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Original Research Article

The Use of Freeze-dried Rosella Extract (*Hibiscus sabdarifa* Linn) as Food Additive in Making Se'i Beef

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Abstract: This study aims to examine the use of freeze-dried rosella extract in the processing of se'i beef as a food additive. The experimental design used was a completely randomized design (CRD) consisting of 5 treatment levels of freeze-dried rosella extract where: R0: without freeze-dried rosella (control); R1 = rosella 10 %; R2 = rosella 12 %; R3_rosella 14 % and R4 = rosella 16 %, and 3 replications for each treatment. The research material used was the *Biceps femoris* muscles of Bali cattle. Parameters observed were: L* value (brightness), pH, protein content and fat content. Data were analyzed using analysis of variance (ANOVA) using the SPSS program. The results showed that the higher the level of rosella extract application, the higher the brightness value and protein content which had a highly significant effect (P<0.01) on se'i beef as well as the pH value which had a significant effect (P<0.05) while the fat content decreased which was highly significant (P<0.01). In conclusion, application of roselle extract levels of 10 % -16 % to se'i beef increases the brightness value and protein content and reduces the fat content and pH value so that the resulting meat product is suitable for consumption. Freeze dried rosella products can be used as food additive.

Keyword: se'i beef, rosella extract, freeze drying method, physical and chemical characteristics.

INTRODUCTION

The use of saltpeter (KNO_3) as a food additive in the production of se'i beef (smoked beef) aims to produce attractive processed colors. Meat that is sliced lengthwise (*lalolak*) is seasoned with salt, spices and saltpeter, and curred for ± 12 hours and then smoked and grilled (Malelak *et al*., 2015). Nitrite (NO_2) as a result of saltpeter decomposition will react with meat myoglobin and finally form nitroso-hemochromogen during smoking and roasting as a stable bright red color giver to processed meat (Pearson and Gillett, 1996). Nitrite also functions to provide flavor, antimicrobial to prevent meat spoilage, and antioxidants to prevent fat breakdown (Pearson and Gillett, 1996; Honikel, 2010). The use of nitrites in meat processing also has an unfavorable effect, which results in the formation of nitrosamines (Hermann *et al*., 2014; Lu *et al*., 2023) which are carcinogenic (Colnemero *et al*., 2006). Cyntia *et al.* (2019) found that the nitrosamine content in 20 samples of commercially processed meat in the city of Medan Indonesia exceeded the maximum standard set by WHO, which was 10 µg/kg. Where 5 of the samples contained nitrosamines 501.290 to 4227.492 µg/kg.

Various food additives have been used to reduce the use of nitrites in meat processing, even to make processed meats that are nitrite-free to make the product healthier. Cooked-cured meat pigment (CCMP) and encapsulated CCMP (PCCMP) are used as dyes. Antioxidants and flavor givers include butylated hydroxy-anisole (BHA), tert-butylhydroquinone (TBHQ), sodium tripolyphosphate (STPP), and sodium ascorbate. Ascorbic acid (Vit-C) and its potassium salts, fumaric acid esters, lactic acid and its salt forms, nisin and other bacteriocins are used for the antimicrobial function (Pegg and Sahidi, 2006). Today's society realizes the importance of functional food, namely food as a provider of basic nutrients, also contains active compounds that are important for health. The use of food additives for enrichment and food processing is beneficial for making people live healthier (Aryee and Boye, 2015). Synthetic chemical compounds as additive pose a risk to consumer health, so the use of natural

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ingredients is a better alternative choice, because they have been widely used as herbs that provide health benefits (Campelo *et al*., 2019).

Anthocyanins as the main phenolic pigments have great potential to be used as natural color in the food industry (Konczak and Zhang, 2004; Aishah *et al*., 2013), where these pigments are responsible for red, purple and blue colors in flowers, fruits, vegetables. and cereal grains (Konczak and Zhang, 2004; Martin et al, 2017). Roselle calyx extract has a high anthocyanin content, also contains vitamin C and various other bioactive compounds (Aishah *et al*. 2013; Triyastuti and Anwar, 2022). Apart from being a food coloring agent, rosella flower extract has antioxidant and antimicrobial properties (Purbowati *et al*., 2019; Márquez-Rodríguez *et al*., 2020; Triyastuti and Anwar, 2022). In making beef jerky, rosella extract is effective as an antimicrobial to inhibit the growth of *Staphylococcus aureus* (Nesi *et al*., 2019). Rosella powder was also reported to have higher antioxidant properties than pink oyster mushroom powder (*Pleurotus djamor*) in the manufacture of beef paste (Bermudez *et al*., 2023). Rosella as herb has been shown to provide health benefits such as lowering blood pressure, reducing the risk of liver damage, lowering blood LDL, anti-diabetic, anti-cancer, anti-inflammatory, increasing stamina (Islam *et al*., 2016), and reducing body weight (Herranz -Lopez *et al*., 2018)

Anthocyanin compounds are very sensitive to high temperatures, so this factor needs to be considered to maintain the quality of anthocyanins (Triyastuti and Anwar, 2022). High temperature processing such as drying and roasting can damage bioactive compounds and reduce antioxidant capacity, while processes without heating (non-thermal) preserve natural bioactive compounds (Dauz and Chang, 2015). Freeze drying is a food drying technique that does not result in chemical changes such as starch gelatinization, sugar caramelization and protein denaturation. It is carried out below the freezing point through a sublimation process under vacuum pressure so as to produce a product whose surface is not wrinkled, more porous, lower density, easily refreshed, and maintains normal color, flavor quality and nutritional value (Hariyadi, 2013). Comparing traditional air drying (45^0C for 24 hours) and freeze drying (-23^0C) of purple carrots, Macura *et al*. (2019) reported anthocyanin loss of 37.80% in traditional drying compared to 12.08% in freeze drying.

This study was designed to examine the use of freeze-dried rosella extract as food additive in the manufacture of se'i beef, namely its effect on the physical and chemical properties of processed products.

MATERIALS AND METHODS

Research Materials

The material for se'i beef is Bali beef taken from thigh muscle (*Biceps femoris*), saltpeter ($NaNO_3$), table salt (NaCl), freeze dried rosella powder, distilled water, aluminum foil, plastic clips, label paper and tissue. Chemical testing materials consist of: protein content (concentrated H_2SO_4, 0.1 N HCl, H_3BO_3, green bromine cresol, 40% NaOH) and fat content (hexane, chloroform, petroleum benzene, ether) (AOAC, 2005). Esco brand freeze dryer, Hakasima brand blenders, and equipment used for physical quality evaluation are: Ohaus brand electric scales, Hanna brand digital pH meters, bimetal thermometers, blotting paper, beakers, electric ovens, water filters and kitchen equipment and stationery while chemical test equipment in the form of: porcelain cup, metal cup, erlenmeyer, measuring cup, soxhlet extract tool, Kjeldahl flask, oven, desiccator, fat extraction unit, desiccator, pipette burette, test tube, electric furnace and paper strain.

Research methods

Completely randomized design (CRD) was applied to the trial with 5 treatments and 3 replications. The treatments tried are as follows: R0 = without rosella application (control); R1 = application of rosella powder 10% (10 g/100 ml of solution); R2 = application of rosella powder 12% (12 g/100 ml solution); R3 = application of rosella powder 14% (14 g/100 ml of solution); R4 = application of rosella powder 16% (16 g/100 ml of solution). Roselle Extraction Rosella extraction using freeze drying method (freeze dryer). Rosella flower petals are cleaned, cut/sliced into small pieces to make it easier when blended and then frozen. Frozen rosella is mashed using a blender (Hakashima brand) by adding a little distilled water that has been cooled to get a thick rosella extract. Furthermore, the rosella extraction results are filtered using a water filter cloth, accommodated in a round clear (mica) container (where the cookies are dry) and frozen again before being put into a freeze dryer. The freeze drying process lasts for 24 – 48 hours which produces a powder product.

Se'i Beef Processing and Provision of Rosella Extract

Fresh beef is obtained from the Aldia meat depot (meat that has been trimmed of connective tissue and subcutaneous fat) in the city of Kupang. The meat used comes from the thigh muscle (*Bicep femoris*) as much as 15 kg. Slicing the meat is done lengthwise (lalolak) against the direction of the meat fiber with a thickness of □ 3 cm, then the meat is washed and set aside for 15 minutes. After that, the meat was weighed and divided into 5 equal parts (3 kg each) to determine the amount of salt (NaCl), saltpeter ($NaNO_3$) and rosella powder used. The salt given was 2% and saltpeter as much as 30 mg/kg of meat, as well as rosella powder was weighed according to the treatment respectively 10, 12, 14 and 16 grams. Rosella extract was prepared by means of freeze dried rosella powder 10g, 12g, 14g and 16g dissolved in distilled water until it reached a volume of 100 ml. Four parts of meat, 3 kg each, were treated with rosella by mixing it evenly with

100 ml of solution according to the level of treatment, then currying for 12 hours (overnight). Meat that has been marinated overnight is taken out 30 minutes before being smoked/roasted using a 32-wick Hock oven and stove and preheated oven. The smoking lasts for 45-60 minutes while being controlled by turning the meat so that all surfaces get the same heat and are cooked through. During smoking, the surface of the meat is covered with kusambi leaves to form the distinctive aroma of sei meat. The cooked se'i meat is removed and cooled, then placed in a plastic clip packaging. To test the sample parameters, they were immediately analyzed, namely measuring the pH value, while measuring the L* value (brightness), protein content and fat content was carried out the next day because the samples had to be shipped to analytical laboratory. Samples were vacuum packed, labeled according to treatments, then stored in a styrofoam box covered with dry ice. Before shipping it is stored in the refrigerator at 4^0C.

L* value (brightness)

Measurement of meat color using a Chroma Meter CR-200/CR-210 Minolta to measure the brightness of the meat. The L* value is the brightness value where: L: brightness from white (100) to black (0).

pH of Se'i Beef

Measurements of pH were carried out using a portable pH meter, Hanna brand. As much as 10g of se'i beef sample was thin sliced then crushed until smooth and put into a cup then added distilled water with a ratio of 1:1 (1g meat: 1ml distilled water). Insert the pH meter probe and the pH value will be read.

Protein Content (AOAC, 2005).

A sample of 0.25 grams was placed in a 100 ml Kjeldahl flask and 0.25 grams of the mixture was added (5g K_2SO_4; 0.25g $CuSO_4$; 0.1g selenium) and 3 ml concentrated H_2SO_4. Then do the destruction (heating in a boiling state) for 1 hour until the solution is clear. After cooling, 50 ml of distilled water and 20 ml of 40% NaOH were added, then distilled. The distillation results were collected in an Erlenmeyer flask containing a mixture of 10 ml of H_3BO_3 and 2 drops of pink green bromine cresol. After the volume of the reservoir (distillate) becomes 25 ml and has a bluish color, the distillation is stopped and the distillate is titrated with 0.02 N HCl until it is pink. The same treatment was also applied to blanks. With this method, the total nitrogen and protein content were calculated by the bellow formulae:

$$\% N = \frac{\text{ml HCl} \times \text{N HCl} \times 14.008}{\text{Gram material}} \times 100\%$$

Protein content = % N x Conversion Factor (6.25)

Fat Content (AOAC, 2005).

Dried the fat flask to be used in an oven at 105°C for 1 hour, and cooled in a desiccator for 15 minutes and weighed (W2). Samples of ± 2 grams se,i beef are mashed and then weighed (W1) and wrapped using filter paper formed by a sleeve (thimble). Assemble the extraction equipment from the heating mantle, fat flask, soxhlet to the condenser. The sample is then put into a soxhlet which is then added with sufficient hexane solvent for 1½ cycles. Extraction was carried out for ± 6 hours until the solvent descended again through the siphon into the clear colored fat flask. The results of the extraction from the fat flask are separated between the hexane and the extracted fat using a rotary evaporator (rpm 50, temperature 69°C). The fat that has been separated from the hexane is then heated in the oven at 105°C for 1 hour. The fat flask is cooled in a desiccator for 15 minutes and weighed (W3). Reheat into the oven for 1 hour, if the difference between the weighing results of the last extraction and the previous weighing has not reached 0.0002 gram.

Fat content is calculated by the formula:

$$\% \text{ fat} = \frac{W3 - W2}{W1} \times 100\%$$

W1 = Sample weight (g)
W2 = Weight of empty fat flask (g)
W3 = Weight of flask fat + extracted fat (g)

RESULTS AND DISCUSSION

Brightness (L* Value) of Se'i Beef

The results showed that the beef sei that received the food additive freeze-dried rosella extract treatment had a significantly brighter color ($P<0.01$) than the control as shown in Table 1. Treatment with higher levels of rosella extract resulted in higher L* values as well. and highest in treatment R4, and there was a difference ($P<0.01$) between each treatment. The addition of rosella extract increased the brightness of the color of se'i beef treated with saltpeter and the brightness value increased with increasing levels of rosella extract. This means that the anthocyanin in freeze-dried rosella extract is effective as a beef coloring agent. According to Macura *et al*. (2019) freeze drying produces a much higher anthocyanin content. Aishah *et al*. (2013) suggested that the anthocyanin of rosella calyx extract as a natural pigment has high stability for use as a food and beverage coloring.

Table 1: Average L* values (brightness) and pH of se'i beef

Parameter	Treatments				
	R0	R1	R2	R3	R4
L* values	43.3±4.84[a]	43.9±9.20[b]	44.3±3.27[c]	46.7±5.11[d]	46.9±0.83[e]
pH	5.43±0.23[ab]	5.55±0.13[b]	5.42±0.14[ab]	5.32±0.57[a]	5.31±0.29[a]

Note: Numbers with different superscript letter in the same row and column show highly significant differences (P<0.01)

The results of current study are in line with the results of previous studies. Parinyapatthanaboot and Pinsirodom (2011) reported that the use of dried rosella flower extract along with 4 kinds of sweeteners in the manufacture of Chinese-style sausage produced a product with a higher brightness (L* value) than the sucrose sweetener control without rosella extract. Valencia *et al*. (2008) using natural antioxidants in fresh pork sausages resulted in a higher brightness value (L*) than the control at baseline and after 3 days and 7 days of storage. Antimicrobial and natural antioxidant treatment of rosemary and cloves on fresh chicken meat, Zang *et al*. (2015) reported an increase in L* brightness values during storage for 15 days, while the BHT treatment and control experienced a decrease in L* values.

Several previous studies on the use of rosella flower powder, extracts and by-products of rosella flower extract (byproduct) gave results that were different from this study. Bozkurt and Belibagli (2009) reported that the use of rosella flower powder in making kavurma could not maintain the L* brightness value of meat where the initial processing value was L* 28.79 and the processing result for 50 hours was the L* value 24.52. Marquez-Rodriguez *et al*. (2020) sprayed dried rosella flower extract on the surface of beef slices and stored at 4 ± 10C for 15 days, resulting in a lower L* brightness value than the control. During storage, meat treated with rosella extract experienced a greater decrease in the brightness value of L*. Bermudez *et al*. (2013) added rosella flower extract byproduct flour in the manufacture of beef paste. Treatment of 2% byproduct flour resulted in an L* value of 39, and 5% byproduct flour had an L* value of 32 while the control had an L* value of 41.7.

According to Feiner (2006), an L* value describes the difference between white and black, where an L* value of zero means black while an L* value of 100 means white. Patrascu *et al*. (2013) suggested that an L* value above 50 means the color of the meat is lighter than dark. Images in the report by Bermudez *et al*. (2013) showed that pasta with an L* value of 41.7 was dark red, an L* value of 39 was dark brown red, and an L* value of 32 was dark brown. According to Bozkurt and Belibagli (2009), the change in the color of the meat is due to the Maillard reaction. Echavaria *et al*. (2012) stated that the Maillard reaction is a very complex non-enzymatic browning reaction between reducing sugars and amino acids where one of the end products is melanoidins which is brown in color. In this study, the anthocyanins in freeze-dried rosella extract were able to inhibit the Maillard reaction resulting in a higher L* value than the control.

Se'i Beef pH

In Table 1 it can be seen that the pH of beef se'i beef R4 and R3 was the lowest and then R2, R0 and R1 were the highest which was significantly different from the other treatments (P<0.01). The results showed that the results of the research treatments R2, R3 and R4 could maintain pH while R1 could not. This may be due to the high content of organic acids in rosella extract in treatments R2, R3 and R4 to maintain the pH of the meat. The results of this study are in line with Bozkurt and Belibagli (2009) who reported that the use of rosella flowers in kavurma resulted in a lower pH, although it was not significantly different from the control and BHT and rosemary treatments. It was also stated that the high content of acidic compounds in rosella flowers contributed to the production of kavurma with a pH of 5.86 compared to a pH of 6.35 for the control and other treatments. Da-Costa-Rhoca *et al*. (2014) stated that rosella flower extract contains many organic compounds which are grouped into 3 classes namely 1) four compound of organic acids; 2): two compound of anthocyanins; 3) thirty three compound of flavonoids and phenolic acids

The use of rosella in the manufacture of Chinese-style sausage by Parinyapatthanaboot and Pinsirodom (2011) showed that 4 sausages treated with rosella flower extract along with 4 kinds of sweeteners had a pH of 5.88, 5.89, 5.90, 5.97, respectively, while the control sausage with sucrose sweetener without rosella has a pH of 6.11. Ismail *et al*. (2022) used 3 kinds of natural sources of antioxidants in the manufacture of buffalo meat paste, namely rosella, wolfberry and beets. After cooking, the pH of the resulting paste was rosella 5.82, wolfberry 6.9, beet 5.97 and control 5.97. The use of natural antioxidants and antimicrobials of cloves, rosemary and a combination of both and synthetic BHT in fresh chicken meat was reported by Zang *et al.* (2015), where the initial pH of the control and all treatments was 5.65. After 15 days of storage, the final control pH and BHT increased to 6.66 and 6.37. Meanwhile, the final pH of the clove, rosemary, and clove-rosemary combination treatments were 5.62, 5.58 and 5.48, respectively.

Se'i Beef Protein Content

Table 2: Average protein and fat content of beef se'i beef

Parameter	Treatments				
	R0	R1	R2	R3	R4
Protein (%)	34.8±2.60a	36.7±0.97b	37.4±1.49b	38.0±1.84c	38.7±1.37c
Fat (%)	6.09±0.58c	4.83±0.88b	4.73±0.46b	4.75±0.93b	4.07±0.43a

Note: Numbers with different superscript letter in the same row and column show very significant differences (P<0.01).

In Table 2 it appears that the protein content of the se'i beef that received the freeze-dried rosella extract treatment was higher than the control (P<0.01). This protein content seemed to increase with increasing levels of rosella extract where the treatments R1, R2, R3 and R4 were different from R0 (P<0.01). From the sample treatment, freeze dried rosella extract levels, R4 and R3 were different from R2 and R1 (P<0.01). The increase in crude protein content can be caused by 2 factors. Firstly, it is caused by the contribution of the protein contained in rosella flower extract. According to Anel *et al*. (2016) the crude protein content of rosella flowers is 11.26%. Secondly, anthocyanins play a role in preventing protein breakdown during the process of making se'i beef, and during storage before analysis. This is because rosella flower extract has been shown to be a strong natural antioxidant (Ismail *et.al*., 2022), as well as a strong antimicrobial (Marquez-Rodriguez *et al*., 2020). The results of this study are in line with the results of Sarmento *et al*., (2016) where the addition of rosella extract powder can increase the protein content of se'i beef. However, the previous study reported a higher protein content in se'i beef for the control and the three levels of rosella administration, namely 35.13%, 44.90%, 45.35% and 47.59%, respectively.

Fat Content of Se'i Beef

The fat content of se'i beef in Table 2 show that application of freeze-dried rosella extract resulted in a decrease in fat content so that it was lower than the control (P<0.01). Treatment R4 with the highest level of rosella extract had the lowest fat content compared to R1, R2 and R3 (P<0.01), while the other levels of rosella extract showed no significant difference (P>0.05). Sarmento *et al*., (2016) reported that the fat content of se,i beef without rosella calyx extract treatment was 1.30%, whereas with 6%, 8% and 10% rosella treatment the fat content was 5.17%, 4.04% and 5.07%.

Maloney (2002) stated that the fat content of processed meat is determined by the fat content of the fresh meat and other ingredients used, as well as the loss of fat during cooking. The fat content of beef is influenced by nation, age, body condition and gender. At the same age and body weight, cattle with heavy muscles have a lower fat content, whereas those with light muscles have a higher fat content. The fat content of rosella flower calyx according to Anel *et al*. (2016) is 8.12%. Hassan *et al*. (2017) reported that the fat content of Bali beef in the thigh was 5.51 ± 1.72%. Malelak (2018) analyzed the fat content of *Biceps femoris* muscle in Bali beef with different body condition scores (BCS), where subcutaneous fat and connective tissue were removed. The fat content was BCS2 6.42±0.17%, BCS3 7.05±0.03% and BCS4 8.96±0.02. Beef that had been used in current study also the *Biceps femoris* muscle.

CONCLUSION

Based on the results of the study, it can be concluded that the use of freeze-dried rosella extract at different levels of 10 g%-16 g%% increased the brightness value and protein content as well as decreased the fat content and the pH value did not change. The use of rosella level 10 g% -16 g% can be applied to se'i meat. The best level that can be applied to increase protein levels and decrease levels is 16 g%. Freeze dried rosella products can be used as food additives (BTP).

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