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ARTICLE

Effect of Cacao Bean Husk Powder on the Quality Properties of Pork Sausages

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Abstract Cacao bean husk (*Theobroma cacao* L.) contains a high level of dietary fiber and therefore can be used as raw material in food processing. The objective of the present study was to measure the physicochemical properties and sensory traits of emulsion-type pork sausages with various levels of cacao bean husk powder (0.25%, 0.5%, 0.75%, 1%, and 2%). The moisture content in cooked sausages increased as the level of cacao bean husk power increased, whereas the protein content decreased ($p<0.05$). With respect to color, as the level of cacao bean husk power increased, there was a decrease in lightness and yellowness, but there was a considerable increase in redness ($p<0.05$). Cacao bean husk powder exhibited a positive effect on emulsion stability and apparent viscosity. In the sensory evaluation, increased level of cacao bean husk increased flavor acceptability; the 0.75% and 1% treatment groups showed significantly high overall acceptability ($p<0.05$). The thiobarbituric acid reactive species content of cooked sausages indicated that with the addition of cacao bean husk powder significantly inhibited lipid oxidation in the sausages during refrigerated storage ($p<0.05$). Overall, the findings of the present study suggest that adding 0.75% and 1% cacao bean husk powder as a natural ingredient in sausages can help develop meat products with excellent qualities.

Keywords antioxidant, cacao bean husk, cocoa shell, lipid oxidation, pork sausage

Introduction

Meat and meat products have a high nutritional value, as they are rich in high-quality proteins and vitamins, and the consumption of meat has increased consistently owing to their savory taste and flavor (Korea Centers for Disease Control, 2010). However, with growing interest in healthy food among consumers, there are concerns about additives, such as preservatives, added to processed meat products (Han and Ahn, 1998). Moreover, there is a demand for healthy and functional meat and meat products (Jimenez-Colmenero et al., 2001). Health concerns about synthetic antioxidants have encouraged the use of natural ingredients with antioxidant characteristics (Gil et al., 2001), and the use of plant extracts rich in phenolic compounds has extended the storage life and enhanced the quality of meat products (Mancini et al., 2015; Misumoto et al., 2005; Zhang et al., 2017). Meanwhile, dietary fibers have various functions, such as water-holding capacity improvement in meat products, tissue deformation and stabilization, and water/oil absorption; these functions can alter the tissue properties of meat products (Thebaudin et al., 1997). In addition to enhancing the quality of meat products, dietary fibers offer several other health benefits, and low-fat foods rich in fiber help lower the risk of various diseases, including colorectal cancer, obesity, and cardiovascular disease (CVD) (Tungland and Meyer, 2002). Several studies have enhanced the quality of various meat products using natural ingredients rich in phenolic compounds and dietary fiber. Cuong et al. (2016) investigated the effects of annatto seed powder containing a high level of polyphenols on pork patties, while Ribas-Agusti et al. (2014) reported the antioxidant activity of cacao and grape seed extracts added to fermented sausages. Cofrades et al. (2008) investigated the effects of edible seaweeds containing a high level of dietary fiber on meat emulsion, while Choe et al. (2013) reported the quality characteristics of frankfurter-type sausages containing wheat fiber. Moreover, lotus rhizome rich in polyphenol and dietary fiber enhanced the physicochemical characteristics and inhibitory effect on lipid oxidation of sausages (Ham et al., 2017).

Cacao (*Theobaroma cacao* Linn.) is an ingredient in chocolate, which is one of the favorite foods worldwide, and it can enhance the quality characteristics of meat products. It is also known to be a functional food ingredient that can lower the risk of CVD-related mortality and possess antioxidant and antimicrobial activities (Djouss et al., 2011; Ferrazzano et al., 2009; Lotito and Frei, 2006). Cacao beans consist of nibs and husk. Cacao nibs are used as a component of cocoa, whereas cacao bean husk (approximately 15 wt% of total cacao bean) is discarded as a co-product. Globally, the annual production of cacao bean is 2.5×10^6 tons with an estimated 4×10^5 tons of cacao bean husk being discarded (Kim et al., 2004). However, cacao bean husk contains similar amounts of polyphenol and physiologically active substances as those in cacao nibs, and studies have reported the presence of phenolic compounds and various functions of such cacao co-products, including antioxidant, anti-diabetic, and anti-inflammatory functions (Dayane et al., 2018; Kim et al., 2004; Martinez et al., 2012). Moreover, because cacao bean husk contains a high level of dietary fiber, they can be used as a raw material in food processing (Martinez et al., 2012). Because of depleting natural resources and serious environmental issues, researchers have attempted to use such co-products for other value-added applications (Fernfern-Lopez et al., 2009; Viuda-Martos et al., 2010; Viuda-Martos et al., 2011).

In the present study, we aimed to measure the physicochemical properties and sensory traits of emulsion-type pork sausages with various levels of cacao bean husk powder and determine the optimal level of addition to enhance processing quality of meat products using natural resources and create value-added foot products using co-products.

Materials and Methods

Preparation of pork sausage with cacao husk powder

Raw meat (Hongju Meat Co., Korea, Hongseong) used was chilled pork lion at 24 h after slaughter. Raw meat and back fat crushed using a grinding machine (PA-82; Mainca, Barcelona, Spain) equipped with a 3 mm plate. The mixing ratios of the ingredients are shown in Table 1. Raw meat, back fat, and ice were ground using a bowl cutter (K-30; Talsa, Valencia, Spain). Subsequently, sugar, nitrite-pickling-salt and mixed spice were added. Cacao bean husk powder (*Theobroma cacao* Linn; TreeToBar Co. Namyang, Korea; moisture 5.51%, crude protein 15.60%, crude fat 27.62%, crude ash 5.78%, total dietary fiber 40.7%, pH 5.15, CIE L* 34.52, CIE a* 10.01, and CIE b* 14.24) was added at concentrations of 0.25%, 0.5%, 0.75%, 1%, and 2% to the sample (Table 1). After preparing the emulsions, the mixture was filled into a natural pork intestine

Table 1. Formulation of various sausages formulated with different amounts of cacao bean husk powder

Ingredients (%)		Adding levels of cacao bean husk powder (%)					
		0 (control)	0.25	0.5	0.75	1	2
Main	Meat	60	60	60	60	60	60
	Back fat	20	20	20	20	20	20
	Ice	20	20	20	20	20	20
Additive	NPS	1.2	1.2	1.2	1.2	1.2	1.2
	Sugar	1	1	1	1	1	1
	Mixed spice	0.6	0.6	0.6	0.6	0.6	0.6
	Cacao bean husk powder	-	0.25	0.5	0.75	1	2

NPS, nitrite picking salt.

casing by a Filling Machine (EM-12, Mainca, Barcelona, Spain). The filled meat emulsion was heated in an 80℃ water bath (10.10ESI/SK; Alto Shaam, Menomonee Falls, WI, USA) for 30 min. The cooked sausages were cooled at 10℃ for 30 min and used in the experiment

Proximate analysis

According to the AOAC method (2000), the cooked sausages were analyzed to determine the content of crude protein, crude fat, crude ash, and total dietary fiber, with three replicates per experiment.

pH

The pH of the sausage before and after heating was measured using a pH meter (Coring 340; Mettler Toledo AG, Kusnacht, Switzerland) in a homogenate of 5 g of sample and 20 mL of distilled water for 60 s (PT 2500 E; Mettler Toledo AG, Kusnacht, Switzerland). All treatments were performed in quintuples.

Color measurement

The color was measured directly on the inner surface of the sausage using a Hunter color reader (CR-400; Minolta, Osaka, Japan). The results are expressed as CIE L*, CIE a*, and CIE b*. A white standard plate with lightness of +94.65, redness of –0.43, and yellowness of +4.12 was used as the reference.

Emulsion stability

The emulsion stability of the sample was evaluated by placing a wire mesh (4×4 cm) on a centrifuge tube, filling the pork emulsion into the tube, and sealing the inlet. The centrifuge tube was heated in a chamber (10.10ESI/SK; Alto Shaam, Menomonee Falls, WI, USA) at 80℃ for 40 min and cooled at 10℃ for 30 min. The oil and water layer (mL) exuded from the sausage were divided by the weight of sausage dough before cooking to obtain a percentage. The emulsion stability was calculated using the following formula:

$$\text{Fat release (\%)} = [\text{The oil layer (mL)} / \text{Weight of batter before heating (g)}] \times 100$$

$$\text{Water release (\%)} = [\text{The water layer (mL)} / \text{Weight of batter before heating (g)}] \times 100$$

Cooking loss

The sausages were weighed before and after heating, and the cooking loss was calculated as percentage using the following formula:

$$\text{Cooking loss (\%)} = [\{\text{Weight of sausage before heating (g)} - \text{Weight of sausage after heating (g)}\} / \text{Weight of sausage before heating (g)}] \times 100$$

Apparent viscosity

The apparent viscosity of the row meat emulsion was measured using a rotary viscometer (MerlinVR; Rheosys, Hamilton, NJ, USA). it was mounted 30 mm parallel plates with a 2.0 mm gap and the head speed was set at 20℃ at 20 rpm for 60 s.

Texture profile analysis (TPA)

The samples sized approximately 2.5×2.0 cm were cut from the central portion of each pork sausages. The texture of the sausages was analyzed using a texture analyzer (TA-XT2; Stable Micro System Ltd., Haslemere, UK) equipped with a round probe (75-mm diameter). The conditions of texture analysis were as follows: test speed 3.0 mm/s, post-test speed 5.0 mm/s, pre-test speed 5.0 mm/s; trigger force 5 g, test distance 7.0 mm. The texture of pork sausages were analyzed 10 times per replication.

Thiobarbituric acid reactive species (TBARS) analysis

Fatty acids were determined by distillation. 10 grams of the heated sample was homogenized for 2 min at 10,000 rpm using a homogenizer (AM-5; Nihonseiki kasha, Osaka, Japan), and then transferred to a TBA instrument and mixed with 47.5 mL of distilled water, 2.5 mL of HCl, three boiling stones, and 1 mL of defoamer. The samples were distilled in a distiller (MS-E102, MTOPS, Seoul, Korea) for 10 min to collect the distillate. Then, 5 mL of the collected distillate and 5 mL of TBA reagent were mixed and heated in a constant temperature water bath (JSWB-30T; JSR, Seoul, Korea) at 100℃ for 35 min, and then cooled for 10 min. The absorbance of the sample was measured using a spectrophotometer (Libra S22; Biochrom, Nottingham, England) at 538 nm. The measured value (OD) was multiplied by a factor value of 7.8 to represent the TBARS value. The samples were refrigerated and tested at 0, 3, and 7 d.

Total microbial counts

For the total aerobic microbial count determination, 10 g of sample and sterile 90 mL of sterile 0.1% peptone water (Difco, NJ, USA) were transferred into a sterile stomacher bag. The samples were then homogenized in the stomacher (HG300V, MAYO, Milano, Italy) for 4 min at normal speed. And the serial dilutions of the homogenate were prepared with 0.1% peptone water. After serially diluting each sample, 0.1 mL of liquid amount was outspread on an aerobic count petrifilm (AC petrifilm; 3M Health Care, Minnesota, USA). The total bacterial count was determined on the petrifilm incubated at 37℃ for 24–48 h. The samples were refrigerated and tested at 0, 3, and 7 d. Microbial colonies were counted using a digital colony counter (KT0074A, S&N, Seoul, Korea) and expressed as Log CFU/g sample of sausages.

Sensory evaluation

Each sample was doubly evaluated by a sensory panelist. Twenty panelists were screened from 25 potential panelists using a basic taste identification test. Using a commercial pork sausage product for 2 min (30–40 min sessions per wk), the panelists familiarized themselves with the pork sausage characteristics that were to be evaluated. The scoring of each sample was done on a single sheet with a nine-point descriptive scale (with 1 being extremely undesirable and 9 being extremely desirable). The color, flavor, tenderness, juiciness, and overall acceptability were then scored.

Statistical analysis

The data were analyzed using IBM SPSS (SPSS Ver. 25.0). One-way or two-way analysis of variance and Duncan's multiple range comparison test were used; the level of significance was set at $p<0.05$. All experiments were repeated three times or more. The data are expressed as mean value±SD.

Results and Discussion

Proximate analysis

Table 2 shows the proximate analysis results of cooked sausages prepared with various levels of cacao bean husk powder. As the level of cacao bean husk powder increased, the moisture content in sausages tended to increase ($p<0.05$). The protein content in the treated sausages was lower than that in the control group, and as the level of cacao bean husk powder increased, the moisture content in sausages decreased ($p<0.05$). While the fat content in the sausages did not show significant differences between the groups, it tended to increase with increase in the cacao husk content, which might have been affected by the fat content (27.62%) in cacao husk. In previous studies (Choi et al., 2008; Ham et al., 2017), the moisture content increased in sausages containing lotus rhizome powder and dietary fiber of rice bran. It was attributed to increase in the water-holding capacity of meat products as dietary fiber increases the binding ability of water molecules. Cacao bean husk powder contains a high level of dietary fiber (40.7%), and it is believed that dietary fiber increases water-holding capacity, affecting the moisture content in cooked sausages. Moreover, Turhan et al. (2005) reported that the moisture content in hamburger patties increased with the hazelnut pellicle content, while Joo and Choi (2014) reported that the moisture content in pork patties containing chestnut inner shell powder was higher than that in the control group. These study results were consistent with the findings of the present study.

Table 2. Proximate composition of cooked sausages formulated with various levels cacao bean husk powder (g/100 g)

Traits (g/100 g)	Adding levels of cacao bean husk powder (%)					
	0 (control)	0.25	0.5	0.75	1	2
Moisture	53.00±0.43^{d}	53.50±0.51^{c}	53.80±0.20^{b}	54.42±0.34^{ab}	54.63±0.21^{a}	54.46±0.32^{a}
Protein	21.10±0.10^{a}	20.06±0.21^{b}	19.30±0.30^{c}	18.53±0.25^{d}	18.50±0.10^{d}	18.56±0.12^{d}
Fat	22.70±1.05	22.73±1.04	22.72±0.33	23.00±2.72	23.07±0.73	23.83±2.10
Ash	1.90±0.20	1.92±0.34	1.88±0.41	1.90±0.17	1.93±0.51	1.90±0.26

All values are mean±SD.

$^{a–d}$ Mean in the same row with different letter are significantly different ($p<0.05$).

pH and color measurement

As shown in Table 3, the pH of raw sausages decreased significantly as the cacao bean husk powder content increased ($p<0.05$). There were no significant differences in the pH of cooked sausages between the control and treatment groups. In all the groups, the pH was high after heating, because of the release of imidazolium from amino acid histidine residue during heating (Choe et al., 2013; Kim et al., 2010). According to Joo and Choi (2014), the pH of pork patties containing chestnut inner shell powder increased, while Park and Kim (2016) reported that the pH of sausages with black rice powder increased before and after cooking. Moreover, the pH of sausages containing a mixture of pig skin and wheat fiber increased (Choe et al., 2013), which can be attributed to the high pH of the material added to the meat products. Therefore, these results can be attributed to the low pH of cacao bean husk powder (pH 5.17±0.03).

In terms of the color values of sausage, the addition of cacao bean husk powder had a significant effect on all color values of emulsion-type sausages before and after heating (Table 3). As the cacao bean husk content increased, the lightness and yellowness of sausages before and after cooking decreased significantly, whereas the redness increased significantly ($p<0.05$). These results might be due to the color values of cacao bean husk powder (CIE L*: 34.52±1.01; CIE a*: 10.01±0.52; CIE b*: 14.24±0.54), affecting the color of sausages. Kim and Kim (2017) enhanced the red color quality of sausages by adding red yeast rice powder with high redness, while Cuong and Chin (2016) added annatto seeds powder to pork patties and obtained results similar to those of the present study. Moreover, Joo and Choi (2014) reported that the lightness and yellowness decreased and redness increased in cooked pork patties containing chestnut inner shell powder and concluded that this was affected by the color values of the ingredient added.

Emulsion stability and cooking loss

The effects of cacao bean husk powder on emulsion stability and cooking loss of meat emulsion are shown in Table 4. The values of water and oil release and cooking loss tended to decrease up to the 1% treatment group ($p<0.05$). There is a correlation between cooking loss and emulsion stability, and low moisture and fat loss in sausages can enhance the sensory traits (Serdaroglu and Degirmencioglu, 2004). Dietary fiber can improve the ability of meat products to bind with water and fat. Choe et al. (2013) and Cofrades et al. (2008) reported that a mixture containing pig skin and wheat fiber increased the

Table 3. pH and color of sausage formulated with different amounts of cacao husk powder

Traits			Adding levels of cacao bean husk powder (%)					
			0 (control)	0.25	0.5	0.75	1	2
pH		Raw	5.89±0.36^{a}	5.87±0.04^{ab}	5.86±0.02^{ab}	5.84±0.02^{b}	5.85±0.01^{b}	5.85±0.02^{b}
		Cooked	6.09±0.04^{a}	6.09±0.01^{a}	6.08±0.02^{a}	6.08±0.03^{a}	6.07±0.01^{a}	6.11±0.01^{a}
Color	Row	CIE L*	76.50±0.85^{a}	74.36±1.61^{b}	71.24±1.50^{c}	69.99±1.01^{d}	66.49±1.39^{e}	64.45±0.98^{f}
		CIE a*	4.38±0.72^{f}	5.30±0.16^{e}	5.72±0.33^{d}	6.10±0.17^{d}	7.18±0.37^{c}	7.81±0.42^{b}
		CIE b*	17.69±0.19^{a}	17.38±0.75^{ab}	17.20±1.11^{ab}	16.75±0.59^{bc}	16.53±0.40^{c}	16.18±0.48^{c}
	Cooked	CIE L*	76.62±0.60^{a}	74.77±1.02^{b}	72.25±1.25^{c}	70.47±1.35^{d}	66.75±1.24^{f}	64.20±0.77^{g}
		CIE a*	3.44±0.2^{g}	3.25±0.16^{g}	3.73±0.33^{f}	4.07±0.19^{d}	5.15±0.34^{c}	5.83±0.35^{b}
		CIE b*	16.88±0.22^{a}	16.49±0.19^{ab}	16.18±0.75^{b}	15.55±0.58^{c}	15.33±0.41^{c}	14.69±0.19^{d}

All values are mean±SD.

$^{a–d}$ Mean in the same row with different letter are significantly different ($p<0.05$).

Table 4. Emulsion stability and cooking loss of sausages formulated with various levels cocoa bean husk powder

Traits		Adding levels of cacao bean husk powder (%)					
		0 (control)	0.25	0.5	0.75	1	2
Emulsion stability (%)	Fat release	1.46±0.07[a]	1.40±0.03[a]	1.35±0.07[a]	1.27±0.02[b]	1.26±0.11[b]	1.42±0.03[a]
	Water release	15.13±0.54[a]	14.74±0.82[ab]	14.66±0.58[ab]	14.13±0.44[bc]	13.54±0.15[c]	13.81±0.12[bc]
Cooking loss (%)		17.73±0.28[a]	17.43±0.69[a]	16.95±0.53[a]	15.21±0.91[b]	15.18±0.94[b]	16.72±0.30[a]

All values are mean±SD.
[a–d] Mean in the same row with different letter are significantly different ($p<0.05$).

emulsion stability and decreased the cooking loss in sausages, because dietary fiber in wheat improves the binding ability of water and fat. The dietary fiber content in cacao bean husk powder used in the present study was 40.7 g/100 g, and it is considered that high fiber content has a positive effect on emulsion stability and cooking loss in sausages. Similar to the present study results, increase in hazelnut pellicles with a high dietary fiber content reduced cooking loss in beef patties (Turhan et al., 2005). Furthermore, cooking loss in beef sausages containing three types of dietary fiber (potato fiber, cellulose, and beta-glucan) was lower than that of the control group (Ktari et al., 2014). On the contrary, in the present study, treatment with 2% cacao bean husk powder resulted in higher fat release and cooking loss than those in the 0.75% and 1% treatment groups (Table 4). Choi et al. (2008) reported that when dietary fiber extracted from rice bran was added to sausages, cooking loss was higher in sausages with 3% and 4% supplementation than that in sausages with 2% supplementation, indicating that the addition of dietary fiber beyond the appropriate amount can actually weaken the binding ability between water molecules to cause greater moisture loss. Moreover, Fernadez-Gines et al. (2004) reported that the addition of excessive amount of dietary fiber reduced the moisture content in sausages. Although cacao bean husk contains a high level of dietary fiber (40.7%), adding more than the appropriate amount might increase emulsion stability and cooking loss. Therefore, it was determined that the cacao bean husk powder content of ≤1% can enhance the binding ability of water and fat in meat emulsion.

Apparent viscosity

Changes in the apparent viscosity of uncooked emulsion-type sausage batter containing different levels of cacao bean husk powder for 60 s are shown in Fig. 1. In the control and treatment groups, sausage batter samples exhibited non-Newtonian thixotropic behavior, with apparent viscosity values that decreased with increase in the rotation time. The apparent viscosity of the control group was lower than that of the treatment groups ($p<0.05$). The apparent viscosity tended to increase with the level of cacao bean husk powder ($p<0.05$), but the apparent viscosity of sausage batter containing 2% cacao bean husk was lower than that of sausage with 0.75% and 1% treatment group cacao bean husk. Emulsion viscosity and cooking loss in meat batter are closely associated with emulsion stability (Choi et al., 2012), and numerous studies have showed that lower cooking loss in meat batter resulted in higher viscosity due to increased binding ability between molecules (Choi et al., 2016; Ham et al., 2017; Lee et al., 2008). These results were consistent with the emulsion stability and cooking loss observed in the present study and it is believed that dietary fiber in cacao bean husk powder increased the binding ability of water molecules, which improved viscosity. Moreover, studies have also reported that ingredients containing dietary fiber increased the apparent viscosity of meat products (Choi et al., 2010; Claus and Hunt, 1991; Ham et al., 2017; Kim et al., 2016).

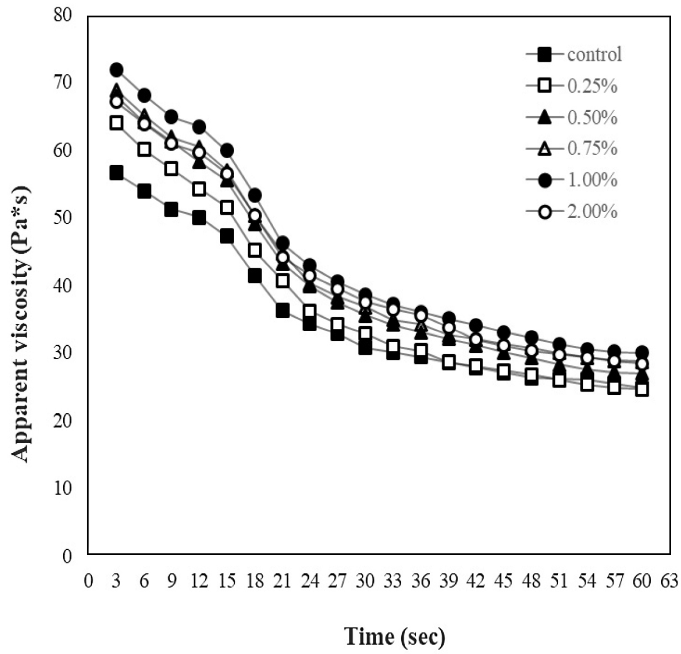

Fig. 1. A change of apparent viscosity on uncooked sausage emulsion-type sausage batter containing cacao bean husk powder for 60 s. ■, meat batter without cacao bean husk powder; □, meat batter with 0.25% cacao bean husk powder ; ▲, meat batter with 0.5% cacao bean husk powder ; △, meat batter with 0.75% cacao bean husk powder ; ●, meat batter with 1% cacao bean husk powder ; ○, meat batter with 2% cacao bean husk powder.

Texture profile analysis (TPA)

As shown Table 5, all texture profile analysis values of cooked sausages tended to show a significant increase as the level of cacao bean husk powder added increased (p<0.05). Dietary fiber can be divided into water-soluble and -insoluble dietary fibers, and generally, the effect of dietary fiber on the texture of meat emulsion varies according to the type and amount added (Cofrades et al., 2000). Contrary to the findings of the present study, Ktari et al. (2014) reported that beef sausages containing three types of water-soluble dietary fiber (potato fiber, cellulose, and beta-glucan) has lower hardness, springiness, adhesiveness, cohesiveness, and chewiness than those of the control group. Moreover, Alvarez and Barbut (2013) reported that cooked meat batter containing water-soluble dietary fiber beta-glucan had lower hardness, springiness, and cohesiveness due to improved water-holding capacity. On the contrary, similar to the present study results, Ham et al. (2017) reported that

Table 5. Texture properties of cooked emulsion-type sausage formulated with various levels cocoa bean husk powder

Parameters	Adding levels of cacao bean husk powder (%)					
	0 (control)	0.25	0.5	0.75	1	2
Hardness (kg)	3.37±0.18[b]	3.47±0.18[ab]	3.46±0.13[ab]	3.50±0.17[ab]	3.66±0.20[a]	3.59±0.23[ab]
Springiness	0.75±0.01[b]	0.75±0.02[b]	0.78±0.03[a]	0.79±0.03[a]	0.80±0.03[a]	0.79±0.04[a]
Cohesiveness	0.48±0.02[c]	0.50±0.02[c]	0.55±0.03[b]	0.61±0.07[a]	0.60±0.03[a]	0.61±0.06[a]
Gumminess (kg)	1.76±0.21[c]	1.78±0.31[bc]	2.27±0.37[ab]	2.37±0.2[ab]	2.16±0.24[a]	2.07±0.21[a]
Chewiness (kg)	1.18±0.19[b]	1.36±0.22[b]	1.61±0.29[a]	1.63±0.24[a]	1.73±0.22[a]	1.71±0.22[a]

All values are mean±SD.
[a–d] Mean in the same row with different letter are significantly different (p<0.05).

sausages containing lotus rhizome powder had increased springiness and chewiness despite having higher moisture content than that of the control group and that this was due to the addition of lotus rhizome consisting mostly of insoluble dietary fiber. Moreover, insoluble fiber extracted from makgeolli increased the hardness, cohesiveness, gumminess, and chewiness of pork frankfurters (Choi et al., 2014). According to Martinez et al. (2012), the total dietary fiber extracted from cacao bean husk was 56.70 g/100 g, of which 42.17 g/100 g was insoluble dietary fiber, indicating that majority of dietary fiber in cacao bean husk powder is insoluble fiber. Joo and Choi (2014) reported that the moisture content in pork patties was higher in groups with up to 3% chestnut inner shell powder, but the hardness, cohesiveness, gumminess, and chewiness were increased. Choi et al. (2010) reported that rice bran fiber increased the binding ability of meat products by improving the water-holding capacity of meat products. These results might be because despite the higher moisture content in cooked sausage than that in the control group, insoluble dietary fiber in cacao bean husk powder can improve the binding ability of sausages.

Thiobarbituric acid reactive species (TBARS)

Table 6 shows that the TBARS content during refrigeration (4℃ for 0, 3, and 7 d) of sausages with cacao bean husk powder. The TBARS content reflects the content of lipid hydro peroxides generated during the oxidation of polyunsaturated fatty acids and malondialdehyde, which is one of the degradation products of lipid hydro peroxides (de Azevedo Gomes et al., 2003). In the present study, the TBARS content significantly increased with storage period in all the groups, except in the 2% treatment group ($p<0.05$), indicating autoxidation of fat in sausage during refrigeration (Wenjiao et al., 2014). On 0 d of storage, the TBARS content in sausages with cacao bean husk powder ranged from 0.17 to 0.46 mg MDA/kg, which was considerably lower than that of the control group (0.79 mg MDA/kg). Moreover, throughout the storage period, the TBARS content in sausages significantly decreased as the level of cacao bean husk powder increased ($p<0.05$). With storage time, the TBARS content in sausage of the control group increased appreciably, from 1.15 mg MDA/kg (3 d) to 2.01 mg MDA/kg (7 d). In contrast, the groups treated with cacao husk bean powder showed no significant difference for up to 3 d with the TBARS content ranging from 0.18 to 0.47 mg MDA/kg, but by 7 d, the TBARS content tended to increase slightly to 0.20–0.53 mg MDA/kg ($p<0.05$). In particular, the 2% treatment group did not show an increase in the TBARS content over time, even on 7 d, while showing the lowest TBARS content throughout the storage period ($p<0.05$). This suggested that cacao bean husk can effectively inhibit lipid oxidation during refrigerated storage.

Fat in meat and meat products is easily oxidized, and hydroperoxides produced during this process generate secondary lipid oxidation products, namely, aldehydes and ketones, causing off-flavor that degrades the food quality (Cuong and Chin 2016). Cacao bean husk contains a high level of polyphenolic compounds that prevents such oxidation, especially monomers

Table 6. Thiobarbituric acid reactive substances (TBARS) value of cooked sausage formulated with different level of cacao bean husk powder during cold storage (4℃)

Storage period (day)	Adding levels of cacao bean husk powder (%)					
	0 (control)	0.25	0.5	0.75	1	2
0	$0.79±0.00^{Ca}$	$0.46±0.01^{Bb}$	$0.37±0.02^{Bc}$	$0.22±0.01^{Bd}$	$0.19±0.01^{Be}$	$0.17±0.01^{Af}$
3	$1.15±0.06^{Ba}$	$0.47±0.01^{Bb}$	$0.38±0.01^{Bc}$	$0.23±0.02^{Bd}$	$0.20±0.00^{Be}$	$0.18±0.01^{Ae}$
7	$2.01±0.01^{Aa}$	$0.53±0.02^{Ab}$	$0.44±0.01^{Ac}$	$0.30±0.01^{Ad}$	$0.25±0.01^{Ae}$	$0.20±0.01^{Af}$

All values are mean±SD.
$^{a–d}$ Mean in the same row with different letter are significantly different ($p<0.05$).
$^{A–C}$ Mean in the same line with different letter are significantly different ($p<0.05$).

catechin and epicatechin and dimer procyanidin B2 (Lamuela-Raventos et al., 2005; Martinex et al., 2012; Mitsumoto et al., 2005). Mitsumoto et al. (2005) reported that catechin extracted from tea can effectively inhibit lipid oxidation in beef and chicken patties, while Jayawadana et al. (2011) and Tackahata et al. (2001) reported that soybean extract containing procyanidin delayed the formation of MDA in meat products. These results suggest that phenolic compounds in cacao bean husk, which are antioxidants, can effectively inhibit lipid oxidation in sausages during storage.

Total microbial counts

As shown in Table 7, the total microbial count increased significantly in all the groups with storage period (0, 3, and 7 d) ($p<0.05$), but there was no significant difference in the total microbial count with the level of cacao husk powder. On 0 d of storage, although there was no significant difference, all the groups showed favorable total microbial count ranging from 3.17 to 3.33 Log CFU/g. On 3 d and 7 d of storage, the range was 3.9–84.10 and 4.18–4.35 Log CFU/g, respectively, with no significant difference between the experimental groups. *Theobroma cacao* is known to have antimicrobial activity against cariogenic microorganisms, such as *Streptococcus muntans*, *S. sanguinis* (Ferrazzano et al., 2009), and *Helicobacter pylori* (Lawal et al., 2014). Tsai et al. (2019) reported that cocoa powder exhibited antimicrobial activity against *Listeria monocytogenes* according to the water activity state under storage, but a low level of cacao powder or low concentration of cocoa extract exhibited very weak antimicrobial activity against *L. monocytogenes.* Takahashi et al. (1999) reported that cocoa extract has antimicrobial activity against enterohemorrhagic *Escherichia coli* at a very high concentration of 8%. Therefore, it was determined that the level of cocoa husk powder used in the present study was not sufficient to exhibit antimicrobial activity, and as a result, adding less than 2% cacao husk powder would not affect the total microbial count in sausages.

Sensory evaluation

The sensory traits of cooked pork sausages with different cacao bean husk powder levels are shown in Table 8. The appearance score was significantly high in the 0.75% treatment group (6.66±1.41). The flavor score was the lowest in the control group and the score tended to increase with increase in the level of cacao bean husk powder ($p<0.05$). The tenderness and juiciness scores did not show significant differences, but the 1% treatment group showed the highest scores. The overall acceptability score was the highest in the 0.75% and 1% treatment groups. These results indicated that the unique flavor and color of cacao husk enhances the quality of sausages and that adding 1% or less cacao bean husk can have a positive effect on the sensory quality of sausages. Similar to the present study findings, Ribas-Agusti et al. (2014) reported that cacao bean

Table 7. Total plate counts (Log CFU/g) of sausage formulated with different amounts of cacao bean husk powder during cold storage (4℃)

Storage period (day)	Adding levels of cacao bean husk powder (%)					
	0 (control)	0.25	0.5	0.75	1	2
0	3.17±0.11^{C}	3.23±0.17^{C}	3.25±0.22^{C}	3.23±0.14^{C}	3.28±0.21^{C}	3.27±0.15^{C}
3	3.98±0.14^{B}	4.01±0.27^{B}	3.99±0.33^{B}	4.10±0.24^{B}	4.21±0.22^{B}	4.06±0.65^{B}
7	4.18±0.09^{A}	4.23±0.16^{A}	4.24±0.21^{A}	4.29±0.13^{A}	4.34±0.14^{A}	4.35±0.11^{A}

All values are mean±SD.
$^{a–d}$ Mean in the same row with different letter are significantly different ($p<0.05$).
$^{A–C}$ Mean in the same line with different letter are significantly different ($p<0.05$).

Table 8. Sensory properties of sausage formulated with different amounts of cacao bean husk powder

Traits	Adding levels of cacao bean husk powder (%)					
	0 (control)	0.25	0.5	0.75	1	2
Color	4.95±2.58[bc]	4.42±2.24[cd]	5.65±1.63[abc]	6.66±1.41[a]	5.94±2.09[ab]	3.40±1.63[d]
Flavor	3.70±2.10[c]	5.05±1.24[a]	5.73±1.85[a]	5.75±2.24[a]	5.88±1.23[a]	6.21±2.25[a]
Tenderness	6.10±2.29	5.26±2.21	5.84±1.92	6.15±1.49	6.27±1.07	4.80±2.39
Juiciness	4.20±2.46	5.70±1.45	4.89±1.76	6.20±2.16	6.94±1.30	3.89±1.99
Overall acceptability	4.45±2.62[c]	4.78±2.01[bc]	5.80±1.57[ab]	6.94±1.95[a]	6.61±1.28[a]	3.75±1.71[c]

All values are mean±SD.
[a–d] Mean in the same row with different letter are significantly different ($p<0.05$).

extract enhanced the overall sensory quality of fermented sausages. Moreover, Joo and Choi (2014) reported that pork patties containing 1% chestnut inner shell powder had the highest scores for all sensory traits assessed. Turhan et al. (2005) reported that meat products with 1% hazelnut pellicle had the highest overall acceptability.

Conclusions

Cacao bean husk powder, as functional additives, was added to emulsion-type sausage to investigate the effects of cacao bean husk on the processing quality characteristics of meat products. Cacao bean husk powder at levels 1% or lower had a positive effect on the moisture content, emulsion stability, apparent viscosity, and color value of sausages. In the sensory evaluation, the 0.75% and 1% treatment groups received the highest scores. Moreover, the TBARS content of cooked sausages indicated that the addition of cacao bean husk powder can significantly inhibit lipid oxidation. Thus, we suggest that cacao bean husk powder (0.75% and 1%) can be used as a natural ingredient to develop quality meat products.

Conflict of Interest

The authors declare no potential conflict of interest.

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Author Contributions

Conceptualization: Han YS. Data curation: Choi HY. Formal analysis: Choi HY. Methodology: Han YS. Software: Choi HY. Validation: Choi JH. Investigation: Choi JH. Writing - original draft: Choi JH. Writing - review & editing: Choi JH, Kim NM, Choi HY, Han YS.

Ethics Approval

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) of Kongju National

University (KNU-IRB-2018-75).

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