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Application of natural colorant from black rice bran for fermented Thai pork sausage – Sai Krok Isan

[1*]Loypimai, P., [2]Moongngarm, A. and [1]Naksawat, S.

[1]Division of Food Science and Technology, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok 10600, Thailand
[2]Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Mahasarakham 44000, Thailand

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Abstract

This study investigated the natural colorant from black rice bran regarding the color characteristics, lipid oxidation, bioactive compounds, and antioxidant activity of fermented Thai pork sausage (Sai Krok Isan). Results indicated that the black rice bran colorant powder (BCP) had potential as a natural functional food colorant and antioxidant. The BCP showed a fivefold increase in cyanidin-3-O-glucoside (9,986.8 µg/g) and a fourfold increase in the total content (13,048.3 µg/g), compared with raw black rice bran. Addition of BCP to the fermented sausages improved color formation and increased the level of bioactive compounds. Sausages containing BCP levels of 0.8% and 1.0% showed similar C^* to those prepared with 120 ppm of nitrite. Sausages produced using BCP contained higher levels of anthocyanins, total phenolics, and antioxidants than those of the sausage with 0% or 120 ppm of nitrite. Consequently, the addition of BCP also retarded lipid oxidation and showed a comparable overall acceptance score to the sausage with 120 ppm of nitrite. Results from this study suggest that BCP is an excellent source of anthocyanin pigments and could partially replace nitrite in the fermented sausage product.

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Introduction

Sai Krok Isan or Sai Krok Prew is one of the most popular varieties of fermented Thai pork sausage, with a long history in several areas of the country, especially the northeastern. Sai Krok Isan is made from pork meat, lard, cooked rice, salt, pepper, anise, spices, and food additives including nitrate and nitrite. During fermentation, the attractive color and flavor of the sausage are produced by the growth of microorganisms and the metabolism of carbohydrates, proteins, and lipids in combination with added spices (Montel *et al.,* 1998; Bruna *et al.,* 2000). Most consumers prefer the intense pink-reddish color of cured meat products where nitrate/nitrite is used to develop the color through the formation of nitrosomyoglobin ($Mb(Fe^{2+})NO$) (Kim *et al.,* 2015). Furthermore, nitrate and nitrite are generally used to retard the growth of pathogenic bacteria such as Listeria spp. and Clostridium botulinum (Sebranek and Bacus, 2007; Hospital *et al.,* 2012). However, residual nitrite/nitrate content in the product adversely affects human health, reacting with a secondary amine in the stomach to form nitrosamine which is carcinogenic (Kim *et al.,* 2015). The addition of nitrate and/or nitrite to meats and meat products is, therefore, currently restricted by Regulation no. 1129/2011 of the EU (European Commission, 2011). Therefore, research into a new natural pigment source which fulfills the function of the nitrite, e.g. the prevention of lipid oxidation, growth inhibition of microorganisms, and the enhancement of consumer acceptability with an attractive color, has attracted attention in the cured meat manufacturing business.

Black waxy rice (*Oryza sativa* L.) has been noted as an excellent source of the dark purple anthocyanins and polyphenols, distributed in the bran fraction (i.e. the pericarp and aleurone layers) of the grain (Abdel-Aal *et al.,* 2006; Loypimai *et al*., 2016). As well as providing the anthocyanin pigments, black rice bran is also an excellent source of bioactive lipophilic compounds such as tocopherols, tocotrienols, and γ-oryzanol (Qureshi *et al.,* 2002; Ryynänen *et al.,* 2004). These have been recognized as health-enhancing substances with their antioxidant, anti-inflammatory, and hypoglycemic properties (Philpott *et al.,* 2004; Nam *et al.,* 2006), as well as other biological effects, including antimutagenic and anticarcinogenic activities (Hyun and Chung, 2004; Nam *et al.,* 2005). Natural food coloring agents are now in high demand by the food industry, not only because of their appearance and attractive consumer acceptability, but also their health benefits and

*Corresponding author.
Email: *patiwit.loypimai@gmail.com*

safety aspects (Chou *et al.,* 2007). Thus, this study was carried out to prepare a natural food colorant and antioxidant from black rice bran to improve the color, retard lipid oxidation, and increase the level of the bioactive compounds in the sausage (Sai Krok Isan). Experimental results were compared against a commercial sausage (CS) containing 120 ppm nitrite.

Materials and Methods

Raw materials and chemicals

The black waxy rice bran (*Oryza sativa* L.) sample was obtained from a local rice-milling factory (10% degree of milling) in Roi-Et Province, Thailand. The raw bran from the milling process was immediately passed through a 20-mesh sieve to remove the broken pieces of rice and husks (Loypimai *et al.,* 2009). The moisture content of the bran sample was determined according to the method of AOAC (2000) before subjection to ohmic heating assisted-solvent extraction.

The common ingredients used for making Sai Krok Isan sausages are fresh pork mince, pork back fat, cooked sticky rice (rinsed with water to remove the sticky layer), fresh garlic, iodized salt, fresh chili, and flavor enhancers (monosodium glutamate). These were all obtained from a local grocery store. Nitrite salt, a commercial sodium nitrite, was purchased from the Thai Food and Chemical Corp., Bangkok, Thailand.

Standards of cyanidin-3-O-glucoside chloride, delphinidin, pelargonidin, cyanidin, and maltodextrin (DE 4-7) were purchased from Sigma-Aldrich Chemical Co., (St. Louis, Mo, USA). High-performance liquid chromatography (HPLC) grade methanol, acetonitrile, acetic acid, and ethanol were purchased from BHD (Poole, UK). All chemicals and reagents were of analytical grade.

Preparation of black rice bran colorant powder (BCP)

The preparation of the colorant powder from black rice bran using ohmic heating-assisted solvent extraction was carried out following the method employed in our previous study (Loypimai *et al.,* 2015). Briefly, the bran sample was added to deionized water to adjust the moisture content to 40% (wet basis) following optimal conditions. It was then placed in a chamber for ohmic heating. Immediately after heating, the bran sample was removed from the chamber and cooled to room temperature. The ohmically-treated bran was extracted following the method reported by Duangmal *et al.,* (2008) and Loypimai *et al.,* (2016). Twenty grams of treated bran was extracted with 100 mL of acidified hydroalcoholic solution (water: 95%, ethanol: 1:1, acidified with 0.1 M HCl to obtain a pH value of 2.5). The bran and solution were mixed and shaken in an orbital shaker (Gerhardt LS500, UK) at 100 rpm for 3 h. The slurry was then filtered through a V-700 vacuum pump (Buchi, Switzerland) using Whatman No. 4 filter paper. The extract was added to maltodextrin (2 g/100 mL) and frozen at –50°C before freeze-drying in a freeze dryer (FTS system Dura-DryTm, USA) at a condenser temperature of –50°C for 20 h. The dried sample was ground into a powder and passed through a 50 mesh sieve. The colorant powder was kept in a brown glass bottle (45 mL), and placed in a desiccator for storage at 4°C until required for analysis.

Manufacturing Sai Krok Isan

Six fermented Thai sausage samples were prepared following the method of Phromraksa *et al*. (2004), replacing the nitrite salts with six different levels of the BCP (per 100 g of sample): 0, 0.2, 0.4, 0.6, 0.8, and 1.0 g. The results were compared with a commercial sausage (containing 120 mg/ kg of sodium nitrite). The fresh pork meat was first trimmed and ground and then thoroughly mixed with the BCP or sodium nitrite, and all the other ingredients (i.e. 73.2% pork mince, 5.5% pork back fat, 18.5% cooked rice, 1% fresh garlic, 1% iodized salt, 0.6% fresh chili, and 0.2% flavor enhancers), using a mechanical mixer for 20 min at room temperature. The mixture was allowed to rest for 30 min and then stuffed into cylindrical double-layer plastic casings (2.25 cm in diameter and 30 cm in length) using a vertical sausage stuffer and tied with aluminum string. Forty casing samples were prepared for each batch and kept in a refrigerator at 4°C for 4 days before testing.

Measurement of visual color

The color of the sausage sample was determined using a Minolta Chroma Meter CR-300 (Konica Minolta, Japan; illuminate calibrated with a white plate, CIE L^* = +97.83, a^* = -0.43, b^* = +1.98), according to Kim *et al.,* (2015) with minor modifications. At three storage times (1st, 3rd, and 5th days), each sausage sample was measured at both cut ends (six replicates), and the average was taken as the reading for the sample. The results were expressed as Hunter color values of L^*, a^*, and b^*. The L^* represents lightness (L^*=0 yields black and L^*=100 indicates diffuse white). The Chroma (C^*) represents color intensity, which is the distance of a color from the origin (a^*=b^*=0) in the a^* and b^* plane. Hue angle (h°) expressed in degrees ranges from 0º to 360º,

where 0º (red) is located on the $+a^*$ axis, then rotating anticlockwise to 90º (yellow) for the $+b^*$ axis, 180º (green) for $-a^*$, and 270º (blue) for $-b^*$. The color of the BCP before addition was also measured.

Measurement of pH

The pH of the sausage was measured following the method described by Tosukhowong *et al.,* (2011). Sausage samples (5 g) were homogenized thoroughly with 10 mL of distilled water. The pH of the homogenated samples was then measured using a pH meter (Mettler Toledo, SevenEasy, USA).

Determination of peroxide value (PV)

The PV was determined in lipids extracted from both stored and non-stored sausage samples by the iodometric assay following IUPAC standard method 2.501 (IUPAC, 1992). PV was calculated and expressed as milliequivalent (meq) peroxide per kg of sample using the equation:

$$PV\ (meq/kg) = ((S \times N)/W) \times 1000,$$

where S is the volume of titration (mL), N is the normality of the sodium thiosulfate solution (0.01 N), and W is the sample weight (kg).

Determination of 2-thiobarbituric acid reactive substances (TBARS)

The TBARS value was determined following the method reported by Mielnik et *al.,* (2003) with some modifications. Five grams of each sample were added to 30 mL of 7.5% aqueous trichloroacetic acid (TCA) solution and then sonicated for 20 min using a sonicator (Vibracell VC 130, Sonics, USA). The slurry was centrifuged at 3,600 rpm for 15 min. The supernatant was collected and mixed with 5.0 mL of 20 mM aqueous thiobarbituric acid (TBA) in a test tube. The samples were incubated at 100°C for 35 min in a water bath and then cooled for 10 min in cold water. Absorbance was measured at 532 nm by spectrophotometer (Spectronic Genesys 5, USA). TBARS values were calculated from the standard curve of malondialdehyde and expressed as milligrams of malondialdehyde (MDA)/kg sample dry matter.

Extraction and determination of anthocyanins

The anthocyanins were extracted according to the method reported by Sarkis *et al.,* (2013), with some modifications. The sample (0.5 g) was added to 50 mL of acidified methanol (methanol/HCl, 100:1 (v/v)), then mixed with a vortex mixer for 1 min and stored overnight at 4°C before immersion in boiling water for 30 min. The sample was then re-cooled in an ice bath, and the supernatant (sub-sample) was filtered through a 0.45 µm nylon syringe filter (Whatman, USA). The content of anthocyanin was quantified using high-performance liquid chromatography (HPLC). The reversed-phase high-performance liquid chromatography (RP-HPLC) system consisted of a Shimadzu (Kyoto, Japan) LC-20AC series pumping system, an SPD-M20A diode array detector, and an SIL-10AD Series auto-injector. The column was an Apollo C18 (Alltech Associates, Deerfield, IL, USA) (ø4.6×250 mm, 5 µm), protected with an Inertsil ODS-3 guard column (ø 4.0×10 mm, 5 µm, GL Science Inc., Tokyo, Japan). A 20 µL aliquot of each sample was used. The elution conditions were performed following the method of Durst and Wrolstad (2001) with some modifications. The mobile phase consisted of solvent A (acetonitrile, CH3CN) and solvent B (4% phosphoric acid, H3PO4) with the following gradient: 94 to 75% B from 0 to 65 min, 75 to 94% B from 65 to 70 min, and isocratic at 94% B from 70 to 75 min to equilibrate the column for the next injection. The chromatograms of anthocyanin were monitored at 520 nm. The operating conditions were column temperature 40°C, injection volume 20 µL, and flow rate of 1.0 mL/min. Calibration curves were constructed using external standards. The anthocyanins in the BCP were also analyzed following the same method as the sausage.

Extraction and determination of DPPH radical scavenging activity

Preparation of extracts was carried out following the method of Loypimai *et al.* (2009) with small modifications. Five grams of the sample were extracted in 80% methanol (25 mL) by placing the mixture in a shaking water bath at room temperature for 2 h. The mixture was then filtered and the residue subjected to the same procedure two more times. The residue was extracted with 0.15 mol/L hydrochloric acid. The extracts were combined, filtered through a filter paper, and then evaporated to dryness under reduced pressure at 45°C using a rotary evaporator (Buchi, Switzerland). The extract samples were tested for antioxidant activity and analyzed for total phenolic content. The DPPH radical scavenging activity assay followed the procedure described by Dasgupta and De (2004).

Total phenolic content (TPC)

The TPC of the extracts was determined using Folin-Ciocalteu reagent following the method of Iqbal *et al.,* (2005). Absorbance was measured at 765 nm by spectrophotometer (CE 2041, England).

Gallic acid was used as the calibration standard and the results were calculated as GAE/ g sample

Sensory analysis

On the first day of storage, the sausages containing different levels of BCP were subjected to sensory analysis. The sausage sample was cooked in a cooker at 180-200ºC for 30 min and then cooled to room temperature for 10 min. The cooked sausage casing was removed and each sausage was cut into slices 4 mm thick before serving. Each sample was randomly labeled with three digit codes and presented on a small white plate at room temperature. A test was carried out using a 9-point hedonic scale labeled from "dislike extremely" to "like extremely". Thirty untrained panel members evaluated appearance (color), odor, taste, juiciness, texture, and overall acceptability. Uncooked sausage samples were also measured for color (Phromraksa *et al.,* 2004).

Statistical analysis

Data were analyzed using the statistical program (SPSS tail version for Windows). Analysis of variance (ANOVA) and Duncan's multiple range tests were applied to determine the significant difference between means. A paired-t-test ($P<0.05$) was also used for data analysis of the effect of storage time (1st day and 4th day) on the PV and TBARS value. Statistical significance was declared at $P<0.05$.

Results and Discussion

Black rice bran colorant powder (BCP) characteristics

The anthocyanins are the major flavonoids in black rice bran. These compounds are responsible for the dark black or purple color, and are located mainly in the aleurone and pericarp layers of the rice bran fraction of the grain. Anthocyanins and the visual color of the raw black rice bran (BRB) and BCP are listed in Table 1. BRB was a rich source of dark-purple anthocyanin pigments. The three major anthocyanins in BRB were cyanidin-3-O-glucoside, delphinidin, and pelargonidin, respectively. Interestingly, the BCP showed a fivefold increase in cyanidin-3-O-glucoside and a fourfold increase in the total content, compared to BRB. In this study, the total anthocyanins at 13,048.3 ± 157.1 µg/g in the BCP gave a higher result than 9,480 µg/g reported by Nontasan *et al.,* (2012). Jang and Xu (2009) also reported that cyanidin-3-O-glucoside was the predominant anthocyanin and could occupy over 90% of the total content in the black rice. In contrast, lower concentrations, and some different anthocyanin profiles were found in Japanese black–purple rice (Pereira-Caro *et al.,* 2013), Chinese and Korean black rice powder (Hou *et al.,* 2011; Frank *et al.,* 2012), and American black rice (Zhang *et al.,* 2013). The variations in anthocyanins may be attributed to different cultivars of pigmented rice, variable growing conditions, and the degree of rice milling.

Table 1. Anthocyanins and visual color value of raw black rice bran (BRB) and black rice bran colorant powder (BCP)

Parameter	BRB	BCP
Anthocyanins[A] (µg/g)		
Cyanidin-3-*O*-glucoside	1,925.5 ± 34.5[b]	9,986.8 ± 45.8[a]
Delphinidin	557.6 ± 54.2[b]	667.3 ± 19.7[a]
Cyanidin	209.4 ± 12.3[b]	610.2 ± 34.9[a]
Pelargonidin	367.5 ± 22.6[b]	1,754.4 ± 65.6[a]
Total contents	3,134.5 ± 76.5[b]	13,048.3 ± 157.1[a]
Visual Color value		
L^*	38.8 ± 0.45[a]	37.4 ± 0.65[a]
C^*	13.4 ± 0.46[b]	18.2 ± 0.87[a]
h^o	307.1 ± 2.72[b]	332.8 ± 4.54[a]

[A] Values are means ± standard deviation of triplicate samples (n=3) (on a wet weight basis).
Mean values in the same row followed by different small letters are significantly different ($P<0.05$).

The visual color measurements in terms of the L*, *C, and hº values of the BCP were 37.4, 18.2, and 332.8, respectively (Table 1). The BCP used in this study was dark purple in color with a darker shade than the raw rice bran. This agreed with the findings of Mozetic *et al.,* (2004), who reported that changes in C* strongly correlated with changes in the anthocyanin content, and can be considered a good indicator of anthocyanin concentration. This may be attributed to the higher number of anthocyanins in the colorant form.

pH value

In this study, the addition of BCP to the sausage did not significantly affect the pH ($P>0.05$) (data not shown). During the initial storage time (first day), the pH values of all sausages ranged between 4.95 and 5.10. As expected, after four days' storage, the pH values of all samples decreased significantly to between 3.98 and 4.22, with the increase in lactic acid during the fermentation process. Phalakornkule and Tanasupawat (2007) opined that lactic acid bacteria played an important role in the ripening process of

Table 2. PV and TBARS value of sausages and sausages added with different levels of black rice bran colorant powder (BCP) during refrigerated storage (4°C)

BCP level (%)	Peroxide value (PV) (meq peroxide/ kg)		TBARS (mg MDA/ kg)	
	1st day	4th day	1st day	4th day
CS	$1.51 \pm 0.01^{a,B}$	$4.42 \pm 0.08^{a,A}$	$1.60 \pm 0.002^{a,B}$	$1.59 \pm 0.004^{b,A}$
0	$1.69 \pm 0.02^{b,B}$	$4.55 \pm 0.09^{a,A}$	$1.61 \pm 0.001^{a,B}$	$1.78 \pm 0.006^{a,A}$
0.2	$1.09 \pm 0.03^{c,B}$	$2.13 \pm 0.06^{b,A}$	$0.36 \pm 0.003^{b,B}$	$1.65 \pm 0.003^{b,A}$
0.4	$0.67 \pm 0.01^{d,B}$	$1.23 \pm 0.07^{c,A}$	$0.34 \pm 0.002^{b,B}$	$1.56 \pm 0.002^{c,A}$
0.6	$0.47 \pm 0.01^{e,B}$	$1.21 \pm 0.06^{c,A}$	$0.15 \pm 0.002^{cd,B}$	$1.47 \pm 0.004^{c,A}$
0.8	$0.46 \pm 0.02^{e,B}$	$0.96 \pm 0.05^{d,A}$	$0.08 \pm 0.005^{d,B}$	$0.36 \pm 0.004^{d,A}$
1.0	$0.34 \pm 0.03^{f,B}$	$0.84 \pm 0.09^{e,A}$	$0.06 \pm 0.001^{d,B}$	$0.24 \pm 0.003^{e,A}$

Values are means ±SD of triplicate samples (n=3).
Mean values in the same column followed by different small letters are significantly different (P<0.05).
Mean values in the same row followed by different capital letters are significantly different (P<0.05).
CS: Commercial sausage; TBARS: Thiobarbituric acid reactive substances.

Table 3. Anthocyanin contents of commercial sausages (CS) and sausages with different levels of added black rice bran colorant powder (BCP)

BCP level (%)	Cy-3-glu (µg/g)	De (µg/g)	Cy (µg/g)	Pe (µg/g)	Total content (µg/g)
CS	-	-	-	-	-
0	-	-	-	-	-
0.2	13.34 ± 1.17^{d}	0.91 ± 0.001^{e}	0.57 ± 0.001^{c}	1.34 ± 0.001^{e}	16.86 ± 1.02^{e}
0.4	19.23 ± 1.61^{c}	1.41 ± 0.002^{d}	0.62 ± 0.001^{c}	1.65 ± 0.001^{d}	22.09 ± 1.13^{d}
0.6	21.24 ± 1.01^{c}	1.96 ± 0.001^{c}	1.18 ± 0.002^{b}	2.38 ± 0.002^{c}	27.54 ± 2.13^{c}
0.8	24.56 ± 1.12^{b}	2.29 ± 0.001^{b}	1.37 ± 0.002^{ab}	3.09 ± 0.002^{b}	32.09 ± 1.96^{b}
1.0	27.43 ± 0.23^{a}	2.58 ± 0.001^{a}	1.41 ± 0.002^{a}	3.88 ± 0.002^{a}	36.10 ± 2.20^{a}

Values are means ±SD of triplicate samples (n=3).
Values with the same letters in the columns are not significantly different (P<0.05).
CS: Commercial sausage with nitrite (120 ppm).
Cy-3-glu: cyanindin-3-O-glucoside; De: delphinidin; Cy: cyanidin; Pe: pelargonidin

raw fermented sausages and noted that most lactic acid bacteria isolated in fermented sausages were *Pediococcus pentosaceus* (31%) and *Lactobacillus plantarum* (35%) In this study, the addition of BCP to the sausages did not influence the ripening process and growth inhibition of the lactic acid bacteria. In low acid fermented sausages (pH<4.6) pathogens such as *Salmonella* spp., *Listeria monocytogenes, Clostridium botulinum*, and *Staphylococcus aureus* may be unable to survive at the end of the ripening process.

PV and TBARS value

The degrees of primary and secondary oxidation of the plain sausages and sausages with different added levels of the BCP were determined by measuring the change in PV and TBARS during refrigerated storage on the first and fourth days. The results are listed in Table 2. The quality of additional BCP significantly influenced the PV and TBARS value of the sausages (P<0.05). After refrigerated storage of four days, the PV and TBARS of all samples changed significantly. As expected, the PV and TBARS of all sausage samples with added BCP showed higher values than the control sample (0% BCP) and commercial sausage (CS). This was because the red-purple color of the anthocyanins and bioactive compounds in the BCP showed strong antioxidant activity, and the capability to retard the lipid oxidation process of the sausage samples. This result agreed with previous studies by Loypimai *et al.,* (2015), they found that BCP prepared from black waxy rice bran using ohmic heating-assisted solvent extraction showed high concentrations of both dark purple anthocyanins and bioactive compounds, namely tocols and γ-oryzanol. Kim *et al.,* (2015) reported that wheat fiber colored with the safflower red pigment carthamin inhibited lipid oxidation and

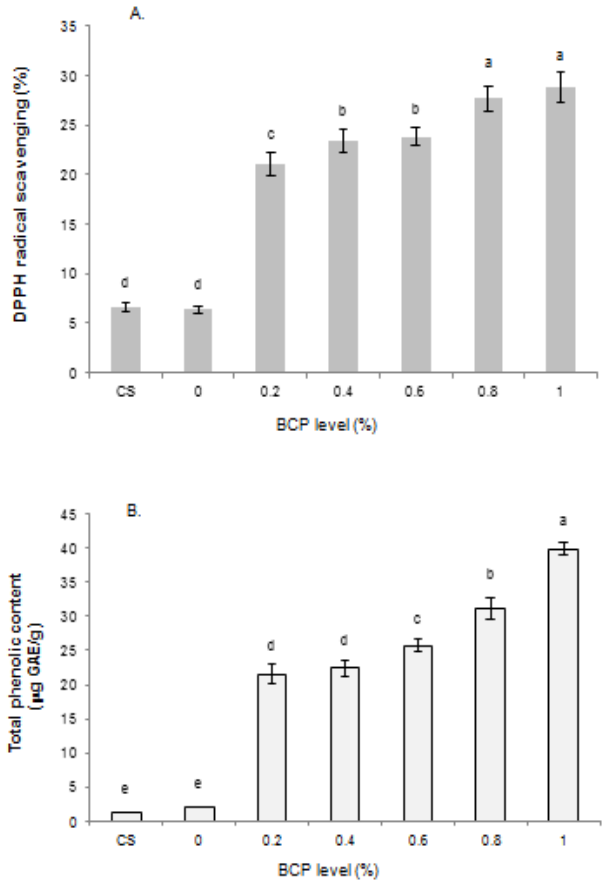

Figure 1. DPPH radical scavenging (A) and total phenolic content (B) of sausages and sausages with different added levels of black rice bran colorant powder (BCP).CS: Commercial sausage.

decomposition of nitrite in cooked sausage, and also had a positive effect on color formation (nitrosoheme pigments).

Anthocyanins

The anthocyanin content of the CS and sausages containing different levels of the BCP are listed in Table 3. In this study, four types of anthocyanins, cyanidin-3-O-glucoside, delphinidin, cyanidin, and pelargonidin were found in the sausages with added BCP. Cyanidin-3-O-glucoside (13.34-27.43 µg/g) was the dominant anthocyanin. The anthocyanins contents were significantly different between sausages containing different levels of BCP, but not detected in CS and sausages with 0% BCP. The addition of BCP to sausages significantly increased individual anthocyanins and total anthocyanin content. The sausage sample with 1.0% BCP recorded the highest total anthocyanin content. This was because of the BCP containing high anthocyanin concentration and more stability in the model fermented pork sausage.

Antioxidant activity

The addition of BCP to the sausage samples significantly increased DPPH radical scavenging antioxidant activity (Figure 1A). The sausage samples with 0.8% and 1% BCP recorded the highest values (27.65 ± 1.3% for 0.8% BCP and 28.82 ± 1.6% for 1% BCP, respectively). This was because the BCP contained higher levels of hydrophilic anthocyanins and lipophilic bioactive compounds such as phenolic acids, tocols, and γ-oryzanol with antioxidant activity. This result agreed with Loypimai *et al.,* (2015). They reported that BCP prepared from black rice bran contained high concentrations of anthocyanins and other bioactive compounds such as phenolic acids, tocols, and γ-oryzanol. Generally, the basic chemical structures of anthocyanins and bioactive compounds such as tocols consist of at least one hydroxyl group linked with a benzene ring which enables them to savage free radicals and singlet oxygen. Antioxidants interact with the DPPH radical either by transfer of an electron or hydrogen atom, thus neutralizing its free radical character (Naik *et al.,* 2003). Zhang *et al.,* (2013) noticed that black or purple rice bran contained both hydrophilic anthocyanins and lipophilic tocols as antioxidants at a significant level. Bhanger *et al.,* (2008) noted that food products containing high concentrations of antioxidants retarded the oxidation caused by free radicals.

TPC

The TPC of CS and sausages containing different levels of the BCP is expressed as the number of equivalents of gallic acid (Figure 1B). The addition of BCP significantly increased the TPC ($P<0.05$). The TPC of sausages containing BCP showed higher levels than sausages without BCP (0%) and CS (120 ppm of nitrite). This was because black rice bran is a potential source of both the dark purple anthocyanin pigments and other compounds including phenolic acids. According to Loypimai *et al.* (2016b) individual phenolic acids namely gallic acid, (+)-catechin, p-coumaric acid, syringic acid, ferulic acid, and caffeic acid were found in black rice bran. In addition to major anthocyanins, tocols, and γ-oryzanol, minor components of carotenoids such as β-carotene, lutein, and zeaxanthin were detected in the BCP (Kong and Lee, 2010; Pereira-Caro *et al.,* 2013; Loypimai *et al.,* 2015; Loypimai *et al.,* 2016b). Phenolic acids (existing in free, soluble, conjugated, and insoluble bound forms) are highly distributed in cereal grains especially in the bran fraction (Martinez-Tome *et al.,* 2004; Adom *et al.,* 2005). The phenolic acids in rice grains are related mainly to the pericarp color. Grains with darker pericarp color such as black rice have higher phenolic content than those with a lighter color like red and brown rice (Pereira-Caro *et al.,* 2013).

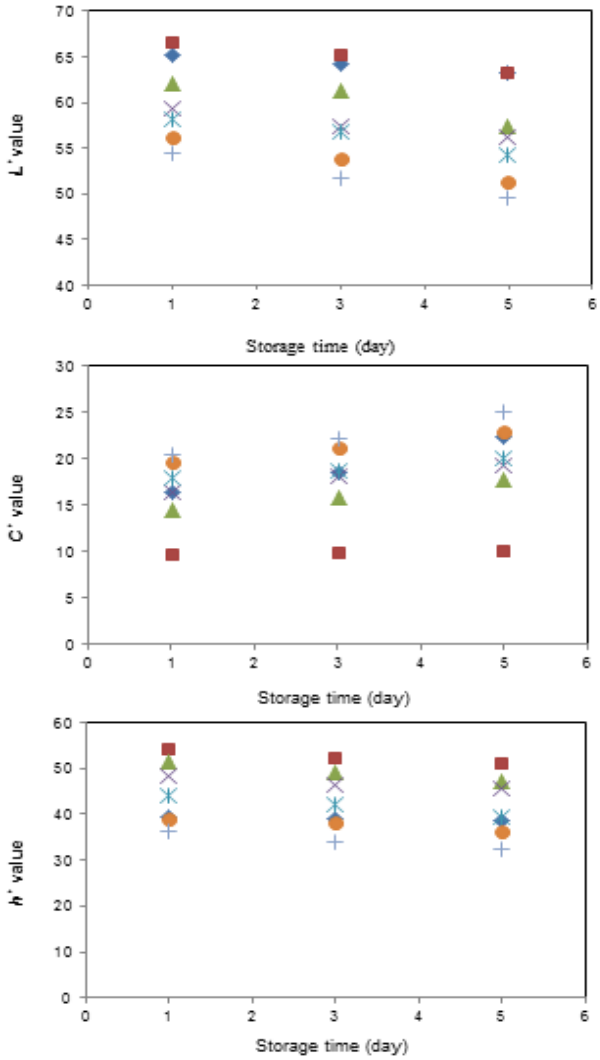

Figure 2. Changes in visual color (L^*, C^*, and $h°$) of sausages and sausages with different added levels of black rice bran colorant powder (BCP) during refrigerated storage for 5 days. CS: Commercial sausage.

Changes in visual color

The level of additional BCP significantly influenced the L*, C*, and hº values of the sausages ($P<0.05$) (Figure 2). The addition of BCP increased the C^* value in the sausage, whereas the L^* and hº values decreased. BCP levels at 0.8% and 1% showed the highest C^* and the lowest values for L^* and hº. Increased storage time increased the value of C^*, while L^* and h^o decreased. However, the C^* of the sausages containing BCP at 0.8% and 1.0% were comparable to sausages prepared with 120 ppm nitrite or CS. Consequently, BCP effectively improved color formation (lightness and redness) of the sausages. Therefore, the addition of BCP promotes the scarlet red color in the sausages. Under the more acidic conditions during the fermentation process, the predominant species of anthocyanins contained in BCP is the red flavylium cation. This contributes to the purple and red colors in the fermented sausage. This result was supported by Torskangerpoll and Anderson (2005) who indicated that the color formation of anthocyanin depended highly on the pH and anthocyanin structure. Anthocyanins can be found in different chemical forms which depend on the pH of the solution (Castañeda-Ovando *et al.,* 2009; Loypimai *et al.,* 2016a). In this study, a change in the C^* value to a positive correlation with addition of BCP was observed. A similar result was reported by Loypimai *et al.,* (2016a); they pointed out that the C^* value showed high correlation with cyanidin-3-O-glucoside ($R^2>0.93$) and total anthocyanins ($R^2>0.92$).

Sensory quality

Overall, both cooked and uncooked sausage samples scored well for all sensory characteristics evaluated (data not shown). Taste (6.25-6.54), odor (7.74-7.85), juiciness (7.11-7.25), and texture (7.34-7.62) of the cooked sausages were not significantly different with increasing levels of BCP, while scores in appearance (color) increased significantly. The sensorial score in terms of overall acceptability was not significantly different between sausages with BCP (0.8 and 1.0%) and CS (120 ppm nitrite). In addition, uncooked sausages containing 0.6, 0.8, and 1.0% BCP gave the highest scores (ranging 7.12-7.34) of color and were comparable to sausage with nitrite (7.36). Therefore, addition of BCP higher than 0.8% (%w/w) increased the color and overall acceptability, comparable to CS. These results were in agreement with Kim *et al.* (2011) who reported that adding tomato powder at 0.5% did not change the sensory characteristics of emulsion type sausages. Application of tomato pomace improved consumer acceptability and preference of frankfurter, beef, ham, and meat-free sausages (Savadkoohi *et al.,* 2014). Moreover, the addition of BCP and its interaction in the sausage may increase water absorption, resulting in improved texture and sensorial quality. Similar results were observed by Hoogenkamp (2012), who reported the application of rice bran as a food ingredient to improve the texture and quality of the sausage.

Results from this study indicated that BCP prepared from black rice bran has potential as a natural functional food colorant and antioxidant in the sausage. Interestingly, the BCP showed a fivefold increase in cyanidin-3-O-glucoside and a fourfold increase in the total content compared with the BRB. The addition of BCP to the sausages resulted in a positive effect on both color formation and bioactive compounds. The sausages containing BCP at 0.8% and 1.0% showed similar redness and lightness to the

commercial sausage (120 ppm of nitrite). The sausages produced by adding BCP contained higher levels of anthocyanins, total phenolics, and antioxidants than those without BCP. Moreover, adding BCP also retarded the lipid oxidation and improved color and overall acceptance scores. Therefore, BCP has potential for use as a natural coloring agent and antioxidant in fermented meat sausages. However, future studies concerning other aspects such as the growth inhibition of microorganisms, the formation of myoglobin, and sensory attributes are still required for further clarification.

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