Dissertation	Factors affecting properties and antioxidant capacities of roselle (*Hibiscus sabdariffa* L.) extracts and their application in meat products
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ABSTRACT

A growing trend in food industry is the development of functional foods or foods from nature. The roselle calyces have attractive color and contain bioactive compounds associated with a number of health benefits that have been confirmed by scientific evidences. Three experiments were conducted to determine: i) the physicochemical and antioxidant properties of roselle extracts prepared by two extraction methods (OG and NG), ii) the antioxidant capacity of roselle extract in Chinese-style sausage as affected by sweeteners and iii) the ability of roselle extract in reactive nitrogen species (RNS) scavenging *in vitro* and nitrite scavenging in meat products.

The properties OG and NG roselle extracts were evaluated under different pH (3.0 and 4.0) with or without sucrose (20 %) and heat treatment (50 to 70 °C). The Delphinidin 3-sambubioside (Dp 3-sam) and Cyaniding 3-sambubioside (Cy 3-sam) were identified as major and minor anthocyanins found in both OG and NG roselle extracts respectively. Significant loss of pH and temperature-dependent anthocyanin pigments were observed in all roselle extract model samples. In addition, results indicated that the highest pigment extraction efficiency was found in the NG roselle at pH 3.0 than at pH 4.0 and then OG roselle. It can be concluded that nano-grinding may favor the extraction of pigments but lead to their serious degradation during storage. *In vitro* antioxidant activities of the OG and NG roselle extracts were evaluated by ferrous ions chelating activity (FICA), trolox equivalent antioxidant capacity (TEAC) and ferric thiocyanate (FTC). Overall results showed that NG samples tended to exhibit higher antioxidant activities for all assay methods compared to the OG samples.

The effect of different sweeteners including sucrose, lactitol, maltitol and xylitol on the physicochemical property and oxidative stability of Chinese-style sausage (CSS) with addition of

roselle anthocyanin extracts (RAE) were evaluated. Xylitol added CSS showed lower moisture content and water activity compared to other sweetener evaluated. No serious changes of color and texture parameters were observed for all CSS samples during 28 day storage. The RAE treated CSS with sucrose addition showed the greatest TBARS values during storage, while the samples with sugar alcohols had significantly lower values. The xylitol can only promote the antioxidant activity of RAE in prevention of lipid oxidation in CSS. Xylitol addition at 16.6 % in the CSS resulted in the sausage with quality characteristics similar to the control CSS. However, xylitol at higher concentration (21.6 %) could cause the pro-oxidant activity in the RAE treated CSS. The sensory evaluation by Quantitative descriptive analysis (QDA) and 7-point hedonic scale revealed a significant higher panel preference for RAE treated CSS with xylitol addition compared to the sucrose added samples and the control CSS (sucrose without RAE). Results also proved the potential use of 16.6 % xylitol with 0.3 % RAE in CSS that ensured the overall quality of the products. It can be concluded that xylitol is a promising alternative sweetener in CSS especially when RAE is used as natural antioxidant.

The ability of RAE in scavenging of reactive nitrogen species (RNS) compared to anthocyanins from black carrot and grape was investigated. These anthocyanin samples exhibited concentration and pH dependent in nitrite scavenging activity. At pH 3.0, the activity increased from 15 to 80 % when the concentration of the anthocyanins increased from 0.1 to 1.0 mg/ml. The nitrite scavenging activity was dramatically decreased when the pH of the reaction system increased from pH 3.0 to 9.0. In addition, the concentration dependent activity was also observed for the nitric oxide scavenging and inhibition of peroxynitrite induced oxidation of Evan-blues dyes. The potency of RNS scavenging activity for the anthoycyanins tested was in the order: grape > roselle > black carrot.

The RAE was then evaluated for its capacity in nitrite reduction in meat products including Vienna pork sausage and traditional Thai fermented pork (Nham). The residual nitrite in RAE treated (0.3 %) Vienna pork sausage with initial 125 and 250 ppm nitrite reduced to 65 and 168 ppm, respectively after refrigerated storage for 24 days. On the other hand, residual nitrite in all Nham samples rapidly decreased > 90 % of the initial nitrite level after 3 days of fermentation at room temperature. Degradation kinetics of nitrite in Nham was the first-order kinetics. The lactic acid fermentation enhanced the reduction of residual nitrite in Nham.

Overall results revealed that roselle calyces can be a good source of anthocyanins with strong antioxidant activity and can potentially be used as natural antioxidant in meat products.

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LIST OF ABBREVIATION

ACN	Anthocyanins
OG	“Original-grinding roselle extracts
Ng	“Nano-grinding roselle extracts
Cy	Cyanidin
Pg	Pelargonidin
Mv	Malvidin
Pt	Petunidin
Pn	Peonidin
Dp	Delphinidin
FICA	Ferrous ions chelating ability
TEAC	Trolox equivalent antioxidant capacity
FTC	Ferric thiocyanate antioxidant assay
TBARS	Thiobarbituric acid reactive substances
TEP	1,1,3,3 tetramethoxypropane
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
$NaNO_2$	Sodium nitrite
$ONOO^-$	Peroxynitrite
NO^-	Nitric oxide
HNO_2	Nitrous acid
N_2O_3	Dinitrogen trioxide
RO_2^-	Peroxyl radicals
CSS	Chinese-style sausage
BHA	Butyric hydroxyanisole
RAE	Roselle anthocyanin extracts
IC_{50}	inhibitor concentration which results in 50% inhibition of activity