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Effectiveness of the Red Dragon Fruit (*Hylocereus polyrhizus*) Peel Extract as the Colorant, Antioxidant, and Antimicrobial on Beef Sausage

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ABSTRACT

This study aimed to evaluate the effectiveness of red dragon fruit (*Hylocereus polyrhizus*) peel extracts addition on beef sausages. Red dragon fruit peel extracts were obtained by maceration using solvent at pH 5. Phytochemical characteristics, total phenols, antioxidant, and antimicrobial activity of the peel extracts were observed. Antioxidant and antimicrobial activities of the extracts were associated with high phytochemical compounds and total phenols contained in the extracts. Red dragon fruit peel extracts with various percentages (0%, 20%, 30%, and 40%) were added on beef sausages, and their physicochemical characteristics, nutrients, antioxidant activity, and microbiological profile were analyzed. The data were analyzed using analysis of variance and Duncan's multiple range test. Results showed that the addition of red dragon fruit peel extracts significantly reduced texture values, but increased intensity of luminosity, intensity of red color, and intensity of yellow color (P<0.05) beef sausages. It could be concluded that red dragon fruit peel extract containing phytochemical compounds was effective as an antibacterial agent and natural antioxidant. The addition of red dragon fruit peel extracts was effective in increasing the antioxidant activity and decreasing TBARS values. The addition of red dragon fruit peel extract did not affect the reddish colorization of beef sausages, but it was capable of increasing the yellowish colorization on beef sausage.

Keywords: antimicrobial, antioxidant, beef sausage, red dragon fruit peel extract

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi efektivitas penambahan ekstrak kulit buah naga merah (*Hylocereus polyrhizus*) pada sosis daging sapi. Ekstrak kulit buah naga merah dihasilkan dengan maserasi menggunakan pelarut pH 5, dan dilakukan pengamatan karakteristik fitokimia, total fenol, aktivitas antioksidan, dan antimikrob. Hasil analisis menunjukkan bahwa ekstrak kulit buah naga merah memiliki aktivitas antioksidan dan antimikrob alami karena memiliki kandungan senyawa fitokimia dan total fenol dalam ekstrak yang tinggi. Ekstrak kulit buah naga merah dengan persentase berbeda (0%, 20%, 30%, dan 40%) ditambahkan pada pembuatan sosis, dan dilakukan pengamatan karakteristik fisikokimia, zat gizi, aktivitas antioksidan, dan mikrobiologi. Data diolah dengan analisis ragam (ANOVA) dan dilanjutkan uji perbandingan berganda menggunakan uji Duncan. Hasil analisis menunjukkan bahwa penambahan ekstrak kulit buah naga merah menurunkan nilai tekstur, tetapi meningkatkan intensitas kecerahan, intensitas warna merah, dan intensitas warna kuning secara signifikan (P<0.05) sosis daging sapi. Dapat disimpulkan bahwa ekstrak kulit buah naga merah yang mengandung komponen fisikokimia, efektif sebagai antimikroba dan antioksidan alami. Penambahan ekstrak kulit buah naga merah mampu meningkatkan aktivitas antioksidan, dan menurunkan nilai *thiobarbituric reactive substance* (TBARS). Penambahan ekstrak kulit buah naga merah belum efektif meningkatkan intensitas warna merah sosis daging sapi, tetapi mampu meningkatkan warna kuning sosis daging sapi.

Kata kunci: *antimikrob, antioksidan, ekstrak kulit buah naga merah, sosis daging sapi.*

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INTRODUCTION

The use of food additives in sausage processing is to provide some advantages such as reddish colorization, antioxidant, and antibacterial activity. In order to control pathogenic bacteria, nitrite and nitrate salts are commonly used in processed meat. These additives may also contribute to produce the red color and antioxidant activity. However, their presence is also associated with formation of carcinogenic compound, nitrosamine. Nitric oxide (NO), formed by nitrites or nitrates, can react with secondary amines in meat to form nitrosamine under certain conditions (eg, high temperature) (Honikel, 2008). This carcinogenic compound is formed in acidic environments, especially in the gastrointestinal tract of human gut (Honikel, 2008). When consumed continuously and exceeds the maximum levels, it can cause health problems such as cancer. Thus, the use of nitrite in food products should be limited.

The use of nitrite or nitrate can be replaced with natural ingredients derived from plants source which extensively studied both function and composition. One of the candidates is dragon fruit peel which is not generally utilized. The dragon fruit peel is potential as a natural colorant (Harivaindaran *et al*., 2008), an antibacterial against nine bacteria of food pathogen (Nurmahani *et al*., 2012), and natural antioxidant (Luo *et al*., 2014). The addition of red dragon fruit peel extracts were expected to give better characteristics based on color, antioxidant, and antibacterial on beef sausage. This study aimed to analyze the effectiveness of red dragon fruit peel extracts (*Hylocereus polyrhizus*) as antibacterial agent and natural antioxidant, as well as to evaluate the effectiveness of the extracts on beef sausages. Other analyses such as physicochemical and nutrient analysis were conducted to obtain the supporting data.

MATERIALS AND METHODS

Experimental design

This experiment used a completely randomized design with 4 treatments of different levels of red dragon fruit peel extract addition (0%, 20%, 30%, and 40%) and three replications.

Procedure

Extraction of red dragon fruit peel. The red dragon fruit peel was extracted by modified maceration from Lourith & Kanlayavattanakul (2013) using distilled solvent at pH 5 adjusted by adding citric acid. Dragon fruit was cleaned and peeled manually. The peels were cut in small size (2 mm), dried at 50°C, and powdered. The powder was macerated with distilled water solvent (1:50) for 60 min and filtered. The solution was evaporated at vacuum evaporator at 60°C. The extract was stored at -20°C.

Sausage processing. The sausage was prepared by following the procedure conducted previously by Arief *et al*. (2014). The meat was added with 15% fat, 3.9% salt, 0.8% sodium tripolyphospate, and 20% ice and ground in a food processor for 30 s. Furthermore, the dough was added with 12% skim milk, 1% garlic powder, 0.5% white pepper powder, 0.5% ginger powder, 0.4% coriander, 0.2% nutmeg, 30% starch, and 40% ice. Percentage of each ingredient was calculated based on the amount of meat used in the dough. Different levels of red dragon fruit peel extracts (0%, 20%, 30%, and 40%, followed by reduction of ice percentage) were added into the dough. The dough was mixed for 90 s, and transferred into casing using a stuffer, then boiled at a temperature of 60-65°C for 60 min.

Analysis of Variables

Analysis of phytochemical components. The phytochemical component test was performed to determine the type of active compounds contained in the extract according to Baxter *et al*. (1998). The tested compounds were alkaloids, flavonoids, phenols hydroquinone, steroids, triterpenoids, tannins, and saponins.

Analysis of total phenolic content. Total phenol content of extracts was determined using spectrophotometric method according to Adnan *et al*. (2011). A total of 100 mL extracts was mixed with 7.9 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent (Sigma-Aldrich Co., USA). After 2 min, 1.5 mL of 7.5% sodium carbonate reagent was added and homogenized. Samples were measured using spectrophotometer at 765 nm (Genequant 1300, Sweden) after being incubated for 2 h. The content of total phenol was expressed as gallic acid equivalent.

Analysis of antioxidant activity. Analysis of antioxidant activity of extracts was conducted by measuring free radicals inhibition against 1,1-diphenyl, 2-picrylhydrazil (DPPH) according to Adnan *et al*. (2011). A total of 150 mL extract with different DPPH concentrations was mixed with 0.9 mL (25 mg L^{-1}) in methanol solution. The mixture was left for 20 min and the absorbance was measured in spectrophotometer with a wavelength of 517 nm. Pure methanol was used as a control. The control was prepared with 100 mL of methanol and dissolved with DPPH solution. Absorbances of samples were measured in a spectrophotometer with a wavelength of 517 nm (Genequant 1300, Sweden). The antioxidant activity was expressed as a percentage of free-radical inhibition which calculated based on the following formula:

% Inhibition = (A control - A sample) × 100 / A control

The percentage of inhibition indicates the antioxidant activity level. Vitamin C (Merck, KgaA, Germany) was used as a standard.

Analysis of antimicrobial activity. Analysis of antimicrobial activity of extracts was performed by the well diffusion method to determine the pattern of inhibition of the extract against pathogenic bacteria according to Rohin *et al*. (2012). Bacterial culture was inoculated in NaCl 0.85% to obtain the bacteria concentration of 10^8 cfu mL^{-1} (compared with 0.5 Mc. Farland standard solution).

Bacterial culture was diluted to obtain a culture concentration of 10^6 cfu mL^{-1}. The other culture was grown in Mueller-Hinton Agar medium (Difco™, USA) and provided with holes as well as a predetermined diameter. The extracts were inserted into the well and covered with filter paper. Grail was stored in the refrigerator for 2-3 h, followed by incubation at 37°C for 24 h and 48 h. The antimicrobial activity was characterized by the formation of clear zones around the wells and measured for its diameter (mm).

Nutritional composition. The proximate analysis of sausages was performed using AOAC (2005) standard, including moisture, protein, fat, and ash contents. Carbohydrate content was determined by difference from the proximate analysis.

Water activity and pH measurement. The pH value of the sausages was measured using pH meter (Hanna HI 99163, HANNA Instruments, USA). Water activity of sausages was measured using a_w meter (Novasiana, Switzerland).

Color measurement. The color of sausages was measured using a Minolta Chroma meter CR 300 (Minolta Co., Ltd. Osaka, Japan). The sample was measured on three different surfaces. L, a, and b values refer to the intensity of luminosity, the intensity of red color, and the intensity of yellow color. A and b values were used to determine °HUE of samples, with the following formula.

°HUE = arc tg (a/b)

Texture measurement. Textural analysis of sausages was performed using texture analyzer Steven-LFRA. The sausages at 2.54 cm in diameter were selected prior to the tests. Texture measurement procedure was carried out in accordance with the manufacturer's instructions.

Emulsion stability. Emulsion stability of sausages was measured by measuring the volume of oil and water emulsion according to Zorba *et al*. (1993). The sample was heated in water bath at 80 °C for 30 min. The samples were centrifuged at 2000 rpm for 15 minutes. The volumes of oil and water were measured to determine the emulsion stability (ES) of samples, with the following formula.

ES_1 (%)= water volume (mL) × 10

ES_2 (%)= oil volume (mL) × d × 10

ES (%)= 100 - (ES1 + ES2)

where: d= density of fat (g/mL) and ES= emulsion stability

TBARS analysis. TBARS (thiobarbituric acid reactive substance) analysis of sausages was conducted with distillation method as reported previously by Sorensen & Jorgensen (1996) with few modifications. Sample (10 g) was blended with 50 mL distilled water and added with propylgallate (PG) and ethylene diamine tetraacetic acid (EDTA) from Sigma (Sigma-Aldrich Co., USA). The homogenization of the sample was added with 2.5 mL HCl 4 N and a few drops of Dow antifoam B (Sigma-Aldrich Co., USA). The mixture was distilled and 50 mL of distillate was collected. The distillate (5 mL) was added with 0.02 M 2-thiobarbituric acid (TBA) from Sigma (Sigma-Aldrich Co., USA) and incubated at 100°C for 40 min. The absorbance was measured in a spectrophotometer at wavelength 532 nm (Genequant 1300, Sweden). The 1,1,3,3-tetraethoxypropane (TEP) (Sigma-Aldrich Co., USA) was used as a standard.

Antioxidant activity. Sausage samples were extracted to obtain a supernatant prior to the determination of antioxidant activity based on the procedure performed previously by Tangkanakul *et al*. (2009). The sausage was extracted with absolute methanol at room temperature with ratio of 1:5. The filter paper was used to separate the supernatant. The supernatant was stored in sealed bottles and stored at -20°C. Antioxidant activity was determined using the same procedure with Adnan *et al*. (2011).

Microbiological analysis. The procedure of microbiological analysis was performed according to FDA (1998). Sausage (25 g) was mixed with 225 mL of buffered peptone water (BPW), and homogenized for 1-2 min to obtain a 10^{-1} solution. Then, 1 mL suspension was transferred with a sterile pipette to 9 mL of BPW solution to obtain a 10^{-2} dilution. The dilution with the same ways was continued until 10^{-4} dilution was obtained. Microbiological analysis consisted of total plate count, *Escherichia coli*, *Salmonella* sp., and *Staphylococcus aureus* analysis.

Statistical Analysis

Data were analyzed using the General Linear Model of Statistical Analysis System's Procedures (SAS Institute Inc., Cary, NC, USA, 2002) and the Duncan's multiple range test was conducted as further analysis. The addition of red dragon fruit peel extracts was set as treatment. Significance range was set at 0.05.

RESULTS

Characteristics of Red Dragon Fruit Peel Extracts

The results indicated that flavonoids, phenols hydroquinone, steroids, triterpenoids, saponin, and tannins were the type of phytochemical compounds contained in red dragon fruit peel extract (Table 1). The antioxidant test was indicated by the phenolic, DPPH scavenging activity, and antioxidant capacity (Table 2). The inhibition zone produced by red dragon fruit peel extracts showed the antibacterial activity (Table 3).

Characteristics of Beef Sausages with Red Dragon Fruit Peel Extract Addition

Nutritional composition (water content, ash content, protein content, fat content, and carbohydrate content) of beef sausage were presented in Table 4. The results revealed that water content, ash content, fat content, and carbohydrate content of sausage were unaltered by incorporation of red dragon fruit peel extracts.

Table 1. Qualitative test of phytochemical compounds in dragon fruit peel extract

Phytochemical compounds	Result
Phenol hydroquinone	++
Flavonoids	++
Triterpenoids	++
Steroids	++
Saponin	++
Tannin	+
Alkaloid	-

Note: +/- indicates the existence of substances in the extract.

Table 2. Total phenolic, DPPH scavenging activity and antioxidant capacity of red dragon fruit peel extracts

Variables	Value
Total phenolic (mg EAG/100 g)	31.12 ± 1.56
DPPH scavenging activity (%)	51.35 ± 0.87
Antioxidant capacity (mg VCE/100 g)	321.78 ± 6.29

However, we found that the treatments affected protein content of beef sausage. Physicochemical characteristics (pH value, water activity, emulsion stability, texture, and color intensity) of beef sausages were presented in Table 5. The data indicated that pH value, water activity, emulsion stability, red color stability, and °HUE were unaffected by incorporation of red dragon fruit peel extracts, but the treatments significantly influenced texture, lightness intensity, and yellowness degree of beef sausage. The result of antioxidant activity and malondialdehyde (MDA) content in beef sausages was presented in Table 6. Significant effects of red dragon fruit peel extracts addition were observed in antioxidant capacity, DPPH scavenging activity, as well as TBARS value. Microbial evaluation including total plate count and some specific bacteria such as *E. coli*, *Salmonella* sp., and *S. aureus* was outlined in Table 7. Different levels of red dragon fruit peel extract unaltered the total plate count on beef sausages.

Table 3. Antibacterial activity of red dragon fruit peel extract

Test bacteria	Diameter of inhibition zone (mm)
Staphylococcus aureus ATCC 25923	12.38 ± 2.36^{a}
Bacillus cereus	8.11 ± 2.85^{b}
Pseudomonas aeruginosa ATCC 27853	10.09 ± 0.96^{ab}
Salmonella enterica ser. Typhimurium ATCC 14028	8.25 ± 1.37^{b}
Escherichia coli ATCC 25922	7.70 ± 2.39^{b}

Note: Means in the same column with different superscripts differ significantly (P<0.05).

Table 4. Nutrient composition of beef sausages with red dragon fruit peel extracts addition

Variables	Treatments				Standard (BSN 1995)
	0% extract	20% extract	30% extract	40% extract	
Water content (%bb)	61.67 ± 1.66	61.05 ± 2.31	61.64 ± 0.80	62.29 ± 0.60	Max. 67
Ash content (%bb)	3.49 ± 0.09	3.46 ± 0.45	3.38 ± 0.15	3.72 ± 0.47	Max. 3
Protein content (%bb)	11.32 ± 0.57^{a}	11.29 ± 0.79^{a}	10.93 ± 0.62^{ab}	10.46 ± 0.81^{b}	Min. 13
Fat content (%bb)	2.77 ± 1.11	2.14 ± 0.11	3.18 ± 0.62	2.51 ± 0.61	Max. 25
Carbohydrates (%bb)	20.51 ± 0.19	21.47 ± 1.44	19.82 ± 1.77	20.51 ± 0.19	Max. 8

Note: Means in the same row with different superscripts differ significantly (P<0.05).

Table 5. Physical characteristics of beef sausages with red dragon fruit peel extracts addition

Variables	Treatments			
	0% extract	20% extract	30% extract	40% extract
pH	5.80 ± 0.14	5.79 ± 0.12	5.74 ± 0.27	5.72 ± 0.24
aw	0.90 ± 0.01	0.90 ± 0.00	0.90 ± 0.01	0.89 ± 0.01
Emulsion stability (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Texture (kg/cm^2)	3.45 ± 0.47^{a}	3.23 ± 0.79^{ab}	2.95 ± 0.68^{b}	2.89 ± 0.49^{b}
Color				
Intensity of luminousity (L*)	40.69 ± 1.79^{ab}	39.49 ± 3.63^{b}	41.62 ± 3.05^{a}	42.60 ± 3.90^{a}
Intensity of red color (a*)	4.46 ± 0.44	4.78 ± 0.35	6.79 ± 3.46	6.82 ± 3.73
Intensity of yellow color (b*)	8.77 ± 1.09^{c}	9.39 ± 1.60^{bc}	10.50 ± 2.07^{ab}	11.16 ± 2.65^{a}
°HUE	62.85 ± 5.02	62.54 ± 5.17	58.83 ± 7.40	59.99 ± 7.59

Note: Means in the same row with different superscripts differ significantly (P<0.05). L* value (+) bright, value (-) dark, a* value (+) red, value (-) green, b* values (+) yellow, value (-) blue.

Table 6. DPPH scavenging activity, antioxidant capacity, and TBARS values of beef sausages with red dragon fruit peel extract addition

Treatments	DPPH scavenging activity (%)	Antioxidant capacity (VCE mg/100 g DM Sausage)	TBARS value (mg/kg DM sausage)
0% extract	49.71 ± 0.61[d]	108.77 ± 1.57[d]	1.28 ± 0.04
20% extract	54.76 ± 1.27[c]	123.67 ± 3.31[c]	1.23 ± 0.53
30% extract	60.89 ± 1.08[b]	137.49 ± 2.77[b]	0.82 ± 0.10
40% extract	72.94 ± 0.77[a]	165.50 ± 1.93[a]	0.77 ± 0.05

Note: Means in the same column with different superscripts differ very significantly (P<0.01).

Table 7. Microbiological analysis of beef sausages with red dragon fruit peel extracts addition (log cfu/g)

Variables	Treatments			
	0% extract	20% extract	30% extract	40% extract
Total plate count	2.94 ± 0.22	2.73 ± 0.53	2.65 ± 0.20	2.56 ± 0.14
Escherichia coli	Negative	Negative	Negative	Negative
Salmonella sp.	Negative	Negative	Negative	Negative
Staphylococcus aureus	Negative	Negative	Negative	Negative

DISCUSSION

Characteristics of Red Dragon Fruit Peel Extracts

Flavonoids, phenols hydroquinone, steroids, triterpenoids, saponin, and tannins were the type of phytochemical compounds contained in red dragon fruit peel extract. Flavonoids and phenols hydroquinone in red dragon fruit extract had the correlation with antioxidant activity. Flavonoids (Nurliyana *et al*., 2010) and phenols (Wu *et al*., 2006) found in dragon fruit peel extract were responsible for antioxidant activity in peel extracts. Steroids and triterpenoids compounds in the red dragon fruit peel had the correlation with antibacterial activity. Amalia *et al*. (2015) also found similar compounds in the screening, which were suspected to have antibacterial activity through reaction with cell wall proteins.

Phytochemicals qualitative test in red dragon fruit peel extract showed positive results in saponins. These results were in contrast to the finding of Amalia *et al*. (2015) using n-hexane as the solvent. This difference could be due to differences in solvents, where polar solvents (e.g water and ethanol) may have more soluble property in comparison with non-polar solvents (e.g n-hexane) (Baxter *et al*., 1998). Red dragon fruit peel extract also contained tannin, while the alkaloids were undetected.

The total phenolic of red dragon fruit peel extract was higher than the results of Nurliyana *et al*. (2010). Differences in solvents for extraction process may account for these different results. This study employed the distilled water, a solvent that is recommended for the food industry because of the extraction contains no residue that makes it is safe for consumption (Kumar *et al*., 2015).

The antioxidant activity of the red dragon fruit peel extract was identified by DPPH scavenging activity and antioxidant capacity. The DPPH scavenging activity (50.14%-52.15%) is not much different from those reported by Fidrianny *et al*. (2014) using n-hexane, ethyl acetate, and ethanol. Antioxidant capacity obtained is 321.78±6.29 mg VCE/100 g. Lourith & Kanlayavattanakul (2013) stated that the red dragon fruit peel extract with solvent water had better antioxidant activity than using ethanol. Harivaindaran *et al*. (2008) suggested that the DPPH scavenging activity and antioxidant capacity was proportional to the total phenol antioxidants in red dragon fruit peel.

The inhibition zone produced by red dragon fruit peel extracts showed the antibacterial activity (Table 3). Gram-positive bacteria, *S. aureus* ATCC 25923, was more sensitive to the antibacterial activity of the red dragon fruit peel extract. High inhibition of Gram-negative bacteria, *P. aeruginosa* ATCC 27853, by red dragon fruit peel extract resulted from antibacterial compounds content such as phenolic compounds. This assumption was supported by Arief *et al*. (2015) which stated that the Gram-positive bacteria were more susceptible to antibacterial activity due to the absence of a lipoprotein wall that capable of preventing antimicrobial compounds.

The bacterial inhibition is also observed in the pathogenic bacteria *S. enterica* ser. Typhimurium ATCC 14 028, according to research conducted by Nurmahani *et al*. (2012) which demonstrates the inhibition of pathogenic bacteria *S. thypii*. Phenolic compounds in the extracts of pomegranate peel studied by Choi *et al*. (2011) were able to inhibit the growth of *Salmonella*. The *B. cereus* and *E. coli* ATCC 25 922 was also inhibited by red dragon fruit peel extracts. Antibacterial activity against both bacteria was also observed by Tahera *et al*. (2014).

Characteristics of Beef Sausages with Red Dragon Fruit Peel Extract Addition

The water content and fat content of each beef sausage were in accordance with the established standard,

but ash and carbohydrate levels exceeded the standards of meat sausage set by BSN (1995). The protein content of beef sausage did not meet the minimum standards set by BSN (SNI 01-3820:1995), as influenced by the low levels of non-meat protein content used in the sausages (Nurul *et al*., 2010).

A higher level of red dragon fruit peel extracts addition was responsible for the lower pH value of beef sausage. The change in pH was in the range of 5.72-5.80, which could be caused by the same pH of distilled water used to dissolve the extract. The solvent was obtained by the addition of citric acid in distilled water according to Harivaindaran *et al*. (2008). The water activity of this sausage is due to the percentage of the addition of dragon fruit peel extract in sausages followed by the reduction of ice on the dough. Emulsion stability of a product is influenced by the degree of meat tenderness (Aminlari *et al*., 2009). The meat used in the study came from the same part i.e., topside.

A higher level of extract addition in the sausage associated with a reduction of its hardness, which could be linked with moisture and protein contents of sausages. The higher protein content resulted in higher harsh texture (Youssef & Barbut, 2010). Youssef & Barbut (2010) showed that the higher addition of protein (8% to 14%) increased the value of hardness because protein formed a denser complex.

HUE values of sausages in this study were 58.83-62.85, thus producing a yellowish red color of the product (ºHUE = 54º-90º). Totosaus (2009) stated that sausages with the addition of natural colorant resulted in the value of ºHUE which almost the same with sausage using nitrate and nitrite. Sausage with the extract addition had higher red intensity value than those without addition. This is a noticeable result, that addition of red dragon fruit peel extract could produce red intensity on the sausage due to the presence of natural pigments contained in dragon fruit peel. Jamilah *et al*. (2011) reported that red dragon fruit peel contained betacyanin pigment which contributes to a natural red dye. Betacyanins pigment is the extraction product of betalains compound (Harivaindaran *et al*., 2008). This pigment is capable of holding the red color in acid condition with the range of pH from 4 to 7.

However, the intensity of red color on sausages with the extract addition was quite low. Stability of the color pigment betacyanin may decrease as a result of the rising temperature and long enough heating process. Faridah *et al*. (2015) stated the stability of betacyanin pigmentin dragon fruit peel decreased along with the increased heating temperature (70°C and 100°C). Harivaindaran *et al*. (2008) stated that the red dragon fruit peel had the highest color stability by heating at 100°C for 5 min and would affect the colors produced when the heating carried out was long enough. The intensity of the yellow color in this study increased with the increasing level of the extract used. Beef sausages with the addition of red dragon peel extract as much as 40% have the highest value of yellow color. According to Woo *et al*. (2011), besides produces natural red color, betalain pigment also contains betaxantine which capable of producing natural yellow color. In addition, the intensity of yellow color in sausages is higher compared to red color. This difference is due to the quite long heating time conducted in the processing which can affect betasianin pigment content. Herbach *et al*. (2004) stated that the effect of heating time with high temperature led to the degradation of betacyanin and production of yellow pigment.

The antioxidant activity increased along with the increased level of dragon fruit peel extracts addition. Nurliyana *et al*. (2010) found that the higher concentration of red dragon fruit peel extract resulted in a higher DPPH scavenging activity. The value of antioxidant activity found in the analysis was high. This result due to the polyphenolic compounds contained in the red dragon fruit peel extract (Harivaindaran *et al*., 2008) and the addition of spices of sausage as garlic, coriander, and pepper which have antioxidant activities (Suryati *et al*., 2014).

Sausage with the addition of red dragon fruit peel extracts had smaller TBARS values than the sausage without the extract addition. This value was associated with the high antioxidant activity correlated with the phenolic in sausages, thus it could inhibit the oxidation of sausage (Wu *et al*., 2006). Phenolic compounds donated a hydrogen atom to the free radicals that enhanced the stability of radical phenolic derivative (Jongberg *et al*., 2013).

The addition of the red dragon fruit peel extract could not decrease the total plate count of the sausages (Table 7), eventhough the red dragon fruit peel extract could inhibit the pathogenic bacteria by *in vitro* methods (Table 3). The total plate count of all sausages were in 2 log 10 cfu/g. It was still in Indonesian National Standart for sausage /01-3820:1995 (BSN, 1995). Bacteria *Escherichia coli*, *Staphylococcus aureus,* and *Salmonella* sp. were not found in the results of microbiological analysis of beef sausage with the addition of red dragon fruit peel extract. The absence of *E. coli* on the sausage was caused by the heat treatment. The acceptable condition for *E. coli* bacteria and *S. aureus* growth was at 7-50°C and 7-48°C, respectively, but their optimum growth was at 37ºC (Adams & Moss, 2008). *Salmonella* sp. and *S. aureus* also were not found in the results of microbiological analysis of meat used in this study. *Salmonella* sp. was unable to grow at high temperature such as heating at 65-74ºC (Jay, 2012).

CONCLUSION

Red dragon fruit peel extract (*Hylocereus polyrhizus*) containing phytochemical compounds was effective as an antibacterial agent and natural antioxidant. The addition of red dragon fruit peel extracts on beef sausages effectively increased antioxidant activity and lowered TBARS values. Microbiological quality of the sausages met in Indonesian National Standard for sausage /SNI 01-3820:1995. Red dragon fruit peel extract at a concentration of up to 40% was ineffective in increasing the reddish intensity of beef sausages, but it was capable of improving the yellow color in beef sausage.

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REFERENCES

Adams, M. R. & M. O. Moss. 2008. Food Microbiology. 3rd ed. RSC Publising, Cambridge, UK.

Adnan, L., A. Osman, & A. A. Hamid. 2011. Antioxidant activity of different extracts of red pitaya (*Hylocereus polyrhizus*) seed. Int. J. Food Prop. 14: 1171-1181. https://doi.org/10.1080/10942911003592787

Amalia, S., S. Wahdaningsih, & E. K. Untari. 2015. Antibacterial activity testing of n-hexane fraction of red dragon (*Hylocereus polyrhizus* Britton & Rose) fruit peel on *Staphylococcus aureus* ATCC 25923. Trad. Med. J. 19: 89-94.

Aminlari, M., S. S. Shekarforoush, H. R. Gheisari, & L. Golestan. 2009. Effect of actinidin on the protein solubility, water holding capacity, texture, electrophoretic pattern of beef, and on the quality attributes of a sausage product. J. Food Sci. 74: 221-226. https://doi.org/10.1111/j.1750-3841.2009.01087.x

AOAC. 2005. Official Methods of Analysis of AOAC International. 18th ed. Assoc. Off. Anal. Chem., Arlington.

Arief, I. I., C. Budiman, B. S. L. Jenie, E. Andreas, & A. Yuneni. 2015. Plantaricin IIA-1A5 from *Lactobacillus plantarum* IIA-1A5 displays bactericidal activity against *Staphylococcus aureus*. Beneficial Microb. 6: 603-613. https://doi.org/10.3920/BM2014.0064

Arief, I. I., T. Suryati, D. Afiyah, & D. Wardhani. 2014. Physicochemical and organoleptic of beef sausages with teak leaf extract (*Tectona grandis*) addition as preservative and natural dye. Int. Food Res. J. 21: 2033-2042.

Baxter, H., J. B. Harborne, & G. P. Moss. 1998. Phytochemical Dictionary: A Handbook of Bioactive Compounds from Plants. CRC Press, London, UK.

BSN. 1995. Standar Nasional Indonesia: Sosis Daging (SNI 01-3820:1995). Badan Standardisasi Nasional, Jakarta.

Choi, J. G., O. H. Kang, Y. S. Lee, H. S. Chae, Y. C. Oh, O. O. Brice, M. S. Kim, D. H. Sohn, H. S. Kim, H. Park, D. W. Shin, J. R. Rho, & D. Y. Kwon. 2011. In vitro and in vivo antibacterial activity of punica granatum peel ethanol extract against Salmonella. Evid. Based Comp. Alternat. Med. 2011: 1-8. https://doi.org/10.1093/ecam/nen085

Faridah, A., R. Holinesti, & D. Syukri. 2015. Betalains from red pitaya peel (*Hylocereus polyrhizus*): extraction, spectrophotometric and HPLC-DAD identification, bioactivity and toxicity screening. Pakistan J. Nutr. 14: 976-982. https://doi.org/10.3923/pjn.2015.976.982

FDA. 1998. Bacteriological Analytical Manual. 8th ed. Assoc. Off. Anal. Chem., Arlington.

Fidrianny, I., M. Harnovi, & M. Insanu. 2014. Evaluation of antioxidant activities from various extracts of sweet orange peels using DPPH, FRAP assays and correlation with phenolic, flavonoid, carotenoid content. Asian J. Pharmaceutical & Clinical Res. 7: 186-190.

Harivaindaran, K., O. Rebecca, & S. Chandran. 2008. Study of optimal temperature, pH and stability of dragon fruit (*Hylocereus polyrhizus*). Pakistan J. Biol. Sci. 11: 2259-2263. https://doi.org/10.3923/pjbs.2008.2259.2263

Herbach, K., F. Stintzing, & R. Carle. 2004. Impact of thermal treatment on color and pigment pattern of red beet (*Beta vulgaris* L.) preparations. J. Food Sci. 69: 491-498. https://doi.org/10.1111/j.1365-2621.2004.tb10994.x

Honikel, K. O. 2008. The use and control of nitrate and nitrite for the processing of meat products. Meat Sci. 78: 68-76. https://doi.org/10.1016/j.meatsci.2007.05.030

Jamilah, B., C. E. Shu, M. A. Kharidah, & A. Noranizan. 2011. Physico-chemical characteristics of red pitaya (*Hylocereus polyrhizus*) peel. Int. Food Res. J. 18: 279-286.

Jay, J. M. 2012. Modern Food Microbiology. 5th ed. Aspen Publisher Inc. Gaithersburg, Maryland.

Jongberg, S., M. A. Torngren, A. Gunvig, L. H. Skibsted, & M. N. Lund. 2013. Effect of green tea or rosemary extract on protein oxidation in Bologna type sausages prepared from oxidatively stressed pork. Meat Sci. 93: 538-546. https://doi.org/10.1016/j.meatsci.2012.11.005

Kumar, Y., D. N. Yadav, T. Ahmad, & K. Narsaiah. 2015. Recent trends in the use of natural antioxidants for meat and meat products. Compr. Rev. Food Sci. & Food Safety 14: 796-812. https://doi.org/10.1111/1541-4337.12156

Lourith, N. & M. Kanlayavattanakul. 2013. Antioxidant and stability of dragon fruit peel colour. Agro. Food Ind. Hi-Tech 24: 56-58.

Luo, H., Y. Cai, Z. Peng, T. Liu, & S. Yang. 2014. Chemical composition and in vitro evaluation of the cytotoxic and antioxidant activities of supercritical carbon dioxide extracts of pitaya (dragon fruit) peel. Chem. Central J. 8: 1-7. https://doi.org/10.1186/1752-153X-8-1

Nurliyana, R., I. Syed Osman, I. Syed Zahir, K. Mustapha Suleiman, M. R. Aisyah, & K. Kamarul Rahim. 2010. Antioxidant study of pulps and peels of dragon fruits: a comparative study. Int. Food Res. J. 17: 367-375.

Nurmahani, M. M., A. Osman, A. A. Hamid, F. M. Ghazali, & M. P. Dek. 2012. Antibacterial property of *Hylocereus polyrhizus* and *Hylocereus undatus* peel extracts. Int. Food Res. J. 19: 77-84.

Nurul, H., T. Alistair, H. Lim, & I. Noryati. 2010. Quality characteristics of Malaysian commercial beef frankfurters. Int. Food Res. J 17: 469-476.

Rohin, M. A. K., A. Bakar, C. Abdullah, & A. M. Ali. 2012. Antibacterial activity of flesh and peel methanol fractions of red pitaya, white pitaya and papaya on selected food microorganisms. Int. J. Pharmacy & Pharmaceutical Sci. 4: 185-190.

Sorensen, G. & S. S. Jorgensen. 1996. A critical examination of some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products. Zeitschrift fur Lebensmittel-Untersuchung und Forschung. 202:205-210.

Suryati, T., M. Astawan, H. N. Lioe, T. Wresdiyati, & S. Usmiati. 2014. Nitrite residue and malonaldehyde reduction in dendeng-Indonesian dried meat-influenced by spices, curing methods and precooking preparation. Meat Sci. 96: 1403-1408. https://doi.org/10.1016/j.meatsci.2013.11.023

Tahera, J., F. Feroz, J. D. Senjuti, K. K. Das, & R. Noor. 2014. Demonstration of anti-bacterial activity of commonly available fruit extracts in Dhaka, Bangladesh. American J. Microb. Res. 2: 68-73. https://doi.org/10.12691/ajmr-2-2-5

Tangkanakul, P., P. Auttaviboonkul, B. Niyomwit, N. Lowvitoon, P. Charoenthamawat, & G. Trakoontivakorn. 2009. Antioxidant capacity, total phenolic content and nutritional composition of Asian foods after thermal processing. Int. Food Res. J. 16: 571-580.

Totosaus, A. 2009. Handbook of Processed Meats and Poultry Analysis: Colorants. L. M. L. Nollet, & F. Toldra (Eds). CRC Press, London, UK.

Woo, K. K., F. H. Ngou, L. S. Ngo, W. K. Soong, & P. Y. Tang. 2011. Stability of betalain pigment from red dragon fruit (Hylocereus polyrhyzus). Am. J. Food Technol. 6:140-148.

Wu, L. C., H. W. Hsu, Y. C. Chen, C. C. Chiu, Y. I. Lin, & J. A. A. Ho. 2006. Antioxidant and antiproliferative activities of red pitaya. Food Chem. 95: 319-327. https://doi.org/10.1016/j.foodchem.2005.01.002

Youssef, M. K. & S. Barbut. 2010. Physicochemical effects of the lipid phase and protein level on meat emulsion stability, texture, and microstructure. J. Food Sci. 75: 108-114. https://doi.org/10.1111/j.1750-3841.2009.01475.x

Zobra, O., H. Y. Gokalp, H. Yetim, & H. W. Ockerman. 1993. Model system evaluations of the effects of different levels of K_2HPO_4, NaCl and oil temperature on emulsion stability and viscosity of fresh and frozen Turkish style meat emulsion. Meat Sci. 34: 145-161. https://doi.org/10.1016/0309-1740(93)90024-C