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Physicochemical Properties and Antioxidant Activity of Chicken Sausage with Addition of Roselle Extract (*Hibiscus sabdariffa*) and Different Filler Ratio

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ABSTRACT

This study was done to determine the effect of Roselle extract level (*Hibiscus sabdariffa*), and the wheat flour and mocaf flour ratio, as well as the interaction between the Roselle extract level and the wheat flour and mocaf flour ratio on the chemical, physical, and antioxidant activity of chicken sausage. The ingredients used were chicken meat, wheat flour, mocaf flour, Roselle extract, skim milk powder, and spices. Roselle extract levels were 0, 4, and 8% (v/w of total dough) and ratio of wheat flour : mocaf flours were 100:0, 50:50 and 0:100. Parameters observed included chemical composition (moisture, protein, and fat contents), physical properties (pH value and tenderness), and antioxidant activity of chicken sausage. Data were analyzed by analysis of variance of 3x3 factorial and continued by Duncan's new multiple range test. The results of statistical analysis showed that Roselle extract level gave a significant effect ($P<0.05$) on moisture content, pH value, and antioxidant activity of chicken sausage. Ratio of wheat flour : mocaf flour gave a significant effect ($P<0.05$) on protein content, tenderness and antioxidant activity of chicken sausage. There was no interaction between Roselle extract level and the wheat flour : mocaf flour ratio on the physicochemical properties and antioxidant activity of chicken sausage. The conclusion of this study is the addition of Roselle extract up to 8% will increase moisture content and antioxidant activity, but it decreases the pH value and tenderness of chicken sausage. Substitution of wheat flour with mocaf flour will reduce protein content, tenderness and antioxidant activity of chicken sausage.

Keywords: Antioxidant activity, Chicken sausage, Filler ratio, Physical properties, Roselle extract

Introduction

Roselle (*Hibiscus sabdarifa* L.) is an important medicinal plant originated from tropics, and is now widely cultivated throughout the tropics and subtropics. Bioactive compounds relevant in the context of its pharmacological are organic acids, anthocyanine, polysaccharides, and flavonoids (Lin *et al*., 2011). Roselle extract also contains high percentage of organic acids including citric acid, hydroxycitric acid, hibiscus acid, malic and tartaric acids as major compounds, and oxalic and ascorbic acid as minor compounds. Some studies have reported that Roselle is effective for decreasing the level of total lipids, cholesterol and triacylglycerol, suggesting the possibily that Roselle functions as hypolipidemic agent (Hirunpanich *et al*., 2006). Previous research also reported that calyx of Roselle has potentials as antioxidative agent (Ologundudu and Obi, 2005; Ologundudu *et al*., 2009; Olusola *et al*., 2012). The highest component in Roelle is anthocyanine, such as delphinidin-3-0-glucoside, delphinidin-3-0-sambubioside, and cyaniding-3-0-sambubioside (Christian *et al*., 2006). Besides anthocyanine, calyx of Roselle also contains alkaloids, L-ascorbic acid, β-carotene, β-sitosterol, citric acid, galactose, gossypetin, hibiscetin and mucopolysaccharides (Hirunpanich *et al*., 2005). Antioxidants are considered to be effective inhibitors of carcinogenics as well as other condition that is pathogenically associated with oxidative mechanisms. Many studies demonstrated that antioxidant nutrients play a protective role in human health (Fiedor and Burda, 2014). Therefore, as calyces are consumed, they will show potential for therapeutic uses.

Wheat is the most source of materials used in the making of flour for bread, biscuit, cookies, cake, breakfast cereal, pasta noodle (Malomo *et al*., 2011), for fermentation to make beer (Palmer, 2001), other alcohol beverage (Richard, 2002). There are two kinds of wheat flour, i.e. hard flour,

which has high gluten content (12 to 14%), and elastic toughness that hold the shape well one baked, and soft flour is comparatively low in gluten content and so results in a finer crumbly texture. A lot of studies have been conducted concerning to the partial substitution of wheat flour with other flour food materials. Composite flour with cassava has been evaluated in bread making and general observations are reduced loaf volume, crust color and impaired sensory quality as the level of substitution of wheat with other flours increased (Eriksson *et al*., 2014). Effect of substitution of wheat flour with Taro flour on some properties of weaning food formula also was investigated (Ali *et al*., 2013). Another study also investigated the effect of partial substitution of wheat flour with Riceberry flour on quality of noodles (Sirichokworrakit *et al*., 2015), and the substitution of wheat flour with mixed-cassava and red beans flour toward the characteristics of instant noodles (Novelina *et al*., 2014). The objective of the study was to investigate the effect of Roselle extract level (*Hibiscus sabdariffa*), and the wheat flour and mocaf flour ratio, as well as the interaction between the Roselle extract level and the wheat flour and mocaf flour ratio on the chemical composition, physical quality, and antioxidant activity of chicken sausage.

Materials and Methods

Materials

The ingredients used were chicken breast meat (obtained from Supermarket in Yogyakarta), Roselle flower (obtained from traditional market in Yogyakarta), wheat flour, mocaf flour, skim milk, spices consisting of salt, garlic, pepper, cooking oil(obtained from Supermarket in Yogyakarta), and sausage casing. Materials for testing chemical composition, physical quality and antioxidant activity include aquades, H_2SO_4, $CuSO_4$, K_2SO_4, NaOH, H_3BO_3, HCl, benzene petroleum, buffer pH 7.0, buffer pH 4.0, methanol and 2, 2-diphenyl-1-picrylhydrazyl (DPPH).

Methods

Extraction of Roselle flower. Dried Roselle flower was ground and powder was filtered by 60 mess filter casa. Roselle flower powder was put into beaker glass with the addition of water. The ratio of water and Roselle flower powder was 3:1, and then was heated at the temperature of 40^{o}C for 20 minute.

Chicken sausage manufacture. Chicken breast meat was cut into small then finely ground using a grinder. Ground meat at about 100 g was then added with skim milk powder 4.5 g, salt 2.5 g, garlic 3.5 g, pepper 2.5 g, cooking oil 2 g, and filler 16 g until all the ingredients were mixed evenly. The dough was divided into 3 levels of filler ratio consisting of wheat flour: mocaf flour (unbranded flour obtained from a Supermarket in Yogyakarta) is 100:0, 50:50 and 0:100. Each level of filler ratio was devided again into 3 levels of Roselle extract addition with level of 0, 4, and 8% (v/w). Sausage dough was then put into a plastic casing with 12 mm of diameter, 100 mm of length. The sausages were then steamed for 30 minutes at 85^{o}C. Sausages were then drained and cooled.

Chemical composition test. The chemical composition of chicken sausages included moisture content, protein content and fat content. The moisture content was tested graphymetrically (AOAC, 2007). The moisture content is the difference of sample weight before heated at 105^{o}C for 12 hours and after heated at 105^{o}C for 12 hours. The protein content was determined by the Kjeldahl method (AOAC, 2007). The Kjeldahl method included destruction with H_2SO_4 to destroy all organic materials, then distillation with NaOH to release ammonium, and titration with HCl to determine the amount of nitrogen. The protein content was obtained by multiplying the nitrogen content (%) by the nitrogen conversion factor (6.25). Fat content was determined by using Soxhlet extraction method (AOAC, 2007). Soxhlet fat extraction used benzene petroleum for 16 hours or until the solution becomes clear. Fat is the material left in the soxhlet flask after dried at 105^{o}C for 8 hours. Fat content was obtained by dividing the fat weight by the sample weight and multiplying by 100%.

Physical qualities test. The physical quality of chicken sausages included pH value and tenderness. The pH value was tested according Bouton and Harris (1972). Chicken sausage samples were weighed at about 10 g and finely ground, and then added with 10 ml of distilled water, and stirred until homogeneous. The pH value of the sample was measured with a pH meter calibrated with phosphate buffer pH 7.00 and phosphate buffer pH 4.00. Tenderness was measured according Kartika *et al*. (1988). A 5 cm long sausage sample was placed under a KIC penetrometer needle with a 50 g loader, before the loader was released the pointer number was returned to the position of number 0. When the loader loaded needle will enter the sausage. The depth of the needle through the sausage multiplied by 0.1 mm, will produce the value of tenderness (mm/50 g).

Antioxidant activity test. Free radical scavenging activity was determined according to the method of Zainoldin and Baba (2009). A sample of yoghurt (4 mL) was homogenized with 1 mL of water. One mL of methanol (blank) or homogenized yoghurt was added to 2 mL of 0.02 g/L of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution in methanol. The mixture was then shaken vigorously and kept standing in the darkroom for 30 min at room temperature. The reduction of DPPH was measured at 517 nm against a blank at 30 min. Antioxidant activity was expressed as free radical scavenging activity using the following equation:

Free radical scavenging activity as expressed as following:

$$\text{Inhibition (\%)} = 100 \times [(A_{control} - A_{sample})/A_{control}]$$

Statistical analysis

Data of chemical composition, physical quality and antioxidant activity of chicken sausage were analyzed by analysis variance of 3x3 factorial, ie 3 levels of Roselle extract 0, 4, 8% and 3 wheat flour: mocaf flour ratio of 100: 0, 50:50 0: 100). The average differences were tested by Duncan's new multiple ranges test (Steel and Torrie, 1980).

Result and Discussion

Chemical composition

The chemical composition consisting of moisture, protein and fat contents of chicken sausage with the addition of Roselle extract and differences in the ratio of wheat flour and mocaf flour was presented in Table 1.

Moisture content. The statistical analysis showed that there was no interaction between addition level of Roselle extract and wheat flour: mocaf flour ratio on moisture content of chicken sausage. The moisture content of chicken sausage with addition of Roselle extract and wheat flour: mocaf flour ratio ranged from 64.51 to 72.66% (Table 1). The moisture contents were around normal moisture content of sausage according to National Standard of Indonesia (SNI) at amount of maximal of 67% (BSN, 2015). The results showed that the addition of Roselle extract significantly affected the moisture content of chicken sausage (P<0.05). The higher level of Roselle extract addition lead to increase moisture content of chicken sausage. This was due to the Roselle has a high moisture content. The addition of Roselle extract will increase the amount of moisture present in the dough, so the moisture content of the sausage becomes increased. Accordings to Ismail *et al.* (2008), fresh calyx of Roselle has moisture content of 83.4%, protein 1.9%, fat 0.1%, carbohydrates 12.3% and fiber 2.3%.

The statistical analysis showed that the wheat flour : mocaf flour ratio had no significant effect on moisture content of chicken sausage. The moisture content of chicken sausage was affected by the moisture content of wheat and mocaf flour. Novelina *et al.* (2014) reported that cassava flour contains moisture content 11.28%, protein 1.52%, fat 0.47% and ash 1.46%. Meanwhile, wheat flour contains moisture content 11.96%, protein 11.87%, crude fat 1.15%, and ash 0.74% (Sirichokworrakit *et al*., 2015). Both fours, wheat flour and mocaf flour have similar moisture content, so the moisture content of chicken sausage was not significantly different.

Protein content. The statistical analysis showed that there was no interaction between addition level of Roselle extract and wheat flour : mocaf flour ratio on protein content of chicken sausage. The protein contents of chicken sausage with addition of Roselle extract and wheat flour : mocaf flour ratio ranged from 17.60 to 20.09% (Table 1). The protein contents were higher than range of the SNI with the amount of minimum of 13% (BSN, 2015). The results showed that the addition of Roselle extract had no significant effect on protein content of chicken sausage. This was because of the protein content of Roselle extract is low. Previous study reported that fresh calyces of Roselle contains protein 1.9% (Ismail *et al*., 2008), so the increase in addition level of Roselle extract did not affect the protein content of the chicken sausage.

The substitution ratio of wheat flour : mocaf flour affected significantly the protein content of chicken sausage (P<0.05). The increase of substitution level of wheat flour with mocaf flour leads to increase protein content. This was due to the higher protein content of wheat flour compared to mocaf flour. Wheat flour contains 11,82% protein (Sirichokworrakit *et al*., 2015), while the mocaf flour contains 1.52% protein (Novelina *et al*., 2014). It was clear that protein content of

Table 1. Chemical composition of chicken sausage with addition of Roselle extract and different wheat flour : mocaf flour ratio

Variables	Roselle level (%)	Wheat flour : Mocaf flour ratio			Average
		100:0	50:50	0:100	
Moisture content (%)	0	67.10±1.17	65.63±1.39	64.51±1.63	65.75±1.65[a]
	4	67.03±0.78	66.63±0.43	67.46±0.96	67.04±0.74[a]
	8	72.66±4.18	68.02±0.83	71.96±4.40	70.88±3.75[b]
	Average[ns]	68.93±4.27	66.76±1.33	68.84±3.31	
Protein content (%)	0	20.07±1.04	18.86±0.20	18.51±0.48	19.15±0.92
	4	20.03±0.01	17.73±1.70	17.58±1.07	18.45±1.56
	8	20.09±0.49	18.94±0.22	17.60±0.84	18.88±1.19
	Average	20.06±0.57[q]	18.51±1.04[p]	17.89±0.85[p]	
Fat content (%)	0	7.21±0.95	6.54±1.15	6.78±0.93	6.84±0.92
	4	7.17±1.51	6.58±0.83	6.36±0.88	6.70±0.03
	8	6.67±1.45	6.09±0.54	5.73±0.46	6.16±0.90
	Average[ns]	6.29±0.82	6.40±0.79	7.01±1.18	

[a,b] Different superscript at the same column indicated significant difference (P<0.05).
[p,q] Different superscript at the same row indicated significant difference (P<0.05).
ns = not significant.

wheat flour was higher than that of mocaf flour, therefore substitution of wheat flour with mocaf flour would slightly decrease the protein content of chicken sausage; the decrease of protein content was proportional to the substitution level. This results was in association with the previous study by Ali *et al*. (2013) stated that substitution of wheat flour with taro flour result in decrease of protein content of weaning food. The wheat flour contains a specific protein called glutein. Biesiekierski (2017) reported that gluten is a complex mixture of hundreds of related but distinct proteins, mainly gliadin and glutenin. Moreover, the wheat kernel contains 8 - 15% of protein, from which 10 - 15% is albumin/globulin and 85 - 90% is gluten.

Fat content. The statistical analysis showed that there was no interaction between addition level of Roselle extract and wheat flour : mocaf flour ratio on fat content of chicken sausage. The fat content of chicken sausage with addition of Roselle extract and wheat flour : mocaf flour ratio ranged from 5.73 to 7.21% (Table 1). The fat content of chicken sausage was lower than the range of SNI with the amount of maximum of 20% (BSN, 2015). The results showed that level of Roselle extract addition did not significantly affect fat content of chicken sausage. This was in association with the fat content of Roselle extract in which has low fat content. Previous study reported that fat content of Roselle extract was 0.1% (Ismail *et al*., 2008), so the increase of Roselle extract addition up to 8% did not affect the fat content of chicken sausage.

The results showed that wheat flour : mocaf flour ratio had no effect on fat content of chicken sausage. The fat content of chicken sausage was affected significantly by amount of fat used in sausage making. The fat content of sausage originally comes from cooking oil addition in sausage making. Chicken meat has fat content of 0.9% (Soeparno, 2011). Both, wheat flour and mocaf flour had similar in fat content 1.15% (Sirichokworrakit *et al*., 2015) and 0.47% (Novelina *et al*., 2014), respectively.

Physical quality

The physical quality of chicken sausage, consisting of pH value and tenderness, of chicken sausage with the addition of Roselle extract and differences in the ratio of wheat flour and mocaf flour was presented in Table 2.

pH value. The statistical analysis showed that there was no interaction between addition level of Roselle extract and wheat flour : mocaf flour ratio on pH value of chicken sausage. The pH value of chicken sausage with addition of Roselle extract and wheat flour : mocaf flour ratio ranged from 6.13 to 6.76 (Table 2). The result showed that addition of Roselle extract significantly affected pH value of chicken sausage ($P<0.05$). The increasing of additional level of Roselle extract results in decreasing pH value of chicken sausage. The Roselle extract used in this study had pH of 3.1. Moreover, Roselle extract contains a higher percentage of organic acids, including citric acid (12 - 20%), hydroxylcitric acid, hibiscus acid (13 - 24%), malic acid (2 - 9%) and tartaric acid (8%) as major compounds, and oxalic and ascorbic acid (0.02 - 0.05%) as minor compounds (Heba *et al*., 2014).

The statistical analysis showed that ratio of wheat flour : mocaf flour had no effect on the pH value of chicken sausage. This was due to the pH value of wheat flour and mocaf flour used in this study was similar, that wheat flour and mocaf flour pH value of 6.0 and 5.8, respectively. Previous study reported that wheat flour has pH value of 6.42, and mocaf flour has pH value of 5.8 (Eriksson *et al*., 2014). Mocaf flour has slightly lower pH value than wheat flour, because mocaf flour is a fermented product of cassava flour. However, the substitution of wheat flour with mocaf flour did nor affect the pH value of chicken sausage.

Tenderness. The statistical analysis showed that there was no interaction between addition level of Roselle extract and wheat flour: mocaf flour ratio on tenderness of chicken sausage. The tenderness of chicken sausage with addition of Roselle extract and wheat flour: mocaf flour ratio ranged between 22.50 to 28.67 mm/50g (Table 2). The statistical analysis showed that addition of Roselle extract had significantly effect on tenderness of chicken sausage ($P<0.05$). The increasing of the addition level of Roselle extract will increase the tenderness of chicken sausage. This was due to the Roselle extract contains high concentration of organic acid, such as citric acid, hibiscus acid, and ascorbic acid (Heba *et al.,* 2014), so that the addition of Roselle extract will decrease pH value. Accordings to Watanabe *et al.* (1996) the decrease of pH tend to increase tenderness of meat.

The statistical analysis showed that wheat flour: mocaf flour ratio had significant effect on the tenderness of chicken sausage ($P<0.05$). The increasing of wheat flour: mocaf flour ratio leads to decrease tenderness of chicken sausage. The decreasing of tenderness was due to the difference of carbohydrate characteristics of both flours. Carbohydrate consists of the two water-insoluble homoglucans amylose and amylopectin. The amount of amylose and the amylose/amylopectin ratio have critical effects on the properties of the starch, influencing the gelatinization, solubility, and loaf volume (Lee *et al*., 2001; Blazek and Copeland, 2008). Immaningsih (2012) reported that wheat flour contains amylose 10.23% and amylopectin 89.77%, while mocaf flour contains amylose 8.06% and amylopectin 91.94%. Moreover, Winarno (2004) stated that lower the amylose content and the greater the amylopectin content the processed products will be more elastic.

Antioxidant activity

The statistical analysis showed that there was no interaction between addition level of

Table 2. Physical properties of chicken sausage with addition of Roselle extract and different wheat flour : mocaf flour ratio

Variables	Roselle level (%)	Wheat flour : Mocaf flour ratio			Average
		100:0	50:50	0:100	
pH value	0	6.76±0.06	6.76±0.06	6.70±0.00	6.74±0.05[c]
	4	6.40±0.10	6.36±0.06	6.40±0.10	6.38±0.08[b]
	8	6.13±0.06	6.13±0.06	6.13±0.11	6.13±0.07[a]
	Average[ns]	6.43±0,29	6.42±0.28	6.39±0.26	
Tenderness (mm/50g)	0	25.67±0.29	26.67±0.29	22.50±2.78	24.95±2.35[a]
	4	27.33±0.29	26.83±0.29	24.00±0.50	26.06±1.59[ab]
	8	28.67±0.58	27.16±1.04	25.00±1.32	26.94±1.83[b]
	Average	27.22±1.35[q]	26.89±0.60[p]	23.83±1.90[p]	

[a,b,c] Different superscript at the same column indicated significant difference (P<0.05).
[p,q] Different superscript at the same row indicated significant difference (P<0.05).
ns = not significant.

Table 3. Antioxidant activity (di-phenyl picryl hydracyl value) of chicken sausage with addition of Roselle extract and diffirent wheat flour : mocaf flour ratio

Variables	Roselle level (%)	Wheat flour : Mocaf flour ratio			Average
		100:0	50:50	0:100	
DPPH value (%)	0	26.99±6.77	10.72±1.41	12.82±4.65	16.85±8.73[a]
	4	32.86±11.13	28.25±3.27	20.10±0.30	27.09±8.05[b]
	8	36.19±5.09	34.64±7.23	28.85±2.20	33.23±5.66[c]
	Average	32.02±8.07[q]	24.54±11.45[p]	20.60±7.41[p]	

[a,b,c] Different superscript at the same column indicated significant difference (P<0.05).
[p,q] Different superscript at the same row indicated significant difference (P<0.05).

Roselle extract and wheat flour : mocaf flour ratio on antioxidant activity (DPPH) of chicken sausage. The result showed that wheat flour : mocaf flour affected significantly the antioxidant activity of chicken sausage (P<0.05). Increasing of substitution of wheat flour with mocaf flour leads to decrease antioxidant activity. This was because of the antioxidant activity of wheat flour is higher than that of mocaf flour.

The results of antioxidant activity of chicken sausage with addition of Roselle extract and wheat flour: mocaf flour ratio expressed in DPPH value was presented in Table 3. DPPH is a free radical compound and has been widely used to test the free radical- scavenging ability of various samples (Shimoji *et al*., 2002; Sakanaka *et al*., 2005). The DPPH value of chicken sausage with addition of Roselle extract and wheat flour : mocaf flour ratio ranged from 10.72 to 36.19% (Table 3). The result showed that addition level of Roselle extract had significantly effect on the antioxidant activity (P<0.05). The increasing of additional level of Roselle extract increased significantly on antioxidant activity. This was due to the antioxidant activity in Roselle extract used in this study has a high antioxidant activity that is 69%. Roselle extract contains high concentration of antioxidative compounds, such as organic acids, ascorbic acid, anthocyanins and flavonoids (Da-Costa-Rocha *et al*., 2014).

Conclusions

There is no interaction between addition level of Roselle extract and wheat flour: mocaf flour ratio on chemical composition, physical quality and antioxidant activity of chicken sausage. The addition of Roselle extract at 8% result in highest antioxidant activity, and substitution of wheat flour with mocaf flour tends to reduce protein content, tenderness and antioxidant activity of chicken sausage.

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