DOI: http://dx.doi.org/10.14334/Proc.Intsem.LPVT-2016-p.514-519

Evaluation of Using *Hibiscus sabdariffa* Calyces in the Production of Rotenesse Smoked Beef (Se'i)

Malelak GEM, Sipahelut GM, Jelantik IGN

Department of Animal Science, Nusa Cendana University
Jl. Adisucipto Penfui, Kupang 85001, East Nusa Tenggara, Indonesia
geminimalelak@yahoo.com.au

ABSTRACT

Aims of this experiment were to evaluate the use of *Hibiscus sabdariffa* extract on the production of *se'i* (Rotenesse smoked beef). A completely randomized design was used in this study. Treatments include: Po = *se'i* was without *Hibiscus sabdariffa* extract as control, P_1 = s*e'i* given *Hibiscus sabdariffa* extract 1% (w/v); P_2 = s*e'i* was given *Hibiscus sabdariffa* extract 2% (w/v); P_3 = s*e'i* was given *Hibiscus sabdariffa* extract 3% (w/v); P_4 = s*e'i* was given *Hibiscus sabdariffa* extract 4% (w/v); and P_5 = s*e'i* was given *Hibiscus sabdariffa* extract 5% (w/v), each treatment had three replication. Variables for this experiment include: flavour, color, taste, tenderness, pH, total plate count, *Coliform, Escherichia coli, Salmonella* and *Staphylococcus aureus*. Result showed that *Hibiscus sabdariffa* extract was not significantly affected the flavour, color, tenderness and pH. However, *Hibiscus sabdariffa* extract enhanced the taste of *se'i* ($P<0.05$). Also, the extract significantly reduced both total plate count and *Staphylococcus aureus* numbers ($P< 0.05$). As a matter of fact, C*oliform, Escherichia coli* and *Salmonella* were not detected in all *se'i*. In conclusion *Hibricus sabdariffa* was able to enhanced the taste of *se'i* and inhibit the growth of bacteria.

Key Words: Se'i, *Hibiscus sabdariffa*, Organoleptics, Bacterial

INTRODUCTION

Se'i is a dry-cured smoked meat product which is usually made from ruminant meat or pork. At present, *se'i* has been produced as a home industry in Kupang and the products have been distributed to many culinary markets in many parts of Indonesia.

Se'i is not completely dry but it still contains 40-60% water, consequently, it is easy to be contaminated by bacteria. Some of the contaminating bacterial in the product may cause food borne illness, shorten shelf-life of food and finally could lead to economic loss for food producers.

Using of natural ingredients to inhibit bacterial growth in food, including meat products, is an interesting option to solve the problem. One of the natural ingredients that usually used as antibacterial agent is *Hibiscus sabdariffa* or roselle.

It has been reported that roselle extract could inhibit *Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus* and *Bacillus cereus* both in ground beef and apple juice (Chao & Yin 2009). Roselle calyces contain antibacterial compounds such as: organic acids, phenolic acids, alkaloids and athocyanins (Christian & Jackson 2009); ascorbic acids and flavonoids (Morales-Cabrera et al. 2013); anthocyanins, flavonoids and polyphenols (Lin et al. 2007). In meat products, roselle calyces could reduce lipid oxidation of kavurma (Bozkurt & Belibagl 2009) and sucuk (Karabacak & Bozkurt 2008). In addition, many beneficial properties have been obtained from roselle calyces in food, drink and also medicine industry throughout the world. Thus, the roselle plant is now widely grown in numerous countries in the world. However, in West Timor, Indonesia, Roselle (*Hibiscus sabdariffa*) just grows naturally in agricultural land and home yard. The utilization of this plant in West Timor is only for spices in kitchen. The objective

of the study was to investigate the effects of *Hibiscus sabdariffa* calyces extract on the organoleptic aspects and microbial activity of *se'i*.

MATERIAL AND METHODS

Materials

A total of 30 kg of beef from butt and rump of Bali cattle was obtained from a meat shop in Kupang. Salt (2%) and Kalium nitrate (KNO_3) (300 mg of kg^{-1}) were added. *Hibiscus sabdariffa* calyces were obtained from Naimata village, Kupang, East Nusa Tenggara Province, Indonesia. *Schleichera oleosa* wood and leaf was obtained around Kupang regency.

Hibiscus sabdariffa extract

The calyces were dried in oven at 60°C for 3 days, then blended with Philips blender to abtain the mass. To obtain 1%, 2%, 3% and 4% (w/v) of roselle extract, each of 1, 2, 3 and 4 g of roselle mass poured into volumetric glass and added distilled water up to 100 ml, stirred at 60°C for 5 minutes and filtered with Whatman (No. 41) (Karabacak & Bozkurt 2008) with modification.

Treatment and *se'i* processing

Completely randomized design (CRD) with 6 treatments was assigned for this experiment. Beef was trimmed off the excessive fat then sliced in rope-shape *(lalolak*), mixed well with salt and Kalium nitrat. The mixture was split into six batches namely: Po = *se'i* without rossele/ as control; P_1 = Rossele extract 1% (w/v); P_2 = Rossele extract 2% (w/v); P_3 = Rossele extract 3% (w/v); P_4 = Rossele extract 4% (w/v); P_5 = Rossele extract 5% (w/v), mixed well and marinated for 12 hours.

After marinating, the sliced-meat was arranged in frame, covered with *Schleichera oleosa* leaf and then smoked using *Schleichera oleosa* wood for 45-60 minutes or until well done. A distance between the meat and the wood was approximately 45 cm.

Parameter measured

Organoleptic test

The organoleptic test was carried out using hedonic scale. Twenty five untrained panelists from Animal Science Faculty students were chosen for sensory analysis (flavour, color, taste and tenderness). To examine the flavour 30 g of samples were sliced, put into small glass jars and allowed to stand for several hours (Bensink et al. 1973). On opening the jars the panelists immediately sniffed the flavour and was asked to give "score 1" if no flavour to "score 5" very strong flavour. The color score was as follows: 5 = very bright red color; 4 = bright red color (specific *se'i* color), 3 = dark red, 2 = moderately dark red, 1 = light red. Whereas, the taste score was 5 = like very much, 4 = like moderately, 3 = like slight, 2 = dislike moderately and 1 = dislike very much. The tenderness score was 5 = very tender, 4 = moderately tender, 3 = slight tender, 2 = tough, 1 = very tough. For each treatment, each penelist had three pieces of *se'i* to be evaluated. An average of the three measurements to calculate mean score for each of the color, flavour, taste and tenderness of *se'i* sample.

Determination of pH

Se'i sample was about 10 grams was chopped and mixed well with 10 ml of distilled water then the probe of pH was placed into the dilution and the pH of sample was read. Determination of pH value was measured using a Hanna digital pH-meter at ambient temperature. Each sample was measured 3 times.

Bacterial analyses

Ten grams finely chop of *se'i* from each treatment were homogenized with 100 ml sterile 0.1% peptone water containing 0.85% NaCL and 1% Tween 80 as emulsifier, at 40-45°C for 2 minutes in a Stomacher 400 Lab Blender. Ten ml of the preceding was diluted with 0.1% sterile peptone water. Total plate count were enumerated in standard plate count agar (Oxoid agar) after incubation at 35°C for 48 h, then all colonies on plates were counted (Harrigan & McCance 1976; Elliot et al. 1978). Coliform counts were determined using violet red bile agar (Oxoid CM 107) after incubation at 37°C for 24 h. Coliform colonies were identified as round, red to pink, 0.5-2 mm in diameter, surrounded with a red to pink halo. *Escherichia coli* were isolated by using EC broth and EMB agar (Harrigan & McCance 1976). For *Salmonella* sp, a 50 g of homogenisate was transferred to a sterile Erlenmeyer flask with 200 ml buffered peptone water (Oxoid CM 509) and incubated at 37°C for 24 hours. After that a combination of bismuth sulphite agar (Oxoid, CM 201), modified brilliant green agar (Oxoid, CM 329) and hectoen enteric agar (Oxoid, CM 419) was used for selective plating. Presumptive salmonella colonies selected from each of selective plate were confirmed by using API 20 E identification system as described by the manufacturer (Bio Meri eux, Basingstoke, UK). (Harrigan & McCance 1976; Elliot et al. 1978). *S. aureus* counts predried Baird-Parker agar (Oxoid, CM 275), incubated at 37°C for 24 to 48 hours. Typical colonies were counted. Colonies were selected from agar surfaces to be examined under microscope for gram strain and tested for catalase reaction, and also coagulate activity by using rabbit plasma with EDTA (Elliot et al. 1978).

Statistical analysis

The data of flavour, color, taste and tenderness were analyzed using non parametric, Kruskas-Wallis test. Mann-Whitney test was used to test for difference between means (significance $P<0.05$; highly significant was $P<0.01$). pH and bacterial data were analysed with analysis of variance (ANOVA). Least Significant Differences test was used to determine the differences among means (significantly different was $P<0.05$; highly significant was $P<0.01$) (SPSS, 20).

RESULTS AND DISCUSSION

Organoleptic test

Table 1 showed that as roselle level increased the score of *se'i* taste also increased ($P<0.05$). However, *se'i* flavour, color and tenderness were not significantly increased ($P>0.05$) with increased of roselle level. To some extent, the organoleptic aspects of meat product are influenced by kind of ingredients used during processing. Roselle calyces used in this experiment contained: 0.1% ascorbate acid, 2.167% tartaric acid, 2.025% citrate acid, 1.89% oxalic acid, 1.76% succinic acid and 1.66% malic acid. The presence of the organic acids are responsible for acid taste (Cisse et al. 2009). Taste of *se'i* samples were

slightly acid when givien 1-3% of roselle extract and the acid taste became stronger at the addition of 4-5% extract. Other experiment reported that addition of roselle calyces extract enhanced sensory attribute (flavour, color and tenderness) of meat processing product "kavurma" (Bozkurt & Belibagl 2009).

Table 1. Average score flavour, color, taste, tenderness and pH of s*e'i* treated with *Hibiscus sabdariffa* calyces extract

Variables	Treatment					
	Control	Roselle 1%	Roselle 2%	Roselle 3%	Roselle 4%	Roselle 5%
Flavour	5.00±0.01	5.00±0.11	4.55±0.04	4.25±0.01	3.75±0.11	3.05±0.09
Color	3.80±0.11	4.35±0.13	4.00±0.07	4.00±0.04	4.00±0.02	4.75±0.05
Taste	3.95±0.12^{a}	4.40±0.10^{b}	4.60±0.02^{b}	4.65±0.02^{b}	4.80±0.01^{c}	4.95±0.07^{c}
Tenderness	4.30±0.10	4.05±0.03	4.45±0.07	4.25±0.07	5.00±0.07	4.95±0.01
Ph	5.86±0.01	5.88±0.01	6.08±0.03	5.65±0.01	5.74±0.02	5.71±0.01

± deviation standard. Means within the row followed by different letters were significantly different at P<0.05

Roselle calyces contains anthocyanins which are responsible for the red color (Cisse et al. 2009). Roselle calyces used in this experiment contained 184 ppm anthocyanins. Meat color was mainly determined by the chemical state of the myoglobin molecules. In this experiment, addition of roselle calyces extract did not affect the color of *se'i* which means that the anthocyanins did not able to change myoglobin molecules. The results provided a good information for *se'i* industry, since addition of roselle calyces extract did not change the flavour and color but expand the taste. Thus the *se'i* product could be offered to the consumer with other taste beside conventional taste. Since the taste score of treatment samples were higher than control.

Bacterial characteristic

Statistical analysis showed that addition of 3-5% roselle extract was significantly effect the total bacteria number as well as *Staphylococcus aureus* of *se'i* samples (P<0.05) (Table 2). In all *se'i* samples total plate count were less than 4 log CFU/g. It meant that the total plate count met SNI standard for smoked meat (5 log CFU/g). An addition of roselle extract 3-5% reduced total plate count by 0.03-0.52 CFU.

Table 2. The number of total bacteria (cfu), *Coliform, E.coli, Salmonella* and *Staphylococcus aureus* (log cfu/g ± SD) of *se'i* treated with *Hibiscus sabdariffa* calyces extract

Variables	Treatment					
	Control	Roselle 1%	Roselle 2%	Roselle 3%	Roselle 4%	Roselle 5%
Total bacteria	3.38±0.12^{a}	3.18±0.01^{a}	2.91±0.04^{a}	2.86±0.01^{b}	2.88±0.11^{b}	2.87±0.10^{b}
Coliform (MPN/g)	Negative	Negative	Negative	Negative	Negative	Negative
E. coli	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative	Negative	Negative
Staphylococcus aureus	1.82±0.12^{a}	1.67±0.01^{a}	1.63±0.04^{a}	1.10±0.01^{b}	1.29±0.11^{b}	1.21±0.10^{b}

± standard deviation. Means within the row followed by different letters were significantly different P<0.05

Staphylococcus aureus is a Gram-positive facultative anaerobic cocci and can grow in food with pH values ranging from 4 to 9.8 (Medvedova & Valik 2012). pH of all *se'i* samples were similar (P>0.05), range between 5.65-6.08. Thus it is suitable for *Staphylococcus aureus* to grow. However, in all *se'i* samples *Staphylococcus aureus*, less than 2 log CFU/g and this number is below the SNI for smoked meat 2 log CFU/g for *Staphylococcus aureus* (SNI 2009). An addition of roselle extract 3-5% reduced *Staphylococcus aureus* 0.05-0.72 CFU.

Coliform, *E. coli* and *Salmonella* are gram-negative. Coliform are used as sanitary quality indicator of food and water. The cut-off value for smoked meat is 10 mpn for Coliform, <3/g for *E. coli* and negative/25g for *Salmonella* sp. (SNI 2009). In all *se'i* samples Coliform, *E. coli* and *Salmonella* were not detected, indicating *se'i* produced in this experiment is save to be consumed.

Hibiscus sabdariffa extract have antimicrobial effect against a wide range of pathogens such as *Staphylococcus aureus*, Coliform and *Escherichia coli* (Olaleye 2007*), E Coli* and *Salmonella* (Fullerton et al. 2011), *Salmonella* (Nwaiwu et al. 2012; Morales-Cabrera et al. 2013). The ability of *Hibiscus sabdariffa* extract to reduce total plate count and *Staphylococcus aureus* indicated that *Hibiscus sabdariffa* extract contain antimicrobial activity. It was reported that *Hibiscus sabdariffa* extract contains organic acids and anthocyanins. Beside organic acids, anthocyanins, water-soluble flavonoid pigments also have antimicrobial activity (Morales-Cabrera et al. 2013).

The organic acids could penetrate cell wall of bacteria in the undissociated form which causes the growth of bacteria is limited or death by dissociating and acidifying the cytoplasm of the microorganism (Samelis & Sofos 2000). Anthocyanins have ability to form a combined complex with bacterial cell walls and also increased hydroxylation which result in increased toxicity (Cowan 1999) thus can kill the bacteria.

CONCLUSION

Roselle calyces extract can be utilzed in *se'i* to create a new taste as well as to reduce total plate count and *Staphylococcus aureus.*

ACKNOWLEDGEMENT

The authors wish to thank the financial support of Directorate for Higher Education, the Ministry of National Education.

REFERENCES

Bensink JC, Ford AL, Yates JR. 1973. Properties and performance of a range of commercial vacuum packaging films used for packing chilled beef. Meat Research Report. 4/73. p.1-1.

Bozkurt H, Belibagl KB. 2009. Use of Rosemary and Hibiscus sabdariffa L in production of Kavurma, a cooked meat product. J Sci Food Agric. 89:1168-1173.

Christian KR, Jackson. 2009 JC. Changes in total phenolic and monomeric anthocyanin composition and antioxidant activity of three varieties of sorrel (*Hibiscus sabdariffa*) during maturity. J Food Comp Anal. 22:663-667.

Chao CY, MC. Yin.2009. Antibacterial effects of Roselle Calyx extracts and protocatechuic acid in ground beef and apple juice. Foodborne Pathog Dis. 6:201-206.

Cisse M, Dornier M, Sakho M, Ndiaye, A, Reynes M, Sock O. 2009. Le bissap (*Hibiscus sabdariffa* L.): Composition et principales utilisations. Fruits. 64:179-193.

Cowan MM. 1999. Plant products as antimicrobial agents. Clin Microbial Rev. 12:564-582.

Elliot RP, Clark KH, Lewis H, Lundbeck JC, Olson JR, Simonsen B. 1978. Microorganisms in Food 1. Their Significance and Methods of Enumeration. 2nd ed. Toronto (Canada): University of Toronto Press.

Fullerton M, Khatiwada J, Johnson JU, Davis S, Williams LL. 2011. Determination of antimicrobial activity of sorrel (*Hibiscus sabdariffa*) on *Esherichia coli* O157:H7 isolated from food, veterinary, and clinical samples. J Med Food. 14:950-956.

Harrigan WF, McCance ME. 1976. Laboratory methods in food and dairy microbiology. London (UK): Academic Press. p. 452.

Karabacak S, Bozkurt H. 2008. Effects of *Urtica dioca* and *Hibiscus sabdariffa* on the quality and safety of sucuk (Turkish dry-fermented sausage). Meat Sci. 78:288-296.

Lin TL, Lin HH, Chen CC, Lin MC, Chou MC, Wang CJ. 2007. *Hibiscus sabdariffa* extract reduces serum cholesterol in men and women. Nut Res. 27:140-145.

Medvedova A, Valik L. 2012. *Staphylococcus aureus*: Characterization and quantitative growth description in milk and artisanal raw milk cheese production. In: Eissa AA, editor. Intech. Available from http://www.Intechopen.com/books/structure-and-function-of-food-engineering.

Morales-Cabrera M, Hernandez-Morales J, Leyva-Ruelas G, Salinas-Moreno Y, Soto-Rojas L, Castro-Rosas J. 2013. Influence of variety and extraction solvent on antibacterial activity of roselle (*Hibiscus sabdariffa* L.) calyces. J Med Plants Res. 7:2319-22.

Nwaiwu NE, Mshelia F, Raufu IA .012. Antimicrobial activities of crude extracts of *Moringa Oleifera*, *Hibiscus S* and *Hibiscus Esculentus* seeds against some enterobacteria. J Appl Phytotechnol Environ Sanit. 1:11-16.

Olaleye M. T. 2007. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. J Med Plant Res. 1:9-13.

Samelis J, Sofos J. 2000. Organic acids. In: Roller S, editor. Natural antimicrobials for the minimal processing of foods. CRC, Cambridge UK. Great Abington (UK): Woodhead Publishing Ltd.

SNI. 2009. Maximum Limited of Microbial Contamination of food. SNI: 7388: 2009. Jakarta (Indonesia): Indonesian National Standard.