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Effects of Anthocyanin-rich Colored Potato Flakes on Lipid Oxidation, Instrumental Color Evaluation and Sensory Characteristics in Cooked Pork Sausages

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We investigated lipid oxidation, instrumental color evaluation, and sensory characteristics in cooked pork sausages containing anthocyanin rich colored potato (*Solanum tuberosam cv*. Shadow Queen) flakes (CPF). According to the thiobarbituric acid reactive substance values, addition of 2% CPF suppressed lipid oxidation ($P < 0.05$) by 80% compared to the control. The antioxidant activity of 2% CPF was comparable to the synthetic antioxidant butylated hydroxytoluene (0.1%) in cooked pork sausages. The *L, a values and odor, flavor, taste, texture and overall sensory quality did not significantly differ between control and 2% CPF. Overall, cooked pork sausage with 2% CPF was as acceptable to consumers as the control. Thus, the present study indicated that the addition of 2% CPF was effective as a natural antioxidant for suppressing lipid oxidation in cooked pork sausages.**

Keywords: anthocyanin, colored potato flake, lipid oxidation, natural antioxidant, pork sausage.

Introduction

Lipid oxidation may produce changes in meat quality parameters such as color, flavor, odor, texture and even the nutritional value, thereby limiting the product shelf life (Aguirrez'abal *et al.*, 2000; Madsen and Bertelsen, 1995). Lipid oxidation can be accelerated by several factors, such as increased levels of unsaturated fats, polyunsaturated fatty acids, oxygen, heat, UV light, metal ions, meat/heme pigments, and oxidized enzymes (Frankel, 1998; Morrissey *et al.*, 1998). Minced meat and meat products undergo oxidative changes more quickly as grinding exposes lipid membranes to metal oxidation catalysts.

The addition of synthetic phenolic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), retard lipid oxidation and extend shelf life in meat products (St. Angleo *et al.*, 1990; Güntensperger *et al.*, 1998; Ahn *et al.*, 2002). However, synthetic antioxidants currently used have been found to exhibit potential mutagenic and carcinogenic activity (Wanasundara and Shahidi, 1998; Huang and Ferraro, 1992). In addition, there has been growing interest in natural antioxidants because of their safety, consumer acceptability and greater application in extending the shelf life of foods, as well as potential alternatives to conventional antioxidants. Efforts towards identifying safe and natural sources of antioxidants of plant origin have intensified considerably in recent years. Compounds obtained from natural sources such as grains, oilseeds, spices, fruits and vegetables have been investigated (Chen *et al.*, 1996). Britt *et al.* (1998) researched the antioxidant effects of cherry fruits in pork patties and sausages. Formanek *et al.* (2003) studied rosemary extracts in irradiated ground beef and found that both lipid oxidation and color change were inhibited by the addition of rosemary. The addition of bearberry in pork products was observed to improve oxidation stability (O'Grady *et al.*, 2008). More recently, Devatkal *et al.* (2010) reported on the antioxidant effects of extracts of kinnow rind, pomegranate rind and seed powders in cooked goat meat patties, whereas Naveena *et al.* (2008) reported on the antioxidant activity of pomegranate rind powder extract in cooked chicken patties.

Anthocyanins are plant-derived natural food colorants that have attracted interest in the food industry due to their safety and efficacy. Recently, several researchers have re-

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ported that anthocyanins have remarkable antioxidant and free radical scavenging activities (Wang *et al.*, 1997; Tsuda *et al.*, 2000), and can inhibit LDL oxidation *in vitro* (Kähkönen and Heinonen, 2003). Thus, anthocyanin rich berries, grapes and purple sweet potato may provide possible health benefits, such as reduction of coronary heart disease (Zern *et al.*, 2005), anti-inflammatory activity (Afaq *et al.*, 2005) and anti-cancer activity (Hagiwara *et al.*, 2002).

On the other hand, some of the newly developed potato cultivars are also considered to be good sources of anthocyanins (Sorenson, 1992). Among these, colored potatoes have attracted special interest in many countries (Johnson, 1995) due to their color appeal and excellent taste (Sorenson, 1992; Rodriguez-Saona *et al.*, 1998). Studies on anthocyanin rich colored potato (*Solanum tuberosam* cv. Shadow-Queen (SQ) = Hokkai no.92) flakes showed antioxidant functions with regard to radical scavenging activity and inhibition of linoleic acid oxidation, and improved antioxidant potentials in rats via enhanced hepatic Mn-SOD, Cu/Zn-SOD and GSH-Px mRNA expression were shown (Han *et al.*, 2006).

The overall objective of the experiments reported here was to establish the effectiveness of a newly developed anthocyanin rich colored potato flake (CPF) in preventing spoilage of cooked pork sausages, which are highly dependent on antioxidants to maintain flavor stability. Hence, in the present study, the effectiveness of natural CPF to reduce lipid oxidation, as well as its acceptance as a natural food additive, in cooked pork sausages were evaluated.

Materials and Methods

Purple sweet potato powder (PSPP) PSPP (SAN RED YM Powder, San-Ei Gen F.F.I., Inc., Osaka, Japan) was used as a colorant and is derived from purple sweet potato, which contains anthocyanins similar to colored potato anthocyanins, because we were unable to isolate sufficient amounts of anthocyanins from colored potato flakes for use in food processing. PSPP is a commercially available powder from purple root tubers of the edible purple sweet potato (*Ipomoea batatas cv. Ayamurasaki*). According to previous studies in our lab, PSPP anthocyanin has a similar antioxidant potential to CPF *in vivo* (Han *et al.*, 2007). Since we failed to completely separate the anthocyanin from starch in CPF using food grade chemicals, we used PSPP to evaluate the degree of antioxidant activity compared to the synthetic antioxidant BHT. During the experiment, 0.1, 0.2, 0.3 and 0.4 % (w/w) concentrations of PSPP were mixed with the sausage mixture and compared with the positive control, 0.1% butylated hydroxytoluene (BHT), a synthetic antioxidant.

Colored potato flakes (CPF) CPF of *S. tuberosam* cv. SQ was a kind gift from House Foods Cooporation's Somatech Center (Yotsukaido, Japan). The preparation of potato flakes was as follows: cultivar SQ potatoes were thoroughly washed with water and air dried on filter paper, and they were then peeled and sliced. The sliced potatoes were treated with steam blanching to minimize enzymatic reactions that cause anthocyanin degradation. Next, they were mashed and dried in a drum dryer, and finally ground to flakes. Dietary fiber, protein, lipid, carbohydrate, moisture, and ash contents, as well as total polyphenol, anthocyanin and flavonoid contents of cultivar SQ CPF were reported in our previous study (Han *et al.*, 2007). Different concentrations of CPF (2%, 50% and 100%) on w/w basis were mixed with the sausage mixture and tested for thiobarbituric acid reactive substances (TBARS) values.

Measurement of anthocyanin content in PSPP PSPP (100 mg) was solubilised in 100 ml of distilled water and this solution was used to measure anthocyanin concentration using the pH differential method (Giusti and Wrolstad, 2001). A Shimadzu 1600-UV spectrophotometer (Shimadzu Co., Kyoto, Japan) was used to determine the absorbance at 510 nm for PSPP and 700 nm in buffer at pH 1.0 and 4.5. Anthocyanin contents were calculated using the molar extinction coefficient of cyanidin 3-glucoside (26900l/cm per mg) and absorbance was determined using the equation

$$A = [(A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}].$$

Anthocyanin concentration of PSPP was converted into mg per 100 g powder weight.

Preparation of model pork sausages Model pork sausages were prepared for testing the antioxidant efficacy of both CPF and PSPP by measuring TBARS value. Sausage processing was performed in the meat processing plant of the Obihiro University of Agriculture and Veterinary Medicine, Obihiro under strict hygienic conditions. Model sausages were prepared with minced lean meat (3 mm diameter) mixed with NaCl (2% w/w), lard (20% w/w) and ice (20% w/w). The mixture was ground using a silent cutter. The meat batter was manually stuffed into collagen casings (# 300, Nippi Inc., Tokyo, Japan) and cooked by boiling for 30 minutes at 63°C core temperature.

Pork sausages for sensory evaluation and instrumental color assessment

Pork sausages for the sensory evaluation and instrumental colored assessment were formulated using the same procedure mentioned in the above section (Preparation of model pork sausages). The minced meat was supplemented with 0.5% sugar, 0.6% pepper, 0.14% allspice, 0.14% coriander, 0.04% cardamom, 0.05% onion powder, 0.05% garlic powder, 0.1% glutamine acid, 0.1% sodium ascorbate, 0.1% sodium polyphosphate N2 (75% sodium polyphosphate,

20% sodium pyrophosphate, 5% sodium acid pyrophosphate; Takeda Pharmaceutical Company Limited, Osaka, Japan) and 0.008% (w/w) sodium nitrite. For the treatment group, 2% CPF was added to the sausage mixture in addition to the ingredients mentioned above. After cooking, sausages were stored at 4°C overnight and the sensory evaluation and instrumental color assessment were carried out the next day.

Determination of TBARS The effect of anthocyanins on the oxidative stability of cooked pork sausages, during storage at 37°C, was evaluated by measuring TBARS values. Measurements were taken at 1, 3 and 5 days of storage. Sausage samples (0.2 g) were taken and TBARS values were extracted for 1 h at 4°C with 4.25 mL of TBARS solution containing 0.28% TBARS, 0.009% BHT, 0.4% SDS, 1.2 M acetate buffer, pH 3.5, and the extract was heated in a boiling water bath at 90°C for 60 min. After cooling, 1 mL of distilled water and 5 mL of *n*-butyl alcohol: pyridine (15:1) were added to the extracts and mixed using a vortex mixer. The mixtures were centrifuged at 3,000 rpm for 10 min at room temperature. After centrifugation, the upper phase was pipetted into test tubes. Sample absorbance was read against the appropriate blank at 532 nm. TBARS was expressed as μmol of malondialdehyde (MDA) per kilogram of meat.

Instrumental color evaluation The effect of CPF on the color properties (L^*, a^*, b^*) of cooked pork sausages was evaluated by a Chroma Meter Minolta CM-2600d Spectrophotometer (Konica Minolta Optics, Inc., Tokyo, Japan). The white standard was a piece of tile of known reflectance; the light source D_{65} and the standard observer angle 10° were used.

Sensory analysis The sensory evaluation was performed by a sensory panel composed of 47 members by using paired test for color, flavor, taste, texture and overall acceptability of the cooked pork sausages. Sausages were pre-warmed before serving and water was available for rinsing the mouth between samples.

Statistical analysis The study was performed in triplicate and measurement of all parameters was made in duplicate. Significant differences between the two groups were determined by the Student's *t*- test. Significant differences among treatment groups were determined by ANOVA with the Turkey-Kramer test (IBM PASW statistics version 18.0, IBM Inc., New York, USA).

Results and Discussion

Results for the TBARS analyses of cooked pork sausages supplemented with PSPP during 5 days storage at 37°C are shown in Fig. 1. TBARS of the negative control (0% PSPP) reached the maximum value of 115 MDA μmol/kg on the first day and a more or less similar value was maintained throughout the 5-day storage period. In contrast, the PSPP treated samples and positive control (synthetic antioxidant BHT treated sample) showed significantly lower ($P < 0.05$) TBARS values compared to the negative control for the entire storage period (Fig. 1). Furthermore, according to Fig. 1, the positive control (0.1% BHT) and 0.2% PSPP showed an almost equivalent TBARS value at 5-days storage. Thus, with the addition of 0.2% PSPP, which contained the natural antioxidant, anthocyanins reduced the level of lipid oxidation in cooked pork sausages similar to that observed with the synthetic antioxidant BHT. The BHT concentration employed (0.1%) was a very effective dosage and well over the legal limit for use in sausages (USDA, 2000).

The anthocyanin content of PSPP was 2.4 g/100 g powder (data not shown). Therefore, the 0.2% PSPP, which imparted a similar antioxidant effect as with 0.1% BHT, contained 0.0048% anthocyanins. According to our previous study (Han *et al.*, 2006), the anthocyanin concentration in CPF was 0.214 g/100 g CPF. Thus, to achieve the same antioxidant activity as with 0.2% PSPP, approximately 2.2% CPF was mixed with the pork sausage mixture.

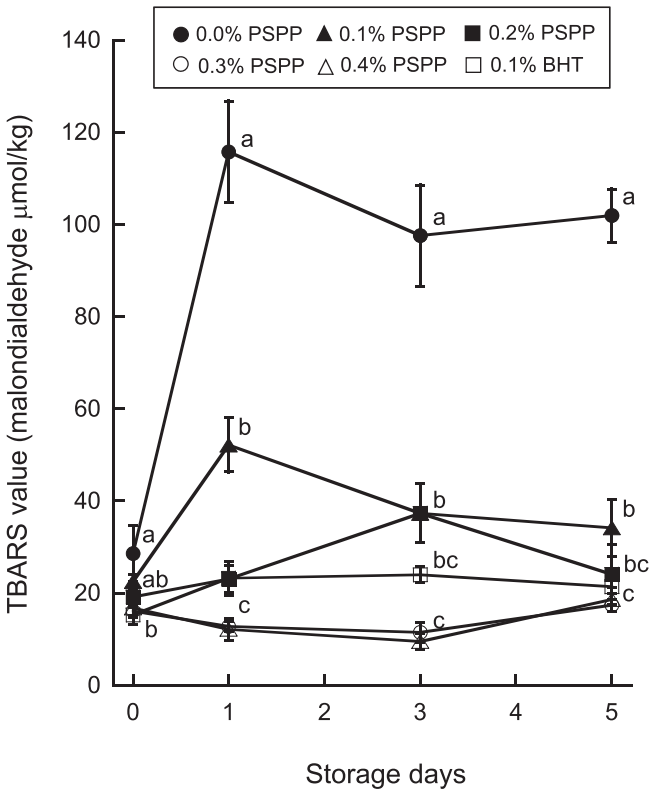

Fig. 1. TBARS values (malondialdehyde μmol/ kg) of model pork sausages with different concentrations of purple sweet potato color powder (PSPP) or 0.1% butylated hydroxytoluene (BHT) during the 5-day storage period at 37°C. Values are expressed as the mean ± SEM ($n = 3$). [a,b,c] Mean values with unlike letters were significantly different ($P < 0.05$). PSPP, purple sweet potato color powder; BHT, butylated hydroxytoluene.

Figure 2 shows the TBARS values of the pork sausages mixed with different concentrations of CPF during 5 days storage at 37°C. Addition of 2% CPF reduced the TBARS values by 80% compared to the negative control (0 % CPF). All three concentrations of CPF (2%, 50 % and 100 %) reduced the TBARS values significantly compared to the negative control. Lipid oxidation, as represented by TBARS values, was reduced with increasing concentrations of CPF. Thus, the order of the antioxidant activity was 0 % < 2 % < 50% = 100% concentrations of CPF. The antioxidant activities of anthocyanins are well documented (Wang *et al.*, 1997; Tsuda *et al.*, 2000), and the observed lipid oxidation reduction in cooked pork sausages could be attributed to its high concentration in CPF (Han *et al.*, 2006). Similar to these findings, Han *et al.* (2006) also reported the reduced hepatic lipid oxidation in rats fed CPF. Therefore, we conclude that the addition of 2% CPF, which was nearly equal to 2.2% as above, was sufficient to suppress lipid oxidation in cooked pork sausages.

Color (CIE L^*, a^* and b^*) values of cooked pork sausage samples are shown in Table 1. There were no significant differences ($P > 0.001$) in L^* values (lightness) and a^* values (redness) in a comparison of control and 2% CPF pork sausage samples. This indicates that the addition of 2% CPF, which significantly reduced TBARS values during storage, did not affect the L^* or a^* value of the pork sausages. Since the predominant color of pork sausages is red and there were no significant differences in a^* value, this may encourage the addition of low levels of CPF in pork sausages. However, CIE b^* values (yellowness) were significantly ($P < 0.001$) lower in the CPF treated (2%) sausage samples. Fat content is the main factor affecting yellowness (Pietrasik, 1999); however, in the present study, the addition of 2% CPF did not significantly alter the fat percentage of the sausage mixture. According to the composition of CPF, starch comprises 83% (Han *et al.*, 2007). However, the addition of 2% CPF to the sausage mixture increased the total starch component in the mixture by only 1.64%. Furthermore, Pietrasik (1999) explained that the addition of starch would enhance many functional properties, including binding, emulsion stabiliza-

Table 1. Effect of colored potate flake (CPF) addition on the CIE L* a* b* values of cooked pork sausages (n = 3).

	Control	2% CPF	Level of significance
L*	73.53 ± 0.35	74.07 ± 0.68	NS
a*	5.12 ± 0.37	5.17 ± 0.19	NS
b*	7.02 ± 0.09	6.21 ± 0.17	**

Level of significance: NS = not significance; ** $p < 0.01$.
CPF = color potato flake

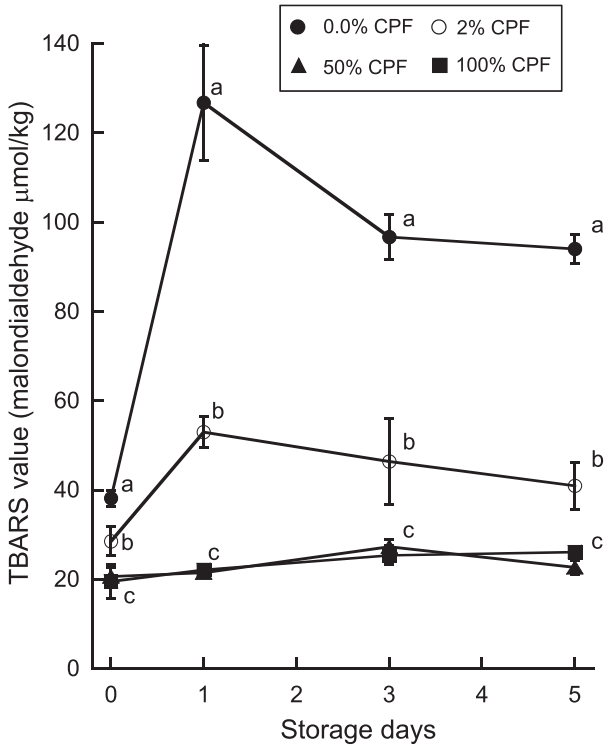

Fig. 2. TBARS values (malondialdehyde µmol/ kg) of model pork sausages with different concentrations of colored potato flake (CPF) during the 5-day storage period at 37°C. Values are expressed as the mean ± SEM ($n = 3$). [a,b,c] Mean values with unlike letters were significantly different ($P < 0.05$). CPF, colored potato flake.

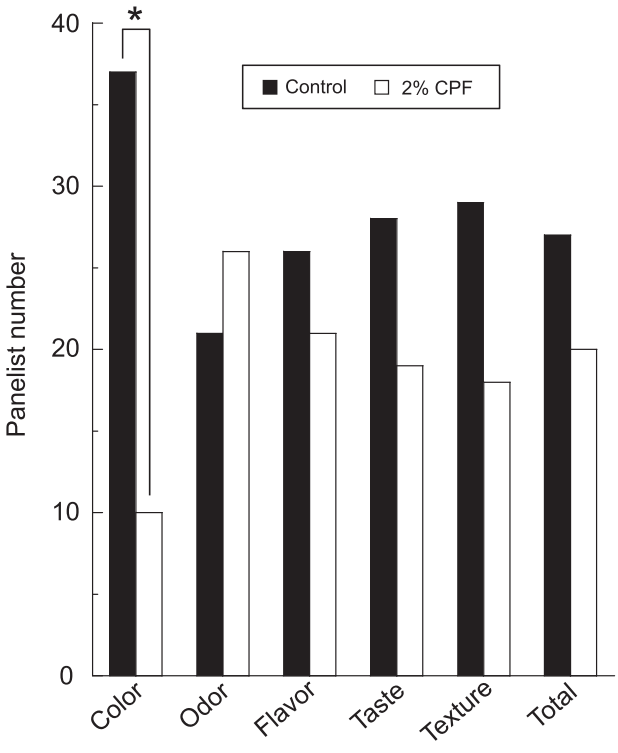

Fig. 3. Mean sensory scores of cooked pork sausages prepared with 2% CPF and control (0% CPF).

*Significantly different at $P < 0.001$

Total panelist number, 47

tion, gelling and moisture retention in sausages.

Sensory panel results are shown in Fig. 3. Addition of 2% CPF had no effect ($P > 0.05$) on odor, flavor, taste, texture and total acceptability, and only the perception of color was significantly different ($P < 0.05$) in 2% CPF supplemented pork sausages compared to the control (0% CPF). However, overall, the panellists appeared to like the pork sausages with 2% CPF as well as the control.

Conclusions

A significant observation in this study was that 2% CPF was as effective as the synthetic antioxidant BHT (0.1%) in retarding lipid oxidation in cooked pork sausages. In addition, odor, flavor, taste, texture and overall sensory quality were not affected by the addition of 2% CPF to cooked pork sausages. Therefore, it is suggested that, as a natural product, CPF could be used to suppress lipid oxidation and extend the shelf life of meat products, providing the consumer with food containing natural additives that might be seen as more healthful than those of synthetic origin.

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