Title **Changing of Antioxidant Activity and Phenolic Content of Mango Seed Kernel Extract during Storage**

ชื่อเรื่อง **การเปลี่ยนแปลงกิจกรรมแอนติออกซิแดนท์และปริมาณฟีนอลิกของสารสกัดจากเนื้อเมล็ดมะม่วงในระหว่างการเก็บรักษา**

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Abstract

The changes in antioxidant activity using DPPH and thiocyanate methods and total phenolic content using Folin-Ciocalteu's phenol reagent of mango (*Mangifera indica* Linn.cultivar Chok-Anan) seed kernel extract during storage at freezing (-20°C), refrigerated (7°C) and room (28-32°C) temperatures were monitored for 182 days. The extracts were packed in Al. foil and PE bag during storage. Water activity of extract samples was also evaluated during storage. The antioxidant properties (AP) and total phenolic content (TPC) of mango seed kernel (MSK) extract were decreased during storage. The higher temperature during storage, the higher loss of AP and TPC were obtained. The water activity of extract packing in PE bag was higher than that packing in Al. foil. At the end of 182-day storage, AP of extract in Al. foil stored at -20°C was not different from initial value. The results indicated that storage temperature and type of packaging affected to phenolic antioxidant properties of mango seed kernel extract.

Keywords : pH, oil, water, partition coefficient, phenolic compound.

Introduction

Mango has been cultivated for about 4,000 years and its production and consumption has gradually increased as its popularity has grown. At least 87 countries grow over 26,286,255 MT per annum. Among internationally traded tropical fruits, mango ranks only second to pineapple in quantity and value. Mango production is highest in India, at 41% of the world's production (10,800,000 MT), followed by China, Thailand, Mexico, Pakistan, Indonesia, the Philippines, Nigeria, and Brazil (Youngmok et al., 2004). Mangoes are consumed as fresh fruits and after processing into pickles, chutneys, canned or dried goods, juices, or nectars. During processing of mango, by-products such as peel and kernel are generated. Kernel contributes to about 17-22% of the fruit (Soong and Barlow, 2004).

Mango seed kernel was also shown to be a good source of phytosterols as campesterol, β-sitosterols, stigmasterol and also contains tocopherols (Soong and Barlow, 2004). Gallotannins and condensed tannin-related polyphenols detected by thin-layer chromatography were reported to be present in mango seed kernel (MSK) (Arogba, 1997). In addition, tannins from dry MSK meal were reported to contain tannic acid, gallic acid and epicatechin in the ratio 17:10:1, respectively (Arogba, 2000). Abdalla, et al. (2006) has recently characterized the phenolic compounds in Egyptian MSK. They are tannins, gallic acid, coumarin, caffeic acid, vanillin, mangiferin, ferulic acid, cinamic acid and unknown compounds. These data show that MSK contains various phenolic compounds.

Limitation of using plant extracts as antioxidant is relevant to safety, availability, cost and shelf life. The shelf life of extract is defined as the time from manufacture to customer, where an extract remains same quantity of phenolic constituents as labeling under recommended conditions (Piljac-Žegarac et al., 2009). This experiment was undertaken with the aim of determining how refrigerated and frozen storage affected the antioxidant activity and total phenolic content of mango seed kernel extracts which were packed in Al. foil and PE bag. The stability of extract polyphenols is influences by many factors such as exposure to light, air or different storage temperatures (Piljac-Žegarac et al., 2009).

Materials and Methods

Materials. Folin Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and sodium carbonate were purchased from Sigma Chemical Co., Ltd (St. Louise, USA). Tannic acid was purchased from Acros Organics (New Jersey, USA). The other chemicals and solvents used in this experiment were analytical grade purchased from Sigma-Aldrich Co., Ltd (Steinheim, Germany).

Three batches of sun dried seeds from ripened mango (*M. indica* cultivar Chok-Anan) were donated by Woraporn Co., Ltd., a mango processing manufacturer in Thailand from March to June in 2008 as by-products. The seeds were washed and sun dried in the greenhouse for three days and the kernels were removed manually from the seeds for further extraction. Moisture content on a dry weight basis according to AOAC (1990) of dried mango seed kernel (MSK) equaled to 9.81±0.34%. The dried material was kept in freezer at -20°C no longer than two months.

Extraction of MSK antioxidant. The freezing kernel (80 g) were blended for 1 min with ethanol at -20°C and the containers were then flushed with nitrogen and shaken for 4.5 hours in the dark at 30°C (Maisuthisakul et al., 2007). The supernatant, after filtration through cheesecloth and Whatman No 4 filter paper, was evaporated under vacuum. Sample was dried in a freeze dryer and stored in Al. foil after flushing with nitrogen at -20°C until analysis by determining the antioxidant properties through using the DPPH and the thiocyanate methods. The MSK extracts were also used to evaluate total phenolic content and water activity.

Storage condition of MSK extract. All dried extracts were stored at room temperature (28-32°C), 7°C and -20°C in PE bag and Al. foil for 6 months. All samples were sampling out every 14 days by determining the antioxidant properties through using the DPPH and the thiocyanate methods. The MSK extracts were also used to evaluate total phenolic content and water activity.

Determination of antioxidant properties. The total free radical-scavenging capacity of MSK was determined by using the DPPH (Masuda et al. (1999). The antioxidant activity in a linoleic acid emulsion system (Hu et al., 2004) was also determined.

The free radical scavenging activity of MSK was evaluated using the stable radical DPPH according to the method of Masuda et al. (1999). The radical scavenging activity (%) was plotted against the plant extract concentration (μg/mL) to determine the concentration of extract that reduces activity by 50% (EC_{50}). These values were changed to antiradical activity (A_{AR}) defined as $1/EC_{50}$, since this parameter increases with antioxidant activity. All determinations were performed in triplicate.

The antioxidant activity in a linoleic acid emulsion system of MSK was determined using the thiocyanate method with some modifications. The 0.5 ml of each sample in absolute ethanol was mixed with 0.5 ml of 5.21% linoleic acid, 1 ml of 0.05 M phosphate buffer (pH 7), and 0.5 m of distilled water and placed in a screw capped tube. The reaction mixture was incubated in the dark at 40°C in an oven. Aliquots of 0.1 ml were removed every 24 h during incubation and the degree of oxidation was measured by sequentially adding ethanol (9.7 ml, 75%), ammonium thiocyanate (0.1 ml, 30%) and ferrous chloride (0.1 ml, 0.02 M in 3.5% HCl). After the mixture was rested for 3 min, the peroxide value was determined by monitoring absorbance at 500 nm until the absorbance of the control reached the maximum. The degree of linoleic acid peroxidation was calculated using the following formula: antioxidant activity = $[(A_{control} - A_{sample})/A_{control}] \times 100$. The antioxidant activity was plotted against sample concentration in order to determine the concentration required to achieve a 50% inhibition of linoleic acid oxidation [AA_{50}]. All tests and analyses were carried out in triplicate and averaged.

Determination of total phenolic content. The total phenolic content of extracts was determined using the Folin-Ciocalteu's phenol reagent (modified from Kähkonen et al., 1999). The concentration of total phenolic compounds in all plant extracts was expressed as mg of tannic acid equivalent per g dry weight of MSK using a linear equation. All determinations were performed in triplicate.

Determination of water activity. The dried extracts were determined the water activity by using Thermoconstanter NOVASINA instrument at 25°C.

Statistical Analysis. Each experiment, from sample preparation to analysis, was repeated in triplicate, and the data were then analyzed by SPSS software program (SPSS Inc., Chicago, IL). The general linear model procedure was applied and Duncan's New Multiple Range Tests was used to compare the mean values at $P < 0.05$. Mean values and pooled standard error of the mean (SEM) were calculated.

Results and Discussion

Changing of antioxidant property of mango seed kernel extract during storage. The free radical scavenging activity and the antioxidant activity in a linoleic acid emulsion system of MSK during storage at -20°C (freezing temperature), 7°C (refrigerated temperature) and 28-32°C (room temperature) in aluminum foil (Al. Foil) and PE (PE) bag were shown in Table 1, 2 and 3. In this study, storage temperature and packaging affected to antioxidant properties of MSK. The antioxidant properties of MSK in Al. foil at freezing temperature were not change during storage for 182 days (Table 1). While that in PE bag were slightly change during storage at -20°C. Unfortunately, there is no report on shelf life of plant extracts. There are interested only using plant extracts to extend shelf life of food (DeJong and Lanari, 2009; O'Keefe and Wang, 2006).

Table 1 Antioxidant properties of mango seed kernel extract during 6-month storage at -20°C *

Storage time (days)	DPPH• scavenging activity (A_{AR}, $1/EC_{50}$)		Antioxidant efficiency ($1/AA_{50}$)	
	Al.Foil ^{NS}	PE bag	Al. Foil ^{NS}	PE bag
0	4.71±0.06	4.73 ^{a} ±0.11	0.019±0.002	0.019 ^{a} ±0.003
14	4.70±0.05	4.70 ^{a} ±0.10	0.019±0.002	0.019 ^{a} ±0.003
28	4.71±0.03	4.68 ^{a} ±0.08	0.019±0.002	0.019 ^{a} ±0.004
42	4.70±0.09	4.66 ^{a} ±0.07	0.019±0.002	0.018 ^{a} ±0.002
56	4.71±0.10	4.65 ^{a} ±0.05	0.019±0.002	0.018 ^{a} ±0.003
70	4.69±0.11	4.66 ^{a} ±0.03	0.019±0.002	0.018 ^{a} ±0.003
84	4.70±0.08	4.65 ^{a} ±0.04	0.019±0.002	0.017 ^{a} ±0.003
98	4.69±0.04	4.63 ^{ab} ±0.09	0.019±0.002	0.017 ^{a} ±0.003
112	4.68±0.09	4.60 ^{ab} ±0.11	0.019±0.002	0.016 ^{a} ±0.002
126	4.67±0.08	4.61 ^{ab} ±0.12	0.019±0.002	0.015 ^{a} ±0.001
140	4.68±0.07	4.58 ^{b} ±0.05	0.019±0.002	0.014 ^{ab} ±0.003
154	4.68±0.08	4.54 ^{b} ±0.02	0.019±0.002	0.014 ^{ab} ±0.003
168	4.70±0.02	4.50 ^{bc} ±0.04	0.019±0.002	0.013 ^{b} ±0.003
182	4.70±0.04	4.44 ^{c} ±0.06	0.019±0.002	0.012 ^{b} ±0.003

* Data followed by different letters within each column are significantly different according to Duncan's New Multiple Range Test at $P < 0.05$. Data obtained from three replicates.

When the extracts were stored at refrigerated temperature, the antioxidant activity of MSK extract decreased during storage (Table 2). These may explain that the degradation of phenolic compounds in MSK extract was dependent on storage time and the type of phenolics. The degradation rate increased if storage temperature was increased. Hence, the antioxidant activity of MSK extract stored at 30°C was changed very fast (Table 3) comparing to the other temperature storage conditions. The storage condition and packaging material should be considered when the extract was kept for a long time. When considered only temperature, the appropriate temperature for extract storage was -20°C or freezing zone. This condition can keep the extract longer than 182 days or more than six months, while the extract did not change the antioxidant properties during storage.

Table 2 Antioxidant properties of mango seed kernel extract during 6-month storage at 7°C *

Storage time (days)	DPPH• scavenging activity (A_{AR}, $1/EC_{50}$)		Antioxidant efficiency ($1/AA_{50}$)	
	Al.Foil	PE bag	Al. Foil	PE bag
0	4.70 ^{a} ±0.02	4.70 ^{a} ±0.02	0.018 ^{a} ±0.002	0.019 ^{a} ±0.002
14	4.68 ^{ab} ±0.03	4.68 ^{ab} ±0.03	0.018 ^{a} ±0.004	0.018 ^{a} ±0.001
28	4.68 ^{ab} ±0.01	4.65 ^{b} ±0.01	0.018 ^{a} ±0.001	0.017 ^{ab} ±0.002
42	4.66 ^{ab} ±0.04	4.60 ^{c} ±0.04	0.018 ^{a} ±0.004	0.016 ^{b} ±0.001
56	4.60 ^{c} ±0.02	4.50 ^{d} ±0.02	0.017 ^{a} ±0.003	0.014 ^{c} ±0.002
70	4.62 ^{c} ±0.01	4.42 ^{e} ±0.01	0.017 ^{a} ±0.002	0.014 ^{c} ±0.001
84	4.59 ^{c} ±0.03	4.35 ^{f} ±0.03	0.016 ^{a} ±0.001	0.012 ^{cd} ±0.002
98	4.56 ^{cd} ±0.03	4.21 ^{g} ±0.03	0.016 ^{a} ±0.001	0.011 ^{d} ±0.001
112	4.50 ^{d} ±0.02	4.10 ^{h} ±0.02	0.016 ^{a} ±0.002	0.010 ^{de} ±0.003
126	4.48 ^{d} ±0.04	4.02 ^{i} ±0.04	0.015 ^{a} ±0.001	0.009 ^{e} ±0.001
140	4.42 ^{e} ±0.02	3.91 ^{j} ±0.02	0.014 ^{a} ±0.002	0.007 ^{f} ±0.001
154	4.37 ^{f} ±0.01	3.80 ^{k} ±0.01	0.013 ^{ab} ±0.002	0.007 ^{f} ±0.001
168	4.32 ^{g} ±0.03	3.65 ^{l} ±0.03	0.012 ^{b} ±0.002	0.006 ^{f} ±0.002
182	4.26 ^{h} ±0.01	3.41 ^{m} ±0.01	0.011 ^{b} ±0.002	0.005 ^{f} ±0.002

* Data followed by different letters within each column are significantly different according to Duncan's New Multiple Range Test at $P < 0.05$. Data obtained from three replicates.

Changing of the total phenolic content and water activity of mango seed kernel extract during storage. To better understand the effect of temperature storage and type of package on antioxidant activity of MSK, the total phenolic content and water activity of MSK needs to be evaluated. The total phenolic content of MSK extract in PE bag was lower than that in Al. foil (Figure 1). These can be explained why the antioxidant activity of MSK extract in Al. foil showed higher value when stored at different temperature storage. Moreover, the water activity of MSK extract in Al. foil was slightly changed (Figure 2a) comparing to that in PE bag (Figure 2b). Julkunen-Tiito (1985) reported that prolong exposure at 40°C can also cause phenolic degradation in grape pomace, whereas 1-8 hour, no degradation was observed (Moure et al., 2001). The quantity of total phenolics during storage for dried samples was decreased due to phenolic decomposition from higher water activity (a_w) as Figure 2b. The water activity increased from 0.3 to 0.55 can cause rapidly deteriorative reactions from oxidation. These results showed that the extract packing in Al. foil was appropriate for keeping a good quality of the extract during storage better than PE bag. The water transmission and oxygen permeability rate of Al. foil is lower than PE bag that is the reason why using Al. foil as packaging material is better than using PE bag for keeping the extract. The future work focusing on the effect of light on phenolic stability of MSK extract will be further investigated.

Table 3 Antioxidant properties of mango seed kernel extract during 6-month storage at room temperature (28-32ºC) *

Storage time (days)	DPPH• scavenging activity (AAR, $1/EC_{50}$)		Antioxidant efficiency ($1/AA_{50}$)	
	Al.Foil	PE bag	Al. Foil	PE bag
0	4.71 ^{a} ±0.03	4.71 ^{a} ±0.02	0.018 ^{a} ±0.002	0.019 ^{a} ±0.002
14	4.66 ^{b} ±0.02	4.62 ^{b} ±0.04	0.018 ^{a} ±0.004	0.018 ^{a} ±0.001
28	4.64 ^{c} ± 0.02	4.60 ^{b} ±0.03	0.017 ^{a} ±0.001	0.017 ^{ab} ±0.002
42	4.62 ^{cd} ±0.04	4.51 ^{c} ±0.04	0.017 ^{a} ±0.004	0.016 ^{b} ±0.001
56	4.60 ^{d} ±0.01	4.41 ^{d} ±0.04	0.017 ^{a} ±0.003	0.014 ^{c} ±0.002
70	4.56 ^{e} ±0.02	4.34 ^{e} ±0.02	0.016 ^{a} ±0.002	0.013 ^{c} ±0.001
84	4.51 ^{f} ±0.01	4.22 ^{f} ±0.03	0.016 ^{a} ±0.001	0.012 ^{cd} ±0.002
98	4.46 ^{g} ±0.03	4.09 ^{g} ±0.03	0.015 ^{a} ±0.001	0.010 ^{d} ±0.001
112	4.40 ^{h} ±0.02	3.95 ^{h} ±0.02	0.014 ^{a} ±0.002	0.009 ^{de} ±0.003
126	4.38 ^{i} ±0.04	3.81 ^{i} ±0.04	0.013 ^{ab} ±0.001	0.008 ^{ef} ±0.001
140	4.32 ^{j} ±0.02	3.70 ^{j} ±0.02	0.012 ^{b} ±0.002	0.007 ^{f} ±0.001
154	4.27 ^{k} ±0.01	3.54 ^{k} ±0.03	0.011 ^{b} ±0.002	0.006 ^{fg} ±0.001
168	4.22 ^{l} ±0.01	3.38 ^{l} ±0.03	0.011 ^{bc} ±0.002	0.005 ^{g} ±0.002
182	4.16 ^{m} ±0.02	3.15 ^{m} ±0.03	0.010 ^{c} ±0.002	0.004 ^{g} ±0.002

* Data followed by different letters within each column are significantly different according to Duncan's New Multiple Range Test at $P < 0.05$. Data obtained from three replicates.

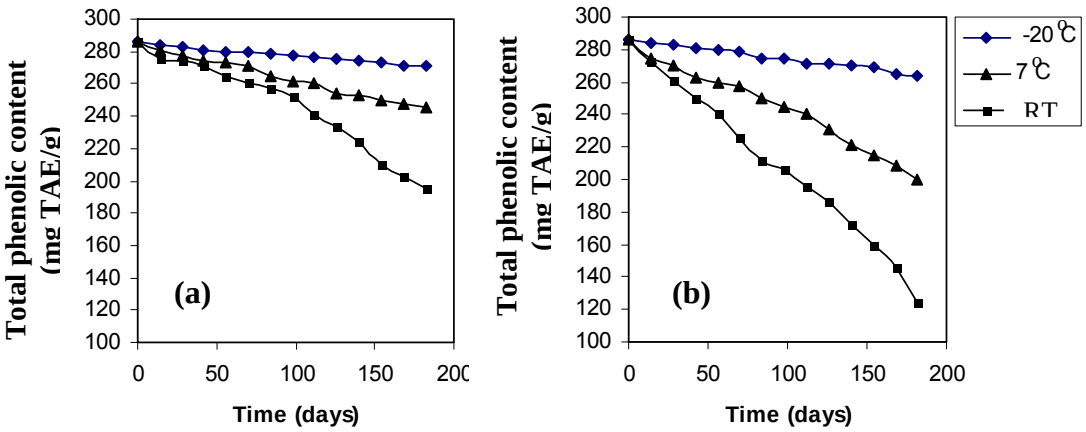

Figure 1 Total phenolic content of mango seed kernel extract in (a) Al. foil and (b) PE bag during storage at -20°C, 7°C and RT (28-32°C).

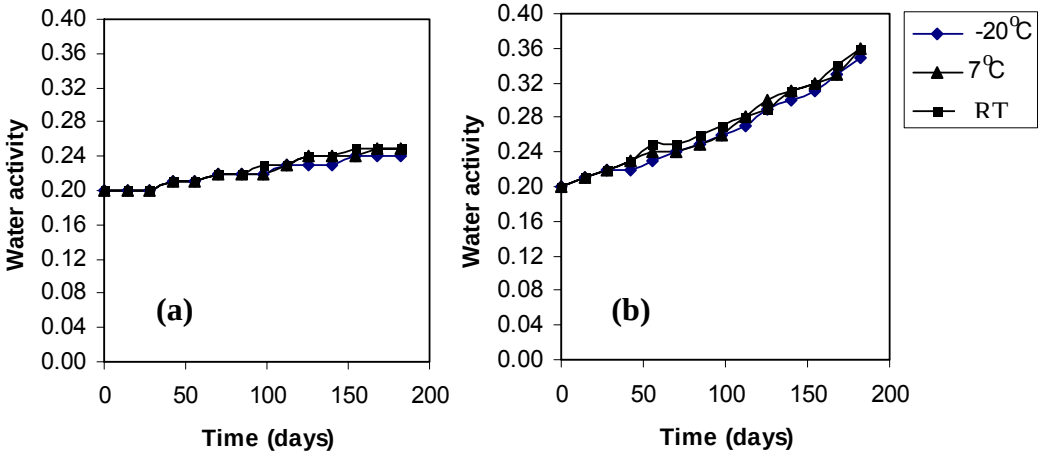

Figure 2 Water activity of mango seed kernel extract in (a) Al. foil and (b) PE bag during storage at -20°C, 7°C and RT (28-32°C).

Conclusion

The present study demonstrates for the first packaging and temperature affects on phenolic antioxidants of mango seed kernel extracts. The appropriate condition for keeping the MSK extract was packing in Al. foil and stored at -20°C. This condition can keep a good quality of the extracts more than 182 days.

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