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THESIS

EFFECTS OF SUGARS ON LIPID CHANGES IN GROUND PERILLA (*PERILLA FRUTESCENS* L.) SEED

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A Thesis Submitted in Partial Fulfillment of
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Chemical composition, bioactive compounds and antioxidant activities of brown perilla and white perilla seeds were comparatively studied. Brown perilla seed had higher protein, fat and ash contents than those of white perilla seed (p<0.05). Additonally, brown perilla seed had greater Ca, Mg, and P contents than those of white perilla seed (p<0.05). Brown perilla seed was rich in polyunsaturated fatty acid, in particular γ-linolenic acid and α-linolenic acid. Higher total phenolic, total flavonoid, and total flavonol contents were observed in brown perilla seed (p<0.05), which might correlate with higher 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS)-radical scavenging activities as well as ferric reducing antioxidant power and reducing power. From these results, brown perilla seed was selected for further study. In addition, chemical and physical properties of seven 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) were also investigated. All commercial sugars showed slightly differences in pH, A_w, and total sugar content. S1 had the highest reducing sugar content, intermediate (OD_{285}) and browning (OD_{420}) products, as well as total phenolic content (p<0.05). However, DPPH-radical scavenging activity of S1, S3, S6 and S7 were higher than other samples. These sugars were chosen for study on lipid changes in ground perilla seed during 30-day storage at room temperature. A decrease in pH and A_w of ground perilla seed added with four different sugars were observed during storage (p<0.05). Generally, changes in L*, a* and b* of all samples were found throughout the storage. Free fatty acid increased with increasing storage time and the rate of increase was more pronounced in sample added with S7 (p<0.05). During storage, peroxide value, conjugated diene and thiobarbituric acid reactive substances (TBARS) of all samples decreased. This was postulated to be due to the loss of volatile oxidation compounds from samples stored for longer time. Lower acceptance in all attributes (appearance, color, odor, taste, sweet, texture, flavor and overall liking) was observed in sample added with S3, than in other samples, particularly when storage time increased (p<0.05). However, sample added with S1, S6, and S7 showed slight decreases in overall liking scores within 20 days. The results revealed that sugars paste, especially coconut sugar paste can be used to retard the lipid oxidation in ground perilla seed products.

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Sikaret Kongkaew

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INTRODUCTION

Perilla seed, also known as "Nga-Kee-Mon" is an oil seed grown in Northern of Thailand, particularly Prae, Nan, Meahongson, and Chiangmai. This seed was also reported as a food plant commonly used in Asia cuisine, especially in Korea (kaennip) and Japan (shiso) (Bassoli *et al*., 2009). In Thailand, perilla seed is used as an ingredient for traditional snack foods such as, "nga-tum-aoi" (ground perilla seed added with cane sugar paste), "khao-nuk-nga" (cooked glutinous rice mixed with roasted perilla seed), and "nga-ud-tang" (sweetened perilla seed bar). Recently, perilla seed has been paid more attention on nutritional value improvements for bakery and Thai dessert products because perilla seed is a good source of protein (17%) and fat (51%) (Longvah and Deosthale, 1991). Gunstone *et al*. (1994) reported that perilla seed has been known as a rich source of α-linolenic acid (ALA, C18:3, *n*3), accounting for approximately 60% of total fatty acids. Siriamornpun *et al*. (2006) also reported the high ration of polyunsaturated fatty acid to saturated fatty acid in perilla seed. Additionally, perilla seed contains many phenolic compounds, namely, phenolic acids, flavonoids, and triterpenoids, and is known to exhibit several health beneficial activities including antioxidant, antibiotic, and antipyretic properties (Kosuna and Haga, 1997; Nakazawa and Ohsawa, 2000).

Lipid changes, lipolysis and lipid oxidation, are important biochemical changes affecting quality and stability of food products. These changes are influenced by several factors, especially reactive oxygen species and free radicals. Generally, the vegetable oils are good sources of linoleic acid, but only few vegetable oils contribute significant amount of α-linolenic acid (ALA) as in perilla oil (Kashima *et al*., 1991). This indicated that perilla seed is sensitive to auto-oxidation. Many studies have been attempted to retard lipid oxidation of perilla oil by adding natural antioxidant such as phenolic compounds, phospholipids and α-tocopherol (Kashima *et al*., 1991). Recently, thermal degradation products of sugar like furfural, caramel or Maillard reaction products

(MRPs) are well known to be browning agents and have been found to be involved in antioxidative activity. In addition, non-enzymatic browning reaction products have been found to exhibit antioxidative activity due to radical scavenging activity (Marales and Jimenez-Perez, 2001; Yen and Hsieh, 1995). Although, some traditional snack foods produced in northern Thailand usually used perilla seed as an ingredient are widely consumed. Nga-tum-aoi, ground perilla seed added with cane sugar paste, is one of many products which is used sugar for sweetening and flavouring. This product is served immediately mixing or kept in refrigerator; however, its rancid flavour occurred during storage. From the point of view, perilla seed is a new source as food ingredients and their nutritional and properties information are not intensively studied. Furthermore, no information has been reported concerning the effect of sugar as antioxidant in ground-perilla seed. Therefore, this study aimed to evaluate nutritional values, some bioactive compounds and antioxidative activities of Thai perilla seed extract. Also, effect of sugar on lipid changes in ground perilla seed, “nga-tum-aoi”, during storage at ambient temperature for 30 days were also investigated.

OBJECTIVES

1. To comparative study on nutritional value and some bioactive compound content of brown perilla and white perilla seeds.

2. To investigate theantioxidative activity of brown perilla and white perilla seeds.

3. To study on lipid changes of ground perilla seed as affected by different commercial sugars during 30-day storage at room temperature.

LITERATURE REVIEW

1. Perilla (*Perilla frutescens*) seed

Perilla seed or "Nga-Kee-Mon" or "Nga-Ki-Mong" is an herb of mint family (Labiatae/Lamiaceae) and native to East Asia (Przybylski, 2005; Asif and Kumar, 2010**)**. It is generally and edible medicinal plant and cultivated in China, India, Japan, Korea, Thailand, and United States (Hagemann *et al*., 1967; Siriamornpun *et al.*, 2006). In Thailand, perilla is widely cultivated in northern, especially Chiang Mai, Chiang Rai, Maehongson, Phrae and Nan (Ampanchai *et al*., 2008). Ito *et al.* (2008) reported that perilla cultivated in northern Loas has two different pericarp colors which were brown and white. The most perilla cultivation is the brown-grain type (Ito *et al.*, 2008). In Loas, pericarps from perilla seed could be used for cooking via roasted and mixed into steamed sticky rice with or without cane sugar to make "Cao-Tom" (Ito *et al.*, 2008). Additionally, perilla seed can be consumed after cooking and roasting along with foods such as cereals, vegetables, roots, or tubers (Longvah and Deosthale, 1998). Przybylski (2005) reported that oil from perilla seed can be used as a fuel, a drying oil, or cooking oil for garnish and flavor builder. The color of perilla seed oil was light yellow and transparent oily liquid (Asif and Kumar, 2010).

1.1 Nutritional properties and some bioactive compounds in perilla seed

Recently, the composition of perilla seed from many regions has been reported. Perilla seed is a potential source of nutrients and bioactive compounds. Peiretti (2011) reported that perilla seed contained 95.3% dry matter, 23.9% protein and 52.3% crude fiber. Also, Longvah and Deosthale (1991) reported that perilla seed is rich in protein (17%) and fat (51%).

Generally, protein content in perilla seeds is varied. The protein in whole perilla seed ranged from 15.7 to 23.7% (Sharma *et al.,* 1989). Longvah and Deosthale (1998) studied the protein content of perilla seed (whole, kernel, and hull) before and after defatted with *n*-hexane. The results showed that defatted perilla seed had higher protein content than perilla seed without defatting. For amino acids profiles, the valine was the limiting amino acid of perilla protein (Longvah and Deosthale, 1991). Furthermore, the protein efficiency ratio of the seed protein (2.07) was lower than of casein (2.99), but comparable to common oilseeds. Different essential amino acids of meal from perilla whole seed and kernel were 39% and 42% of their total amino acids content, respectively (Longvah and Deosthale, 1998) (Table 1). Longvah and Deosthale (1998) concluded that the essential amino acids of perilla seed was 39% of total amino acid were lower than animal protein. Thus, perilla seed could be a nutritious food and used as ingredient for functional foods.

Lipid content, composition and fatty acid profile of perilla seed have been studied. Differences cultivated perilla seed, difference in lipid content and fatty acids were found. Total lipid content of perilla seed from Thailand and India ranged from 34 to 37% (Table 2). (Siriamornpun *et al.,* 2006). Also, perilla seed from Manipur and Dehradum contained 51.7% and 30% to 35% lipid content, respectively (Longvah and Deosthale, 1991; Asif and Kumar, 2010). Additionally, Siriamornpun *et al.* (2006) studied lipid composition of perilla seed oil from different locations in Thailand by thin layer chromatography/flamed ionized detector (TLC/FID). Triglyceride (TG) was the most predominant lipid, followed by phytosterols (Table 3).

Table 1 Amino acid composition of perilla seed (mg/g protein).

Amino acid	Perilla seed	
	whole seed	Kernel
Valine	33	39
Isoleucine	41	34
Leucine	62	74
Lysine	37	38
Tryptophan	13	15
Threonine	41	38
Tyrosine	35	39
Phenylalanine	55	67
Cysteine	16	20
Methionine	26	23
Histidine	31	27
Aspartate	97	84
Serine	51	55
Glutamate	206	199
Proline	41	38
Glycine	53	50
Alanine	45	53
Arginine	109	101
Total essential amino acids	390	413.2
Total amino acids	992	991.6

Source: modified from Longvah and Deosthale (1998)

Table 2 Lipid content of perilla seed oil from different countries.

Country	Lipid content (g/100g)	Reference
Thailand (Maehongson)	34.62	(1)
Thailand (Chiang Mai)	37.14	(1)
India (Manipur)	51.7	(2)
India (Dehradun)	30-35	(3)

Source: modified from (1): Siriamornpun *et al.* (2006); (2): Longvah and Deosthale (1991); (3): Asif and Kumar (2010)

Table 3 Lipid composition of perilla seed oil from different locations in Thailand.

Locations	Lipid composition*(% of total lipids)				
	CE	TAG	DAG	FFA	PTS
Maehongson	ND	96.48	ND	ND	3.52
Chiang Mai	ND	97.18	ND	ND	2.82
Commercial	ND	97.02	ND	ND	2.98

*CE = Cholesterol ester, TAG = Triglycerides, DAG = Diglycerides, FFA = Free fatty acids, PTS = Phytosterol, ND = not detected

Source: modified from Siriamornpun *et al.* (2006)

Both saturated and unsaturated fatty acids were found in perilla oil. Perilla oil is a rich source of α-linolenic acid (ALA), accounting for approximately 60% of total fatty acids (Gunstone *et al*., 1994; Ihara *et al*., 1998). In addition, perilla oil composed of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1*n*9), linoleic acid (C18:2*n*6) and α-linolenic acid (C18:3*n*3) (Kwak, 1994; Lee *et al*., 2002). Kim *et al*. (2007) reported that palmitic acid, stearic acid were a minor composition in seed oil. For Thai perilla oil, the ratio of saturates:monounsaturates:polyunsaturates was

approximately 1:1:8 (Siriamornpun *et al.*, 2006) (Table 4). Among lipid components, linolenic acid, precursor of the long chain *n*3 fatty acids in human, antioxidants and phytosterols has been paid the most attention as dietary ingredients effectively lowering risk of heart diseases (Schmitz and Ecker, 2008). Ciftci *et al*. (2012) suggested that the lack of fish oil as *n*3 fatty acid source, the useful of this fatty acid from plant source could be recognized. Additionally, ALA is a precursor of long chain *n*3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA, C20:5*n*3) and docosahexaenoic acid (DHA, C22:6*n*3). These PUFA had been reported as the beneficial effects for chronic disease control such as the lowering the plasma lipid level and the increase in EPA and DHA in the hepatic membranes of rats (Connor, 2000; Kim and Choi, 2001; Kim *et al*., 2004). However, highly unsaturated fatty acids in perilla oil has limited for quality of food both characteristics and shelf-life stability. Kashima *et al*. (1991) also reported that perilla oil was sensitive to auto-oxidation due to high ALA levels. Longvah and Deosthale (1991) concluded that perilla oil had abundant of PUFA which two essential fatty acids (linoleic acid and linolenic acid).

Table 4 Fatty acid composition of perilla seed oils grown at different locations in Thailand.

Source of perilla seed	Saturated fatty acids				Monounsaturated fatty acids			Polyunsaturated fatty acids		
	16:0	17:0	18:0	Total	16:1	18:1	Total	18:2 (*n*6)	18:3 (*n*3)	Total
Maehongson	6.86	0.03	3.16	10.05	0.11	11.55	11.66	18.45	59.84	78.29
Changmai	6.54	0.16	2.67	9.37	0.17	11.41	11.58	22.09	56.96	79.05
Commercial	7.33	0.09	3.32	10.74	0.08	12.66	12.74	22.26	54.26	76.52

Source: modified from Siriamornpun *et al.* (2006)

The fat-soluble vitamin (vitamin A and vitamin E) are normally found in seed oils. Pawel *et al*. (2013) reported that the contents of β-carotene, provitamin A, in flaxseed and sesame seed were 1.87 μg/g and 0.37 μg/g, respectively. Generally, the antioxidant activity of carotenoids has been reported. Carotenoids can be acted as primary antioxidant or as secondary antioxidant (Halliwell and Gutteridge, 1998). The vitamin E or tocopherol in perilla seed was 276.78 μg/g dry weight, which was higher than black sesame and white sesame seeds (Sangaroon *et al*., 2011). Eventually, tocopherol is well known as the most widely used as an antioxidant in foods (Pokorny, 1987). The α-tocopherol is the most important antioxidant due to their high antioxidant activity (Yanishlieva-Maslarova, 2001).

Many bioactive compounds from plants are intensive studies. Generally, phenolic compounds are known as secondary metabolites and widely distributed in both leaves and seeds (Barros *et al.,* 2011). To date, the phenolic compounds in perilla seed also have been paid more attention. Perilla seed contained many phenolic compounds, namely, phenolic acid, flavonoids, and triterpenoids. Their health beneficial activities including antioxidant, antibiotic, and antipyretic properties were observed (Kosuna and Haga, 1997; Nakazawa and Ohsawa, 2000). Several active ingredients in perilla seed have been reported. Peng *et al*. (2005) also reported that the perilla seed had flavonoids and phenolic acid which were composed of catechin, ferulic acid, apigenin, luteolin, rosmarinic acid, and caffeic acid (Table 5). Lee *et al*. (2013) studied identification, characterization, and quantification of phenolic compounds in Korean perilla seeds. It was found that four phenolic acids and five flavonoids in perilla seed were observed. Then nine compounds were elucidated as caffeic acid-3-*O*-glucoside, caffeic acid, luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, rosmarinic acid-3-*O*-glucoside, rosmarinic acid, luteolin, apigenin, and chrysoeriol. Rosmarinic acid-3-*O*-glucoside and rosmarinic acid were the predominant compounds in Korean perilla seeds. Additionally, the difference flavonoid content in perilla might resulted from the harvesting time (Peng *et al*., 2005)

Table 5 Phenolic compound in *Perilla frutescens* L seed (10^{-5} g/g).

Compound	*Perilla frutescens* L seeds (Code)		
	040905	040925	041015
(+)-catechin	5.3	9.3	13.4
ferulic acid	5.7	6.6	12.9
apigenin	4.0	5.9	11.6
luteolin	8.1	14.3	16.0
rosmarinic acid	63.7	116.1	162.5

Source: modified from Peng *et al*. (2005)

2. Changes in lipid of oilseed during storage

Generally, oilseeds, such as soybean, cottonseed, rapeseed (canola), flaxseed, sunflower seed and peanut, are the largest source of vegetable oils even though most oil-bearing tree fruits provide the highest oil yields (*e.g.*olive,coconut and palm trees) (Gunstone, 2002).Oilseeds are used chiefly to produce vegetable oil and oilseed meal, which in turnare used to produce food fats and oil products. The oilseeds can be stored for a long time before being processed (Macrae *et al*., 1993). Although, oilseed can be stored for a long time, but oil seed are sources of oil, particulary unsaturated fatty acids. Thus, it caused changes of lipid.

Some seed oils contain significant amounts of the essential fatty acids such as ALA and linoleic acid. Lipids belong to the most labile components and labile to alterations under conditions of storage and processing of food. These changes can be desirable or can lead to adverse effects on the quality of various commodities. The adverse effects on quality of lipid may caused by lipid degradation by three different mechanisms of fatty acid oxidation yielding different products as follows: auto-oxidation, photo-oxidation and lipooxygenase (Wasowicz *et al.*, 2004). Deleterious changes in foods caused by lipid oxidation include not only loss of flavor or development of off-flavors, but also loss of color, nutrition value, and the accumulation

of compounds, which may be detrimental to the health of consumers (Wasowicz *et al.*, 2004).

2.1 Mechanism of lipid oxidation

Lipid oxidation in foods is a major problem and difficult to overcome often and leads to loss of shelf-life, palatability, functionality, and nutritional quality (Reische *et al*., 2002). Auto-oxidation is a spontaneous reaction of molecular oxygen with lipids, leading to oxidative deterioration. Auto-oxidation must be induced by performed or primary hydroperoxides. Edible oil is oxidized during processing and storage via auto-oxidation and photosensitized oxidation, in which triplet oxygen ($^3O_2$) and singlet oxygen ($^1O_2$) react with the oil, respectively. Auto-oxidation of oils requires radical forms of acylglycerols, whereas photosensitized oxidation does not require lipid radicals since $^1O_2$ reacts directly with double bonds. Lipid hydroperoxides formed by $^3O_2$ are conjugated dienes, whereas $^1O_2$ produces both conjugated and nonconjugated dienes. The hydroperoxides are decomposedto produce off-flavor compounds and the oil quality decreases. Auto-oxidation of oil is accelerated by the presence of free fatty acids, monoglycerides and diglycerides, metals such as iron, and thermally oxidized compounds (Choe and Min, 2006).

The auto-oxidation of oils, free radical chain reaction, includes initiation, propagation, and termination steps (Choe and Min, 2006) are shown as follows;

Initiation

$$RH \longrightarrow R^{\bullet} + H^{\bullet}$$

Propagation

$$R^{\bullet} + {}^3O_2 \longrightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$$

Termination

$$ROO^{\bullet} + R^{\bullet} \longrightarrow ROOR$$

$$R^{\bullet} + R^{\bullet} \longrightarrow RR$$

The lipid alkyl (R) radical reacts with $^3O_2$ and forms lipid peroxy radical, another reactive radical. Reaction between lipid alkyl radical and $^3O_2$ occurs very quickly at normal oxygen pressure and, consequently, the concentration of lipid alkyl radical is much lower than that of lipid peroxy radical (Aidos *et al*., 2002). The lipid peroxy radical abstracts hydrogen from other lipid molecules and reacts with the hydrogen to form hydroperoxide and another lipid alkyl radical. These radicals catalyze the oxidation reaction, and auto-oxidation is called the free radical chain reaction.

The hydroperoxide formation in the auto-oxidation of linoleic acid. The rates for the formation of lipid peroxy radical and hydroperoxide depend only on oxygen availability and temperature (Velasco *et al*., 2004). When radicals react with each other, non-radical species are produced and the reaction stops.

The primary oxidation products, lipid hydroperoxides, are relatively stable at room temperature and in the absence of metals. However, in the presence of metals or at high temperature they are readily decomposed to alkoxy radicals and then form aldehydes, ketones, acids, esters, alcohols, and short-chain hydrocarbons. The most likely pathway of hydroperoxide decomposition is a homolytic cleavage between oxygen and the oxygen bond, in which alkoxy and hydroxy radicals are produced. The alkoxy radical then undergoes homolytic β-scission of the carbon-carbon bond and produces oxo-compounds and saturated or unsaturated alkyl radicals (Figure 1). After electron rearrangement the addition of hydroxyl radical, or hydrogen transfer, the ultimate secondary lipid oxidation products are mostly low molecular weight aldehydes, ketones, alcohols, and short-chain hydrocarbons, as shown in Table 6.

Another mechanism of oxidation occurs in the presence of sensitizer and UV-light. Photo-oxidation pathway is an alternative route leading to the formation of hydroperoxides instead of the free radical mechanism. Excitation of unsaturated fatty acid or oxygen may occur in the presence of light and a sensitizer. There are two types of photo-oxidation (Gordon, 2001): I–an electron or a hydrogen atom transfers between an excited triplet sensitizer and a substrate (PUFA), producing free radicals or radical ions; and II–triplet oxygen ($^3O_2$) can be excited by light to singlet oxygen ($^1O_2$), which

reacts with the double bond of unsaturated fatty acids, producing an allylic hydroperoxide (Frankel, 1985). This reaction results in a formation of a *trans* configuration. Products of oleate oxidation are 9- and 10-hydroperoxides, linoleate produces a mixture of 9-10-(*trans*, *cis*), 12-13-(*cis*, *trans*) isomers (Frankel, 1998). The third mechanism of oxidation is based on lipoxygenase activity. Lipoxygenase is an enzyme which is a very important source of hydroperoxides formed during oil extraction. Lipoxygenase produces similar flavor volatiles to those produced during auto-oxidation. A molecule of lipoxygenase contains an iron atom, which is in high spin state Fe (II) and must be oxidized to Fe (III) by fatty acid hydroperoxides or hydrogen peroxide. The active enzyme abstracts a hydrogen atom from the methylene group of a polyunsaturated fatty acid with the iron being reduced to Fe (II) (Gordon, 2001). A conjugated diene system is formed, followed by reaction with oxygen. Peroxyl radical and finally hydroperoxide are generated. The second type of enzyme reacts with an esterified substrate, before the release of fatty acids by lipase, additionally ketodiene fatty acids are formed (Belitz and Grosch, 1999).

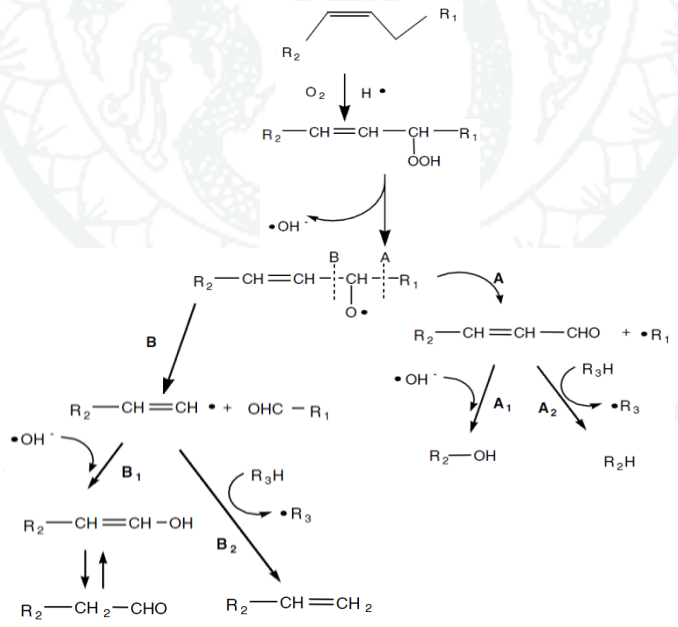

Figure 1 Mechanisms of hydroperoxide decomposition to form secondary oxidation products

Source: modified from Choe and Min (2006)

Table 6 Secondary oxidation products of fatty acid methyl ester by auto-oxidation.

Class	Oleic acid	Linoleic acid	Linolenic acid
Aldehydes	Octanalb	Pentanal	Propanal
	Nonanal	Hexanal	Butanal
	2-Decenal	2-Octenal	2-Butenal
	Decanal	2-Nonenal	2-Pentenal
		2,4-Decadienal	2-Hexenal
			3,6-Nonadienal
			Decatrienal
Carboxylic acid	Methyl heptanoate	Methyl heptanoate	Methyl heptanoate
	Methyl octanoate	Methyl octanoate	Methyl octanoate
	Methyl 8-oxooctanoate	Methyl 8-oxooctanoate	Methyl nonanoate
	Methyl 9-oxononanoate	Methyl 9-oxononanoate	Methyl 9-oxononanoate
	Methyl 10-oxodecanoate	Methyl 10-oxodecanoate	Methyl 10-oxodecanoate
	Methyl 10-oxo-8-decenoate		
	Methyl 11-oxo-9-undecenoate		
Alcohol	1-Heptanol	1-Pentanol	
		1-Octene-3-ol	
Hydrocarbons	Heptane	Pentane	Ethane
	Octane		Pentane

Source: modified from Frankel (1985)

Another mechanism of oxidation occurs in the presence of sensitizer and UV-light. Photo-oxidation pathway is an alternative route leading to the formation of hydroperoxides instead of the free radical mechanism. Excitation of unsaturated fatty acid or oxygen may occur in the presence of light and a sensitizer. There are two types of photo-oxidation (Gordon, 2001): I–an electron or a hydrogen atom transfers between an excited triplet sensitizer and a substrate (PUFA), producing free radicals or radical ions; and II–triplet oxygen ($^3O_2$) can be excited by light to singlet oxygen ($^1O_2$), which reacts with the double bond of unsaturated fatty acids, producing an allylic hydroperoxide (Frankel, 1985). This reaction results in a formation of a *trans* configuration. Products of oleate oxidation are 9- and 10-hydroperoxides, linoleate

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2.2 Changes in lipid during storage

Several methods have been used to evaluate the extent of oxidative deterioration, which are related to the measurement of the concentration of primary or secondary oxidation products. Breaking of ester linkages of triglycerides in oil caused free fatty acid. The percentage of free fatty acids is an important indicator of quality throughout the processing of oils and fats. The most frequently used are peroxide value (PV) (Frankel, 1998). PV is related to the concentration of hydroperoxide. The formation of peroxides is concurrent with conjugation of double bonds in polyunsaturated fatty acids, which can be measured using the specific absorptivity of Conjugated dienes (Rohman and Che Man, 2011). Thiobarbituric acid reactive substances (TBARS) are naturally present in biological specimens and include lipid hydroperoxides and aldehydes which increase in concentration as a response to oxidative stress (Armstrong and Browne, 1994). TBARS assay values are usually reported in malonaldehyde equivalents, a compound that results from the decomposition of polyunsaturated fatty acid lipid peroxides.

2.2.1 Free fatty acid

During the storage of oilseed, the lipid fraction is slowly hydrolyzed by natural lipolytic enzymes or those produced by bacteria and/or fungi, contributing to the hydrolytic rancidity of the product (Araujo, 2004). Increase in the content of free fatty acids from lipids occurs by the action of lipase and phospholipase enzymes present in the oilseed or produced by the associated microflora, which contribute to the breaking of ester linkages of triglycerides (Zadernowsi *et al.*, 1999). Thus, the percentage of free fatty acids is an important indicator of quality throughout the processing of oils and fats. O'Brien (2004) stated that hydrolytic rancidity can affect taste, odor and other characteristics of oil.

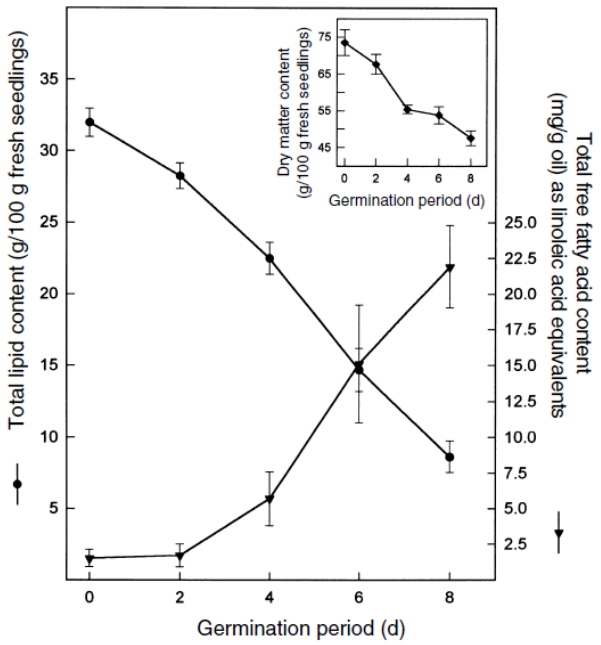


Figure 2 Quantitative changes in total lipids and free fatty acids of flaxseeds during 8 days of germination. Inset: changes of dry matter content during the same germination period

Source: modified from Wanasundara *et al.* (1999)

Wanasundara *et al.* (1999) studied changes in flaxseed lipids during germination. Flaxseeds were germinated for 8 days under laboratory conditions. From the results, it was found that decrease in the content of total lipids as germination progressed and increased free fatty acid content after day 2 of germination and on the day 8 the content of free fatty acid was 2.2% of total lipids (Figure 2). Additionally, lipase activity of germinated flaxseeds at day 0 exhibited 160 U/g thereafter, it plateaued at day 2 of germination.

2.2.2 Peroxide value (PV)

PV is a common method used to measure lipid oxidation, and is a measure of the amount of peroxides and hydroperoxides formed in the initial stages of lipid oxidation which associated with rancidity in lipid-containing food products expressed as milliequivalents of free iodine per kg of lipid. (Nouros *et al*., 1999; Wabner, 2002)

Malcolmson *et al.* (2000) studied storage stability of milled flaxseed, two samples of flaxseed are Linott and a mixture of several varieties, were milled and stored at 23±2°C for 128 days in paper bags with plastic liners. Samples were taked for evaluation at day 0, 33, 66, 96, and 128. It was found that neither the mixed variety nor Linott samples showed a significant increase in PV throughout the 128 days of storage.

Abramovic and Abram (2005) studied the effect of storage conditions on the formation of primary oxidation products of camelina oil versus time of storage. At the beginning of the experiment, the peroxide value of 2.38 meq O_2/kg for camelina oil was observed. Camelina oil exposed to daylight for 1 month at ambient temperature PV rose sharply to 21.0 meq O_2/kg, and that after 10 months it reached 50.6 meq O_2/kg. An increase in the PV during the first month storage was observed. PV of the oil stored in darkness did not show such a sharp increase after the first month of storage. During 10 months PV increased, it was concluded that the oxidation process in the first period of storage was more affected by light than by temperature (Figure 3).

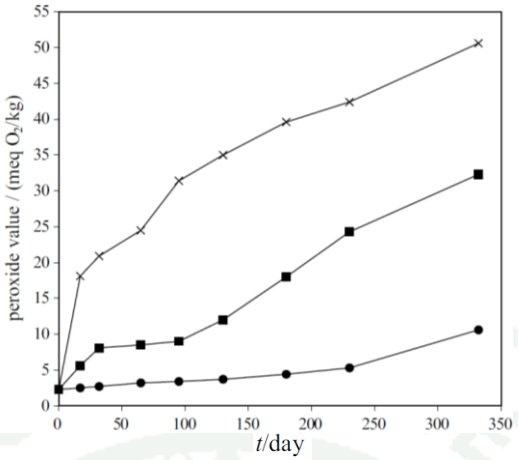


Figure 3 Effect of light and temperature on the peroxide value of *Camelina sativa* oil during storage (×–daylight, room temperature; ■–darkness, room temperature; ●–darkness, temperature 8°C)

Source: modified from Abramovic and Abram (2005)

2.2.3 Conjugated dienes (CD)

Conjugated dienes are almost immediately after peroxides are formed, the non-conjugated double bonds (C=C–C–C=C) that are present in natural unsaturated lipids are converted to conjugated double bonds (C=C–C=C) (Gunstone and Norris, 1983). Conjugated dienes exhibit an intense absorption at 234 nm (Hamed and Abo-Elwafa, 2012)

Ni Eidhin *et al*. (2003) studied oxidative stability of plant and fish oil found that CD of the sample increase when increased heating time oilseed. Camelina oil had higher CD values than rapeseed, sesame, sunflower, corn, and olive oils from day 5 through 16 of storage and had lower values than fish oil (day 0 to 16) (Figure 4) and correlation with PV.

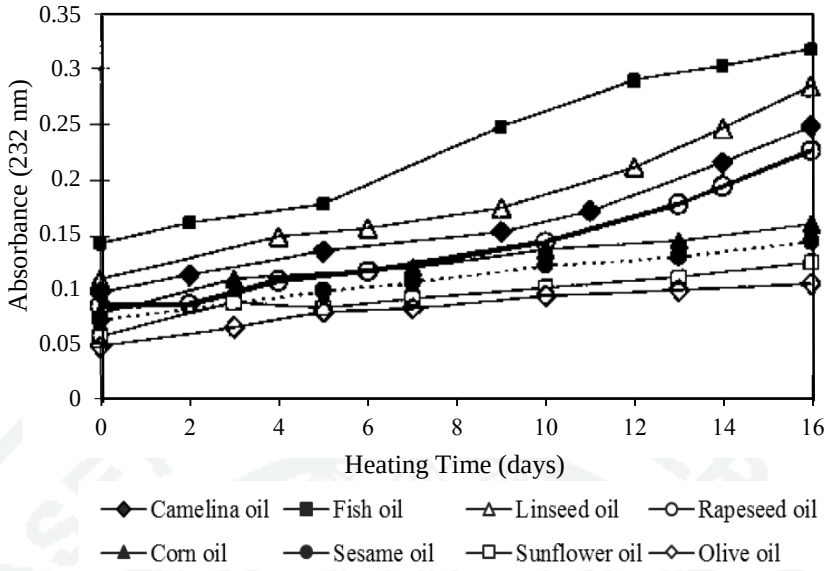


Figure 4 Conjugated diene (CD) values of plant oils and fish oil stored at 65°C

Source:modified Ni Eidhin *et al*. (2003)

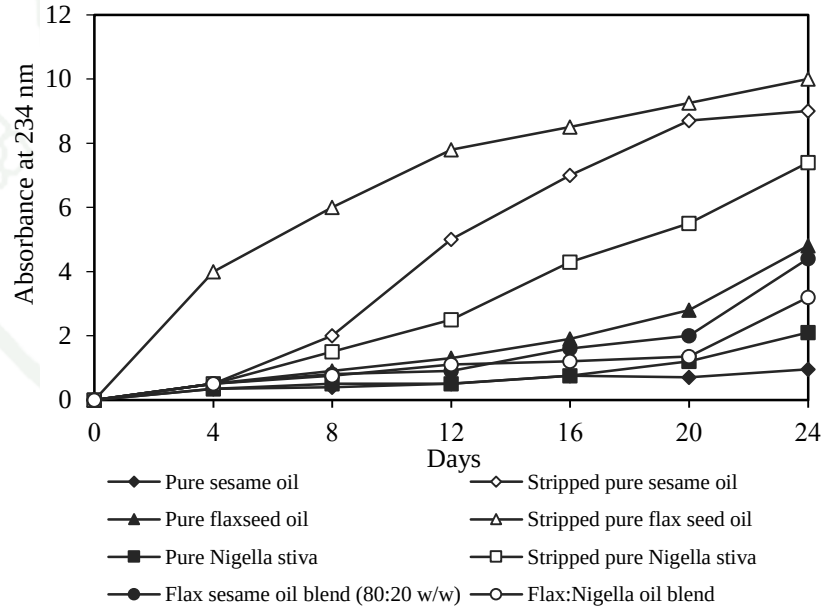


Figure 5 Conjugated diene value of pure oils, stripped oils and blends

Source: modified Hamed and Abo-Elwafa (2012)

Hamed and Abo-Elwafa (2012) studied enhancement of oxidation stability of flaxseed oil by blending with stable vegetable oils found that CD of stripped oils, pure oils or oil blends went in the same trend as peroxide value. CD increased gradually with time and pure oils or oil blends showing significantly less values than stripped oils did. Oxidative stability of flaxseed oil mixed with 20% Nigella stiva oil (FNO) was better than either pure flaxseed oil (FO) oil alone or flax sesame oil blend (FSO) blend containing 20% sesame oil (Figure 5).

2.2.4 Thiobarbituric acid reactive substances (TBARS)

The presence of TBARS in a sample of oil or meat indicates that lipid peroxidation has taken place and the level of TBARS shows the amount of peroxidation that has already occurred (Lukaszewicz *et al.,* 2004). The resulting TBARS value is measured as the malonaldehyde, which is a secondary product formed as a result of lipid peroxidation (Ulu, 2004). TBARS values tend to have a good correlation with sensory testing when being used to detect rancidity of foods (Fernandez *et al.,* 1997, Babalola and Apata, 2011), making it a good choice of an assay to pair with sensory testing.

Ni Eidhin *et al*. (2003) reported that TBARS of the camelina oil increase when increased heating time oilseed. Camelina oil had higher TBARS values than rapeseed, sesame, sunflower, corn, and olive oils from day 5 through 16 of storage and had lower values than fish oil (day 0 to 16) (Figure 6), which correlation with PV and CD. Increasing PV, CD and TBARS value reflect increasing levels of primary and secondary products of lipid oxidation. These values have been used extensively to estimate the progress of lipid oxidation in oils and food products. When the stability of several oils is being compared, higher PV, CD and TBARS values indicate a greater susceptibility to degradation by lipid oxidation.

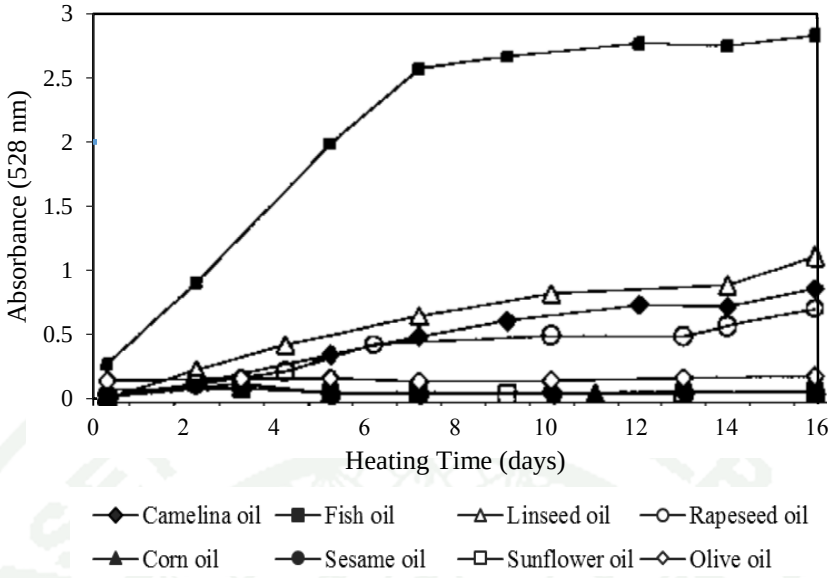


Figure 6 TBARS values of plant oils and fish oil stored at 65°C

Source: modified Ni Eidhin *et al*. (2003)

3. Sugar and their antioxidant properties

3.1 Type of sugar

Sugar or 'culinary sugar' is often used for food cookery and production. In generally, sugar are manufactured from sugarcane, sugar beet, and other (i.e. palm sap, coconut sap, maple sap) (Harish Nayaka *et al*., 2009; Phaichamnan *et al*., 2010). The different types of sugar are based on the method of production, crystal size, color, and taste as well as raw materials (Harish Nakaya *et al.*, 2009). However, the most common types of sugar that are widely consumed are white, refined, brown, and raw sugar. Gul and Harasek (2012) concluded the process of conventional sugar production process (Figure 7). The extraction of juice is the first step, followed by preheating and purification. Many sugar usually used for food production are listed as followed (James, 1990; Harish Nakaya *et al.*, 2009).

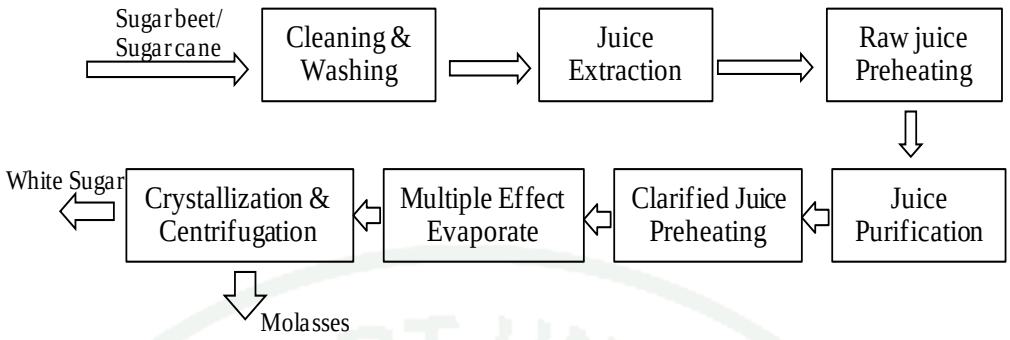


Figure 7 Simplified flow sheet diagram of conventional sugar manufacturing process

Source: modified from Gul and Harasek (2012)

a. White sugars can be produced by phosphatation technique which is precipitation many impurities out of the cane juice.

b. Refine sugar is made by dissolving brown sugar and purifying it with a phosphoric acid. After filtration through activated carbon or bone char for decolorization, the crystal of refined sugar is dried and sold as granulated sugar.

c. Raw sugar is comprised of yellow to brown sugar. The clarified cane juice is boiled until a crystalline solid occurs. This mineral salts and phytochemicals in this salt could be retained.

d. Brown sugar is made by extent process of refining sugar and molasses occurred. Then the fine crystal sugars are coated with molasses. The brown sugar is basically divided into two types. The first raw sugar which is produced by the sugar factories and it has good flavor. However, raw sugar may suffer from variation in quality, particularly regarding hygiene and foreign matters. The second type is produced in sugar refineries with evaporation by boiling. Asikin *et al*. (2014) reviewed that cane brown sugar is valuable nutritional product which has many different names across country, for example, kokuto in Japan, panela in South America, and jaggery in South Asia and Africa. Jaggery is generally produced from sap of palm or coconut. It

is usually sold as loaf form rather than as a crystalline power. The cane brown sugar also called non-centrifugal sugar, has various biological functions with beneficial effects on human health, including anticariogenic antitoxic-cytoprotective, anticarcinogenic, and antioxidation effects (Asikin *et al*., 2014).

3.2 Chemical composition of sugars

3.2.1 Non-enzymatic browning reaction

Many foods undergo browning due to non-enzymatic browning reactions that 23 ccurred during processing or storage (Ibarz *et al.,* 1999). These reactions are the most complex reaction in food industry and are composed of Maillard reaction and caramelization (Eskin, 1990).

3.2.1.1 Formation of color

The heating process is generally used to evaporate water from juice of extract from sugar plants. During the development of brown color caused by the Maillard reaction and caramelization can occur simultaneously (Buera *et al.,* 1987). Caramelization reactions contribute to overall non-enzymatic browning reaction, especially in the alkaline pH ranges, leading to an overestimation of the Maillard reaction in foods (Ajandouz and Puigserver, 1999; Ajandouz *et al*., 2001). The development of color is extremely important and is associated with the extent of Maillard reaction (Martins and Van Boekel, 2003) and caramelization. The Maillard reaction involved in the formation of brown pigments due to the condensation between the carbonyl groups of reducing sugars and aldehydes and ketone, the free amino group of lysine and/or other amino acids, peptides, and proteins or any nitrogenous compound (Jing and Kitts, 2002; Yoo *et al.,* 2004). The color produced range from pale yellow to dark brown, depending on the type of food and/or the extent of the reactions. Color occurs due to the formation of high molecular weight polymeric compounds known as melanoidins (Coca *et al.,* 2004).

Guerra and Mujica (2010) reported the L*, a* and b* value of granulated cane sugar "panelas" were 50.82 to 69.40, 3.88 to 9.51 and 14.31 to 15.69, respectively. Phaichamnan *et al.* (2010) studied color value of palm sugar concentrate 30 samples were collected from primary producers in Songkhla Province, Thailand. The L* value ranges from 1.78 to 53.93, a* value ranges from 9.87 to 34.75 and b* value from 3.09 to 78.94. Additionally, Naknean and Meenune (2011) showed color value of palm sugar syrup produced in Songkhla Province, Southern Thailand, ranges in L*, a* and b* value between 5.34 to 30.14, 21.13 to 36.14 and 8.19 to 56.12, respectively.

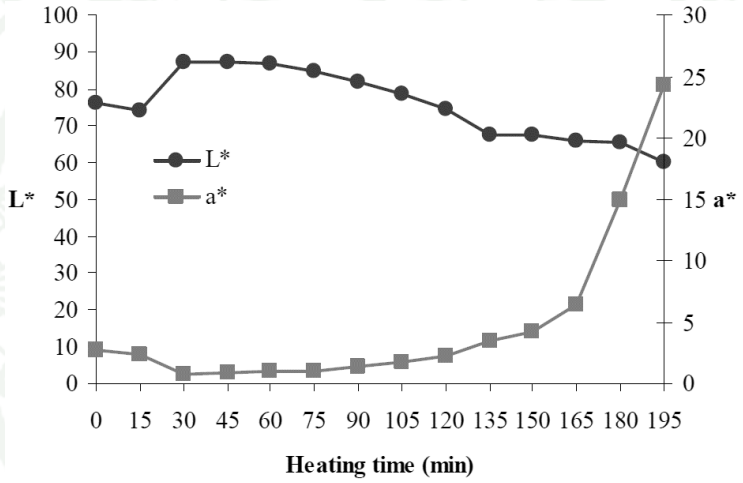


Figure 8 Changes in L*and a* values during the production of palm sugar syrup

Source: modified from Naknean *et al*. (2009)

Naknean*et al*. (2009) studied changes in L* and a* value during the production of palm sugar syrup. During heating, decrease in L* and increase in a* value (Figure8). The L* values decreased indicates that samples were turning darker, which correlated well with increases in the browning of food materials (Maskan, 2006). The a* value continued to change, and the color of sample changed towards orange-yellow, indicating the onset of caramelization and Maillard reactions. The decrease of L* values and increased a* values may contribute to the non-enzymatic browning reaction during the heating process such as heating temperature and time

(Apriyatono *et al*., 2002). Therefore, these values can be used to indicate the rate of non-enzymatic browning reaction. The increase of a* value during heating was responsible for the brown color that corresponded to a decrease in L* value (Naknean *et al*., 2009; Naknean and Meenune, 2011; Naknean *et al*., 2013).

Naknean *et al*. (2013) studied changes in color of palm sugar syrup during storage by using the CIE color system (L*, a*and b*). The L* value decreased by increasing the storage temperature and storage time. The decreasing of this value indicates that the color of palm sugar syrup changed to brown (Burdurlu and Karadeniz, 2003). The greatest decrease in L* values during storage occurred in palm sugar syrup samples stored at 30°C, followed by those stored at 4°C. The a* values can be used to evaluate browning of palm sugar syrup. The a* value increased by increasing storage temperature and storage time. Furthermore, palm sugar syrup samples stored under 30°C presented higher a* values than those stored under 4°C. Changes in b* values decreased by increasing the storage temperature and time. The greatest decrease in b* value during storage occurred in the palm sugar syrup samples stored under 30°C, followed by those stored under 4°C.

Apriyantono *et al*. (2002) studiedrate of browning reaction during preparation of coconut and palm sugars showed that volatile composition of these sugars was dominated by browning reaction products, e.g. furans and pyrazines, and lipid degradation products (fatty acids and ketones). They also contained organic acids, i.e., citric, succinic and lactic acids. The presence of pyrazines in palm and coconut sugars indicates that not only caramelization, but also the Maillard reaction took place during preparation of both sugars.

Generally, the degree of browning could be measured via the absorbance at 420 nm which was used to assess the extent of the Maillard reaction products (Amin *et al*., 2010). Apriyantono *et al*. (2002) reported the increases resulting products from Maillard reaction in coconut and palm sugars were furans and pyrazines. Also, decreases in sucrose, glucose, and fructose content during coconut sap juice heating occurred, whereas the browning intensity (OD_{420}) increased markedly (Table

7). Beside reducing sugar, the free amino acid, 0.01% lysine, also affected in browning intensity (OD_{420}) (Figure 9).

Table 7 Changes in sucrose, glucose, fructose, and brown color intensity of coconut sap during preparation of coconut sugar.

Parameters	Heating time (min)				
	0	22.5	45	67.5	90
Sucrose (g)	345	344.2	342.9	319.8	288.7
Glucose (g)	45.7	41.3	32.4	20.7	12.6
Fructose (g)	35.8	23.2	21.9	13.9	12.8
Total N (g)	0.7	nd.	nd.	nd.	nd.
Brown color intensity (OD_{420})	0.127	0.163	0.363	0.451	0.562

nd= not detected

Source: modified from Apriyantono *et al*. (2002)

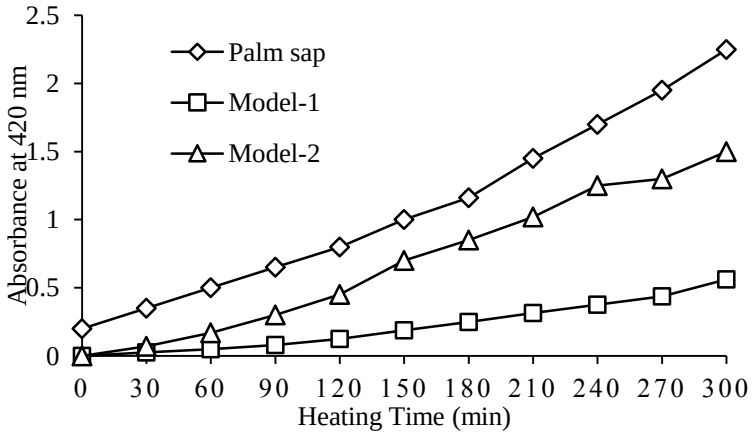


Figure 9 Rate of brown color reaction during heating of palm sap, Model-1 (containing3.42% glucose, 1.56% fructose and 7.22% water), and Model-2 (containingsugars as in Model-1 plus 0.01% L-lysine)

Source:modified from Apriyantono *et al*. (2002)

Amin *et al*. (2010) reported that the browning intensity of palm sugar-like flavoring depends on the sugar concentration which was influenced by the type of reducing sugar involved in reaction. Different types of reducing sugar affected on the browning intensity of palm sugar-like flavoring and commercial palm sugars. Therefore, temperature and duration of heating, pH and concentration of reactant are important parameters for browning reaction.

3.2.2 Polyphenols

Sugars are generally produced from fruits and vegetables which are valuable natural sources of bioactive compounds including phenolic compounds. Many researches, the phenolic acids, polyphenols and flavonoids in sugar cane were reported (McGhie, 1993; Nutt *et al*., 2004; Colombo *et al*., 2006). Phenolic compounds in liquid sugar from cane molasses or in brown sugars were observed (Godshall and Roberts, 1982; Palla, 1982). Also, sugarcane (*Saccharum officinarum*) contains phenolic compounds (Fontaneilla *et al*., 2003) and these compounds have also been found in sugar products such as syrup or molasses and in brown sugar (Palla, 1982).

Harish Nayaka *et al*. (2009) reported that the total phenolic content and phenolic composition of sugar were varieties. The total phenolic content of white and refined sugars was found to be 31.5 and 26.5 µg GAE/g, respectively. Higher phenolic content was observed in brown sugar (372 µg GAE/g) and the presence of gallic acid, protocatechuic, gentisic, 4-hydroxyohenylacetic, vanillic, syringic, *p*-couaric and ferulic acids in brown sugar were found. This concluded that the presence of high total phenolic content in jaggery and brown sugars compared to white and refined sugar might due to 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 (Godshall *et al.,* 2002). The difference techniques used in cane processing to remove color and impurities affect the amount of polyphenols in different sugars and this may resulted in the low phenolic content of white and refined sugars (Harish Nayaka *et al*., 2009).

Payet *et al*. (2006) investigated the total phenolic content of cane sugar manufacturing products by the Folin-Ciocalteu assay and their free radical scavenging capacity to 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. It was total polyphenol content of cane sugar manufacturing products ranged from 0.7 GAE/kg (by dry weight) to 27.21 GAE/kg (by dry weight) (Payet *et al*., 2006). Additionally, Payet *et al.* (2005) reported that the total phenolic content of the cane brown sugar ranged from 108.1 to 418.1 mg GAE/kg of sample, which these variations could be explained by both the different raw materials and the different manufacturing processes. However, antioxdative activity of brown sugars aqueous solutions exhibited weak free radical scavenging activity in the DPPH assay and higher antioxidant activity in the ABTS assay at relatively high concentration. The brown sugar extracts showed interesting free radical scavenging properties despite the low concentration of phenolic. Thus, sugar is a common foodstuff traditionally used for its sweetening properties, which might be accompanied by antioxidant properties arising from molecules (polyphenols, Maillard reaction products) other than sucrose of the cane brown sugars.

3.3 Antioxidative activity

Antioxidant activity is one of the functional properties of MRPs, which impact on the overall acceptability of food by consumer. Many researchers interested in antioxidative properties of sugars, particularly MRPs and CPs as well as polyphenols. Thermal degradation products of sucrose like furfural, caramel, or other MRPs are also well known to be browning agent and furfural had been found to be involved in antioxidant properties. Among non-enzymatic browning reactions, the Maillard reaction has been the most intensively studied. Because MRPs have been found to exhibit antioxidative activity due to radical scavenging (Marales and Jimenez-Perez, 2001; Yen and Hsieh, 1995), metal chelating activity (Wijewickreme *et al*., 1997), scavenging of active oxygen species (Yoshimura *et al.*, 1997), as well as decomposition of hydroperoxide (Tanaka *et al*., 1992). MRPs can be used to prevent lipid oxidation in food systems.

DPPH is one of compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on the exposure to proton radical scavengers (Yamaguchi *et al*., 1998). 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 (Shon *et al*., 2003). The color changed from purple to yellow by acceptance of a hydrogen radical and it became a stable diamagnetic molecule.

Benjakul *et al*. (2005a) studied antioxidant activity of MRPs from a porcine plasma protein–sugar model system. MRPs derived from galactose showed slightly greater activity than fructose with a heating time of 3-5 h, while MRPs derived from glucose showed the lowest radical-scavenging activity. Yen and Hsieh (1995) also found the DPPH-radical scavenging activity of xylose–lysine MRPs. Additionally, Benjakul *et al*. (2005b) who reported that DPPH-radical scavenging activity of CPs under neutral condition (pH 7.0) increased as the heating time of sugars increased. CPs from ribose showed the highest activity, while CPs from glucose were found to exhibit to lowest activity. Phongkanpai *et al*. (2006) exhibit the DPPH-radical scavenging activity of CPs from fructose and glucose increased with increasing heating time and pH levels. It was found that MRPs and CPs were able to reduce the DPPH-radical to the yellow-colored diphenylpicrylhydrazine. Therefore, MRPs and CPs possessed hydrogen-donating ability, suggesting potency to react with free radicals. However, Kirigaya *et al*. (1968) found that antioxidant activity increased with increasing color intensity and Rhee and Kim (1975) also reported that effective antioxidant compounds were formed at an earlier stage of browning reactions. In addition, Benjakul *et al*. (2005a) found correlation between DPPH-radical scavenging activity correlated well with browning intensity (OD_{420}) and absorbance at 294 nm. Thus, it was suggested that either intermediates or the final brown polymer could function as hydrogen donors.

Harish Nayaka *et al.* (2009) evaluated antioxidant activity of jaggery, brown, refined and white sugars by DPPH-radical scavenging and reducing power assays. The results of the free radical scavenging ability of jaggery, brown, refined, and white sugar are shown in Figure 10. Both jaggery and brown sugars showed higher free radical scavenging ability than other sugars. These indicated the potential electron

donating ability of jaggery and brown sugars. Naknean and Meenune (2011) reported DPPH-radical scavenging activity of palm sugar syrup produced in southern Thailand ranged from 13.27-18.49 μmol Trolox equivalents/g sample.

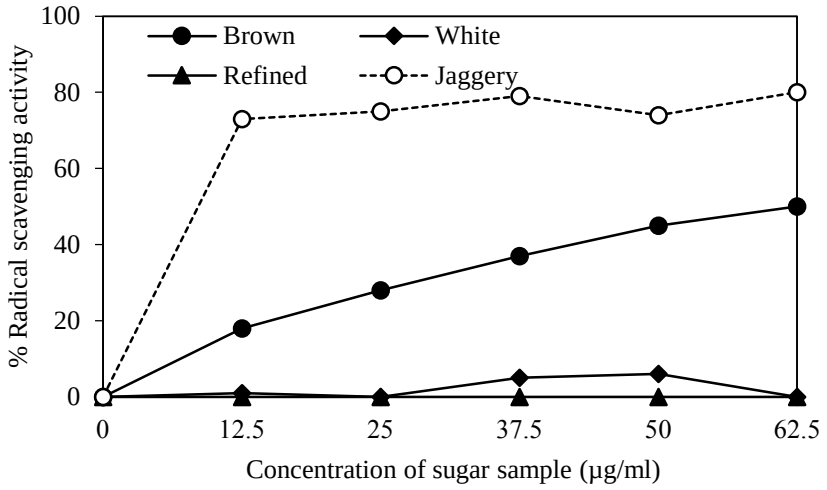


Figure 10 DPPH-radical scavenging activity of jaggery and other sugar sample

Source: modified from Harish Nayaka *et al*. (2009)

Amin *et al*. (2010) reported the DPPH-radical scavenging activity of 5 palm sugar-like flavoring (PSLF) and commercial palm sugars. The commercial palm sugar showed that the lowest DPPH-radical scavenging activity associated with low browning intensity (Figure 11). Hayase *et al*. (1989) speculated that melanoidins showed a greater rate of scavenging hydroxyl radicals as a result of the presence of reductones, enamides or pyrrole-like structure in the melanoidins. In general, the melanoidins were reported to have relatively stable free radicals in their molecules. As the free radicals are very important, this was considered to be a scavenging the radicals.

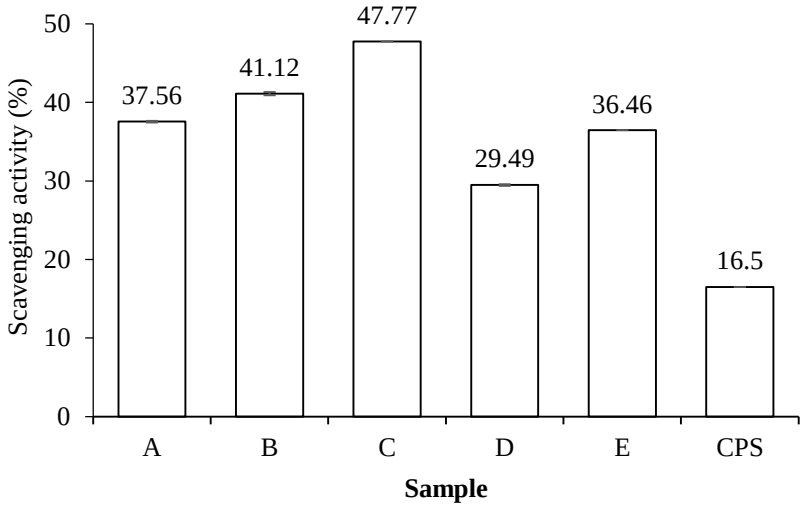


Figure 11 Percentage of DPPH-radicalscavenging activity of various formulations of palm sugar-like flavoring (A-E) and commercial palm sugar (CPs)

Source: modified from Amin *et al*. (2010)

The reducing power is a primary assay undertaken to determine the primary antioxidant activity of antioxidants obtained from components of food products and plant and fruit extracts (Vhangani and Wyk, 2013). The presence of an antioxidant reduces the ferric chloride/ferricyanide complex to its ferrous form. The ferrous form is monitored spectrophotometrically by measuring the formation of a Perl's Prussian blue color at 700 nm (Tafulo *et al*., 2010; Zeng *et al*., 2011). The higher reducing power of the reaction mixture was determined by the higher absorbance (Yuanxia *et al*., 2007). This assay particularly measures the antioxidative activity of MRPs since the hydroxyl groups of MRPs play a role in the reducing activity through their redox potential of transferring electrons (Berker *et al*., 2010; Gu *et al*., 2010; Hwang *et al*., 2011).

To data, the studies about reducing power of MRPs. MRPs from xylose–lysine (Yen and Hsieh, 1995), glucose–glycine (Yoshimura *et al*., 1997), sugar–lysine (Wijewickreme *et al*., 1999) and PPP–sugar (Benjakul *et al*., 2005a) model systems possessed reducing power. Benjakul *et al*. (2005a) showed reducing power of MRPs increased as the heating time increased. MRP samples prepared with galactose showed

the greatest reducing power. MRPs derived from glucose exhibited the lowest reducing power, especially with increasing heating time. MRP samples containing a higher level of sugar had a greater reducing power than those with a lower level. It was suggest that MRPs could function as electron donors.

Lertittikul *et al*. (2007) reported reducing powers of all MRPs with different initial pHs and heating times sharply increased within the first 2 h of heating. No difference in reducing power were observed between MRPs with the initial pHs of 10-12 when the heating time was more than 2 h. MRPs with the initial pH of 8 showed the lowest reducing power at all heating times (compared with those with the higher pHs). Thus, MRPs from the PPP–glucose model system, especially with high initial pH, had hydrogen-donating activity. The hydroxyl groups of MRPs play an important role in reducing activity (Yoshimura *et al*., 1997). However, the intermediate reductone compounds of MRPs were reported to break the radical chain by donation of a hydrogen atom (Eichner, 1981).

Benjakul *et al*. (2005b) studied antioxidative activity of caramelization products of different sugars (D-glucose, D-fructose, D-ribose, and D-xylose). During the heating sugars at pH 7.0, the reducing power of CPs increased when the heating time increased. The CPs referred the reducing compounds might be formed and could exhibit antioxidative activity. The reducing power of CPs might be due to hydrogen-donating ability (Shimada *et al*., 1992).

Harish Nayaka *et al*. (2009) studied reducing power of jaggery, refind, white and brown sugars (Figure 12). Reducing power, a dose dependent increase in absorbance for jaggery and brown sugars. The increased absorbance at 700 nm indicated the presence of reducing power. High antioxidation activity may the contribution of polyphenols in bioactivity. However, the availability of phenolic components in sugarcane juice and their antioxidant activity (Duarte-Almeida *et al*., 2006) also substantiates the bioactivity observed in different sugar varieties. In addition, Naknean and Meenune (2011) also reported the reducing power of palm sugar syrup sample was found in range of 0.85-1.45. The presence of antioxidant activity in

palm sugar syrup was probably due to the presence of phenolic compounds and the formation of MRPs and CPs during heating process. The antioxidant activity of phenolic compounds is clearly related to free radical scavenging and hydrogen-donating ability. The MRPs and CPs could function as electron donors. The hydroxyl groups of MRPs or CPs play an important role in reducing activity. Additionally, the intermediate reductone compounds of the MRPs were reported to break the radical chain by donation of hydrogen atoms (Rufian-Henares and Morales, 2007; Kim and Lee, 2009).

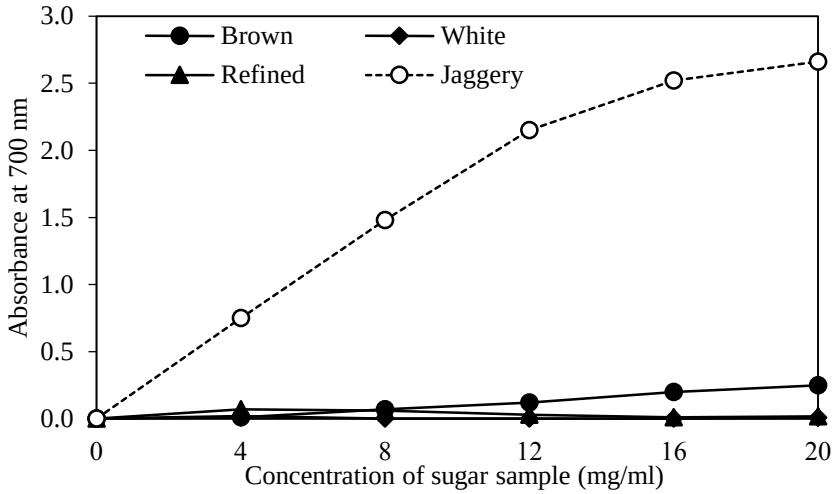


Figure 12 Reducing power of jaggery and other sugars samples

Source: modified from Harish Nayaka *et al*. (2009)

MATERIALS AND METHODS

Materials

1. Raw materials
   1.1 Black perilla and white perilla seeds (Maw Hongson, Thailand)
   1.2 Cane sugar paste (Chiang Mai, Thailand)
   1.3 Unrefined cane sugar
   1.4 Brown cane sugar
   1.5 Caramel crystal cane sugar
   1.6 Refined white crystal cane sugar
   1.7 Coconut sugar paste (Chumphon, Thailand)
   1.8 Palm sugar paste was purchased (Phetchaburi, Thailand)

2. Chemicals

Reagents	Source/Supplier
β-carotene type I	Sigma-Aldrich (St. Louis, Mo, USA)
Gallic acid	Fluka, (Buxchs, Switzerland)
Quercetin	Sigma-Aldrich (St. Louis, Mo, USA)
2, 2-diphenyl-1-picrylhydrazyl (DPPH)	Sigma-Aldrich (St. Louis, Mo, USA)
2,2´-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)	Sigma-Aldrich (St. Louis, Mo, USA)
(±)-6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid (Trolox)	Sigma-Aldrich (St. Louis, Mo, USA)
2,4,6-tris (2-pyridy)-S-triazine (TPTZ)	Sigma-Aldrich (St. Louis, Mo, USA)
Heptadeconoic acid	Sigma-Aldrich (St. Louis, Mo, USA)
3,5-Dinitrosalicylic acid (DNS reagent)	Sigma-Aldrich (St. Louis, Mo, USA)
1,1,3,3-tetramethoxypropane	Sigma-Aldrich (St. Louis, Mo, USA)
Palmitic acid	Sigma-Aldrich (St. Louis, Mo, USA)
2,6-di-tert-butyl-4-methylphenol (BHT)	Panreac (Barcelona, Spain)
Folin-Ciocalteu reagent	Sigma-Aldrich (St. Louis, Mo, USA)
Thiobarbituric acid	Sigma-Aldrich (St. Louis, Mo, USA)
Pyridine	Loba-Chemie (Bombay, India)
Fatty acid methyl ester (FAMEs) C4-C24	Supelco (Bellefonte, PA, USA)

3. Instruments

3.1 Blender (AT710131, Moulinex, France)

3.2 Digital electric balance (GT 4100, OHAUS, Switzerland)

3.3 Hot air oven dryer (FD-115, Binder, Germany)

3.4 Furnace (EF 100478, Lenton, UK)

3.5 Soxhlet apparatus (SER148 Solvent Extraction, VELP Scientifica, Italy)

3.6 Kjeldatherm digestion apparatus (Vapodest 20, Generator, Germany)

3.7 Fiber analyzers (FIRE, VELP Scientifica, Italy)

3.8 Ultrasonic bath (US-1R,AS ONE, Japan)

3.9 Water bath (SW B1-30,WELLAB LIMITED, China)

3.10 Homogenizer (T18 basic, IKA, Germany)

3.11 Vortex mixer (Vortex-Genie 2,Scientific Industries Inc, USA)

3.12 Coupled plasma optical emission spectrophotometer (ICP-OES) (3300 DV, Perkin-Elmer Norwalk, USA).

3.13 Rotary evaporator (N-100, EYELA, Japan)

3.14 Spectrophotometer (Gene Quant 1300, GE Healthcare, Sweden)

3.15 Water activity (A_w) meter (S40003131, AQUA LAB, USA)

3.16 Refrigerated centrifuge (Allera X-15R centrifuge, BECKMAN COULTER, USA)

3.17 pH meters (UB 10, Denver Instrument, USA)

3.18 High Performance Liquid Chromatography (1200, Agilent technologies, USA)

3.19 Gas chromatography (6890N Network GC system, Agilent technologies, USA)

Methods

1. Comparative studies on nutritional characteristics and some bioactive compounds of brown perilla and white perilla seeds

1.1 Perilla seed collection

Brown perilla and white perilla seeds were purchased from Meahongson, Thailand. Samples were vacuum packed in aluminum bag (100g/bag) and transferred to Food and Nutrition Laboratory, Department of Home Economics, Kasetsart University. Samples were kept at -80°C until analyses.

1.2 Analyses

1.2.1 Proximate analysis

Perilla seed sample was ground in a grinder (AT710131, Moulinex, France) for 1 min. Ground samples were analyzed for moisture, protein, fat, ash and crude fiber contents as described by the methods of AOAC (2000). Moisture content was determined by oven drying at 105°C until constant weight. Protein content was determined according to the Kjeldahl method. The values for protein were calculation from the amount of total nitrogen (N). The factor 6.25 was used for conversion of nitrogen to crude protein content. Fat content was determined by the Soxhlet extraction system. Petroleum ether was used to extract total fat content. Ash content was determined by heating in furnace at 550°C until constant weight. Crude fiber was determined by using the defatted sample and digestion with weak acid and base. Total carbohydrate content was determined by difference (AOAC, 2000). Total carbohydrates = [100 - (moisture + ash + fat + protein + crude fiber)].

1.2.2 Determination of mineral content

The mineral analyzed includes, calcium, magnesium, iron, phosphorus, zinc and potassium, were determined by inductively coupled plasma emission spectrometer (Optima 3300 DV, Perkin-Elmer, United States) according to the AOAC (2000). One hundred and fifty milligrams of ground perilla seed was mixed well with 4 ml of nitric acid. The mixture was heated on hot plate until digestion was complete. 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 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, phosphorus, zinc and potassium were 317.933, 285.213, 238.204, 213.617, 206.200 and 766.490 nm, respectively.

1.2.3 Determination of β-carotene content

The amount of β-carotene of perilla seed was determined according to method of Kurilich and Juvik (1999) with a slight modification. Two grams of sample was mixed with 6 ml of 0.1% ethanolic butylated hydroxyl toluene (BHT) (w/v), mixing for 20 sec by vortex, and placed in an 85°C water bath for 5 min. Potassium hydroxide (0.5 ml, 80% w/v) was added to the heated ethanol-sample mixtures, to saponify the potentially interfering oils. Then, mixed by vortex and returned to the 85°C water bath for 10 min with an additional mixing at 5 min. After saponification, sample was immediately placed in ice, and added 3 ml of cold deionized water. Three millilitres of hexane was then added to the cooled sample and centrifuged at 1,200 ×g at 25°C for 10 min using a centrifuge. The supernatant was adjusted to 3 ml with hexane. β-carotene content was measured absorbance at 450 nm by spectrophotometer (GeneQuant 1300, GE Healthcare, Sweden). A standard curve of β-carotene type I ranging from 0-7 µg/ml was prepared. β-carotene contentwas expressed as µg β-carotene/g dry weight of the sample.

1.2.4 Determination of α-tocopherol content

The content of α-tocopherol in both perilla seeds was measured by high performance liquid chromatography (HPLC) according to the method of Qian and Sheng (1998). Sample (1g) and ascorbic acid (1 g) were dissolved in distilled water (2 ml), then the mixture was added with 3 ml potassium hydroxide and 3 ml ethanol and mixed well. Sample was incubated at 85°C for 30 min, cooling and extract with 3 ml of hexane:ethyl acetate solution (8:2 v/v) (4 times). Pipette supernatant from extraction were then combined, washed with distilled water until lye out the hexane phase was drained off into an Erlenmeyer flask. Then, add about 2.5 g of anhydrous sodium sulphate, shaken very well and decanted into round bottom flask through a Whatman No.4 filter paper. The solvent was evaporated at 50°C to dryness. The residue was redissolved in 1 ml hexane before being inject into the HPLC system.

The HPLC system used for the separation was an Agilent Series 1200 LC Systems and Modules with a Diode-Array Detector (DAD) (Agilent Technologies, New York, USA). Chromatographic separation was performed on a 250×4.6 mm platinum C_{18} column heated to 40°C. Methanol was used as mobile phase at a flow-rate of 1 ml/min. A 290 nm wavelength, where vitamins presented a relatively high absorbance, was used for the analysis.

1.2.5 Determination of lipid composition and fatty acid profiles

1.2.5.1 Lipid extraction

Lipid of perilla seed was extracted by the method of Bligh and Dyer (1959). Sample (25-50 g) was homogenized with 200 ml of a chloroform:methanol:distilled water mixture (50:100:50) at the speed of 9,500 rpm for 2 min at 4°C using homogenizer (T18 basic, IKA, Germany). The homogenate was treated with 50 ml of chloroform and homogenized at 9500 rpm for 1min. Then, 25 ml of distilled water was added and the homogenized again for 30 sec. The homogenate was centrifuged at 3000 rpm at 4°C for 15 min using a Allera X-15R centrifuge and

transferred into a separating flask. The chloroform phase was drained off into a 125 ml Erlenmeyer flask containing about 2-5 g of anhydrous sodium sulfate, shaken very well, and decanted into a round-bottom flask through a Whatman No.4 filter paper. The solvent was evaporated at 25°C, using an EYELA rotary evaporator N-100 (Tokyo, Japan), and the residual solvent was removed by flushing with nitrogen. The resulting lipid was kept in amber vial at -20°C until analyses.

1.2.5.2 Lipid compositions

Lipid classes were determined using a thin layer chromatography/flame ionization detection analyzer (IATROSCAN TLC/FID Analyser, IATRON Laboratories, Inc., Tokyo, Japan). One microliter of lipid (0.25 mg/ml) was spotted onto the scanned quartz rod (silica power coated Chromatorod-S III, IATRON Laboratories, Inc., Tokyo, Japan) and was separated using a mixture of benzene:chloroform:acetic acid (50:20:0.7) for 35 min. The develop sample was dried in an oven at 105°C for 5 min and immediately scanned with the TLC-FID analyzer with a scanning speed of 30 sec/scan. The analytical conditions were: H_2, flow rate of 160 ml/min; air, flow rate of 2000 ml/min. Retention times of lipid composition standards were used to identify chromatographic peaks of the samples. Each lipid was calculated, based on peak area ratio and expressed as % of total lipid.

1.2.5.3 Fatty acid profile

Fatty acid profile of perilla seed was determined as fatty acid methyl ester (FAMEs). The FAMEs were prepared according to the method of AOAC (2000). The 300 µl heptadecanoic acid (0.5 µg/µl), used as internal standard, were added. The prepared methyl ester was injected to the gas chromatography (6890N Network GC system, Agilent technologies, USA) equipped with auto-sample and on the flame ionization detector (FID) at a split ratio of 1:100. Separation was accomplished on a 30 m BPX70 capillary column (SGE Australia, Ltd., Australia) with 0.25 mm i.d. and 0.25 micrometers phase thickness. The initial temperature of the column was set at 140°C and was increased to 200°C at a rate of 4°C/min and then

increasing to 220°C at a rate of 1°C/min. Carrier gas was helium (He) at flow rate of 29 cm/sec. The detector temperature was set at 260°C, while the temperature at the injection port was maintained at 220°C. Retention time of FAME standard was used to identify chromatographic peaks of the sample. Fatty acid content was calculated, based on the peak area ration and expressed as g fatty acid/100 g lipid.

1.2.6 Determination of bioactive compounds

1.2.6.1 Preparation of perilla seed extract

Perilla seed extract was prepared according to the method of Peng *et al.* (2005). Perilla seed was dried at 60°C for 2h. Then, sample was blended into powder by blender (model AT710131, Moulinex, France). Two grams of sample power was extracted with 10 ml of 80% ethanol for 2h in an ultrasonic bath (US-1R, AS ONE, Japan) at ambient temperature and centrifuged at 7,000 rpm at 25°C for 15 min using a centrifuge. The supernatant was filtered through a 0.22 µm nylon filter and adjusted volume up to 10 ml with 80% ethanol. The extract was kept at 4°C in the dark until analyses.

1.2.6.2 Determination of total phenolic content

Total phenolic content of perilla seed extract was determined by the method of the Folin-Ciocalteu micro-method (Saeedeh and Asna, 2007). Twenty microliters of extract was mixed with 1.16 ml of distilled water and 100 µl of Folin-Ciocalteu reagent, followed by the addition of 300 µl 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 from the standard curve of gallic acid equivalent (GAE) and expressed as mg gallic acid equivalent (GAE)/g dry weight of the sample.

1.2.6.3Determination of total flavonoid content

Total flavonoid content of perilla seed extract was determined according to method of Ordoñez *et al*. (2006). One millilitres of perilla seed extract was mixed with 1 ml of 2% $AlCl_3$ ethanolic solution using a vortex. After incubation in water bath at room temperature for 1 h, the absorbance at 420 nm was measured using spectrophotometer. The presence of flavonoid in sample was indicated by a yellow color. Total flavonoid content was calculated as quercetin equivalent (QE) from standard curve and expressed as µg quercetin equivalent (QE)/g dry weight of the sample.

1.2.6.4 Determination of total flavonol content

Total flavonol content of perilla seed extract was determined according to method of Kumaran and Joel (2007). Two millilitres of perilla extract was mixed with 2.0 ml of 2% $AlCl_3$ ethanolic solution. Then, added 3.0 ml sodium acetate solutions (50 g/l). After incubation in water bath at 20°C for 2.5 h, the absorbance of mixture was measured at 440 nm using spectrophotometer. Total flavonol content was calculated from the standard curve of quercetin equivalent (QE) and reported as µg quercetin equivalent (QE)/g dry weight of the sample.

2. Comparative studies on antioxidative activity of brown perilla and white perilla seeds

2.1 Preparation of perilla seed extract (as mentioned in 1.2.6.1)

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

DPPH radical scavenging activity of perilla seed extracted was determined according to method of Yen and Hsieh (1995) with a slight modification. Four hundred microliters of extract (concentration 15 mg/ml) 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, except that 80% methanol was used instead of perilla seed extract. The radical scavenging activity was measured as a decrease in the absorbance of DPPH-sample mixture. The percentage of DPPH radicals scavenging activity was calculated as follows (Singh and Rajini, 2004).

$$\text{Radical scavenging activity (\%)} = [1-(A_{\text{sample(517 nm)}} / A_{\text{control(517 nm)}})] \times 100$$

where; $A_{\text{sample (517 nm)}}$ is the absorbance of sample

$A_{\text{control (517 nm)}}$ is the absorbance of control

2.3 Determination of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS)-radicalscavenging assay

ABTS radical scavenging activity was determined as described by Re *et al*. (1999) and modified slightly by Khantaphant and Benjakul (2008). ABTS radical ($ABTS^{\bullet+}$) 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, $ABTS^{\bullet+}$ solution was diluted with methanol to obtain an absorbance of 1.1 ($\pm$0.02) at 734 nm. To initiate the reaction, one hundred and fifty microliters of perilla seed extracted was mixed with 2850 µl of $ABTS^{\bullet+}$ solution. The mixture was incubated at room temperature for 2h (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.

2.4 Determination of ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power of perilla seed extracted was determined following the method of Benzie and Strain (1996). 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 $FeCl_3.6H_2O$. A working solution was prepared freshly by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3.6H_2O$ solution. The mixed solution was incubated at 37°C for 30 min and was referred to as FRAP solution. One hundred fifty microliters of perilla seed extracted 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.

2.5 Determination of reducing power

Reducing power of perilla seed extracts was determined by the method of Oyaizu (1986) with a slight modification. Five hundred microliters of extract was mixed with 500 µl of 0.2 M sodium phosphate buffer (pH 6.6) and 500 µl of 1% potassium ferricyanide. The reaction mixtures were incubated in water bath at 50°C for 20min, followed by addition of 500 µl of 10% trichloroacetic acid. The mixtures were treated with 2 ml of distilled water and 400 µl of 0.1% ferric chloride. The absorbance at 700 nm of the mixture was measured by spectrophotometer. The standard curve was prepared using BHT ranging from 0-100 µg/µl. and expressed as BHT equivalents /100 g dry weight of the sample.

3. Effect of sugars on lipid oxidation of ground perilla seed

3.1. Study on antioxidant activity of sugars

3.1.1 Commercial sugars collection

Seven commercial sugars were procured from supermarkets and local markets (as shown in material section). Each sugar was vacuum packed in aluminum bag (500 g/bag). For cane sugar paste, coconut sugar paste and palm sugar paste, the packed samples were kept in freezer. The other sugar samples were kept at room temperature until analysis (within 10 days).

3.1.2 Physicochemical properties of commercial sugars

3.1.2.1 Determination of pH value

The pH of sugar was determined according to the method of Benjakul *et al*. (1997). Sample was homogenized with 10 volumes of distilled water (w/v), measured using a pH meter.

3.1.2.2 Determination of water activity (A_w) value

A_w was measured using water activity meter (S40003131, AQUA LAB, USA)

3.1.2.3 Determination of color value

Color was measured by Hunter lab and reported in CIE system, L* a* and b* parameters indicate lightness, redness/greenness and yellowness/blueness, respectively.

3.1.2.4 Determination of total sugar and reducing sugar contents

Total sugar content was determined following the method of James (1995) 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 the cooled with ice and water for 5 min. Twelve milliliter 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 µl) was pipetted into a test-tube and 500 µl of 3,5-dinitrosalicylic acid (DNS) solution was added then vortex. Distilled water (1 ml) was added and tranfered 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 standard curve 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 (1995) with a slight modification. Sample (50 g) was mixed 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.

3.1.2.5 Determination of mineral content (as mentioned in 1.2.2)

3.1.2.6 Determination of intermediate browning product and browning intensity

The intermediate browning product and browning intensity of sugar was modified the method of Ajandouz *et al.* (2001). Sugar 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.

3.1.2.7 Determination of total phenolic content (as mentioned in 1.2.6.2)

3.1.3 Determination of antioxidative activity of sugars

3.1.3.1 Preparation of extracts from sugars

Solutions of sugar were prepared at 0.5% (dry weight/volume), dissolved in distilled water and 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. Antioxidative activity of all samples were measured as described in section 2.

3.2 Study on lipid changes of ground perilla seed as influenced by different sugars

3.2.1 Production of sugar-ground perilla seed

The sugar-ground perilla seed was produced as the method of "nga-tum-aoi" production (Chiang Mai University Library, 2012). Perilla seed was roasted at 50°C for 10 min and ground into powder. Sugar (130 g) was added to ground perilla seed (65 g), then mixed well and add water (5 g). The mixture (2 g) was shaped in spherical and wrapped with cellophane. The resulting product was packed in plastic box and stored at room temperature. During storage, sample was taken at day 0, 5, 10, 15, 20, 25 and 30 for analysis.

3.2.2 Analyses

3.2.2.1 Determination of pH value (as mentioned in 3.1.2.1)

3.2.2.2 Determination of moisture content (as mentioned in 1.2.1)

3.2.2.3 Determination of water activity (A_w) value (as mentioned in 3.1.2.2)

3.2.2.4 Determination of color value (as mentioned in 3.1.2.3)

3.2.2.5 Determination of lipolysis and lipid oxidation

3.2.2.5.1 Lipid extraction (as mentioned in 1.2.5.1)

3.2.2.5.2 Determination of free fatty acid content

Free fatty acid content was determined according to the method of Lowry and Tinsley (1976). The extracted lipid (100 mg) was treated with 5 ml of isooctane and swirled vigorously to dissolve the sample. The mixture was then treated with 1 ml of 5% (w/v) cupric acetate–pyridine reagent, prepared by dissolving reagent grade cupric acetate, filtering and adjusting the pH to 6.0-6.2 using pyridine. The mixture was shaken vigorously for 90 sec using a Vortex and allowed to stand for 10-20 sec. The upper layer was subjected to absorbance measurement at 715 nm, using a spectrophotometer. A standard curve was prepared using palmitic acid in isooctane at concentrations ranging from 0-50 µmol/5ml. Free fatty acid content was expressed as g free fatty acid/g lipid.

3.2.2.5.3 Determination of peroxide value

Peroxide value was determined according to the method of Low and Ng (1978). The extracted lipid (150 mg) was treated with 10 ml of organic solvent mixture (chloroform:acetic acid mixture, 2:3). The mixture was shaken vigorously, followed by addition of 1 ml of saturated potassium iodide solution. The mixture was kept in the dark for 5 min and 20 ml of distilled water was added and the mixture was shaken. To the mixture with 0.5 ml of starch solution (1%, w/v) was added as an indicator. The peroxide value was determined by titrating the iodine liberated from potassium iodide with standardized 0.01 N sodium thiosulfate solution. The peroxide value was expressed as milliequivalents of free iodine/kg lipid.

3.2.2.5.4 Determination of conjugated diene

Conjugated diene was measured according to the method of Frankel and Huang (1996). The extracted lipid (10 mg) was dissolved in 5 ml of isooctane and the absorbance were measured at 234 nm using a spectrophotometer.

3.2.2.5.5 Determination of thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Buege and Aust (1978). Two hundred milligram of sample was homogenized for 1 min with 25 ml of a solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl, using an homogenizer. The mixture was heated in a boiling water bath (95-100°C) for 10 min, cooled with running tap water and centrifuged at 5500 rpm at 25°C for 25 min using a centrifuge. The absorbance of the supernatant was measured at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at concentrations ranging from 0-10 ppm. TBARS was calculated and expressed as mg malonaldehyde/g sample.

3.3.2.6 Effect of sugars on acceptability of ground perilla seed during 30-days storage at room temperature

The sugar-ground perilla seed was evaluated for acceptance by an untrained 40-membered panel. The panelists were students and staffs in Department of Home Economics of age ranging from 22 to 35 years, Faculty of Agriculture, Kasetsart University. Panelists had sensorial acquaintance with perilla seed products. A nine-point hedonic scale: in which a score of 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely, was used for evaluation (Chamber IV and Wolf, 1996). Samples were randomly selected and coded with three-digit random number and presented to the panelists at room temperature. During evaluation, the panelists were situated in private booths. Room temperature water was given to rinse the mouth between samples. Sensory preferences on appearance, color, odor, taste, sweetness, texture, flavor, and overall acceptability.

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 and Independent samples t-test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

1. Comparative studies on nutritional characteristics and some bioactive compounds of brown perila and white perilla seeds

1.1 Proximate composition

Differences in compositions of brown perilla and white perilla seeds were observed ($p<0.05$). Generally, brown perilla seed had lower moisture content than did in white perilla seed ($p<0.05$). Saklani *et al*. (2011) reported that perilla seed from Uttarakhand, India contained 8.00% moisture content. For other oilseed, moisture content of varietal different sesame seed was between 5.00-8.75% (Adebowale *et al*., 2011). According to the farmer information, they usually dry perilla seed after harvesting by sun-drying for 2-3 days. The moisture content could be used as indicator for storage time. The lowering moisture content could be used for shelf-life extension. Higher moisture content can enhance the microbial and enzymatic activity, leading to deteriorate or lower nutritional value of the oil seed (Goli *et al.,* 2013). From the results, differences in moisture content of both samples might be affected by variety and cultivation as well as after harvest handling, however, their low moisture contents could be considered as positive property for long storage time of perilla seed.

Higher protein content in brown peilla seed was found ($p<0.05$). Ingeneral, protein in perilla seed is located in seed kernel (Longvah and Deosthale, 1998), which contained 15.7–23.9% protein content (Longvahand Deosthale, 1991; Longvah and Deosthale, 1998). Additionally, protein content of perilla seed samples was similar to plants in the same family (Labiatae/Lamiaceae) such as basil seed (14.76-20.16%) (Razavi *et al*., 2009), but perilla seed was an inferior source protein to other oil seeds such as soybean (40%) (Agostoni *et al*., 2006), black sesame 27.65% and white sesame (26.94%) (Gandhi and Srivastava, 2007). In addition, Longvah and Deosthale (1998) reported that the essential amino acids of perilla seed represent 39% of total amino acid but limiting amino acid, lysine, can be found. However, total essential amino acids are lower than most animal proteins (Longvah and Deosthale, 1998).

Obviously, brown perilla seed had higher fat content than this in white perilla seed for 5.6 times (p<0.05). Perilla seed is a source of edible vegetable oil, but in this study brown and white perilla seeds had fat only 20.27% and 3.59% respectively, which was lower than other reports. Siriamornpun *et al.* (2006) reported that total lipid content of perilla seed ranged from 34-37%, while Longvah and Deosthale (1991) and Asif and Kumar (2010) reported that perilla seed from Manipur and Dehradum had 51.7% and 30-35% total lipid content, respectively. Difference fat content depends on many factors such as the maturation period, harvesting time, ripening stage of the seed, environmental conditions, cultivation climate, season and different location (Siriamornpun *et al.*, 2006). However, perilla seed fat content was comparable with those of commercial oilseeds such as soybean (20%), cottonseed (18%), and higher than that of raspberry (10.7%), grape seed (8.2%), and maize (2.39-3.92%) (Kamel *et al*., 1985; Oomah *et al*., 2000; Ali *et al*., 2010). The results showed that the perilla, particularly brown seed can be regarded as a good source of edible oil.

Ash content of brown perilla and white perilla seeds were 3.37% and 3.34%, respectively. The ash content is an indication of the mineral content of the perilla seed. It has been reported that ash content in basil seed (4.72-5.54%) (Razavi *et al*., 2009) and sesame (5.56-5.59%) (Gandhi and Srivastava, 2007). From the results, ash content in both perilla seed samples was lower than sesame and basil seeds.

Generally, brown perilla seed had high content of crude fiber than this in white perilla seed (p<0.05). Crude fiber is mostly in husk or seed coat, which is consisted of cellulose, hemicellulose, pectin and chitin (Narasinga Rao, 1985). Crude fiber plays an important role in promotion of normal blood cholesterol concentration, modulation of blood glucose concentration and maintenance of healthy bowel function (Sizer and Whitney, 2008). It has been discovered that high content of fiber can improve digestibility and absorption processes (Miranda *et al*., 2010). Several researches reported that crude fiber of perilla seed was 20.68-23.27%, which was higher than that of sesame (3-6%) (Gopalan *et al*., 1982; Taha *et al*., 1987).

The amount of total carbohydrate in perilla seed was 33.96% of brown perilla seed and 56.88% of white perilla seed. In general, oilseed carbohydrate, such as sesame seed, contained sugars (D-glucose, D-galactose, D-fructose and planteose) and most of which are reducing type (Hegde, 2004). Furthermore, no starch was found in oilseed (Wankhede and Tharanathan, 1976). Thus, the different carbohydrate content of both perilla seed samples might different sugar content.

From the results, perilla seeds are good source of fat and protein, especially brown perilla seed. Lower fat content in white perilla seed might affect on lower crop production. However, total carbohydrate in white perilla seed is an interesting composition which was found to be high content.

Table 8 Proximate composition of brown perilla and white perilla seeds.

Composition	Brown perilla seed	White perilla seed
Moisture (%)	$4.82 \pm 0.02^{*b**}$	6.82 ± 0.10^{a}
Protein (% dry weight)	19.13 ± 0.19^{a}	15.51 ± 0.09^{b}
Fat (% dry weight)	20.27 ± 0.31^{a}	3.59 ± 0.07^{b}
Ash (% dry weight)	3.37 ± 0.02^{a}	3.34 ± 0.02^{b}
Crude fiber (% dry weight)	23.27 ± 0.05^{a}	20.68 ± 0.18^{b}
Total carbohydrate (% dry weight)	33.96 ± 0.40^{b}	56.88 ± 0.12^{a}

* Mean $\pm$ SD from triplicate determinations.

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

1.2 Mineral content

The contents of different minerals in both perilla seeds, brown and white seeds, are shown in Table 9. Brown perilla seed had higher content of Ca, Mg, and P determined than had white perilla seed (p<0.05). No difference in Fe content was observed (p>0.05). Both perilla seeds contained slightly difference of Zn content, while white perilla seed exhibited higher K than had brown perilla seed (p<0.05). From the results, it was found that Ca and P were the dominant minerals in both samples. Generally, lower Ca content in perilla seed comparable with sesame seed for 2-3 folds was reported by Gopalan *et al*. (1982) and Weiss (1983). However, these result showed that the contents of Ca and P in both sample were higher than that reported by Saklani (2011) (0.24 mg/g Ca and 0.21 mg/g P). For nutritional characteristics, P is needed for bone growth, kidney function and cell growth, and also plays a role in maintaining the body's acid-alkaline balance. In addition, Ca assists in teeth and bone development (Brody, 1994). Fe is a transition metal ion which has been known as the major catalyst for oxidation (Thanonkaew *et al*., 2006). Thus, this mineral might contribute to the oxidation of perilla seed lipid.

Table 9 Mineral contents (mg/g dry weight) in brown perilla and white perilla seeds.

Composition	Brown perilla seed	White perilla seed
Ca	5.13 ± 0.05^{a}	4.00 ± 0.04^{b}
Mg	2.76 ± 0.00^{a}	2.35 ± 0.03^{b}
Fe	0.12 ± 0.00^{a}	0.12 ± 0.00^{a}
P	6.54 ± 0.05^{a}	5.14 ± 0.12^{b}
Zn	0.03 ± 0.00^{b}	0.04 ± 0.00^{a}
K	2.05 ± 0.05^{b}	2.34 ± 0.02^{a}

* Mean ± SD from triplicate determinations.

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

1.3 β-carotene and α-tocopherol contents

The content of β-carotene in brown perilla and white perilla seeds are shown in Table 10. Generally, β-carotene content in brown perilla seed was found to be 0.62 μg/g dry weight. Pawel *et al.* (2013) reported that β-carotene content in flaxseed and sesame seed were 1.87 μg/g and 0.37 μg/g, respectively. However, the content of β-carotene in white perilla seed was not detectable. Generally, β-carotene can act as provitamin A, which is an essential nutrient required for maintaining immune function, eye health, vision, growth and survival in human being (Rich *et al*., 2004). In addition, carotenoid is acting as primary antioxidant by trapping free radicals or as secondary antioxidant by quenching singlet oxygen (Halliwell and Gutteridge, 1998).

Brown perilla seed had higher 5.5-fold α-tocopherol content than did in white perila seed ($p<0.05$). Difference content of α-tocopherol in oilseed and other oil crops had been reported. Codex (1999) reported that the α-tocopherol content of sunflower, sesame, soybean, and corn was 40.3-93.5 mg/100 g of fat, 0.33 mg/100 g of fat, 0.9-35.2 mg/100 g of fat, and 2.3-57.3 mg/100 g of fat, respectively. In general, tocopherol or vitamin E in perilla seed was 276.78 μg/g dry weight (Sangaroon *et al*., 2011). From the results, α-tocopherol content of brown perilla seed was higher than did in sesame. According to nutritional characteristic of α-tocopherol, it showed an important form of vitamin E for human's and animal's health (Traber and Sies, 1996). Principal function of α-tocopherol is being an antioxidant in plant cell, and it is also a powerful biological antioxidant (Lee *et al*., 2008). From the results, the content of α-tocopherol could be one of key factors for health food product development. Thus, brown perilla seed is an interesting source for using health food development.

1.4 Lipid composition and fatty acid profiles

Lipids from both brown perilla and white perilla seeds had triglyceride as a major component (93-97%) (Table 11). In addition, a little amount of monoglyceride was found in brown perilla seed (2.19%) and white perilla seed (6.08%). No diglyceride was found in both perilla seeds. In previous studies, it has been that found triglyceride

was the most predominant lipid in perilla seed (96.48-97.02%) and diglyceride was not detected (Siriamornpun *et al.*, 2006).

Table 10 Thecontents of β-carotene and α-tocopherol (µg/g dry weight) in brown perilla and white perilla seeds.

Samples	β-carotene	α-tocopherol
Brown perilla seed	0.62 ± 0.02*	10.14 ± 0.37a**
White perilla seed	nd***	1.85± 0.07b

* Mean ± SD from triplicate determinations.

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

***nd = not detectable

Table 11 Lipid compositions of brown perilla and white perilla seeds.

Composition (% of total lipid)	Brown perilla seed	White perilla seed
Triglyceride	97.81	93.92
Diglyceride	nd*	nd
Monoglyceride	2.19	6.08

* nd = not detected

The fatty acid profiles of both perilla seeds are shown in Table 12. Fatty acids were significantly different in terms of quality and quantity of brown perilla and white perilla seeds (p<0.05). The nine identified fatty acids in perilla seed lipid can be devided into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Brown perilla seed lipid had higher content of fatty acids than did in white perilla seed. The ratio of SFA:MUFA:PUFA was approximately 1.00:1.22:7.89 and 1.00:1.37:8.30 for brown perilla seed and white perilla seed,

respectively. These results were similar to previous study of Longvah *et al.* (1991) and Kim *et al*. (2004) who reported that the SFA:MUFA:PUFA ratio of perilla seed of 1.00:1.00:5.73 and 1.00:1.77:8.50, respectively. Also, Siriamornpun *et al*. (2006) and Ding *et al*. (2012) reported the ratio of 1:1.10:7.76, and 1.00:2.00:6.02, respectively. From the results, predominant unsaturated fatty acidof both perilla seeds was polyunsaturated fatty acids, particularly α-linolenic acid. Considering the MUFA, oleic acid was a major monounsaturated fatty acid in brown perilla seed (11.81%) and white perilla seed (11.18%), which was similar to Siriamornpun *et al*. (2006); Peiretti (2011) and Ciftci *et al.* (2012). Predominant PUFA of both perilla seeds was α-linolenic acid, followed by γ-linolenic acid. Linolenic acid in brown perilla and white perilla seeds was 59.08% and 50.79%, respectively. In general, linolenic acid is considered as essential fatty acids (Sizer and Whitney, 2008). This result was similar to those reported in previous studies on perilla seed where α-linolenic acid content of the seed was about 52.58-62.00% (Longvah and Deosthale, 1991; Shin and Kim, 1994; Siriamornpun *et al*., 2006; Peiretti, 2011; Ciftci *et al.*, 2012; Ding *et al.*, 2012). Additionally, γ-linolenic acid (18.87% in brown perilla seed and 17.93% in white perilla seed) and eicosadienoic acid (0.13% in brown perilla seed and 0.14% in white perilla seed) were also found. Furthermore, the contents of these fatty acids were higher than the results of Ciftci *et al*. (2012).

According to nutritional quality, the *n*6 and *n*3 ratio is importance to lipid metabolism. The ratios of *n*6:*n*3 fatty acid of brown perilla and white perilla seeds were 0.32 and 0.35, respectively (Table 12). The results were similar to the ratio (0.3-0.4) in perilla lipid which was reported by Siriamornpun *et al*. (2006). However, Ciftci *et al*. (2012) reported a lower of this ratio for perilla seed (0.22). This might be due to the differences in species and cultivation as well as season. From the results, the *n*3 to *n*6 ratio of fatty acid are typically low, but *n*3 fatty acid, particular α-linolenic acid, were very high. However, perilla seed could be used as α-linolenic acid rich source to enrich diet, which currently is often deficient in *n*3 fatty acids. However, the α-linolenic acid is very susceptible to oxidation and the seed handling and storage must be done carefully to avoid oil rancidity and off-flavor.

Table 12 Fatty acid compositions (g/100 g lipid) of brown perilla and white perilla seeds.

Fatty acids	Brown perilla seed	White perilla seed
Saturated fatty acid		
Palmitic acid	6.33 ± 0.01[*a**]	5.54 ± 0.00[b]
Stearic acid	3.22 ± 0.00[a]	2.57 ± 0.00[b]
Heneicosanoic acid	0.22 ± 0.00[a]	0.19 ± 0.00[b]
Behenic acid	0.12 ± 0.00	nd[***]
Monounsaturated fatty acid		
Oleic acid	11.81 ± 0.01[a]	11.18 ± 0.00 [b]
Eicosenoic acid	0.22 ± 0.00[a]	0.17 ± 0.00[b]
Polyunsaturnted fatty acid		
γ-linolenic acid	18.87 ± 0.01[a]	17.93 ± 0.01[b]
α-linolenic acid	59.08 ± 0.02[a]	50.79 ± 0.01[b]
Eicosadienoic acid	0.13 ± 0.00[a]	0.14 ± 0.01[a]
Total saturated fatty acid (SFA)	9.89	8.30
Total monounsaturated fatty acid (MUFA)	12.03	11.35
Total polyunsaturated fatty acid (PUFA)	78.08	68.86
Total unsaturated fatty acid	90.11	80.21
Total fatty acid	100.00	88.51
SFA:MUFA:PUFA	1.00:1.22:7.89	1.00:1.37:8.30
*n*6 PUFA	19.00	18.07
*n*3 PUFA	59.08	50.79
*n*6/*n*3 PUFA ratio	1: 3.1 (0.32)	1:2.81 (0.36)

[*] Mean ± SD from triplicate determinations.

[**] Different superscripts letters in the same row indicate significant differences ($p<0.05$).

[***] nd = not detected

1.5 Bioactive compounds

Total phenolic, flavonoid and flavonol contents from the extract of perilla seed, brown perilla and white perilla seeds, are shown in Table 13. Higher total phenolic, total flavonoid and total flavonol contents in brown perilla seed extract were observed ($p<0.05$). These compounds are important secondary metabolites in plants affecting appearance, taste, odor, and oxidative stability of plant based foods (Singh *et al*., 2012). Phenolic compounds are not only abundant in fruits, cereals, and vegetables but also found in seed oils (Hoed, 2010). In general, brown perilla seed extract has been reported for its bioactive compound content (Peng *et al*., 2005). From the results, both brown perilla and white perilla seed extracts had higher total phenolic content than those reported by Santosa *et al*. (2012). Furthermore, the phenolic compounds were characterized as catechin, ferulic acid, apigenin, luteolin and rosmarinic acid of perilla seed which were major active ingredients in perilla seed (Peng *et al*., 2005)

Flavonoids are the most common phenolics generally including anthocyanins, flavanols, flavones, flavanones and flavonols (Kasote, 2013). Total flavonoid content of brown perilla and white perilla seed extracts were 196.96 µg QE/g dry weight and 7.28 µg QE/g dry weight, respectively. Similarly, total flavonol content were found to be 225.20 µg QE/g dry weight and 0.45 µg QE/g dry weight for brown perilla and white perilla seed extracts, respectively. Both flavonoids and flavonols have been reported as possed a broad spectrum of chemical and biological activity including radical scavenging properties. In the present study, total flavonoid and flavonol contents of both perilla seed were found to be lower than sesame cake (880 µg QE/g dry weight and 400 µg QE/g dry weight, respectively) (Mohdaly *et al*., 2011)

Table 13 Bioactive compounds of brown perilla and white perilla seeds.

Bioactive compounds	Brown perilla seed	White perilla seed
Total phenolic content (mg GAE/g dry weight)	2.95 ± 0.22[*a**]	1.29 ± 0.11[b]
Total flavonoid content (µg QE/g dry weight)	196.96 ± 0.34[a]	7.28 ± 0.08[b]
Total flavonol content (µg QE/g dry weight)	225.20 ± 0.67[a]	0.45 ± 0.13[b]

[*] Mean ± SD from triplicate determinations.

[**] Different superscripts letters in the same row indicate significant differences (p<0.05).

2. Comparative studies on antioxidant activity of brown perilla and white perilla seeds

2.1 DPPH radical scavenging activity

Antioxidative activities of the extract from perilla seed, brown perilla and white perilla, are shown in Table 14. Both samples exhibited different antioxidant activity as measurement with DPPH radical scavenging activies (p<0.05). DPPH is a stable free radical that shows a maximum absorbance at 517 nm in ethanol. The DPPH method was generally selected for measuring the primary antioxidant activity of the seed extracts because it is one of the most effective methods for evaluating the concentration of radical-scavenging materials actively by a chain-breaking mechanism (Maisuthisakul *et al*., 2005). The results showed that DPPH-radical scavenging activity of brown perilla seed extract was more pronounced than those from white perilla seed (p<0.05). These results were similar to sesame seed reported by Shahidi *et al*. (2006) and Choi *et al*. (2007) study antioxidant activity of methanolic extracts from grains consumed in Korea reported grains has high pigmented showed relatively higher radical scavenging activity than non-pigmented. Additionally, the DPPH-radical scavenging

activity assay suggest that brown perilla seed could act as hydrogen donors more effectively than white perilla seed at a concentration of 15 µg/ml, because the values obtained for DPPH-radical scavenging capacity corresponded well with those of the total phenolic, flavonoid and flavonol content, which those hydrogen donation ability (Yen *et al.,* 1993). From the results, the different DPPH-radical scavenging activity of both extracts might be affected by different contents of total phenolics, total flavonoids, and total favonols (Table 13).

2.2 ABTS-radical scavenging activity

The extracts from brown perilla and white perilla seeds posses ability to quence ABTS radicals (Table 14), ABTS assay is an excellent tool for determining the antioxidative activity, in which the radical is quenched to form ABTS-radical complex (Binsan *et al.,* 2008). It is also widely employed for measuring the relative radical scavenging activity of hydrogen donating and chain breaking antioxidants in many plant extracts (Brand-Williams *et al*., 1995; Netzel *et al*., 2003). Results of ABTS radical scavenging activity showed that the extract fron brown perilla seed was 10 times higher than those from white perilla seed. Sargi *et al.* (2013) reported that antioxidant capacity ($ABTS^{\bullet+}$assay) of *Perilla frutescens* L. was 4.06 and 3.32 mmol Trolox equivalent antioxidant capacity/g sample for brown perilla and white perilla seeds, respectively. They concluded that the variation may have been due to the dependence of the sample composition on factors such as climate conditions and geographic location (Mezadri *et al*., 2008; Vasco *et al*, 2008). From the results, ABTS radical scavenging activity might affected by differences bioactive compounds (Table 13) and variety of seeds.

2.3 Ferric reducing antioxidant power (FRAP)

FRAP of brown perilla and white perilla seed extracts is shown in Table 14. The results showed that FRAP of both samples was slightly diffences (p<0.05). This indicated that perilla seed extract contained some compounds which can reduce FRAP reagent from ferric to ferrous form, so they are regarded as antioxidants. This reaction

involves an electron transferring from an oxidizable compound to TPTZ-Fe III complex resulting in the formation of blue colored TPTZ Fe-II complex that absorbs strongly at 593 nm (Amarnath, 2004; Siddhuraju and Becker, 2007). As the results obtained, brown perilla seed extract showed the pronounced effect in donating electrons, in which propagation of lipid oxidation could be retarded. According to ABTS and DPPH assays, the radial scavenging ability of antioxidants is obtained, while FRAP gives reducing ability of antioxidants (Amarnath, 2004). Flavonoids can inhibit metal-initiated lipid oxidation by forming complexes with metal ions (Lee *et al*., 2004). Therefore, the extract from both brown perilla and white perilla seeds posses ferric reducing, suggesting ability to react with free radicals, especially ferric ion.

2.4 Reducing power

Reducing power of extracts from brown perilla and white perilla seeds is shown in Table 14. Generally, extract from brown perilla seed was higher (2 times) than that from white perilla seed ($p<0.05$). The reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay *et al*., 2003). This is directed correlation between antioxidant activity and reducing power of certain plant extracts (Duh, 1998; Tanaka *et al*., 1988). The reducing properties are generally associated with the presence of reductones (Duh, 1998), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. From the results, the reducing power of the extracts indicated that is likely to contribute significantly towards the observed antioxidant effect. The presence of antioxidants in the samples causes the reduction of the Fe^{3+}/Ferricyanide complex to the ferrous form. Additionally, Fe^{2+} can be monitored by measuring of the formation of Perl's Prussian blue at 700 nm (Zou *et al*., 2004). The reducing power of perilla seed extracts might be due to hydrohen-donating ability (Shimada *et al*., 1992).Therefore, higher reducing power of brown perilla seed extract might contribute to bioactive compounds and chemical composition.

Table 14 Antioxidant activity of brown perilla and white perilla seeds.

Antioxidant activity	Brown perilla seed	White perilla seed
DPPH (% scavenging)	77.83 ± 0.58[*a**]	20.86 ± 0.26[b]
ABTS (µmol Trolox/g dry weight)	3.70 ± 0.01[a]	0.36 ± 0.00[b]
FRAP (µmol Trolox/g dry weight)	2.46 ± 0.03[a]	2.26 ± 0.02[b]
Reducing power (BHT equivalent/ 100 g dry weight)	0.47 ± 0.01[a]	0.26 ± 0.00[b]

* Mean ± SD from triplicate determinations.

** Different superscripts letters in the same row indicate significant differences (p<0.05).

3. Effect of sugars on lipid oxidation of ground perilla seed

3.1 Study on antioxidant activity of sugars

3.1.1 Physicochemical properties of commercial sugars

3.1.1.1 pH values

Table 15 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 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 values of sugar can be used as an indicator for the product safety. Furthermore, the pH value can be used to classify the food products into acid food and low acid food (Britt, 2008). Guerra and Mujica (2010) reported that the pH values of cane sugar "Panela" ranged from 5.58-6.90. Naka (1996)

showed that the pH value of coconut sugar was 5.57. Naknean and Meenune (2011) and Phaichamnan *et al*. (2010) 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 (Guerra and Mujica, 2010). Also, Phaichamnan *et al*. (2010) 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 production (Apriyantono *et al*., 2002). 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 (Brands and Van Boekel; 2002; Lertittikul *et al*., 2007; Vhangani and Van Wyk, 2013). From the results, the differences in pH of sugar might be resulted from the different raw materials and processes used.

Table 15 pH value of commercial sugars

Samples	pH
Cane sugar paste	$5.57 \pm 0.03^{*a**}$
Unrefined cane sugar	5.53 ± 0.05^{ab}
Brown cane sugar	5.14 ± 0.08^{d}
Caramel crystal cane sugar	5.19 ± 0.06^{d}
Refined white crystal cane sugar	5.47 ± 0.01^{b}
Coconut sugar paste	5.50 ± 0.04^{ab}
Palm sugar paste	5.27 ± 0.01^{c}

* Mean ± SD from triplicate determinations.

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

3.1.1.2 Water activity (A_w) values

Different A_w value of seven commercial sugar were observed (p<0.05) (Table 16). Except refined white crystal cane sugar, the A_w values of commercial sugar produced from sugar cane were slightly different (0.61-0.65). Coconut sugar and palm sugar had the highest A_w values (p<0.05). The marked lowest A_w value was found in refined white crystal cane sugar (0.48). The heating time for sugar production (dehydration process) might affect the A_w value of final product. Apriyanoto *et al*. (2002) reported that the weight of coconut sap and palm sap decreased with increasing heating time. Generally, the Aw value can be used to indicate the shelf-life stability of food products (Mathlouthi, 2001). Since A_w value refers to free water that can promote microbial deterioration and biochemical degradation reaction. Concluded that different commercial granulated cane sugar (Panel) had different A_w value (Guerra and Mujica, 2010) Naknean and Meenune (2011) reported the A_w value of palm sugar syrup produced in southern Thailand was between 0.79-0.81. The low $A_w$value (0.65-0.80) may allow the growth of osmophillic yeast which can spoil food containing high concentration of sugar (Naknean, 2010).

Table 16 Water activity (A_w) value of commercial sugars.

Samples	A_w
Cane sugar paste	0.61 ± 0.00^{d}
Unrefined cane sugar	0.62 ± 0.01^{c}
Brown cane sugar	0.65 ± 0.00^{b}
Caramel crystal cane sugar	0.61 ± 0.00^{d}
Refined white crystal cane sugar	0.48 ± 0.01^{e}
Coconut sugar paste	0.72 ± 0.01^{a}
Palm sugar paste	0.73 ± 0.00^{a}

* Mean ± SD from triplicate determinations.

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

3.1.1.3 Color values

The color values (L*, a* and b*) of seven commercial sugars were varied depending on types of sugar as shown in Figure 13. 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 different 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 (Guerra and Mujica, 2010).

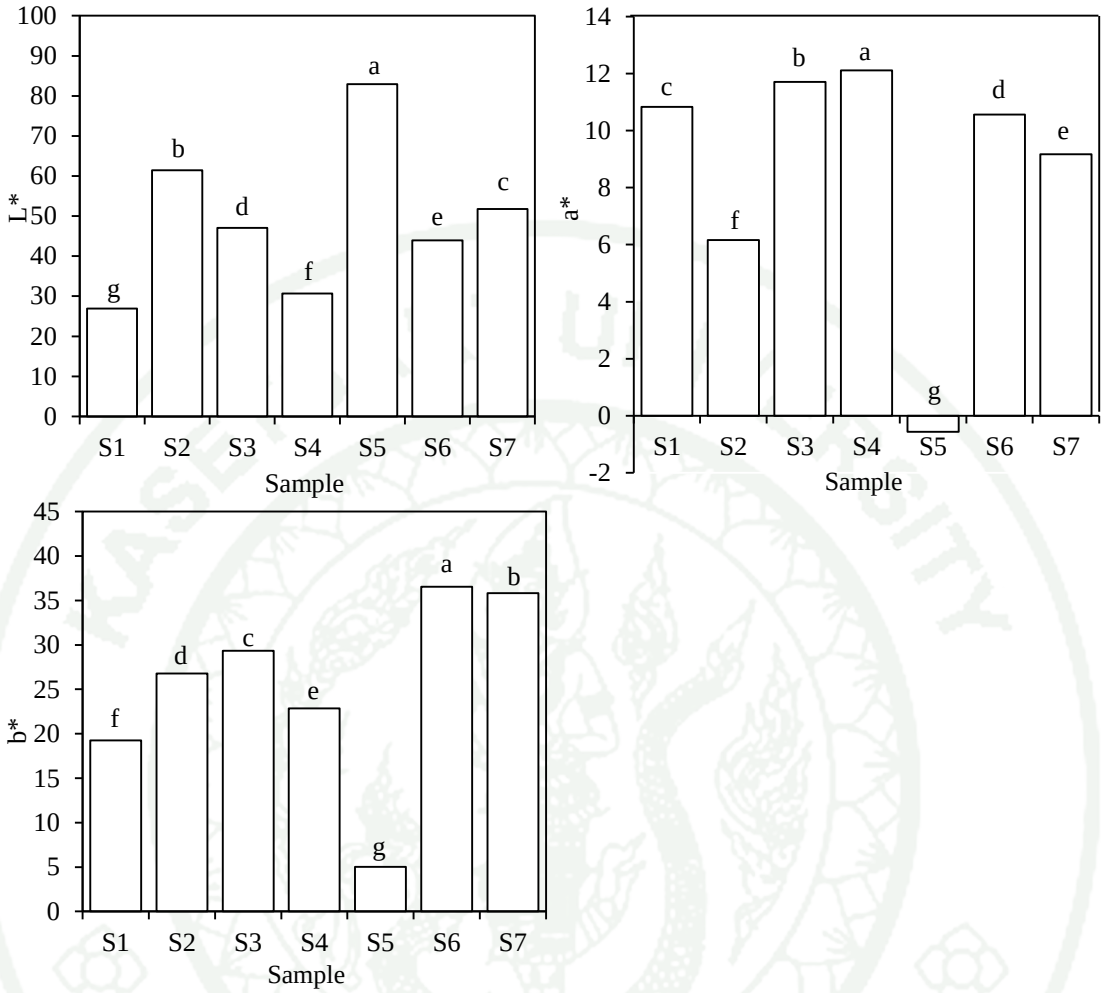


Figure 13 L*, a* and b* of commercial sugars. Bars represent the standard deviations from triplicate determinations. Different letters in the same value the different samples indicate the significant differences (p<0.05).

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

3.1.1.4 Total sugar and reducing sugar contents

Total sugar contents of seven commercial sugars were slightly different, while reducing sugar contents were significantly different ($p<0.05$). Commercial sugars had total sugar content between 761.96-841.23 mg/g dry weight, (Figure 14). The highest total sugar content was found in caramel crystal cane sugar, followed by brown cane sugar, while coconut sugar paste had the lowest. Reducing sugar content of commercial sugars ranged from 0.15-122.07 mg/g dry weight. 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 *et al*. (2001) 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 (Naknean and Meenune, 2011). 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 (Naknean *et al*., 2009). 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 *et al.* (2010) reported that difference of total sugar and reducing sugar contents might be due to the effect of contamination from micro-organisms in sugar. The microrganisms can convert sucrose to glucose and fructose (invert sugar) and finally to organic acids or alcohols (Willits and Hills, 1976).

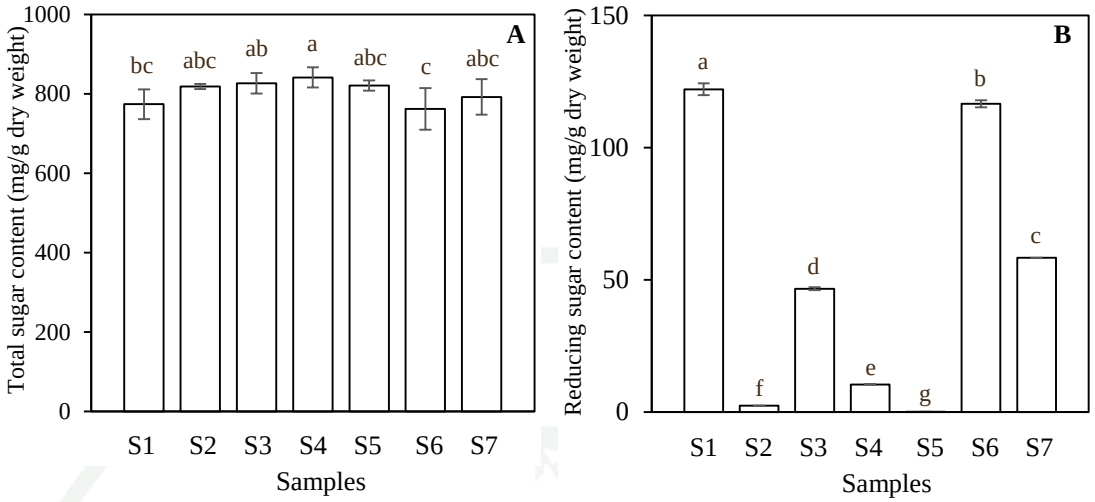


Figure 14 Total sugar (A) and reducing sugar (B) contentsin commercial sugars. Bars represent the standard deviations from triplicate determinations. Different letters in the same value the different samples indicate the significant differences (p<0.05).

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

3.1.1.5 Mineral content

The mineral contents of seven commercial sugars are shown in Figure 15. Different commercial sugars, different level of minerals were observed (p<0.05). The levels of Ca, Mg,Fe, K, and Na were found to be high 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 brown cane sugar production (Gul and Harasek, 2012). The pattern of mineral level of sugars produced from sugar cane was difference, especially Fe, K, and Na. In this research, the cane sugar paste was obtained from retail market (traditional product of northern of Thailand), while other were purchased from supermarket (industrial products). The production processes might affect on levels of minerals. Guerra and Mujica (2010) found that different brands of granulated sugar “Panel” had different contents of Ca, Mg, Fe, and K. Olaiya (2006)

reported the calcium is highly implicated in the maintenance of firmness of fruits and it requirements in fruits are related to cell wall stability and membrance 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, and handling, level of juice extraction, clarifying method, lime purity and impurity removal efficiency in manufacturing process (Guerra and Mujica, 2010). From the results, cane sugar paste was abundant in minerals.

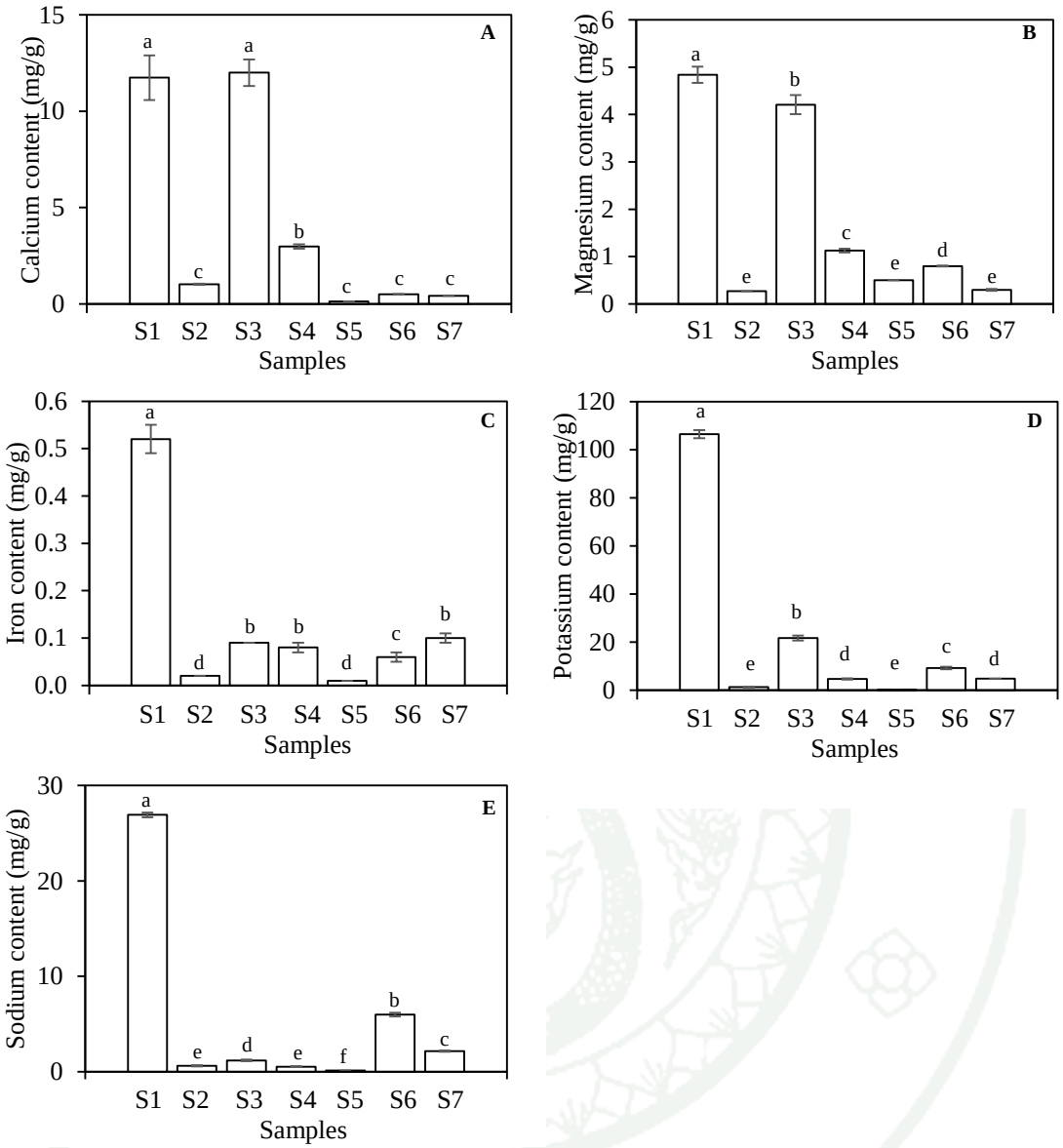


Figure 15 Calcium (A), magnesium (B), iron (C), potassium (D), sodium (E) contents incommercial sugars. Bars represent the standard deviations from triplicate determinations. Different letters in the same value within the different samples indicate the significant differences (p<0.05).

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

3.1.1.6 Intermediate browning product (OD_{285}) and browning intensity (OD_{420})

Intermediate browning product and browning intensity of seven commercial sugars were significantly different ($p<0.05$) (Figure 16). 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 (Ajandouz *et al*., 2001; Benjakul *et al*., 2005b). The high absorbance at 285 nm suggested the formation of an uncoloured compound, which could be the precursor of the Maillard reaction (Ajandouz *et al*., 2001; Benjakul *et al*., 2005a). The intermediate degradation products via enolization known as color precursors include methylglyoxal, glyceraldehyde, hydroxymethyl furaldehyde, furfural and hydroxyacetylfuran (Kroh 1994; Homoki-Farkas *et al*. 1997; Cammerver *et al*. 1999; Phongkanpai *et al*., 2006). Naknean *et al*. (2013) 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 (Hodge 1953; Mauron 1981; Ajandouz *et al*., 2001). From the study in the model system of Ajandouz *et al*. (2001); Benjakul *et al*. (2005a, 2005b); Phongkanpai *et al*. (2006); Lertittikul*et al*. (2007), intermediate degradation products increased as the heating time and pH increased. In addition, Benjakul *et al*. (2005b) reported that the browning of sugars was developed increasingly with the increase in pH. From the results, high absorbances at 285 nm and 420 nm of cane sugar paste might correlated to pH values (Table 15). Thus, different intermediate browning products and browning intensity depend on the heating time and pH levels.

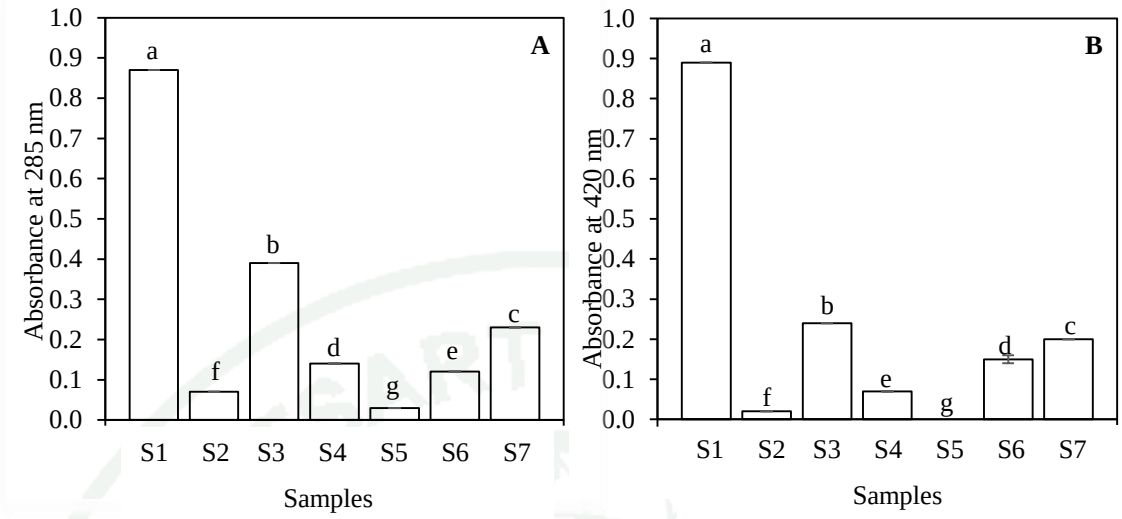


Figure 16 Intermediate browning product (A) and browning intensity (B) in commercial sugars. Bars represent the standard deviations from triplicate determinations. Different letters in the same value within the different samples indicate the significant differences (p<0.05).

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

3.1.1.7 Total phenolic content

Total phenolic content of seven commercial sugars extracted with distilled water or methanol were investigated. Total phenolic content of commercial sugars 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 (Onwuka *et al*., 2012). 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 *et al*. (2009) 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 (2011) 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 *et al*. (2010) 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 *et al*. (2009) 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 (Godshall *et al*., 2002). 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 suar paste, coconut sugar paste, and palm sugar paste) had high total phenolics content. Brown cane sugar also contained high total phenolics content. Most phenolic compounds found in distilled water extract. Therefore, sugar paste in generally had higher total phenolic compounds than granulated and powdered sugars.

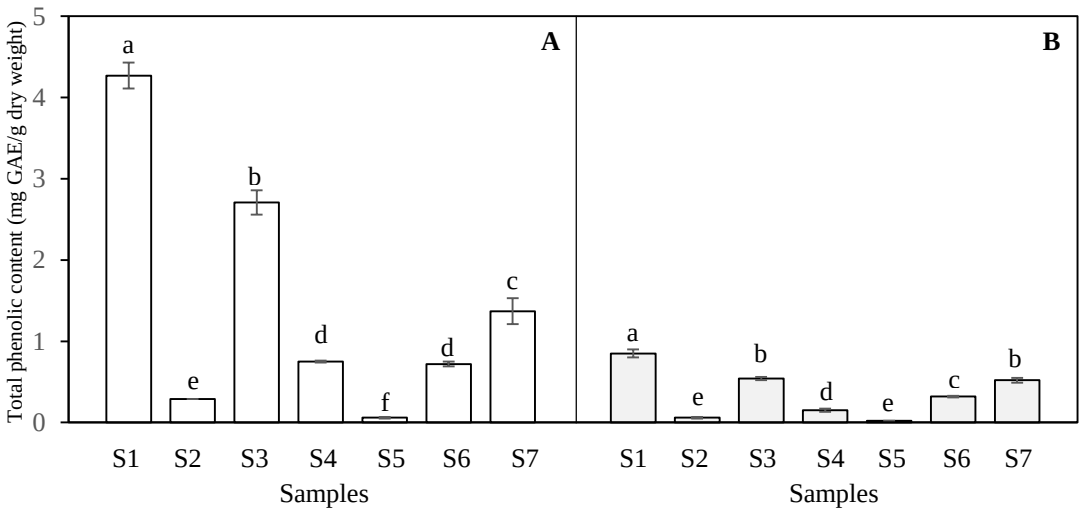


Figure 17 Total phenolic contentin sugar dissolved in distilled water (A) and 95% methanol (B). Bars represent the standard deviations from triplicate determinations. Different letters in the same solution within the different samples indicate the significant differences (p<0.05).

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

3.1.2 Antioxidative activity

3.1.8.1 DPPH-radical scavenging activity

DPPH-radical scavenging activities of seven commercial sugars extracted with distilled water or methanol were investigated. DPPH-radical scavenging activities of commercial sugars extracts were different (p<0.05) (Figure 18). The results show that the DPPH-radical scavenging activity of commercial sugars extracted with distilled water was greater than that of commercial sugars extracted with methanol. 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 extract 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 (Yamaguchi *et al*., 1998). Lertittikul *et al.* (2007) and Benjakul *et al.* (2005b) 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 (Shon *et al*., 2003). 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 *et al*. (2013) reported that intermediates or the final brown polymer could function as hydrogen donors. From the results, commercial sugars, especially can sugar paste can function as antioxidant through the donation of hydrogen atom.

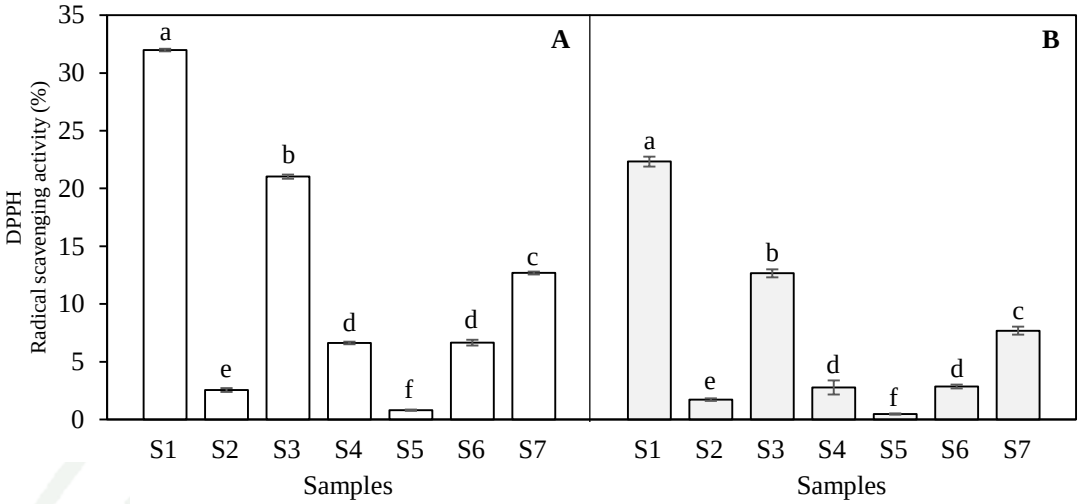


Figure 18 DPPH radical scavenging activity in sugar dissolved in distilled water (A) and 95% methanol (B). Bars represent the standard deviations from triplicate determinations. Different letters in the same solution within the different samples indicate the significant differences (p<0.05).

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

3.1.8.2 ABTS-radical scavenging activity

The antioxidant activities of sugars, using the ABTS-radical scavenging activity assay are shown in Figure 19. ABTS-radical scavenging activities of seven commercial sugars extracted with distilled water or methanol were investigated. ABTS-radical scavenging activity of commercial sugars extracts were different (p<0.05). The results showed that the ABTS-radical scavenging activity of commercial sugars extracted with distilled water was greater than that of commercial sugars extracted with methanol. Regardless of extraction solvent, cane sugar paste showed the highest ABTS-radical scavenging activity, compared with other sugars (p<0.05). ABTS-radicalscavenging activity of water extract from cane sugar paste and refined white crystal cane sugar was 13.19 and 0.97 µmol Trolox/g dry weight, respectively whereas the water extract of cane sugar paste and refined white crystal

cane sugar was 9.87 and 0.37 µmol Trolox/g dry weight, respectively. The results showed that higher ABTS-radical scavenging activity was coincidental with higher DPPH-radical scavenging. However, ABTS radical assay employed for measuring the relative radical scavenging activity of hydrogen-donating and chain breaking antioxidants (Brand-Williams *et al*., 1995: Netzel *et al*., 2003). The decolorization assay method was to screen the antioxidative activity, and it is applicable to both lipophilic and hydrophilic antioxidants (Re *et al*., 1999). Thus, commercial sugars, especially cane sugar paste can act as hydrogen donating and chain breaking antioxidants. From the results, ABTS-radicalscavenging activity correlated well with DPPH radical scavenging, intermediate browning product, browning intensity and total phenolic content.

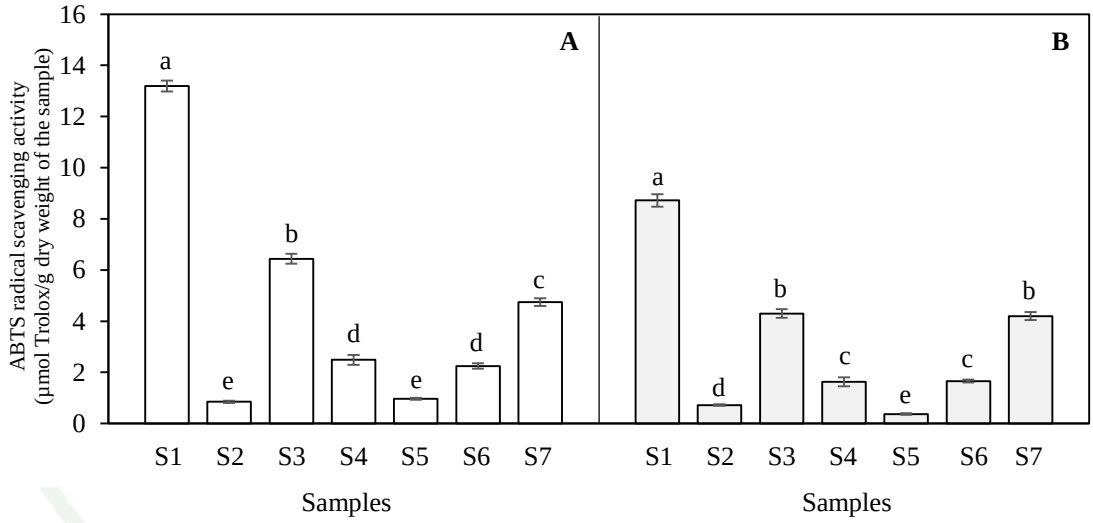


Figure 19 ABTS radical scavenging activity in sugar dissolved in distilled water (A) and 95% methanol (B). Bars represent the standard deviations from triplicate determinations. Different letters in the same solution within the different samples indicate the significant differences (p<0.05).

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

3.1.8.3 Ferric reducing antioxidant power

Ferric reducing antioxidant powers of seven commercial sugars are extracted with water or methanol shown in Figure 20. 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 µmol 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 µmol 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 assay. 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.

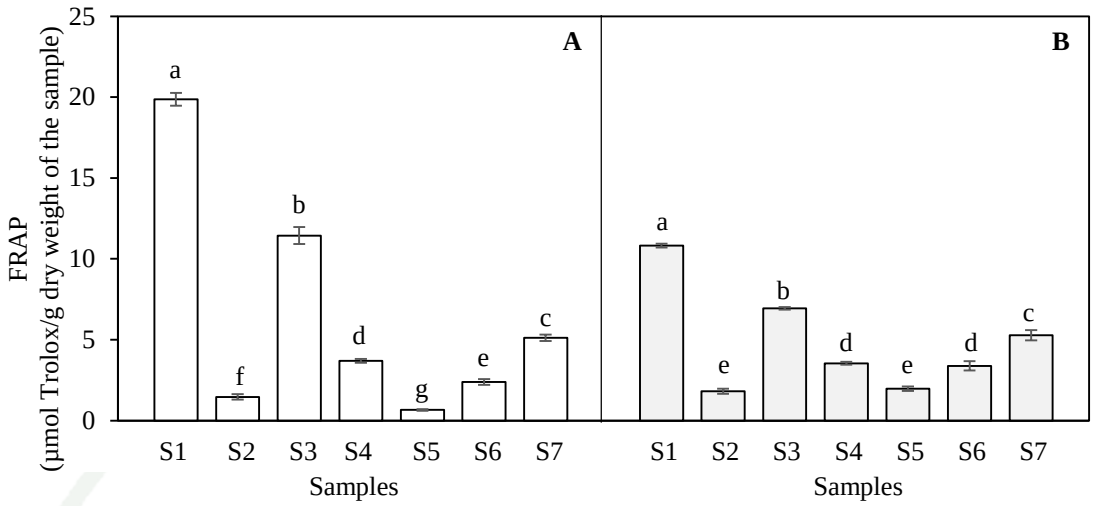


Figure 20 FRAP in sugar dissolved in distilled water (A) and 95% methanol (B). Bars represent the standard deviations from triplicate determinations. Different letters in the same solution within the different samples indicate the significant differences (p<0.05).

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

3.1.8.4 Reducing power

Reducing powers of seven commercial sugars extracted with water or methanol were investigated. Reducing powers of commercial sugars extracts were different (p<0.05). The results exhibited that the reducing power of commercial sugars extracted with distilled water was greater than that of commercial sugars extracted with methanol. However, reducing power of commercial sugar extracted with distilled water was slightly different from commercial sugars extracted with methanol. Distilled water extract of cane sugar paste had the highest reducing power, followed by brown cane sugar and palm sugar paste, respectively. For the methanol extract cane sugar paste had the highest reducing power, followed by palm sugar paste and brown cane sugar, respectively. Refined white crystal cane sugar with

both extracting media had the lowest reducing power (Figure 21). Benjakul *et al*. (2005a; 2005b) and Shimada *et al*. (1992) reported that reducing power was used to indicate hydrogen-donation ability in the Maillard reaction products and caramelization products Hydroxyl groups of the Maillard reaction products play a role in reducing activity (Yoshimura *et al*., 1997). From the results of intermediate browning products and browning intensity, it was in accordance with Phongkanpai *et al*. (2006) who reported that the increase in reducing power was coincidental with the development of browning as well as with the formation of intermediate products. Thus, intermediate browning products and browning intensity had hydrogen-donation properties. In addition, the higher the reducing sugar content of commercial sugars the greater the reducing power was observed.

From the results, reducing power correlated well with DPPH and ABTS-radical scavenging activities, intermediate browning product, browning intensity and total phenolic content of commercial sugars. Therefore, commercial sugars with high antioxidant activity (cane sugar paste, brown cane sugar, coconut sugar paste, and palm sugar paste) were chosen for further study.

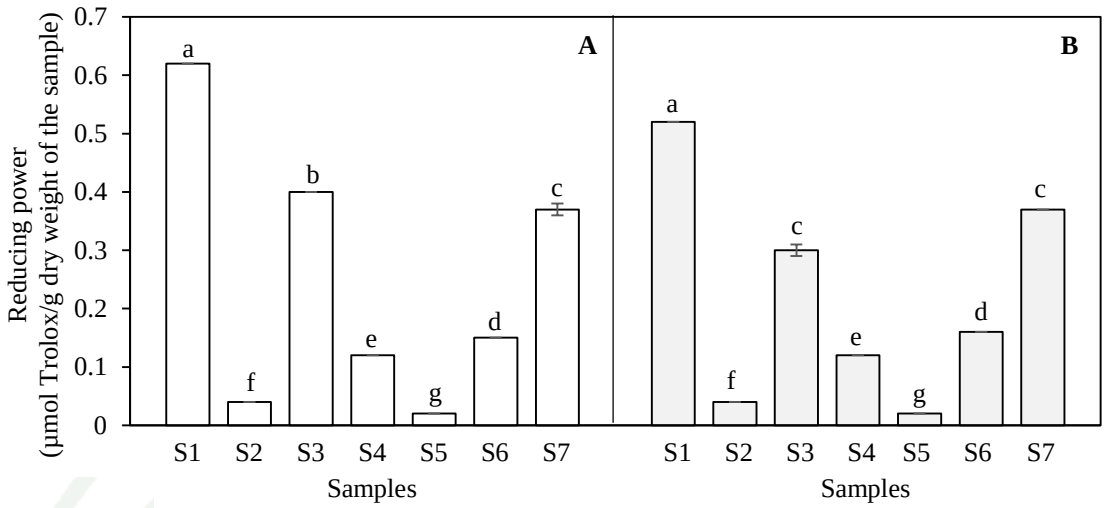


Figure 21 Reducing power in sugar dissolved in distilled water (A) and 95% methanol (B). Bars represent the standard deviations from triplicate determinations. Different letters in the same solution within the different samples indicate the significant differences (p<0.05).

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 Study on lipid changes of ground perilla seed as influenced by different sugars

3.2.1 pH values

Changes in pH values of ground perilla seed added with different four commercial sugars during 30 days of storage are shown in Figure 22. At 0 day of storage, all samples had different pH value (p<0.05). Sample added with cane sugar paste exhibited the highest pH value (5.96), whereas sample added with brown cane sugar had the lowest (5.41). The pH values of samples added with coconut sugar paste and palm sugar paste were 5.69 and 5.76, respectively. Difference in pH value of ground perilla seed with all samples might be due to the different pH value of raw

materials, particularly sugar. The sample added with different commercial sugars was found in the range of low-acid foods in which the pH value was greater than 4.6 (Britt, 2008). During storage up to day30, the pH values of ground perilla seed with all sugars slightly decreased (p<0.05). At the end of storage, the pH values of ground perilla seed with cane sugar paste, brown cane sugar, coconut sugar paste and palm sugar paste were 5.88, 5.35, 5.59 and 5.61, respectively. Decrease of pH value in ground perilla seed during long-term storage may cause by certain chemical reactions which can produce hydrogen ions. Additionally, reduction of pH was due to the formation of organic acids, such as formic and acetic acid in ground perilla seed (Phaichamnan *et al*., 2010).

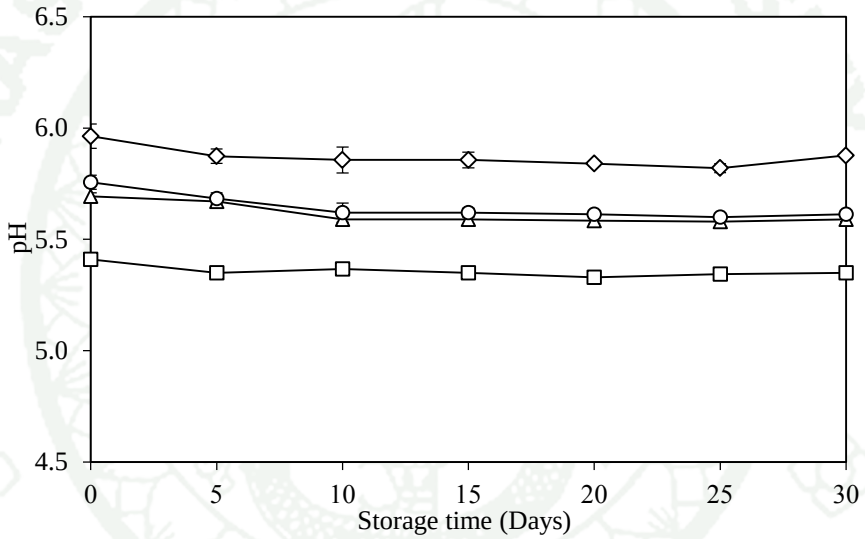


Figure 22 Changes in pH of ground perilla seed added with different sugars including cane sugar paste (◇), brown cane sugar (□), coconut sugar past (△), palm sugar paste (○) during 30-day ofstorage. Bars represent the standard deviations from triplicate determinations.

3.2.2 Moisture content

Changes in the moisture content of ground perilla seed added with different four commercial sugars during 30 days of storage are shown in Figure 23. The moisture contents of all treatments were different depending on type of sugars ($p<0.05$). Ground perilla seed added with commercial sugars produced from sugar cane including cane sugar paste and brown cane sugar exhibited the lower moisture content, compared with other samples ($p<0.05$). Ground perilla seed with brown cane sugar had the lowest moisture content, while ground perilla seed with palm sugar paste had the highest ($p<0.05$). The initial moisture content of ground perilla seed with cane sugar paste, brown cane sugar, coconut sugar paste and palm sugar paste were 8.89%, 3.43%, 9.51% and 10.78%, respectively. Differences in moisture content of ground perilla seed might be resulted from the different moisture content of sugar raw materials (Table 16). Additionally, brown cane sugar is in powder from and its properties is higher hygroscopic than others. Then sample added with this sugar was dryer than other samples. The moisture content of all samples slightly decreased with increasing storage time. After 30 days of storage,moisture content of ground perilla seed with cane sugar paste, brown cane sugar, coconut sugar paste and palm sugar paste were 8.31, 3.10%, 9.08 and 9.82, respectively. In general, sugar is effective in preservation because it reduces the amount of water availability (Karel and Lund, 2003). Additionally, moisture plays a critical role in determining the quality and shelf life of food products. Thus, as long as the moisture contents of these products remained unchanged, their qualities would stable over a storage period.

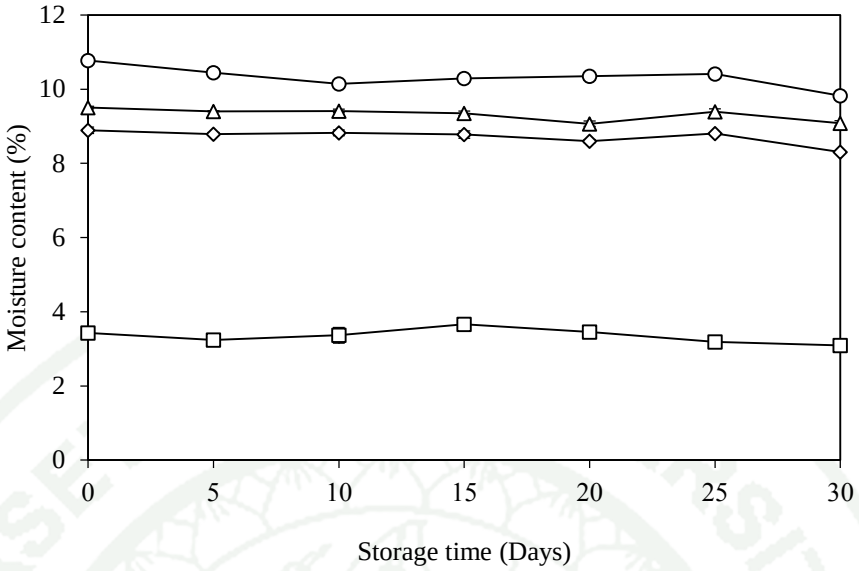


Figure 23 Changes in moisture content of ground perilla seed added with different sugars including cane sugar paste (◇), brown cane sugar (□), coconut sugar past (△), palm sugar paste (○) during 30-day of storage. Bars represent the standard deviations from triplicate determinations.

3.2.3 Water activity (A_w) values

Changes in A_w values of ground perilla seed added with different four commercial sugars during storage are shown in Figure 24. The A_w values of samples added with four commercial sugars were slightly different during storage up to 30 days (p<0.05). Ground perilla seed with commercial sugars produced from sugar cane exhibited a lower A_w value than other sample. Ground perilla seed with coconut sugar paste had the highest A_w value (p<0.05). The initial Aw value of ground perilla seed with commercial sugars produced from sugar cane was 0.65-0.66, while ground perilla seed with coconut sugar paste and palm sugar paste were 0.71 and 0.70, respectively. During storage up to 30 days, as light decrease in A_w value of all samples was noticeable. After 30 days of storage, A_w value of ground perilla seed with cane sugar paste, brown cane sugar, coconut sugar paste and palm sugar paste were 0.65, 0.62, 0.70 and 0.69, respectively. The decrease in A_w value was probably due to the action of sugar which canreduce the amount of water availability (Karel and Lund,

2003). Generally, the A_w value can be used to indicate shelf-life stability of food products (Mathlouthi, 2001). Since A_w value is used to refer free water that can promote microbial deterioration and biochemical degradation reaction (Guerra and Mujica, 2010). However, ground perilla seed classified as intermediate moisture foods having A_w values ranging from 0.90-0.60 (Leistner and Rodel, 1976). It can be seen that in the intermediate moisture range, many deteriorative reactions can occur including lipid oxidation, browning, enzyme activity and microbial growth. Thus, the changes of A_w may affect the acceptability and storage stability of ground perilla seed product.

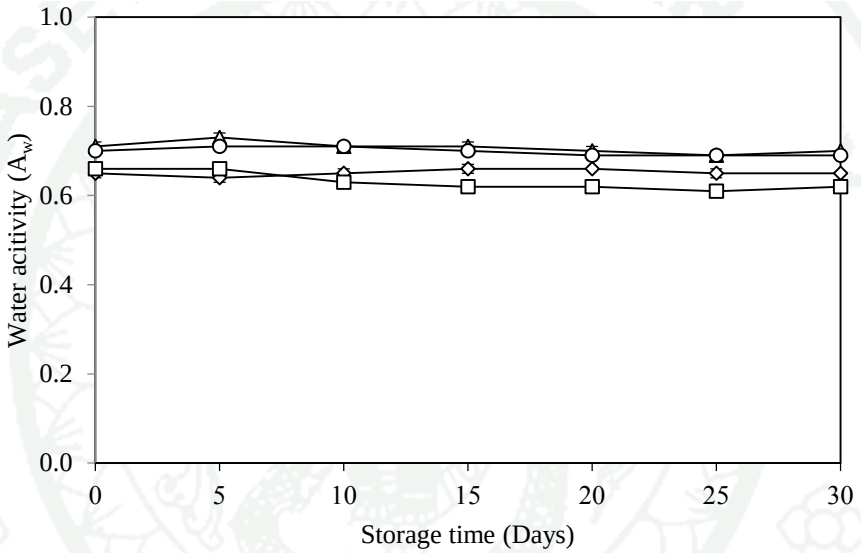


Figure 24 Changes in water activity of ground perilla seed added with different sugars including cane sugar paste (◇), brown cane sugar (□), coconut sugar past (△), palm sugar paste (○) during 30-day of storage. Bars represent the standard deviations from triplicate determinations.

3.2.4 Color values

The color values (L*, a* and b*) of ground perilla seed added with different four commercial sugars were different during 30-day storage (p<0.05) (Figure 25). The highest L* value was found in ground perilla seed with brown cane sugar, while the lowest was found in sample added with cane sugar paste. The L* values were initially found to be 16.23, 31.85, 21.27 and 17.79 for ground perilla seed with cane sugar paste, brown cane sugar, coconut sugar paste and palm sugar paste, respectively. The initial a* values of ground perilla seed added with cane sugar paste, brown cane sugar, coconut sugar paste and palm sugar pastewere 8.27, 10.2, 6.04 and 9.79, respectively. The initial b* values of ground perilla seed added with cane sugar paste, brown cane sugar, coconut sugar paste and palm sugar pastewere 13.47, 21.43, 7.91 and 16.95, respectively. Ground perilla seed added with brown cane sugar had the highest a* and b* values, followed by those added with palm sugar paste and cane sugar paste, respectively. Ground perilla seed with coconut sugar paste had the lowest a* and b* values. During storage of ground perilla seed added with all sugars, the increase in L* value with the decrease in a* and b* values were observed (p<0.05). After 30 days of storage, the a* and b* values of ground perilla seed added with brown cane sugar tended to slightly decrease (9.9 and 23.08, respectively), while those added with cane sugar paste, coconut sugar paste and palm sugar paste markedly increased at the first 5 days of storage and tended to decrease till the end of storage period (day 30).

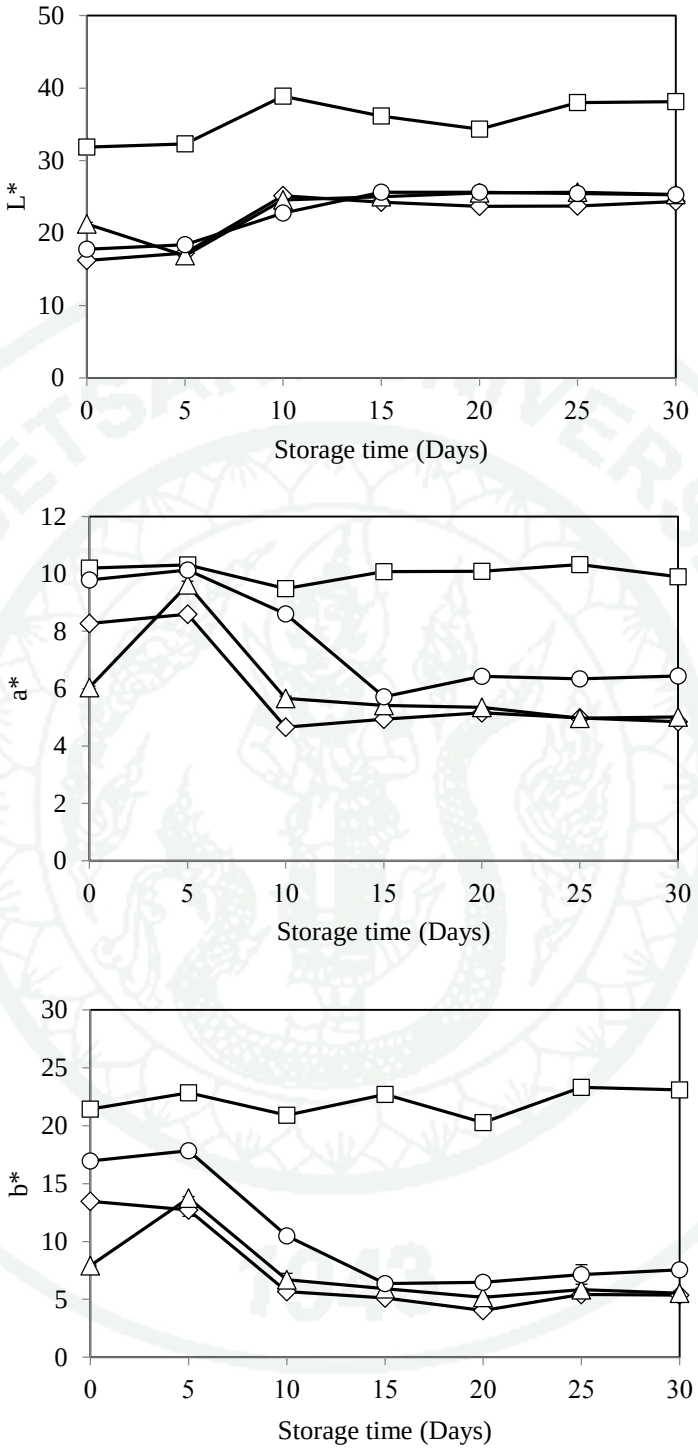


Figure 25 Changes in L*, a* and b* values of ground perilla seed added with different sugars including cane sugar paste (◇), brown cane sugar (□), coconut sugar paste (△), palm sugar paste (○) during 30-day of storage. Bars represent the standard deviations from triplicate determinations.

3.2.5 Lipid changes

3.2.5.1 Free fatty acid content

Changes in free fatty acid content of ground perilla seed added with different four commercial sugars during storage are depicted in Figure 26. At 0 day of storage, the free fatty acid contents of ground perilla seed with different commercial sugars were found in the ranges of 10.20-19.45 g fatty acid/g lipid. Ground perilla seed with cane sugar paste had the lowest free fatty acid content, while that with palm sugar paste had the highest, compared with other samples (p<0.05). At 5 days of storage, except ground perilla seed with coconut sugar paste, the free fatty acid of ground perilla seed with different commercial sugars slightly decreased, while that of coconut sugar paste increased. At day 5, sample added with cane sugar paste had the lowest, while that with palm sugar paste had the highest (p<0.05). During day 5 to day 25, it was found that the free fatty acid contents of ground perilla seed added with cane sugar paste, brown cane sugar and coconut sugar increased (p<0.05). The free fatty acid content of ground perilla seed added with palm sugar paste decreased at day 15 and day 25 of storage (p<0.05). At the end of storage, it was noted that the free fatty acid contents of ground perilla seed added with all sugar decreased (p<0.05). Decrease of free fatty acid content may because of the progressive oxidation of ground perilla seed lipid. Generally, free fatty acid contents of ground perilla seed with different sugars at day 30 were 22.38-26.14 g fatty acid/g lipid. Accumulation of free fatty acid during storage of foods influences the quality of the final product and the period of useful life (Bernardez *et al*., 2005). Free fatty acids are formed in foods as a result of hydrolytic rancidity (Goffman and Bergman, 2003). Hydrolytic rancidity occurs when free fatty acid are split from fat molecules as a result of the action of lipases, phospholipases or lipoxidase (enzymes catalyzing the breakdown of fat) during storage (Mcwilliams, 2006). Troni *et al.,* 2013 showed that the formation of free fatty acids increased for higher temperatures and longer heating times. Normally, free fatty acids are prone to oxidation.

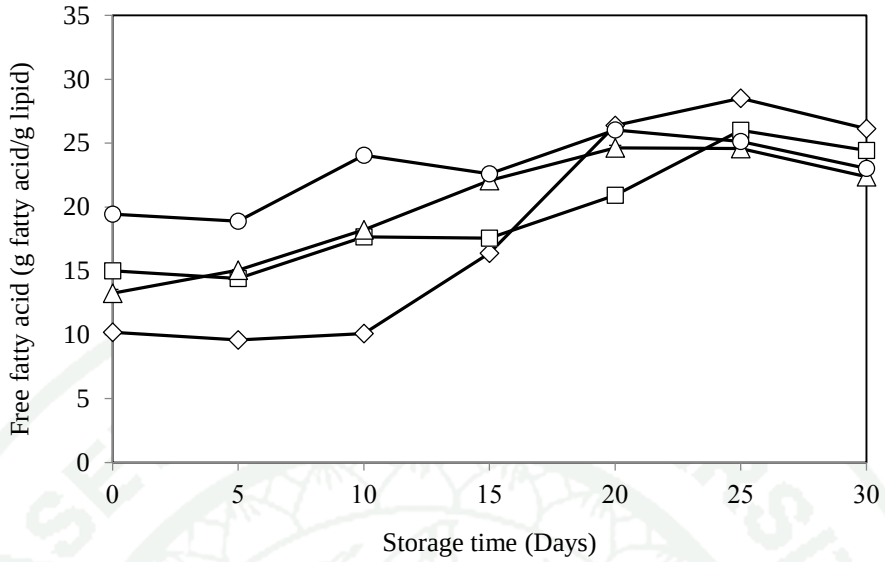


Figure 26 Changes in free fatty acid of ground perilla seed added with different sugars including cane sugar paste (◇), brown cane sugar (□), coconut sugar paste (△), palm sugar paste (○) during 30-day of storage. Bars represent the standard deviations from triplicate determinations.

3.2.5.2 Peroxide value

Changes in peroxide value in ground perilla seed added with different sugar during storage are depicted in Figure 27. Peroxide values of ground perilla seed added with different commercial sugar were different during storage up to 30 days (p<0.05). At 0 day of storage, sample added with cane sugar paste had the highest peroxide value, while that added with brown cane sugar had the lowest. The peroxide value of all samples decreased at day 5 of storages except for ground perilla seed with cane sugar paste. Ground perilla seed with cane sugar paste has the highest peroxide value throughout the storage. At day 10 and 15, the peroxide values of ground perilla seed added with different commercial sugar slightly decreased. Sample with cane sugar paste has the highest peroxide value, followed by sample added with coconut sugar paste and brown cane sugar, respectively, while sample added with palm sugar paste had the lowest (p<0.05). At 20 days of storage, sample added with all sugar had a marked increase in peroxide value (p<0.05) and it turned to slightly decrease at day

25. Sample added with cane sugar paste had the highest peroxide value, while palm sugar paste sample had the lowest. At the end of storage, the peroxide values of ground perilla seed with brown cane sugar and palm sugar paste slightly decreased (p<0.05), while those of cane sugar paste and coconut sugar paste were unchanged. The decreased peroxide value observed with extended storage time was presumed to be due to the decomposition of hydroperoxide. Hydroperoxides break down in several steps, yielding a wide variety of decomposition products, including aldehydes (Nawar, 1996). Lipid oxidation is a complex process in which unsaturated fatty acids react with molecular oxygen, usually via a free radical mechanism, to form hydroperoxides, the primary oxidation products (Simic and Taylor, 1987). However, peroxide value is a common parameter in the chemistry of lipids. It is a measure of the amount of peroxides and hydroperoxides formed in the initial stages of lipid oxidation (Wabner, 2002) which associated with rancidity in lipid-containing food products (Nouros*et al*., 1999).

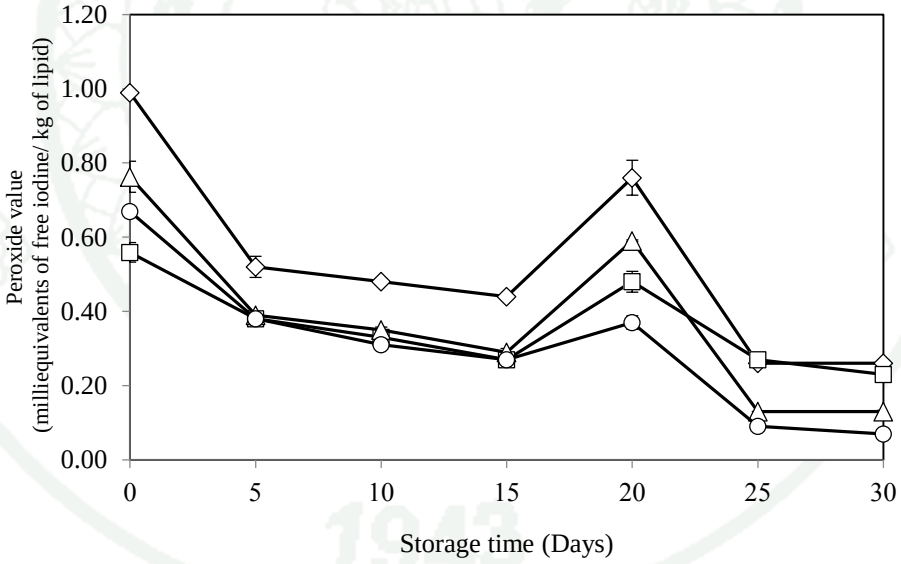


Figure 27 Changes in peroxide value of ground perilla seed added with different sugars including cane sugar paste (◇), brown cane sugar (□), coconut sugar paste (△), palm sugar paste (○) during 30-day of storage. Bars represent the standard deviations from triplicate determinations.

3.2.5.3 Conjugated diene

Changes in conjugated diene of ground perilla seed added with different commercial sugars during storage are depicted in Figure 28. Conjugated diene values of ground perilla seed added with different commercial sugar were different (p<0.05). Conjugated diene of ground perilla seed with all sugar samples tended to slightly decrease up to 30 days of storage. Ground perilla seed with cane sugar paste, brown cane sugar and coconut sugar paste had the highest conjugated diene at day 0 while ground perilla seed with palm sugar paste had the highest conjugated diene at day 15. The lowest conjugated diene at day 25 and day 30 was found in ground perilla seed with cane sugar paste and ground perilla seed with coconut sugar paste, respectively. In general, conjugated dienes are formed as intermediates through a shift of a double bond of polyunsaturated acids (Houhoula *et al*., 2002). Conjugated dienes are almost immediately after peroxides are formed, the non-conjugated double bonds (C=C–C–C=C) that are present in natural unsaturated lipids are converted to conjugated double bonds (C=C–C=C)(Gunstone and Norris, 1983).

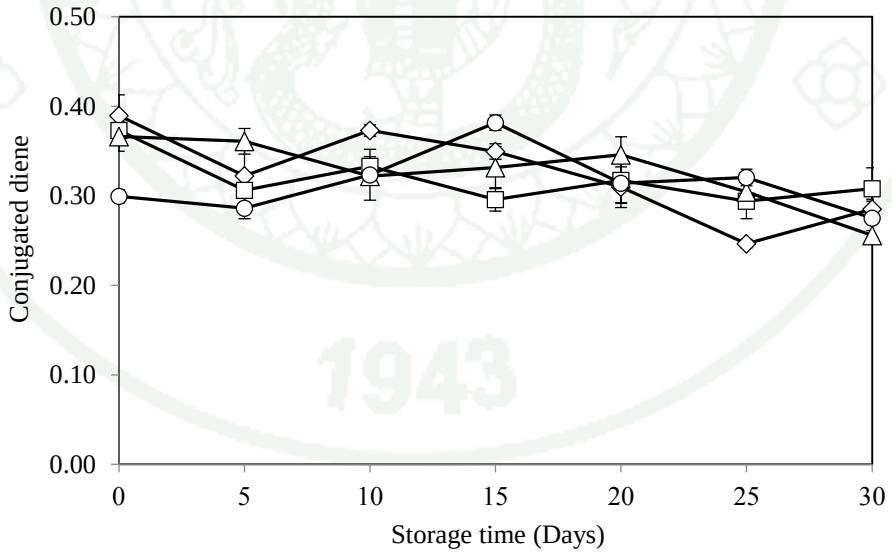


Figure 28 Changes in conjugated diene of ground perilla seed added with different sugars including cane sugar paste (◇), brown cane sugar (□), coconut sugar paste (△), palm sugar paste (○) during 30-day of storage. Bars represent the standard deviations from triplicate determinations.

3.2.5.4 Thiobarbituric acid reactive substances (TBARS) value

The TBARS values of ground perilla seed added with different sugar during storage were different, indicating the different degree of lipid oxidation among samples (Figure 29). TBARS values of ground perilla seed added with different sugar were different (p<0.05). At 0 day of storage, ground perilla seed with palm sugar paste had the lowest TBARS value, followed by coconut sugar paste and cane sugar paste, respectively, while that with brown cane sugar had the highest TBARS value. The TBARS value of ground perilla seed with all samples decreased at day 5 of storage in which sample added with coconut sugar paste had the lowest TBARS value. At day 10, TBARS value of ground perilla seed with all sugar samples increased. Ground perilla seed with coconut sugar paste had the lowest TBARS value, followed by palm sugar paste and cane sugar paste, respectively, while that with brown cane sugar had the highest TBARS value. After 15 days of storage, TBARS value of ground perilla seed with all sugar tended to decrease. Ground perilla seed with brown cane sugar had the highest TBARS value, followed by sample added with cane sugar paste and palm sugar paste, respectively. Ground perilla seed with coconut sugar paste had the lowest TBARS value. In general, TBARS value had been used to measure the concentration of relatively polar secondary reaction products, especially aldehydes (Nawar, 1996). Secondary oxidation products are indicators of oil rancidity (Uluata and Ozdemir, 2012). From the results of Uluata and Ozdemir (2012), TBARS value in seed oil increased as the storage time increased. However, in this study, it was noted that TBARS value of ground perilla seed added with different sugars tended to decrease with increasing the storage time (p<0.05). This indicated that sugar may involve in the antioxidative activity of ground perilla seed during storage.

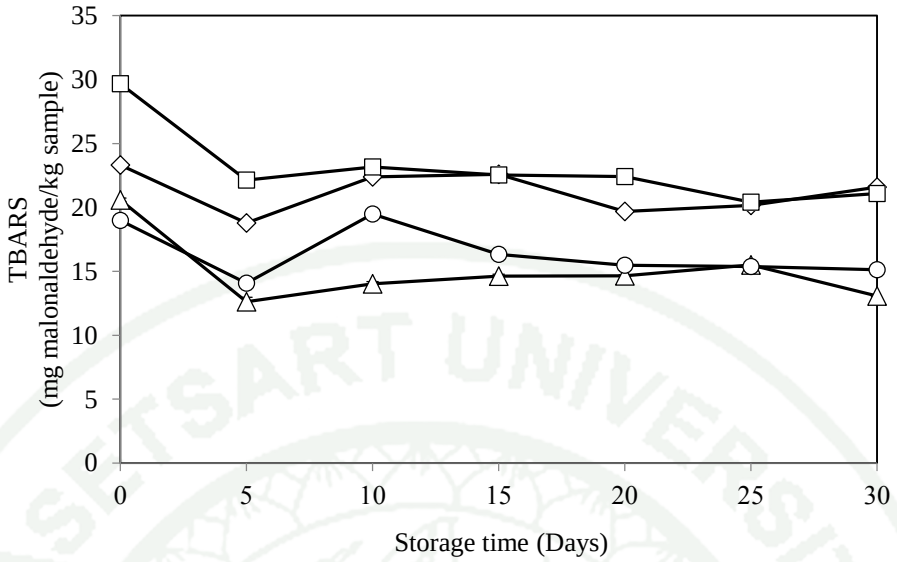


Figure 29 Changes in thiobarbituric acid reactive substances (TBARS) values of ground perilla seed added with different sugars including cane sugar paste (◇), brown cane sugar (□), coconut sugar past (△), palm sugar paste (○) during 30-day of storage. Bars represent the standard deviations from triplicate determinations.

3.2.6 Effect of sugars on acceptability of ground perilla seed during 30-day storage at room temperature

The acceptance scores for ground perilla seed added with different four commercial sugars stored for 30 days at room temperature are shown in Table 17. At the 0 day of storage, sample added with cane sugar paste had the highest score for all attributes, compared with those added with other commercial sugars ($p<0.05$). Sample added with cane sugar paste (the control) is traditionally produced and consumed in northern of Thailand and it is called “nga-tum-aoi”. Sample added with palm sugar paste had no differencein likeness scores for color, odor, taste, sweetness, texture, flavor and overall liking when compared with the control ($p>0.05$). The lowest appearance likeness score for sample added with brown cane sugar was observed ($p<0.05$). In general, ground perilla seed added with sugar paste is a paste-like product, but the sample added with brown cane sugar (powder form) had no moisture on its

surface. This might be resulted from water absorption by sugar occurred during preparation.

Appearance, color, odor, taste, sweetness, texture and flavor likeness scores of all samples decreased when the storage time increased ($p<0.05$). However, the degree of decrease varied with the samples. In general, the sample added brown cane sugar showed the lowest overall liking score compared with other samples ($p<0.05$). Sample added with coconut sugar paste, palm sugar paste, and the control were acceptable throughout the storage, whereas sample added with brown cane sugar was unacceptable at day 20 of storage. This might be due to the changes in moisture content (Figure 28), L* a* b* (Figure 30), and lipid oxidation products (peroxide value, conjugated diene, TBARS). Furthermore, the changes in lipid might be resulted from high level of ALA in ground perilla seed. Lipid oxidation products from ALA are generally aldehydes, carboxylic acid and hydrocarbons. These might contributed to flavor of sample. From the results, it could be concluded that ground perilla seed might be prone to oxidation as affected by different sugars. However, the use of coconut sugar paste might be used to extend shelf-life and as alternative sugar for cane sugar paste.

Table 17 Acceptance score of ground perilla seed added with different sugars during 30-day of storage.

Storage time (Days)	Samples**** S1	S3	S6	S7
Appearance				
0	7.89 ± 0.83*a**A***	5.91 ± 0.89^{aC}	7.20 ± 0.83^{aB}	7.46 ± 0.82^{aB}
5	7.69 ± 0.63^{abA}	5.86 ± 0.65^{aC}	7.17 ± 0.38^{aB}	7.37 ± 0.55^{abB}
10	7.51 ± 0.85^{abcA}	5.63 ± 0.94^{abC}	7.09 ± 0.61^{aB}	7.23 ± 0.69^{abcAB}
15	7.49 ± 0.89^{abcA}	5.57 ± 0.95^{abC}	7.06 ± 0.68^{aB}	7.14 ± 0.77^{abcAB}
20	7.31 ± 0.63^{bcA}	5.37 ± 0.81^{bB}	6.94 ± 0.80^{aA}	7.00 ± 0.84^{bcA}
25	7.17 ± 0.95^{cdA}	4.86 ± 0.94^{cC}	6.60 ± 0.81^{bB}	6.89 ± 0.72^{cAB}
30	6.86 ± 0.70^{dA}	4.06 ± 0.96^{dB}	7.06 ± 0.76^{aA}	7.20 ± 0.80^{abcA}
Color				
0	7.80 ± 0.68^{aA}	5.77 ± 0.94^{aB}	7.40 ± 0.81^{aA}	7.66 ± 0.76^{aA}
5	7.77 ± 0.55^{aA}	5.69 ± 0.83^{abC}	7.34 ± 0.54^{aB}	7.49 ± 0.70^{abAB}
10	7.51 ± 0.66^{abA}	5.54 ± 0.89^{abB}	7.29 ± 0.46^{abA}	7.37 ± 0.65^{abcA}
15	7.37 ± 0.97^{bcA}	5.49 ± 0.98^{abB}	7.17 ± 0.45^{abA}	7.26 ± 0.51^{bcA}
20	7.14 ± 0.60^{bcdA}	5.29 ± 0.89^{bcB}	7.00 ± 0.34^{bcA}	7.09 ± 0.37^{cdA}
25	7.00 ± 0.91^{cdA}	4.94 ±0.97^{cdB}	6.77± 0.65^{cA}	6.91 ± 0.66^{dA}
30	6.89 ± 0.84^{dA}	4.69 ± 0.98^{dB}	7.26 ± 0.82^{abA}	7.17 ± 0.83^{bcdA}
Odor				
0	7.57 ± 0.98^{aA}	6.43 ± 0.73^{aB}	7.40 ± 0.98^{aA}	7.34 ± 0.91^{aA}
5	7.37 ± 0.81^{abA}	6.17 ± 0.86^{abB}	7.09 ± 0.89^{abA}	7.31 ± 0.80^{aA}
10	7.23 ± 0.77^{abcA}	5.91 ± 0.89^{bcB}	6.80 ± 0.96^{bcA}	7.17 ± 0.89^{abA}
15	7.11 ± 0.80^{bcA}	5.60 ± 0.88^{cdC}	6.54 ± 0.82^{cB}	7.03 ± 0.71^{abA}
20	6.91 ± 0.70^{cdA}	5.46 ± 0.74^{dC}	6.37 ± 0.69^{cdB}	6.77 ± 0.94^{bcA}
25	6.60 ± 0.85^{dA}	5.03 ± 0.98^{eC}	6.00 ± 0.94^{dB}	6.46 ± 0.66^{cA}
30	7.00 ± 0.83^{bcdA}	4.43 ± 0.96^{fC}	6.40 ± 0.95^{cdB}	6.57 0.93^{cAB}

Table 17(Continued)

Storage time (Days)	Samples**** S1	S3	S6	S7
Taste				
0	7.51 ± 0.95^{aA}	6.17 ± 0.98^{aB}	7.43 ± 0.88^{aA}	7.57 ± 0.98^{aA}
5	7.46 ± 0.82^{abA}	5.89 ± 0.80^{abB}	7.34 ± 0.80^{abA}	7.40 ± 0.95^{abA}
10	7.43 ± 0.56^{abA}	5.77 ± 0.91^{abcB}	7.23 ± 0.73^{abA}	7.37 ± 0.84^{abA}
15	7.34 ± 0.59^{abcA}	5.63 ± 0.73^{bcB}	7.14 ± 0.49^{abA}	7.20 ± 0.72^{abcA}
20	7.20 ± 0.63^{abcA}	5.40 ± 0.60^{cdB}	7.03 ± 0.92^{bcA}	7.09 ± 0.82^{bcA}
25	7.09 ± 0.78^{bcA}	5.06 ± 0.97^{dC}	6.69 ± 0.53^{cB}	6.80 ± 0.80^{cAB}
30	7.00 ± 0.55^{cA}	4.40 ± 0.89^{eB}	7.06 ± 0.65^{abA}	7.11 ± 0.54^{bcA}
Sweet				
0	7.63 ± 0.84^{aA}	5.97 ± 0.86^{aB}	7.49 ± 0.98^{aA}	7.60 ± 0.98^{aA}
5	7.54 ± 0.56^{abA}	5.80 ± 0.96^{abB}	7.43 ± 0.56^{aA}	7.49 ± 0.85^{aA}
10	7.46 ± 0.51^{abcA}	5.77 ± 0.81^{abB}	7.40 ± 0.60^{aA}	7.43 ± 0.85^{abA}
15	7.37 ± 0.60^{abcA}	5.46 ± 0.95^{bcB}	7.17 ± 0.66^{aA}	7.29 ± 0.75^{abcA}
20	7.23 ± 0.81^{bcdA}	5.20 ± 0.68^{cdB}	6.77 ± 0.77^{bA}	7.00 ± 0.65^{bcA}
25	7.14 ± 0.94^{cdA}	5.00 ± 0.94^{dB}	6.57 ± 0.50^{bA}	6.86 ± 0.88^{cA}
30	6.97 ± 0.74^{dA}	4.29 ± 0.96^{eB}	7.17 ± 0.52^{aA}	7.00 ± 0.74^{bcA}
Texture				
0	7.80 ± 0.83^{aA}	5.20 ± 0.96^{aB}	7.51 ± 0.74^{aA}	7.54 ± 0.82^{aA}
5	7.54 ± 0.66^{abA}	5.17 ± 0.75^{aB}	7.31 ± 0.72^{abA}	7.37 ± 0.55^{abA}
10	7.40 ± 0.55^{bA}	5.09 ± 0.78^{abB}	7.20 ± 0.83^{abcA}	7.31 ± 0.72^{abA}
15	7.31 ± 0.8^{3bcA}	4.94 ± 0.91^{abB}	7.06 ± 0.64^{bcdA}	7.14 ± 0.94^{bcA}
20	7.09 ± 0.51^{cdA}	4.83 ± 0.66^{abB}	6.89 ± 0.80^{cdA}	6.91 ± 0.56^{cdA}
25	6.94 ± 0.48^{deA}	4.69 ± 0.76^{bcC}	6.40 ± 0.98^{eB}	6.69 ± 0.72^{dAB}
30	6.77 ± 0.43^{eA}	4.31 ± 0.91^{cB}	6.74 ± 0.67^{deA}	6.60 ± 0.70^{dA}

Table 17 (Continued)

Storage time (Days)	Samples**** S1	S3	S6	S7
Flavor				
0	7.71 ± 0.99^{aA}	6.43 ± 0.98^{aB}	7.63 ± 0.77^{aA}	7.40 ± 0.81^{aA}
5	7.43 ± 0.61^{abA}	6.06 ± 0.64^{abB}	7.37 ± 0.55^{abA}	7.29 ± 0.71^{aA}
10	7.37 ± 0.94^{abcA}	5.83 ± 0.95^{bB}	7.11 ± 0.76^{bcA}	7.14 ± 0.88^{abA}
15	7.14 ± 0.85^{bcdA}	5.63 ± 0.91^{bB}	7.00 ± 0.59^{cA}	7.09 ± 0.70^{abA}
20	7.00 ± 0.59^{cdeA}	5.20 ± 0.83^{cC}	6.51 ± 0.82^{dB}	6.89 ± 0.58^{bcA}
25	6.89 ± 0.63^{deA}	4.71 ± 0.86^{dC}	5.74 ± 0.74^{eB}	5.91 ± 0.89^{dB}
30	6.69± 0.64^{eA}	4.54 ± 0.90^{dB}	6.77 ± 0.55^{cdA}	6.60 ± 0.82^{cA}
Overall liking				
0	7.80 ± 0.47^{aA}	6.06 ± 0.64^{aC}	7.40 ± 0.60^{aB}	7.57 ± 0.50^{aAB}
5	7.57 ± 0.61^{abA}	5.83 ± 0.89^{abB}	7.34 ± 0.64^{abA}	7.46 ± 0.56^{aA}
10	7.43 ± 0.92^{bA}	5.74 ± 0.85^{abB}	7.23 ± 0.91^{abcA}	7.31 ± 0.58^{abA}
15	7.29 ± 0.67^{bA}	5.43 ± 0.85^{bcB}	7.03 ± 0.45^{bcdA}	7.14 ± 0.55^{bcA}
20	6.97 ± 0.66^{cA}	5.06 ± 0.80^{cdB}	6.74 ± 0.92^{dA}	6.89 ± 0.58^{cA}
25	6.77 ± 0.73^{cA}	4.74 ± 0.92^{deC}	6.31 ± 0.80^{eB}	6.46 ± 0.95^{dAB}
30	6.66 ± 0.49^{cB}	4.46 ± 0.93^{eC}	6.97 ± 0.52^{cdA}	6.86 ± 0.36^{cAB}

* Mean + SD from thirty five determinations.

** Different superscripts letters in the same column within the same attribute indicate the significant differences (p<0.05).

*** Different superscripts capital letters in the same row within the same storage time indicate significant differences (p<0.05).

**** Samples: ground perilla seed added with cane sugar paste (S1), brown cane sugar (S2), coconut sugar paste (S6), and palm sugar paste (S7).

CONCLUSION

Differences in nutritional characteristics of brown and white perilla seeds were found. Both perilla seeds showed differences in chemical compositions, particularly fat and carbohydrate. Higher protein, fat, ash, crude fiber, β-carotene and α-tocopherol contents were found in brown perilla seed. Additionally, brown perilla seed contained high total unsaturated fatty acid, especially essential fatty acid, α-linolenic acid (C18:3). For bioactive compounds, brown perilla seed had high contents of total phenolic, total flavonoids, and total flavonols. As a result, brown perilla seed showed more scavenging activity against DPPH radical, ABTS radical and also showed more FRAP and reducing power.

Different commercial sugars showed different physicochemical properties. High reducing sugar and mineral contents (Fe, Na, and K) were found in cane sugar paste (S1). High content of non-enzymatic browning reaction products, intermediate product and browning intensity, were found in cane sugar paste (S1), brown cane sugar (S3), coconut sugar paste (S6) and palm sugar paste (S7). Also, S1, S3, S6 and S7 had high total phenolic content with high antioxidant activity. Therefore, S1, S3, S6 and S7 were chosen for the study the effect of sugars on lipid oxidation of ground perilla seed during storage at room temperature.

During storage, slight changes in pH, A_w, L*, a* and b* of all samples were observed. Lipid changes, lipolysis and lipid oxidation, of ground perilla seed added with different sugars occurred during 30-day of storage at room temperature. Increased free fatty acids content with increased storage time was noted. Generally, high peroxide values of all samples were observed at the beginning of the storage and tended to decrease throughout the storage. Among samples, different TBARS values were observed, however, slight changes in TBARS were observed during 5 to 30 days of storage. Lower acceptance in all attributes was observed in sample added with brown cane sugar, than in other samples, particularly when storage time increased. Sample added with cane sugar paste, coconut sugar paste, and palm sugar paste showed slight decreases in overall liking scores within 20 days. The results revealed that the different sugars used might affect the lipid oxidation differently.Therefore, commercial sugar

pastes could be mixed with ground black perilla seed to produce “nga-tam-oi” with antioxidant capability.

LITERATURE CITED

Abramovic, H. and V. Abram. 2005. Physico-chemical properties, composition and oxidative stability of *Camelina sativa* oil**. Food Technology and Biotechnolgy** 43(1): 63-70.

Adebowale, A.A., S.A. Sanni and O.A. Falore. 2011. Varietal difference in the physical properties and proximate composition of elite sesame seeds. **World Journal of Agriculture Science** 7(1): 42-46.

Agostoni, C., I. Axelsson, O. Goulet, B. Koletzko, K.F. Michaelsen, J. Puntis, D. Rieu, J. Rigo, R. Shamir, H. Szajewska and D. Turck. 2006. Soy protein infant formulae and follow-on formulae: A commentary by the ESPGHAN committee on nutrition. **Journal of Pediatric Gastroenterology and Nutrition** 42: 352-361.

Aidos, I., S. Lourenco, A. Van Der Padt, J.B. Luten and R. M. Boom. 2002. Stability of crude herring oil produced from fresh by products: influence of temperature during storage. **Journal of Food Science** 67: 3314-3320.

Ajandouz, E.H. and A. Puigserver. 1999. Nonenzymatic browning reaction of essential amino acids: effect of pH on caramelization and Maillard reaction kinetics. **Journal of Agricultural and Food Chemistry** 1(15): 928-943.

_____, L.S. Tchiakpe, F. Dalle-Ore, A. Benajiba and A. Puifserver. 2001. Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model system. **Journal of Food Science** 66: 926-931.

Ali, Q., M. Ashraf and A. Farooq. 2010. Seed composition and seed oil antioxidant activity of maize under water stress. **Journal of the American Oil Chemistry's Society** 87: 1179-1187.

Amarnath, B. 2004. **A study on antioxidant nature of petai (*Parkia speciosa)***. M.E. Thesis, National University of Singapore.

Amin, N.A.M., W.A.W. Mustapha, M.Y.Maskat and H.C. Wai. 2010. Antioxidative activities of palm sugar-like flavouring. **The Open Food Science Journal** 4: 23-29.

Ampanchai, S., S. Nuglor, P. Puddhanon, P. Supapornhemin and V. Champa. 2008. Effect of storage conditions on seed quality and oil content of *Perilla frutescens* (L.) Britt. in the upper North of Thailand. **Thai Journal of Agricultural Scicence** 39(3): 421-424.

AOAC. 2000. **Official Method of Analysis**. 17th ed. The association of official analytical chemistry, Washing DC.

Apriyantono, A, A. Aristyani, Nurhayati, Y. Lidya, S. Budiyanto and S.T. Soekarto. 2002. Rate of browning reaction during preparation of coconut and palm sugar. **International Congress Series** 1245: 275-278.

Araujo, J.M.A. 2004. **Quimica de Alimentos: Teoria e Pratica.** 3rd ed. Editora UFV, Sao Paulo.

Armstrong, D. and R. Browne. 1994. The analysis of free radicals, lipid peroxidases, antioxidant enzymes and compounds related to oxidative stress as applied to the clinical chemistry laboratory. **Free Radicals in Diagnostic Medicine** 366:43-58.

Asif, M. and A. Kumar. 2010. Nutritional and functional characterizations of *Perilla frutescens* seed oil and evaluation of its effect on gastrointestinal motility. **Malaysian Journal of Pharmaceutical Sciences** 8(1): 1-12.

Asikin, Y., A. Kamiya, M. Mizu, K. Takara, H. Tamaki and K. Wada. 2014. Changes in the physicochemical characteristics, including flavor components and Maillard reaction products, of non-centrifugal cane brown sugar during storage. **Food Chemistry** 149: 170-177.

Babalola, T.O.O. and D.F. Apata. 2011. Chemical and quality evaluation of some alternative lipid sources for aqua feed production. **Agriculture and Biology Journal of North America** 2(6): 935-943.

Barros, L., M. Duenas, I.C.F. R. Ferreira, A. M. Carvalho and C. Santos-Buelga. 2011. Use of HPLC-DAD-ESI/MS to profile phenolic compounds in edible wild greens from Portugal. **Food Chemistry** 127: 169-173.

Bassoli, A., G. Borgonovo, S. Caimi, L. Scaglioni, G. Morini, A. Schiano Moriello, V. Di. Marzo and L. De. Petrocellis. 2009. Taste-guided identification of high potency TRPA1 agonists from *Perilla frutescens*. **Bioorganic and Medicinal Chemistry** 17: 1636-1639.

Belitz, H.D. and W. Grosch. 1999. **Food Chemistry**. Springer-Verlag Berlin Heidelberg, New York.

Benjakul, S., T.A. Seymour, M.T. Morrissey and H. An. 1997. Physicochemical changes in pacific whiting muscle proteins during iced storage. **Journal of Food Science** 62: 729-733.

_____, W. Lertittikul and F. Bauer. 2005a. Antioxidant activity of Maillard reaction products from a porcine plasma protein–sugar model system. **Food Chemistry** 93: 189-196.

_____, W. Visessanguan, V. Phongkanpai and M. Tanaka. 2005b. Antioxidative activityof caramelisation products and their preventive effect on lipid oxidation in fish mince. **Food Chemistry** 90: 231-239

Benzie, I.F.F. and J.J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. **Analytical Biochemistry** 239: 70-76.

Berker, K.I., K. Guclu, I. Tor, B. Demirata and R. Apak. 2010. Total antioxidant assay using optimized ferricyanide Prussian blue method. **Food Analytical Methods** 3:154-168.

Bernardez, M., L. Pastoriza, G.Sampedro, J.J.R. Herrera and M.L. Cabo. 2005. Modified method for the analysis of free fatty acids in fish. **Journal of Agricultural and Food Chemistry** 53(6): 1903-1906.

Binsan, W., S. Benjakul, W. Visessanguan, S. Roytrakul, M. Tanaka and H. Kishimura. 2008. Antioxidative activity of mungoong, an extract paste,from the cephalothorax of white shrimp (*Litopenaeus vannamei*). **Food Chemistry** 106: 185-193.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. **Canadian journal of biochemistry and physiology** 30: 911-917.

Brand, C.M.J. and M.A.J.S. Van Boekel. 2002. Kinetics modeling of reactions in heated monosaccharide-casein system. **Journal of Agricultural and Food Chemistry** 50: 6725-6739.

Brand-Williams, W., M.E. Cuvelier and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. **LWT-Food Science and Technology** 28: 25-30.

Britt, I.J. 2008. Thermal Processing, pp 63-79.*In* G.S. Tucker, ed.**Food Biodeterioration and Preservation.** Blackwell Publishing Ltd., Oxford.

Brody, T. 1994. **Nutritional Biochemistry**. Academic Press, New York.

Buege, J.A. and S.D. Aust. 1978. Microsomal lipid peroxidation. **Method in Enzymology** 52: 302-304.

Buera, M.D.P., J. Chirife, S. Resnik and G. Wetzler. 1987. Nonenzymatic browning in liquid model systems of high water activity: kinetics of color changes due to Maillard's reaction between different single sugars and glycine and comparison with caramelization browning. **Journal of Food Science** 52: 1063-1067.

Burdurlu, S.H. and F. Karadenzi. 2003. Effect of storage on nonenzymatic browning of apple juice concentrates. **Food Chemistry** 80: 91-97.

Camerver, B., B.L. Wedzicha and L.W. Kroh. 1999. Nonenzymatic browning reactions of retro-aldol degradation products of carbohydrates. **European Food Research and Technology** 209: 261-265.

Chamber IV, E. and M.B. Wolf. 1996. **Sensory testing methods**. 2nd ed. American Society for Testing and Materials, West Conshohocken, Philadelphia.

Chiangmai University Library. 2007. **Nga tam oi**. Available Source: http://library.cmu.ac.th/ntic/en_lannafood/detail_lannafood.php?id_food=31, October 5, 2011

Choe, E. and D.B. Min. 2006. Mechanisms and factors for edible oil oxidation. **Comprehensive Reviews in Food Science and Food Safety** 5: 169-186.

Choi, Y., S.H. Jeong and J. Lee. 2007. Antioxidant activity of methanolic extracts from some grains consumed in Korea. **Food Chemistry** 103: 130-138.

Ciftci, O.N., R. Przybylski and M. Rudzinska. 2012. Lipid components of flax, perilla, and chia seeds. **European Journal of Lipid Science and Technology** 144: 794-800.

Coca, M., M.T. Garcia, G. Gonzalez, M. Pena and J.A. Garcia. 2004. Study of colored compounds formed in sugar beet processing. **Food Chemistry** 86*:* 421-433.

Codex Standard. (1999). **Codex Standard for named vegetable oils**. Codex Alimentarius Comission.

Colombo, R., F.M. Lancuas and J.H. Yariwake. 2006. Determination of flavonoids in cultivated sugarcane leaves, bagasse, juice and in transgenic sugarcane by liquid chromatography-UV detection. **Journal of Chromatography A** 1103: 118-124.

Connor, W. E. 2000. Importance of n-3 fatty acids in health and disease. **The American Journal of Clinical Nutrition** 71: 171-175.

Ding, Y., N.C. Mokgolodi, Y. Hu, L. Shi, C. Ma and Y-J. Liu. 2012. Characterization of fatty acid composition from five perilla seed oils in China and its relationship to annual growth temperature. **Journal of Medicinal Plants Research** 6(9): 1645-1651.

Duarte-Almeida, J. M., A.V. Novoa, A.F. Linares, F.M. Lajolo, and M.I. Genovese. 2006. Antioxidant activity of phenolics compounds from sugarcane (*Saccharum officinarum* L.) juice. **Plant Foods for Human Nutrition** 61: 187-192.

Duh, P-D. 1998. Antioxidant activity of burdock (*Arctium lappa* Linné): its scavenging effect on free-radical and active oxygen. **Journal of the American Oil Chemists' Society** 75: 455-461.

Eichner. K. 1981. Antioxidative effect of Maillard reaction intermediates. **Progress in Food and Nutrition Science**5:441-446.

Eskin, N.A.M. 1990. Biochemistry of food processing: browning reactions in foods, pp. 240-295. *In*: N.A.M. Eskin, ed. **Biochemistry of Foods**. Academic Press, London.

Fernandez, J., J.A Perez-Alvarez and J.A. Fernandez-Lopez. 1997. Thiobarbituric acid test for monitoring lipid oxidation in meat. **Food Chemistry** 59: 345-353.

Fontaniella, B., C. Vicente, M.E. Legaz, R. De Armas, C.W. Rodriguez, M. Martinez,D. Pinon, R. Acevedo and M.T. Solas. 2003. Yellow leaf syndrome modifies the composition of sugarcane juices in polysaccharides, phenols and polyamines. **Plant Physiology and Biochemistry** 41: 1027-1036.

Frankel, E.N. 1985. Chemistry of autoxidation: mechanism, products and flavor significance, pp 1-34. *In*D.B. Min, T.H. Smouse, eds. **Flavor chemistry of fats and oils**. American Oil Chemistry's Society, Washington.

_____. 1998. Photooxidation of unsaturated fats, pp. 43-54. *In* E.N. Frankel, ed. **Lipid Oxidation**. The Oily Press LTD, Scotland.

_____, and S. Huang. 1996. Evaluation of antioxidant activity of rosemary extracts, carnosol and carnosic acid in bulk vegetable oils and fish oil and their emulsions. **Journal of the Science of Food and Agriculture** 72: 201-208.

Gandhi, A.P. and J. Srivastava. 2007. Studies on the production of protein isolates from defatted sesame seed (*Sesamum indicum*) flour and their nutritional profile. **ASEAN Food Journal** 14 (3): 175-180.

Godshall, M. A. and E.J. Roberts. 1982. Phenolics in sugar products: their role in flavor and color production, pp. 47-72. *In* **Proceedings of the 1982 Sugar Processing Research Conference**, SPRI, New Orlesns.

_____, J.R. Vercellotti and R. Triche. 2002. Comparison of cane and beet sugar macromolecules in processing. **International Sugar Journal** 104: 228-233.

Goffman, F.D. and C. Bergman. 2003. Relationship between hydrolytic rancidity, oil concentration and esterase activity in rice bran. **Cereal Chemistry** 80(6): 689-692.

Goli, S.A.H., S.M. Sahafi, B. Rashidi and M. Rahimmalek. 2013. Novel oilseed of *Dracocephalum kotschyi* with high n-3 to n-6 polyunsaturated fatty acid ratio. **Industrial Crops and Products** 43: 188-193.

Gopalan, C., B.V. Ramasastri and S.C. Balasubramanian. 1982. **Nutritive value of Indian foods**. National institute of nutrition-Indian council of medical research, Hyderabad, India.

Gordon, M. H. 1990. The mechanism of antioxidant action in vitro, pp. 1–18. *In* B. J. F. Hudson, ed. **Food Antioxidants**. Elsevier Applied Science, London:

_____. 2001. The development of oxidative rancidity, pp. 7-22. *In* J. Pokorny, N. Yanishlieva and M. Gordon, eds. **Antioxidants in Food-Practical Applications.** CRC Press, Washington.

Guerra, M.J. and M.V. Mujica. 2010. Physical and chemical properties of granulated cane sugar “panelas”. **Ciencia e Tecnologia de Alimentos** 30(1): 250-257.

Gu, F.L., J.M. Kim, S. Abbas, X.M. Zhang, S.Q. Xia and Z.X. Chen. 2010. Structure and antioxidant activity of high molecular weight Maillard reaction products from casein-glucose. **Food Chemistry** 120: 505-511.

Gul, S. and M. Harasek. 2012. Energy saving in sugar manufacturing through the integration of environmental friendly new membrane processes for thin juice pre-concentration. **Applied Thermal Engineering** 43: 128-133.

Gunstone, F.D. 2002. Production and trade of vegetable oils. *In* F.D. Gunstone, ed. **Vegetableoils in food technology composition, properties and uses.** Blackwell Publishing Ltd, Oxford, UK.

_____, F.A. Norris. 1983. Oxidation, pp. 58-65. *In* F.D. Gunstone and F.A. Norris, eds. **Lipid in foods: Chemistry, biochemistry and technology**. Pergamon Press, New York.

_____, J. Harwood, and F. B. Padley. 1994. **The lipid handbook** (2nd eds.), Chapman and Hall, London.

Hagemann, J.M., F.R. Earle, I.A. Wolff and A.S. Barclay. 1967. Search for new industrial oils. XIV. Seed oils of labiatae. **Lipids** 2(5): 371-380.

Haghparast. S., B. Shabanpour, H. Kashiri1, GH. Alipour and M. Sodagar. 2013. 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: 87-99.

Halliwell, B and J.M.C. Gutteridge. 1998. Free radicals, other reactive species and disease, pp. 617-784. *In* B. Halliwell and J.M.C. Gutteridge, eds. **Free radicals in biology and medicine**. Oxford UP, Oxford.

Hamed, S.F. and G.A. Abo-Elwafa. 2012. Enhancement of oxidation stability of flax seed oil by blending with stable vegetable oils. **Journal of Applied Sciences Research** 8(10): 5039-5048.

Harish Nayaka, M.A., U.V. Sathisha, M.P. Manohar, K.B. Chandrashekar and S.M. Dharmesh. 2009. Cytoprotective and antioxidant activity studies of jaggery sugar. **Food Chemistry** 115: 113-118.

Hayase, F, S. Hirashima, G. Okamota and H. Kato. 1989. Scavenging of active oxygen by melanoidins. **Journal of Agricultural and Biological Chemistry** 53: 3383-3385.

Hegde, D.M. 2004. Sesame. *In* K.V. Peter., eds. **Handbook of herbs and spices, Volume 2**. TJ International, Padstow, Cornwall, England.

Hodge, J. E. 1953. Chemistry of browning reactions in model systems. **Journal of Agricultural and Food Chemistry** 1: 928-936.

Hoed, V.V. 2010. Phenolic compounds in seed oils**. Lipid Technology** 22(11): 247-249.

Homoki-Farkas, P., F. Orsi and L.W. Kroh. 1997. Methylglyoxal determination from different carbohydrates during heat processing. **Food Chemistry** 59: 157-163.

Houhoula, D. P., V. Oreopoulou and C. Tzia. 2002. A kinetic study of oil deterioration during frying and a comparison with heating. **Journal of the American Oil Chemists' Society** 79:133-137.

Hwang, I.G., H.Y. Kim, K.S. Woo, J. Lee and H.S. Jeong. 2011. Biological activities of Maillard reaction products (MRPs) in a sugar-amino acid model system. **Food Chemistry** 126: 221-227.

Ibarz, A., J. Pagan and S. Garza. 1999. Kinetic models for color changes in pear puree during heating at relatively high temperatures. **Journal of Food Enginerring** 39: 415-422.

Ihara, M., H. Umekawa, T. Takahashi and Y. Furuichi. 1998. Comparative effects of short and longterm feeding of safflower oil and perilla oil on lipid metabolism in rats. **Comparative Biochemistry and Physiology-Part B: Biochemistry & Molecular Biology** 121(2): 223-231.

Ito, M., G. Honda and K. Sydara. 2008. *Perilla frutescens* var. *frutescens* in northern Laos. **Journal of Natural Medicines** 62:251-258.

Jackson, E.B. 1990. **Sugar Confectionery Manufacture**. Chapman & Hall, London, New York.

James, C. S. 1995. **Analytical chemistry of foods**. Chapman and Hall, Glasgow, UK.

Jing, H. and D.D. Kitts. 2002. Chemical and biochemical properties of casein-sugar Maillard reaction products. **Food and Chemical Toxicology** 40: 1007-1015.

Kamel, B.S., H. Dawson and Y. Kakuda. 1985. Characterestics and composition of melons and grape seeds oils and cakes. **Journal of American Oil Chemist's Society** 62(5): 881-883.

Karel, M. and D.B. Lund. 2003. **Physical principles of food preservation**. Marcel Dekker. New York, USA.

Kashima, M., G.S. Cha, Y. Isoda, J. Hirano and T. Miyazawa. 1991. The antioxidant effects of phospholipids on perilla oil. **Journal of the American Oil Chemistry's Society** 68: 119-122.

Kasote, D.W. 2013. Flaxseed phenolics as natural antioxidants. **International Food Research Journal** 20(1): 27-34.

Khantaphant, S. and S. Benjakul. 2008. 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: 410-419.

Kim, H-K. and Choi, H. 2001. Dietary α-linolenic acid lowers postprandial lipid levels with increase of eicosapentaenoic and docosahexaenoic acid contents in rat hepatic membrane. **Lipids** 36: 1331-1336.

_____, S. Choi and H. Choi. 2004. Suppression of hepatic fatty acid synthase by feeding α-linolenic acid rich perilla oil lowers plasma triacylglycerol level in rats**. Journal of Nutritional Biochemistry** 15: 485-492.

Kim, K.S., S.H. Park and M.G. Choung. 2007. Nondestructive determination of oil content and fatty acid composition in perilla seeds by near-Infrared spectroscopy. **Journal of Agricultural and Food Chemistry** 55 (5): 1679-1685.

Kim, S.J. and S.Y. Lee. 2009. Antioxidant activity of Maillard reaction products derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time. **Food Chemistry** 116: 227-232.

Kirigaya, N., H. Kato and M. Fujimaki. 1968. Studies on antioxidant activity of nonenzymic browning reaction products: 1. Relations of color intensity and reductones with antioxidant activity of browning reaction products. **Agricultural and Biological Chemistry** 32: 287-290.

Kosuna, K. and M. Haga. 1997. The development and application of perilla extract as an anti-allergic substance, pp. 143-148. *In* H. C. Yu, ed. **Perilla-The Genus Perilla.** Harwood Academic Publishers, Amsterdam, London.

Kroh, L.W. 1994. Caramelization in food and beverages. **Food Chemisty** 51: 373-379.

Kumaran, A. and K.R. Joel. 2007. In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. **LWT-Food Science and Technology** 40: 344-352.

Kurilich, A. C. and J.A. Juvik. 1999. Simultaneous quantification of carotenoids and tocopherols in corn kernel extracts by HPLC. **Journal of Liquid Chromatography and Related Technologies** 22: 2925-2934.

Kwak, T.S. 1994. Major growth characters and fatty acid composition of Korean native perilla collections. **The Korean Journal of Breeding Science** 26: 148-154.

Lee, B.H., S.N. Ryu, and T.S. Kwak. 2002. Current status and prospects of quality evaluation in perilla. **Korean Journal of Crop Science** 47: 150-162.

Lee, B-K., S-L. Kim, K-H. Kim, S-H. Yu, S-C. Lee, Z. Zhang, M-S. Kim, H-M. Park and J-Y. Lee. 2008. Seed specific expression of perilla gamma-tocopherol methyltransferase gene increases alph-tocopherol content in transgenic perilla (*Perilla frutescens*). **Plant Cell Tissue and Organ Culture** 92: 47-54.

Lee, J., C.E. Finn and R.E. Wrolstad. 2004. Anthocyanin pigment and total phenolic content of three vaccinium species native to the Pacific Northwest of North America. **Hortscience** 39: 959-964.

_____, K.H. Park, M-H. Lee, H-T. Kim, W.D. Seo, J.Y. Kim, I-Y. Baek, D.S. Jang and T.J. Ha. 2013. Identification, characterisation, and quantification of phenolic compounds in the antioxidant activity-containing fraction from the seeds of Korean perilla (*Perilla frutescens*) cultivars. **Food Chemistry** 136: 843-852.

Leistner, L. and W. Wodel. 1976. The stability of intermediate moisture food with respect to microorganisms, pp. 120-137. *In* R. Davies, G.G. Birch and K.J. Paker, ed. **Intermediate moisture food**. Elsevier Applied Science, London.

Lertittikul, W., S. Benjakul and M. Tanaka. 2007. Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein-glucosemodel system as influenced by pH. **Food Chemistry** 100: 669-677.

Longvah, T. and Y.G. Deosthale. 1991. Chemical and nutritional studies on Hanshi (*Perilla frutescens*) a traditional oilseed from Northeast India. **Journal of the American Oil Chemistry's Society** 68: 781-784.

_____, _____. 1998. Effect of dehulling, cooking and roasting on the protein quality of perilla frutescens seed. **Food Chemistry** 63(4): 519-523.

Low, L.K. and C.S. Ng. 1978. Determination of peroxide value, pp. C7.1-C7.3. *In* H. Hasegawa, ed. **Laboratory manual on analytical methods and procedures for fish and fish products**. Marine Fisheries Research Department, Southeast Asian Fisheries Development Center, Singapore.

Lowry, R. and I. Tinsley. 1976. Rapid colorimetric determination of free fatty acids. **Journal of the American Oil Chemistry's Society** 53: 470-472.

Lukaszewicz, M., J. Szopa and A. Krasowska. 2004. Susceptibility of lipids from different flax cultivars to peroxidation and its lowering by added antioxidants. **Food Chemistry** 88: 225-231.

Macrae, R., R.K. Robinson and M.J. Sadler. 1993. **Encyclopaedia of Food Science, Food Technology and Nutrition**. Academic Press, London.

Maisuthisakul, P, M. Suttajit and R. Pongsawatmanit. 2005. Assessment ofphenolic content and free radicalscavenging capacity of some Thaiindigenous plants. **Food Chemistry**100: 1409-1418.

Malcolmson, L.J., R. Przybylski and J.K. Daun. 2000. Storage stability of milled faxseed. **Journal of the Americal Engineering** 77(3): 235-238.

Marales, F. J. and S. Jimenez-Perez. 2001. Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. **Food Chemistry** 72: 119-125.

Martins, S.I.F.S and M.A.J.S. Van Boekel. 2003. Melanoidins extinction coefficient in the glucose/glycine Maillard reaction. **Food Chemistry** 83:135-42.

_____, W.M.F. Jongen and M.A.J.S. Van Boekel. 2001. A review of Maillard reaction in food and implications to kinetic modeling. **Trends in Food Science and Technology** 11: 364-373.

Maskan, M. 2006. Production of pomegranate (*Punica granatum L*.) juice concentrate by various heating methods: color degradation and kinetics. **Journal of Food Engineering** 72: 218-224.

Mathlouthi, M. 2001. Water content, water activity, water structure and the stability of foodstuffs. **Food Control** 12: 409-417.

Mauron, J. 1981. The Maillard reaction in food: a critical review from the nutritional stand point. **Progress in Food and Nutrition Science** 5: 5-18.

McGhie, T. K. 1993. Analysis of sugarcane flavonoids by capillary zone electrophoresis. **Journal of Chromatography A** 634*:* 107-112.

McWilliams, M. 2006. **Nutrition and Dietetics**, 8th Edition. Pearson Education, Inc, publishing as Prentice Hall.

Meilgaard, M., G. Civilleand B**.** Carr. 1991. **Sensory Evaluation Techniques**. 2nd edn. CRC Press, Boca Raton, FL.

Mezadri, T., D, Villano, M.S. Fernandez-Pachon, M.C. Garcia-Parrilla and A.M. Troncoco. 2008. Antioxidant compounds and antioxidant activity in acerola (*Malpighia emarginata* DC.) fruits and derivatives. **Journal of Food Composition and Analysis** 21: 282-290.

Miranda, M., A. J. Vega-Galvez, G. Lopez, M. Parada, M. Sanders, M. Aranda, E. Uribe and K.D. Scala. 2010. Impact of air-drying temperature on nutritional properties, total phenolic content and antioxidant capacity of quinoa seeds *(Chenopodium quinoa* Willd.). **Industrial Crops and Products** 32(3): 258-263.

Mohdaly, A. A. A., I. Smetanska, M.F. Ramadan, A. Mohamed, M.A. Sarhan and A. Mahmoud. 2011. Antioxidant potential of sesame (*Sesamum indicum*) cake extract in stabilization of sunflower and soybean oils. **Industrial Crops and Products** 34: 952-959.

Naka, P. 1996. Potential of producing sugar from coconut. *In* **Proceedings of promoting multi-purpose uses and competitiveness of the coconut.** 26-29 September 1996. IPGRI.

Nakazawa, T. and K. Ohsawa. 2000. Metabolites of orally administered *Perilla frutescens* extract in rats and humans. **Biological and Pharmaceutical Bulletin**. 23: 122-127.

Naknean, P. 2010. **Factors affecting browning and crystallisation of palm sugar syrup and palm sugar cake**. Ph.D. Thesis, Prince of Songkla University.

_____. 2011. Characteristics and antioxidant activity of palm sugar syrup produced in Songkhla Province, Southern Thailand. **Asian Journal of Food and Agro-Industry** 4(4): 204-212.

_____, M. Meenune and G. Roudaut. 2009. 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: 448-456.

_____, _____, _____. 2013. Changes in properties of palm sugar syrup produced by an open pan and a vacuum evaporator during storage. **International Food Research Journal** 20(5): 2323-2334.

Narasinga Rao, M.S. 1985. Nutritional aspect of oil seeds, pp. 625–634. *In* H.C. Srivastava, S. Bhaskaran, B. Vatsya and K.K.G. Menon, eds., **Oil Seed Productions–contraints and opportunities**. Oxford and IBH Publishing Co. Ltd, New Delhi.

Nawar, W.W. 1996. Lipids, pp. 225-264. *In* O. R. Fennema, ed. **Food Chemistry,** 3rd edn. Marcel Dekker. New York.

Netzel, M., G. Strass, I. Bitsch, R. Konitz, M. Christmann and R. Bitsch. 2003. Effect of grape processing on selected antioxidant phenolics in red wine. **Journal of Food Engineering** 56: 223-228.

Ni Eidhin, D., J. Burke and D. O'Beirne. 2003. Oxidative stability of ω3–rich camelina oil and camelina oil-based spread compared with plant and fish oils and sunflower spread. **Journal of Food Science** 68(1): 345-353.

Nouros, P.G., C.A. Georgiou and M.G. Polissiou. 1999. Direct parallel flow injection multichannel spectrophotometric determination of olive oil peroxide value. **Analytica Chimica Acta** 389(1): 239-245.

Nutt, K. A., M.G. O'Shea and P.G. Allsopp. 2004. Feeding by sugarcane whitegrubs induces changes in the types and amounts of phenolics in the roots of sugarcane. **Environmental and Experimental Boany**51: 155-165.

O'Brien R.D. 2004. **Fats and oils formulating and processing for applications**, CRC Press, Boca Raton, United States.

_____, W.E. Farr and P.J. Wan. 2000. **Introduction to Fats and Oil Technology**, 2nd edn. AOCS Press, Champagin, IL, USA.

Oktay, M., I. Gulcin and O.I. Kufrevioglu. 2003. Determination of in vitro antioxidant activity of fennel *(Foeniculum vulgare)* seed extracts. **Lebensmittel-Wissenschaft und-Technologie** 36: 263-271.

Olaiya, C.O. 2006. Effects of three plant bioregulators on somebiochemical properties of *Lycopersicon esculentum* (L.) Mill. Ph.D. Thesis, University of Ibadan.

Onwuka, G.I., H. Clifford and N. Onuegbu. 2012. Development of extraction protocol for phenolic compounds in African pear seed (*Dacryodes edulis*). **International Journal of Innovative Pharmaceutical Research** 3(2): 212-216.

Oomah, B.D, S. Ladet, D.V. Godfrey, J. Liang and B. Girard. 2000. Characteristics of raspberry (*Rubis idaeus* L.) seed oil. **Food Chemistry** 69: 187-193.

Ordoñez, A.A.L., J.D. Gomez, M.A. Vattuone and M.I. Isia. 2006. Antioxidant activities of *sechium edule* (Jacq.) Swartz extracts. **Food Chemistry** 97: 452-458.

Oyaizu, M. 1986. Antioxidant activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. **Journal of the Japanese Society for Food Science and Technology** 35: 771-775.

Palla, G. 1982. Isolation and identification of phenolic glucosides in liquid sugars from cane molasses. **Journal Agricltural and Food Chemistry** 30: 764-766.

Pawel, G., A. Siger, K. Juhnevica, G. Lacis, E. Sne and D. Seglina. 2014. A comparison of new cold-pressed Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach) seed oil and nine other plant oils. **European Journal of Lipid Science and Technology** DOI: 10.1002/ejlt.201300425.

Payet, B., A. Shum Cheong Sing and J. Smadja. 2005. Assessment of antioxidant activity of cane brown sugars by ABTS and DPPH radical scavenging assays: determination of their polyphenolic and volatile constituents. **Journal Agricltural and Food Chemistry** 53: 10074-10079.

_____, _____, _____. 2006. Comparison of the concentrations of phenolic constituents in cane sugar manufacturing products with Their Antioxidant Activities. **Journal Agricltural and Food Chemistry** 54: 7270-7276.

Peiretti, P.G. 2011. Fatty acid content and chemical composition of vegetative parts of perilla (*Perilla frutescens* L.) after different growth lengths. **Research Journal of Medicinal Plant** 5(1): 72-78.

Peng, Y.Y., J.N. Ye and J.L. Kong. 2005. Determination of phenolic compounds in *Perilla frutescens* L. by capillary electrophoresis with electrochemical detection. **Journal Agricltural and Food Chemistry** 53: 8141-8147.

Phaichamnan, M., W. Posri and M. Meenune. 2010. Quality profile of palm sugarconcentrate produced in Songkhla province, Southern Thailand. **International Food Research Journal** 17: 425-432.

Phongkanpai, V., S. Benjakul and M. Tanaka. 2006. Effect of pH on antioxidative activity and other characteristics of caramelization products. **Journal of Food Biochemistry** 30: 174-186.

Pokorny, J. 1987. Major factors affecting the autoxidation of lipids, pp. 141-206. *In* H.W.S. Chan, ed. **Autoxidationof Unsaturated Lipids**. Academic Press, London.

Przybylski, R. 2005. Flax oil and high linolenic oils. pp. 281-301. *In* F. Shahidi, eds. **Bailey's industrial oil and fat products**, 6th edition, vol. 2. John Wiley & Sons Inc, New York.

Qian, H. and M. Sheng. 1998. Simultaneous determination of fat-soluble vitamins A, D and E and pro-vitamin D_2 in animal feeds by one-step extraction and high-performance liquid chromatography analysis. **Journal of Chromatogr A.** 825: 127-133.

Razavi, S.M.A., S.A. Mortazavi, L. Matia-Merino, S.H. Hosseini-Parvar, A. Motamedzadegan and E. Khanipour. 2009. Optimisation study of gum extraction from basil seeds (*Ocimum basilicum* L.). **International Journal of Food Science and Technology** 44: 1755-1762.

Reische, D.W., D.A. Lillard and R.R. Eitenmiller. 2002. Antioxidants, pp. 489. *In* C.C. Akoh and D.B. Min, eds. **Food Lipids: Chemistry, Nutrition and Biotechnology**. Marcel Dekker, New York.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radical Biology and Medicine** 26: 1231-1237.

Rhee, C. and D.H. Kim. 1975. Antioxidant activity of acetone extracts obtained from a caramelization-type browning reaction. **Journal of Food Science** 40: 460-462.

Rich, A.L., K.P. West Jr. and R.E. Black. 2004. Vitamin A deficiency, pp. 212-256. *In* M. Ezzati, A.D. Lopez, A. Rodgers and C.J.L. Murray, eds. **Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attributable to Selected Major Risk Factors.** World Health Organization, Geneva, Switzerland.

Rohman, A. and Y.B. Che Man. 2011. Application of gas chromatography and FTIR spectroscopy for anaysis of palm oil in adulterated sesame oil. **European Journal of Lipid Science and Technology** 133: 522–527.

Rufian-Henares, J.A. and F.L. Morales. 2007. Functional properties of melanoidins: in vitro antioxidant, antimicrobial and antihypertensive activities. **Food Research International** 40: 995-1002.

Saeedeh, A. and U. Asna. 2007. Antioxidant properties of various solvent extracts ofmulberry (*Morus indica* L.) leaves. **Food Chemistry** 102: 1233-1240.

Saklani, S., S. Chandra and A.K. Gautam. 2011. Phytochemical investigation and contribution of perilla frutescence as spices in traditional health care system. **International Journal of Pharmacy and Technology** 4(3): 3543-3554.

Sangaroon, T., K. Sringarm and D. Karladee. 2011. Vitamin E in post-harvested grains of purple rice white sesame black sesame and perilla. **Agricultural Science Journal** 42(3): 408-411.

Santosa, H.M.C.D., P.F. Montanherb, B.C.E. Silva, J.S. Boeingb, T. Clausb, M. Matsushitab, J. Vergilio and V. Visentainer. 2012. Antioxidant capacity and phenolic compounds of oil and defatted waste from *Perilla Frutescens*. The 16th World Congress of Food Science & Technology (*IUFoST)*, August 5-9, 2012, Parana state, Barzia.

Sargi, S.C., B.C. Silva, H.M.C. Santos, P.F. Montanher, J.S. Boeing, O.O. Santos Junior, N.E. Souza and J.V. Visentainer. 2013. Antioxidant capacity and chemical composition in seeds rich in omega-3: chia, flax, and perilla**. Food Science and Technology** 33(3): 541-548.

Schmitz, G and J. Ecker. 2008. The opposing effects of n-3 and n-6 fatty acids. **Progress in Lipid Researc**h 47(2): 147–155.

Shahidi, F., C.N. Liyana-Pathirana and D.S. Wall. 2006. Antioxidant activity ofwhite and black sesame seeds and their hull fractions. **Food Chemistry** 99: 478-483.

Sharma, B. D., D.K. Hore and S. Mondal. 1989. Perilla: an oil and protein rich underexploited crop of northeast hill. **Journal of Oilseeds Research** 6:386-389.

Shimada, K., K. Fujikawa, K. Yahara and T. Nakamura. 1992. Antioxidative properties of xanthan on the antioxidation of soy bean oil in cyclodextrin emulsion. **Journal Agricltural and Food Chemistry** 40: 945-948.

Shin, H.S and S.W. Kim. 1994. Lipid composition of Perilla seed. **Journal of the American Oil Chemistry's Society** 71: 619-622.

Shon, M.Y., T.H. Kim and N.J. Sung. 2003. Antioxidants and free radical scavenging activity of *Phellinus baumii* (Phellinus of Hymenochaetaceae) extracts. **Food Chemistry** 82: 593-597.

Siddhuraju, P. and K. Becker. 2007. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) walp.) seed extracts. **Food Chemistry** 101: 10-19.

Simic, M. and K. Taylor. 1987. Free radical mechanisms of oxidation reactions, pp. 69-72. *In* A.J.St. Angelo and M.E. Bailey, eds. **Warmed-Over Flavor of Meat**. Academic Press. New York.

Singh, N. and P.S. Rajini. 2004. Free radical scavenging activity of anaqueous extract of potato peel. **Food Chemistry** 85(4): 611-616.

Singh, R., P.K. Verma and G. Singh. 2012. Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium.* **Journal of Intercultural Ethnopharmacology** 1(2): 101-104.

Siriamornpun, S., D. Li, L. Yang, S. Suttajit and M. Suttajit. 2006. Variation of lipid and fatty acid compositions in Thai Perilla seeds grown at different locations, Songklanakarin. **Journal of Science and Technology** 28: 17-21.

Sizer, F.S. and E. Whitney. 2008. **Nutrition concepts and controversies**, 11th ed. Thomson Wadsworth, United States of America.

Steel, R.G.D. and J.H. Torrie. 1980. Principle and procedure of statistics, 2nd ed. McGraw-Hill, New York.

Tafulo, P.A.R., R.B. Queiros, C.M. Delerue-Matos and M.G.F. Sales. 2010. Control and comparison of antioxidant capacity of beers. **Food Research International** 43: 1702-1709.

Taha, F.S., M. Fahmy and M.A. Sadek. 1987. Low phytate protein concentrates and isolates from sesame seed. **Journal of Agricultural and Food Chemistry** 35: 1289-1292.

Tanaka, M., C.W. Kuie, Y. Nagashima and T. Taguchi. 1988. Applications of antioxidative Maillard reaction products from histidine and glucose to sardine products. **Nippon Suisan Gakkaishi** 54: 1409-1414.

_____, W.K. Chiu, Y. Nagashima and T. Taguchi. 1992. Inhibitory effect of the Maillard reaction products towards lipid oxidation. **Journal of the Tokyo University of Fisheries** 79: 135-141.

Thanonkaew, A., S. Benjakul, W. Visessanguan and E.A. Decker. 2006. The effect of metal ions on lipid oxidation, colour and physicochemical properties of cuttlefish (*Sepia pharaonis*) subjected to multiple freeze-thaw cycles. **Food Chemistry** 95: 591-599.

Traber, M.G. and H. Sies. 1996. Vitamin E in humans: demand and delivery. **Annual Review of Nutrition** 16: 321-437.

Troni, K., S.M. Silva, A.J.A. Meirelles and R. Ceriani. 2013. Study of fatty acid and fatty alcohol formation from hydrolysis of rice bran wax. **Chemical Engineering Transactions** 32: 1747-1752.

Uluata, S. and Ozdemir. 2012. Antioxidant activities and oxidative stabilities of some unconventional oilseeds. **Journal of the Amercian oil Chemists' Society** 89(4): 551-559.

Ulu, H. 2004. Evaluation of three 2-thiobarbituric acid methods for the measurement of lipid oxidation in various meats and meat products. **Meat Science** 67: 683-687.

Vasco, C., J. Ruales and A. Kamal-Eidin. 2008. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. **Food Chemistry** 111: 816-823.

Velasco, J., M.L. Andersen and L.H. Skibsted. 2004. Evaluation of oxidative stability of vegetable oils by monitoring the tendency to radical formation. A comparison of electron spin resonance spectroscopy with the Rancimat method and differential scanning calorimetry. **Food Chemistry** 85(4): 623-632.

Vhangani, L.N. and J. Van Wyk. 2013. Antioxidant activity of Maillard reaction products (MRPs) derived from fructose-lysine and ribose-lysine model systems. **Food Chemistry** 137(1-4): 92-98.

Wabner, D. 2002. The peroxide value-A new tool for the quality control of essential oils. **Internation Journal of Aromatherapy** 12: 216-218.

Wanasundara, P.K.J.P.D., U.N. Wanasundara and F. Shahidi. 1999. Changes in flax (*Linum usitatissimum* L.) seed lipids during germination**. Journal of the American Oil Chemists' Society** 76: 41-48.

Wankhede, D. B. and R.N. Tharanathan. 1976. Sesame (Sesamun Indicum) arbohydrates. **Journal of Agriculture and Food Chemistry** 24: 655-659.

Wasowicz, E., A. Gramza, M. Hes, H.H. Jelen, J. Korczak, M. Matecka, S. Mildner-Szkudlarz1, M, Rudzinska, U, Samotyja and R. Zawirska-Wojtasiak. 2004. Oxidation of lipid in food. **Polish Journal of Food and Nutrition Sciences** 13/54(1): 87-100.

Weiss, E. A. 1983. **Oilseed Crops**. Longman Inc., New York.

Wijewickreme, A. N., D.D. Kitts and T.D. Durance. 1997. Reaction condition influence the elementary composition and metal chelating affinity of nondialyzable model Maillard reaction products. **Journal Agricltural and Food Chemistry** 45: 4577-4583.

_____, Z. Krejpcio and D. D. Kitts. 1999. Hydroxyl scavenging activity of glucose, fructose, and ribose-lysine model Maillard products. **Journal of Food Science** 64(3): 457-461.

Willits, C.O. and C.H. Hills. 1976. **Maple Syrup Producers Manual**. AgriculturalResearch Service, United States Department of Agriculture, Government Printing office, Washington, D.C.

Yamaguchi, T., H. Takamura, T. Matoba and J. Terao. 1998. HPLC methodfor evaluation of the free radical-scavenging activity of foods by using1,1,-diphenyl-2-picrylhydrazyl. **Bioscience Biotechnology and Biochemistry** 62: 1201-1204.

Yanishlieva-Maslarova, N.V. 2001. Inhibiting oxidation, pp. 22-70. *In* J. Pokorny, N. Yanishlieva and M. Gordon, eds. **Antioxidants in Food Practical** Applications. Woodhead Publishing Ltd, Cambridge.

Yen, G.C., P.D. Duh and C.L. Tsai. 1993. The relationship between antioxidant activity and maturity of peanut hulls. **Journal of Agricltural and Food Chemistry** 41: 67-70.

_____, and P.P. Hsieh. 1995. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction-products. **Journal of the Science of Food and Agriculture** 67: 415-420.

Yoo, M.A., H.W. Kim, K.H. Kim and M.H. Kang. 2004. Antioxidant effect of brown substances separated from defatted roasted sesame dregs. **Food Science and Biotechnology** 13: 274-278.

Yoshimura, Y., T. Iijima, T. Watanabe and H. Nakazawa. 1997. Antioxidative effect of Maillard reaction products using glucose-glycine model system. **Journal Agricltural and Food Chemistry** 45: 4106-4109.

Yuanxia, S., H. Shigeru, O. Masahiro and I. Ken. 2007. Antioxidative propertiesof custard pudding dessert containing rare hexose, D-psicose. **Journal of Food Control** 18: 220-227.

Zadernowski, R., H. Nowak-Polakowska and A.A. Rashed. 1999. The influence of heat treatament on the activity of lipo and hydrophilic components of oat grain. **Journal of Food Processing and Preservation** 23(3): 177-191.

Zeng, Y., X. Zhang, Y. Guan and Y. Sun. 2011. Characteristics and antioxidantactivity of Maillard reaction products from psicose-lysine and fructose–lysinemodel systems. **Journal of Food Science** 76: 398-403.

Zou, Y., Y. Lu and D. Wei. 2004. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. in vitro. **Journal of Agricultural and Food Chemistry**, 52, 5032-5039.

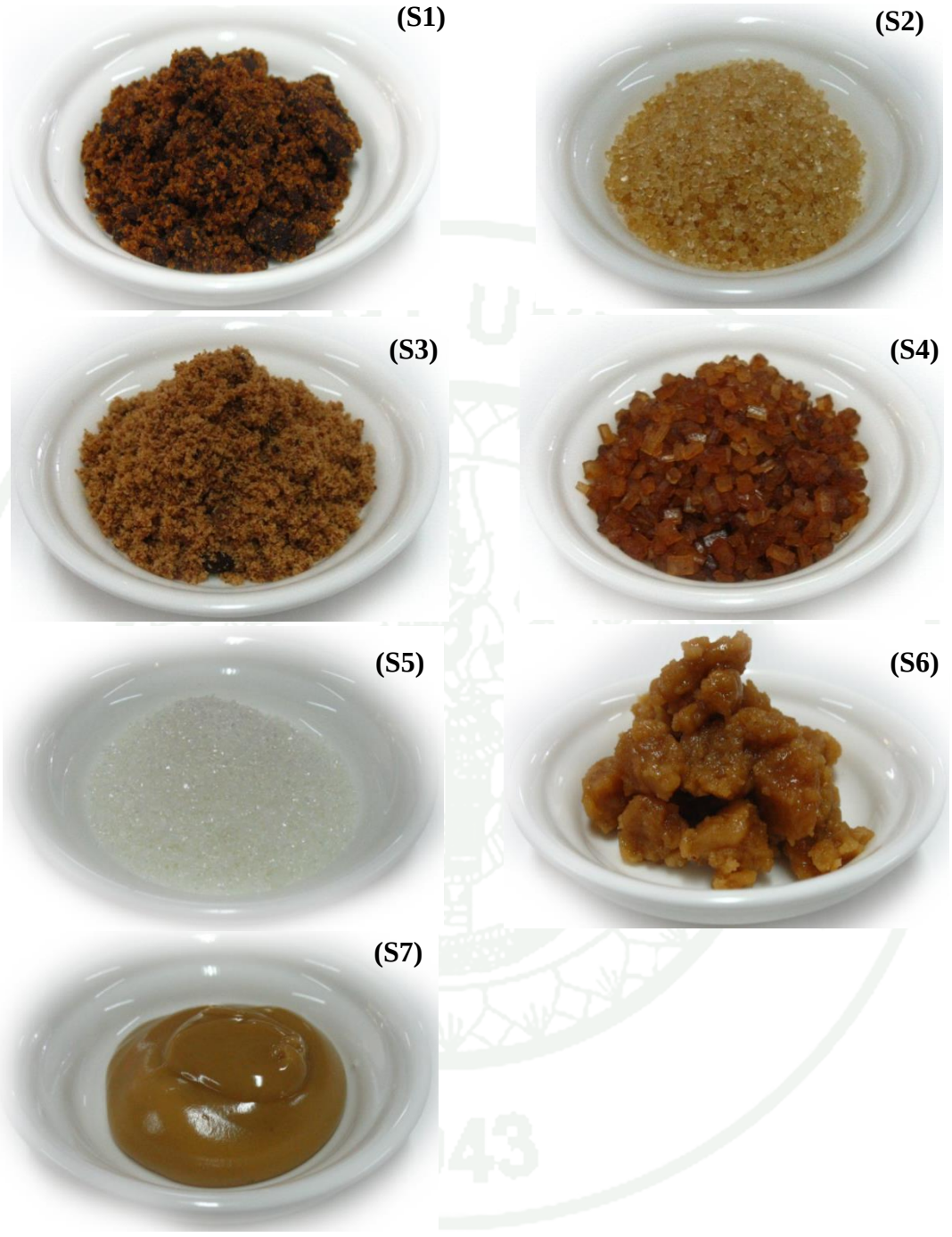
APPENDICES

Appendix A

Illustrations of perilla seed, perilla seed extracts, and ground perilla seed added with different commercial sugars

Appendix Figure A1 Seeds of brown perilla (A) and white perilla (B)

Appendix Figure A2 Extracts from brown perilla seed (A) and white perilla seed (B)



Appendix Figure A3 Seven 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)

Appendix Figure A4 Ground perilla seed added with cane sugar paste (A), brown cane sugar (B), coconut sugar paste (C) and palm sugar paste (D).

Appendix B

Production of sugar-ground perilla seed

Sugar ground perilla seed (nga-tum-aoi) (Chiang Mai University Library, 2007; with a slight modification)

Raw materials

1. 65 g of perilla seed
2. 130 g of sugar
3. 5 g of water

Method

1. Heat perilla seed at 50°C for 10 min.
2. Weigh 65 g of perilla seed and then crush till it is fine.
3. Add 130 g of sugar and 5 g of water into smashed perilla seed. Keep smashing them till the texture is well integrated.
4. Pack 2 g of the mixture with cellophane.
5. Store sample at room temperature.

Appendix C

Analytical Methods

1. Determination of moisture content (AOAC, 2000)

Method

1. Dry moisture can and lid in the hot air oven at 105°C for 3 h and transfer to desiccator to cool. Then weigh.
2. Weigh about 2 g of sample (W_1) to the moisture can and spread the sample to uniformity.
3. Place the moisture can with sample in the hot air oven. Dry at 105°C for 3 h.
4. After drying, transfer the moisture can with partially covered lid to the desiccator to cool. Reweigh the moisture can with dried sample (W_2).

Calculation

Moisture content (%) = $[(W_1-W_2) \times 100] / W_1$

Where:

W_1 = Weight (g) of sample before drying

W_2 = Weight (g) of sample after drying

2. Determination of protein content (AOAC, 2000)

Regents

1. Kjedahl catalyst : K_2SO_4:$CuSO_4.5H_2O$ (9:1) (w:w)
2. Sulfuric acid (H_2SO_4)
3. 40% Sodium hydroxide (NaOH) solution (w/v)
4. 0.1 N Hydrochloric acid (HCl) solution
5. 4% Boric acid (H_3BO_3) solution (w/v)
6. Mixed indicator solution: Mix 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% bromocresol green (in 95% ethanol)

Method

1. Place sample 0.5 g in digestion tube.
2. Add 5 g Kjedahl catalyst and 20 ml of conc. H_2SO_4.
3. Prepare a tube containing the above chemical except sample as blank. Place flask in inclined position and heat gently until frothing ceases. Boil briskly until the solution becomes clear.
4. Cool and add 60 ml of distilled water cautiously.
5. Immediately connect flask to digestion bulb on condenser and with tip of condenser immersed in standardized 0.1 N HCl solution and 5-7 drops of mixed indicator in receiver. Rotate flask to mix content thoroughly; then heat until all NH_3 is distilled.
6. Remove receiver, wash tip of condenser and titrate excess standard acid distilled with standard NaOH solution.

Calculation

Protein content (%) = $[(A-B) \times N \times 14.007 \times 6.25 \times 100] / [W \times 1000]$

Where:

A	=	Volume (ml) of 0.1 N HCl used for sample titration
B	=	Volume (ml) of 0.1 N HCl used for blank titration
N	=	Normality of HCl
W	=	Weight (g) of sample
14.007	=	Atomic weight of nitrogen
6.25	=	The protein-nitrogen conversation factor

3. Determination of fat content (AOAC, 2000)

Reagent

Petroleum ether

Method

1. Place the extraction cup in the hot air oven at 105°C overnight to ensure that weight of the extraction cup is stable (W_1).
2. Weigh about 2 g of sample(W_2) to ashless filter paper.
3. Take the sample into extraction thimble and transfer into a soxhlet apparatus.
4. Fill 50 ml of petroleum ether into the extraction cup and place it on heating mantle.
5. Connect the soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle.
6. Heat the sample about 30 min at 105°C.
7. Evaporate the solvent by using the vacuum condenser.
8. Incubate the extraction cup at 80-90°C until solvent is completely evaporated and extraction cup is completely dry.
9. After drying, transfer the extraction cup to the desiccator to cool. Reweigh the extraction cupwithdried sample content (W_3).

Calculation

Fat content (%) = $[(W_3-W_1) \times 100] / W_2$

Where:

W_1 = Weight of extraction cup

W_2 = Weight of sample before extraction

W_3 = Weight of extraction cup after extraction

4. Determination of ash content (AOAC, 2000)

Method

1. Place the crucible and lid in the furnace at 550°C overnight to ensure that impurities on the surface of crucible are burned off.
2. Cool the crucible in the desiccator (30 min).
3. Weigh the crucible and lid to 3 decimal points (W_1).
4. Weigh about 1 g sample (W_2) into the crucible. Heat with hot plate. When fumes are no longer produced, place crucible and lid in furnace. Heat at 550°C. During the heating, do not cover the lid.
5. Place the lid after complete heating to prevent loss of fluffy ash. Cool down in the desiccator.
6. Weigh the ash with crucible and lid when the sample turns to grey (W_3). If not, return the crucible and lid to the furnace for the further ashing.

Calculation

Ash content (%) = $[(W_3-W_1) \times 100] / W_2$

Where:

W_1 = Weight of crucible

W_2 = Weight of sample before burned

W_3 = Weight of crucible after burned

5. Determination of crude fiber content (AOAC, 2000)

Reagent

1. 1.25% Sulfuric acid (H_2SO_4)
2. 1.25% Sodium hydroxide (NaOH)
3. Anti-foaming reagent

Method

1. Weigh about 1 g of defatted sample (W_1).
2. Digested with 100 ml of 1.25% H_2SO_4 in a beaker under reflux for 30 min.
3. Filter through fritted glass crucible.
4. Residue was then washed with hot distilled water till neutralized.
5. Wash material and transfer to beaker and reflux for 30 min with 100 ml of 1.25 % NaOH.
6. Digested material and wash with hot water until neutralized.
7. Dry material at 130°C for 1 h, cool in a desiccator and weigh (W_2).
8. Ignite residue at 550°C for 6 h and reweigh the crucible with burnt material (ash) (W_3).

Calculation

Crude fiber content (%) = $[(W_2-W_3) \times 100] / W_1$

Where:

W_1 = Weight of sample before digested

W_2 = Weight of sample afterdigested

W_3 = Weight of sample after burned

6.Determination of total carbohydrate content (AOAC, 2000)

Calculation

Total carbohydrate content(%)

=100-(moisture+protein+fat+ash+crude fiber)

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