealing patterns of correlation and variation among the sample properties (Table 5). This approach helps illustrate how these factors were distributed across different samples, giving a more comprehensive view of their interactions. Before performing PCA, we assessed the dataset for its suitability and sampling adequacy for chemometric analysis. This included conducting Bartlett's test for sphericity, which determines whether the

Table 5 Factor loading, eigenvalues, variability, cumulative variability, Keiser-Meyer-Olkin (KMO) test, and Barlett's sphericity test associated with each principal component

Variables	PC1	PC2
Eigenvalues	3.771	2.315
Variability (%)	37.709	23.151
Cumulative variability (%)	37.709	60.860
Kaiser-Meyer-Olkin (KMO) Test	0.623, KMO > 0.5 is indicated as adequate for performing principal component analysis	
Bartletts's sphericity test	$p < 0.0001$, there is at least one of the correlations between the variables is significantly different from 0.	
Factor loadings (FL)*		
Total phenolic content	-0.058	**0.813**
Total flavonoid content	**0.807**	0.321
DPPH IC_{50}	-0.052	-0.721
Ferric reducing antioxidant power	-0.320	0.436
Conjugated dienes	**0.757**	0.160
Anisidine value	**-0.782**	0.327
Total soluble protein	0.030	-0.039
Carbonyl content	**0.951**	-0.214
Creatine content	0.072	-0.543
Creatinine content	-0.696	0.380

*FL ≥|0.750| = strong factor loading;|0.500| < FL<|0.749| = moderate factor loading, and FL≤|0.499|= weak factor loading. FL with bold value indicated strong factor loading in the principal component

dataset is appropriate for multivariate analysis. With a result of $p < 0.0001$, the findings confirmed that at least one of the variable correlations was significantly different from zero at a 0.05 significance level, validating the dataset's readiness for further analysis. To evaluate whether the dataset was adequate for chemometric analysis, we conducted a Kaiser-Meyer-Olkin (KMO) test, yielding an index of 0.623 (Table 5). Since this result is above 0.5, it indicates that the sampling was sufficiently robust for analysis. Consequently, our dataset was considered suitable and adequate for performing PCA. To explore the dataset using PCA, two principal components (PCs) with eigenvalues greater than 1 were selected. Together, these components accounted for 60.9% of the total variance, with PC1 explaining 37.7% and PC2 capturing 23.2% of the data variability.

The factor loadings, which quantify each variable's contribution to the principal components, revealed distinct groupings related to antioxidant capacity, lipid oxidation, and protein oxidation. PC1, which accounted for the largest portion of variance (37.7%), was heavily loaded by variables associated with both lipid and protein oxidation. Carbonyl content (0.951) and conjugated dienes (0.757) exhibited strong positive loadings on PC1, highlighting their association with protein and lipid oxidation, respectively. High values for these variables indicate greater oxidative degradation of proteins and lipids in the samples. On the other hand, anisidine value (AV, -0.782) and creatinine content (-0.696) had negative loadings on PC1. This suggests that samples with higher levels of primary lipid oxidation (CD) and protein oxidation (carbonyl) tend to have lower secondary oxidation (AV) and creatinine levels. These relationships imply a dynamic interplay where primary oxidative changes may limit secondary product formation under specific conditions, such as the presence of high antioxidant levels or shorter cooking times. Also, total flavonoid content (TFC, 0.807) had a positive loading on PC1, indicating that higher flavonoid levels were linked to increased lipid and protein oxidation. As discussed in earlier analyses, this relationship may reflect the pro-oxidant effects of flavonoids at high concentrations or when exposed to prolonged cooking. PC2, accounting for 23.2% of the variance, was primarily associated with antioxidant properties. Total phenolic content (TPC, 0.813) and ferric reducing antioxidant power (FRAP, 0.436) had positive loadings on PC2. This indicates that PC2 represents antioxidant characteristics, where high TPC aligns with strong reducing power (FRAP), reflecting enhanced antioxidant efficacy in beef rendang samples. Meanwhile, DPPH IC_{50} (-0.721) had a negative loading on PC2, further affirming that higher phenolic and antioxidant levels correlate with lower (favourable) DPPH IC_{50} values, indicating stronger free radical scavenging capacity.

Figure 2 presents the Principal Component Analysis (PCA) results, which explored clustering patterns among antioxidant and oxidation parameters based on coconut milk concentration and cooking time. The PCA biplot revealed that the first two principal components (PC1 and PC2) accounted for more than 75% of the total variance, demonstrating that these two dimensions effectively summarised the dataset's key variations. The PCA biplot (Fig. 2A) and loading plot (Fig. 2B) illustrate how coconut milk concentration and cooking time influence antioxidant and oxidation markers. In general, beef rendang samples with higher coconut milk concentrations and shorter cooking times tend to cluster in regions associated with high TPC, FRAP, and lower DPPH IC_{50}, indicating enhanced antioxidant profiles. Meanwhile, prolonged cooking times, particularly in samples with lower coconut milk levels, aligned with increased carbonyl content and conjugated dienes, marking higher oxidative stress and protein degradation. Following this, the PCA score plot (Fig. 2C) and HCA dendrogram (Fig. 2D) elucidate sample groupings, revealing underlying patterns influenced by coconut milk levels and cooking durations. The HCA dendrogram (Fig. 2D) used Ward's method to cluster samples based on similarity in their antioxidant and oxidation profiles. The dendrogram confirmed the clustering pattern identified by the PCA model, revealing three distinct clusters. These clusters are color-coded as red (R0, R25, R50, R75, R100, and R125 samples with 0-hour cooking

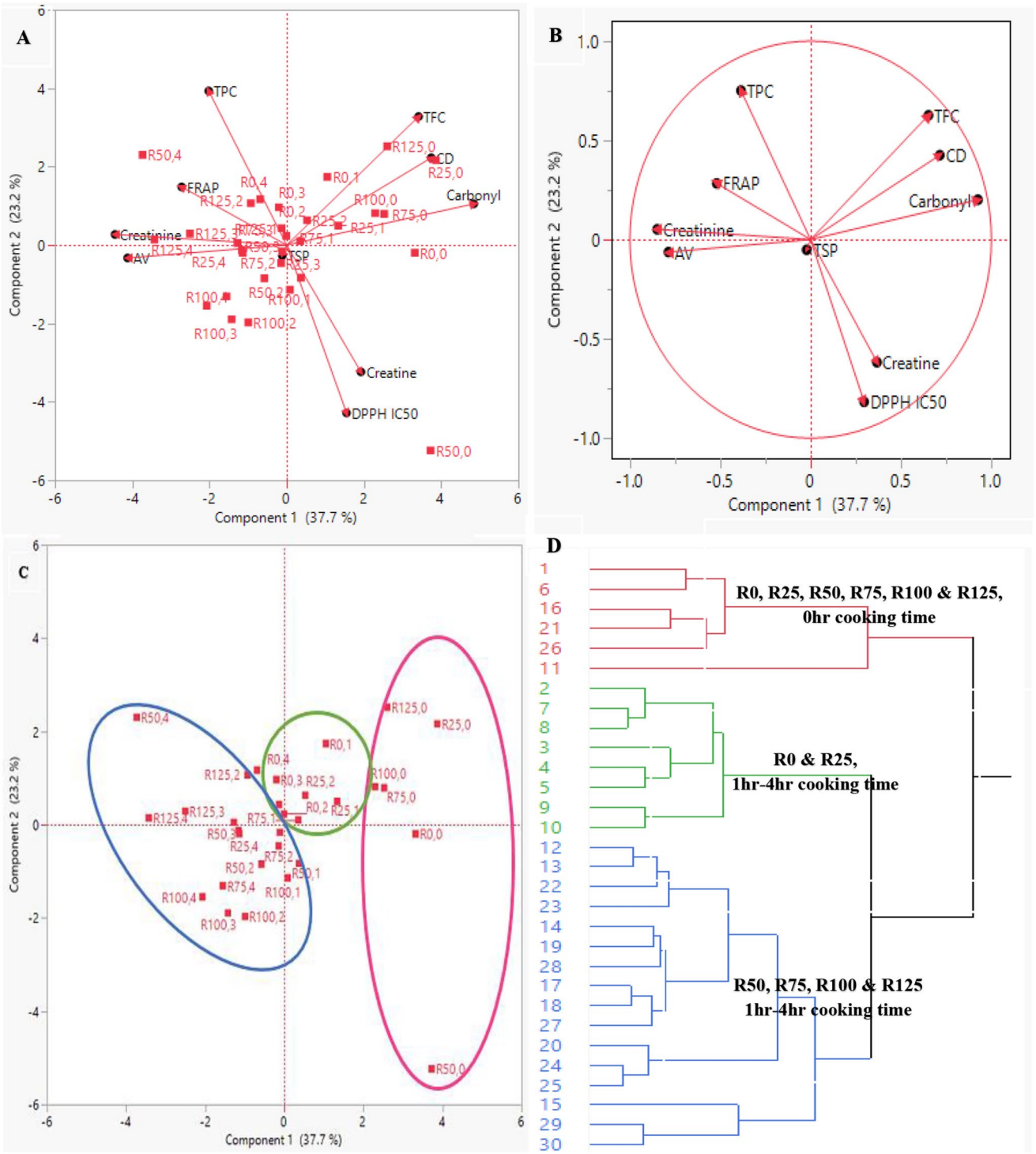


Fig. 2 Chemometric analysis of antioxidant properties, oxidation products, creatine and creatinine content in beef rendang. **A** PCA biplot; **B** PCA loading plot; **C** PCA score plot; **D** Dendrogram of HCA using Ward's method. *R0* = 0% coconut milk, *R25* = 25% coconut milk, *R50* = 50% coconut milk, *R75* = 75% coconut milk, *R100* = 100% coconut milk, *R125* = 125% coconut milk. The numerical values of 0, 1, 2, 3, & 4 indicate cooking times of 0 h,1 h,2 h,3 h, and 4 h, respectively. The variance (%) per PC is provided in parentheses on the x- and y-axes for PCA models

time), green (R0 and R25 samples cooked for 1 to 4 h), and blue (R50, R75, R100, and R125 samples cooked for 1 to 4 h). The clustering results indicate that grouped samples share similar antioxidant properties, lipid and protein oxidation markers, and creatine and creatinine levels. The red cluster consists of fresh, uncooked samples with intact antioxidants, minimal oxidation, and high creatine levels. The green cluster is marked by elevated oxidation and creatine breakdown, resulting from low antioxidant levels and extended cooking times. Lastly, the blue cluster represents a balanced profile, with moderate antioxidant retention and some oxidation, as higher coconut milk concentrations offer partial protection during cooking.

The conclusion of the present study on beef rendang's lipid and protein co-oxidation highlights the effects of cooking time and coconut milk concentration on its antioxidant capacity and oxidative stability. Increasing coconut milk concentrations, combined with prolonged cooking, enhances antioxidant properties, as indicated by higher total phenolic content (TPC) and flavonoid content. However, extended cooking also raises the levels of lipid and protein oxidation markers, such as conjugated dienes and carbonyl content, suggesting that while coconut milk initially bolsters antioxidant stability, its protective capacity diminishes over time due to oxidation. Although the study used precise cooking conditions, real-world variations in heat distribution, ingredient sourcing, and preparation methods could affect outcomes.

The findings of this study have significant implications for food manufacturers, particularly in the production of ready-to-eat, frozen, and packaged rendang products. Optimal antioxidant preservation in beef rendang is achieved with moderate coconut milk levels (50-75%) and shorter cooking durations (2–3 h), which together reduce oxidative degradation. This can help home cooks and food manufacturers refine traditional cooking methods for improved nutritional quality. The interaction between coconut milk and cooking duration, as shown by chemometric analysis, demonstrates a nuanced balance, where prolonged cooking with high coconut milk percentages can maximise antioxidant release from spices, yet extended heating or excessive coconut milk increases oxidation, thereby reducing the effectiveness of antioxidants. These findings are also beneficial for the development of functional food products, allowing food scientists to refine traditional cooking techniques while enhancing health benefits. By adjusting cooking time and coconut milk composition, manufacturers can produce products with richer antioxidant profiles while minimising oxidative damage, catering to health-conscious consumers and specialised dietary needs.

From a public health and nutritional standpoint, this study highlights the effects of prolonged cooking on protein and lipid oxidation, which have implications for dietary health. Higher levels of lipid oxidation products have been linked to inflammatory diseases and oxidative stress-related conditions. Understanding these changes allows nutritionists and dietitians to recommend cooking practices that optimise the retention of beneficial antioxidants while reducing the formation of harmful oxidation by-products. This is particularly important for individuals with dietary concerns regarding fat intake, oxidative stress, and cardiovascular health. By incorporating these practical applications, this study bridges the gap between scientific findings and real-world usage, providing actionable insights for the food industry, home cooks, and health professionals alike.

The study assessed oxidation immediately after cooking, but future work should explore oxidative stability during storage to better reflect commercial applications. In addition, the study primarily focused on antioxidant activity and oxidative stability, but future research should incorporate sensory evaluation to assess the impact of cooking parameters on taste and texture. While this study provides valuable insights, future research should explore alternative cooking techniques, such as sous vide or controlled-temperature cooking, which may further enhance oxidative stability and nutrient retention in beef rendang. Additionally, further investigations into the synergistic effects of spices and coconut milk on oxidation and antioxidant activity during prolonged heating would help refine our understanding of how ingredient interactions influence food stability. Expanding this research to different meat types and plant-based alternatives could also broaden the applicability of these findings, providing insights for the development of more sustainable and health-conscious food products. By addressing these areas, future studies can build on the current findings, offering enhanced strategies for food preservation, oxidative stability, and nutrient retention, ultimately contributing to improved food quality and consumer health outcomes.

Nonetheless, this study provides the first integrated analysis of coconut milk concentration and prolonged heating on beef rendang's oxidative stability using a chemometric approach. The novel application of chemometric techniques serves as a valuable tool for food scientists and manufacturers to refine traditional cooking practices for improved product stability and consumer health benefits. The findings of this study have significant implications for both food industry professionals and home cooks, as well as nutritionists and public health experts seeking to optimise the nutritional value and oxidative stability of beef rendang.

Acknowledgements The authors would like to 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.

Author contributions Siti Nabilah Karim: Data curation, formal analysis, validation, visualisation, writing—original draft, writing—review and editing; Rashidah Sukor: Conceptualisation, data curation, formal analysis, validation, visualisation, supervision, project administration, writing—review and editing. Nuzul Noorahya Janbari, Maimunah Sanny, and Alfi Khatib: Supervision. Wan Zunairah Wan Ibadullah: data curation and formal analysis. Nur'ain Sharifuddin, Nur Huwaidah Ithinin, Nur Azlin Zulhaimi and Nor Rabi'atul 'Adawiyah Zuhir: Data curation, formal analysis and validation. All authors have read and agreed to the publication of the manuscript.

Funding Open access funding provided by The Ministry of Higher Education Malaysia and Universiti Putra Malaysia
This research was funded by the Ministry of Higher Education Malaysia under the Fundamental Research Grant Scheme (FRGS/1/2020/STG04/UPM/01/1).

Data availability All data generated or analysed during this study are included in this published article.

Declarations

Ethical approval Not applicable.

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

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