EFFECT OF ANTHOCYANINS FROM DIFFERENT PLANT SOURCE ON THE OXIDATIVE STABILITY OF VACUUM-PACKED CHINESE-STYLE SAUSAGES DURING STORAGE

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Abstract— The purpose of this study was to evaluate the effect of natural anthocyanins from different sources, including roselle, black carrot and grape, on the physicochemical parameters and oxidative stability of vacuum packed Chinese-style sausages during storage. Results showed that all Chinese-style sausage samples with anthocyanins added regardless of plant sources had moisture content, a_W and pH similar to the control. However, roselle anthocyanins added sausages showed all color parameters and visual color vary close to the control sausages. In addition, higher values of PV and TBARS were found during storage in all samples with anthocyanins added compared to the control. This finding indicates that the addition of anthocyanins can cause the lipid pro-oxidation effect in Chinese-style sausages.

***Index Terms*—Anthocyanins, Chinese-style sausages, Oxidative stability, Natural antioxidant**

I. INTRODUCTION

Food with higher fat content such as Chinese-style sausage, a non-fermented semi-dry meat product, is preferred, because fat improves taste, flavor and aroma of products and makes them more attractive for consumers. Apart from these positive factors, fat consumption in food is accompanied by some negative factors with direct or indirect impact on human health. Lipid is mainly sensitive to various changes, such as hydrolysis and oxidation. Lipid oxidation is an autocatalytic process occurring in food, which leads to significant damage of food quality (Niki et al., 2005). The main catalysts of oxidation are highly reactive free radicals as hydroxyl radical ($OH^{\bullet}$), peroxyl radicals ($ROO^{\bullet}$) and superoxide anion (O^{2-}), which contain one or more free electron. Other factors, which support lipid oxidation, are substances as myoglobin, hemoglobin, and transition metals (Fe, Cu, etc) (Baron & Andersen, 2002). They are produced during the process of meat ripening. Level of oxidation is also increased, because of the damage of muscle membrane during processes such as grinding, mixing and tumbling (Wanous et al., 2008).

To maximize the oxidative stability of meat and meat products, mostly synthetic antioxidants; BHA, BHT, and some well known natural antioxidant; α-tocopherol and ascorbic acid, are added to meat products. The beneficial effect of this supplementation for the subsequent enhanced stability of lipids in muscle foods has been extensively reported for raw meat, patties and sausage (Mielnik et al., 2003; Estévez et al., 2006). In addition, an increasing number of studies have reported on the antioxidant properties of plant extracts and compounds *in vitro* or when added during food processing (Schwarz et al., 2001). Furthermore, several studies have confirmed the potential use of natural plant extracts to improve oxidative stability of meat or meat products (Jayathilakan et al., 2007).

Anthocyanins have long been the study of botanists due to their phytoprotective capabilities. To date, over 300 distinct types of anthocyanins have been identified. Based upon research, anthocyanins belong to one class of flavonoids and different classes of flavonoids have different oxidation states. Thus, from an oxidative standpoint, different fruits and vegetables offer different oxidative degrees (Steyn, 2009). Generally, anthocyanins have antioxidant properties, which could protect and enhance our body system with bioactive abilities such as anticancer, antimicrobial and anti-inflammatory and others (Kong et al., 2003). Antioxidative activity of anthocyanins has been confirmed by reported of Kong et al (2003), Castañeda-Ovando et al. (2009). Anthocyanins extract from fruits and vegetables have been evaluated for its antioxidative effect on meat products and have been reported to improve the oxidative stability of cooked chicken (Sáyago-Ayerdi et al., 2009), dry-fermented sausage (Karabacak & Bozkurt, 2008), cooked beef (Ismail & Yee, 2006).

The objective of this work was to compare the effectiveness of anthocyanins obtained from different sources including roselle, grape skin and black carrot in delaying lipid oxidation in Chinese-style sausages.

II. MATERIALS AND METHODS

A. Materials

Dried roselle calyces (*Hibiscus sabdariffa* L.) were purchased from a local market in Bangkok, Thailand. Roselle anthocyanin extract was prepared in the laboratory by ethanol extraction. Briefly, dried roselle calyces were crushed finely by using a blender (Moulinex type AAW9, Indonesia). The roselle powder (20 g) was extracted by soaking for 24 hr in 95% ethanol (1:10 w/v) with an occasional shaking to increase the extraction capacity. The extract was filtered through Whatman No.1 filter paper, and the filtrates were evaporated in rotary evaporator (Büchi model R210, Switzerland) at 30 °C until the ethanol was removed. The crude extract was kept in a tightly closed brown bottle at -18 °C until use. The anthocyanin powder from grape skin (AC 12 WSP) and black carrot (ColorFruit Carrot 12 WSP) was purchased from The East Asiatic (Thailand) Public Company Limited, Thailand. All chemicals used in this study were analytical grade.

B. Preparation of Chinese-style sausage

Fresh lean pork and back fat purchased from a local market in Bangkok, Thailand. The lean pork and immediate subcutaneous fat, which was trimmed out of connective tissue and external fat, were ground through a 4 mm grinder plate (SevenFive, Thailand). The mixture of lean pork (65%) and pork fat (16%) was formulated to contain 0.3% for each anthocyanins. All other ingredients were added in equal amounts to the 3 formulations of sausage: 0.2% potassium nitrite (prague powder), 0.1% Chinese five-spice blend, 0.3% monosodium glutamate and 16.5% sucrose. The sausage mixtures were stuffed into collagen casing (Nippi casing, Japan), linked into 10 cm length and then dried at 60 °C for 24 hr. Sausages were packed under vacuum in nylon/PE vacuum bag (PA/LLDPE/LDPE, thickness: 80 micron) stored at room temperature (29±2 °C) and analyzed every week for 4 weeks. Three replicates with batches were conducted in this study.

C. Physicochemical parameters

The moisture content of sausages was determined using the hor air oven method at 105±1 °C (Bozkurt & Bayram, 2006). Water activity (a_W): a_W of the ground sausages was measured by a Novasina Humidat-IC II unit (Novasina AG, Zurich, Switzerland). The values of pH were measured by the method of Bozkurt & Erkmen (2002). Color parameters (L*, a*, b* value) were analyzed by a colormeter (Minolta CR-400, Japan). The color measurements of Chinese-style sausage were performed at room temperature (29±2 °C) in triplicate. Total color difference (TCD; Equation 1), Hue angle (Equation 2), chroma (saturation index; Equation 3), and browning index (BI; Equation 4) were calculated using Hunter L*, a*, and b* values (Bozkurt & Bayram, 2006) as:

$$TCD = \sqrt{(Lo - L)^2 + (ao - a)^2 + (bo - b)^2} \quad (1)$$

$$Hue\ angle = \arctan(b/a) \quad (2)$$

$$Chroma = \sqrt{a^2 + b^2} \quad (3)$$

$$BI = \frac{[100\ x\ (X - 0.31)]}{0.17} \quad (4)$$

Where: $X = \dfrac{(a + 1.75\ x\ L)}{(5.645\ x\ L + a - 3.012\ x\ b)}$

D. Assessment of lipid oxidation

Measurement of TBARS: Lipid oxidation was assessed by measurement of 2-thiobarbituric acid reactive substances using the distillation method as described by Ferrari & Torres (2002) and was expressed as mg of malonaldehyde (MDA) per kg of sausage, which is determined by using spectrophotometer (Shimadsu UV-1200, Japan) at 532 nm. Measurement of peroxide value: Peroxide value (PV) was determined according to the AOAC 965.33 (AOAC, 1995). PV was calculated and expressed as milliequivalent of peroxide per kg of sausages.

E. Effect of roselle anthocyanins concentration on oxidative stability of Chinese-style sausages

Chinese-style sausage samples were prepared according to the method in *B*. With the addition of roselle anthocyanins at 0, 0.05, 0.1, 0.2 and 0.3%, respectively. The samples were packed and stored at the same conditions as mentioned in *B*. Peroxide value and TBARS were analyzed every week for 4 weeks for each sample to detrmine the oxidative stability of the sausages.

F. Statistical analysis

All measurements were carried out in triplicate (n=3), and results were subjected to analysis of variance (ANOVA) using SPSS software. Differences between means were determined by the least significant difference test, and significance was defined at P≤0.05.

III. RESULTS AND DISCUSSION

A. Physicochemical parameters

Physicochemical parameters of the control sample and anthocyanin-added Chinese-style sausages during storage are shown in Table 1. Significant different were found for moisture content, pH, a_W, and color parameters (TCD, Hue, Chroma and BI) between the control and anthocyanins-added samples ($p \leq 0.05$) during storage. All samples had moisture content in the range of 24-29%. These agree with the standard according to the Thai Industrial standard (TISI, 103/2003) which mentioned that moisture content of Chinese-style sausages can not be higher than 29%. Water activity of all samples ranged from 0.81 to 0.86 and corresponded to a typical intermediate moisture food. Lowest pH was observed in the sample with the addition of roselle anthocyanins extract. This was due to the crude roselle extract contained other organic acids that could reduced the pH of the product. The effect of anthocyanins contributing to a*-value of the sausages is shown in Figure 1. Both control and anthocyanins-added Chinese-style sausages showed the tendency of an increased a*-value during storage.

Considering the results of all color parameters analyzed, the Chinese-style sausages with roselle anthocyanins addition had a*, TCD, Hue, Chroma and BI values more similar to the control sausages than the samples containing anthocyanins from black carrot and grape. The finding was also agreed with the apparent color of the samples. Roselle anthocyanins added sausages exhibited almost the same reddish color to the control sample, while the visual color observed for black carrot or grape anthocyanins added sausages was dark red.

Table 1. Physicochemical properties of the control and anthocyanins added Chinese-style sausages during storage.

	Control		Roselle		Black carrot		Grape	
	0 day	28 day	0 day	28 day	0 day	28 day	0 day	28 day
Moisture (%)	26.72±0.08d	28.81±0.25a	25.94±0.31e	27.05±0.44c	29.03±0.08a	27.61±0.15b	24.31±0.15f	27.08±0.11c
Water activity	0.84±0.00c	0.86±0.00a	0.84±0.00c	0.83±0.00d	0.85±0.00b	0.84±0.00c	0.81±0.00e	0.84±0.00c
pH	5.87±0.01b	5.87±0.01b	5.65±0.01d	5.64±0.01e	5.88±0.00a	5.87±0.01b	5.82±0.00c	5.82±0.01c
Color								
TCD	55.16±1.85cd	46.91±1.40e	54.04±1.75d	46.56±1.43e	56.28±1.69c	53.93±1.26d	60.68±1.13a	58.04±1.19b
Hue	49.48±2.63c	56.26±1.69b	55.52±1.66b	59.25±1.22a	19.23±2.66f	37.37±0.86d	10.97±1.99g	21.48±1.11e
Chroma	9.39±0.46d	13.47±0.61b	9.12±0.49d	14.27±0.89a	8.03±0.48e	11.09±1.07c	5.37±0.23g	6.63±0.58f
BI	28.07±0.89d	34.39±1.24b	26.87±1.43d	36.30±1.67a	19.19±1.14e	30.45±2.41c	12.73±0.39g	17.27±1.64f

Value in the same row with different letter are significantly different ($p \leq 0.05$).

B. Lipid oxidation

Peroxide values (PV) and TBARS, which are indicators of lipid oxidation of sausages samples with the addition of anthocyanins are shown in Figure 2. The PV and TBARS of the Chinese-style sausage samples increased during storage, indicating that lipid oxidation had occurred. In this study higher values of PV and TBARS were observed in all samples of anthocyanins-added Chinese-style sausages compared to the control (no anthocyanins) at all sampling dates. The result from this study demonstrated that the addition of anthocyanins clearly showed a lipid pro-oxidation added effect in Chinses-style sausages.

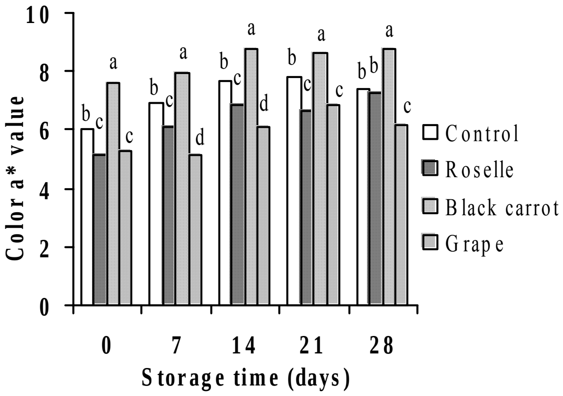

Figure 1. Color a*-value during storage display of sausage without anthocyanins and addition roselle, black carrot and grape. Samples for the same storage time followed by the same letter were not significantly different ($p \leq 0.05$).

Effect of roselle anthocyanins concentration on the PV and TBARS of Chinese-style sausages is shown in Figure 3. The result clearly showed that samples are higher concentration of roselle anthocyanins addition tended to exhibit higher PV and TBARS during storage. This finding confirmed the pro-oxidant properties of anthocyanins in Chinese-style sausages. There is evidence to suggest that in some situations antioxidants can also act as pro-oxidants. For example, ascorbic acid was promoted as pro-oxidant in the presence of iron overload (Carr & Frei, 1999). Moreover, at high oxygen partial pressures β-carotene may become pro-oxidant (Edge & Truscott, 1997).

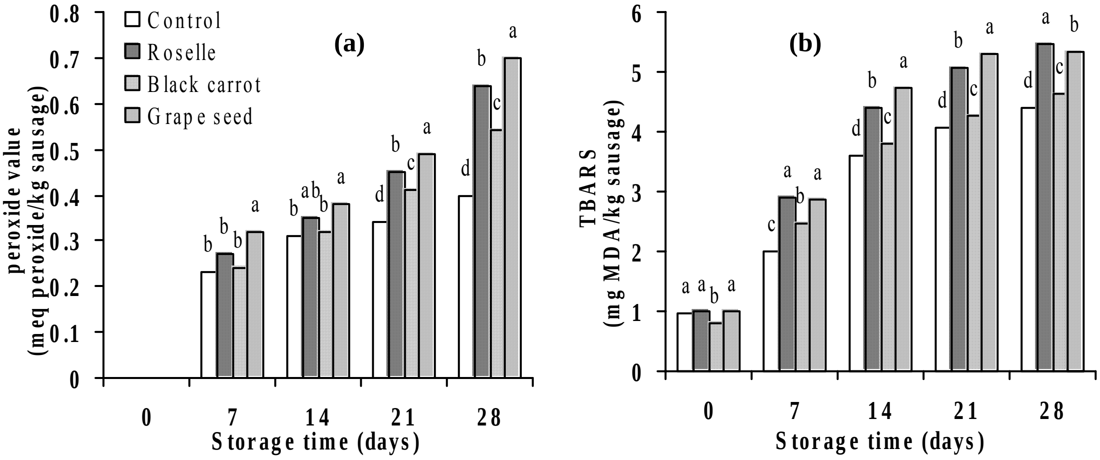

Figure 2. Effect of anthocyanins on PV (a) and TBARS (b) of Chinese-style sausages during storage. Samples for the same storage time followed by the same letter were not significantly different (p≤0.05).

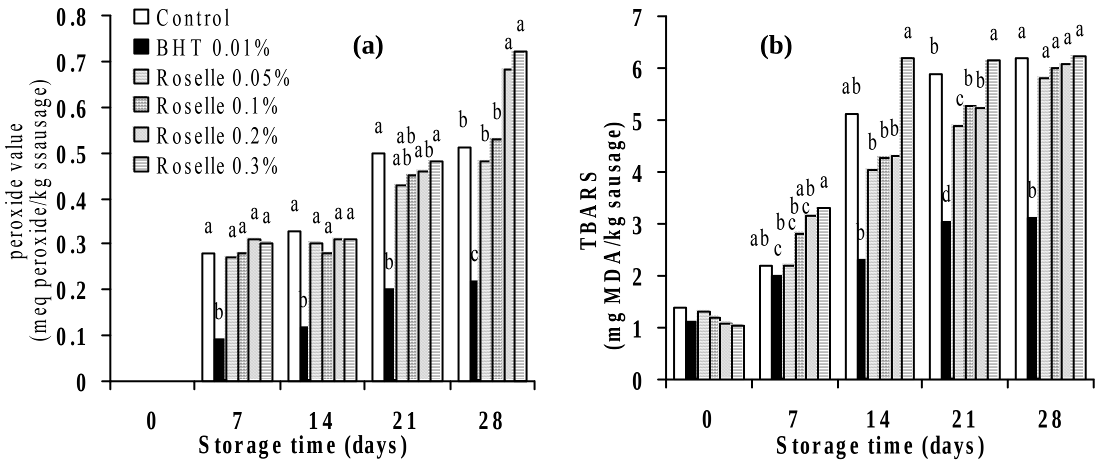

Figure 3. Effect of roselle anthocyanins added on PV (a) and TBARS (b) of Chinese-style sausages during storage. Samples for the same storage time followed by the same letter were not significantly different (p≤0.05).

IV. CONCLUSION

Among the three plant sources, anthocyanins from roselle extract when added to Chinese-style sausages, yielded the product with physicochemical and all color parameters more closely to the control sample compared to black carrot and grape anthocyanins. All Chinese-style sausage with anthocyanins addition had PV and TBARS values higher than those of control sausages at all storage times. Moreover, the sausages with higher content of roselle anthocyanins addition showed higher PV and TBARS values. Results reveal that anthocyanins can cause lipid pro-oxidation effect in Chinese-style sausages.

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