

ANTIOXIDANT PROPERTIES OF ROSELLE EXTRACT AND ITS ANTILIPOPEROXIDANT EFFICIENCY IN MEAT PRODUCTS AS AFFECTED BY SUCROSE

Thanawoot Parinyapatthanaboot¹, Supamach Musika², and Praphan Pinsirodom^{1*}

¹Faculty of Agro-industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

²Center for Advanced Agriculture and Food Research, Faculty of Agriculture, Kasetsart University, Jatujuk, Bangkok 10900, Thailand

*Corresponding author (phone: (662) 326-4112, (662) 326-4091; fax: (662) 326-4112, e-mail: kppraph@kmitl.ac.th)

Abstract—The main purposes were to evaluate *in vitro* antioxidant properties of roselle extract and its antilipoperoxidant efficiency in Chinese-style sausages and pork chips as affected by sucrose concentrations. Total anthocyanins, total polyphenol contents, diphenyl picryl hydrazyl (DPPH) radical scavenging, hydroxyl radical scavenging, inhibition of linoleic acid oxidation, hydrogen peroxide scavenging and Trolox equivalent antioxidant activity were used as antioxidant capacity determinants with ascorbic acid and Trolox[®] used as references. Among all methods assayed, the roselle extract exhibited the highest ability and as efficient as Trolox in inhibition of linoleic acid oxidation by ferric thiocyanate (FTC) assay. Antilipoperoxidant efficiency of the roselle extract in Chinese-style sausages and pork chips as affected by sucrose concentrations at 0, 7, 13, 16 and 20 percent (w/w) were investigated by measuring the changes of TBARS values during storage. A decreased antilipoperoxidant efficiency of the roselle extract in the products toward an increase of sucrose concentration was significantly observed. The finding indicates that roselle extract could be used as a natural antioxidant in meat products with low sucrose concentration.

Index Terms—Roselle extract, Antioxidant activity, Chinese-style sausages, Pork chips

I. INTRODUCTION

Anthocyanins are natural colorants belonging to the flavonoid family. They give rise to the orange-red-blue color of many flowers, fruits and vegetables. Anthocyanins have been used for several therapeutic treatments as review by Kong et al. (2003) and Thomasset et al. (2009). Roselle (*Hibiscus sabdariffa* L.), an herb that belong to malvaceae family, is widely used to prepared cold and hot beverages in many world's tropical and subtropical countries. Moreover, roselle extracts are currently marketed as supplements due to their apparent potential health benefits and as a natural colorant to replace some synthetic colorants.

Non-fermented processed pork products, Chinese-style sausages and pork chips, are popular meat products in Thailand. These products are obtained by first mincing the meat, ingredient mixing, shaping and drying. These processing methods are susceptible to oxidation and off flavor development in the products. Natural pigments such as carotenoids have been reported to maintain the oxidation stability of the sausage (Mercadante et al., 2010). Moreover, natural antioxidant and/or by-products of food processing such as plum, apple pomace, grape seed, citrus peel etc. were of great potential antioxidant and could be developed as functional ingredients for meat industries (Mercadante et al., 2010; Sasse et al., 2009).

Many researchers have indicated that oxidation in meat products can be controlled or minimized by the addition of commercial synthetic antioxidants or natural antioxidants (Mercadante et al., 2010; Sasse et al., 2009; McCarthy et al., 2001). However, few studies have been carried out to evaluate the potential anthocyanins in preventing oxidation in meat products.

The objectives of this study were to evaluate the antioxidant capacities of roselle anthocyanins extract and investigate the effect of sucrose on antilipoperoxidant efficiency in Chinese-style sausages and pork chips.

II. MATERIALS AND METHODS

A. Sample preparation

To prepare roselle extract, the dried calyces of roselle were purchased from local herbal marketed brand (Dr. Green; Thailand) and were cleaned and powdered. The extract was prepared by soaking dried roselle calyces powder in 95% ethanol (1:10 w/v) in the dark for 24 hr with an occasional shaking to increase the extraction capacity. The extract was

rapidly filtered through a Buchner funnel with Whatman no 1 filter paper and the filtrate was evaporated under reduced pressure at 30 °C. The crude extract were kept in amble glass vial with screw cap at -18 °C before assays.

B. Analysis of total anthocyanins, total polyphenol contents and antioxidant activities

The quantification of total anthocyanins of the roselle extract was measured by the pH differential method (Giusti & Wrolstad, 2001) using UV-Visible spectrophotometer (UV-1601 Shimadzu, Japan). The content of total anthocyanins was express in mg of cyaniding-3-*O*-glucoside equivalents per 100 g of sample. Total polyphenol content was determined following method describes by Singleton et al. (1999). The antioxidant activities of roselle extract were measured by using DPPH assay which was assessed by the method of Murakami et al. (2004). Hydroxyl radicals ($\cdot\text{OH}$) scavenging was determined using the method described by Aruoma (1994). The ferric thiocyanate (FTC) method was determined according to the method of Larrauri et al. (1996). The ability of roselle extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Yen & Chen (1995). The Trolox equivalent antioxidant activity (TEAC) was evaluated applying the improved ABTS radical cation decolorization assay (Arts et al., 2004).

C. Effect of sucrose on roselle antioxidation measured by the ferric thiocyanate (FTC) method

The effect of sucrose on antioxidant activity of roselle extract was testing by FTC method to confirm the amount of peroxide at the beginning of lipid peroxidation of the reaction mixture. The FTC method was determined according to the method of Larrauri et al. (1996). Sucrose solution prepared in 0.05 M phosphate buffer (pH 7.0) was added to the reaction mixture at the final concentrations of 0-50 percent.

D. Preparation of Chinese-style sausage and pork chips

Chinese-style sausages and pork chips were processed according to the formulation shown in Table 1. Lean pork and back fat were separately cut into small pieces and minced using small scale grinder (SevenFive, Thailand) equipped with 4 mm plate, then kept at 4 °C for 2 hr. To compare the effect of sucrose on antioxidant activity of roselle extract in the products, the concentration of sucrose was assigned as 0, 7, 13, 16 and 20 percent (w/w).

Preparation of sausages: minced back fat was mixed with roselle extract in a bowl using an electrical mixer (Moulinex Mixer BM8, USA) at medium speed for 1 min. The minced lean pork was added and mixed well at low speed for 2 min. All other ingredients were then added and mixed well at low speed for another 5 min. The sausage mixture was stuffed into collagen casing (Nippi casing, Japan) and linked into 10 cm length. The linked sausages were dried at 60 °C for 24 hr in a tray dryer.

Preparation of pork chips: minced lean pork was mixed with roselle extract and other ingredients using an electrical mixer at low speed for 2 min and the mixture was aged at ambient temperature for 2 hr. The pork mixture (100 g) was then spread on a 21x30 cm plastic sheet to form one millimeter thickness film, the pork film was then dried at 65 °C for 30 min (for each side) in a tray dryer. The dried pork film was cut into pieces with the size of 7x10 cm.

The raw Chinese-style sausages and pork chips were packed under vacuum in Nylon/PE vacuum bag (PA/LLDPE/LDPE, thickness: 80 micron) and stored at room temperature (29±2 °C) and analyzed every week for 4 weeks. The 2-thiobarbituric acid reactive substances (TBARS) assay was carried out according to the procedure of Shahidi et al. (1985). Three replicates with batches will be conducted in this study

E. 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 $P \leq 0.05$.

Table 1. Formulation of the experimental Chinese-style sausages and pork chips

Ingredients (% by weight)	Chinese-style sausages	Pork chips
Lean pork	65	86.83
Pork back fat	16	-
Potassium nitrite (prague powder)	0.2	0.2
Chinese five-spice powder	0.1	-
Salt (NaCl)	1.8	-
Sucrose	16.6	7.23
Monosodium glutamate (MSG)	0.3	-
Soy sauce	-	4.34
Water	-	1.4
Roselle extracts	0.3	0.3

III. RESULTS AND DISCUSSION

A. Total anthocyanins, total polyphenol contents and antioxidant capacities

In vitro antioxidant capacity assays (DPPH, $\cdot\text{OH}$, FTC, H_2O_2 and TEAC) showed that roselle extract exhibited considerable antioxidant properties (Table 2 and 3). However, the ability of roselle extract in inhibition of linoleic acid oxidation was as efficient as Trolox[®].

Table 2. Total anthocyanins^a, total polyphenol contents^a, TA/TP ratio^b and antioxidant activity (TEAC)^a

	Total anthocyanins (TA) (mg/100g sample)	Total polyphenol (TP) (mg GAE/100g sample)	TA/TP	TEAC value (μM Trolox)
Roselle calyx	4.35 \pm 0.52	207.87 \pm 1.02	0.02	NT
Roselle extract	45.00 \pm 0.80	2139 \pm 11.00	0.02	0.37 \pm 0.03

^a values are means \pm SD (n=3), ^b the portion of anthocyanins in total polyphenol concentration, NT = not tested

Table 3. Free radical-scavenging activity (EC_{50}) of roselle extract

	Free radical scavenging activity (EC_{50})			
	DPPH (mg/mg DPPH)	$\cdot\text{OH}$ (mg/mg $\cdot\text{OH}$)	FTC (mg/mg linoleic acid)	H_2O_2 (mg/mg H_2O_2)
Roselle extract	1.16	7.12	0.02	4.44
Ascorbic acid	0.17	0.04	1.12	1.78
Trolox	0.26	4.27	0.01	0.80

EC_{50} = the concentration of antioxidant needed to decrease the initial substrate concentration by 50%

In order to elucidate the contribution of sucrose to the antioxidant capacity of roselle extract in the model system, the experiment was carried out by FTC assay to evaluate the inhibition of lipid peroxidation. As seen in Figure 1, antioxidant activity of roselle extract at all concentrations of sucrose showed no difference.

B. Effects of sucrose concentration on the antilipoperoxidant efficiency of roselle extract in meat products

The TBARS values of Chinese-style sausages and pork chips during storage at room temperature under vacuum packed in plastic bag are presented in Figure 2. The analysis of variance for the TBARS data indicates that the TBARS values were significantly affected ($p \leq 0.05$) by both the sucrose concentration and the storage time. Initial TBARS values for all samples were significantly higher than those for the control (0 % sucrose) ($p \leq 0.05$). During storage lipid oxidation of all samples started to increased rapidly after 7 days and 21 days for the Chinese-style sausages and pork chips, respectively. Obviously, samples containing higher amount of sucrose showed greater lipid oxidation and all samples with sucrose addition gave higher TBARS values over the control during storage. The lower TBARS values found in pork chips comparing to Chinese-style sausages might be due to the lower fat content of pork chips (Table 1) and consequently, less lipid oxidation occurred.

IV. CONCLUSION

In vitro antioxidant property assays showed that roselle extract exhibited excellent antioxidant activity, especially for the inhibition of linoleic

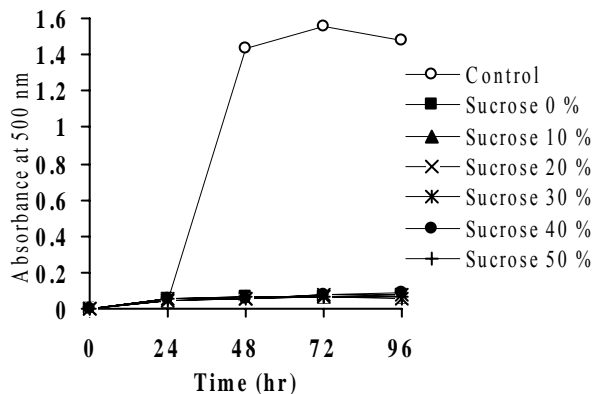


Figure 1. Antioxidant properties of roselle extract with different sucrose concentration determined with the FTC

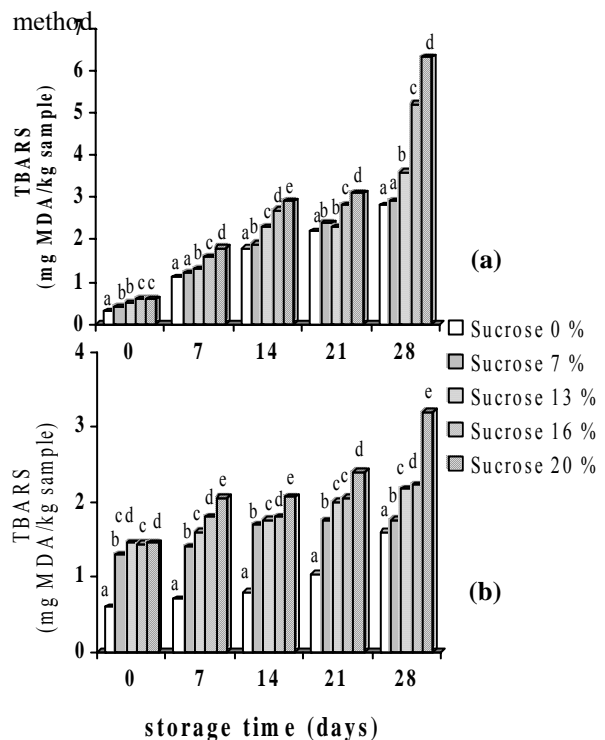


Figure 2. Development of TBARS during storage of Chinese-style sausages (a) and pork chips (b) containing with different concentrations of sucrose. Samples for the same storage time followed by the same letter were not significantly different ($p \leq 0.05$).

oxidation. Although sucrose showed no effect on inhibition of linoleic oxidation in model reaction, it significantly affected the ability of roselle extract in inhibition of lipid oxidation in Chinese-style sausages and pork chips. Results indicate that roselle extract could be used as a natural antioxidant in certain meat products with low sucrose concentration.

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