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Inhibitory Activity of Cashew Apple (*Anacardium Occidentale*) Extract Marinade on the Meat Total Bacterias

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Abstract

Microbial activities cause physical and functional alteration, which can decrease the quality and shelf life of meat. Inhibiting the bacterial growth becomes a crucial effort to prevent spoilage of meat. Previous studies reported that human pathogen bacterias could be inhibited by using cashew apple juice. It was interested to explore the utilization of cashew apple as marinade solution to suppress the meat bacteria. Current study was aimed to get more insight the effectiveness of cashew apple extract marinade (CAM) as growth inhibitor for spoilage bacteria on beefs and chickens. Results indicated that inhibition zone of 20% CAM were significant higher than 0% CAM on the medium of *E. coli*, and *S. aureus*. The pretty high polyphenols content in CAM was considered as an active compound responsible for antibacterial activity. Furthermore, total bacterial count assay showed that 10 to 30% CAM significantly reduced the beef total bacteria in comparison to 0% while 15 % CAM significantly decreased the chicken total bacteria. It was suggested the range of effective level of CAM to reduce the meats bacteria is 10 to 30%. Thus, cashew apple can be a promising natural food preservative in the future, especially for extending the shelf life of meat.



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Introduction

Meat is a perishable food and one of factors that led to meat spoilage is microbial growth¹. Commonly, meat supply in Indonesia is organized by abattoirs where its yields afterward slaughtered, distributed or consumed allows potentially contaminated with microbes. Microbial activities on meat cause physical

and functional alteration with the result that decrease in quality and shelf life.

According to the National Standardization Agency of Indonesia, microbiological quality requirement by using Total Plate Count (TPC) test on meat is a maximum of 10⁶ CFU/g. When the total bacteria

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of meat are higher than an established standard, meat is not feasible anymore as food because it was perishable. In addition, pathogenic bacteria exposed meat will be a potential source for food borne disease when it was consumed by human. Therefore, to get more optimum usefulness and quality of meat especially in the point of view of microbiological aspect, addition of an active ingredient on meat through the marinating process is necessary to be done.

In the beginning, marinating was just as seasoning. In the latter case, marinating is also play a role for suppressing meat bacteria due to the bioactive constituent containing in the marinade^{2,3}. Thus marinating can be advantaged as a method to prolong the shelf life, increase the addedvalue, and assure the food safety on meat or its products. It has been known that kind of organic acid, flavonoid, and polyphenol contained in the fruits extract commonly used as a natural marinades ingredient that is safe for health⁴. Principally, vitamin C and flavonoid have a function as antioxidant while polyphenol can play a role as antibacterial. In this study, we used cashew apple as a promising marinade because of its highly polyphenol and organic acids content⁵. All this time, cashew apple only has been being a waste in cashew nut industry. When cashew nut became the high valuable food, cashew apple was often considered as by-product with estimation of worldwide production about 30 million ton⁶. Previous studies reported that cashew apple juice contain antibacterial property having potential to inhibit human pathogens *Staphylococcus aureus*^{7,8}. It was interested to explore the utilization of cashew apple as marinade solution to suppress the meat bacteria.

Therefore, current study was aimed to get more insight the effectiveness of cashew apple extract marinade (CAM) as growth inhibitor for spoilage bacteria on beefs and chickens. This investigation was contributed in order to get an alternative ingredient for fresh meat preservative. Moreover, this study also provided the scientific basic regarding to the application of natural preservative material for food that was biodegradable, non-residue, and safe for consumer health. Overall, this finding was expected can promote the economic and social development in the light of Indonesia as one of the

biggest cashew nut producer in the world overtake Mozambique, India, and Brazil.

Materials and Methods

Collection of Cashew Apples

The ripe cashew apples were collected from some farmers located at the district of Wonogiri, Central Java Province, Indonesia. Those samples were brought immediately to laboratory and washed well with clean water to release cashew apples from of impurities.

Preparation of Cashew Apples Extracts (CAE)

Extraction of cashew apple was performed by using percolation method. Solvent used in that percolation extraction was 70% ethanol. Extraction was initiated by grinding of cashew apple simplisia followed by percolation stage and finally obtained cashew apple in ethanol extract. The entire of extraction process was illustrated in the Figure 1. The yields obtained from that process were calculated for its total phenolic and flavonoids content by using Folin-Ciocalteu method⁹. Briefly, 200 μ l of the appropriately diluted crude extract were added to 1.9 mL of freshly diluted 10-fold Folin-Ciocalteu reagent. The same volume (1.9 mL) of a sodium carbonate solution (60 g/L) was used to neutralize the mixture. After a 120-min reaction in the dark, at room temperature, the absorbance of the mixture was measured at 725 nm. Ferulic acid was used as a standard, and results were expressed as mg of ferulic acid equivalents (FAE) per g of extract. Meanwhile the total flavonoid content of CAE was determined using the Dowd method¹⁰. 5 mL of 2 % aluminiumtrichloride (AlCl₃) in methanol was mixed with the same volume of the extract solution (0.4 mg/mL). Absorption readings at 415 nm using Perkin Elmer UVVIS lambda 25 spectrophotometer were taken after 10 minutes against a blank sample consisting of a 5 mL extract solution with 5 mL methanol without AlCl₃. The total flavonoid content was determined using a standard curve with catechin (0 - 100 mg/L) as the standard. Total flavonoid content is expressed as mg of catechin equivalents (CE) per g of extract.

Antibacterial Activity Assay of CAE

Before being applied as marinade solution, antibacterial potency of CAE wastested by using disc diffusion method¹¹. First of all, medium of nutrient

agar (NA) and bacteria culture equipments were sterilized by autoclave at 121°C for 15 minutes. Bacteria cultures such as *E. coli*, *S. aureus*, *S. typhi*, and *Bacillus sp* in nutrient broth (NB) medium were shaken out by vortex, poured into NA (ratio of indicated bacteria and NA was 1:20 in v/v) and homogenized thoroughly. Bacteria cultures were incubated at optimum growth temperature for 24 hours until mediums were turbid with 10⁸-10⁹ colony /ml indensity. Inoculated NA-mediums were poured for 15-20 ml on petri dish and allowed to solidify at the room temperature. Discs in 5 mm diameter were passed away over the Bunsen burner and then dipped into the solution of 20% cashew apple extract. Those discs were laid on the bacteria inoculated NA-mediums surface and then incubated at the room temperature for 24 hours. Finally the diameter of inhibition zones located around the discs was measured.

Inhibitory Activity Assay of Cashew Apple Extract Marinades (CAM)

First of all, beef and chickens were prepared as meat samples in this study. Chicken breast fillets were purchased from a local retail outlet and cut into pieces of similar dimensions weighing about 40 g each. Beef samples (tenderloin) were purchased from a major butchery in the city. All fat were removed from the sample and then cut manually about 1 cm in thick and weighed about 60 g each. Before marinating process was done, all samples were kept in the refrigerator at 5 °C.

Cashew apple marinated (CAM) was prepared in serial dilution by dissolving CAE into the sterile distillate water. Concentration level of CAM for chicken meat marinating was determined to 0, 5, 10, and 15 % while beef marinating was 0, 10, 20, and 30 %. Simultaneously, each sample was soaked in the dish with indicated concentration of CAM for 4 hours. No addition of CAE (0 % concentration) was considered as a control in this study. Post marinating step, samples were taken out from the dish, drained, and kept in plastic bags before total bacteria of meat was evaluated.

Total bacteria were calculated by total plate count (TPC) method. Principle of this method is calculating all coloni formed by bacteria. Activities related to TPC technique was under sterile condition. First of each

samples were dipped in 45 ml of destilatte water for 10 minutes. Sample solution was dilluted to desired final dillution level. 1 ml of each samples solution was poured into petri dishes followed by pouring on of 20 ml of NA medium. It was allowed to coagulate at the room temperature. Petri dishes were incubated at 37 °C in upside down condition. After 24 hours incubation, the number of colony was calculated by colony counter assistance. Counted colony number was corrected by its dilution factor. Total bacteria were expressed as log CFU/mL.

Statistical Analysis

One-way ANOVA was used to identify the difference levels of CAM for some indicated parameters. For group differences, post hoc multiple comparison Duncan multiple range tests (DMRT) were used to analyse meat total bacteria, while least significant difference (LSD) was used to analyse antibacterial activity of CAM. Statistical analyses were performed using SPSS for Windows. $P < 0.05$ was considered statically significant¹².

Results and Discussion

Antibacterial Activity of CAE

Previous study successfully revealed the potency of cashew apple as antibacterial to human pathogens. Cashew apple juice showed inhibit zone of *Staphylococcus aureus* isolates. Inhibition zone reached cashew apple juice was equal with antibiotic of Tetracycline in that study. However, bioactive components responsible for antibacterial activity of cashew apple as well as its level of efficacy have not been elucidated clearly. Through this study, we extracted cashew apple in order to get the bioactive constituent possesses the antibacterial activity (Fig. 1). The yield obtained from extraction process was determined phenolic and flavonoid content subsequently. It has been known that both compound are board spectrum antibacterial produced from plants¹³. Based on the spectrophotometer analysis, total phenolic content of CAE was quite higher than total flavonoids content (Table 1). It was suggested that phenolic compound likely a substance responsible for antibacterial activity of cashew apple extract. That fact was more clarified by antibacterial activity test of CAE in this study wherein 20 % concentrations extract compare with distillate water as a control, able to increase the inhibition zone toward *E coli* and *S aureus* significantly

(Fig.2). The biggest inhibition zone was visible occur to *S aureus*, suggested that cashew apple inhibits bacterial growth effectively so that ingredients can be applied as food preservative.

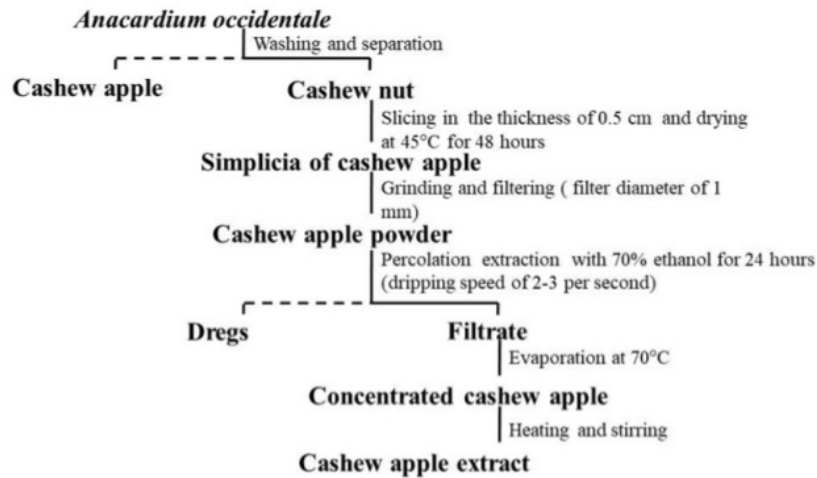


Fig. 1: The scheme of percolation extraction guidance of cashew apple

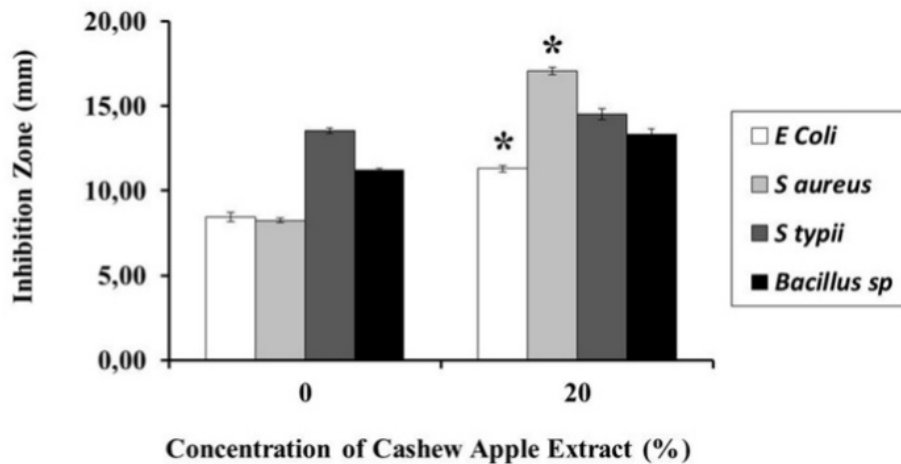


Fig. 2: Graphic of antibacterial activity of cashew apple extracts (CAE). Inhibitory efficacy of CAE was expressed as inhibition zone in mm unit. Data were mean \pm SD of 4 replicate analyses.*Differ significantly ($P < 0.05$) versus the control group (0%) according to the least significant difference (LSD)

Table 1: Level of bioactive component of CAE

Active compound	Concentration
Total flavonoids (mg of CE/g of extract)	0.14
Total phenolic (mg of TAE/g of extract)	13.3
CE : Catechin Equivalents	
TAE : Tannic Acid Equivalents	

As a pathogen bacteria, *Staphylococcus aureus* (Staph) is found on the skin and in the nose of about 25% of healthy people and animals¹⁴. It usually does not cause illness in healthy people, but Staph has the ability to make toxins that can cause food poisoning. Staphylococcal food poisoning is a gastrointestinal illness caused by eating foods contaminated with toxins produced by the bacterium *Staphylococcus aureus*¹⁵. Meanwhile inhibition activity of cashew apple toward *E coli* showed that material was potent to control pathogenic bacteria that threaten human health. Although most types of *E. coli* are harmless, some types can make people sick especially when

bacteria play a role as food contaminant, for example undercooked ground meat¹⁶.

Inhibitory Activity of CAM on the Total Meat Bacteria

In order to establish the application of cashew apple related to food safety, its extract was used as marinade solution (CAM) for beef and chicken in this study. This study revealed the effectiveness of CAM to inhibit the total bacterial count (TBC) of meat. From the data showed that beef marinating only using distillate water (0% CAE) within 4 hours have the TBC more than 10^6 CFU/g (Fig. 3). Such condition has indicated the presence of bacterial contamination on beef that allegedly came from the abattoir since slaughtering, handling, and processing time¹⁶. CAM in the level of 10 up to 30 % concentration within 4 hours significantly decreased the TBC of beef (Fig. 3). It suggested that meat marinating by using CAM could reduce the risk of microbial contamination although lowering of contamination was not reaching the microbial quality requirement in this case wherein the status of contaminant has been high since the early stage.

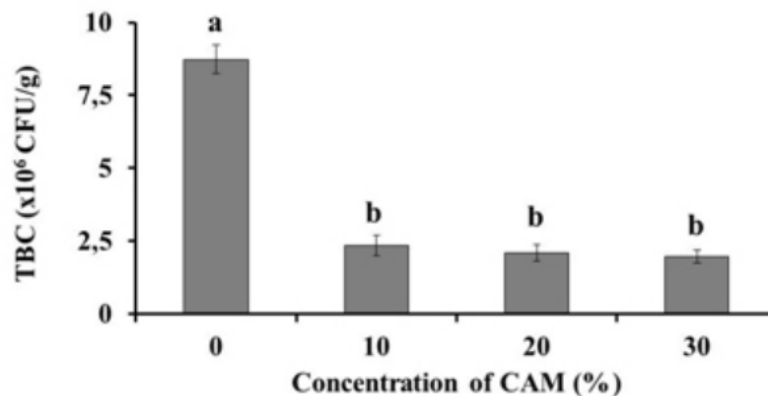


Fig. 3: Graphic of the effect of cashew apple marinades (CAM) on the beef total bacteria. Total bacterial count (TBC) was expressed as $\times 10^6$ CFU/g unit. Data were mean \pm SD of 4 replicate analyses. ^{a,b}Differ superscripts above the bar chart show differ significantly ($P < 0.05$) according to the Duncan's multiple-range test

In the other hand, TBC of chicken meat significantly reduced by 15% concentration of CAM within 4 hours (Fig. 4). All data suggested that effective concentration of CAM to inhibit the meat bacterial growth could be reached less than 30%. Inhibition capacity of CAM toward some meat bacteria likely was caused by direct contact of phenolic compound contained extract around the meat surface. It was assumed that phenolic as one of bioactive constituents responsible for bactericidal properties of cashew apple. Phenol will establish the protein complex when it was bounded by cell. As a

consequence, enzymes of bacteria will be inhibited and finally die. However, the antibacterial mechanism of cashew apple still needs further investigation.

Bacterial Inhibition activity of cashew apple in the concentration rank of 10 to 30% proved that fruit as potential natural ingredient to prolong the shelf life of meat. Thus cashew apple is promising as natural food preservative in the future. This finding is expected can give a little bit contribution to solve the food safety problem related to the food borne diseases caused by microorganism.

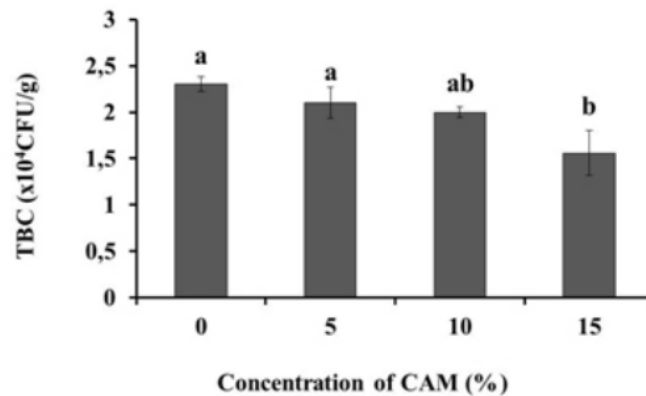


Fig. 4: Graphic of the effect of cashew apple marinades (CAM) on the chicken meat total bacteria. Total bacterial count (TBC) was expressed as $\times 10^4$ CFU/g unit. Data were mean \pm SD of 4 replicate analyses.^{a, b} Differ superscripts above the bar chart show differ significantly ($P < 0.05$) according to the Duncan's multiple-range test

Conclusions

Application of cashew apple extract marinade within four hours in duration time can effectively inhibit the meat bacterial growth wherein the optimum concentration of extract as a bacterial inhibitor on beef is lower than chicken meat. Thus, cashew apple can be a promising natural food preservative in the future, especially to the prevention of early spoilage in meats.

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