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Influence Marination Length Using Evaporated Roselle (Hibiscus sabdariffa Linn) Extract on Microbiological Quality of Beef Se'i

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ABSTRACT: Experiment was conducted to determine the influence of marination length using evaporated roselle extract (*Hibiscus sabdariffa* Linn) on microbiological aspects of beef se'i. The completly randomized design (CRD) with 4 treatments 4 replicates was applied in this experiment. Those treatments were P₀ marination length of 12 hours without roselle extract; P₁ marination length 6 hours with 5% roselle extract; P₂ marination length 12 hours with 5% roselle extract, and P₃ marination length 18 hours with 5% roselle extract. The parameters measured were pH, moisture, water activity and total plate count. Data compilled were analyzed using variance analysis and Duncan's multiple range test. The result of analysis showed that pH and moisture of beef se'i significantly affected by marination length using evaporated roselle extract (P<0.05) but no significant (P>0.05) on water activity and total plate count. It was concluded that beef se'i produced through the marination process with different lengths of time (6, 12 and 18 hours) using evaporated rosel extract, showed characteristics of decreased pH, increased water content with high water activity and total plate count tended to decrease. The best marination length of this study was 12 hours using rosela extract.

KEYWORDS: beef se'i, evaporated roselle extract, marination length, microbiological quality

INTRODUCTION

One of the commonly recognised traditional meat products in East Nusa Tenggara province is se'i, which is processed through smoking techniques. Malelak et al. (2014) describe the process of making se'i starting from slicing the meat lengthwise in a cylindrical shape (*lalolak* = local designation), seasoned, marinated, and then smoked using kusambi wood (*Schleichera oleosa*) and during smoking, the surface of the meat is covered with kusambi leaves to get a distinctive flavour characteristic in se'i. Similarly, Djando and Beyleto (2018) stated that se'i is a processed meat product typical of Kupang City, East Nusa Tenggara Province (NTT) which is traditionally processed by curing then followed by smoking using kusambi wood (*Schleiechera oleosa*) as fuel. Hutasoit et al. (2013) reported that the shelf life of se'i is only 3-7 days, this is due to the chemical activity and microorganism contamination that occurs in se'i. One of the stages in the process is marination, which is the process of soaking meat in certain ingredients (marinade) before further processing. The ingredients used are flavourings, organic acids and enzymes that can improve the sensory quality of meat such as flavour, tenderness, juice impression (Nurwantoro et al., 2012), improve the physical properties of meat and extend the shelf life (Brooks, 2011).

A potential herbal material that contains organic acids and has not been widely applied is rosella (Hibiscus sabdarifa Linn). The presence of organic acids such as citric acid, ascorbic acid and pectin as well as polyphenols in the form of anthocyanins, phenolic acids and flavonoids in rosella petals as reported by Baba and Malik (2015) is expected to be a practical solution in an effort to improve the quality of se'i while extending its shelf life. Several rosela products have been utilised as functional food ingredients in meat processing such as freeze-dried rosela powder (Sipahelut and Rihi Kale, 2018) rosela flour (Sarmento et al., 2016; Sipahelut, 2021) and evaporated rosela extract (Ramli et al., 2024). The results of the study on the use of evaporated roselle extract with levels of 1, 3 and 5% in the manufacture of se'i gave a significant effect on cholesterol and antioxidant activity in goat se'i (Ramli et al., 2024). Further proving whether there is an effect on the reduction of germ numbers as an indicator of microbiological quality in order to predict the shelf life is important to be studied. Based on this premise, research was conducted to study the effect of using evaporated roselle extract in the marination process with different lengths of time on the microbiological aspects of beef se'i.

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MATERIAL AND METHODS

The research was conducted from January-February 2024, where the beef sei process was carried out at the Animal Products Technology Laboratory-Faculty of Animal Science, Marine and Fisheries, Nusa Cendana University and sample testing at the Chem-Mix Pratama Laboratory-Yogyakarta. The materials used consisted of 10 kg of lean fresh beef, table salt (NaCl), saltpeter (KNO₃), rosela flower extract (*Hibiscus sabdariffa* Linn), kesambi wood and kesambi leaves (*Schleichera oleosa*). equipment includes scales, electric scales, evaporators, refrigerators (refrigerators), blenders, baking sheets, measuring cups, plastic clips, vacuum plastic and smoking drums.

The completly randomized design (CRD) with 4 treatments 4 replicates was applied in this experiment. Those treatments were P_0 marination length of 12 hours without roselle extract; P_1 marination length 6 hours with 5% roselle extract; P_2 marination length 12 hours with 5% roselle extract, and P_3 marination length 18 hours with 5% roselle extract. The collected data were processed according to the variance analysis procedure using SPSS 23.

Research Procedure

The roselle petals were selected, cleaned, cut into pieces, weighed, blended with distilled water in a 1:1 ratio, then filtered. The filtrate was concentrated using a vacuum rotary evaporator until a thick extract was obtained due to solvent evaporation. Evaporation was carried out by putting 250ml filtrate in an erlenmeyer flask and then evaporated for \pm 6 hours to produce a thicker extract of 73 ml.

The preparation of se'i refers to the modified procedure of Sipahelut and Kale (2018), where fresh lean beef is cut lengthwise against the direction of the meat fibre (*lalolak*) with a thickness of 3cm. The meat was washed and drained for 30 minutes, then weighed to calculate the amount of salt and saltpeter (KNO₃) used as curing ingredients. The proportion of salt was set at 2% by weight of meat and saltpeter at 30 mg/kg, both ingredients were added and mixed until homogeneous. The meat was divided into 4 parts according to the number of treatments, added with roselle extract, put in a plastic clip and stored in a refrigerator at 4°C for the marination process, namely: 6, 12, and 18 hours. After the marination process, the meat was removed and smoked for 45 minutes at a temperature of $\pm 75^{\circ}$ C - 90°C. After smoking, the meat was removed, cooled and then laboratory testing included pH, moisture, water activity and total plate count.

Variables studied

- 1. pH Calculation of the pH value is carried out by using a pH meter (Kosim, 2015).
- 2. Moisture The calculation of moisture content refers to the procedure used by Utari et al (2024

$$Moisture = \frac{(a+b)-c}{b}x \ 100$$

Description: a = weight of porcelain cup after drying (g); b = sample weight (g); c = weight of cup + contents (g)

3. Water activity-Water activity testing on beef se'i was conducted by referring to the procedure used by Kusnadi et al. (2012). $aw = \frac{\rho}{2}$

 $aw = \frac{\rho}{\rho o}$

Description: ρ = water vapour pressure inside the food material' ρ 0: vapour pressure of pure water at the same temperature

4. Total plate count- Total bacteria in se'i samples were determined using the Total Plate Count method, following the National Standard Agency procedure (SNI 2908: 2013).

RESUL AND DISCUSSION

Data of pH, moisture, water activity and total plate count of beef se'i produced by different of marination length using evaporated rosselle extract are presented in Table 1.

Variables	Treatment				 P value
	P ₀	P ₁	P ₂	P ₃	- r value
pH	5.37±0.14 ^b	5.15±0.04 ^a	5.12±0.02ª	5.11±0.04 ^a	0.001
Moisture (%)	58.52±0.63ª	60.46 ± 0.60^{b}	61.02 ± 1.22^{b}	60.06 ± 0.86^{b}	0.009
Water activity	0.98 ± 0.01	0.99 ± 0.00	0.98 ± 0.02	0.98 ± 0.01	0.404
Total plate count (10^2 cfu/g)	$0.70{\pm}1.40$	$0.53{\pm}1.05$	0.00 ± 0.00	0.00 ± 0.00	0.581

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Notes: different superscripts on the same line indicate significant differences (P<0.05); $P_0 = 12$ -hour beef marination time without roselle extract; $P_1 = 6$ -hour beef marination time with roselle extract; $P_2 = 12$ -hour beef marination time with roselle extract; $P_3 = 18$ -hour beef marination time with roselle extract;

Effect of manination length on pH of Beef Se'i

The results of variance analysis showed that marination time using evaporated roselle extract had a significant effect (P<0.05) on the pH value of beef se'i. In Table 1, it can be seen that the pH of beef se'i decreased where the highest was in P₀ (control), at 5.37 and the lowest pH was in P₃ at 5.11. Duncan test results showed that the pH of beef se'i in the 12-hour marination treatment without roselle (P₀) was different from that using roselle for 6 hours (P₁), 12 hours (P₂) or 18 hours (P₃), while the three treatments using roselle showed no difference. This condition means that the use of evaporated roselle extract in the longer marination process provides an opportunity for more organic acids contained in roselle to be absorbed into the meat tissue, thus reducing pH. Mardiah et al. (2009) stated that roselle petals are a source of organic acids, which will penetrate the beef tissue, resulting in a decrease in the pH value of the beef. Gunanda et al. (2021) reported that the longer the marination process, the more acid diffuses into the meat tissue, resulting in a decrease in pH. Supporting this statement, Oktasari et al. (2020) stated that meat marinated in organic acids such as citric acid, malic acid, tartric acid, or lactic acid has a lower pH so that the quality becomes better.

The pH value obtained in this study (5.11 - 5.37) is lower than some previous studies with pH values ranging from 5.50-5.63 in beef se'i using lime extract (Malelak et al., 2015); 5.40 - 6.37 in beef se'i using blimbing wuluh (Zainal et al., 2021); 5.31 - 5.55 in beef se'i using freeze-dried rosela extract (Sipahelut et al., 2023). The low pH of se'i in the 6 -18 hours marination treatment using evaporated rosela extract indicates that the acidic atmosphere created in the se'i product can help inhibit the growth rate of microorganisms so that it is expected that the product will be safe and the shelf life can be extended.

Effect of manination length on Moisture Content of Beef Se'i

The results of variance showed that the length of marination had a significant effect (P<0.05) on the water content of beef se'i. Table 1 shows that there was an increase in the water content of se'i produced where the lowest was in the 12-hour marination treatment without roselle (P_0) at 58.52% and increased at 6 hours of marination and the highest at 12 hours (61.02%) then decreased again at 18 hours. Duncan's test results showed the water content of beef se'i in the treatment of 12 hours marination without roselle (P_0) was different from the marination treatment for 6 hours (P_1), 12 hours (P_2) and 18 hours (P_3) using roselle extract, while among the three treatments using roselle extract showed no difference. This means that the use of evaporated roselle extract in the longer marination process resulted in higher water content. This condition occurs because the longer the marination process opens the opportunity for more marinade solution to diffuse into the meat tissue. Salsabella (2022) stated that the length of marination time can increase the water content of beef. The same thing was also explained by Gamage et al. (2017) that the increase in water content occurs due to the penetration of the marinating solution into the material, where the penetration continues until an equilibrium condition is reached between the water content inside the material and outside the marinated material.

The moisture content of se'i obtained in this study (58.52 - 61.02%) indicates that specific handling is needed so that the product can avoid damage due to microorganism activity. This is quite reasonable considering that water is one of the factors supporting the growth of microorganisms besides pH, nutrients and oxygen. Hamida (2010) states that the water content in foodstuffs will affect the resistance of foodstuffs to microbial attack expressed by aw, which is the amount of free water that can be used by microorganisms for growth. Previous studies reported by Aoetpah et al. (2023) using different smoking methods produced se'i with water content ranging from 41.18 - 64.64% while Malelak (2010) stated that the water content of se'i ranged from 40 - 60%.

Effect of manination length on Water Activity (aw) of Beef Se'i

The results showed that the length of marination had no significant effect (P>0.05) on water activity. This means that the length of marination with and without using evaporated roselle extract showed that the water activity of beef se'i produced tended to be the same. This tendency occurs because the period or volume of meat used in this study is the same so that it has the same water holding capacity. According to Salsabella (2022) the marination process did not affect the water activity because the beef may have reached a saturation point in absorbing the liquid from the rosela extract, so that even though the marination time was increased, the water activity remained constant. Barbosa-Cánovas et al. (2020) stated that after the equilibrium condition between the moisture content inside and outside the material is reached, changes in water activity tend to be small.

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Water activity in foodstuffs affects the resistance of foodstuffs to the attack of microorganisms for growth. Foodstuffs that have a high a_w value generally spoil quick therefore water activity is a very important consideration in the food industry. The higher the aw in a foodstuff, the more likely microorganisms live in that environment. Buckle, et al. (2009), that different types of microorganisms require different amounts of water for growth. Bacteria generally grow and multiply only in media with high water activity (0.91), yeasts require even lower water activity values (0.87 2 0.91) and moulds even lower (0.80 2 0.87).

Previous studies reported water activity of 0.70 in beef se'i and 0.75 in buffalo se'i (Jahidin, 2016) which is lower than the results obtained from this study which ranged from 0.98 - 0.99. The higher water activity in this study illustrates that this condition will be favourable for bacteria and moulds to grow if the product is not handled properly.

Effect of manination length on TPC of Beef Se'i

The results of variance analysis showed that the length of marination had no significant effect (P>0.05) on the TPC (total plate count) of beef se'i. This means that beef se'i produced from the marination process with different lengths of time either using evaporated roselle extract or without roselle extract showed the same TPC. It is suspected that the presence of organic acids in roselle that are absorbed in the meat and form an acidic atmosphere with a pH of 5.11 - 5.37 (Table 1) can be an inhibitor for microorganisms (bacteria) to grow and develop freely. Agustiyani et al. (2004) stated that microorganisms generally like a neutral pH for optimal growth, but can grow at a more acidic pH. Most microorganisms stop growing at pH 5.0, and some microorganisms can grow up to pH 4.6 or even down to 4.

The decrease in TPC in this study is also suspected that the longer the marination process, the stronger the phytochemical compounds in rosela that act as antibacterials work to inhibit the growth rate of bacteria in se'i. Table 1 shows the highest TPC in 12 hours marination without roselle extract which is 0.7 x 102cfu/g and the lowest in marination for 12 - 18 hours using evaporated roselle extract, even able to eliminate bacteria with the result obtained is 0.00. Bohm (2009) stated that roselle petals contain phenol compounds such as anthocyanins and flavonoids that act as antimicrobials. Furthermore, Yani (2010) stated that the use of roselle petal solution can inhibit bacterial activity in foodstuffs and its use at a concentration of 10% can kill eschrichia coli bacteria, which is a common bacterial in meat.

CONCLUSIONS

It was concluded that beef se'i produced through the marination process with different lengths of time (6, 12 and 18 hours) using evaporated rosela extract, showed characteristics of decreased pH, increased water content with high water activity and total plate count tended to decrease. The best marination time in this study was 12 hours using rosela extract. Microbiologically, the beef se'i obtained had good quality after processing, but specific handling is needed if the product is to be stored considering the many factors that play a role in changes in quality such as pH, moisture content and water activity.

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