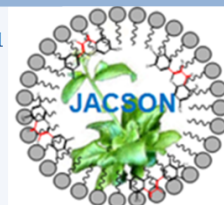
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### Effect of Concentration of Soursop (*Annona muricata*) Leaf and Soaking Time on Protein and Fat Contents and Sensory Quality of Raw Chicken Meat

Diana A. Wuri<sup>1</sup>, Jublin F. Bale-Therik<sup>2</sup>, and Gomera Bouk<sup>2</sup>

<sup>1</sup>Department of Public Health Veterinary, Faculty of Veterinary Medicine Nusa Cendana University, Kupang 85361;

<sup>2</sup>Department of Animal Science, Post Graduate School, Nusa Cendana University, Kupang 85361, INDONESIA

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#### ABSTRACTS

The purpose of this study was to find out the effect of concentration of soursop (*Annona muricata*) leaf and soaking time on protein and fat contents and sensory quality of raw chicken meat during storage. This research was conducted in Laboratory of Animal Products Technology, Faculty of Animal Husbandry Nusa Cendana University and Laboratory of Veterinary Public Health, East Nusa Tenggara Province Livestock Services Kupang, Indonesia. The experiment was arranged in completely randomized design in a 4x3 factorial lay out with three replications. The first factor was concentration of soursop leaf in water (C) with four levels: 0, 10, 20, and 30 g/L water. The second factor was soaking time of chicken meat (T) with three levels: 0, 10, and 20 minutes. The quality of chicken meat changes were monitored chemically and organoleptically at hours 12 of storage time. Data collected of protein and fat contents of chicken meat were analyzed using Analysis of Variance and continued with Duncan's Multiple Range Test, while data of organoleptic properties (color and aroma) characteristics were compared using the Kruskal Wallis Test. It was found that both treatment factors concentration of soursop leaf and soaking time with the interactions had significant effect ( $P < 0.01$ ) on protein and fat content of chicken meat. These results also indicated that color characteristics of chicken meat detected by the descriptive panelist were significantly affected by the both factors concentration of soursop leaf and soaking time ( $P < 0.05$ ), while aroma characteristics were insignificantly affected by the treatments.

**Keywords:** *Annona muricata* leaves, protein of chicken meat, chemical composition, sensory quality

\* Corresponding author: [jublinbale@staf.undana.ac.id](mailto:jublinbale@staf.undana.ac.id)

#### 1. Introduction

Chicken meat is an excellent source of protein and recognized as one of the most perishable foods. This is due to its chemical composition that favours microbial growth and contributing significantly to meat quality deterioration. Meat property changes and contaminations may occur during the process operations and storage. In fact, during and after slaughtering, the bacteria from animal microbiota, the slaughterhouse environment, and the equipment used contaminate carcasses, their subsequent cuts, and processed meat products. These bacterial contaminants can grow or survive during food processing and storage that some of which are pathogens (Rouger et al., 2016).

There are three main mechanisms for the spoilage the meat and meat product: microbial spoilage, lipid oxidation and autolytic enzymatic spoilage (Dave and Ghally, 2011). In decay of meat, microorganism that produces proteolytic enzymes capable of breaking down proteins or so called protein denaturation. With the occurrence of the denaturation process, the protein gradually loses its ability to hold fluids. As a result, the body fluids will come loose and flow out of the meat. The most common form of chemical damage in meat is

lipid oxidation which is initiated in the unsaturated fatty acids fraction in subcellular membranes (Meza et al., 2014). Lipid oxidation is a major cause of the meat quality deterioration that may generate changes in meat quality parameters such as colour, flavour, aroma, texture, and nutritive value (Kolakowska, 2003; Devatkal and Naveena, 2010). It was also reported that in muscle cells of slaughters animals, enzymatic actions are taken place naturally and the enzymes act as catalysts for chemical reaction that finally end up in meat shelf life deterioration. In addition, the process of decay occurs due to the activity of enzymes that remodeling the components of food to form a compound whose aroma is not preferred.

Meat preservation became necessary to inhibit the microbial spoilage and minimize the oxidation and enzymatic spoilage. Many synthetic preservatives are currently being used to reduce microbial growth and thereby extend the shelf-life of meat. Because of the increasing consumer demand for "healthier" meals (free of conventional chemical preservatives), the use of natural preservatives and environmentally friendly technologies has been suggested (Artes, 2007). The oxidation of lipids, and of unsaturated fatty acids in particular, leads to the formation of hydroperoxides whose cleavage is accompanied by the formation of secondary

products such as pentanal, hexanal, 4-hydroxynonenal, malondialdehyde and other oxygen compounds including aldehydes, acids and ketones (Fernández et al., 1997). These secondary products may cause loss of colour and nutritional value and a negative smell and taste (Min and Ahn, 2005; Resconi et al., 2013) due to their effect on lipids, pigments, proteins, saccharides, and vitamins (Dave and Ghaly, 2011).

Utilizing natural ingredients as antibacterial and antioxidants can be recommended to maintain the quality of chicken meat during storage. Several studies have found a reduction in oxidation in different meats after the addition of different natural compounds such as those found in oregano, ginger, and grape (Botsoglou et al., 2003; Olatidoye, 2015; Nardoia et al., 2017). Experimental results indicated that several natural antioxidants from plants also showed positive effects in improving meat quality and extending shelf life (Hamied et al., 2009; Kare et al., 2013; Bale-Therik et al., 2016).

Soursop (*Annona muricata*) leaves were reported to possess antimicrobial and antioxidant activities. Some extracts of phytochemical compounds of leaf, stem, root, and seeds from the *Annona muricata* such as alkaloids, flavonoids, carbohydrates, cardiac glycosides, saponins, tannins, phytosterol, and terpenoids have shown antibacterial activity against several pathogen micro-organisms (Vijayameena et al., 2013). On the other hand, antioxidant compounds such as phenols (gallic and chlorogenic acid), flavonoids (myricetin, fisetin, morin, quercetin, kaempferol, and isorhamnetin), anthocyanins, ascorbic acid, tocopherols, tocotrienols, carotenoids, and acetogenins have been found in soursop leaf, seed, and pulp (Mazilla et al., 2015; Sayuti et al., 2015). Among these compounds, tannins have metal-chelating, antioxidant, and protein precipitating activities and show positive effects on meat color stability and extend their shelf life (Maqsood et al., 2010; Hagerman, 2011). Antioxidants from a natural source provide a good alternative to conventional anti-oxidants because of high phenolics and other active ingredients, which can effectively prevent initiation or propagation of lipid oxidation reactions (Ahn et al., 2002, 2007; Brannan, 2008). Various natural antioxidants have also been shown to exert a positive or negative effect on the color and sensory properties of the meat products (Kumar et al., 2015).

There are several methods that are commonly used to prevent growth of spoilage and pathogenic microorganism in foods. Decoction is a method of extraction by boiling parts of herbs to dissolve the chemicals of the material. The process can also be applied to meat (Montagné, 2001). Therefore, the objective of this study was to determine the effects of concentrations of soursop (*Annona muricata*) leaf and soaking time on protein and fat contents and sensory quality of raw chicken meat during storage.

## 2. Materials and Methods

### 2.1. Materials and Site Study

This research was conducted at Laboratory of Animal Products Technology, Faculty of Animal Husbandry Nusa Cendana University and Laboratory of Veterinary Public

Health, NTT Province Livestock Services. These laboratories were located in Kupang city, Indonesia.

A total of 72 chicken carcasses obtained from a commercial processor were used for meat evaluation. Chicken carcasses were directly acquired to the Laboratory 4 hours after slaughtering the animals. The carcasses were washed, weighed, and dissected into retail cuts to obtain breast meat.

The leaves of soursop were purposively collected from Kupang. The fourth and the fifth leaves were picked out of the point of a young leaf. The leaves were weighed and chopped after washing to ease the drying process. Soursop leaves were dried in open air for 3 days. The dry products were then crushed to become a powder mass. Then, the dry powder of soursop leaf was dissolved into water and boiled for about 20 minute to obtain the decoction concentrations of 10, 20, and 30 g/L, respectively. The decocted soursop was cooled down at room temperature (26-28°C) before using as a medium for soaking the meat.

Chicken meat samples were prepared for soaking in decocted soursop leaves. Samples were then stored at room temperature until subsequent analysis after 12 hours of storage.

## 2.2. Methods

### 2.2.1. Experimental Design

The experiment was arranged in completely randomized design in a 4x3 factorial lay out with three replications. The first factor was concentration of soursop leaf in water (C) with four levels: 0, 10, 20, and 30 g/L water. The second factor was soaking time of chicken meat (T) with three levels: 0, 10, and 20 minutes. There were 12 combinations of treatment in this experiment.

### 2.2.2. Chemical and Sensory Analysis

Chemical analysis was done to determine the content of protein and fat of chicken meat. Proximate compositions of chicken meat samples were determined using the procedures of AOAC (2000). All chemical analyses were carried out in triplicate.

Sensory characteristics of samples were investigated to evaluate color and aroma of chicken meat appearance. A panel of seven judges trained in chicken evaluation performed sensory analysis. Panelists were asked to evaluate the color and aroma of the chicken breast meat samples. The samples were randomly presented to the trained panelist. Acceptability as a composite of color and aroma was estimated using a 6 point hedonic scale, where 6=high and 1=low. The scale points for color as follows: 6= creamy yellow; 5= slightly yellow; 4= yellow; 3= slightly dark yellow; 2= moderately dark yellow; 1= very dark yellow. The scale points for aroma as follows: 6= very good; 5= good; 4= acceptable; 3= poor (first off-odour); 2= very poor; 1= extreme poor. The panel evaluated each treatment within each replication in triplicate.

### 2.2.3. Statistical Analysis

Data collected of protein and fat contents were analyzed using Analysis of Variance (ANOVA) and continued with Duncan's Multiple Range Test, while data of organoleptic

properties (color and aroma) were compared using the Kruskal Wallis Test.

### 3. Results and Discussion

#### 3.1. Protein and Fat Contents of Chicken Meat

##### 3.1.1. Protein content

The amount of protein content of chicken meat samples are demonstrated in Table 1. Experimental results showed a significant interaction was found between concentration of soursop leaves (C) and soaking time (T) on protein content of chicken meat. This would suggest that the effect of concentration depended on the soaking time. The results showed that there was significantly different between the treatments of soaking time in C10, C20, and C30. Furthermore, the results also found that there was significantly different between the treatments of concentration especially for C0, C10, and C20 in all levels of soaking time. In general, this study can be explained that protein content of chicken meat decreased with the addition of soaking time and concentration of soursop leaves. During storage time, bacteria in chicken meat increased and in the way would damage the protein. This study indicated that soursop leaves substances was not able to act as antimicrobial. According to Farag et al. (1989), antimicrobial activity of plant substances depends on several factors such as composition and concentration.

Ristianty (2017) reported that total bacteria of fresh chicken meat increased during storage at room temperature. It was also reported that at 2 hours of storage, the total bacteria of chicken meat reached about  $15.8 \times 10^7$  cfu/g, but at 6 hours of storage total bacteria increased to  $20.33 \times 10^7$  cfu/g. Furthermore, protein will be damaged due to the decomposition of proteins caused by bacteria and enzymes. Proteolytic enzyme would hydrolyze proteins into smaller peptides while proteolytic bacteria would form soluble nitrogen compounds (Muliati et al., 2014). The process of proteolysis by a bacterial activity caused the formation of  $\text{NH}_3$  gas decreasing protein content. In addition, there was relationship between protein denaturation and protein content during food processing. Asrullah et al. (2012) reported that the higher levels of food processed denaturation caused a greater decrease in protein content. On the other hand, secondary metabolite compounds produced by the plants generally can inhibit the growth of pathogenic microorganisms. Wisdom et al. (2014) reported the phytochemical analysis of soursop leaves indicated that soursop leaves have various secondary metabolites such as tannin/polyphenols, steroids, saponins and flavonoids. Flavonoid compounds in extracts of soursop has the potential as an antibacterial for being able to inhibit the growth of bacteria by destroying the cell wall permeability, microsomes, lysosomes and bacterial cells a result of interaction between flavonoids with DNA (Sabir et al., 2005). While the tannins/polyphenolic able to act as an antibacterial in the way that it reacts with cell membranes, inactivate enzymes, function and metabolism of the cell's genetic material so hampered and disrupted cell wall synthesis

(Roslizawaty et al., 2013). Saponins might disturb the permeability of the bacterial outer membrane (Arabski et al., 2011).

Table 1. Effects of different concentrations and soaking times on protein and fat contents of chicken meat

Variable	Concentration* of Soursop leaves	Soaking Time**		
		T0	T10	T20
----- (%) -----				
Protein Content	C0	20.1733 <sup>a(A)</sup>	19.7600 <sup>b(A)</sup>	20.1533 <sup>a(A)</sup>
	C10	19.8367 <sup>a(B)</sup>	19.4167 <sup>b(B)</sup>	18.6367 <sup>c(B)</sup>
	C20	20.3600 <sup>a(C)</sup>	19.0300 <sup>b(C)</sup>	18.5367 <sup>c(C)</sup>
Fat Content	C0	20.1733 <sup>a(A)</sup>	19.9333 <sup>b(D)</sup>	18.7100 <sup>c(D)</sup>
	C10	3.4633 <sup>a(A)</sup>	3.3833 <sup>b(A)</sup>	3.2500 <sup>c(A)</sup>
	C20	3.2467 <sup>a(B)</sup>	3.5667 <sup>b(B)</sup>	3.0533 <sup>c(B)</sup>
Fat Content	C20	3.9300 <sup>a(C)</sup>	4.0267 <sup>b(C)</sup>	2.9400 <sup>c(C)</sup>
	C30	3.5267 <sup>a(D)</sup>	4.0300 <sup>b(C)</sup>	2.9300 <sup>c(C)</sup>

Value with different superscript raw wise (small alphabets) and column wise (capital alphabets differ significantly ( $P < 0.05$ )). \*C0, C10, C20, C30 = 0, 10, 20, 30 g/L; \*\*T0, T10, T20 = 0, 10, 20 minutes

##### 3.1.2. Fat Content

Fat content of chicken meat parameter results are presented in Table 1. In this study, a significant interaction was detected between concentration of soursop leaves (C) and soaking time (T) on fat content of chicken meat. Table 1 showed analysis results for soaking time indicated that C0, C10, and C20 were significantly different for T0, T10, and T20. While analysis for concentration indicated that T0, T10 and T20 were significantly different, in which T20 had lower fat content compared to T0 and T10 for all levels of concentration.

Those results can be explained that the higher concentration and soaking time caused the lower value in fat content. The decline in value of fat content of the meat due to the role of compounds such as tannin and alkaloid contained in soursop leaves. Tannin and alkaloid serve as a preservative and antioxidants. According to Soeparno (2009), antioxidants can inhibit rancidity due to fat oxidation and can increase fat content but at high concentrations can reduce fat content of meat. Those reasons are in line with previous study results showing that broiler meat boiled with mayana (*Solenostemon scutellarioides*) leaf, degrade fat content on broiler meat (Indriastuti and Praptiwi, 2014). It was also reported that mayana leaf possesses antioxidant activity (40.77%), because of its high content of phenolic compound. In addition, it was also reported that the tannin contained in mayana leaves that is equal to 1.19% potentially reduce the fat content of chicken meat (Indriastuti and Praptiwi, 2014). Similarly, Ojezele (2016) reported that soursop leaves had the potential to reduce fat content on meat because of a tannin content in soursop leaf (1.60%), while Sayuti (2015), reported that soursop leaves extract possesses antioxidant activity of 57.51%.

#### 3.2. Sensory Quality of Chicken Meat

##### 3.2.1. Color Characteristics

The results of color analysis presented in Table 2. There were significant differences in color amongst the treatments, concentration of soursop leaves (C) and soaking time (T). The

Table 2. Effects of different concentrations and soaking times on the color and aroma of chicken meat

Variable	Concentration* of Soursop leaves	Soaking Time**			Average
		T0	T10	T20	
Color	C0	3.38	4.38	2.95	3.57
	C10	4.28	4.14	4.09	4.17
	C20	4.28	3.76	3.90	3.98
	C30	4.23	4.29	2.86	3.79
	Average	4.04	4.14	3.45	3.87
Aroma	C0	4.43	4.57	4.57	4.52
	C10	4.43	4.57	4.57	4.57
	C20	4.57	4.57	4.57	4.57
	C30	4.57	4.57	4.57	4.57
	Average	4.50	4.57	4.57	4.55

\*Concentration: C0; C10; C20; C30 = 0; 10; 20; g/L ; \*\* Soaking time: T0; T10; T20 = 0; 10; 20 minute

The scale points for color as follows: 6= creamy yellow; 5= slightly yellow; 4= yellow; 3= slightly dark yellow; 2= moderately dark yellow; 1= very dark yellow. The scale points for aroma as follows: 6= very good; 5= good; 4= acceptable; 3= poor (first off-odour); 2= very poor; 1= extreme poor

results of the sensory evaluation showed that the panelists gave a higher meat color assessment for the concentration of soursop leaf on C10 and the lower meat color on C20 with an average score of 4.17 each (yellow) and 3.45 (slightly dark yellow), respectively. In addition, for soaking time, the panelists found that T10 had a higher meat color score and T0 had a lower meat color score with an average score of 4.17 (yellow) and 3.57 (slightly dark yellow), respectively.

It was known that concentration of soursop leaf C10 and soaking time T10 showed the highest mean value of meat color. It could be due to the tannin content in soursop leaf for concentration C10 and soaking time T10 do not seem to affect the color of the meat. The changes of meat color were seen in the treatments with higher concentration of soursop leaves and the longer soaking time. It was indicated that darker color of meat was caused by the tanin brown color of soursop leaves that reinforce the color of chicken meat. This is in agreement with the results of Pensera et al. (2004) who found that longer brewing time would also optimize the amount of dissolved tannin compounds. Ajijah (2016) also indicated that volatile compound contained in the extracts of the kecombrang flower was able to seep into the meat.

### 3.2.1. Aroma Characteristics

Effect of different concentration and soaking time on the aroma of chicken meat are presented in Table 2. The results of aroma analysis did not differ amongst the treatments concentration of soursop leaves (C) and soaking time (T) indicating that the treatments did not change acceptance by panelists. It is important to point out that the parameters analyzed obtained scores from 4.43 to 4.57, which represent a classification of "acceptable" according to the hedonic scale. It can be explained that there is similarity in response to the treatments on aroma, although it was seen the meat treated with soursop leaf tend to be better results compared to the control. Similar results were reported by Ajijah (2016) who found that concentration of kecombrang flowers extract, soaking time, and their interactions had no effect to the aroma of fresh beef on the 2<sup>nd</sup> day of storage. It was then also reported that on the 6<sup>th</sup> day of storage, the higher the

concentration of the extract, then the higher valuation was given by panelists; it is because of the volatile compounds contained in the extracts of the flowers of kecombrang already seeped into the meat, so that it can cover the stench that of the beef. In addition, Das et al. (2011) found no significant difference in colour and aroma scores of control and curry leaf powder treated raw ground goat meat (stored at 4±1°C for 9 days). These findings are not in agreement with the results of Singh et al. (2014) who reported that there was significant difference on aroma between the treatments, control, and meat emulsion incorporated with clove powder, ginger, and garlic paste on raw chicken meat refrigerated at 4±1°C.

## 4. Conclusion

The treatment for concentration of soursop leaves up to 30 g/L water and soaking time up to 20 minutes on raw chicken meat stored 12 hours at room temperature indicated that protein and fat contents, sensory quality especially the meat color are affected by the treatments, while aroma of chicken meat is not affected by the treatment.

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