

# Physical and Chemical Characteristics of Beef Marinated by Cashew Apple Extract

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## Abstract

Marination is one of the methods that are often used in beef processing in an attempt to obtain high quality of beef. Cashew apple extract marinade (CAM) improves the microbiological characteristics of meat by inhibiting the growth of meat bacteria. The effect of CAM on other aspects such as physical (microbiological, tenderness, cooking loss, water holding capacity and pH) and chemical (moisture, fat and protein content) characteristics of meat have not been evaluated, which would be beneficial for the utilisation of agroindustry waste in the meat industry. In this study, the effect of CAM on the physical and chemical characteristics of beef, including microbiological characteristics, tenderness, cooking loss and water holding capacity, was evaluated. CAM (0%, 10%, 20% and 30%) was used during beef processing for 4 h at refrigeration temperature. Significant effects of CAM were observed on the physical and chemical characteristics of beef. CAM increased beef tenderness and reduced total bacteria, cooking loss, moisture, fat, and protein content. The optimum concentration of CAM for which significant changes were observed in the physical and chemical characteristics of beef was 20%. Thus, cashew apple can be utilised as a promising marinade agent in beef processing with the criteria of food for specific health use. This approach will help reduce cashew apple waste and is an eco-friendly approach.

**Keywords:** Beef; Cashew apple extract; Marination; Marinade; Cashew nut

## 1 Introduction

Physical, chemical and microbiological characteristics of beef are one of the main appeals of this meat for consumers (Henchion et al., 2017). To obtain good quality beef, several methods are used during its processing and one of them is marination. Earlier, marination was used only for seasoning of beef (Smith & Acton, 2010). With the development of the processing

technique, marination evolved to function as a flavouring and tenderising agent, and to extend the shelf life of beef (Çetinkaya, 2017). The liquid used for marination is known as a marinade, which can be either acidic or enzymic or neutral in pH (Yusop et al., 2011). Recently, a juice obtained from some fruits was used as a marinade for improving the quality of meat (Guo et al., 2020). In a previous study, cashew apple extract marinade (CAM) was used to improve the micro-

biological characteristics of meat by inhibiting meat bacteria (Susanti et al., 2018). However, further studies about the effect of CAM on meat quality including physical and chemical properties are required.

Cashew apple is a waste product produced in the cashew nut industry; it is abundant in quantity reaching approximately 30 million tonnes per year globally (Oliveira et al., 2020). Although availability of cashew apple is high, only 10% of cashew apples are utilised commercially, whereas the rest (90%) remain un-utilised (Oliveira et al., 2020). This pseudo fruit is generally left on the ground in the shade to rotting after separation of the nuts. Cashew apple contains phytochemical compounds containing acidic groups, polyphenols and flavonoids (Rufino et al., 2010). This study aimed to evaluate the effect of CAM on the physical and chemical characteristics of beef. It showed the potential use of cashew apple as a marination agent in the meat processing industry.

## 2 Materials and Methods

### 2.1 Preparation of CAM

Cashew apples were collected from several farm areas along the Java Island. After washing and drying, the fruits were extracted using the percolation method as described earlier by Susanti et al. (2018). CAM was prepared by mixing the indicated concentration of cashew apple extract with mineral water to obtain final concentrations of 10%, 20% and 30%. Mineral water without any extract (0%) was used as the control.

### 2.2 Beef Marination Process

Beef samples were prepared by cutting tenderloin into  $2 \times 2 \times 2$  cm parts. CAM was prepared by diluting the indicated concentration of cashew apple extract in the required volume of mineral water. The samples were soaked in CAM for 4 h in 4 concentrations: 0% (control), 10%, 20% and 30% (Figure 1). The samples were stored in the refrigerator. After the marination time, the samples were flushed once with mineral water

for neutralisation and then physical and chemical characteristics were determined.

### 2.3 Evaluation of Physical Characteristics of Beef

#### Microbiological Test

Total bacteria present in beef was determined by counting the colonies under sterile conditions. The beef samples were soaked in 45 mL of distilled water for 10 min. Samples were diluted by serial dilution and 1 mL of each pipetted into petri dishes followed by 20 mL of nutrient agar medium. The medium was allowed to solidify at the room temperature. The petri dishes were then incubated at 37 °C for 24 h. The number of colonies was counted using a colony counter, and the dilution factor was used to calculate log CFU/mL.

#### Tenderness Test

Beef tenderness was measured using a texture analyser (Brookfield CT-03, USA). The samples were placed under a cylindrical probe of 10-mm diameter. The probe was moved downwards at  $2 \text{ mm.s}^{-1}$ . The probe continued moving downward until penetration of 75% of the sample thickness was attained, retracted to the initial point of contact with the sample, and stopped for 2 s of set time period before initiation of the second compression cycle. During the test, the force-time data of the sample was recorded and plotted on the force-time plot (de Huidobro et al., 2005). Tenderness ( $\text{mm/g}/10\text{s}$ ) was calculated using the standard procedure (Honikel, 1998; Railton & Aronstam, 1987).

$$\text{Tenderness} = \frac{\text{Mean of recorded data}}{10 \text{ seconds}} \quad (1)$$

#### Cooking Loss Test

Beef cooking loss was evaluated to determine the loss of beef mass during the cooking period. The samples were drained for 10 min at room temperature and then kept in trays after marination for 4 h. The samples were weighed, kept inside polypropylene plastic bags, and then heated in

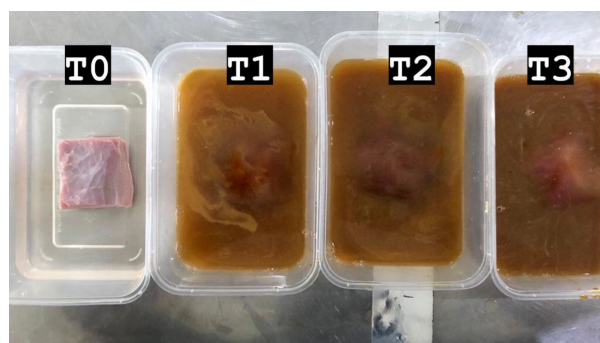


Figure 1: Cashew apple extract marinated (CAM) beef with the concentrations of 0% (T0), 10% (T1), 20% (T2) and 30% (T3) CAM.

water bath (Memmert, Germany) at a temperature of 60 °C for 60 min. Cooking loss was expressed as the percentage of difference between weights before and after cooking.

#### Water-holding capacity (WHC) Test

The WHC of beef was estimated as reported by Miller et al. (1980) by determining expressible juice using a modification of the filter paper press method. The sample (300 mg) was weighed and kept on an 11-cm diameter filter paper between Plexiglas plates and pressed at 200 psi for 1 min. The outline areas of the meat film and the expressible juice were traced, and both the areas were determined using a compensating planimeter. The percentage of expressible juice was calculated as described by Qiao et al. (2001). The increase in expressible juice percentage is related to the decrease in beef WHC.

$$\text{WHC}(\%) = \text{Moisture}(\%) - \text{Moisture}_{\text{wet area}}(\%) \quad (2)$$

$$\text{Moisture}_{\text{wet area}}(\%) = \frac{(\text{Area}_{\text{total surface}} - \text{Area}_{\text{meat film}})}{\text{Weight}_{\text{Sample}}} \times 100\% \quad (3)$$

#### pH Test

The pH of beef was measured by using a pH meter (pH1120x, Mettler Toledo, USA) according to the manufacturer's instructions. First, the pH meter was calibrated by using buffer solutions of pH 7 and 4. Beef was minced and then loaded

into a plastic tube filled with 10 mL of Aquadest. The tip of the pH meter was dipped into the sample and the pH recorded.

## 2.4 Evaluation of Chemical Characteristics of Beef

### Determination of Moisture

An empty porcelain cup was kept in an oven (100–105 °C) for 1 h, transferred into a desiccator, cooled for 30 min, and then weighed. Approximately 2–3 g of beef sample was taken into the cup, weighed, and then dried in an oven (100–105 °C) for 3 h. Drying and weighing were performed continuously. After a constant weight was obtained, the sample was transferred into a desiccator, cooled for 30 min, and then weighed. Moisture content was calculated by using the following formula.

$$\text{Moisture}(\%) = \frac{(\text{Weight}_{\text{initial sample}} - \text{Weight}_{\text{dried sample}})}{\text{Weight}_{\text{Sample}}} \times 100\% \quad (4)$$

### Determination of Protein Content

Protein content was measured using the Kjeldahl method. The sample (10 g) was put into a Kjeldahl flask and 2 g of  $\text{K}_2\text{SO}_4$  and 20 mL of  $\text{H}_2\text{SO}_4$  were added. The digestion was performed for 30 min until a clear, light-green solution was obtained. Digestion solution (10 mL) was added

to distilled water and shaken to mix thoroughly. NaOH (20 mL) and phenolphthalein indicator (3 drops) were added to the solution and then distilled using Erlenmeyer flasks.  $H_3BO_3$  (3%, 20 mL) and 2 drops of red/blue methyl, respectively, were used as the indicators. The yield of the distillation process was determined by titration with 0.1 N HCl until the solution turned light purple. Blank solution was prepared just before the determination of protein content as reported by Kolawole et al. (2020).

$$\text{Proteint Content(\%)} = \text{Total Nitrogen} \times 6.25 \quad (5)$$

$$\begin{aligned} \text{Total Nitrogen (\%)} = \\ \frac{(\text{Mol}_{NH_4Cl} \times \text{Equivalent Weight}_N)}{\text{Sample weight}} \times 100\% \end{aligned} \quad (6)$$

### Determination of Fat Content

Fat content was measured by the Soxhlet method. Approximately 1-2 g of beef sample was weighed into a paper thimble coated with cotton. The paper sleeve was plugged with cotton and dried in an oven at 105 °C for 1 h. The sleeve was inserted into the Soxhlet apparatus connected to the fat extraction flask containing dry boiling stones and having known weight. The samples were extracted using hexane for 6 h until the fat extract was obtained. The fat extract was cooled and weighed. Fat content was determined by using the following formula.

$$\begin{aligned} \text{Fat content (\%)} = \\ \frac{\text{Weight}_{\text{Flask with fat extract}} - \text{Weight}_{\text{Empty flask}}}{\text{Weight}_{\text{Sample}}} \times 100\% \end{aligned} \quad (7)$$

### 2.5 Statistical Analysis

One-way analysis of variance was performed to determine the significance of effects shown by CAM for the indicated parameters. Each parameter consisted of 4 treatments with 6 replicates per treatment. Significant differences among the treatment groups were confirmed by post hoc multiple comparison Duncan multiple range tests. Statistical analyses were performed using

SPSS for Windows. A *p-value* of <0.05 was considered statistically significant (Dawson & Trapp, 2001).

## 3 Results and Discussion

The quality of meat is the measure of characteristics that determine the suitability of fresh or stored meat for consumption for a reasonable period without deterioration (Elmasry et al., 2012). Usually, the quality of meat is assessed by consumers using sensory/organoleptic testing. To avoid subjectivity, quality assessment evolved as an assessment of these characteristics by physical and chemical analysis. In the present study, the quality of marinated meat as a highly nutritional food was evaluated. Ideally, marination is the soaking of beef in the marinade (solution or sauce), which facilitates the passive transport of nutrients from the marinade to the beef by osmosis (Çetinkaya, 2017).

The effect of CAM on the microbiological characteristic, tenderness, cooking loss and water holding capacity (WHC) of beef was evaluated in this study (Table 1). The effect on the microbiological characteristics was significant; concentrations of CAM between 10% to 30% showed a significant reduction in bacterial growth (Figure 2). Phenolic compounds present in the cashew apple extract might have inhibited the bacterial growth in beef. Phenol destroys the bacterial cell membrane and enzymes, thus resulting in bacterial death (Lima et al., 2019). Thus, CAM ameliorated the microbiological status of beef and also extended the shelf life of beef.

CAM beef was more tender than the control, and tenderisation increased with the increase in CAM concentration. The optimum level of tenderisation was found at 20% CAM; however, no significant difference was found between tenderisation at 20% and 30% CAM. Thus, CAM improved beef tenderness effectively. This might have been because of the acid content of the cashew apple. Acidic compounds hydrolyse and break the cross-linking of connective tissues in marinated beef. Akinwale (2000) reported the highest quantity of ascorbic acid in the cashew apple juice (203.5 mg 100 mL<sup>-1</sup>). This finding showed that the ascorbic acid content of cashew apple juice

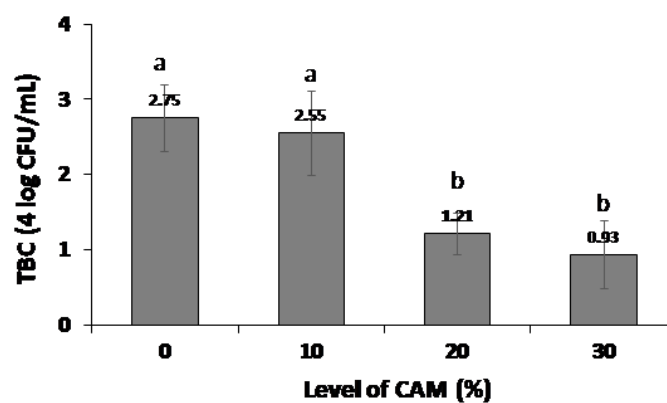


Figure 2: Effect of cashew apple extract marination (CAM) on the total bacterial count (TBC) of beef. TBC was expressed as a mean of 4 log CFU/mL unit  $\pm$  SD of 5 replicate analyses. Different superscripts letters show significant differences ( $p \leq 0.05$ ).



Figure 3: Beef appearance after the cooking process expressed as cooking loss. T0 is beef without cashew apple extract marination (CAM) and T1-3 are beef with CAM concentration of 10%, 20% and 30%, respectively.

Table 1: Physical characteristics of cashew apple extract (CAE) marinated beef

Parameters	CAE			
	0%	10%	20%	30%
Tenderness (g)	95.80 ± 9.38 <sup>a</sup>	82.50 ± 8.28 <sup>b</sup>	74.70 ± 6.69 <sup>b,c</sup>	68.60 ± 4.72 <sup>c</sup>
Cooking loss (%)	56.46 ± 6.56 <sup>a</sup>	47.14 ± 6.46 <sup>b</sup>	42.38 ± 8.41 <sup>c</sup>	36.40 ± 4.91 <sup>d</sup>
WHC (%)	26.69 ± 0.77 <sup>a</sup>	25.45 ± 0.55 <sup>b</sup>	23.63 ± 0.41 <sup>c</sup>	23.04 ± 0.23 <sup>c</sup>
pH	5.92 ± 0.13 <sup>a</sup>	5.56 ± 0.08 <sup>b</sup>	5.34 ± 0.05 <sup>c</sup>	5.24 ± 0.06 <sup>c</sup>

Data are expressed as mean ± standard deviation. Different superscripts letters on the same line show significant differences ( $p \leq 0.05$ ) between treatments. WHC, water-holding capacity

Table 2: Chemical characteristics of cashew apple extract (CAE) marinated beef

Parameters	CAE			
	0%	10%	20%	30%
Moisture (%)	77.18 ± 3.38 <sup>a</sup>	77.68 ± 4.99 <sup>a</sup>	74.67 ± 3.04 <sup>b</sup>	71.59 ± 3.56 <sup>c</sup>
Protein content (%)	20.89 ± 2.55 <sup>a</sup>	19.10 ± 2.52 <sup>b</sup>	15.10 ± 1.16 <sup>c</sup>	14.71 ± 3.33 <sup>c</sup>
Fat content (%)	8.54 ± 0.31 <sup>a</sup>	7.80 ± 0.15 <sup>b</sup>	7.23 ± 0.25 <sup>c</sup>	6.57 ± 0.36 <sup>d</sup>

Data are expressed as mean ± standard deviation. Different superscripts letters on the same line show significant differences ( $p \leq 0.05$ ) between treatments.

was almost four times higher than that of popular citrus fruits (54.7 mg 100 mL<sup>-1</sup>). Based on this finding, the tenderisation observed in our study was attributed to the high ascorbic acid content. Besides acid content, the sodium content of cashew apple (12 mg 100 g<sup>-1</sup>) may improve the beef texture by activating the binding of the water component to the proteins in the beef (Ahmad et al., 2020). Furthermore, sodium reduces fluid loss when beef is cooked at a high temperature under vacuum. Its manifestation was evident in the physical characteristics of beef, including cooking loss, which describe the degree of meat shrinkage during cooking (Figure 3). The percentage of cooking loss of CAM beef significantly decreased with the increasing concentration of cashew apple extract (Table 1). WHC describes the ability of meat to hold its native and added moisture during fabrication and processing. The WHC of beef progressively decreased ( $p \leq 0.05$ ) with the increasing concentration of CAM from 10% to 30%. WHC is the ability of proteins to prevent water release from the

three-dimensional structure of proteins. This is probably because of the proteolysis of myofibrillar proteins by ascorbic acid present in CAM and pH shifting towards the isoelectric point of myofibrillar proteins. The pH of CAM-treated beef decreased significantly with the increase in CAM concentration up to 20% (Table 1). Although the pH tended to decrease as an effect of treatments, the pH of CAM-treated beef was higher than its isoelectric point (5.0–5.1) and cooking loss was also low (Table 1).

Furthermore, CAM also influenced the moisture, protein and fat content of the beef. As described in Table 2, a decrease in moisture and protein content of the marinated beef was observed up to 20% CAM, whereas a decrease in fat content was observed up to 30% CAM.

The decrease in beef moisture and protein content in this study was thought to be correlated with lower WHC and increased concentration of cashew apple extract because of proteolysis caused by ascorbic acid. The fat content of beef decreased significantly with the increase in CAM

concentration because of the action of phenolic compounds in cashew apple extract. Beef fat, an ester of fatty acids, is hydrolysed into fatty acids during the marination process that involves water. Fatty acids are broken down into constituent esters by phenol through the esterification reaction (Figueroa-Espinoza & Villeneuve, 2005), and this could be the reason for the decrease in total fat content of the marinated beef.

Beef processing by the marination method in this study showed that cashew apple extract application as a meat marinade was able to reduce the fat and protein content of beef. The presence of low fat-protein beef can be useful for providing diet for humans with specific health conditions such as renal failure, coronary heart disease, diabetic mellitus, obesity and breast cancer (Rhee et al., 2018; Rubio-Patino et al., 2018; Shai et al., 2008). In addition, a low fat-protein diet can also be a clinically relevant lifestyle-intervention strategy for delaying the onset of cognitive impairment and dementia, especially in females (Buccarello et al., 2017).

#### 4 Conclusions

CAM increased beef tenderness and reduced the bacterial growth, cooking loss, moisture and protein content of beef. The optimum level of CAM for significant changes in the physical and chemical characteristics of beef was 20%. Thus, cashew apple extract could be utilised as a promising marinade agent in beef processing.

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