

Some Characteristics and Antioxidant Activity of Commercial Sugars Produced in Thailand

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ABSTRACT

Seven commercial sugars produced in Thailand (cane sugar paste (S1), unrefined cane sugar (S2), brown cane sugar (S3), caramel crystal cane sugar (S4), refined white crystal cane sugar (S5), coconut sugar paste (S6) and palm sugar paste (S7)) were analyzed for chemical and physical properties as well as antioxidative activity. The pH values of all samples ranged from 5.17 to 5.57. S5 exhibited the highest L*value, whereas S1 showed the lowest L* value ($p < 0.05$). Among sugars from sugar cane, S1 had the highest amount of Ca, Mg, Fe, K, and Na ($p < 0.05$). Additionally, differences in total sugar and reducing sugar contents of all samples were observed ($p < 0.05$). The intermediate browning products and browning intensity of all samples were also determined. S1 had the highest intermediate and browning products ($p < 0.05$). Higher total phenolic content was observed in sample extracted with water compared with that did with 95% methanol ($p < 0.05$). Regardless of extraction solutions, S1 extract exhibited the highest total phenolic content ($p < 0.05$). Antioxidant activities of both extracts were evaluated. S1 exhibited the highest DPPH and ABTS radical scavenging activities and ferric reducing power, compared with other samples ($p < 0.05$). From the results, sugar with high browning products and total phenolic content contributed to their high antioxidant activities.

Keywords: sugar, browning intensity, total phenolic content, antioxidative activity

1. INTRODUCTION

Sugar as a basic food carbohydrate primarily comes from sugar cane and from sugar beet. It is a natural sweetener, and it has been intensively used in many food industries for example, candy, soft drinks and canned fruit. Sugar enhances the taste, texture,

color, and aroma of many kinds of foods. Additionally, sugar is also used as traditional preservatives such as sugar syrup, sweetmeat and pickling etc. because sugar can function as antimicrobial preservative, which inhibit the growth of bacteria and fungi. In Thailand, sugar are manufactured from many type of plant, based on raw materials available in each local such as sugar palm from the sap of flowering stalks, coconut palm from the sap from coconut tree blossoms and juice from stem of sugar cane. There are various types of sugar categorized by their use and their characteristics such as size of the crystals, color and taste. In the production of sugar, syrup must heat until it is concentrated. During the heating process, physical and chemical changes occur which impart the unique color and flavor features. Major reactions occur during the process of heating, including the inversion reaction and non-enzymatic browning reactions (Maillard reaction and caramelization) [1]. Maillard reaction products and caramelization products have been found to exhibit antioxidative activity due to their radical scavenging activities and reducing power [2]. Sugars in form of powder granulate and paste can be found commercially. Although, studies on some characteristics and antioxidative activity of certain sugars have been mentioned but no information on characteristics and antioxidative activities of commercial sugars produced in Thailand has been systematically reported. Thus, this study aimed to investigate the chemical and physical properties as well as antioxidative activities of different commercial sugars produced in Thailand.

2. MATERIALS AND METHODS

2.1 Collection of commercial sugars

Cane sugar paste (S1) was procured from Chaingmai, Thailand. The manufactured sugars including unrefined cane sugar (S2), brown cane sugar (S3), caramel crystal cane sugar (S4), and refined white crystal cane sugar (S5) were purchased from supermarkets with the same manufacturer. Coconut sugar paste (S6) and palm sugar paste (S7) were obtained from Chumphon and Petchaburi, Thailand (Figure 1). Sugar samples

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were vacuum packed in aluminum bag (500 g/bag) and kept at -20°C until used within 1 week.



Figure 1 Commercial sugars. Cane sugar paste (S1), unrefined cane sugar (S2), brown cane sugar (S3), caramel crystal cane sugar (S4), refined white crystal cane sugar (S5), coconut sugar paste (S6) and palm sugar paste (S7)

2.2 Physicochemical properties of commercial sugars

2.2.1 Determination of pH value

The pH of sugar was determined according to the method of Benjakul, Seymour, Morrissey and An [3]. Sample was homogenized with 10 volumes of distilled water (w/v), and the pH of the homogenate was measured using a pH meter (UB-10 UltraBasic, Denver, USA).

2.2.2 Determination of water activity (a_w) value

The a_w of sugar was measured using a water activity meter (S40003131, AQUA LAB, USA)

2.2.3 Determination of color

Color of sugar was measured by Hunter lab (ColorFlex, Hunter, USA) and reported in CIE system, L^* , a^* and b^* parameters indicate lightness, redness/greenness and yellowness/ blueness, respectively.

2.2.4 Determination of total sugar and reducing sugar contents

Total sugar content was determined following the method of James [4] with a slight modification. Sample (1 g) was added with 10 ml of 1.5 M sulphuric acid and mixed well with a vortex. The mixture was incubated at 100°C for 20 min in water bath then cooled with ice and water for 5 min. Twelve milliliters sodium hydroxide solution (10%) was added and mixed with a vortex. The mixture was filtered through a Whatman No.4 filter paper into a 50 ml volumetric flask and made up to volume with distilled water. The filtrate (500 μ l) was pipetted into a test-tube and 500 μ l of 3,5-dinitrosalicylic acid (DNS) solution was added and then mixed well. Distilled water (1 ml) was added and transferred to incubate at 100°C for 20 min then cooled with ice and water for 5 min. The total sugar content was measured absorbance at 540 nm by spectrophotometer. A D-glucose solution (0-1.5 mg/ml) was used as a standard for total sugar and expressed as mg glucose/g dry weight of sample.

The content of reducing sugar was determined according to the method of James [4] with a slight modification. Sample (5 g) was added with 50 ml distilled water and mixed well. The mixture was incubated with water bath at 70°C for 10 min. Then, the mixture was filtered with Whatman No.4 filter paper. The reducing sugar content in filtrate was measured as described in total sugar content determination.

2.2.5 Determination of mineral content

The minerals including, calcium, magnesium, iron, potassium, and sodium were determined by inductively coupled plasma emission spectrometer (Optima 3300 DV, Perkin-Elmer, United States) according to the AOAC [5]. Sample (150 mg) was mixed well with 4 ml of nitric acid. The mixture was heated on hot plate (100 - 120°C) until digestion was completed (5 h). The digested sample was transferred to a volumetric flask and the volume was made up to 50 ml with deionized water. The solution was subjected to inductively couple plasma-optical emission spectrometer (ICP-OES) analysis. Flow rates of argon to plasma, auxiliary and nebulizer were kept at 15, 0.2 and 0.8 l/min, respectively. Sample flow rate was set at 1.5 ml/min. The wavelengths for analysis of calcium, magnesium, iron, potassium, and sodium were 317.933, 285.213, 238.204, 766.490, and 589.592 nm, respectively.

2.2.6 Determination of intermediate browning product and browning intensity

The intermediate browning product and browning intensity of sugar was determined according to the modified method of Ajandouz and Puigserver [6]. Sample was dissolved in distilled water (10% w/v). Prior to analysis, fifty-fold and five-fold dilution was made for determination of intermediate browning product and browning intensity, respectively using distilled water as the diluent. The intermediate browning product and browning intensity was measured at a wavelength of 285 nm and 420 nm, respectively using a spectrophotometer.

2.2.7 Determination of total phenolic content

Total phenolics content of sugar solution was determined by the Folin-Ciocalteu micro-method (Saeedeh and Asna [7]). Twenty microliters of sugar solution was mixed with 1.16 ml of distilled water and 100 μ l of Folin-Ciocalteu reagent, followed by the addition of 300 μ l of 20% Na_2CO_3 solution. Then, the mixture was incubated at 40°C in a shaking water bath (model D-91126, Memmert, Schwabach, Germany) with speed 6 for 30 min. The absorbance at 760 nm of the mixture was measured by spectrophotometer. Total phenolic content was calculated and expressed as mg gallic acid equivalent (GAE)/g dry weight of the sample.

2.3 Determination of antioxidant activities of sugars

2.3.1 Preparation of extracts from sugars

Solutions of sugar were prepared at 0.5% (dry weight/volume), dissolved in distilled water or

methanol using a stirrer for 2 h. Then, the mixture was filtered through a Whatman No.4 filter paper. The extracts were stored at 4°C in the dark until analyses.

2.3.2 Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH)-radical scavenging activity

DPPH-radical scavenging activity of sugar solution was determined according to the method of Yen and Hsieh [8] with a slight modification. Four hundred microliters of sugar solution was mixed with 2 ml of DPPH solution (0.12 mM in 95% methanol). The reaction mixture was mixed well and incubated at room temperature for 30 min. The absorbance of mixtures was measured at 517 nm by spectrophotometer. The control was prepared in the same manner, excepted that 80% methanol was used instead of sugar solution. The radical scavenging activity was measured as a decrease in the absorbance of DPPH-sample mixture. The percentage of DPPH radical scavenging activity was calculated as follows (Singh and Rajini [9])

$$\text{Radical scavenging activity (\%)} = \left(1 - \frac{As}{Ac}\right) \times 100$$

where;

As is the absorbance at 517 nm of sample
Ac is the absorbance at 517 nm of control

2.3.3 Determination of 2,2'-azino-bis (3-ethylbenzthiazoline -6-sulphonic acid) (ABTS)-radical scavenging activity

ABTS radical scavenging activity was determined as described by Re, Pellegrini, Proteggente, Pannala, Yang and Rice-Evans [10] with a slight modification by Khantaphant and Benjakul [11]. ABTS radical (ABTS^{•+}) was produced by reacting ABTS stock solution (7.4 mM) with 2.6 mM potassium persulfate at the ratio of 1:1 (v/v). The mixture was allowed to react in dark for 12 h at room temperature. Prior to assay, ABTS^{•+} solution was diluted with methanol to obtain an absorbance of 1.1 (±0.02) at 734 nm. To initiate the reaction, sugar solution (150 µl) was mixed with 2850 µl of ABTS^{•+} solution. The mixture was incubated at room temperature for 2 h (dark reaction). The absorbance was then read at 734 nm of the mixture was measured by spectrophotometer. A standard curve of Trolox ranging from 0-600 µM was prepared. The activity was expressed as µmol Trolox equivalents (TE)/g dry weight of the sample.

2.3.4 Determination of ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power of sugar solution was determined following the method of Benzie and Strain [12]. Stock solutions contained 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM FeCl₃·6H₂O. A working solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of FeCl₃·6H₂O solution.

The mixed solution was incubated at 37°C for 30 min and was referred to as FRAP solution. One hundred fifty microliters of sugar solution was mixed with 2850 µl of FRAP solution and kept for 30 min in the dark. The ferrous tripyridyltriazine complex (colored product) was measured by reading the absorbance at 593 nm. The standard curve was prepared using Trolox ranging from 0-600 µM. The activity was expressed as µmol Trolox equivalents (TE)/g dry weight of the sample.

2.4 Statistical analysis

The data was subjected to One-way Analysis of Variance (ANOVA) and the differences between means were evaluated by Duncan's Multiple Range Test or Independent samples t-test (Steel and Torrie [13]).

3. RESULTS AND DISCUSSION

3.1 Physicochemical properties of commercial sugars

3.1.1 pH values

Table 1 shows the pH values of seven commercial sugars. Differences in pH values of commercial sugars were observed (p<0.05). In general, five commercial sugars produced from sugar cane used in this study are cane sugar paste, unrefined cane sugar, brown cane sugar, caramel crystal cane sugar and refined white crystal cane sugar. The pH values of those cane sugars were found in the ranges of 5.14 to 5.57. Cane sugar paste exhibited the highest pH value (5.57). Beside cane sugar sample, the pH values of coconut sugar paste and palm sugar paste were 5.50 and 5.27, respectively. Many researchers suggested that the pH of sugar can be used as an indicator for the product safety [1]. Furthermore, the pH value can be used to classify the food products into acid food and low acid food [14]. Guerra and Mujica [15] reported that the pH values of cane sugar "Panela" ranged from 5.58-6.90. Naka [16] showed that the pH value of coconut sugar was 5.57. Naknean and Meenune [1] and Phaichamnan, Posri and Meenune [17] also reported the pH value of palm sugar which was in the ranges of 4.49-5.42 and 4.5-5.37, respectively. The pH value of the sugar is related to the quantity of lime used in the clarifying process of the juice during sugar preparation [15]. Also, Phaichamnan, Posri and Meenune [17] found that the organic acids, particularly lactic acid, produced by lactic acids bacteria were observed in palm sugar concentrate. Generally, the decrease in pH value of palm sap and coconut sap occurred during heating for palm sugar and coconut sugar productions [18]. In addition, the reduction of pH was probably due to the occurrence of organic acids such as formic and acetic acid via the Maillard reaction [19-21]. From the results, the differences in pH of sugar might be resulted from the different raw materials and processes used.

TABLE 1 PH VALUE OF SEVEN COMMERCIAL SUGARS

Samples	pH
Cane sugar paste	5.57 ± 0.03 ^c
Unrefined cane sugar	5.53 ± 0.05 ^c
Brown cane sugar	5.14 ± 0.08 ^c
Caramel crystal cane sugar	5.19 ± 0.06 ^c
Refined white crystal cane sugar	5.47 ± 0.01 ^c
Coconut sugar paste	5.50 ± 0.04 ^c
Palm sugar paste	5.27 ± 0.01 ^c

Mean ± SD from triplicate determinations.

**Different superscript letters in the same column indicate significant differences ($p < 0.05$).

3.1.2 Water activity (a_w) values

The difference a_w values of seven commercial sugars were observed ($p < 0.05$) (Table 2). The a_w values of commercial sugars produced from sugar cane were slightly different (from 0.61 to 0.65). For sugars in paste form, coconut sugar and palm sugar showed the highest a_w values ($p < 0.05$). The marked lowest a_w value was found in refined white crystal cane sugar (0.48). Generally, a_w is a crucial factor determining the quality and storage time. The heating time for sugar production (dehydration process) might affect the a_w value of final product. Apriyanoto, Aristyani, Nurhayati, Lidya, Budiyanoto and Soekarto [18] reported that the weight of coconut sap and palm sap decreased with increasing heating time. Generally, the a_w value can be used to indicate the shelf-life stability of food products [22]. Since a_w value refers to free water that can promote microbial deterioration and biochemical degradation reaction. Guerra and Mujica [15] concluded that different commercial granulated cane sugar had different a_w value. Naknean and Meenune [1] reported the a_w value of palm sugar syrup produced in southern Thailand was between 0.79-0.81. Low a_w value (0.65-0.80) may allow the growth of osmophilic yeast which can spoil food containing high concentration of sugar [23].

TABLE 2 WATER ACTIVITY (a_w) OF SEVEN COMMERCIAL SUGARS

Samples	a_w
Cane sugar paste	0.61 ± 0.00
Unrefined cane sugar	0.62 ± 0.01
Brown cane sugar	0.65 ± 0.00
Caramel crystal cane sugar	0.61 ± 0.00
Refined white crystal cane sugar	0.48 ± 0.01
Coconut sugar paste	0.72 ± 0.01
Palm sugar paste	0.73 ± 0.00

† Mean ± SD from triplicate determinations.

† Different superscripts letters in the same column indicate significant differences ($p < 0.05$)

3.1.3 Color

The color (L^* , a^* and b^*) of seven commercial sugars were varied depending on types of sugar as shown in Fig 2. Among all samples, the refined white crystal cane sugar (S5) had the highest L^* value ($p < 0.05$). The difference in a^* and b^* values were noticeable in all samples ($p < 0.05$). Generally, the color of sugars from sugar cane could be found in light yellow to dark brown, depending on type of raw materials, heating process (temperature and time) and chemical process. Most of the commercial sugar appeared in yellow-brown to brown color, except for the refined white crystal cane sugar which appeared in white. The different color of all samples might result from the difference in type of plants raw material, temperature and heat as well as manufacturing process. Additionally, the color of the sugar depends on the amount of dark compounds generated during extraction and heating of the cane juice, which could be derived from oxidation of phenolic compounds; caramelization of sucrose, glucose and fructose; Maillard reaction and alkaline decomposition of sucrose [15].

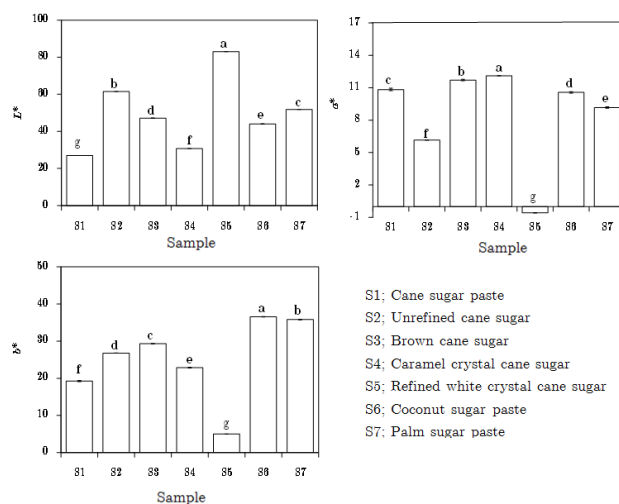


Figure 2 L^* , a^* and b^* of seven commercial sugars. Bars represent the standard deviations from triplicate determinations. Different letters within same value indicate the significant differences ($p < 0.05$).

3.1.4 Total sugar and reducing sugar contents

Total sugar contents of seven commercial sugars were slightly different ($p < 0.05$) (Fig.3). Commercial sugars had total sugar content between 761.96 - 841.23 mg/g dry weight. The highest total sugar content was found in caramel crystal cane sugar, followed by brown cane sugar, while coconut sugar paste had the lowest. A wide range of reducing sugar content of commercial sugars (0.15-122.07 mg/g dry weight) was noticeable. Cane sugar paste contained the highest reducing sugar content, followed by coconut sugar paste and palm sugar paste, respectively. Refined white crystal cane sugar had the lowest reducing sugar content. Martins, Jongen and Van Boekel [24] reported that during heating in the manufacturing process, especially at high temperature

and long heating time, it could accelerate the hydrolysis of sucrose yielding reducing sugars. However, high reducing sugar content presented in sugar also influences the browning color of sugar afterward, due to the Maillard reaction [1]. Cane sugar paste, coconut sugar paste and palm sugar paste were traditional produced by evaporating the juice in a large opened pan under heating with the wood fired stove until the concentrated paste was obtained [25]. Those sugar pastes had higher reducing sugar content than other industrially manufactured sugars. Thus, low reducing sugar in refined white crystal cane sugar compared to cane sugar paste, coconut sugar paste and palm sugar paste may be due to the chemical refining process which can eliminate the reducing sugar. In addition, Phaichamnan, Posri and Meenune[17] reported that the difference of total sugar and reducing sugar contents might be due to the effect of contamination from micro-organisms in sugar. The microorganisms can convert sucrose to glucose and fructose (invert sugar) and finally to organic acids or alcohols [26].

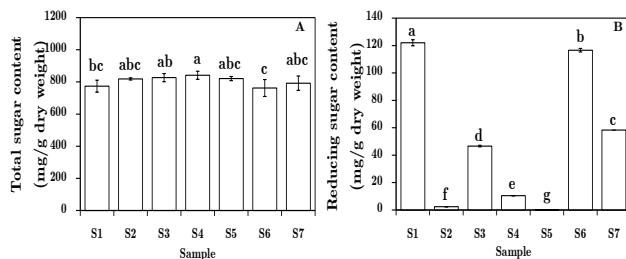


Figure 3 Total sugar (A) and reducing sugar (B) contents in commercial sugars. Bars represent the standard deviations from triplicate determinations. Different letters in the same value the different samples indicate the significant differences ($p < 0.05$). S1: cane sugar paste; S2: unrefined cane sugar; S3: brown cane sugar; S4: caramel crystal cane sugar; S5: refined white crystal cane sugar; S6: coconut sugar paste; S7: palm sugar paste

3.1.5 Mineral contents

The mineral contents of seven commercial sugars are shown in Fig 4. Different commercial sugars contained different levels of minerals ($p < 0.05$). The levels of Ca, Mg, Fe, K, and Na were found to be highest in cane sugar paste ($p < 0.05$). The contents of Ca in brown cane sugar was similar to that in cane sugar paste. This was probably due to the molasses addition during brown cane sugar production [27]. The pattern of mineral level of sugars produced from sugar cane was different, especially Fe, K, and Na. In this research, the cane sugar paste was obtained from retail market (traditional product of northern of Thailand), while others were purchased from supermarket (industrial products). The production processes might affect the levels of minerals. Guerra and Mujica [15] found that different brands of granulated sugar “Panel” had different contents of Ca, Mg, Fe, and K. Olaiya [28] reported that Ca is highly implicated in the maintenance of firmness of fruits and its requirements in fruits are related to cell

wall stability and membrane integrity. According to the nutrition, Ca, Mg, and K are elements in macronutrient for function of human body. Several factors have been reported to affect the amount of some minerals in sugar such as cane variety, climate, soil, crop, handling, level of juice extraction, clarifying method, lime purity and impurity removal efficiency in manufacturing process [1]. From the result, cane sugar paste was abundant in minerals.

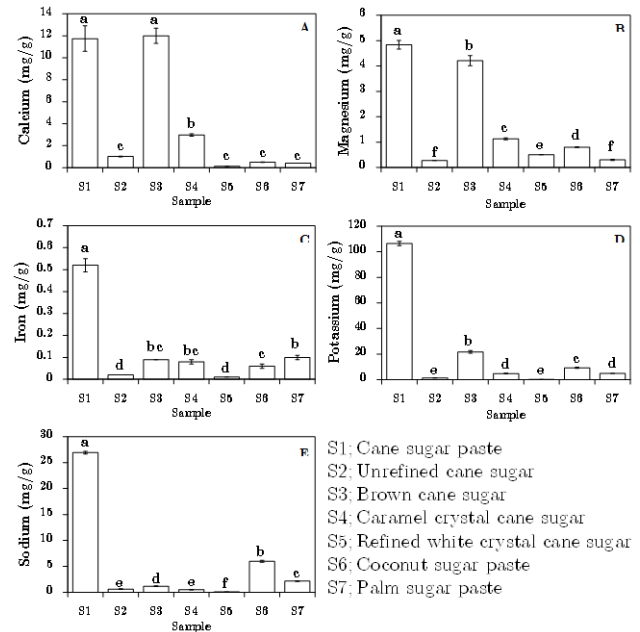


Figure 4 Calcium (A), magnesium (B), iron (C), potassium (D), and sodium (E) contents in commercial sugars. Bars represent the standard deviations from triplicate determinations. Different letters in the same value the different samples indicate the significant differences ($p < 0.05$).

3.1.6 Intermediate browning product and browning intensity

Intermediate browning product and browning intensity of seven commercial sugars were significantly different ($p < 0.05$) (Fig 5). Cane sugar paste exhibited the highest intermediate browning product and browning intensity, followed by brown cane sugar and palm sugar paste, respectively whereas refined white crystal cane sugar exhibited the lowest. UV absorbance at 285 nm was used to monitor the intermediate degradation product of non-enzymatic browning reaction in commercial sugars [6], [29]. The high absorbance at 285 nm suggested the formation of an uncoloured compound, which could be the precursor of the Maillard reaction [6], [30]. The intermediate degradation products via enolization known as color precursors include methylglyoxal, glyceraldehyde, hydroxymethylfuraldehyde, furfural and hydroxyacetyl furan [31-34]. Naknean, Meenune and Roudaut [35] suggested that an intermediate product was converted to a brown polymer and brown pigments were formed proportionately with the intermediate products generated. The degree of browning, usually measured

using the absorbance value at 420 nm, is often used to determine the browning intensity in final stages [6], [36], [37]. From the study in the model system of Ajandouzand Puigserver [6], Lertittikul, Benjakul and Tanaka [20], Benjakul, Visessanguan, Phongkanpai and Tanaka [29], Benjakul, Lertittikul and Bauer [30] and Phongkanpai, Benjakul and Tanaka [34], intermediate degradation products increased as the heating time and pH increased. In addition, Benjakul, Visessanguan, Phongkanpai and Tanaka [29] reported that the browning of sugars was developed increasingly with the increase in pH. From the results, high absorbance at 285 nm and 420 nm of cane sugar paste might correlate to pH values. Generally, different intermediate browning products and browning intensity depend on the heating time and pH levels.

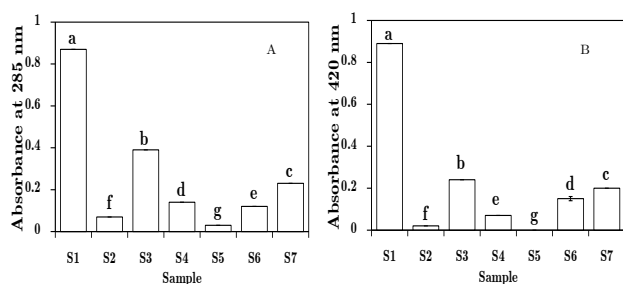


Figure 5 Intermediate browning product (A) and browning intensity (B) in commercial sugars. Bars represent the standard deviations from triplicate determinations. Different letters in the same value the different samples indicate the significant differences ($p < 0.05$). S1: cane sugar paste; S2: unrefined cane sugar; S3: brown cane sugar; S4: caramel crystal cane sugar; S5: refined white crystal cane sugar; S6: coconut sugar paste; S7: palm sugar paste

3.2 Total phenolic content

Total phenolic contents of seven commercial sugars extracted with distilled water or 95% methanol were investigated. Total phenolic content of commercial sugar extracts were different ($p < 0.05$). Higher total phenolic content was observed in commercial sugar extracted with distilled water. In general, the solubility of phenolic compound depends on polarity of solvent [38]. Regardless of extraction solvent, cane sugar paste had the highest total phenolic content, followed by brown cane sugar and palm sugar paste, respectively, whereas the lowest was found in refined white crystal cane sugar. Harish Nayaka, Sathisha, Manohar, Chandrashekar and Dharmesh [39] reported that total phenolic content of brown cane sugar, refined sugar and jaggery (palm sugar) extracted with distilled water were 0.37, 0.03 and 3.84 mg GAE/g, respectively. Naknean and Meenune [1] studied the phenolic content of palm sugar syrup produced in southern Thailand. It was found that the total phenolic content of distilled water extract was 1.35-2.21 mg GAE/g. Phaichamnan, Posri and Meenune [17] reported that the phenolic compound was naturally found in the palm sap itself and some was dissolved from *Kiam* wood during the

palm sap collection. In addition, Harish Nayaka Sathisha, Manohar, Chandrashekar and Dharmesh [39] reported that jaggery and brown sugar had higher phenolic content compared to white and refined sugar. This was probably due to the minimal chemical processing in the manufacture of jaggery and brown sugar which retains more polyphenols. The phenolic compounds impart color as well as taste to the sugar and its removal is an important problem associated with sugar manufacture [40]. The different techniques used in sugar processing to remove color and impurities affect the amount of polyphenols in sugars and this may explain the low phenolic content of white and refined sugars. From the results, commercial sugar pastes (cane sugar paste, coconut sugar paste and palm sugar paste) and brown cane sugar exhibited high total phenolics content. Most phenolic compounds found in distilled water extract. Therefore, sugar paste composed of higher total phenolic compounds than granulated and powdered sugars.

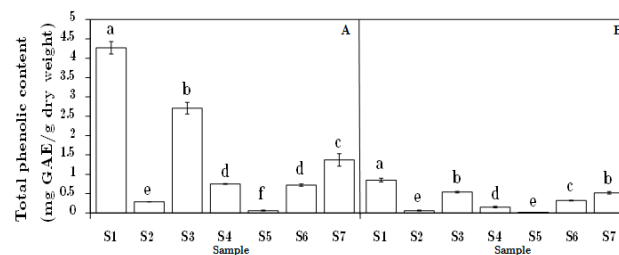


Figure 6 Total phenolic content in sugar dissolved in distilled water (A) and 95% methanol (B). Bars represent the standard deviations from triplicate determinations. Different letters in the same value the different samples indicate the significant differences ($p < 0.05$). S1: cane sugar paste; S2: unrefined cane sugar; S3: brown cane sugar; S4: caramel crystal cane sugar; S5: refined white crystal cane sugar; S6: coconut sugar paste; S7: palm sugar paste

3.3 Antioxidative activities

3.3.1 DPPH-radical scavenging activity

DPPH-radical scavenging activities of seven commercial sugars extracted with distilled water or 95% methanol were elucidated. DPPH-radical scavenging activities of commercial sugar extracts were different ($p < 0.05$) (Fig 7). Distilled water extracts had higher DPPH-radical scavenging activity than methanol extract. Regardless of extraction solvent, cane sugar paste exhibited the highest percentage scavenging activity, followed by brown cane sugar, palm sugar paste and coconut sugar paste, respectively. Refined white crystal cane sugar had the lowest percentage scavenging activity. The higher percentage scavenging activity of commercial sugars extracts was coincidental with the higher reducing sugar, intermediate browning product, browning intensity and total phenolic content. DPPH is one of compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on the exposure to proton radical scavenging [41]. Lertittikul, Benjakul and Tanaka [20] and Benjakul, Visessanguan, Phongkanpai and Tanaka [29] reported that Maillard reaction products

(MRPs) and caramelization products (CPs) from fructose showed the highest radical-scavenging activity, compared with glucose. The reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant is due to the formation of the non-radical form, DPPH-H. When the DPPH-radical is scavenged by antioxidants through the donation of hydrogen to form a stable DPPH-H molecule, the color is changed from purple to yellow [42]. However, sugar consists primarily of sucrose containing glucose and fructose. Therefore, the higher the reducing sugar the greater the percentage scavenging activity was observed. In addition, Haghparast, Shabanpour, Kashirila Alipour and Sodagar [43] reported that intermediates or the final brown polymer could function as hydrogen donors. From the results, commercial sugars, especially cane sugar paste can function as antioxidant through the donation of hydrogen atom.

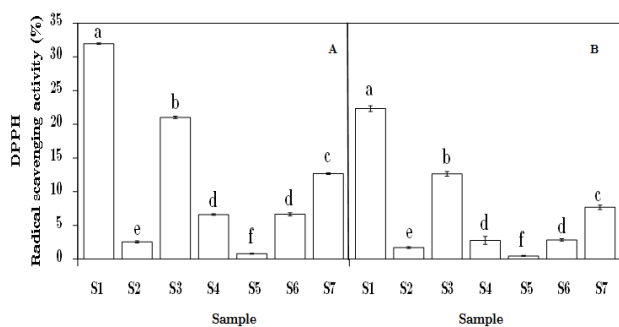


Figure 7 DPPH radical scavenging activity in sugar dissolved in distilled water (A) and 95% methanol (B). Bars represent the standard deviations from triplicate determinations. Different letters in the same value the different samples indicate the significant differences ($p < 0.05$). S1: cane sugar paste; S2: unrefined cane sugar; S3: brown cane sugar; S4: caramel crystal cane sugar; S5: refined white crystal cane sugar; S6: coconut sugar paste; S7: palm sugar paste

3.3.2 ABTS-radical scavenging activity

The antioxidant activities of sugars, using the ABTS-radical scavenging activity assay are shown in Fig 8. ABTS-radical scavenging activities of seven commercial sugars extracted with distilled water or 95% methanol were tested. ABTS-radical scavenging activity of commercial sugars extracts were different ($p < 0.05$). The results showed that the ABTS-radical scavenging activity of distilled water extract was greater than that of methanol extract. Regardless of extraction solvent, cane sugar paste showed the highest ABTS-radical scavenging activity, compared with other sugars ($p < 0.05$). ABTS-radical scavenging activity of water extract from cane sugar paste and refined white crystal cane sugar was 13.19 and 0.97 $\mu\text{mol Trolox/g}$ dry weight, respectively whereas the water extract of cane sugar paste and refined white crystalcane sugar was 9.87 and 0.37 $\mu\text{mol Trolox/g}$ dry weight, respectively. The results showed that higher ABTS-radical scavenging activity was in agreement with higher DPPH-radical scavenging.

However, ABTS radical assay employed for measuring the relative radical scavenging activity of hydrogen-donating and chain breaking antioxidants [44], [45]. The decolorization assay method was to screen the antioxidative activity, and it is applicable to both lipophilic and hydrophilic antioxidants [10]. Thus, commercial sugars, especially cane sugar paste can act as hydrogen donating and chain breaking antioxidants. From the results, ABTS-radical scavenging activity correlated well with DPPH radical scavenging, intermediate browning product, browning intensity and total phenolic content.

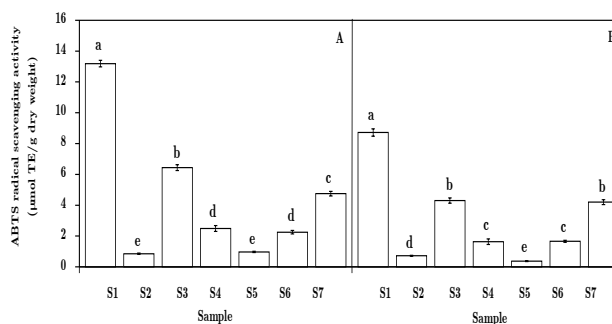


Figure 8 ABTS radical scavenging activity in sugar dissolved in distilled water (A) and 95% methanol (B). Bars represent the standard deviations from triplicate determinations. Different letters in the same value the different samples indicate the significant differences ($p < 0.05$). S1: cane sugar paste; S2: unrefined cane sugar; S3: brown cane sugar; S4: caramel crystal cane sugar; S5: refined white crystal cane sugar; S6: coconut sugar paste; S7: palm sugar paste

3.3.3 Ferric reducing antioxidant power

Ferric reducing antioxidant powers of seven commercial sugars extracted with water or 95% methanol are shown in Fig 9. Ferric reducing antioxidant powers of commercial sugars extracts were different ($p < 0.05$). The results showed that the ferric reducing antioxidant power of commercial sugars extracted with distilled water was greater than that of commercial sugars extracted with methanol. Regardless of extraction solvent, the ferric reducing antioxidant power was found in the order of cane sugar paste > brown cane sugar > palm sugar paste > coconut sugar paste > caramel crystal cane sugar > unrefined cane sugar > refined white crystal cane sugar ($p < 0.05$). Ferric reducing antioxidant powers of cane sugar paste were 19.87 and 10.82 $\mu\text{mol Trolox/g}$ dry weight, when extracted with distilled water and methanol, respectively whereas, those of refined white crystal cane sugar extracted with distilled water and methanol of were 0.66 and 1.97 $\mu\text{mol Trolox/g}$ dry weight, respectively. These results were in agreement with those obtained for the antioxidant activities determined by the DPPH and ABTS-radical scavenging activity assays. Generally, the correlation between ferric reducing antioxidant power with the DPPH-radical scavenging, ABTS-radical scavenging activity, intermediate browning

product, browning intensity and total phenolic content was noticeable.

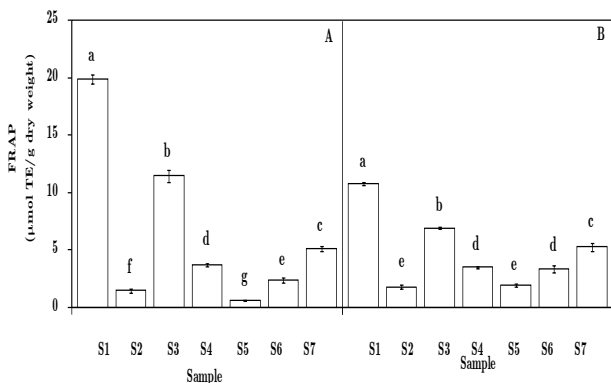


Figure 9. FRAP in sugar dissolved in distilled water (A) and 95% methanol (B). Bars represent the standard deviations from triplicate determinations. Different letters in the same value the different samples indicate the significant differences ($p < 0.05$). S1: cane sugar paste; S2: unrefined cane sugar; S3: brown cane sugar; S4: caramel crystal cane sugar; S5: refined white crystal cane sugar; S6: coconut sugar paste; S7: palm sugar paste

4. CONCLUSION

Seven commercial sugars showed different physicochemical properties and antioxidant activities. High reducing sugar and mineral contents (Fe, Na, and K) were found in cane sugar paste (S1). Also, cane sugar paste (S1) with high browning intensity exhibited the greatest antioxidant activity. Therefore, sugar particularly cane sugar paste could be used as a sweetener with antioxidant property for food production.

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REFERENCES

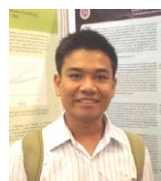
- [1] Naknean, P., and Meenune, M. Characteristics and antioxidant activity of palm sugar syrup produced in Songkhla Province, Southern Thailand, *Asian Journal of Food and Agro-Industry*, 4 (4), 2011, 204-212.
- [2] Marales, F. J. and Jimenez-Perez, S. Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence, *Food Chemistry*, 72, 2001, 119-125.
- [3] Benjakul, S., Seymour, T. A., Morrissey, M. T. and An, H. Physicochemical changes in pacific whiting muscle proteins during iced storage, *Journal of Food Science*, 62, 1997, 729-733.
- [4] James, C. S. *Analytical chemistry of foods*, Chapman and Hall, Glasgow, 1995.
- [5] AOAC. *Official Method of Analysis*. 17th ed. The association of official analytical chemistry, Washing DC, 2000.
- [6] Ajandouz, E. H. and Puigserver, A. Nonenzymatic browning reaction of essential amino acids: effect of pH on caramelization and Maillard reaction kinetics,

- Journal of Agricultural and Food Chemistry*, 1(15), 1999, 928-943.
- [7] Saeedeh, A. and Asna, U. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves, *Food Chemistry*, 102, 2007, 1233-1240.
- [8] Yen, G. C. and Hsieh, P. P. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction-products, *Journal of the Science of Food and Agriculture*, 67, 1995, 415-420.
- [9] Singh, N. and Rajini, P. S. Free radical scavenging activity of anaqueous extract of potato peel, *Food Chemistry*, 85 (4), 2004, 611-616.
- [10] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radical Biology and Medicine*, 26, 1999, 1231-1237.
- [11] Khantaphant, S. and Benjakul, S. Comparative study on the proteases from fish pyloric caeca and the use for production of gelatin hydrolysate with antioxidative activity, *Comparative Biochemistry and Physiology Part B*, 151, 2008, 410-419.
- [12] Benzie, I. F. F. and Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay, *Analytical Biochemistry*, 239, 1996, 70-76.
- [13] Steel, R. G. D. and Torrie, J. H. *Principle and procedure of statistics*, 2nd ed. New York, McGraw-Hill, 1980.
- [14] Britt, I. J. *Thermal Processing*. In: Tucker, G.S. *Food Biodeterioration and Preservation*. Oxford, Blackwell Publishing Ltd, 2008, pp. 63-79.
- [15] Guerra, M. J. and Mujica, M. V. Physical and chemical properties of granulated cane sugar "panelas", *Ciencia e Tecnologia de Alimentos*, 30 (1), 2010, 250-257.
- [16] Naka, P. Potential of producing sugar from coconut. In: *Proceedings of promoting multi-purpose uses and competitiveness of the coconut*, 26-29 September 1996. IPGRI, 1996.
- [17] Phaichamnan, M., Posri, W. and Meenune, M. Quality profile of palm sugarconcentrate produced in Songkhla province, Southern Thailand, *International Food Research Journal*, 17, 2010, 425-432.
- [18] Apriyantono, A., Aristyani, A., Nurhayati, Lidya, Y., Budiyanto, S. and Soekarto, S. T. Rate of browning reaction during preparation of coconut and palm sugar, *International Congress Series*, 1245, 2002, 275-278.
- [19] Brand, C. M. J. and Van Boekel, M. A. J. S. Kinetics modeling of reactions in heated monosaccharide-casein system, *Journal of Agricultural and Food Chemistry*, 50, 2002, 6725-6739.
- [20] Lertittikul, W., Benjakul, S. and Tanaka, M. Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein-glucosemodel system as influenced by pH, *Food Chemistry*, 100, 2007, 669-677.
- [21] Vhangani, L. N. and Van Wyk, J. Antioxidant activity of Maillard reaction products (MRPs) derived from fructose-lysine and ribose-lysine model systems, *Food Chemistry*, 137 (1-4), 2013, 92-98.
- [22] Mathlouthi, M. Water content, water activity, water structure and the stability of foodstuffs, *Food Control*, 12, 2001, 409-417.
- [23] Naknean, P. Factors affecting browning and crystallisation of palm sugar syrup and palm sugar cake. Ph.D. Thesis, Prince of Songkla University, 2010.
- [24] Martins, S. I. F. S. Jongen, W. M. F. and Van Boekel, M. A. J. S. A review of Maillard reaction in food and

- implications to kinetic modeling, *Trends in Food Science and Technology*, 11, 2001, 364-373.
- [25] Naknean, P., Meenu, M. and Roudaut, G. Changes in physical and chemical properties during the production of palm sugar syrup by open pan and vacuum evaporator, *Journal Asian of Food and Agro-Industry*, 2, 2009, 448-456.
- [26] Willits, C. O. and Hills, C. H. *Maple Syrup Producers Manual*. Agricultural Research Service, United States Department of Agriculture, Government Printing office, Washington, DC, 1976.
- [27] Gul, S. and Harasek, M. Energy saving in sugar manufacturing through the integration of environmental friendly new membrane processes for thin juice pre-concentration, *Applied Thermal Engineering*, 43, 2012, 128-133.
- [28] Olaiya, C. O. Effects of three plant bioregulators on some biochemical properties of *Lycopersicon esculentum* (L.) Mill. Ph.D. Thesis, University of Ibadan, 2006.
- [29] Benjakul, S., Visessanguan, W., Phongkanpai, V. and Tanaka, M. Antioxidative activity of caramelisation products and their preventive effect on lipid oxidation in fish mince, *Food Chemistry*, 90, 2005, 231-239.
- [30] Benjakul, S., Lertittikul, W. and Bauer, F. Antioxidant activity of Maillard reaction products from a porcine plasma protein-sugar model system, *Food Chemistry*, 93, 2005, 189-196.
- [31] Kroh, L. W. Caramelization in food and beverages, *Food Chemistry*, 51, 1994, 373-379.
- [32] Homoki-Farkas, P., Orsi, F. and Kroh, L. W. Methylglyoxal determination from different carbohydrates during heat processing, *Food Chemistry*, 59, 1997, 157-163.
- [33] Camerver, B., Wedzicha, B. L. and Kroh, L. W. Nonenzymatic browning reactions of retro-aldol degradation products of carbohydrates, *European Food Research and Technology*, 209, 1999, 261-265.
- [34] Phongkanpai, V., Benjakul, S. and Tanaka, M. Effect of pH on antioxidative activity and other characteristics of caramelization products, *Journal of Food Biochemistry*, 30, 2006, 174-186.
- [35] Naknean, P., Meenu, M. and Roudaut, G. Changes in properties of palm sugar syrup produced by an open pan and a vacuum evaporator during storage, *International Food Research Journal*, 20 (5), 2013, 2323-2334.
- [36] Hodge, J. E. Chemistry of browning reactions in model systems, *Journal of Agricultural and Food Chemistry*, 1, 1953, 928-936.
- [37] Mauron, J. The Maillard reaction in food: a critical review from the nutritional stand point. *Progress in Food and Nutrition Science*, 5, 1981, 5-18.
- [38] Onwuka, G. I., Clifford, H. and Onuegbu, N. Development of extraction protocol for phenolic compounds in African pear seed (*Dacryodes edulis*), *International Journal of Innovative Pharmaceutical Research* 3 (2), 2012, 212-216.
- [39] Harish Nayaka, M. A., Sathisha, U. V., Manohar, M.P., Chandrashekar, K. B. and Dharmesh, S. M. Cytoprotective and antioxidant activity studies of jaggery sugar, *Food Chemistry*, 115, 2009, 113-118.
- [40] Godshall, M. A., Vercellotti, J. R. and Triche, R. Comparison of cane and beetsugar macromolecules in processing, *International Sugar Journal*, 104, 2002, 228-233.
- [41] Yamaguchi, T., Takamura, H., Matoba, T. and Terao, J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl, *Bioscience Biotechnology and Biochemistry*, 62, 1998, 1201-1204.
- [42] Shon, M. Y., Kim, T. H. and Sung, N. J. Antioxidants and free radical scavenging activity of *Phellinus baumii* (*Phellinus* of *Hymenochaetaceae*) extracts, *Food Chemistry*, 82, 2003, 593-597.
- [43] Haghparast, S., Shabanpour, B., Kashiri, H., Alipour, G. H. and Sodagar, M. A Comparative study on antioxidative properties of carameled reducing sugars; inhibitory effect on lipid oxidative and sensory improvement of glucose carameled products in shrimp flesh, *Journal of Agricultural Science and Technology*, 15, 2013, 87-99.
- [44] Brand-Williams, W., Cuvelier, M. E. and Berset, C. Use of a free radical method to evaluate antioxidant activity, *LWT-Food Science and Technology*, 28, 1995, 25-30.
- [45] Netzel, M., Strass, G., Bitsch, I., Konitz, R., Christmann, M. and Bitsch, R. Effect of grape processing on selected antioxidant phenolics in red wine, *Journal of Food Engineering*, 56, 2003, 223-228.



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