Original Article

Total Phenolic Content and Antioxidant Activity of Fresh and Preserved Fruits of *Ellaeocarpus hygrophilus* Kurz.

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ABSTRACT

Objective: To determine the total phenolic content and antioxidant activities of fresh Spanish plum fruits (*Ellaeocarpus hygrophilus* Kurz., family Elaeocarpaceae.) in various ages and to examine the influence of preserved methods which were chilling and pickling on the total phenolic content and antioxidant activities of the fruit. **Method:** The meat of Spanish plum fruit was preserved by storage at -70, -4, 0 and 4 °C for 1, 4 and 6 months. **Results:** The duration and temperature of storage resulted in no significant effect on pH of the fruit juice. The various durations of chilling showed no significantly different effect on % brix of the fruits, but lower chilling temperature was associated with increased % brix. The younger fruit contained higher amount of total phenols in its crude extracts and juice than the older ones. The duration of chilling showed no effect on total phenols of the fruits. The duration and temperature of chilling of the fruits before juice squeezing gave significant decreasing effect on the amount of total phenols in the juices. The antioxidant activities of the fruits with all ages were low. The duration and temperature of chilling and prickle duration of the fruits had no significant effect on antioxidant activities. **Conclusion:** The preserved condition could save pH, % brix, amount of total phenol and antioxidant activities of the Spanish plum fruit.

Keywords: Spanish plum, *Ellaeocarpus hygrophilus* Kurz, antioxidant activity, phenolic compounds, preserved fruit

Thai Pharm Health Sci J 2008;4(1):21-28[§]

Introduction

In 1992, Harman et al reported the involvement of reactive oxygen species (ROS) in aging process.[1] The ROS also plays an important role in oxidative stress in the elderly that relates to severe vascular and neurodegenerative diseases.[2] Vegetables and fruits are well known as sources of redox-active secondary metabolites (i.e. antioxidants) such as polyphenols. These substances could prevent human body from oxidative damage. A large cumulative body of evidences show that a diet rich in cereal, vegetable and fruits is related to lower incidence of coronary disease, neurodegenerative illnesses and cancer.[3]

Among various kinds of fruit produced in Thailand, Spanish plum is one of the most commonly consumed among Thais. Spanish plum or *Ellaeocarpus hygrophilus* Kurz., family Elaeocarpaceae, is a foreign plant that has been widely grown in local orchards of Thailand. Its fruit has a sour and astringent taste. The mature, sugar preserved (glazed fruit) and saline pickled fruits are famous as a snack with dipping. In Thai traditional medicine, the fruit has anti-thirst effect by stimulating saliva secretion and reducing body overheat.[4] Its bark also has an astringent taste. Digestion of the bark is

[§] 14th year of Srinakharinwirot Journal of Pharmaceutical Science

used as haematotonic for women after giving birth.[5] The fruit of Spanish plum is a rich source of nutritional substances. As reported by the Ministry of Public Health of Thailand in 1987, nutritional value of Spanish plum per 100 g of edible part of the fresh fruit included 86 calories energy, 0.3 g fat, 22.3 g carbohydrate, 1 g protein, 0.5 g fiber, 14 mg calcium, 35 mg phosphorus, 0.9 mg iron, 375 I.U. vitamin A, 0.09 mg vitamin B1, 0.05 mg vitamin B2, 49 mg vitamin C, and 0.4 mg niacin.[6] Furthermore, its kernel has vegetable oil that is under study for commercial usage.

With its rich nutritional source and potential for commercial product development, this study aimed to determine a value added of preserved Spanish plum fruits in addition to the fresh fruit. Specific objectives of this study were 1) to determine the total phenolic content and antioxidant activities of fresh Spanish plum fruits in various ages, and 2) to examine the influence of preserved methods where were chilling and pickling on the total phenolic content and antioxidant activities of the Spanish plum fruits.

Material and Methods

Plants

Spanish plum fruits with various ages (1, 2.5, 3 and 4 months) were purchased from local market and collected from orchards in Nakhon-Pathom province by the researchers, during July 2005. The fruit was identified by a researcher (P. Wetwitayaklung). The dry voucher specimens were deposited in the Department of Pharmacognosy, Silpakorn University in Nakhon-Pathom, Thailand.

Chemicals

$ABTS^{2-}$,2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) was obtained as sulfonic acid from Sigma (St. Louis, USA). Trolox (or (+/-)–6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid) was purchased from Aldrich (Steinheim, Germany). Potassium persulfate ($FeSO_4$x $7H_2O$) and sodium acetate were from Asia Pacific Specialty Chemicals Limited (Seven Hills, Australia). Folin-Ciocalteu reagent, $FeCl_3$ x $6H_2O$ and NaCl were purchased from CarLo ErbaReagenti (Milano, Italy). 2,4,6-tri-pyridyl-s-triazine (TPTZ) was from Fluka Chemie GmbH (Switzerland) and methanol was purchased from Merck (Darmstadt, Germany).

Preservation preparations

A) Chilling conditions and determination of physical properties for *E. hygrophilus* (Spanish plum) fruit

Seeds of four-month old fresh fruits were removed. The seedle°ss fruits were chilled either by chilling at 4, 0 and -4 °C (SJ-D58L SHARP, Sharp Appliance, Thailand) or by freezing at and -70 °C (MDF-U50V SANYO, Sanyo Electric Co., Japan) for 1 week, and 1, 4 and 6 months. Once the chilling period ended, the fruits were blended using a household blender and squeezed for their juice. The acidity was determined by a hand-held refractometer (Pocket PAL, Japan) (n = 3). The pH results were compared to that of the juice squeezed from fresh fruits of the same age.

B) Pickling conditions for *E. hygrophilus* fruit

Fresh fruits of 2.5 months old were pickled in salt water (15% w/w NaCl) for 3, 15 and 30 days. Fresh fruits of four months old were pickled in the same concentration of salt water for 30 days.

Methanolic extract of *E. hygrophilus* fruit meat

The meat of the fresh fruit with various ages, the meat of the fruits undergoing chilling for 1 week or 1 month, and the meat of the pickled fruit were dried at 55 °C for 72 hrs before grinding and passing through sieve No. 30. The samples were kept at 4 °C until used. The methanolic extraction was performed by maceration of the dried samples in methanol in a ratio of sample to methanol of 1:4 for 72 hrs. The filtrates from the extracts were evaporated using a rotary evaporator (Buechi R205, Switzerland).

Quantification of total polyphenols[7]

The 0.5 mL of methanolic extract (10 μg/mL) of Spanish plum juice was mixed with 0.5 mL of Folin-Ciocalteu reagent and 0.5 mL 10% Na_2CO_3. The mixture was shaken and placed at room temperature for 1 hr before measuring the absorbance at 760 nm. The calibration curve was prepared using gallic acid with a concentration range of 2 - 8 mg/L. The total polyphenols was calculated and reported as gallic acid equivalent (GEA), g of gallic acid in 100 g of methanolic extract.

Determination of antioxidant activity

An antioxidant activity was performed by determination of scavenging effect on $ABTS^{\bullet+}$ radical. The $ABTS^{\bullet+}$ solution was prepared by mixing equal volume of 7 mM $ABTS^{2-}$ in water with 4.9 mM potassium persulfate in water. The solution was protected from light and stored at room temperature for 12 – 16 hrs. $ABTS^{\bullet+}$ formation was checked for its absorbance (A) at 734 nm using UV-Vis-Spectrophotometer, (Agilent 8453E UV-Visible Spectroscopy System, Agilant Technology, USA.). The absorbance of $ABTS^{\bullet+}$ was equilibrated to 0.7 (± 0.02) by diluting with water at room temperature.

All samples were prepared in a concentration range of 50 - 500 μg/50 μL, except for the extract from fresh fruit of one month old which was in a range of 25 – 250 μg/50 μL. A portion of each dilution (50 μL) was mixed with 3 mL of $ABTS^{\bullet+}$ solution. After the mixture had been allowed to stand for 6 minutes at room temperature, its absorbance was measured at 734 nm using a spectrophotometer. Trolox was used as a standard. The antioxidant capacity was calculated as an average of four replicate absorbance measurements, and reported as % inhibition along with IC_{50} and also as Trolox equivalent antioxidant capacity (TEAC).

Calculation of antioxidant capacity

Percent inhibition could be calculation as follows;

$$\% \text{ inhibition } T = \frac{A \text{ (solvent)} - A \text{ (compound)}}{A \text{ (solvent)}} \times 100 \quad ;$$

where A (solvent) was an absorbance of the sole solvent (no extract added) and A (compound) was an absorbance of the mixture of samples (or of Trolox) and $ABTS^{\bullet+}$ solution at 734.

The curve was plotted between % inhibition and concentration of sample or Trolox solutions. The regression coefficient (r^2) was calculated from the linear curve. The IC_{50} was determined from the concentration that resulted in 50% inhibition. TEAC was the ratio of % inhibition of the sample to % inhibition of Trolox at the given concentration.

Statistical analysis

Statistical analyses were performed using one-way ANOVA, two-way ANOVA and linear regression analysis. A significance level of less than 0.01 was considered to indicate a statistically significant change. SPSS® Statistics Package version 11.5 (SPSS Inc.) and MS Excel® Software with CORREL statistical function (Microsoft Corp.) were used in the analysis.

Results and Discussions

1. Acidity and sugar content of preserved *E. hygrophilus* fruits

After Spanish plum fruits were preserved by chilling at -70, -4, 0 and 4 °C for 1, 4 and 6 months in the refrigerator, the increase in pH was found in each of all chilled fruit samples with different chilling periods and temperatures (Table 1). The 1-month chilling fruits showed reduction of acid property (from pH 3.5 to 5) in every chilling temperature, except 6 month fruit after chilling at 0 °C (from pH 3.5 to 6). The differences in duration and temperature of chilling were not associated with pH change. This result was similar to the one reported by Blanda et al (2008) that the frozen, the chilled frozen and the osmodehydrated strawberries at -18 °C for 1 month resulted in a small range of pH change from the fresh fruits.[8] For sugar content of Spanish plum, the condition of chilling caused a small

increase of sugar content. Differences in durations of chilling caused no significantly different effect on % brix change, while various temperatures of preservation resulted in a significant effect on % brix change (P = 0.001). In each of the chilling periods, % brix was found highest with the chilling temperature of -70 °C. While % brix of fresh fruit was 6.7, those of the preserved fruits under -70 °C chilling temperature were 9.9, 10.1 and 9.9, for the chilling periods of 1, 4 and 6 months respectively. From this result, it may conclude that a lower temperature of chilling (-70 °C) was better than a higher one since it resulted in a higher % brix. Our results agreed with the study of Bartolomé et al (1996) that the soluble solid (or % brix) of one year storage of frozen pineapple cultivar named Smooth Cayenne stored at -18 °C was increased (% brix = 12.48 and 15.17 for fresh and one-year stored samples respectively).[9]

Table 1 Average pH and sugar content of 4 month Spanish plum fruits under various preserving condition.

Preserved condition		Acidity (pH)	Sugar content (% Brix)
Chilling duration (month)	Chilling temperature (°C)		
1	-70	5	9.9
	-4	5	6.6
	0	5	7.9
	4	5	7.7
4	-70	5	10.1
	-4	5	6.8
	0	4.5	7.9
	4	5	8.0
6	-70	4.5	9.9
	-4	4.5	7.1
	0	6	6.3
	4	4.5	8.0
0 (Fresh fruit)	-	3.5	6.7

2. Methanolic extractive value

The % yields of dried methanolic extracts of the fruits in this experiment are shown in Table 2. Fruits with ages of 1 and 2 months did not differ in amount of % yield from the extracts. However, the more the fruits matured, the higher % yield of the extracts was given. The meat of the 4-month fruit gave the highest % yield from methanol extract (49.47%), while skin of the fruit gave the lowest % yield (20.93%). The pickle condition of fruits probably presented a little lowering effect to the amount of % yield of the extracts, however the chilling condition did not.

3. Total phenolic compounds

The amount of total phenols of the crude extracts of the fruits is shown in Table 3. The total phenol amounts were lower in the extracts of older fruits. The result also indicated that the phenolic compounds accumulated more in the fruit skin than in the meat. The chilling and prickling condition caused a small decrease of phenolic content of the fruit. The various temperatures of chilling and various prickling durations gave significant effect on amount of total phenols in the preserved fruit (P < 0.05), but the chilling duration did not (P = 0.296). This result agreed with the report of Blanda et al (2008) that the frozen strawberries at -18 °C for 1 month showed a slight decrease of total phenols.[9]

Table 2 Yield of methanolic extract (%) of fresh and preserved fruits with various ages.

Fruit condition			Preservation condition			% Yield of methanolic extract
Condition	**Part**	**Age**	**Method**	**Duration**	**Temp.**	
Fresh fruit	Whole	1 month				23.53
	Whole	2.5 months				23.20
	Whole	3 months				35.93
	Whole	4 months				40.39
	Meat	4 months				49.47
	Skin	4 months				20.93
Preserved fruit (whole)		4 months.	Chilling	1 week	-70 °C	41.91
		4 months	Chilling	1 week	0 °C	43.54
		4 months	Chilling	1 week	4 °C	42.91
		4 months	Chilling	1 month	-70 °C	43.82
		4 months	Chilling	1 month	0 °C	40.37
		4 months	Chilling	1 month	4 °C	38.68
		2.5 months	Pickling	3 days		24.51
		2.5 months	Pickling	15 days		21.33
		2.5 months	Pickling	30 days		18.28
		4 months	Pickling	30 days		33.86

Table 3 Amount of total phenols in fresh and preserved Spanish plum fruits with various ages.

Fruit condition (Age of fruit))	Preservation condition (Duration, Temperature)	Amount of total phenols (GAE, g of gallic acid / 100g extract)				
		Sample 1	**Sample 2**	**Sample 3**	**Average**	**SD**
Fresh fruit						
Whole fruit (1 month)		2.3612	2.5359	2.3887	2.4286	0.09
Whole fruit (2.5 months)		1.0502	1.0701	1.0456	1.0553	0.01
Whole fruit (3 months)		0.6821	0.5978	0.6015	0.6271	0.05
Whole fruit (4 months)		0.5573	0.5686	0.5710	0.5656	0.01
Meat (4 months)		0.4885	0.4908	0.4850	0.4881	0.00
Skin (4 months)		1.0625	0.9693	0.9291	0.9870	0.07
Preserved whole fruit						
Preserved (4 months)	Chilling (1 week, -70 °C)	0.5292	0.5199	0.5269	0.5253	0.00
	Chilling (1 week, 0 °C)	0.4624	0.4569	0.4424	0.4539	0.01
	Chilling (1 week, 4 °C)	0.4888	0.4876	0.4836	0.4867	0.00
	Chilling (1 month, -70 °C)	0.4822	0.4953	0.6245	0.5340	0.08
	Chilling (1 month, 0 °C)	0.5290	0.5389	0.5472	0.5384	0.01
	Chilling (1 month, 4 °C)	0.4516	0.4567	0.4172	0.4418	0.02
Preserved (2.5 months)	Pickling (3 days)	1.6484	1.5584	1.5443	1.5837	0.06
	Pickling (15 days)	1.4824	1.3862	1.3898	1.4195	0.05
	Pickling (30 days)	1.1354	1.3024	1.3750	1.2710	0.12
Preserved (4 months)	Pickling (30 days)	0.9188	0.8094	0.6963	0.8082	0.11

Note:

The mean differences of chilling condition which were significant at the 0.05 level by two-way ANOVA include:

1) Duration (1-month chill* fresh whole 4-month fruit) and (1-week chill* fresh whole 4-month fruit)

2) Temperature (fresh whole 4-month fruit*0 °C), (fresh whole 4-month fruit*4 °C) and (4 °C*-70 °C)

The mean differences of prickling condition which were significant at the 0.05 level by one-way ANOVA include (fresh fresh whole 2.5-month fruit* 3-day prickle), (fresh fresh whole 2.5-month fruit* 15-day prickle), (fresh fresh whole 2.5-month fruit* 30-day prickle) and (3-day prickle* 30-day prickle).

The amount of total phenols of the fruits juice is shown in Table 4. The amount of total phenols in the juice was lower with increasing age similar to the results found in the fruit extract. The chilling condition caused a decreasing of total phenols of fruit juice after storage. The duration, chilling temperature and the two factor together (interaction term of duration*temperature in two-way ANOVA) significantly affected the amount of total phenols in the juice ($P < 0.01$). This means that at -70 °C total phenol decreased with longer duration, while at 0 °C it increased with longer duration.

Table 4 Amount of total phenols in juice of fresh and preserved Spanish plum fruits with various ages.

Fruit condition (Age of fruit)	Preservation condition (Duration, Temperature)	The average total phenols (GAE, g of gallic acid /L juice)
Fresh fruit		
Fresh (1 month)	-	26.94 ± 1.51
Fresh (2.5 months)	-	21.89 ± 0.23
Fresh (3 months)	-	14.04 ± 0.31
Fresh (4 months)	-	5.87 ± 0.33
Preserved fruit (4 months)		
	Chilling (1 month, -70 °C)	5.33 ± 0.04
	Chilling (1 month, 0 °C)	0.22 ± 0.01
	Chilling (1 month, 4 °C)	0.25 ± 0.04
	Chilling (4 months, -70 °C)	1.10 ± 0.12
	Chilling (4 months, 0 °C)	0.28 ± 0.01
	Chilling (4 months, 4 °C)	0.28 ± 0.048
	Chilling (6 months, -70 °C)	0.97 ± 0.10
	Chilling (6 months, 0 °C)	0.31 ± 0.06
	Chilling (6 months, 4 °C)	0.25 ± 0.01

Note:

The mean differences of chilling condition which were significant at the 0.05 level by two-way ANOVA include these interaction terms:

1) Duration: (fresh whole 4-month fruit*1 month chill), (1-month chill* 4-month chill), (1-month chill* 6-month chill), (fresh whole 4-month fruit*4-month chill), and (fresh whole 4-month fruit*6-month chill)

2) Temperature: (fresh whole 4-month fruit*0 °C), (fresh whole 4-month fruit*4 °C), (fresh whole 4month fruit*-70 °C), (0 °C*-70 °C) and (4 °C*-70 °C)

4. Antioxidant activity

The % inhibition equations of the antioxidant activities of all Spanish plum extracts, their IC_{50} and TEAC values are shown in Table 5. The antioxidant activities (TEAC value) of Spanish plum fruits with all ages were rather low. The antioxidant activity of the meat of fruits with various ages and preserved fruit meat exhibited the linear relationship with the concentration of the extract with r^2 in a range of 0.9624 - 0.9951. The younger fruit meat possessed a higher amount of antioxidant activities than the older fruits. This could be owing to the higher amount of phenolic compounds in the younger fruits (Table 3 and 4). Both temperature and period of chilling of 4-month fruit meat showed no significant effect on antioxidant activity. The prickle duration showed no significant effect on antioxidant activity of 2.5-month fruits. The TEAC and total phenolic content exhibited linear relationship correlation with r^2 of 0.9521 (Figure 1).

Table 5 The slope, intercept and r^2 of % inhibition equations, IC_{50} and TEAC values of the Spanish plum fruit extracts.

Fruit condition (Age of fruit)	Preservation condition (Duration, Temperature)	Equations			Antioxidant activity	
		Slope	Intercept	r^2	IC_{50} (μg)[#]	TEAC[$]
	Trolox	5.0248	-0.9708	0.9985	10.14	1.0000
Fresh fruit						
Whole fruit (1 month)		0.3731	8.1254	0.9665	112.23	0.0906
Whole fruit (2.5 months)		0.1514	5.2554	0.9834	295.54	0.0407
Whole fruit (3 months)		0.0507	1.9220	0.9812	948.28	0.0139
Whole fruit (4 months)		0.0369	0.9877	0.9896	1328.25	0.0093
Meat (4 months)		0.0405	1.0486	0.9920	1208.68	0.0101
Skin (4 months)		0.0759	1.6764	0.9917	636.67	0.0185
Preserved whole fruits						
Preserved (4 months)	Chilling (1 week, -70 °C)	0.0449	1.2421	0.9899	1085.92	0.0114
	Chilling (1 week, 0 °C)	0.0383	0.8760	0.9729	1282.61	0.0094
	Chilling (1 week, 4 °C)	0.0435	1.2582	0.9673	1120.50	0.0112
	Chilling (1 month, -70 °C)	0.0447	1.2560	0.9827	1090.47	0.0114
	Chilling (1 month, 0 °C)	0.0339	1.3118	0.9806	1436.23	0.0094
	Chilling (1 month, 4 °C)	0.0239	1.3070	0.9624	2037.36	0.0074
Preserved (2.5 months)	Pickling (3 days)	0.1880	7.3249	0.9714	227.00	0.0521
	Pickling (15 days)	0.1461	4.8195	0.9752	309.24	0.0387
	Pickling (30 days)	0.1350	2.5960	0.9951	351.14	0.0321
Preserved (4 months)	Pickling (30 days)	0.0601	2.2000	0.9832	795.34	0.0164

[#] IC_{50} = The concentration of the fruit extract at 50% inhibition.

[$] TEAC = Trolox equivalent antioxidant capacity value at the same concentration of the fruit extract and trolox.

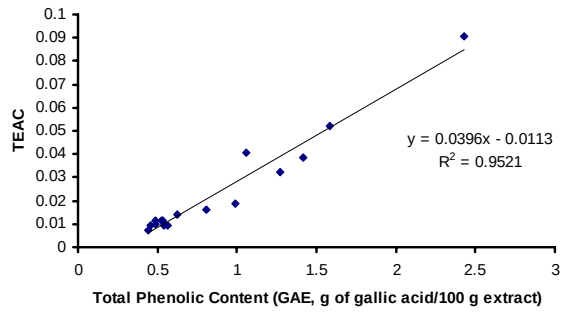

Figure 1 Linear correlation between Trolox equivalent antioxidant capacity value (TEAC) and total phenol content.

Conclusion

The duration and temperature of chilling showed no significant effect on the pH of preserved Spanish plum fruits. For sugar content (% brix) the duration of chilling had no significant effect on the property, but the temperature did exert significant augmenting effect. The mature fruits gave higher % yield of methanolic extract than the younger ones. The chilling, freezing and pickling condition of fruits might cause lowering effect on % yield of the extracts.

The phenolic compound of the fruit markedly accumulated in skin of the fruit. The amount of total phenols of crude extracts of the fruits and of fruit juice were lowering upon the age of the fruits. The duration of chilling did not significantly affect the amount of total phenols of the fruit, but apparently affected that of the juice. The temperature of chilling showed significant effect on amount of total phenols in the fruit and the juice. The longer duration of pickling resulted in a significant decreasing effect on total phenol.

The antioxidant activities of the fruits in all ages in this experiment were rather low. The younger fruits presented an amount of antioxidant activities higher than that in the older ones. The chilling condition caused no significant change of antioxidant activity of the fruit, so the preservation by freezing could protect the decrease of

antioxidant activities. The TEAC and total phenolic content showed a high correlation. We could conclude that the amount of antioxidant activities of fresh and preserved Spanish plum fruits had a direct proportion to their total phenolic compounds.

For selection of the best condition for preservation of *E. hygrophilus* fruits, the data did not show outstanding difference between duration and temperature of chilling on pH, % brix, amount of total phenol and antioxidant activities. The pickle duration also did not show considerable effect on total phenol and antioxidant activities. This may mean that the preserved condition could maintain pH, % brix, amount of total phenol and antioxidant activities of the fresh fruit. So the household refrigerator that could chill to 4 °C and 0 °C may be able to maintain these properties of the fruit kept in a certain constant temperature. Unfortunately the antioxidant activity of the mature fruit (4-month old) that are consumed as snack was quite low. Then the extra value of the fruit as free radical scavenger that we expected was not fulfilled.

Acknowledgement

This research work was kindly supported by the Faculty of Pharmacy, Silpakorn University. We would like to thank Mr. Sarawut Sukjindasathien for his tidy and active laboratory work.

References

1. Harman D. Role of free radicals in aging and disease. *Ann NY Acad Sci* 1992;673:126-141.
2. Mariani E, Polidori MC., Cherubini A and Mecocei P. Oxidative Stress in Brian Aging, Neurodegenerative and vascular Diseases: An Overview. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005;827(1):65-75.
3. WHO/FAO. Diet, Nutrition and the Prevention of Chronic Diseases. (Accessed on Aug. 22, 2008, at http://whqlibdoc. who.int/trs/WHO TRS 916.pdf).
4. Pongbunrod S. Mai-ted Mung Thai. Properties of foreign and Thai crude drugs. Bangkok. Feang-aksorn Publishing, 1971: pp.395-396.
5. Poopatpong L. Sa-mund-phai Thai Part.5. Bangkok. Chutima Kanprim Ltd., 1987: pp.632.
6. The Nutritional Table of Thai Foods in 100 g of their Edible Part. Department of Health, Nutrition Division, Ministry of Public Health, Nonthaburi. 1987: pp.48.
7. Kumazawa S, Taniguchi M, Suzuki Y, et al. Antioxidant activity of polyphenols in carob pods. *J Agric Food Chem* 2002;50(2):373-377.
8. Blanda G, Cerretani L, Cardinali A, Barbieri S, Bendini A, Lercker G. Osmotic dehydrofreezing of strawberries: polyphenolic content, volatile profile and consumer acceptance. *LWT-Food Sci Technol* 2009;42(1):30-36.
9. Bartolomé AP, Rupérez P, Fúster C. Changes in soluble sugars of two pineapple fruit cultivars during frozen storage. *Food Chem* 1996;56(2):163-166.