THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Food Science)

DEGREE

Food Science | Food Science and Technology

FIELD | **DEPARTMENT**

TITLE: Changes in Odor Characteristics of Canned Coconut Milk during Storage

NAME: Miss Patcharaporn Tinchan

THIS THESIS HAS BEEN ACCEPTED BY

(Associate Professor Siree Chaiseri, Ph.D.) **THESIS ADVISOR**

(Professor Keith R. Cadwallader, Ph.D.) **THESIS CO-ADVISOR**

(Assistant Professor Wannee Jirapakkul, Ph.D.) **THESIS CO-ADVISOR**

(Miss Sumitra Boonbumrung, Ph.D.) **THESIS CO-ADVISOR**

(Assistant Professor Wannee Jirapakkul, Ph.D.) **DEPARTMENT HEAD**

APPROVED BY THE GRADUATE SCHOOL ON _____

(Associate Professor Gunjana Theeragool, D.Agr.) **DEAN**

THESIS

CHANGES IN ODOR CHARACTERISTICS OF CANNED COCONUT MILK DURING STORAGE

PATCHARAPORN TINCHAN

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Food Science)
Graduate School, Kasetsart University
2014

Patcharaporn Tinchan 2014: Changes in Odor Characteristics of Canned Coconut Milk during Storage. Doctor of Philosophy (Food Science), Major Field: Food Science, Department of Food Science and Technology. Thesis Advisor: Associate Professor Siree Chaiseri, Ph.D. 209 pages.

The changes of the volatile profiles in the canned coconut milk, stored under the tropical condition (32-35 °C) for 6 months, were investigated in the first experiment. Two stages of major changes in volatile profiles were observed. First, there was the major change in month 2 identified by the increases of alcohols, acids and lactones. The second change was observed in month 5 when lactones and acids extensively increased corresponded with the notification of the coconut-like, sweet, fatty and rancid odors. To gain better understanding of the odor development during the canning process and the storage of canned coconut milk, the second experiment was performed. The aroma characteristics of the fresh coconut milk (FCM), the canned coconut milk immediately after processing (CCM0), the canned coconut milk after 3 months of storage at ambient temperature of 23 °C (CCM3-AT) and those after 3 months of storage at 40 °C (CCM3-40C) were investigated. Thirty-seven odorants were detected in FCM, with the dominance of δ-octalactone (FD = 486), δ-decalactone (405), guaiacol (378), 2-phenylethanol (54), 4-ethylguaiacol (54), methional (45), capric acid (18) and octanal (15). After canning process, 51 odorants were detected in CCM0, and most of them had FD values higher than those in FCM, correlating to the stronger odor of CCM0. Methional (FD =729), 2-actyl-1-pyrroline (27), dimethyl trisulfide (18) and 2-methyl-3-furanthiol (9) associated with the potato, popcorn, sulfury and meaty odors that led to the off-odor of canned coconut milk. The non-terminological odor, described as stale, rancid-green, sweet, fatty and astringency, was detected by the trained panelists in CCM3-AT and CCM3-40C. Storing at 40 °C for 3 months reduced FD values of most odorants comparing to those of CCM3-AT. Indole and skatole were firstly detected in CCM3-40C and could contribute to the off-odor. Quantification of volatile compounds and lipid profiles of coconut milks were also investigated in this study.

_____ _____ __ / __ / ____

Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

It has been a worthy journey of being Ph.D. student in Kasetsart University. I would like to give my deeply sincerely gratitude and grateful thanks to Assoc. Prof. Siree Chaiseri. This thesis would not be accomplished without her support, encouragement, valuable advices and knowledge wisdom. Her patient and invaluable constructive criticism have led me to be improved and be a better person. I am most thankful to Prof. Keith R. Cadwallader for his invaluable support, encouragement, academic advices and for everything he had done helping me. I would like to give a grateful thank to Asst. Prof. Wannee Jirapakul who guided and encouraged me to study in Ph.D. Her kind supports are always helping me to keep focus and not to give up. I would like to thank Dr. Sumitra Boonbumrung for her valuable time, advices and suggestions. My sincere thanks to Asst. Prof. Tanaboon Sajjaanantakul for being chair person, as well as for his incomparable advices he gave me. I would like to express my grateful appreciation to Dr. Kanjana Mahattanatawee for her valuable time and suggestions. I would like to give a grateful thanks to my teachers for their priceless knowledge and support. A special thanks to Dr. Yaowapa Lorjaroenphon, Dr. Inthawoot Suppavorasatit, Miss Pattong Sawadikiat, Dr. Rattakorn Jumnongpon, Miss Mayoonkarn Dechkunchon, Miss Mantana Buttara, Miss Nawaporn Larpsongphon, lab mates, staffs and friends from KU and UIUC, everything they taught and helped me were nothing compared. Our friendship is more than anything I can imagine for. I would like to gratefully acknowledge the RGJ-Ph.D. Program for the scholarship. I am most thankful to Mr. Prachaya Treesutthacheep for his support and encouragment to pursue this degree. Last but not least, I would like to give my deepest love and respect to my father, my mother, my step-father, my brother, my sisters and family members for their unconditioned love and sacrifice. Without the wisdoms, hard works and understanding from them, this dream would not be possible to be true. Thanks to my brother for being the best brother I have never dreamt about. I am most thankful to them, my family who own my deepest love.

Patcharaporn Tinchan

April, 2013

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

AEDA	=	aroma extraction dilution analysis
AF	=	acid fraction
2AP	=	2-acetyl-1-pyrroline
°C	=	degree Celsius
CCM0	=	canned coconut milk after caning process
CCM3-AT	=	canned coconut milk stored for 3 months at ambient temperature
CCM3-40C	=	canned coconut milk stored for 3 months at 40 °C
DDI-H_2O	=	doubled deionized water
DSE	=	direct solvent extraction
FCM	=	fresh coconut milk
FD	=	dilution factor
FFA	=	free fatty acid
g	=	gram
GC-FID	=	gas chromatography-flame ionization detector
GC-MS	=	gas chromatography-mass spectrometry
GC-O	=	gas chromatography-olfactometry
h	=	hour
HCl	=	hydrochloric acid
HS	=	head space
H_2SO_4	=	sulfuric acid
HVD	=	high vacuum distillation
kcal/mole	=	kilocalorie/mole
mg	=	milligram
min	=	minute
mL	=	milliliter
N/B	=	neutral/basic fraction
NaCl	=	sodium chloride
Na_2SO_4	=	sodium sulfate
O_2	=	oxygen

LIST OF ABBREVIATIONS (Continued)

ppb	=	part per billion
ppm	=	part per million
$R^{\cdot}$	=	free radical
RH	=	unsaturated fatty acid
$RO^{\cdot}$	=	alkoxyl radical
$ROO^{\cdot}$	=	peroxy radical
ROOH	=	hydroperoxide
SAFE	=	solvent assisted flavor evaporation
SPME	=	solid phase micro-extraction
µg	=	microgram
µL	=	microliter

CHANGES IN ODOR CHARACTERISTICS OF CANNED COCONUT MILK DURING STORAGE

INTRODUCTION

Coconut milk is an essential ingredient in Asian and Thai cuisines, especially in various kinds of curries and desserts. The flavor of coconut milk is described as coconut-like, creamy, sweet and mild-pleasant. Emerging of Asian and Thai foods worldwide encourages the food manufacturers to deliver more of processed coconut milk. Different kinds of processed coconut milk have been launched including UHT, powdered and canned coconut milk. Among these products, canned coconut milk is the most popular product due to the advantages on shipment, easy usage and long shelf storage.

Previous studies in the literatures have been focused on the emulsion property and compositions of coconut milk. Despite the fact that the odor is one of the criterions on the canned coconut milk quality, studies on coconut milk odor have not been investigated thoroughly. Canned coconut milk has been reported to have a distinctive cooked odor after canning process and a long storage. The difference in the odor perceptions between the fresh and the canned coconut milk is immediately noticed after canning process. The thermal related reactions play the dominant role on the occurrence of the cooked odor and the alteration of canned coconut milk odor. The aroma alteration in canned coconut milk continually occurs during the storage period. The change of aroma leads to the decline of consumer acceptance. To gain further insight of this problem, the odor characteristics of coconut milk were monitored. In this study, the odor characteristics of the fresh, after processed- and stored-canned coconut milks were studied. The odor profile, aroma and volatile compounds were identified. Lipid is considered as one of the most important pools for aroma and volatile generation. The changes of lipid compositions of coconut milks were investigated.

OBJECTIVES

Experiment 1: Changes in volatile profile of canned coconut milk during 6 months of storage

1. To monitor the alteration of volatile profile of canned coconut milk during 6 months of storage at tropical temperature (32-35 °C).

Experiment 2: The Odor characteristics of canned coconut milk

1. To study the odor profile of fresh and canned coconut milk after canning process and canned coconut milk after 3 months of storage at ambient temperature (23 °C) and 40 °C using a consensus descriptive analysis.

2. To identify the aroma and volatile compounds of fresh, canned coconut milk immediately after canning process and canned coconut milk after 3 months of storage at ambient temperature (23 °C) and at 40 °C.

3. To monitor the change of fatty acid compositions in canned coconut milks immediately after canning process and after 3 months of storage at ambient temperature (23 °C) and at 40 °C.

LITERATURE REVIEW

1. Terminology of coconut milk

Coconut milk is one of the most important products of coconut fruit. The scientific name of coconut is *Cocos Nucifera* Linn. in the family of Arecaceae or Palmae. Asian and Pacific Coconut Community (APCC) estimated the total world coconut growth area in 1996 of 110,000 km^3, and around 93% was found in Asian and Pacific region (APCC, 2003). The terminology of coconut milk is easily confused among literatures. The terms "coconut water", "coconut cream" and "coconut milk" can be misinterpreted in different geographical regions. This dissertation uses the definition of coconut milk given by the Asian and Pacific Coconut Community (APCC 1994).

The term "coconut milk" is referred to the aqueous product extracted from the solid coconut endosperm without fiber, and coconut water or potable water can be added. "Coconut water" is referred to the natural aqueous liquid endosperm of the drupe of *Cocos nucifera* L. "Coconut milk" can be distinguished from "coconut cream" by the contents of fats, proteins and water. Coconut milk contains not less than 30% fat and 3% protein, and not more than 55% water, whereas coconut cream contains not less than 50% fat and 5% protein derived from coconut (Seow and Gwee, 1997).

2. Chemical composition of coconut milk

The chemical compositions of coconut milk are varied due to different factors, such as geographical location, the maturity, cultural practice, the method of preparation, extraction and the degree of dilution (Seow and Gwee, 1997). The compositions of coconut milk without any water added from different regions are shown in Table 1. The main compositions of coconut milk are lipids, proteins, carbohydrates and small amount of minerals.

2.1 Lipid

Lipid is the major composition in coconut milk. The lipid content obtained from literatures is in the range of 32.2-40.0% (Table 1), which is higher than the lipid content of processed coconut milk produced in Thailand. The lipid contents of processed coconut milks noted from their nutrition labels are in the range of 16-31% (Table 2). Coconut milk is claimed as an excellent source of saturated fatty acids with more than 90% in coconut oil. The primary saturated fatty acids are lauric acid, myristic acid, palmitic acid and caprylic acid, respectively (Table 3). Monounsaturated fatty acids found in coconut oil are pamitoleic and oleic acid, while polyunsaturated fatty acids are linoleic and linolenic acids.

Table 1 Proximate composition of undiluted whole coconut milk.

Constituent (%)	**Nathaneal (1954)**	**Popper *et al*. (1966)**	**Jeganathan (1970)**	**Anon (1984)**
Moisture	50.0	54.1	50.0	53.9
Lipid	39.8	32.2	40.0	34.7
Protein (N x 6.25)	2.8	4.4	3.0	3.6
Ash	1.2	1.0	1.5	1.2
Carbohydrate	6.2	8.3	5.5	6.6

Source: Seow and Gwee (1970)

The reviews of the acylglycerol composition of coconut oil have been reported, but limited in coconut milk. Because of the same original source of lipids, these reviews are useful to predict the acylglycerol profile. Crude coconut oil contains approximately 95% of triacylglycerol, and the other compositions are free fatty acids, monoacylglycerols, diacylglycerols, phospholipids, free sterols, acylated sterols, tocopherols, tocotrienols and hydrocarbons. For triacylglycerol, tri-saturated triacylglycerols possesses 84% in total, follows by di-saturated triacylglycerols (12%) and mono-saturated triacylglycerol (4%), respectively (O'Brien, 2009). The refining

and bleaching processes are able to remove phospholipids, color bodies, metals, ions, phosphoric acid, free fatty acid and volatile components from crude coconut oil (Amri, 2011). Pham *et al.* (1998) determined the contents of acylglycerol and fatty acid compositions of coconut oil using the silica gel impregnated with 5% boric acid thin layer chromatography. They reported that triacylglycerols had the major abundance of 85.36%, followed by diacylglycerols, complex lipids and monoacylglycerols of 6.55%, 4.62% and 3.45%, respectively. Many studies have reported the carbon atom of triacylglycerol in coconut oil with wide ranges of 22-54 (Kuksis, *et al*., 1964); 28-52 (Bezard *et al*., 1971); 30-54 (Pham *et al.*, 1998) and 30-44 (Laureles, *et al*., 2002). In agreement of all studies, triacylglycerols with carbon numbers of 32, 34, 36, 38, 40 and 42 are found in major abundance. Lauric acid is the major acylglycerol fatty acid that contributes 50% of total triacylglycerol fatty acids, and distributes mainly in the *sn*-1,3 position (Pham *et al.*, 1998). Mono- and di-acylglycerols are minor lipids found in coconut oil. Their contents vary depending on plant materials, processing, storage and the age of oil. Dayrit *et al*. (2008) used ^{31}P NMR spectroscopy technique to identify mono- and di-acylglycerols in the refined coconut oil and the virgin coconut oil. Monoacylglycerols were found in small amounts of 0.000-0.052% (w/w), whereas 1,2-diacylglycerols and 1,3-diacylglycerols were found at the level of 0.807-3.107% and 0.000-2.712% (w/w), respectively. The refined coconut oil has lower content of monoacylglycerols, but higher content of diacylglycerols than those in the virgin coconut oil.

Sterols have been found in a small amount at the average of 0.015% (w/w) in the refined coconut oil and 0.096% (w/w) in the virgin coconut oil (Dayrit *et al*., 2008).

Coconut oil is also claimed as a rich source of glycerol with 13.9% of total fat, and has low cholesterol level of 5-24 ppm (Kumar *et al*., 2006). Many aspects of health concerns in coconut oil have been debated. For example, high content of short and medium chain saturated fatty acid is preferable for body digestion and fast source of energy (Kumar *et al*., 2006). However, some studies have pointed out that the high level of saturated fatty acids could lead to the higher blood

cholesterol levels that associated with the cardiovascular diseases (El-Anany and Ali, 2012). In this work, the issue of health effect will not be discussed in detail. Both benefits and drawbacks of coconut oil and their related products have to be carefully studied and interpreted the data.

Table 2 Fat content (%, w/w) of UHT and canned coconut milk manufactured in Thailand (data from the labels).

Product	Brand	%Fat content
UHT coconut milk (100% coconut milk)	Aroy-D	22
UHT coconut milk (100% coconut milk)	Chaokoh	18
Canned coconut milk (100% coconut milk)	Chef's Choice	20
Canned coconut milk (100% coconut milk)	Chaokoh	17
Canned coconut cream (100% coconut milk)	Chaokoh	22
Canned coconut milk (100% coconut milk)	Maeploy	20
Canned coconut milk (100% coconut milk)	TCC	17
Canned coconut milk (100% coconut milk)	Aroy-D	26
Canned coconut milk (100% coconut milk)	Savoy	31
Canned coconut milk (100% coconut milk)	Thai Kitchen	17

2.2 Proteins

Proteins are natural emulsifiers in coconut milk that play a role on the emulsion stability. It has been considered as the crucial compositions, although they are presented only 3-4% in the undiluted coconut milk (Table 1). According to many studies, the difference in protein content has been influenced by the nature of coconut and the processing. Balasubramaniam and Sihotang (1979) have reported that 90% of proteins in coconut milk are in the aqueous phase after applying the centrifugal force. The other 10% of proteins is found in the layer of cream and in the small pellets in the bottom layer. Another study of coconut press cake after coconut milk extraction pointed out that only 46% of protein was in coconut milk, while 54% were left in the

coconut press cake. Of this coconut milk protein, 65% was in the aqueous layer (Chambal *et al*., 2012).

In coconut meal, globulin is the major protein that accounted for 61.9% followed by albumins (30.6%), glutelins (4.7%) and prolamines (1.1%; Samson *et al*., 1971). Tangsuphoom and Coupland (2008a) reported that the storage 11S globulin named, cocosin, had the highest abundance in coconut meat. Cocosin is a hexamer of the 55 kDa subunit globulin. The slightly different contents of proteins were reported in the defatted coconut meal. Globulins (40.1%) and albumins (21.0%) with molecular weights ranged from 22 to more than 100 kDa were predominant in the defatted coconut meal. Meanwhile, acetic acid soluble-glutelins, NaOH soluble-glutelins and prolamines were found in minor concentrations of 14.4%, 4.8% and 3.3%, respectively (Kwon *et al.*, 1996). The difference in the recovery of proteins in the coconut meal and the defatted coconut meal was influenced by the presence of lipids. Balasubramaniam and Sihotang (1979) have noticed that phospholipid enhanced the protein solubility in skimmed coconut milk.

Chemical and functional properties of proteins in coconut milk and their related products have been broadly studied in order to gain higher yields and stabilize the emulsion. Those properties will not be discussed extensively in this review. Only the important functional properties that related to the volatile extraction and the deterioration of coconut milk will be discussed.

Protein characterization is obtained by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Kwon *et al*. (1996) has reported the protein composition of coconut flour. Fractions of globulins, albumins, prolamines, acetic and acid-soluble-glutelins were extracted from the defatted coconut flour. Albumin and globulin were the predominant fractions. The polypeptides in coconut flour were linked by one or more disulfide bonds. The molecular weight of proteins in coconut flour ranged from 14 to 66 kDa. In the presence of β-mercaptoethahol, the major polypeptides in the reduced form of albumin, had the molecular weights of 52, 28 and 18 kDa, whereas polypeptides in the globulin fractions were dominant with

proteins that had the molecular weights of 61 and 44 kDa. The polypeptides of acetic acid-soluble-glutelins had the similar pattern to globulin, but with more disulfide linkages than albumin.

Table 3 Fatty acid profile of coconut oil.

Fatty acid	Fatty acid content (%)			
	Jayadas and Nair (2006)	Kumar *et al.* (2006)	Kumar (2011)	El-Anany and Ali (2012)
C6:0 (Caproic)	-	0.2-1	0.08-0.49	-
C8:0 (Caprylic)	9.5	3-8	2.77-7.21	5.12
C10:0 (Capric)	4.5	3-7	3.46-5.94	4.30
C12:0 (Lauric)	51	41-53	42.42-52.52	42.9
C14:0 (Myristic)	18.5	16-22	18.12-23.05	20.20
C16:0 (Palmitic)	7.5	7-11	7.59-12.99	10.76
C16:1 (Pamitoleic)	-	0.019-0.4	0.05-3.90	-
C18:0 (Stearic)	3.0	2-4	2.45-4.07	3.00
C18:1 (Oleic)	5.0	5-9	4.92-10.86	9.50
C18:2 (Linoleic)	1.0	1-4	0.14-2.80	4.22
C18:3 (Linolenic)	-	-	0.05-0.14	-
C20:0 (Arachidic)	-	0.01-0.50	0.02-0.31	-
C22:0 (Behenic)	-	0.02-0.12	0.02-0.19	-
C24:0 (Lignoceric)	-	0.01-1.5	0.02-1.36	-

Source: Adapted from Jayadas and Nair (2006), Kumar *et al.* (2006), Kumar (2011) and El-Anany and Ali (2012)

Amino acids in cream and coconut skimmed milk layers were similar (Table 4; Gunetileke and laurentius, 1974). The predominant amino acids were glutamic acid, arginine, and aspartic acid. The limited amino acids were methionine, cystine and tryptophan with the lower concentrations (Gunetileke and Laurentius,

1974). The amino acid profiles of globulin and albumin were slightly different. Phenylalanine, valine and aspartic acid in the globulin fraction were found in higher contents than in those of the albumin fraction (Table 4). The difference in amino acids influences the emulsifying properties of proteins. Kwon *et al.* (1996) reported the hydrophobicity of protein fractions that could contributed to the emulsion stability. Albumins have more proportion of the polar side chain than globulins implying less hydrophobicity property. Seow and Gwee (1997) stated that only 30% of proteins in the filtered coconut milk were dissolved in the aqueous phase. The undissolved proteins gathered surrounding the oil globules and acted as emulsifiers.

One of the factors that affect the solubility of protein is the pH of food system. The pH of coconut milk is about 6.2 (Waisundara *et al*., 2007). The protein solubilities at different pH in the aqueous phase were observed by Samson *et al*. (1971) and (Balasubramaniam and Sihotang (1979). Coconut proteins showed lower solubility at pH 2.5-6.5. Hence, the higher solubility was observed when the pH was lower than 2.5 and greater than 7.0. The least point of protein solubility was at pH 3.9 (Samson *et al*., 1971) and 5.8 (Balasubramaniam and Sihotang, 1979). The maximum solubility was at pH 10.3-10.5. At the natural pH of coconut milk, proteins have low solubility in the aqueous phase. Proteins locate at the surface of oil droplets instead of the aqueous phase resulting in the emulsifying property. The solubility of proteins in the aqueous phase is affected by many factors. The average hydrophobicity and the charge frequency play the dominant role on protein solubility. However, the nature of protein surface and the thermodynamic of their interaction with the surrounding solvent have the most influence (Damodaran, 1991).

Enzyme activity is the major concern in the fresh and the processed coconut milk. Inactivation of the enzymes is crucial to maintain chemical property, appearance and odor profile of coconut milk. The effect of pH and protein solubility on enzymatic activity has been reported. Two factors responsible for enzymatic activity when pH has changed are (1) change in protein structure leading to denaturation and (2) quality of electrostatic charge on enzyme active site (Belitz *et al*., 2009). At pH 6.3, lipase, extracted from coconut meat, had no enzymatic activity

measured by the colorimetric method and titration of fatty acid released (Balasubramaniam and Sihotang, 1979). High lipase activity was obtained at pH 10.3 that was the maximum pH of protein solubility (Balasubramaniam and Sihotang, 1979). On contrary, the optimum pH of lipase in olive oil is range of 5-8 (Belitz *et al*., 2009). The different in food matrix could affect the optimum pH for enzyme activity. In addition to lipase, minor enzymes; amylase, dehydrogenase, acidphosphatase, pyrophosphatase and phospholipase were also presented in the aqueous phase of coconut milk that had been adjusted the pH to 6.3 and 10.3 (Balasubramaniam and Sihotang, 1979). Up to now, two most discussed enzymes in coconut meat are lipase and lipoxygenase.

Lipase (glycerol ester hydrolase) serves the function of hydrolysis. Ester bond of triacylglycerol is hydrolyzed by lipase yielding free fatty acid and glycerol. Lipase activity is dominant at the lipid–water interface and shows less activity in aqueous solutions (Menzel and Schreier, 2007). The hypothetical model for the alignment of lipase on the water-interface has proposed by Belitz *et al*. (2009). The hydrophobic head of lipase is bound to the oil droplet by the hydrophobic interaction, and the active site is aligned with the hydrophobic head. Lipase activity increases with the maturity of fruit, thus, increasing the free fatty acid content in fruit (Waisundara *et al*., 2007). The optimum pH for lipase activity is at pH 5-8 (Belitz *et al*., 2009). In flavor science, lipase has been used for flavor biosynthesis. The chemical reactions involving in lipase catalyzed flavor synthesis are esterification, transesterification, interesterification and transfer of acyl groups from esters to other nucleophiles, such as amines or thiols (Menzel and Schreier, 2007).

Lipoxygenase (linoleic acid oxygen oxidoreductase) catalyzes the oxidation reaction of unsaturated fatty acids to their corresponding monohydroperoxides. There are 2 types of lipoxygenase in plant, type I and II (deMan, 1999). Type I lipoxygenase reacts only with free fatty acid with a high stereo- and region-selectivity. Only free fatty acid that has been hydrolyzed by lipase can be a substrate of type I lipoxygenase. Type II lipoxygenase directly reacts to triacylglycerol and acts as a general catalyst for autoxidation, due to its less specific for linoleic acid.

Table 4 Amino acid composition of the protein fractions from defatted coconut meal (g/100 g of protein), cream and skim coconut milk layer (g/16 g nitrogen).

Amino acid	Coconut cream layer (g/16 g N)	Coconut skim milk layer (g/16 g N)	Coconut flour (g/100g protein)	Albumin (g/100g protein)	Globulin (g/100g protein)
	Gunetileke and Laurentius (1974)		Kwon *et al.* (1996)		
Isoleucine	5.2	4.3	4.2	2.8	4.1
Leucine	9.7	8.9	7.4	3.9	6.5
Lysine	5.7	5.0	4.7	5.1	3.5
Methionine	1.8	1.9	1.8	1.2	2.9
Phenylalanine	8.7	6.8	5.1	2.7	5.9
Threonine	4.2	4.1	2.5	3.3	3.3
Valine	8.0	4.4	5.4	3.5	7.5
Histidine	-	-	1.8	1.8	1.9
Tyrosine	3.5	3.1	1.8	3.0	3.7
Aspartic acid	12.7	10.7	9.3	5.6	8.9
Proline	4.0	4.5	3.6	2.7	3.4
Serine	5.8	5.8	5.3	3.1	5
Glutamic acid	24.8	22.8	22.4	24.9	17.5
Glycine	3.5	3.1	5.1	4.0	4.9
Alanine	6.0	5.9	4.8	2.9	4.1
Arginine	14.7	15.6	12.3	17.9	15.0
Cystine	0.9	1.7	-	-	-
Tryptophan	1.4	1.2	-	-	-

Source: Adapted from Kwon *et al.* (1996) and Gunetileke and Laurentius (1974)

Lipoxygenase oxidizes only fatty acids that contain a 1-*cis*,4-*cis*-pentadiene. Therefore, the preferred substrates are linoleic, linolenic and arachidonic acids. Thus,

oleic acid is not oxidized by type II lipoxygenase (Belitz *et al*., 2009). The optimum pH for lipoxygenase activity is about 9 (deMan, 1999). The action of lipoxygenase also leads to aroma formation. 1-Octen-3-ol that gives the mushroom odor is a product of lipoxygenase from *Agaricus Sp*. (Konar, 2000). Off-odor compounds in legumes; 2-*n*-pentylfuran, 3-*cis*-hexenal 2,6-nonadienal, 2,4-heptadienal, 3,5-octadien-2-one and 2,4,6-nonatrienal, are the products via actions of lipoxygenase (Sessa, 1979).

2.3 Carbohydrates and other components

Carbohydrates in undiluted coconut milk range from 5-8% (Table 1). The most contributions of carbohydrates in coconut milk are to sweeten and being the precursors of Maillard reactions. The sweetness of coconut meat is largely contributed to sucrose (Jayalekshmy and Mathew, 1990). In coconut water, glucose and fructose have the highest abundance, while sucrose is identified as the major sugar in coconut meat. Increasing in sucrose content in coconut water is associated with coconut maturity. On contrary, sucrose content in coconut meat decreased as the mature state progressed (Santoso *et al*., 1996). Total available carbohydrates, total sugars, reducing sugars, starches and dextrins of dry coconut meat are 11.7, 8.32, 0.82 and 3.36%, respectively (Jayalekshmy and Mathew, 1990). Ribose, rhamnose, fructose, glucose, galactose and sucrose are also identified in coconut meat. Roasting at 130-165 °C resulted in a noticeable reduction in the total available carbohydrates, total sugars, reducing sugars, starches and dextrins.

Mineral content in the undiluted coconut milk is about 1-2% (Table 1). In mature coconut water and mature coconut meat, potassium is claimed to be the highest abundance, followed by phosphorus and magnesium. The other minor minerals are calcium, sodium, sulfur, manganese, iron, zinc, copper, boron and aluminum (Santoso *et al*., 1996). According to Seow and Gwee (1997), the major minerals founded in raw coconut milk are phosphorus, calcium and potassium. Vitamin B1, vitamin B2, vitamin B3, vitamin C, niacin, α-, β- and γ-tocopherol were identified in the mature coconut meat (Santoso *et al*., 1996). Vitamin B and vitamin C

were founded in small amount in the freshly extracted coconut milk (Seow and Gwee, 1997).

3. Coconut milk emulsion

Coconut milk is a protein-oil-water emulsion that has white and opaque characteristic. The effective droplet diameter (d_{43}) of the fresh coconut milk is 13.1±2 μm (Tangsuphoom and Coupland, 2005). The emulsion of coconut milk is naturally stabilized by proteins (globulins and albumins) and phospholipids (lecithin and cephalin; Salunkhe *et al*., 1992) that can be adsorbed at the oil-water emulsion interface (Cancel, 1970). However, coconut milk emulsion is not physically stable due to their large oil droplet size and low content of natural proteins. It starts to separate into a coconut cream (upper part) and a coconut skimmed milk or serum (lower part) within 5 to 10 h after the production and completely separates within 24 h (Tangsuphoom and Coupland, 2005). However, it can be re-emulsified by shaking. Many published papers have studied about how to stabilize the coconut milk emulsion. The factors that affect the stability of processed coconut milk emulsion are pH, fat content, homogenization, type and amount of stabilizing agents, and thermal process conditions. Flocculation, not the coalescence of oil droplets, is observed at the pH value of 3.5 to 4, that are the isoelectric points (pI) of coconut proteins (Tangsuphoom and Coupland, 2008a; Ariyaprakai *et al*., 2013). Micrograph of oil droplet and protein dispersion at pH 4 indicated that protein appeared to clump together when the attaching of oil droplets occurred (Tangsuphoom and Coupland, 2008a).

Fat content has a significant effect on the rheological property of coconut milk. Simuang *et al*. (2004) has reported that increasing in fat content caused the presence of larger number of fat globules. These further increased the resistance to flow of coconut milk and the apparent viscosity of the emulsion system.

Homogenization of coconut milk slightly increases the stability of emulsion by reducing the mean diameter of the droplets, but the fine droplets quickly flocculate,

due to the limited protein content. Thus, homogenization had no significant effect on the effective size of coconut oil droplets (Tangsuphoom and Coupland, 2005). Nevertheless, the high shear forces during homogenization can reduce the droplet sizes, especially at higher homogenizing pressures. Reduction in the fat particle diameters results in an increase of consistency coefficient value (k), and thus improved the product stability (Chiewchan *et al*., 2006).

Heating causes the denaturation of the interface adsorbed proteins and the conformation alteration occurs. The conformational changes increase the attractive forces between fat droplets leading to the aggregation (Jirapeangtong *et al*., 2008). The denaturation temperature of the heat labile protein in coconut is 80 °C. Heating at temperatures over 80 °C, therefore, causes coagulation (Seow and Gwee, 1997). Many researchers have suggested that the cooperation of homogenization and addition of the stabilizing agents help improve the stability of coconut milk emulsion than when using only one approach. The emulsifying and stabilizing agents reduce the interfacial tension between two phases. Therefore, fat globules are able to disperse throughout the water phase (Chiewchan *et al*., 2006; Tangsuphoom and Coupland, 2009). Many stabilizing agents have been introduced to increase the emulsion stability, for example, sodium caseinate, whey protein isolate, sodium dodecyl sulfate and polyoxyethylene sorbitan monolaurate (Tween 20; Tangsuphoom and Coupland, 2009) carboxymethyl cellulose and Montanox 60 with sugar added (Jirapeangtong *et al*., 2008). The stability of coconut milk emulsion is denoted by the type of the emulsifying agent. Tangsuphoom and Coupland (2009) has suggested that the small-molecule surfactants had better stability against heating than the bigger ones, but it was not prevent the coalescence in the freeze–thaw sample due to their thin interfacial layer. The type of the surface-active stabilizer should be carefully selected to get the better emulsion stability of coconut milk products.

4. Thermal processes of coconut milk

The fresh coconut fruit in its shell can be stored for 1-2 months at the temperature of 0-15 °C at the relative humidity of 75% or less (Gundberg, 2008). It is

known that thermal processes, including pasteurization, UHT, drying and canning process, can prolong the shelf-life of coconut milk. Pasteurization at 72 °C for 20 min can prolong the shelf-life of coconut milk up to 5 days at 4 °C. For canning process, F_0 of 5 min at 121 °C is normally obtained to ensure commercial sterility of the canned coconut milk (Seow and Gwee, 1997). During preparation and extraction, coconut kernel is treated with several treatments, including washing, defrosting, bleaching, grading, pressing, homogenizing, preheating, exhausting. During the long time of those steps, many possible physical and chemical alterations occur. For industry, coconut meat itself has to be pretreated and stored. Coconut meat is normally stored before the milk extraction. The whole coconut fruit or dehusked coconut meat can either be stored. Blanching is applied to inactivate enzyme and raise the yield of extracted coconut milk. Even though coconut meat is stored for 8 weeks, blanching at 85, 90 and 95 °C raise the yield of the extracted coconut milk. As mentioned, blanching lowers lipase activity and lower free fatty acid content during storage (Waisundara *et al*., 2007). Nonetheless, the addition of water at 30°C and 90 °C to dilute coconut milk has no impact on the coconut milk composition profile (Cancel 1979).

The flocculation of oil droplet in coconut milk had been observed in freshly prepared or low-thermal processed coconut milk. Applying thermal process and the long period of storage lead the problem of coagulation of oil droplets that were evidenced in many researches focusing on the emulsion stability of coconut milk. The coagulation problem has more impact in the undiluted coconut milk than the diluted milk (Seow and Gwee, 1997). As mentioned above, the combination of homogenization and stabilizing agent helps solving this problem. Nonetheless, pre-heating is applied before the retorting process. The coagulation is not observed when the temperature is below 75 °C in the solvent system (Hagenmaire *et al*., 1973). Conversely, heating over 80 °C consequently leads to the denaturation of the heat-labile proteins that will then coagulate (Cancel 1979). Tangsuphoom and Coupland (2009) have noted that the homogenized coconut milk flocculated and then being coalescence when heating at 90 and 120 °C for 1 h. Addition of whey protein isolate prevents emulsion destabilization when coconut milk is freeze-thawed at -20 °C and

heated up to 120 °C. However, Seow and Gwee (1997) have noted that the pre-heating at 90-95 °C for several minutes before filtration and retorting process produced more smooth and stable emulsion. Agitating retort can be the other option to produce canned coconut milk with more acceptable response.

5. Odor of coconut milk

5.1 Volatile and odor compounds in coconut milk and isolation techniques

The volatile compounds of coconut meat and coconut milk were studied. δ-Octalactone, δ-decalactones and *n*-octanol are the major volatile compounds in coconut meat extracted by distillation approach. In addition, 2-heptanol, hexanol, 2-nonanone, 2-octanol, 2-nonanol, octanol, 2-undecanone, ethyldecanoate, 2-undecanol, 2-phenyl ethanol, benzothiazole, δ-undecalactone and lauric acid are also found in coconut meat (Lin and Wilkens, 1970). Headspace aroma compounds of coconut meat and coconut water from Cameroon were isolated by solid phase microextraction (SPME). The compounds founds in the headspace of the coconut meat and coconut water were 3-methylbutanal, butanol, 2-pentanone, 2-pentanol, 3-methyl butanol, pentanol, 2-hexanone, hexanal, hexanol, heptanol, 2-heptanol, octanal, nonanal, limonene, octanol, 2-nonanol, δ-hexalactone, nonanol, caprylic acid, ethyloctanoate, decanal, δ-octalactone, decanol, nonanoic acid, undecanal, undecanol, capric acid, δ-decalactone, δ-dodecalactone (Jirovetz *et al*., 2003). In 1984, Saittagaroon *et al*. (1984) studied the effect of roasting on volatile compounds of the shredded coconut meat. Fifteen compounds have been positively identified in the unroasted-shredded coconut meat in vacuum-package. These include δ-octalactone, δ-decalactone, δ-dodecalactone, ethyl acetate, ethyl octanoate, ethyl-5-hydroxy-octanoate, ethyl decanoate, ethyl-5-hydroxy-decanoate, ethyl dodecanaote, ethyl tetradecanoate, 2-undecanone, 2-tridecanone, 2-methylpyridine, benzothiazole and furfuryl alcohol. After roasting at 160 °C for 35 min, ethylpyrazine, 2,5-dimethylpyrazine, 2-methyl-5-ethylpyrazine, 2,6-diethylpyrazine, 2,6-diethyl-3-methylpyrazine, 2-acetylpyrrole, 5-methyl-2-formylpyrrole, dimethyl furan and 5-methylfurfural were formed. These compounds have been hypothesized as Maillard reaction products (Saittagaroon *et al*.,

1984). The effecting of heating temperature on the volatile and aroma compounds of coconut milk was studied by Silanoi (2004). Heating at the temperature 0f 80, 90 and 100 °C brought coconut milk with more strong coconut, sweet, creamy and cooked notes than fresh coconut milk. The 49 volatile compounds were detected by Silaoi (2004), which esters, acids, alcohols, lactones, aldehydes, ketones, phenol, thiazoles and sulfides were identified The less number of volatile compounds was detected in fresh coconut milk. The volatile and aroma compounds generated upon heating at 80, 90 and 100 °C for 20 min. The aroma compounds which had increased in their dilution factor (FD) values factor were heptanal, *(E)*-2-hexenal, 2-acetyl-1-pyrroline, 2,3-dimethylpyrazine, dimethyl trisulfide, methyl octanoate, methyldodecanoate, benzothiazole, nonanal, 1,3-butanediol, 2-acetylthiazole, γ-hexalactone, δ-nonalactone, γ-decalactone δ-decalactone, δ-undecalactone, (Z)-6-γ-dodecenolactone and δ-dodecalactone (Silanoi, 2004).

In this work, many extraction techniques would be applied to isolate the volatile compounds, including solid-phase microextraction (SPME), direct solvent extraction (DSE), high vacuum distillation (HVD) and solvent assisted flavor evaporation (SAFE). SAFE was firstly introduced by Engel *et al*. (1999). It was developed to meet 3 demands, including (1) compounds contributing to a certain flavor should not be discriminated, (2) the conditions applied should not alter the structure of key aroma compounds and (3) non-volatile compounds which might interfere with the gas chromatographic separation should be completely removed (Engel *et al*., 1999). This technique has advantages for volatile isolation from the emulsion system in coconut milk. Volatile compounds should be separated from the non-volatile compounds with fewer artifacts. High pressure applied to the system helps to isolate volatile compounds with less structural changes. Figure 1 shows the schematic view and equipment of SAFE.

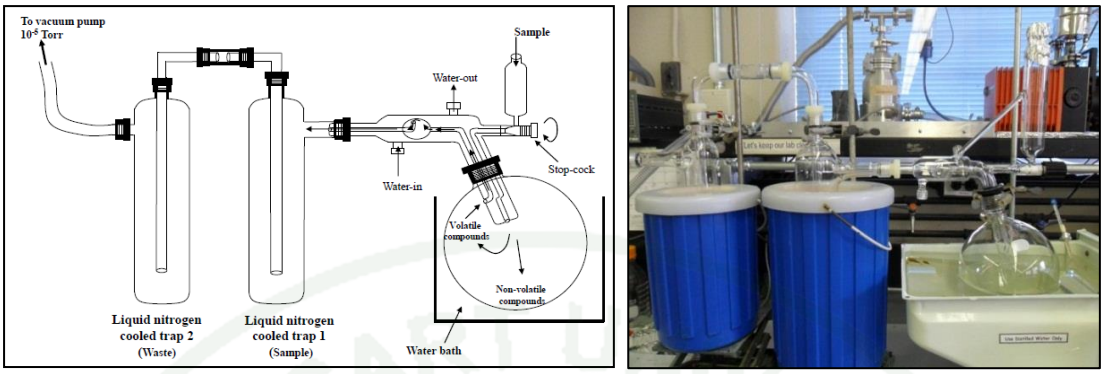


Figure 1 Schematic view (left) and the adapted SAFE apparatus used (right).

Source: Adapted from Engel *et al*. (1999)

5.2 Lactone and enantiomeric property

Lactones are important aroma compounds in coconut and its related products. Lactones represent sweet, fruity and coconut-like aroma. Moreover, lactones are also crucial aroma compounds related to unique fruity and sweet odors in many food products, such as butter milk (Kinsella *et al*., 1967) and wine (Luan *et al*., 2006). The most stable structures of lactone are δ- and γ-lactones (Figure 2).

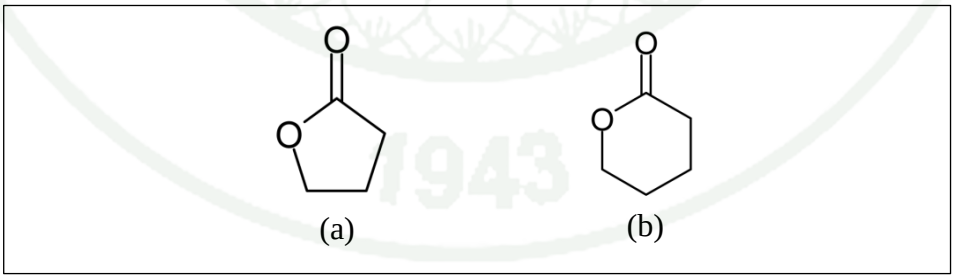


Figure 2 Chemical structure of γ-lactone (a) and δ-lactone (b).

The biogenesis pathway of lactone in plant is complex. Schwab (2000) has mentioned 6 possible biosynthesis pathways of lactones in plants and microorganism, including 1) reduction of keto-acid by NAD-linked reductase, 2) hydration of unsaturated fatty acid, 3) from hydroperoxides, 4) from fatty acid epoxides, 5) from

naturally occurring hydroxy fatty acids, and 6) cleavage of long chain fatty acids. During processing, fatty acids are released upon heating and go further to oxidation reaction to form hydroxy fatty aci as precursors of lactones as shown in Figure 3 (Fisher and Scott, 1997; Reineccius, 2006). This is the major formation pathway of δ- and γ-lactones in butter fat (Kinsella *et al*., 1967). Lactones can be produced by microorganism action in the fermented alcoholic beverage (Reineccius, 2006). In this case, amino acids are precursors of lactones (Figure 4). According to pathway A (Figure 4), the oxidation deamination of glutamic acid yields 2-oxoglutarate, and goes further to be decarboxylated producing 4-oxobutyrate. 4-Oxobutyrate is reduced to 4-hydroxy butyrate. The cyclization of 4-hydroxy butyrate results in the formation of γ-butyrolactone, alkoxy- and acyl-lactones. For pathway B (Figure 4), the 2-oxobutyric acid is converted into 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone that causes the burnt aroma in aged sake (Reineccius, 2006).

The enatiomeric property of aroma compound is very important. The different enantiomer form makes the difference in aroma quality. Enatiomeric ratios also have an impact on the odor characteristic of the aroma compound. Since lactones have been dominant compounds in coconut products, the enatiomeric ratios of lactones have been cited. (R)-δ-oclactone has distinctly coconut-like, while (S)-δ-oclactone has more-fatty and less-intensive coconut notes than its racemate (Nago and Matsumoto, 1993). (R)-δ-decactone has heavier, more lactonic and floral notes than in the (S)-enantiomeric form (Mosandl *et al*., 1992).

Ratios of enatiomeric aroma are characterized by origin-biogenetic pathways, and normally are catalyzed by enzymes (Mosandl, 2007). Percentages of each enatiomeric ratio of lactones in fresh coconut meat, fresh coconut water and canned coconut milk are showed in Table 5. Chiral stationary phase has been used to separate enantiomers in chromatography analysis. Derivertized cyclodextrin have been used as a stationary phase. Bicchi and coworkers (1999) published a great review of research using cyclodextrin derivatives as chiral phase column. They have mentioned the advantages of cyclodextrin derivatives phase with high enatioseletivity,

high stability and high productivity. This phase also has a potential to separate more than 90% racemates of isomers without derivatization.

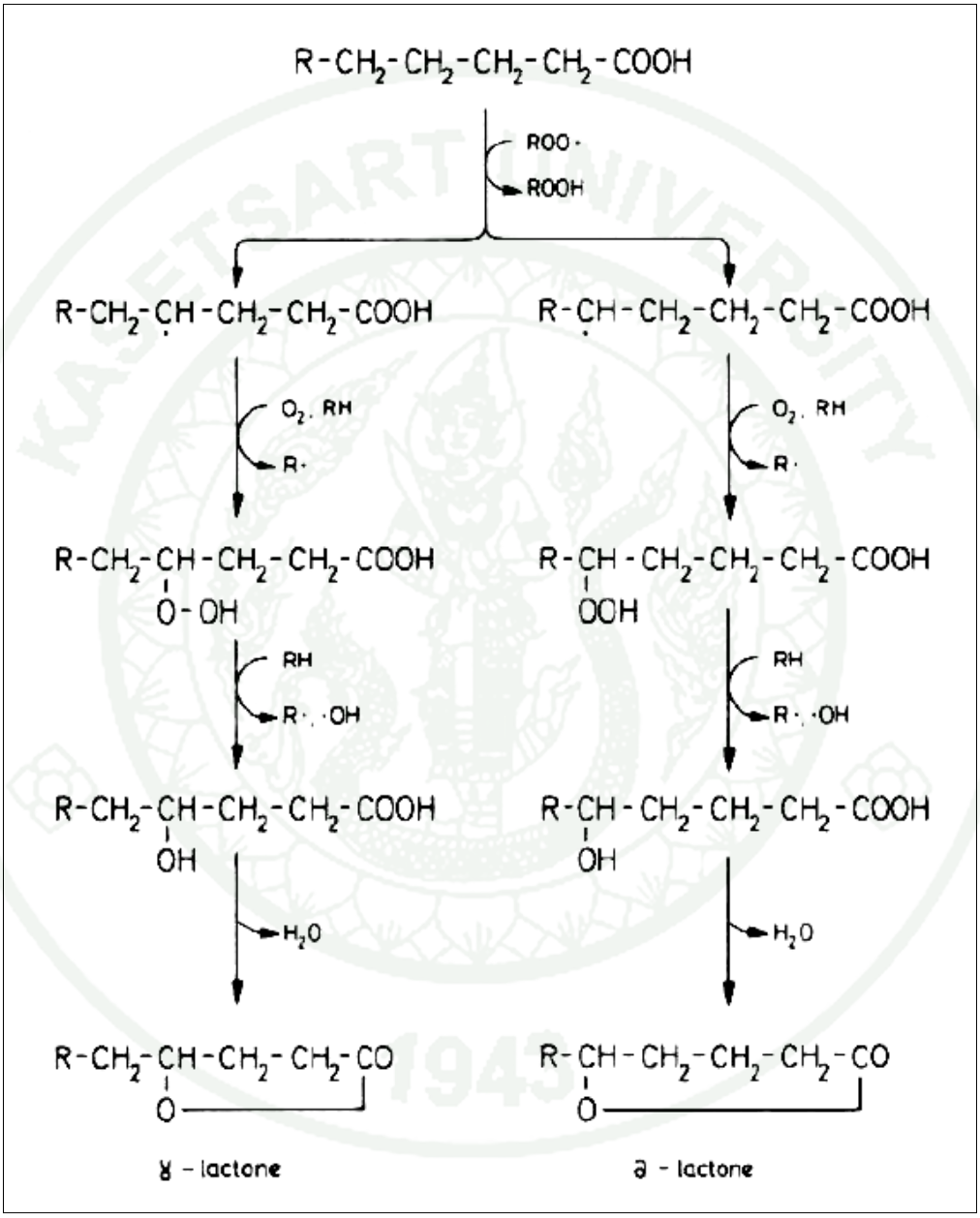


Figure 3 Formation pathway of δ- and γ-lactones via oxidation reaction of hydroxy fatty acid in the present of heat and water.

Source: Fisher and Scott (1997)

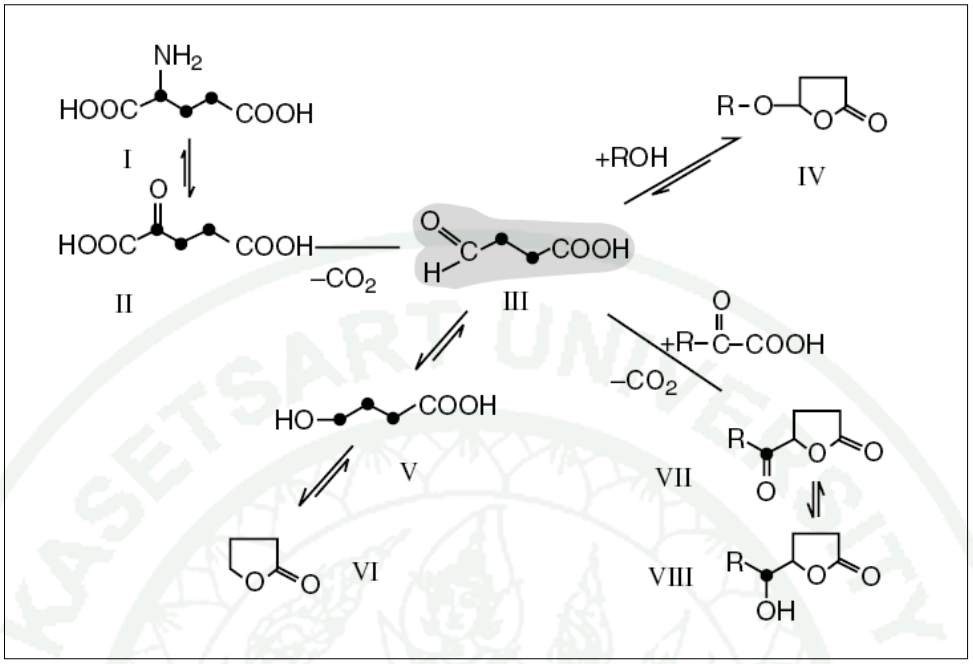


Figure 4 Formation pathway of lactone via microorganisms activity.

Source: Reineccius (2006)

As mentioned above, δ-lactones have been reported as the dominant the compounds representing coconut odor. There are few researches that mentioned about enatiomeric ratios of lactones in coconut as followed. The dominant isomer of δ-lactones (C6, C8, C10 and C12) in coconut meat and coconut water is the (R)-form. Nonetheless, (S)-form tends to increase in their ratio as carbon chain length increases (Jirovetz *et al*., 2003). The enatiomeric ratio of δ-octa- and -deca-lactones in canned coconut milk are close to those found in coconut meat and coconut water. According to Table 5, the ratio of δ-dodecalactone in canned coconut milk seems to be different from those of the other lactones. Nago and Matsumoto (1993) have suggested that it was probably because of the variation among coconut or the heating process.

5.3 Major chemical reactions of canned coconut milk

Evidently, canned coconut milk often contains the distinctive cooked odor that decreases the consumer acceptance. The cooked odor and other off-odor have

occurred after canning process and during storage. Many possible reactions occur at that time of canning process and storage, but three major reactions are reviewed in this part.

Table 5 Enatiomeric ratios of coconut meat, coconut water and canned coconut milk using chiral phase gas chromatography.

Compound	Coconut meat		Coconut water		Canned coconut milk	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
δ-Hexalactone	89.13	10.87	86.92	13.08	-	-
δ-Octalactone	91.24	8.76	79.73	20.27	92.00	8.00
δ-Decalactone	86.33	13.67	84.81	15.19	80.00	20.00
δ-Dodecalactone	63.75	36.25	61.39	38.61	36.00	64.00

Source: adapted from Jirovetz *et al*. (2003) and Nago and Matsumoto (1993)

5.3.1 Lipolysis

Lipolysis is the hydrolysis reaction at the ester bonds in lipid molecules. The word “lipolysis” was referred to this definition throughout this study. Lipolysis is caused by the enzyme action or by the presence of heat and moisture, and results in the liberation of free fatty acids (Nawar, 1996). The release of free fatty acid has major effect on flavors of foods. Short chain fatty acids with carbon numbers less than six is usually responsible for the undesirable rancid flavor in food. For example, butanoic acid (C4) has a sweaty and cheesy note with the threshold in oil of 135 ppb. Moreover, the released unsaturated fatty acids can go further to lipid oxidation or other reactions.

Most literatures on lipolysis have been focused on lipase activity. In fresh and low-heat treatment milks, lipase activity is strongly related the release of free fatty acid. Two types of lipase-lipolysis in milk are categorized, a spontaneous

and an induced lipolysis (Deeth, 2006). For spontaneous lipase-lipolysis, factors that have high influence on the rate of reaction included the amount of lipase in milk, the integrity of the milk fat globule membrane, and the balance of lipolysis-activating and lipolysis-inhibiting factors. This type of lipolysis occurs only at the farm. This occurrence contributes to either the late lactation period or the poor nutrition condition. On the other hand, the induced lipase-lipolysis is initiated by the physical damage of the milk fat globule membrane. The damage allows lipase to access the substrates. The operating actions that initiate the occurrence of the induced lipase-lipolysis are agitation, pumping and homogenization (Deeth, 2006).

Coconut milk has the similar physical characteristic and the emulsion property to those of the milk fat globules. The induced lipase-lipolysis could be similar to that found in milk fat. As mentioned in the section 2.2, lipase plays the dominant role during the early step of preparation. It has been noted that the released short-chain fatty acids, such as butyric and hexanoic acids gave the strong acid and cheesy notes to fresh coconut milk. In the meantime, the medium chain fatty acids produce the distinctive soapy taste in coconut oil (Seow and Gwee, 1997). Blanching of coconut meat at 80 °C for 12 min is adequate to inactivate lipase in coconut meat (Waisundara *et al*., 2007).

Even though natural lipase is considered as a major source of enzymatic hydrolysis, microbial lipases also has a role on the flavor development in coconut milk. Lipases isolated from 5 different fungi were inoculated into the coconut cream (25.4% fat), and 2-phenylethyl ester was formed as an aroma active compound (Tan *et al*., 2011). In pararell with lipase-lipolysis, heat induced lipolysis has been reported in milk and related products (Pereda *et al*., 2008; Bertrand *et al*., 2011). Gervajio (2005) has noted that the increases of temperature and pressure during the thermal process accelerated lipolysis in coconut oil. The condition of high temperature and pressure increased the solubility of the water in the oil phase. The increase of the temperature from 150 °C to 220 °C increased water solubility by two to three times. The presence of small amounts of mineral acids, such as sulfuric acid

or certain metal oxides, such as zinc or magnesium oxide, accelerates the splitting reaction.

5.3.2 Oxidation reaction

Unsaturated fatty acids are susceptible to be oxidized, even they are in free form or are esterified with the acylglycerol molecules. Enzymatic oxidation is relating to lipoxygenase activity. The activity of this enzyme leads to hydroperoxide formation that goes further to produce aroma compounds. In coconut milk, this enzyme plays a crucial role during preparation without heat. Blanching of coconut meat at 80 °C for 12 min is adequate to inactivate lipase and lipoxygenae enzymes in coconut meat (Waisundara *et al*., 2007).

For the auto-oxidation, three steps including initiation, propagation and termination are well known. Auto-oxidation scheme with primary and secondary degradation products are illustrated in Figure 5. In this scheme, many volatile compounds are products from auto-oxidation. Lipid oxidation has been known as a free radical chain reaction. The initiation step of lipid oxidation is not spontaneous. The catalyst is required to start lipid oxidation process by removing an electron from either the lipids or oxygen or by changing the electron spin of the oxygen. The reaction is endothermic and requires about 64 Kcal/mole (Rawls and van Santan, 1970). By the action of the catalysts, the unsaturated fatty acids and hydroperoxides (ROOH) are in the singlet states, while oxygen is in the triplet state. Metals, light and heat are necessary to act as catalysts for the initiation step of lipid oxidation. Trace amount of metal with the concentration very less than micro molar is sufficient for catalyzing the reaction (Schaich, 2005). Free radicals ($R^{\cdot}$) are generated. For propagation, free radical chain reaction and free radical chain branching occur. The propagation starts by the abstraction of hydrogen atoms at the positions α- to the double bond positions of fatty acids, producing free radical species. Addition of oxygen yields peroxyl radicals ($ROO^{\cdot}$) and alkoxyl radicals ($RO^{\cdot}$), and then hydroperoxide abstracts hydrogen from α-methylenic groups of other unsaturated fatty acid molecules to yield hydroperoxides and new free radicals. This step of

propagation is repeated in presence of oxygen (Nawar, 1996). Finally, the radical species may combine with one another to produce the non-radical products in order to terminate the process. In Figure 5, hydroperoxide is considered as the primary product of oxidation that is very unstable, tasteless and odorless. Meanwhile, the secondary products, such as aldehydes, alcohols, ketones, etc. are very important. These compounds are related to the aroma of food products depending on their thresholds and their amounts presented in food (Shahidi, 2000).

For photooxidation, Shahidi (2000) has stated that the products of photooxidation and auto-oxidation are very similar, but the different mechanism pathways are involved. For auto-oxidation, the unsaturated fatty acids and the triplet oxygen along with the initiators (e.g. heat, light, transition metal ions, etc.) are required. On the other hand, the unsaturated fatty acids, the singlet oxygen and the photosensitizers (e.g. chlorophyll, methylene blue, erythrosine, rose benegal, etc.) are required for photooxidation. Photooxidation is faster than auto-oxidation. Rawls and van Santan (1970) noted that the reaction rate of the singlet oxygen with linoleic acid is at least 1,450 times faster than that of the triplet oxygen. The most important pathway to produce singlet oxygen via photooxidation requires the presence of the photosensitizers. Two pathways have been proposed for photosensitized oxidation (Figure 6; Nawar, 1996). In the first pathway, the sensitizer reacts with the substrate (A) in the presence of light to form intermediates that then reacts with the ground-state oxygen to yield the oxidation products. In the second pathway, the molecule of oxygen reacts directly with the sensitizers upon the light absorption.

The oxidation reaction is a source of volatile formations in many food products as well as in coconut milk. Many aldehydes and esters form during the step of preparation and pre-treatment. Time management has to be considered for consistency of canned products. During pre-heating and retorting, many volatile compounds are generated as products of Maillard reaction and thermal decomposition of lipids, proteins and carbohydrates. Because lipid is the major composition in coconut milk, the thermal-induced lipid decomposition is briefly discussed. During pre-heating, oxygen is available in excess, oxidation reaction plays a role as

accelerating factor. Nawar (1989) has mentioned about the auto-oxidation at elevated temperatures. He reported that the rate of hydroperoxide formation has rapidly increased in the early part of heating at 70 °C, and decreased about 6 times as time increased. Moreover, the hydroperoxides went further to yield oxidized volatile compounds.

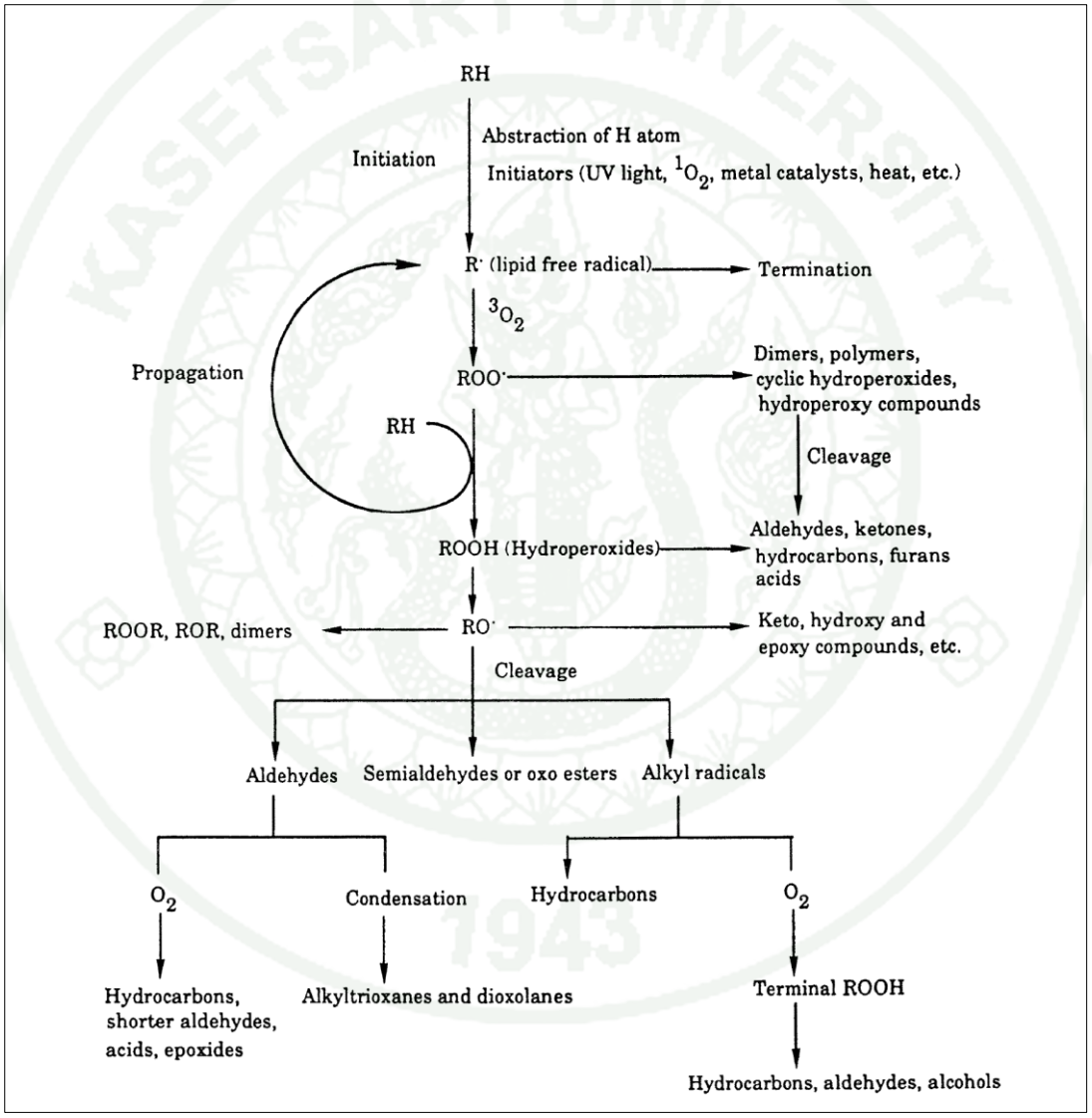


Figure 5 The mechanism of lipid oxidation and formation of the primary and the secondary degradation products.

Source: Shahidi (2000)

Hexanal has been reported as the oxidation product. It is an index of the low temperature oxidation. Meanwhile, 2,4-decadienal is the major aldehyde formed in the model analysis. Thermal conditions also consequences the liberation of free fatty acids from their acylglycerol precursors. These free fatty acids are precursors of methyl ketones, aldehydes, lactones and hydrocarbons (Nawar, 1989).

During storage, the volatile profiles of food products have changed. The environmental condition of the storage, such as temperature, relative humidity, time of storage, and also the food composition has involved in this alteration. In dairy product, lipid and protein are the major source of aroma compounds. Drake *et al*. (2009) reported the sensory profile of whey protein during 12-18 month storage. The score of odor attributes, including cucumber-like, fatty, cereal/grain-like, brothy, sweet aromatic, cardboard-like, cabbage, and astringency has increased with storage time. The gas chromatography (GC) analysis with solid-phase microextraction showed the results of the suspected compound. The aroma compounds originated from lipid oxidation are suspected to the increase of odor attributes in sensory analysis.

The 1st proposed pathway:

$$\text{Sens} + \text{A} + h\nu \longrightarrow \text{Intermediates-I}$$

$$\text{Intermediates-I} + O_2 \longrightarrow \text{products} + \text{sens}$$

The 2nd proposed pathway:

$$\text{Sens} + O_2 + h\nu \longrightarrow \text{Intermediates-II}$$

$$\text{Intermediates-II} + \text{A} \longrightarrow \text{products} + \text{sens}$$

Figure 6 Proposed pathways of photosensitized oxidation.

Source: Nawar (1996)

5.3.3 Maillard reaction

Maillard reaction was firstly described in 1912 by French Chemist, Louis-Camille Maillard. The coherent scheme of this reaction was put forward by John Hodge in 1953, which is usually divided into three stages (Yanlayan, 1997). The early stage starts with a condensation between an amino group and a carbonyl group of reducing sugars, leading to an N-glycosylamine that rearranges into the so-called Amadori products. The advanced stage consists of the breakdown of the Amadori products into numerous fission products of sugar-amino compounds. The final Maillard reaction consists of the condensation of amino compounds and sugar fragments into polymerized proteins and the brown pigment, called melanoidins.

Maillard reaction affects the whole host of sensory properties of food during processing and storage. Yanlayan (1997) has reviewed Maillard reaction in a conceptual way for more visual understanding. In his review, the Maillard reaction was divided as pools of chemical reactions that composed of parent pool, primary fragmentation pool and interaction pool. For the parent pool, three principal precursors are defined as sugars, amino acids and Amadori or Heyns products. Interestingly, Amadori and Heyns products are grouped as the precursors of Maillard reaction products. He explained that the Amadori or Heyns product can act as precursors, and interact to the degradation products of amino acids and reducing sugar residues. Amino acids, sugars and Amadori or Heyns product had undergone the degradation and has been termed as the primary fragmentation pools. Then, these fragments move to the phase of the interaction pool to produce many different compounds as Maillard reaction products. The well-known consequence is the brown color formation that is called the non-enzymatic browning. In addition to brown color, van Boekel (2006) has stated that the distinctive flavors in foods and beverages are the products of Maillard reaction. The Strecker degradation is the most important reaction. The heterocyclic compounds formed via the Strecker degradation usually have a certain impact on food flavors. The nutrition quality of food may be damaged by the Maillard reaction during processing with decreasing protein digestibility and loss of amino acid (Fayle and Gerrard, 2002). Factors affecting the Maillard reaction

products are type of reactants, reaction temperature, time, pH, water activity and the presence of oxygen, metals and inhibitors (Ames, 1998).

In term of food flavor, Maillard reaction products have both desirable and undesirable effects. Many desirable flavor compounds in coffee, chocolate and bakery products are formed via Maillard reaction (Fayle and Gerrard, 2002). For example, 2-methybutanal and 3-methylbutanal are the potent odorants of chocolate. They are formed via Strecker degradation of leucine and isoleucine, respectively (Chen and Robbins, 2000). On contrary, Maillard reaction is considered as the off-odor pathway in dairy products during storage. Colahan-Sederstrom and Peterson (2005) reported that the addition of epicatechin could inhibit the formation of methional, furfural and 2-isopropyl-3-methoxypyrazine that contributed to the undesirable cooked odor in the UHT milk. The residues of reducing sugars and amino acids, such as furosines and furfurals, are the indicators of Maillard reaction in the UHT bovine milk. The increment of these compounds correlates with the occurrence of Maillard reaction (Ferrer *et al*., 2000). Maillard reaction in coconut or related products has not been studied directly. Saittagaroon *et al.* (1984) has studied the effect of roasting on volatile compounds of the shredded coconut meat. Some compounds are presumably classified as Maillard compounds. Twenty pyrazines were later on discovered in roasted coconut meat, and the proposed formation pathway was Maillard reaction. The increases of pyrazine, pyridine and pyrroles are function by the roasting temperature in the range of 130-160 °C (Jayalekshmy *et al*., 1991).

MATERIALS AND METHODS

Materials

1. Commercial coconut grating machine (Sakaya, Thailand)
2. Hydraulic press (Sakaya, Thailand)
3. Hydraulic can seamer (Shin I Machinery Works Co., Ltd, Taiwan)
4. Gas retort (Ngauhaudyu, Thailand)
5. Filter paper No.1 (Whatman, Kent, UK)
6. Vacuum pump (model 35.3AN.18-IP20, M&C Products Analysentechnik, Ratingen, Germany)
7. Magnetic stirrer (model: RCT basic, Ika®, Wilmington, USA)
8. High vacuum distillation pump (model: B62426952, Edward, West Sussex)
9. High vacuum distillation active gauge controller (model D38655000, Edward, West Sussex)
10. Hydraulic hand grater
11. Twin screw press (Hander Inc., Sioux Falls, SD)
12. Steam retort (model #AA3152, Food Machinery and Chemical Corp., Hoopeston, IL)
13. Hot air oven (Memmert, Germany)
14. Solvent assisted flavor extraction (SAFE) glassware (Ace Glassware, Vineland, NJ)
15. SAFE pump (Edward, West Sussex)
16. SAFE active gauge controller (Edward, West Sussex)
17. Water bath (Heetgrid immersion heater, George Ulanet Co., Newark, NJ)
18. HP-5 column (60 m length, 0.25 mm ID and 0.25 µm film thickness, Agilent Technologies, Inc., Palo Alto, CA)
19. FFAP column (60 m length, 0.25 mm ID and 0.25 µm film thickness, Quadrex Corp., New Haven, CT)
20. SAC-5 column (30 m length, 0.25 mm ID and 0.25 µm film thickness, Supelco, PA)

21. Stabil-WAX column (30 m length, 0.25 mm ID and 0.25 µm film thickness, Restek, Bellefonte, PA)

22. DB-WAX column (15 m length, 0.32 mm ID and 0.25 µm film thickness, Restek, Bellefonte, PA)

23. RTX-5 SLIMS column (15 m length, 0.53 mm ID and 1.5 µm film thickness, Restek, Bellefonte, PA)

24. RTX-WAX column (15 m length, 0.32 mm ID and 0.25 µm film thickness, Restek, Bellefonte, PA)

25. Bond elute aminopropyl disposable columns (500 mg) (Varian, Inc., Palo Alto, CA)

26. Gas chromatography-mass spectrometry (GC-MS, (Agilent Technologies, Palo Alto, CA)

27. Gas chromatography-Flame ionization detector (GC-FID, Agilent Technologies, Palo Alto, CA)

28. Sniffing port (DATU Technology Transfer, Geneva, NY)

29. Teflon Sniffing bottle (Nalge Nunc International, Rochester, NY)

30. Analytical balance (4 digits) (Sartorius, Bradford, MA)

31. pH meter (model AB92333171, Fisher Scientific, PA)

32. Centrifuge (model HN-S II, IEC, Forma Scientific Inc., Ohio)

33. Centrifuge rotor (model 901, IEC, Forma Scientific Inc., Ohio)

Methods

This work had been divided into 2 sets of experiments. The first experiment was aimed to have an understanding of the changes in the volatile profile of the canned coconut milk stored for 6 months under the tropical condition. The canned coconut milk samples were analyzed monthly in order to monitor the specific changes of volatile compounds that could lead to the undesirable perception of the canned coconut milk. The first experiment was conducted at Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand.

The second experiment was aimed to study in the more specific details of aroma compounds and the possible reactions. Aroma compounds were identified to gain a better understanding of the odorants in coconut milk. Sensory analysis was conducted to monitor the overall odor profile of canned coconut milk. In addition, the fatty acid profiles of coconut milks were determined to follow the changes of fatty acids that were the possible precursors for aroma compounds. The second experiment was studied at the University of Illinois, Urbana-Champaign, USA.

Because there were two lots of canned coconut milk that were separately prepared, the practical operations during canning process, such as grating procedure, the condition for pre-heating, were controlled to have the same protocol. The F_0 calculated from the different retorts were pre-studied and controlled to achieve the same value of 5 min at 121 °C.

Experiment 1: **Changes in volatile profile of canned coconut milk during 6 months of storage**

1. Chemical

General reagent or HPLC grade chemicals, such as anhydrous diethyl ether, anhydrous sodium sulfate, and sodium chloride, were obtained from Fisher Scientific (Fair Lawn, NJ). Internal standards; 2-methyl-3-heptanone, 6-undecanone and 2-ethyl butyric acid were obtained from Sigma Aldrich, St. Louis, MO). Nitrogen (99.999% purity), and helium (99.999% purity) were purchased from Thai Industrial Gases Public Co. Ltd. (Bangkok, Thailand).

2. Sample preparation and canning process

Coconut meat without brown testa was purchased from local market, Bangkok, Thailand. Coconut meat was washed with the tab water and grated using commercial grating machine (Sakaya, Thailand). Grated coconut meat was added with potable water in the weight ratio of 1:1. Hydraulic press (Sakaya, Thailand) was used

to obtain fresh coconut milk. Fresh coconut milk was preheated at 72 °C for 1 min, and then it was filled in the cans (dimension 307 x 409; diameter x height) that were previously cleaned and blanched in hot water. Each can was filled with approximately 380 g of fresh coconut milk. Water steam was used to heat coconut milk to reach 80 °C for exhausting purposed before can seaming. Canned coconut milk was heated in a gas retort (Knoahaudyu, Thailand) until F_0 at 121 °C equal to 5 min (Seow and Gwee 1997). Canned coconut milk was then cooled to room temperature in an ice bath before extraction and storage.

3. Storage condition

Canned coconut milk samples were stored at ambient temperature (32-35 °C) for 6 months. Two cans of coconut milk were taken every month to be analyzed for volatile compounds**.**

4. Extraction methods

Direct solvent extraction using diethyl ether was conducted as extraction method. Canned coconut milk was filtered through the Whatman filter paper No.1 under vacuum pump for homogenization to avoid heating. Homogeneous canned coconut milk (100 mL) was added with 10 µl of 2-methyl-3-heptanone (12.14 mg/mL), 6-undecanone (10.56 mg/mL), 2-ethyl butyric acid (12.45 mg/mL) in diethyl ether as the internal standard mixture. Diethyl ether (50 mL) was added to extract volatile compounds in a duran bottle with a magnetic stirrer stirred at a low speed for 30 min at room temperature. Extractions were carried out 3 times. Diethyl ether containing volatile compounds was collected by a pastured pipette. High vacuum distillation (vacuum pump model: B62426952, Edward, West Sussex) was applied under 10^{-5} torr at the ambient temperature for 2 h and at 50 °C for 1 h. Extracts in the first trap were collected, purged under N_2 gas to reach 5 mL and dried over 2 g of anhydrous sodium sulfate to remove residual water. Extracts were then concentrated to 250 µL under N_2 gas purging before analysis.

5. Gas chromatography-mass spectrometry (GC-MS) analysis

Concentrated extracts (1 µL) were analyzed on a 6890 GC equipped with a HP 5973 mass selective detector (Agilent Technologies, Palo Alto, CA). The electron-impact ionization of 70 eV ionization power was applied. The range of scan was 30-300 m/Z at the rate of 2.74 scan/s. Samples were injected using the splitless injection mode. Two capillary columns, HP-5 (60 m length, 0.25 mm ID and 0.25 µm film thickness, Agilent Technologies, Inc., CA) and FFAP column (60 m length, 0.25 mm ID and 0.25 µm film thickness, Quadrex Corporation, New Haven, CT) were used. Carrier gas was helium at the flow rate of 1.7 mL/min. Temperatures of the injector and the detector were set at 250 ºC. The oven temperature was initiated at 40 ºC and held at this temperature for 5 min, raised at the rate of 2 ºC/min to 60 ºC, raised at the rate of 20 ºC/min to 90 ºC, raised at the rate of 10 ºC/min to 200 ºC, and held at this temperature for 20 min. Retention index (RI) was calculated correlated to *n*-alkane standards (C6–C30). Concentrations of volatile compounds were calculated as relative concentrations. Identification of volatile compounds was investigated by comparing mass spectrum of volatile compounds with Wiley 275 library, NIST 02 library, and by matching RI from two different columns with RI from literatures. Compounds were identified by comparing the data with those of authentic compounds. Odor activity value (OAV) was calculated from the relative concentration of the volatile compound divided by its odor threshold. Because coconut milk is an oil in water emulsion, the OAV were calculated based on both water and oil media.

6. Statistical analysis

Complete randomize design (CRD) was applied to study the effect of the storage time on volatile compounds of the canned coconut milk. One-way ANOVA was used to indicate the differences of volatile compound concentrations in the samples over 6 months. Significant difference was calculated at 0.05 level using Duncan's multiple range test by SPSS version 12 (IBM Corporation, New York, USA).

Experiment 2: The Odor characteristics of canned coconut milk

1. Chemicals

General reagents or HPLC grade chemicals, included anhydrous diethyl ether, methanol, heptane, hexane, 2-propanol, anhydrous sodium sulfate, sulfuric acid, hydrochloric acid, sodium bicarbonate, sodium hydroxide, and sodium chloride, were obtained from Fisher Scientific (Fair Lawn, NJ). Ultra high purity (UHP) nitrogen and UHP helium were purchased from S.J. Smith (Davenport, IA). BF_3-methanol solution was obtained from Sigma Aldrich (St. Louis, MO).

Standard compounds for identification included ethyl octanoate, ethyl decanoate, ethyl dodecanoate, butyric acid, 3-methyl butyric, caproic acid, caprylic acid, capric, phenyl acetic acid, hexanal, octanal, decanal, δ-hexalactone, δ-heptalactone, guaiacol, ethyl maltol and vanillin were obtain from Sigma Aldrich, St. Louis, MO. δ-Octalactone was obtained from TCI, Chuo-Ku, Tokyo. δ-Nonalactone, δ-decalactone, δ-undecalactone and δ-dodecalactone were obtained from Bedoukian Research Inc. (Danbury, CT). 2-Methoxy-4-vinyl phenol was obtained from Avocado Research Chemical Co. Ltd., (Lancashire, UK). β-Damascenone was obtained from Firmenich (Princeton, NJ). Internal standard compounds for aroma extraction, 2-methyl-3-heptanone 2-ethyl butyric acid and dimethyl trisulfide, were obtained from Sigma Aldrich (St. Louis, MO). Internal standards for lipid analysis, pentadecanoic acid and heptadecanoin, were obtained from Nu-Check Prep, Inc. (Elysian, MN).

Isotope compounds were listed in Table 6. [$^2H_3$]-Acetic acid, [$^2H_7$]-butyric acid, 2-methoxy-[$^2H_3$]-phenol, ([$^2H_3$]-guaiacol), were obtained from CDN (Quebec, Canada). [$^{13}C_2$]-phenylacetic acid was obtained from Isotec, (Miamisburg, OH). [$^2H_3$]-methional, [$^2H_2$]-3-methyl-butanol, [$^2H_2$]-2-methyl-2,3-propanol, [$^2H_4$]-ethyl decanoate, [$^2H_2$]-ρ-vinyl guaiacol, [$^2H_4$]-β-damascenone, [$^2H_5$]-propanoic acid, [$^2H_{11}$]-caproic acid, [$^2H_{11}$]-caprylic acid, [$^2H_2$]-phenyl propanoic acid, [$^2H_2$]-capric acid, [$^2H_3$]-vanillin, [$^2H_{15}$]-ethyl octanoate, [$^{13}C_2$]-phenyl acetaldehyde, [$^2H_3$]-aminoacetophenone and [$^2H_5$]-3,6-dimethyl-2-ethyl pyrazine were supported from

Agricultural Bioprocess Laboratory, Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign. [$^2H_4$]-Octanal, [$^2H_4$]-decanal, [$^2H_4$-2]-nonanone, [$^2H_4$]-2-undecanone and [$^2H_2$]-δ-lactones were synthesized according to the method described in Appendix B.

2. Sample preparation and canning process

Coconuts were purchased from local grocery store in Champaign, IL, USA. They were claimed to be products of California. Coconuts were stored at ambient temperature (25 °C) for 10 days before the production. Coconuts were cut to half and the meat was grated by using a hydraulic coconut grater. Distilled water was added to the grated coconut meat at the ratio of 1:1 before the twin screw pressing (Hander Inc., Sioux Falls, SD). Ten kilograms of fresh coconut milk were kept in the chilling room at 5 °C for chemical analysis as the fresh sample. Fifteen kilograms of the fresh coconut milk was heated to reach 80 °C without holding time in a double-steam, double-jacket kettle (Groen, Unified Brands**,** Jackson, MS**)**. Heated coconut milk was filled into cans (dimension 211 x 400; diameter x height). Each can contained 260 g of coconut milk. Cans were then closed by a hydraulic can seamer. The determination of F_0 was pre-studied to ensure the equal quality of the canned coconut milk to the samples processed in Thailand. The thermograph of coconut milk starting from the pre-heating treatment to the retorting process is shown in Figure 7. The come up time of steam retort ((Model #AA3152, Food Machinery and Chemical Corp., Hoopeston, IL) is 4.30 min. The condition of retort during process was; the temperature of the retort = 249-252 °C and the pressure of 17 psi. The total process time was 55 min to get F_0 at 121 °C as 5.3 min. The ice-water bath was used to cool down the canned coconut milks.

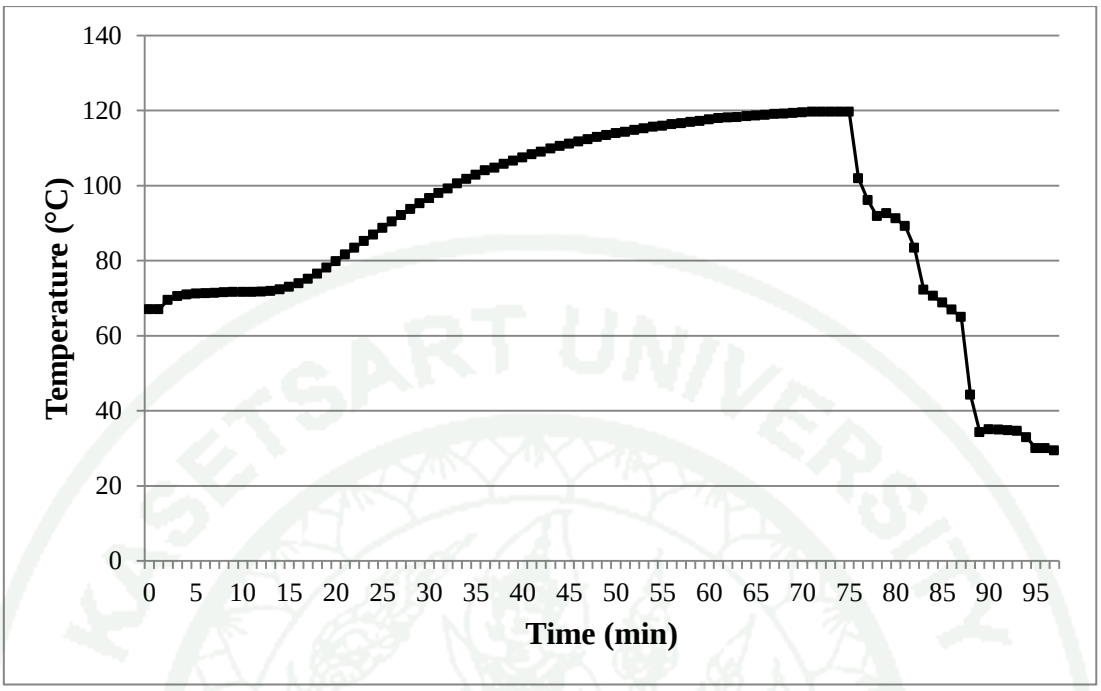


Figure 7 Thermograph of coconut milk during preheating and retorting on canning process optimization.

3. Storage condition

Canned coconut milks were kept under 2 different conditions for 3 months. The first condition is ambient temperature (average temperature = 23.2 °C), and the second temperature is 40±1 °C. The temperature of 40±1 °C was achived by hot air oven. The temperatures of both conditions were checked every 2 days.

4. Extraction method

Solvent assisted flavor evaporation or SAFE was used as extraction method. Fresh and canned coconut milks were extracted. Two hundreds grams of each coconut milk were added with 2-methyl-3-heptanone and 2-ethyl butyric acid as internal standards, and diethyl ether (50 mL). Coconut milk was loaded to SAFE apparatus at the average rate at 16 mL/min. The condition of SAFE was; water bath temperature of 40 °C, pressure of 10^{-5} torr. Volatiles were trapped in the first bath of

liquid N_2. Extraction was carried on for 2.5 h. After that, fractionation was conducted to achieve neutral/basic fraction (N/B) and acid fraction (AF) as the scheme in Figure 8.

5. Gas chromatography-mass spectrometry (GC-MS) and gas chromatography -olfactometry (GC-O) analysis

5.1 GC-MS-SPME

Coconut milk (5 g) and 1 g of NaCl were weighted into glass vial. The analysis was run on auto-sampler injection mode. The pre-incubation time of 10-15 min at 40 °C was conducted. The isolation time was 25 min. The desorption at the GC injection port was 10 min at 250 °C. SPME fiber was carboxen/polydimethylsiloxane (Carb/PDMS)**.** Gas chromatography (model HP 6890, Agilent Technologies, Palo Alto, CA) equipped with the HP 5973 mass selective detector with the Electron-Impact Ionization were used. Stabilwax (30 m length, 0.25 mm ID and 0.25 µm film thickness, Restek, PA) was used. The oven temperature were initiated at 35 ºC, held at this temperature for 5 min, raised at the rate of 4 ºC/min to 225 ºC, held at this temperature for 20 min. Carrier gas was helium at the flow rate of 1.0 mL/min on the cold-splitless mode**.**

5.2 GC-MS of SAFE extracts

The extracts were analyzed on GC-MS using two different columns; a polar and a non-polar. The extract (1 µL) of each fraction was analyzed on a gas chromatography (model HP 6890) equipped with HP 5973 mass selective detector, with Electron-Impact Ionization. Two capillary columns, SAC-5 (30 m length, 0.25 mm ID and 0.25 µm film thickness, Supelco, PA) and Stabilwax (30 m length, 0.25 mm ID and 0.25 µm film thickness, Restek, PA) were used. Carrier gas was helium at flow rate of 1.0 mL/min on the cold-splitless mode. The injected sample was trapped at -50 °C by liquid nitrogen trapping. The oven temperature was programed as in GC-MS-SPME analysis. Volatile compounds were identified by comparing mass

spectrum of volatile compounds with Wiley and NIST 08 libraries. Relative concentrations were calculated by comparing peak areas of total or selected ions of interested compounds to the internal standard compound or isotope labeled compound (Appendix A). Three replications were conducted.

5.3 GC-O Headspace

The fresh and canned coconut milks (after processed) were weighted to have 20 g into round bottom flask. The incubation time of 25 min at 50 °C was conducted. Headspace volume of 25 mL was analyzed. The GC-O system consisted of an HP-6890 GC (Agilent Technologies Inc., Santa Clara, CA) equipped with a flame ionization detector (FID) and a sniff port (ODP2, Gerstel, Germany). A CIS4 programmable temperature vaporizer (PTV) inlet was used to cryofocus the headspace volatiles prior to injection on cold-splitless mode. Initial inlet temperature was programmed as follows: initial temperature, -120 °C (0.1 min hold); ramp rate, 10 °C/s; final temperature, 260 °C (10 min hold). Separations were performed using an RTX-WAX column (15 m length, 0.53mm ID and 1.0 µm film thickness; Restek; Bellefonte, PA). Helium was used as the carrier gas at the constant flow of 5 mL/min. FID temperature was 250 °C. Oven temperature was programmed as follows: initial temperature, 40 °C (5 min hold), ramp rate, 10 °C/min, final temperature, 225 °C (10 min hold). Two trained panelists were asked to sniff via sniffing port, which circulated by humidified air at 35 mL/min. The column effluent was split 1:5 between the FID (250°C) and olfactory detection port (250°C), respectively. Retention times and odor characteristics of aroma were recorded.

5.4 GC-O SAFE extracts

SAFE-aroma extracts (2 µl) were analyzed by aroma extraction dilution analysis (AEDA) on DB-WAX (15 m length, 0.32 mm ID and 0.25 µm film thickness, Restek, Bellefonte, PA), and RTX-5 SLIMS (15 m length, 0.53 mm ID and 1.5 µm film thickness, Restek, Bellefonte, PA) columns. The extracts were diluted

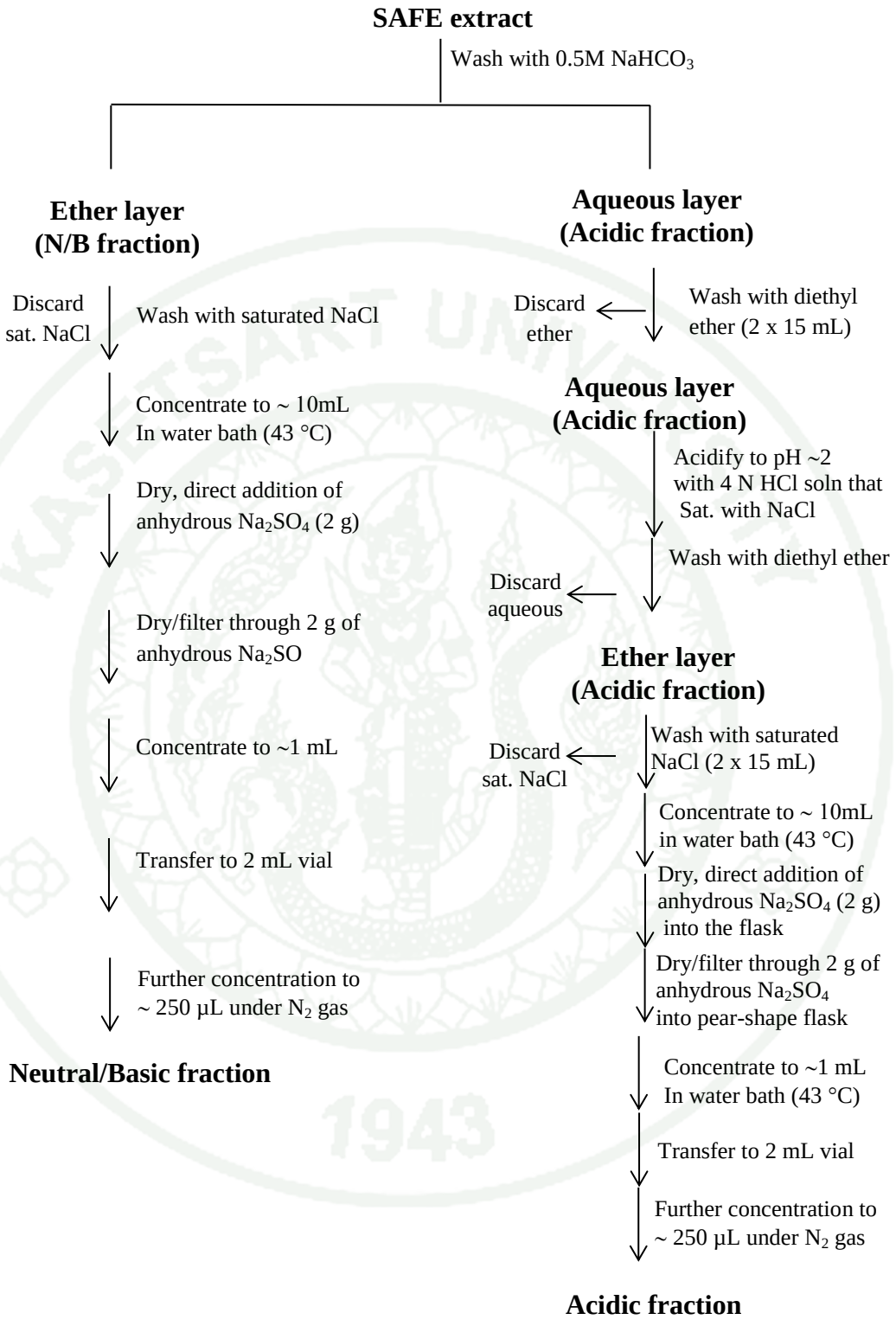


Figure 8 Fractionation procedure of the SAFE extract**.**

with diethyl ether in the ratio of concentrated extract:diluted extract by a series of Log_3 as 1:3, 1:9, 1:27,... until trained panelists could not detect the odor of compounds.

Two trained panelists sniffed the odor of compounds via a sniffing port that had the humidified air circulated at 35 mL/min. The dilution factors of aroma compounds were reported as the mean values from two panelists. The GC-O system consisted of an HP 6890 GC equipped with a flame ionization detector (FID) and a sniffing port (DATU Technology Transfer, Geneva, NY). The column effluent was split at 1:5 between the FID (250 °C) and the olfactory detection port (250 °C), respectively. The GC oven temperature was programmed from 35 to 225 °C at the rate of 10 °C/min for DB-WAX column and at the rate of 6 °C/min for RTX-5 SLIMS capillary column, with the initial and the final holding times of 5 and 20 min, respectively. The FID and the sniffing port were maintained at 250 °C. The extracts were injected to GC-O using the on-column mode injector. The sniffing port was humidified by the water vapor at the temperature of 40 °C.

6. Fatty acid profile analysis

The amounts of short-chain (C2-C6) free fatty acids were analyzed by the Stable Isotope Dilution Assay (SIDA)-GC-MS approach. Concentrations of short chain free fatty acids were calculated relatively to those of acid isotopes analyzed by GC-MS.

For medium- and long-chain free fatty acids and acylglycerol-fatty acids, the analysis was conducted by the method adapted from Cadwallader *et al*. (2007). Ten grams of the homogeneous coconut milk was mixed with 3 mL of aqueous 2.5 M H_2SO_4 and 10 µL of each internal standard (pentadecanoic acid 1.056 mg/mL heptane for free fatty acids and heptadecanoin 1.008 mg/mL of heptane for acylglycerols. Coconut milk fat was extracted by 5 mL of diethyl ether-heptane (1:1 v/v). The mixture was then centrifuged for 10 min at 1,000 x g (IEC centrifuge, model HN-S II, Former Scientific Inc., Ohio). The extraction was repeated for 2 more times. All

organic fractions containing crude fats and free fatty acids were combined together. The mixture was loaded through an aminopropyl-boned phase column, previously equilibrated with 10 mL of heptane. Chloroform:2-propanol (10 mL, 2:1 v/v) was loaded to elute acylglycerols. Formic acid (2% v/v) in diethyl ether was used to elute free fatty acids. Each fraction was collected into glass test tube with screw cap.

To analyze fatty acid profile of coconut milk, fatty acid methyl esters were prepared as the followed procedure. Acylglycerol-fraction was evaporated to dryness under N_2 stream at ambient temperature. 0.5 N Sodium hydroxide in methanol (2 mL) was added, and then vortex for 30 s. After fatty acids were released from their parent glycerol, fatty acid methyl esters were then prepared. BF_3-methanol solution (2 mL) was added, and then vortex for 30 s. The mixture solution was incubated in the oven at 90 °C for 30 min, and waited until the mixture was cooled to the ambient temperature. Hexane (2 mL) was added, and then vortex for 30 s. The mixture solution was incubated at 120 °C for 30 min, and waited until cool to ambient temperature. Hexane layer containing free fatty acid methyl ester was collected. It was dried over anhydrous sodium sulfate. The extract was then analyzed by gas chromatography-flame ionization detector (GC-FID). Methyl esters of separated free fatty acids were prepared by the same procedure of acylglycerol-fatty acids without adding sodium hydroxide solution. Extract (1 μL) of each fraction was injected into GC-FID. RTX-WAX column (15 m length, 0.32 mm ID and 0.25 μm film thickness, Restek, Bellefonte, PA) and a hot-splitless mode were used. The temperature of the injector and the detector was 250 °C. Hydrogen flew at the rate of 1 mL/min. The oven temperature program started from 35 °C, raised at 5 °C/min to 170 °C and to 240 °C at the rate of 3 °C, held at the this temperature for 15 min. The concentration was calculated relatively to internal standard compound. Peak identification of interested compounds was performed by comparing to individual reference. Three replications were done.

7. Consensus descriptive analysis

Trained panelists (3 males and 7 females) were students and staffs of University of Illinois in the age of 20-50. All panelists had the experience in evaluating sensory profiles of various foods. They were trained for 5 h to identify and define terms for coconut milk aroma. Twenty grams of coconut milk and references were presented in the 125-mL Nalgene PTFE wash bottles with siphon tubes removed from the caps. Bottles were labeled with random 3-digit codes. The coconut milk samples were covered with aluminum foil to prevent any visual bias. Samples were presented at ambient temperature (~ 25 °C). Panelists evaluated each sample by gently squeezing the bottle and sniffing the air emitted from the nozzle. Panelists were asked to range the intensity of each term from 0 (none) to 15 (extremely strong), and were discussed to have an agreed value for the intensity of each odor term. The final valuesof odor intensities were recorded.

Table 6 Isotope compounds for SIDA-GC-MS approach.

#	Compound	Conc. (mg/mL)	Selected ions for calculation		Response factor
			Unlabelled	Labelled	
1	[$^{2}H_4$]-Octanal	11.6	100	104	1.03
2	[$^{2}H_4$]-Decanal	17.4	112	115	1.44
3	[$^{2}H_3$]-Methional	0.562	104	107	1.70
4	[$^{2}H_2$]-3-Methyl-butanol	4.14	70	72	0.45
5	[$^{2}H_3$]-ρ-Vinyl guaiacol	2.62	150	153	1.350
6	[$^{2}H_3$]-Guaiacol	2.12	124	127	1.490
7	[$^{13}C_2$]-2-Phenylethanol	10.9	122	124	0.267
8	[$^{2}H_4$-2]-Nonanone	13.1	142	146	1.35
9	[$^{2}H_4$]-2-Undecanone	15.7	170	174	0.86
11	[$^{2}H_4$]-β-Damascenone	0.982	69	73	1.036
12	[$^{2}H_2$]-δ-Octalactone	14.8	99	101	1.299

Table 6 (Continued)

#	Compound	Conc. (mg/mL)	Selected ions for calculation		Response factor
			Unlabelled	Labelled	
13	[$^2H_2$]-δ-Nonalactone	15.1	99	101	1.170
14	[$^2H_2$]-δ-Decalactone	17.6	99	101	1.440
15	[$^2H_2$]-δ-Undecalactone	21	99	101	1.5401
16	[$^2H_2$]-δ-Dodecalactone	13.2	99	101	1.202
17	[$^2H_3$]-4-Vinylguaiacol	2.62	150	153	0.9465
18	[$^2H_3$]-Acetic acid	579.6	60	63	0.9508
19	[$^2H_7$]-Butyric acid	14.1	88	90	1.20
20	[$^2H_2$]-3-Methyl butyric acid	19.2	87	89	0.50
21	[$^2H_5$]-Propanoic acid	20.56	74	78	0.82
22	[$^2H_{11}$]-Caproic acid	14.4	60	63	0.8162
23	[$^2H_{11}$]-Caprylic acid	15	60	63	0.5040
24	[$^2H_2$]-Capric acid	49.6	73	75	0.6751
25	[$^2H_3$]-Vanillin	1.17	152	155	1.250
26	[$^2H_{15}$]-Ethyl octanoate	651.1	88	91	0.8901
27	[$^2H_4$]-Ethyl decanoate	17.9	101	103	0.7845
28	Ethyl maltol	12.1	128	140	1.4592
29	2-Ethyl butyric acid	25.2	Scan	Scan	-
30	2-Methyl-3-heptanone	31	Scan	Scan	-

8. Statistical analysis

Complete randomize design (CRD) was applied as an experimental design for study the effect of canning process and storage conditions on volatile compounds and lipid profile of coconut milks. One-way ANOVA was used to indicate the differences of the amount of volatile compound and fatty acid methyl ester among coconut milk samples. The concentration of volatile compounds and fatty acid methyl

esters of fresh coconut milk, canned coconut milk after canning process, canned cconut milk stored at ambient temperature for 3 months and canned coconut milk stored at 40 °C for 3 months were compared. Significant difference was calculated at 0.05 level using Duncan's multiple range test by SPSS version 12 (IBM Corporation, New York, USA).

RESULTS AND DISCUSSION

In this study, the experiment was divided into two parts. The first experiment had focused on the monitoring of volatile profiles of canned coconut milk during 6 months of storage under tropical conditions. This study was conducted at Kasetsart University. The canning process was set to have F_0 of 5 min at 121 °C (Seow and Gwee, 1997). Several extraction techniques and conditions were attempted to optimize the volatile compound isolation. Volatile compounds of canned coconut milk samples were analyzed by GC-MS.

The second experiment of the study was to further characterize aroma compounds of canned coconut milk. This study was conducted at University of Illinois at Urbana-Champaign by using coconuts from California as raw materials. The canning process was performed under the same protocol conducted in Thailand. The experiment was set to identify and quantify aroma compounds which have impacted on consumer acceptance. The sensory analysis was managed to understand the changes of odor characteristics of coconut milk caused by the canning process and storage conditions.

Experiment 1: Changes in volatile profile of canned coconut milk during 6 months of storage

1. Sample preparation and canning process

1.1 Preheating

Coconut milk was obtained by grating and pressing the endosperm of mature coconut with potable water at the ratio of 1:1 (by weight). The aroma of fresh coconut milk was mild but the acidic odor was noted in the samples kept at room temperature for 3-5 h. The released short chain fatty acids caused by lipases from coconut and microorganisms were hypothesized as the sources of this acidic odor. According to Seow and Gwee (1997), common genera of bacteria found in coconut

milk were *Bacillus*, *Achromobacter*, *Microbacterium*, *Micrococcus*, and *Bravibacterium*, while *Penicillium*, *Geotricum*, *Mucor*, *Furasium* and *Saccharomyces* spp. were the dominant fungi. Among these microorganism, *Penicillium* and *Eurotium* species showed the lipase activities that contributed to the ketonic rancidity in the desiccated coconut meat (Kinderlerer and Kellard, 1984). In addition, the released fatty acids could be modified via β-oxidation, resulting in the formation of aliphatic methyl ketones with one less carbon atom than their fatty acid parents (Kinderlerer and Kellard, 1984; Kinderlerer and Hatton, 1991). As such, fresh coconut milk in this study was immediately preheated at 72 °C for 1 min after being pressed to pasteurize and inactivate enzymes. It was noted that preheating could prevent the formation of the acidic odor in the samples.

1.2 Homogenization

In this study, neither homogenization nor adding food additive was applied to coconut milk. The preliminary study on using a double stage of pressure homogenization showed that the homogenization without an addition of emulsifiers could not improve the emulsion stability. The analytical method of homogenization study was described in detail in Appendix D. The result was in agreement with the work by Tungsuphoom and Coupland (2008b). They reported that oil droplets of coconut milk tended to flocculate after homogenization because there was low amount of natural proteins to act as emulsifiers. Homogenization resulted in an increase of the surface area of oil droplets. The amount of natural proteins was not enough to keep the dispersion between fat globules. High shear force combining with the pasteurization temperature might cause the protein denaturation, change of size and structure of protein, followed by the loss of proteins' stabilizing properties. The proteins that previously located at the surface of oil droplets had evidently moved closer and aggregated (Appendix D).

After 24 h, the creaming indexes of the non-homogenized and the homogenized coconut milks were not significantly different ($P>0.05$). From the results, it was suggested that, if an emulsifier is added, the double-stage 17/4 MPa

was recommended for homogenization. Because this level of pressure did not generate much heat, and the oil droplet sizes were much smaller than those of the non-homogenized coconut milk. In addition, the color of the homogenized coconut milk was preferably lighter than those of the non-homogenized coconut milk.

Coconut milk is an oil-in-water emulsion that has proteins as natural emulsifiers. Figure 9 shows the emulsion system of the undiluted-fresh coconut milk under light and confocal laser scanning microscopes. The particle size distribution (d_{43}) of fresh coconut milk examined by a Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) was 9.88 µm with monomodal distribution that was similar to the data reported by Tungsuphoom and Coupland (2008a, 2008b). Figure 9 (right) shows the arrangement of proteins in red color surrounding the oil droplets. The stabilized emulsion was maintained for 4-5 h before the separation of cream and skim coconut milk occurred. Coconut proteins; albumins and globulins and phospholipids play a major role on stabilizing coconut milk emulsion system (Tungsuphoom and Coupland, 2008b). However, the flocculated emulsion could be re-dispersed by hand shaking because the layer separation was caused by the flocculation of oil droplets, not the coalescence (Seow and Gwee, 1997).

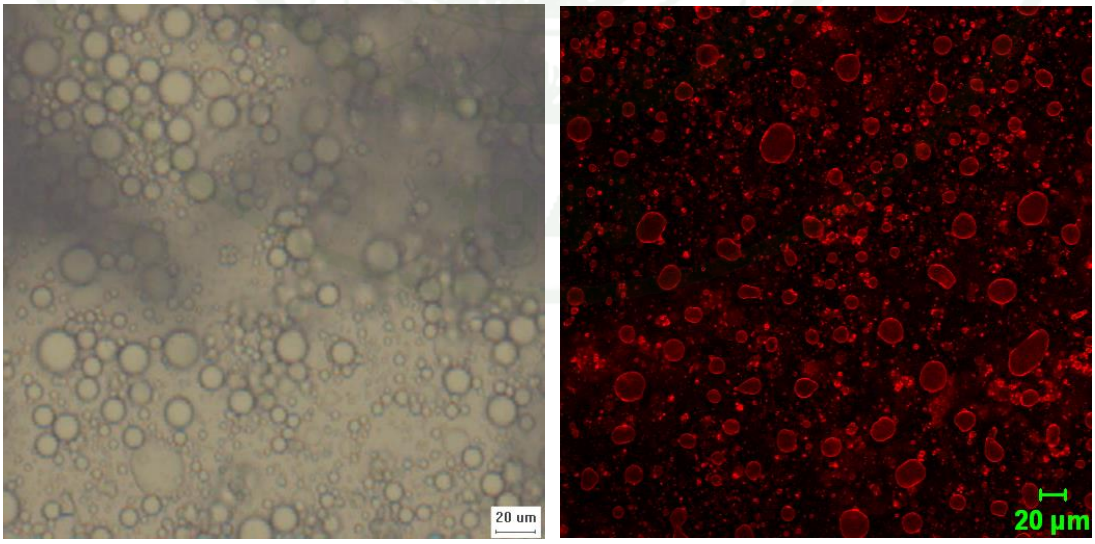


Figure 9 Emulsion systems of the fresh coconut milk under a light microscope (left) and a confocal laser scanning microscope (right).

2. Isolation of volatile compounds

The preliminary study was conducted to choose the method and conditions to isolate volatile components from coconut milk. The isolation methods have largely influenced on odor active compounds analyzed by GC-O (van Ruth, 2001). In addition, there are many variables involving, such as amount of food sample, concentration factor and injected sample volume. These factors have a great impact on volatile compounds isolation. In the preliminary test, the comparative study of extraction techniques was investigated. Two techniques, high vacuum distillation (HVD) and direct solvent extraction (DSE), were compared.

Generally, HVD is selected to isolate volatile compounds from foods with high fat content which is the case for coconut milk. However, this technique alone can isolate lesser amount of volatile compounds than DSE. Moreover, the HVD glassware was found to be unsuitable for directly using with coconut milk. It was because of the bubbling of coconutmilk during the extractionthat could go through the connection tube between the sample flask and the trapping flask. This was resulting in a contamination of the extract.

Solvent extraction is one of the simplest approaches for volatile isolation, and was selected for this study due to a wider range of volatile recovery. In addition, to isolate hydrophobic volatile compounds, the direct contact between oil and solvent has to be achieved. In the preliminary study, pentane, hexane, diethyl ether and dichloromethane were used. Isolation using diethyl ether showed the widest range of volatile compounds (data not shown) and was selected as an extraction solvent for this study.

Even though, diethyl ether was selected as an appropriated solvent, but it was not easy to be separated from the coconut milk matrix. There was a small amount of coconut milk still contaminated in the diethyl ether extracts. Generally, DSE is suitable for non-lipid foods. In case of food containing lipids, the extract must be re-extracted under high vacuum to separate the lipid phase (Reineccius, 2006). As such,

the diethyl ether extracted by SDE was re-extracted under high vacuum distillation to remove the contaminants before GC injection.

The stirring method for DSE was studied. The extensive stirring extraction (stirring speed of level 9, Magnetic stirrer model: RCT basic, Ika®, Wilmington, North Carolina), mild stirring extraction (stirring speed of level 4), and the centrifugation isolation were compared. The centrifugal force and the extensive stirring caused more trapping of diethyl ether-coconut oil mixture within the coconut matrix making it unable to be separated from the coconut milk. Mild stirring, on the other hand, gave a wider range of isolated volatile compounds and easier to be separated from the coconut milk. Therefore, mild stirring of the direct solvent extraction coupled with the re-extracting under high vacuum distillation was used to isolate the volatile compound from canned coconut milk stored for 6 months under the tropical conditions.

3. Changes of volatile profiles of canned coconut milk during storage

Canned coconut milks were stored at ambient temperature (32-35 °C) for 6 months to stimulate the tropical shelf-stored condition. Volatile constituents of canned coconut milk were directly extracted by diethyl ether, and 28 compounds were identified by GC-MS (Table 7). Seven groups of compounds were pronounced, including alcohols, aldehydes, acids, esters, ketones, lactones and others. High abundances of ethyl octanoate, caprylic acid and δ-octalactone were detected as reported in the literatures (Lin and Wilkens 1970; Pai *et al.*, 1979; Saittagaroon *et al.*, 1984; Jirovetz *et al.*, 2003).

The freshly prepared canned coconut milk sample (month 0) contained the lowest concentrations of volatile compounds. Major volatile components in this sample were ethyl octanoate, caprylic acid, δ-hexalactone, δ-octalactone, butyric acid, and 3-hydroxy-2-butanone (Table 7). The compounds were indigenously presented as

Table 7 Relative concentrations of volatile compounds in canned coconut milk during 6-months of storage.

No.	Compound	Retention index		Relative concentration (ŋg/g; ppb)[2]							Iden.[3]
		FFAP	HP-5	M0	M1	M2	M3	M4	M5	M6	
	Alcohols										
1	1-Butanol	1117	n.d.	0.0^{d}	267.8^{b}	613.9^{a}	137.7^{c}	176.0^{bc}	135.7^{c}	539.3^{a}	MS, RI
2	2-Ethyl-1-hexanol	1445	1032	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	10.4^{a}	0.0^{b}	MS, RI
3	1,3-Butanediol	1491	n.d.	39.9^{d}	279.2^{d}	3,167.0^{a}	1,373.0^{c}	1,636.4^{bc}	2,104.4^{b}	1,740.5^{bc}	MS, RI
4	2,3-Butanediol	1528	≤800	26.7^{e}	218.7^{e}	1,609.1^{d}	3,517.4^{a}	2,158.2^{cd}	2,414.2^{bc}	2,912.9^{ab}	MS, RI
5	2-Nonanol	1532	n.d.	11.9^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	MS, RI
6	2-Furanmethanol	1661	n.d.	0.0^{b}	0.0^{b}	1,688.0^{a}	382.1^{b}	357.5^{b}	1,451.2^{a}	1,739.7^{a}	MS, RI
	Aldehydes										
7	Hexanal	1073	n.d.	34.4^{c}	21.9^{c}	70.9^{bc}	107.0^{bc}	173.7^{ab}	234.8^{a}	52.5^{c}	MS, RI, AC
8	Octanal	1283	1008	49.7^{c}	60.7^{c}	94.9^{c}	155.3^{c}	609.1^{b}	547.0^{b}	898.4^{a}	MS, RI, AC
9	Decanal	1496	n.d.	0.0^{ns}	0.0^{ns}	6.2^{ns}	11.8^{ns}	12.6^{ns}	5.3^{ns}	0.0^{ns}	MS, RI, AC

Table 7 (Continued)

No.	Compound	RI[1]		Relative concentration (ŋg/g; ppb)[2]							Iden.[3]
		FFAP	HP-5	M0	M1	M2	M3	M4	M5	M6	
	Ketones										
10	3-Hydroxy-2-butanone	1275	n.d.	238.8^{d}	1,395.0^{c}	1,899.9^{b}	1,990.5^{b}	281.0^{d}	2,448.2^{a}	1,393.6^{c}	MS, RI
11	2-Methyl-5-decanone	1535	1218	7.2^{b}	7.9^{b}	10.4^{b}	7.8^{b}	14.6^{b}	94.2^{a}	87.1^{a}	MS, RI
12	2-Tridecanone	1745	1486	8.1^{c}	108.0^{b}	113.7^{b}	170.8^{a}	26.0^{c}	33.4^{c}	22.3^{c}	MS, RI
	Acids										
13	Acetic acid	1434	n.d.	22.40^{d}	35.76^{d}	5,503.5^{c}	6,543.4^{c}	14,027.9^{b}	23,105.5^{a}	18,239.9^{b}	MS, RI
14	Butyric acid	1620	n.d.	338.9^{cd}	238.3^{d}	924.5^{bc}	1,459.0^{ab}	1,672.4^{a}	1,514.1^{ab}	311.0^{cd}	MS, RI
15	Caproic acid	1840	1030	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	12,774.0^{a}	4,331.7^{b}	MS, RI
16	Caprylic acid	2083	1168	1,621.8^{c}	2,197.9^{c}	2,615.5^{bc}	3,029.3^{b}	5,386.7^{a}	6,616.1^{a}	6,624.5^{a}	MS, RI, AC

Table 7 (Continued)

No.	Compound	RI[1]		Relative concentration (ŋg/g; ppb)[2]							Iden.[3]
		FFAP	HP-5	M0	M1	M2	M3	M4	M5	M6	
17	Lauric acid	2416	1554	34.9^{b}	61.7^{b}	117.1^{b}	1,024.4^{a}	1,180.6^{a}	907.4^{a}	543.8^{ab}	MS, RI
18	Myristic acid	2744	n.d.	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	1,830.1^{a}	2,274.4^{a}	MS, RI
	Esters										
19	Ethyl octanoate	1425	1198	2,137.4^{ab}	2,061.3^{ab}	1,647.8^{ab}	2,247.4^{a}	1,254.7^{b}	1,813.5^{ab}	2,478.4^{a}	MS, RI, AC
20	Ethyl decanoate	1626	1395	17.2^{b}	63.2^{ab}	22.7^{b}	30.1^{b}	38.8^{b}	70.4^{ab}	113.9^{a}	MS, RI, AC
21	Ethyl dodecanoate	1777	1597	12.0^{d}	41.5^{bcd}	211.6^{ab}	23.1^{cd}	323.4^{a}	93.2^{bcd}	200.2^{bc}	MS, RI
22	1,2-Diethylbenzenedicarboxylate	2352	1595	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	176.0^{a}	174.8^{a}	MS, RI
	Lactones										
23	δ-Hexalactone	1766	1086	109.2^{c}	1,096.5^{bc}	3,665.7^{b}	2,834.2^{b}	1,832.4^{bc}	12,434.5^{a}	13,992.1^{a}	MS, RI

Table 7 (Continued)

No.	Compound	RI[1]		Relative concentration (ŋg/g; ppb)[2]							Iden.[3]
		FFAP	HP-5	M0	M1	M2	M3	M4	M5	M6	
24	δ-Octalactone	1951	1285	512.0^{d}	880.5^{d}	8,335.1^{cd}	13,218.4^{cd}	25,103.4^{bc}	36,485.9^{ab}	47,310.9^{a}	MS, RI, AC
25	δ-Decalactone	2176	1498	25.3^{c}	32.5^{c}	318.0^{c}	574.6^{c}	820.7^{c}	4,856.4^{b}	6,448.1^{a}	MS, RI, AC
26	δ-Dodecalactone	2380	1698	34.3^{ns}	20.6^{ns}	28.2^{ns}	66.8^{ns}	56.5^{ns}	40.8^{ns}	24.1^{ns}	MS, RI, AC
	others										
27	3-Methyl-2(5*H*)-furanone	1699	n.d.	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	2,883.2^{a}	3,352.7^{a}	MS, RI
28	Phenol	1992	n.d.	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	578.9^{a}	3,129.9^{a}	MS, RI

[1] RI; retention indices of 2 columns; FFAP (60 m x 0.25 mm x 0.25 µm film thickness) and HP-5 (60 m length, 0.25 mm ID and 0.25 µm film thickness)

[2] Relative concentrations of volatile compounds; M0 = 0 month, M1 = 1 month,…

[3] Iden. = identification; MS = mass spectrometry, RI = retention index, AC = authentic compound

The numbers of compounds were matched to the number on the chromatogram on the Appendix Figure C1-C3 (Appendix C)

Table 7 (Continued)

[a-e] Values with the different letters in the same row were significantly different ($p \leq 0.05$), [ns] = not significant

n.d.= not detected

well as generated from the thermal processing. The complex reactions contributing to rising volatile compound were occurred since the beginning of preparation to retorting operation. Main involved reaction could be related to lipids as reservoir precursors. Lipolysis with or without lipase could be responsible for volatile compound formations in the step of preparation. Lipolysis is defined as the hydrolysis reaction at the ester bonds in lipid molecules (Nawar, 1996). During lipolysis, short- and medium-chain free fatty acids were liberated from their glycerol parents including butyric and caprylic acids. These acids could be an important contributor to acidic, pungent, cheesy and waxy notes in canned coconut milk. The release of free fatty acids could occur during pre-heating. According to Silanoi (2004), heating coconut milk at 80 °C for 20 min increased the concentrations of acetic, butyric, caproic and capric acids from those in the fresh coconut milk. These could be highly contributed to heat-induced lipolysis.

The auto-oxidation could occur during preparation, preheating and exhausting processes. The elevated temperature during pre-heating and exhausting had a major impact on auto-oxidation. Nawar (1989) mentioned on auto-oxidation at elevated temperatures. He reported that the rate of hydroperoxide formation in fats rapidly increased in the early part of heating at 70 °C that closed to the pre-heating temperature (72 °C) in this study. Liberated free fatty acids and hydroperoxides could go further and yield methyl ketones, aldehydes, alcohols, lactones and hydrocarbons as the secondary products (Litman and Numrych, 1978; Nawar, 1989).

During 6 months of storage, concentrations of volatile compounds tended to have 2 stages of the major changes, which had been noticed at 2 and 5 months of storage. Development of volatile profiles during 0-1 month was minimal. Most volatile compounds increased only slightly during month 1. However, 3-hydroxy-2-butanone and 2-tridecanone had a significant increase ($p \leq 0.05$; Table 7). The OAV of 3-hydroxy-2-butanone was more than 1 (Table 8) that could contribute to the detection of creamy and buttery odors in the canned coconut milk after 1 month of storage. The loss of volatile was also detected after 1 month of storage. 2-Nonanol had lost since the first month of storage. This compound has been found in fresh

coconut milk (Lin and Wilkens, 1970). The loss of 2-nonanol might be the result of the heating process that continuously lost in the early period of storage.

Hexanal and octanal that are usually used as an oxidation index, showed no significant increase during 0-3 months ($p>0.05$). This period could be an induction period of oxidation that went slow and steady (Hamilton, 1994). Not only aldehydes, but other oxidative groups of compounds including alcohols, esters, ketones and lactones, also increased slowly. It was noticeable that the occurrence of lipid oxidation products was observed throughout the storage, even in the environment of low oxygen and low amount of unsaturated fatty acids as in the canned coconut milk sample. It suggested that a very low amount of oxygen in the canned products was sufficient for the chain reaction of oxidation. Lipid oxidation is auto-catalytic and the reaction is self-propagating and self-accelerating (Schaich, 2005).

On the second month of storage, certain alcohols, acids and lactones rapidly gained their concentrations in the range 1-5,000 times comparing to those found in the canned coconut milk in the first month (Figure 10). This phenomenon was used as a criterion indicating the first major change of volatile profile in canned coconut milk during storage. One of the major changes was noticed in the group of lactone. Lactones have been reported as the aroma impact compounds in coconut related products representing coconut-like, creamy and sweet odors (Saittagaroon *et al.*, 1984; Jirovetz *et al.*, 2003). Free fatty acids released upon heating could be oxidized to form hydroxy fatty acid as a precursor of lactone, as well as natural occurring hydroxy fatty acids (Fisher and Scott, 1997). Rising 6 times in the total amount and the increase of the OAV of lactones (Table 8) indicated their crucial influence on the aroma of the canned coconut milk in 2 months of storage. That was corresponding to an increase of coconut-like odor noted in month 2.

Acids also gained their concentrations after 2-3 months of storage (Figure 10). Non enzymatic lipolysis might involve in the liberation of free fatty acids during storage. It could be hypothesized that free fatty acids with 4-8 carbon atoms were the major products of lipolysis reaction.

The significant increases of 1-butanol, 1,3-butanediol, 2,3-butanediol and 2-furanmethanol were observed after 2 months of storage ($P \leq 0.05$). Sserunjogi *et al.* (1998) has studied the aroma in ghee and proposed the formation pathway of alcohols via the reduction of aldehydes and diols as the oxidative compounds. It was possible that increases of alcohols in the canned coconut milk after 2 months could contribute to lipid oxidation.

The formation of acetic acid in canned coconut milk during storage was not clearly understood. It is known that acetic acid can be produced via the microbial activity and the thermal degradation of hexose sugar. High temperature and long process time of canned coconut milk are presumably able to destroy microorganisms that are capable of producing acetic acid. Moreover, the reaction of sugar fragmentation requires high temperature up to 120 °C to produce acetic acid (Devidek *et al*., 2006). The condition was similar to the heating condition in the retort. Thus, the high abundance of acetic acid observed during storage could be the result of the progress of sugar fragmentation during retorting but it was still unclear.

The high abundance of acetic and butyric acids had started increasing at month 2. Meanwhile, caprylic and lauric acids increased after 4 months of storage. The released fatty acids might be the important contributors to the flavor of canned coconut milk. Fatty acid with fewer than eight or ten carbon atoms can impart to acidic, pungent and rancid notes in food products, while fatty acids with more than twelve carbon atom are responsible for a soapy taste (Cadwallader *et al*., 2007). More importantly, the high abundance of these acids might be undesirable for canned coconut milk with longer time storage.

3-Hydroxy-2-butanone and 2-furanmethanol that have been previously identified as Maillard reaction products in a serine-monosaccharide model system (Chen and Ho, 1998), increased after 2 months of storage. Moreover, methylketone and furanone could also be from Maillard reaction. These compounds have been previously identified as Maillard reaction products in the UHT milk (Al-Attabi *et al*., 2009).

Even though the nutty, roasted and cooked odors were noted in the canned coconut milk after processing and during storage, pyrazines, pyrroles, furans and furfurals were not detected by a GC-MS in the first experiment. These compounds had been identified as Maillard reaction products in the heated coconut meat (Saittagaroon *et al*., 1984; Jayalekshmy *et al*., 1991) and possess the odor description of nutty, cooked and roasted. After the canning process, the cooked and nutty odors were dominantly perceived in the canned coconut milk. Meanwhile, the pungent, coconut toffee-like and caramel odors were the dominant odors after a longer storage time. The perception of these odors could indicate the presence of Maillard reaction products in the canned coconut milk. Maillard reaction products usually have low odor thresholds; even in low abundance, they can be perceived by human senses.

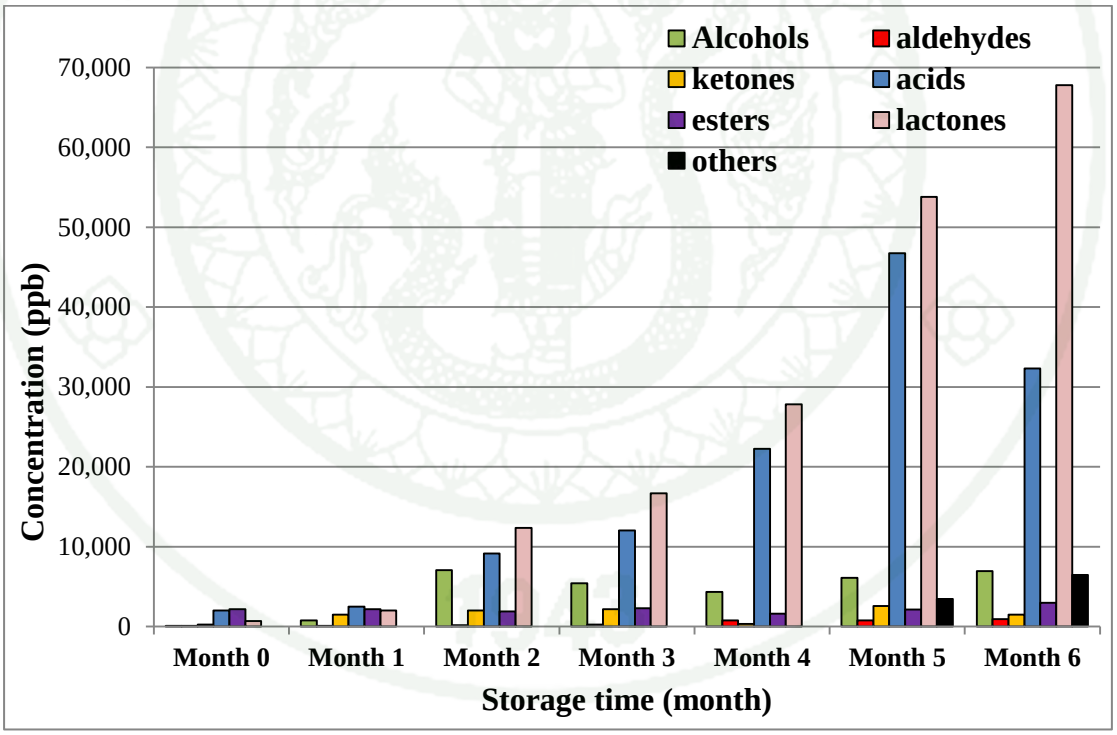


Figure 10 Volatile profile of canned coconut milk storing for 6 months under tropical condition (32-35 °C).

Most of volatile compounds of caned coconut milk during 3-4 months of storage were not significantly different ($P>0.05$; Table 7). The significant increase ($p\leq0.05$) was observed in some compound including octanal, acetic acid, caprylic acid and ethyl dodecanoate (Table 7). Ultimately, the increases of volatile compounds were retrieved after 5 months. 2-Furanmethanol, 3-hydroxy-2-butanone, acetic acid, caproic acid, tetradecanoic acid, ethyl octanoate, 1,2-diethyl-benzenedicarboxylate, δ-hexalactone, δ-octalactone, δ-decalactone, 3-methyl-2(5*H*)-furanone and phenol were significantly increased ($p\leq0.05$). Moreover, some of these compounds were firstly detected after 5 months. It could predict the second stage of reaction. Evidently, the rising of 3-hydroxy-2-butanone, 2-methyl-5-decanone and 3-methyl-2(5*H*)-furanone implied the occurrence of Maillard reaction. Maillard reaction was considered as one of the most important reactions in canned coconut milk after 5 months of storage.

Lipolysis also had an impact on volatile profile of caned coconut milk after 5 months of storage. High abundance of short- and medium-chain fatty acids was observed. The hydroxy fatty acid could be also liberated and contributed to dramatically increase of lactones after 5 months. High abundance of lactones could make a significant difference of volatile profiles between the short and the long time stored canned coconut milk resulting in the different intensities of the sweet coconut-like and peach-like odors. Octanal increased during 4-6 months (Figure 11). On contrary, hexanal and decanal were reduced on month 6. It could be the decline of oxidation due to the limited oxygen in the can's headspace. In this circumstance, lipid precursors were also considered as main factors affecting the occurrence of lipid oxidation. Oleic acid is oxidized to form octanal, and the oxidation product of linoleic acid is hexanal (Lee and Morr, 1994). Small amount of unsaturated fatty acids has been reported in coconut and coconut products. The major unsaturated fatty acids found in mature coconut meat are oleic acid (7.18% of total lipid) and linoleic (1.59% of total lipid), respectively (Santoso *et al.*, 1996). The availability of oleic acid could have more effect on the formation of octanal rather than the limited oxygen content.

The Maillard reaction products have the antioxidant property (Nursten 2005). They might play an important role on this decline phase of lipid oxidation. According

to Yanagimoto *et al.* (2002), pyrroles and furans, the Maillard reaction products, inhibited the oxidation of hexanal in the model system over 40 days of storage. The ability of hydroxyl radical abstraction was proposed as the mechanism of their antioxidant activity.

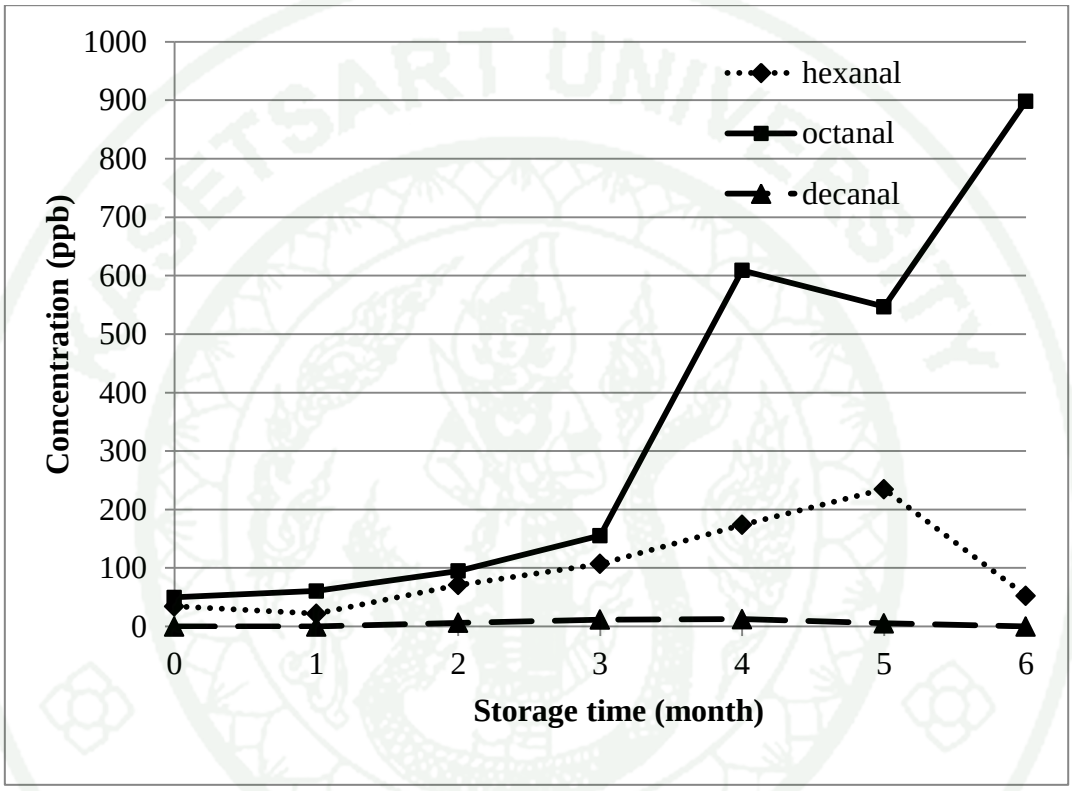


Figure 11 Concentration of aldehydes in canned coconut milk during 6 months of storage.

Odor activity value (OAV) could suggest the capability of odor perception. It is the ratio of compound concentration to its odor threshold. The value which is greater than one contributes to the ability of the compound to be perceived as an aroma of food stuff (Grosch, 2001). To categorize the aroma compounds, OAVs of volatile compounds in canned coconut milk after process and on 2 and 5 months of storage are shown in Table 8. Because coconut milk is the oil-in-water emulsion, OAVs in both oil and water media were calculated (Table 8).

The presence of hexanal, octanal, ethyl octanoate and δ-octalactone with the relatively high OAVs in water could contribute to fat, green, lemon, fruity, and coconut-like odor in fresh canned coconut milk after process (Table 8). Meanwhile, aldehydes, lactones and acids were strongly perceived after 2 and 5 months. Despite the relatively low abundance of aldehydes, high values in OAVs after storage implied that the samples could have a strong note of green, soap, fatty odors. It indicated the impact of the oxidation reaction on the overall aroma of canned coconut milk.

The OAV of acetic acid was greater than one after 5 months in the water medium, and after 2 months in the oil medium (Table 8). With high abundance of acetic acid, the pungent and the vinegar-like was the major concern as off-odors in the canned coconut milk during storage. Likewise, butyric, caproic and caprylic acids had OAVs more than one indicating their importance as aroma compounds. Because of their odor characteristics of cheesy, sweaty, rancid, goat-like and soapy odors, high abundance of these acids possibly resulted in the off-odor in canned coconut milk during storage.

The coconut toffee-like odor was strongly noted after 2-6 months. It was possible that the Maillard compounds were responsible for this occurrence considering the odor characteristics of Maillard compounds. Although, the abundance of Maillard compound was too low to be recovered and detected by the DSE-GCMS, they could be detected by human sense. The color of the canned coconut milk also changed from white in month 0 to slightly yellow after 2-4 months of storage. At month 6, the color of canned coconut milk had and turned into yellow and slightly brown. These indicated the occurring of Maillard reaction of the canned coconut milk during the storage under tropical condition.

High abundance of lactone might also correlate to this coconut toffee-like odor, due to their odor descriptions of sweet, peach-like and coconut-like. Consumers from different continents might be satisfied by different odor characteristics. As mentioned before, Asian food is normally prepared by fresh coconut milk. Therefore, the increase of lactones could make a difference in the odor recognition and being an

off-odor characteristic in the canned coconut milk. The present study was focused on the alteration of volatile profiles of canned coconut milk during storage. The next chapter will focus on the sensory profile and the odorants that could have an impact on the off-odor in canned coconut milk.

Table 8 Odor unit value (OAV) of volatile compounds in canned coconut milk on 0, 2 and 5 months of storage.

Compound	RI[1]		Odor description[2]	Odor threshold[3] (in water, ppb)	Odor threshold[3] (in oil, ppb)	OAV[4] in water			OAV[4] in oil		
	FFAP	HP-5				M0	M2	M5	M0	M2	M5
Alcohols											
Butanol	1117	n.d.	fruity	500[a]	10,500[b]	≤1	1.2	≤1	≤1	≤1	≤1
2-Ethyl-1-hexanol	1445	1032	rose, green	300[b]	n.a.	≤1	≤1	≤1	n.a.	n.a.	n.a.
1,3-Butanediol	1491	n.d.	-	15,000[b]	n.a.	≤1	≤1	≤1	n.a.	n.a.	n.a.
2,3-Butanediol	1528	≤800	-	35,000[b]	n.a.	≤1	≤1	≤1	n.a.	n.a.	n.a.
2-Nonanol	1532	n.d.	fat, green	70[b]	n.a.	≤1	≤1	≤1	n.a.	n.a.	n.a.
2-Furanmethanol	1661	n.d.	-	1,950[b]	n.a.	≤1	≤1	≤1	n.a.	n.a.	n.a.
Aldehydes		n.d.									
Hexanal	1073	n.d.	grass, tallow, fat	10.5[a]	120[a]	3.3	6.7	22.4	≤1	≤1	2.0

Table 8 (Continued)

Compound	RI[1]		Odor description[2]	Odor threshold[3] (in water, ppb)	Odor threshold[3] (in oil, ppb)	OAV[4] in water			OAV[4] in oil		
	FFAP	HP-5				M0	M2	M5	M0	M2	M5
Octanal	1283	1008	lemon, green, fat, soap	0.7[a]	320[b]	71.0	135.6	781.4	≤1	≤1	1.7
Decanal	1496	n.d.	green, soap, orange peel	1[a]	6,700[b]	≤1	6.2	5.3	≤1	≤1	≤1
Ketones											
3-Hydroxy-2-butanone	1275	n.d.	buttery	800[a]	n.a.	≤1	2.4	3.1	n.a.	n.a.	n.a.
2-Methyl-5-decanone	1535	1218	-	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2-Tridecanone	1745	1486	-	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Acids											
Acetic acid	1434	n.d.	pungent, vinegar	22,000[a]	124[a]	≤1	≤1	2.0	≤1	44.4	350.2

Table 8 (Continued)

Compound	RI[1]		Odor description[2]	Odor threshold[3] (in water, ppb)	Odor threshold[3] (in oil, ppb)	OAV[4] in water			OAV[4] in oil		
	FFAP	HP-5				M0	M2	M5	M0	M2	M5
Butyric acid	1620	n.d.	sweaty, cheesy, rancid	1,000[a]	109[b]	≤1	≤1	1.5	3.1	8.5	13.9
Caproic acid	1840	1030	goat-like, sweaty	3,000[a]	29,700[b]	≤1	≤1	4.3	≤1	≤1	≤1
Caprylic acid	2083	1168	sweaty	3,000[a]	161,000[a]	≤1	≤1	2.2	≤1	≤1	≤1
Lauric acid	2416	1554	soapy	1,000[c]	n.a.	≤1	≤1	≤1	n.a.	n.a.	n.a.
Tetradecanoic acid	2744	n.d.	-	10,000[b]	n.a.	≤1	≤1	≤1	n.a.	n.a.	n.a.
Esters											
Ethyl octanoate	1425	1198	fruity, fatty	70[a]	n.a.	30.5	23.5	25.9	n.a.	n.a.	n.a.
Ethyl decanoate	1626	1395	grape	23[b]	n.a.	≤1	≤1	3.1	n.a.	n.a.	n.a.
Ethyl dodecanoate	1777	1597	leaf	400[b]	n.a.	≤1	≤1	≤1	n.a.	n.a.	n.a.

Table 8 (Continued)

Compound	RI[1]		Odor description[2]	Odor threshold[3] (in water, ppb)	Odor threshold[3] (in oil, ppb)	OAV[4] in water			OAV[4] in oil		
	FFAP	HP-5				M0	M2	M5	M0	M2	M5
1,2-Diethyl-benzenedicarboxylate	2352	1595	-	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Lactones											
δHhexalactone	1766	1086	sweet	1,600[a]	n.a.	≤1	2.3	7.8	n.a.	n.a.	n.a.
δ-Octalactone	1951	1285	coconut-like	400[a]	3,000[d]	1.3	20.8	91.2	≤1	2.8	12.2
δ-Decalactone	2176	1498	sweet, peach, coconut-like	100[a]	400[b]	≤1	3.2	48.6	≤1	≤1	12.1
δ-Dodecalactone	2380	1698	sweet	1,000[a]	120[a]	≤1	≤1	≤1	≤1	≤1	≤1
Others											
3-Methyl-2(5*H*)-Furanone	1699	n.d.	-	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Phenol	1992	n.d.	phenolic	5,900[a]	40,000[b]	≤1	≤1	≤1	≤1	≤1	≤1

Table 8 (Continued)

[1] RI; retention indices of 2 columns; FFAP (60 m length, 0.25 mm ID and 0.25 µm film thickness) and HP-5 (60 m length, 0.25 mm ID and 0.25 µm film thickness)

[2] odor description from Rychlik *et al*. (1998)

[3] Odor threshold from [a] Rychlik *et al*. (1998), [b] van Gemert (2003), [c] Fazzalari (1978), [d]Siek *et al*. (1970)

[4] OAV, odor activity value = concentration/threshold; M0 = 0 month 0, M2 = 2 months, M5 = 5 months

n.a. = not available, n.d. = not detected

Experiment 2: The Odor characteristics of canned coconut milk

In the first experiment, the alteration profile of volatile compounds in canned coconut milk during storage was investigated. To retrieve more understanding and clarification about the odor of coconut milk, odor profiles and aroma compounds were determined by using the consensus descriptive analysis, GC-O analysis, GC-MS analysis at the University of Illinois, Urbana-Champaign. The consensus descriptive analysis gave aroma profiles of coconut milk samples. GC-O analysis was aimed to identify aroma compounds in coconut milk and GC-MS analysis was used for compound identification by comparing mass spectrum with Wiley 08 and NIST 08 libraries. The last part was focusing on lipolysis reaction that is considered as one of the pool formation pathways of the off-odor compounds.

The differences between the fresh and the canned coconut milk were not monitored in the first experiment. Therefore, the second study was aimed to determine the effect of canning process on frsh coconut milk, as well as changes during storage. In addition, more intensive identification of volatile and aroma compounds was expected. SAFE technique was used to isolate volatile compounds and showed a better recovery than DSE. In this study, canned coconut samples were stored at ambient temperature in the US (23±2 °C) and at 40 °C for 3 months.

1. Consensus descriptive analysis

The consensus descriptive analysis was aimed to characterize the odor detected in coconut milk. The odor terms had to be unique and not overlapping. For example, the term coconut oil cannot be used, because this term was the overlap of coconut and oily odors. During the process of the terminology creating; fresh, UHT, canned coconut milks, re-dissolved coconut milk powder, and other related coconut products were presented to the trained panelists. The table was set for the sensory group discussion. The room was isolated from the other part of the lab. The temperature was set at 25±2 °C. Samples were contained in Teflon sniff bottles (Nalgene PTFE wash bottle without siphon tube; Nalge Nunc International,

Rochester, NY) at room temperature (25±2 °C) for 30 min before evaluation. The panelists were asked to gently squeeze the bottle, sniffed the expressed air of the coconut milk samples and generated the odor term from samples. Nine odor attributes were developed by the panelists representing aroma profile of coconut milk. These included coconut, creamy, caramel/custard-like, popcorn, nutty, potato, meaty, fruity and fresh (Table 9). The attribute definitions and their references are also shown in Table 9.

In the evaluation of aroma intensities of coconut milk, sample bottles were covered with aluminum foil, and were labeled with randomly generated 3-digit codes. All samples and references were served at the same time. Each panelist evaluated the intensity using a 15-points scale-consensus descriptive starting from none to extremely strong. After self-evaluation, the intensity of each odor attribute was adjusted to agreement by group discussion. Aroma profiles of fresh and canned coconut milks are shown in Figure 12. Aroma profile of fresh coconut milk (FCM) was totally different from canned coconut milks. Intensities of most attributes in all canned coconut milks were higher than those of fresh coconut milk, except for the fresh and the fruity aromas. Lower odor intensities of the fresh coconut milk indicated weak odors, especially the top note odors. It has been known that thermal process has largely impacted on aroma formation. According to Silanoi (2004), heating coconut milk at 80, 90 and 100 °C for 20 min has intensified the odor of coconut-like, sweet, creamy and cooked odors. Coconut milk heated at 100 °C for 20 showed the strongest intensities of the creamy and cooked odors. The result of this study was in agreement with Silanoi (2004), in which thermal process caused the formation of odors. These odors could be desirable or undesirable odor and made the canned coconut milk different from the fresh one.

As mentioned in the experimental part 1, cooked odor was perceived in canned coconut milk. This odor might be undesirable for consumer. In my point of view, the off-odors that contributed to the cooked odor were described as meaty, potato and nutty. Canned coconut milk after canning process (CCM0), canned coconut milk after 3 months of storage at ambient temperature of 23±2 °C (CCM3-

Table 9 Odor attributes and references for consensus descriptive analysis of coconut milk.

Term	Definition	Reference
Coconut	aromatic associated with coconut meat	30 g of fresh mature coconut meat
Creamy	aromatic associated with creamy milk	30 g of evaporated milk; Nestle Carnation®
Caramel/custard-like	aromatic associated with custard	20 g of Jell-O custard dessert, without egg
Popcorn	aromatic associated with popcorn	0.0136 g of popcorn; Orville Redenbacher®
Nutty	aromatic associated with heated nuts	0.06 g of grinned roasted cashew nut; Regency brand
Potato	aromatic associated with cooked potato	1.5 g of 20 min-boiled potato in 30 mL double deionized-H_2O solution
Meaty	aromatic associated with canned chicken broth	0.015 g of broth from canned chicken; Hormel® white and dark chicken
Fruity	aromatic associated with fruit	8.30 g of ripe plum
Fresh	aromatic associated with ethanol	5% of ethanol in 20 mL double deionized-H_2O solution

AT) and canned coconut milk after 3 months of storage at 40 °C (CCM3-40C) had similar aroma profiles. Although meaty and potato notes had the strongest intensity in CCM0, these two odor attributes faded during storage indicating the instability ofaroma compounds contributed to these notes. The aroma profile of CCM3-AT was not much different from CCM3-40C. However, the remaining attributes could be undesirable in Asian market. Since Asian food is likely to be prepared using fresh

coconut milk, the stronger odor attributes could make a difference in odor recognition and in being an off-odor character in canned coconut milk during storage. In this case; the odors of coconut, caramel or custard-like, creamy and popcorn that were remaining after storage could be considered of importance. Furthermore, the nutty odor of CCM3-AT and CCM3-40C had slightly increased after storage. This might impart to the off-odor developing during storage of canned coconut milk.

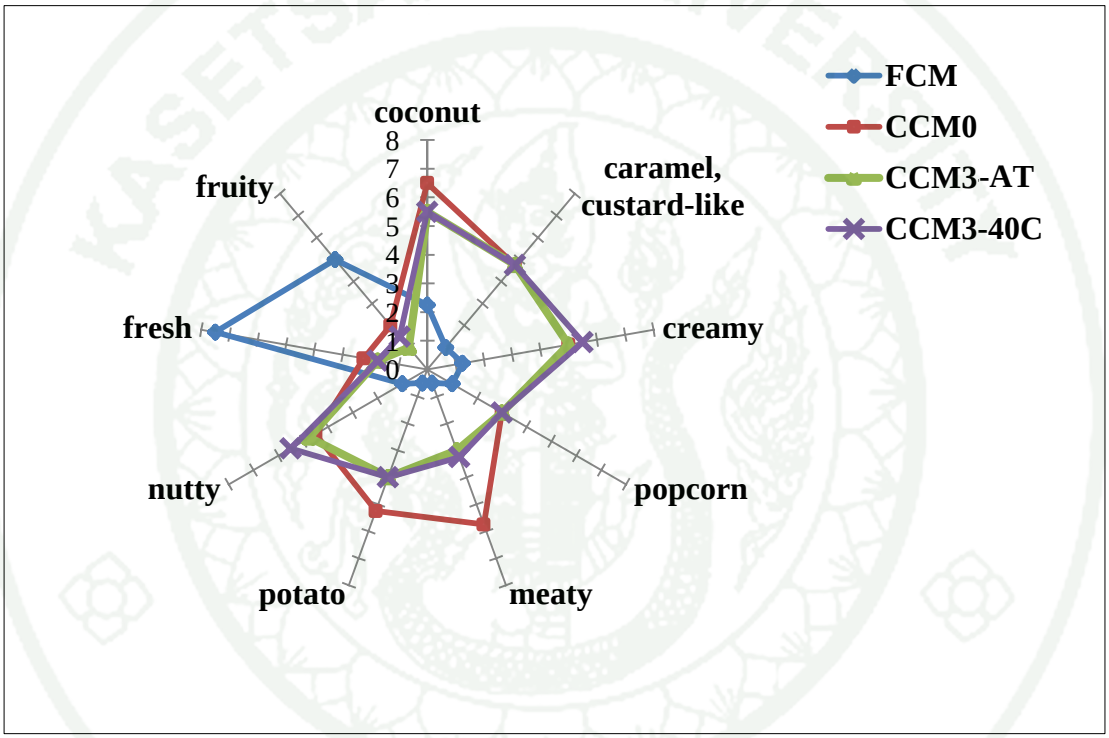


Figure 12 Aroma profiles of fresh and canned coconut milks, FCM = fresh coconut milk, CCM0 = canned coconut milk after canning process, CCM3-AT = canned coconut milk stored for 3 months at ambient temperature (23±2 °C), and CCM3-40C = canned coconut milk stored for 3 months at 40 °C.

Unfortunately, there was also the new odor attribute that was not found during the developing odor attribute session. In this situation, this term was called Unknown odor which was aroma associated with stale, metallic, rancid-green or astringency. The panelists with American nationality had described this new odor as green, oxidative or rancid note. On the other hands, the Asian panelists described this

odor as astringency, rancid, metallic with a weak acidity note. Some of references were brought to the panelists including blue cheese and used fried oil. The best reference for this term was 0.001 ppm of (*E*)-2-nonenal in doubled deionized water (DDI-H_2O). The description of unknown odor was possibly related to the odor characteristic of aldehyde as green, fat and soapy (Rychlik *et al*., 1998). In the first experiment, the amount of aldehydes increased during the first 3 months. This Unknown odor was not detected during the term creating session. Later, some panelists could not range its intensity properly. As such, this unknown odor was not included in aroma profile of coconut milk samples. The intensity of unknown odor was in the range of 0.5-1.5 in CCM3-AT and 2.0-4.0 in CCM3-40C. Future study of the unknown odor is recommended. Regarding to their odor descriptions, this unknown odor could contribute to the off-odor in canned coconut milk during storage, especially under the high temperature condition.

2. GC-O analysis

2.1 Aroma compounds in the headspace of coconut milks

Sensory analysis of canned coconut milk had discovered many odor attributes from the air above the samples. For this circumstance, the static headspace analysis was conducted together with the GC-O analysis to identify aroma compounds of coconut milk. The data from GC-O headspace analysis were compared with the odor that the panelists detected in the consensus descriptive analysis. This study was aimed to identify the meaty and undesirable odor compounds of canned coconut milk that were previously mentioned. This study was, therefore, focused on the canned coconut milk sample immediately after the process (CCM0) and not on the fresh coconut milk that had only 3-methylbutanal, 2-acetyl-1-pyrroline and δ-octalactone detected. Only 11 headspace aroma compounds were detected in CCM0 by this isolation technique. Even though, the headspace-GC-O analysis on CCM0 discovered few aroma compounds with weak odors, the results revealed a clue of headspace aromas. The aroma compounds with lower numbers of RI correlated with the sulfury, meaty and unpleasant notes. These compounds were very important, since they had

low molecular weights and could evaporate easily. Therefore, they could contribute to the headspace odor of canned coconut milk and had an influence on consumer preference. Hydrogen sulfide with rotten egg odor has been reported as the thermal degradation product of amino acid containing SH-group in meat products. The exponential increased of hydrogen sulfide when temperature had increased from 90 to 121 °C was reported in heated meat (Langourieux and Escher, 1998). Amino acids containing SH-group have not been reported in coconut or coconut related products. Only methionine and cystine have been found in coconut milk (Gunetileke and Laurentius, 1974). In canned orange juice, disulfide bond of cystine was readily reduced in the presence of heat, and resulted in cysteine and then thiol radical (Perez-Cacho and Rouseff, 2008). Moreover, Langourieux and Escher (1998) have noted that the thiol radical can be the precursor of hydrogen sulfide in cooked meat. From the literatures, the presence of cystine in coconut milk, even in small amount, could contribute to the formation of hydrogen sulfide. Since hydrogen sulfide has low threshold (odor threshold in air = 0.012 ppb; van Gemert, 2003), it might impart to an undesirable headspace aroma of canned coconut milk after canning process.

2-Methyl-3-furanthiol (Table 10) has the very low threshold (0.004 ppb in water) and a strong characteristic of meaty note (Rychlik *et al*., 1998). 2-Methyl-3-furanthiol is the aroma impact compound in canned tuna (Withycombe and Mussinan, 1988), cooked meat (Mottram and Madruga, 1994) and seafood (Varlet and Fernandez, 2010). The possible pathway of 2-methyl-3-furanthiol formation is Maillard reaction that has cysteine as the precursor, or it can be formed by thiamine degradation (Varlet and Fernandez, 2010). According to Santoso *et al*. (1996), thiamine is reported as the major vitamin in mature coconut meat.

Dimethyl trisulfide brought the sulfury and cabbage-like odor, therefore had a potential as the source of the off-odor in the canned coconut milk. Dimethyl trisulfide is a product of methionine degradation in heated food (Belitz *et al*., 2009).

Table 10 Headspace aroma compounds of canned coconut milk immediately after the canning process.

RI [1]	RI[2]	Compound[3]	Odor description[4]
≤500	≤700[a]	Hydrogen sulfide	fecal, sulfury, rotten egg
611	-	Unknown 1	meaty, sulfury, fecal
804	821[b]	Isobutyraldehyde	dark chocolate, malty
917	912[a]	3-Methylbutanal	dark chocolate
931	935[a]	Pentanal	fatty, oily, pungent
1310	1307[a]	2-Methyl-3-furanthiol	meaty, pungent
1336	1331[c]	2-Acetyl-1-pyrroline	pandan leave, popcorn
1369	1362[d]	Dimethyl trisulfide	sulfur, cabbage, vitamin
1862	1881[a]	γ-Octalactone	coconut, fruity
1877	-	Unknown 2	rancid, metallic, hay
1933	1924[a]	δ-Octalactone	coconut

[1] Retention index of compound from RTX-WAX column (15 m length, 0.32 mm ID and 0.25 µm film thickness, Restek, Bellefonte, PA)

[2] Retention index from reference; [a] from Acree and Arn (2004), [b] from Peterson and Reinccius (2003), [c] from Karagul-Yuceer *et al*. (2001), and [d] from Whetstine *et al*. (2005)

[3] Compound; identified by RI and odor characteristic comparing with references

[4] Odor description; reported by 2 panels on GC-O analysis

2-Acetyl-1-pyrroline (2AP) with the popcorn and pandan leave odor characteristics might have the most impact on popcorn odor on consensus sensory analysis. 2AP is the aroma impact compound in popcorn (Schieberle, 1991), pandan leave (Laksanalamai and Ilangantileke, 1993) and cooked rice (Yoshihashi *et al*., 2002). According to Blank *et al*. (2003), 2AP is the Maillard reaction product of glucose-L-proline and fructose-L-proline system during boiling. 2AP in the canned coconut milk samples could be formed by the same route of Maillard reaction during

thermal process, due to the similar precursors of proline, glucose and fructose found in coconut milk (Seow and Gwee, 1997).

Two lactones were discovered in the headspace of canned coconut milk representing coconut and fruity odors. Two unknown compounds were also reported in Table 10. Regarding to its odor characteristic, the unknown 1 might have influenced on the sulfury and meaty notes. It could be a sulfur-containing compound. Meanwhile, an unknown 2 could contribute to the rancid and oily notes, and might be a product of lipid oxidation.

GC-O headspace analysis for the samples during storage was not investigated because only few numbers of aroma compounds were detected in the GC-O headspace. Most of them, except hydrogen sulfide and acetaldehyde, could be detected in the GC-O- SAFE-extract in the following section.

2.2 Aroma compounds of SAFE extracts

GC-O analysis was conducted to analyze aroma compounds of the extracts from SAFE isolation. Two columns, a DB-WAX (polar column) and an RTX-5 SLIMS (non-polar column) were used to identify aroma compounds by AEDA technique in the neutral/basic fraction of SAFE extracts to get a covered range of polarity for coconut milk's aroma compounds. On the other hand, AEDA of the acid fraction was studied using only the DB-WAX column. This column could separate all compounds in the acid fraction. Furthermore, all compounds that could be isolated on RTX-5 SLIMS were also found on the DB-WAX column. The retention indices of aroma compounds were calculated on both polar and non-polar columns. On the DB-WAX column, 62 aroma compounds in the neutral/basic fraction and 12 aroma compounds in the acid fraction were detected in coconut milks by human sense. There were 6 unknown compounds in the neutral/basic fraction that were separated on the DB-WAX column (Table 11).

2.2.1 The aroma compounds in the neutral/basic fraction of fresh coconut milk analyzed by DB-WAX column

In the neutral/basic fraction of the fresh coconut milk (FCM), 29 aroma compounds were detected, and most of their FD values were the least among the other coconut milk samples. Aroma compounds with the high FD values in FCM were δ-octalactone (486), δ-decalactone (405), guaiacol (378), 2-phenylethanol (54), 4-ethylguaiacol (54), methional (45), octanal (15) and *(E,Z)*-2,4-decadienal (14). Lactones had the highest FD values which contributed to the coconut-like, peachy and fruity notes in the fresh coconut milk sample. Among those aroma compounds, guaiacol, 4-ethyl-guaiacol, phenylethanol and methional were reported for the first time in fresh coconut milk. Some of them were detected only on GC-O analysis, but could not produce a significant mass spectrum on the GC-MS chromatograms.

The biogenesis pathways in plant are complex. Fatty acids, carbohydrates, amino acids, vitamins and minerals are precursors of the fruit aroma. Aldehydes, alcohols, esters, hydrocarbons, ketones, lactones and sulfur containing compounds are developed during ripening, maturation and storage (Salunkhe *et al*., 1976). The formation pathways of important compounds were discussed as followed.

The natural occurrence of lactone in FCM could be formed by many possible reactions. Schwab (2000) mentioned possible biosynthesis pathways of lactones in plants such as 1) reduction of keto-acid by NAD-linked reductase, 2) hydration of unsaturated fatty acid, 3) from hydroperoxides, 4) from fatty acid epoxides, 5) from naturally occurring hydroxy fatty acid, and 6) cleavage of long chain fatty acids. Regarding to Pai *et al.* (1979), Saittagaroon *et al.* (1984) and Jayalekshmy *et al.* (1991), hydroxy fatty acids are the most predominant precursors of lactone in coconut oil and coconut meat. The natural occurring hydroxy fatty acids have been identified in coconut meat (Jayalekshmy *et al.* (1991). Moreover, the oxidation reaction occurring during milk extraction could be the pool of hydroxy fatty acid formation and underwent to form lactone.

Phenylethanol with rosy note was the dominant odorant in FCM. The biogenesis pathway of phenylethanol could be similar to those found in rose flower. Biogenesis pathway of phenylethanol in rose flower is elucidated by Hayashi *et al*. (2004). The possible pathways are the emission from glucoside and the degradation reaction of phenylalanine. Amino acid aminotransferase and 2-phenylacetaldehyde reductase has involved in biogenesis pathway of this compound in rose (Hayashi *et al*., 2012) and tomato (Tieman *et al*., 2007), respectively. However, microbial activity also involves in the formation of 2-phenylethanol which is the similar reaction that plant enzyme involved. Gassenmeier and Schieberle (1995) has proposed the degradation reaction of 2-phenylethanol which phenylalanine is a precursor via the activity of yeast enzymes. The formation of 2-phenylethanol via the microbial activity could occur during coconut milk extraction.

The next interesting compound was 4-ethylguaiacol with clove and sweet odors. This compound could be a released from the glyco-conjugated parent as found in grape, where enzymatic reaction and acid hydrolysis play the dominant role on the freeform release (Jackson, 2008). The release of the compound was then contributing to the aroma when the free-form of the compound presented. 4-Ethylguaiacol and 4-vinylguaiacol are the derivatives of polyphenols. The natural formation pathway of these two compounds has not been well published. It is possible that these two compounds were formed during the preparation or the extraction of coconut milk, where polyphenol could be the precursor of these compounds. Polyphenols in coconut meat, coconut oil and coconut water have been reported (Debmandal and Mandal, 2011). However, the formation route via polyphenol of 4-ethylguaiacol and 4-vinylguaiacol during thermal process is not clearly understood.

Methional in coconut milk was firstly identified in this work by GC-O. The concentration of methional was too low to be detected by GC-MS. Methionine has been reported as the precursor of methional in fruits that had transaminase for the conversion (Eskin and Cogan, 1971). The organic sulfur compounds in fruits and vegetables tend to form during storage (Salunkhe *et al*.,

1976). It was possible that these compounds were found in higher abundance in mature coconut than in young coconut.

Octanal and *(E,Z)*-2,4-decadienal were the lipid autoxidation products. The precursor of 2,4-decadienal was linoleic acid (Shahidi, 2000).

The correlation between sensory and GC-O analysis is discussed as followed. Each odor description on sensory analysis was affected by aroma compounds, especially those with high FD and low threshold values. In FCM, fruity and fresh were the major odor characteristics. The compound that might contribute to fresh aroma in FCM was ethanol. As in sensory test, ethanol solution was used as an odor reference for fresh odor. The odor characteristic of ethanol solution suited well and represented the fresh note in coconut milk. Ethanol was found in high amount in the headspace of FCM (data not shown). It was a breakdown product of sugar by amylase activity that was further converted to ethanol. Amylase activity was found in the undiluted coconut milk (Balasubramaniam and Sihotang, 1979).

The fruity note in FCM might contribute to ethyl-2-methylpropanoate, octanol, octanal, nonanal, nonanol, 2-undecanone, ethyl hexanoate, guaiacol, other ketones and esters (Table 11). For other odor attributes, low intensity scores were observed. Although the odor related compounds had high FD values, their odor attributes was not much detected by the panelists in the sensory analysis. For example, methional (potato note) with FD value of 45 was not detected as a strong potato note by the panelists. The sensory scores of potato note and others were in the range of 1-3 (Figure 12). However, most of the aroma compounds in FCM had the lowest FD values when compared with those of the canned coconut milks. This was agreed with the lower intensities of the other attributes, excepted for the fresh and fruity notes, in FCM. Lactones were highly contributed to the coconut odor. Meanwhile, caramel or custard-like, creamy and popcorn notes could be a results of aldehydes, alcohols and ketones considering their odor characteristics. The intensities

Table 11 Aromatic compounds and FD factors in neutral/basic fraction of coconut milk sniffed by 2 panels on DB-WAX column.

RI[1]		Compound[2]	Odor description[3]	FD Values[4]			
WAX	DB-5			FCM	CCM0	CCM3-AT	CCM3-40C
≤1000	n.d.	Unknown 1	dark chocolate	n.d.	n.d.	0.5	0.5
≤1000	750	Ethyl 2-methylpropanoate	fruity	2.0	n.d.	0.5	4.5
≤1000	≤700	2-Methylbutanal	dark chocolate	n.d.	n.d.	1.5	0.5
≤1000	≤700	3-Methylbutanal	dark chocolate	2.0	81.0	13.5	15.0
≤1000	n.d.	2-Pentanone	fruity	0.5	3.0	1.0	5.0
≤1000	≤700	2,3-Butanedione	buttery	n.d.	2.0	n.d.	n.d.
1033	803	Ethyl butanoate	fruity	n.d.	0.5	n.d.	n.d.
1049	845	Ethyl-3-methylbutanoate	fruity	n.d.	0.5	n.d.	0.5
1083	799	Hexanal*	green, grassy	n.d.	3.0	6.0	15.0
1096	≤700	2-Methyl-1-propanol	malty, dark chocolate	1.0	n.d.	0.5	n.d.
1102	n.d.	*(Z)*-2-Penten-1-Ol	plastic, stale	n.d.	n.d.	0.5	1.0
1152	n.d.	Unknown 2	dark chocolate, malty	n.d.	0.5	n.d.	1.0
1203	n.d.	1-(Methylthio)ethanethiol	sulfurous, cabbage	n.d.	3.0	n.d.	n.d.
1209	740	3-Methyl-1-butanol	malty, dark chocolate	2.0	2.0	n.d.	0.5
1239	n.d.	2-Hexanol	herb, sweet, fruity	6.0	18.0	6.0	n.d.

Table 11 (Continued)

RI[1]		Compound[2]	Odor description[3]	FD Values[4]			
WAX	DB-5			FCM	CCM0	CCM3-AT	CCM3-40C
1284	1004	Octanal*	orange, pungent, green, citrus	15.0	15.0	15.0	0.5
1296	n.d.	Heptanol	mushroom	n.d.	n.d.	1.0	5.0
1300	868	2-Methyl-3-furanthiol*	meaty	n.d.	9.0	n.d.	n.d.
1328	923	2-Acetyl-1-pyrroline	popcorn, cooked rice	2.0	27.0	3.0	6.0
1366	965	Dimethyl trisulfide	cabbage	n.d.	18.0	15.0	162.0
1389	1104	Nonanal*	citrus, green, pungent	2.0	3.0	3.0	n.d.
1420	n.d.	Unknown 3	vitamin, cooked meat	n.d.	6.0	n.d.	n.d.
1424	n.d.	3,5-Octadienone	stale, fatty	n.d.	n.d.	n.d.	4.5
1441	905	Methional	potato	45.0	729.0	729.0	486.0
1481	n.d.	2-Decanone	rosy, floral	n.d.	2.0	n.d.	n.d.
1496	1125	*(Z)*-2-Nonenal*	hay, stale	3.0	9.0	2.0	9.0
1517	1177	2-Nonanol	bug-like, green	n.d.	2.0	n.d.	n.d.
1535	1169	*(E)*-2-Nonenal*	hay, woody, stale	6.0	27.0	18.0	9.0
1555	1074	Octanol*	citrus, lemony, green	6.0	n.d.	n.d.	5.0

Table 11 (Continued)

RI[1]		Compound[2]	Odor description[3]	FD Values[4]			
WAX	DB-5			FCM	CCM0	CCM3-AT	CCM3-40C
1581	1293	2-Undecanone	hay-like, stale, metallic, green	3.0	n.d.	0.5	2.0
1605	1249	(*E*)-2-Decanal	green-waxy	0.5	n.d.	1.0	n.d.
1618	n.d.	2-Acetylpyrazine	roasted nut	n.d.	n.d.	n.d.	162.0
1627	1047	Phenylacetaldehyde	rosy, plastic	2.0	6.0	15.0	6.0
1635	1025	2-Acetylthiazole	roasted nut	n.d.	n.d.	1.0	5.0
1638	1195	3-Mercapto-3-methyl-1-Butanol	stew, soup	n.d.	14.0	n.d.	n.d.
1658	1467	α-Humulene	plastic, rubbery, green	n.d.	6.0	n.d.	5.0
1662	1168	2-Methyl-3-(methyldithio)furan	meaty, vitamin	n.d.	9.0	45.0	162.0
1689	1293	(*E,Z*)-2,4-Decadienal	stale, fatty, beany	14.0	27.0	6.0	18.0
1714	1319	(*E,E*)-2,4-Decadienal	fatty, fried, oily	2.0	2.0	0.5	4.5
1746	1105	2-Acetyl-2-Thiazoleline	popcorn, roasted nut	n.d.	6.0	n.d.	54.0
1748	1372	2-Undecanal	green, cilantro-like	3.0	1.0	n.d.	n.d.

Table 11 (Continued)

RI[1]		Compound[2]	Odor descriptions[3]	FD Values[4]			
WAX	DB-5			FCM	CCM0	CCM3-AT	CCM3-40C
1754	1091	2-Acetyl-5-methylthiophene	cabbage, sulfurous	n.d.	9.0	54.0	n.d.
1800	n.d.	Unknown 4	unripe, metallic, nutty, stale	n.d.	n.d.	42.0	9.0
1819	1388	β-Damascenone*	apple sauce	n.d.	14.0	13.5	5.0
1843	1089	Guaiacol*	smoky	378.0	9.0	405.0	18.0
1851	n.d	Unknown 5	stale, rancid	n.d.	135.0	n.d.	n.d.
1877	n.d.	Unknown 6	burnt, burnt rubber	n.d.	2.0	n.d.	n.d.
1893	1101	2-Phenylethanol*	rosy, wine-like	54.0	6.0	27.0	9.0
1947	1282	δ-Octalactone*	coconut, herbaceous	486.0	1,458.0	3,645.0	135.0
1990	1381	*(E)*-4,5-Epoxy-*(E)*-2-decanal	metallic, unripe	n.d.	n.d.	15.0	5.0
2009	1278	4-Ethyguaiacol	cloves	54.0	2.0	6.0	4.5
2059	n.d.	ρ-Cresol	dung, stable	n.d.	n.d.	1.5	1.5
2129	1276	γ-Decalactone	peach, coconut	2.0	3.0	3.0	1.0
2143	1276	Nonanoic acid*	sweaty, spice	3.0	n.d.	n.d.	n.d.
2171	1492	δ-Decalactone*	peach, coconut	405.0	486.0	10,206.0	1,458.0

Table 11 (Continued)

RI[1]		Compound[2]	Odor description[3]	FD Values[4]			
WAX	DB-5			FCM	CCM0	CCM3-AT	CCM3-40C
2195	n.d.	2-Aminoacetophenone	stale, musky	n.d.	1.0	0.5	2.0
2198	n.d.	Ethylhexadecanoate	waxy	3.0	n.d.	n.d.	n.d.
2246	1380	Capric acid*	soapy, waxy	3.0	n.d.	2.0	2.0
2414	n.d.	*1H*-Indole	indole, fecal	n.d.	n.d.	n.d.	1.0
2423	1705	δ-Dodecalactone*	peach, sweet	n.d.	6.0	15.0	1.5
2462	n.d.	Lauric acid*	waxy, soapy	6.0	1.0	15.0	n.d.
2465	n.d.	Skatole	mothball, skatone, fecal	n.d.	n.d.	n.d.	5.0

[1] Retention index of DB-WAX column (15 m length, 0.32 mm ID and 0.25 µm film thickness, Restek, Bellefonte, PA), and RTX-5 SLIMS column (15 m length, 0.53 mm ID and 1.5 µm film thickness, Restek, Bellefonte, PA)

[2] Compound; identified by comparing RI, odor character, and * with authentic compounds

[3] Odor description; reported by 2 panels on GC-O analysis

[4] FD values, dilution factor calculated by mean value of 2 panels

n.d. = not detected, FCM = fresh coconut milk, CCM0 = canned coconut milk after canning process, CCM3-AT = canned coconut milk stored for 3 months at ambient temperature, and CCM3-40C = canned coconut milk stored for 3 months at 40 °C

of the meaty, potato and nutty notes were 0.5, 0.5 and 1.0, respectively. These odor attributes of meaty, potato and nutty were expected to be strongly detected after canning process. Guaiacol, 4-ethylguaiacol and methional that have the odor of clove and smoky were presented in FCM, but their concentrations were too low to be detected.

2.2.2 Effect of canning process on the aroma compounds in neutral/basic fraction of canned coconut milk analyzed by DB-WAX column

As much as the natural generated pathway, thermal processing has an enormous impact on the volatile and aroma compound formations. After canning process, 42 aroma compounds were detected by panels on GC-O analysis. Comparing to FCM, 22 new compounds were detected on DB-WAX column after canning process. These compounds were 2,3-butanedione, ethylbutanoate, ethyl-3-methylbutanoate, hexanal, 1-(methylthio)-ethanethiol, 2-methyl-3-furanthiol, dimethyl trisulfide, 2-decanone, 2-nonanol, 3-mercapto-3-methyl-1-butanol, α-humulene, 2-methyl-3-(methyldithio)furan, 2-acetyl2-2thiazoline, 2-acetyl-5-methylthiophene, β-damascenone, 2-amino-acetophenone, δ-dodecalactone, lauric acid, and 4 unknown compounds. The aroma compounds that had FD value≥9.0 in canned coconut milk after canning process (CCM0) were, 3-methylbutanal (81), 2-hexanol (18), octanal (15), 2-methyl-3-furantiol (9), 2-actyl-1-pyrroline (27), dimethyl trisulfide (18), methional (729), *(Z)*-2-nonenal (9), *(E)*-2-nonenal (27), 3-mercapto-3-methyl-1-butanol (14), 2-methyl-3-(methyldithio)furan (9), *(E,Z)*-2,4-decadienal (27), 2-acetyl-5-methylthiophene (9), β-damascenone (14), guaiacol (9), unknown 5 (135), δ-octalactone (1,485) and δ-decalactone (486).

δ-Octalactone had the highest FD value that brought the dominant of coconut odor to canned coconut milk, and made a difference between canned and fresh coconut milk. As the FD value of all lactones increased, the sensory intensity scores of coconut odor increased from 2.5 to 6.5 points after canning process. The increase in FD values of lactones after canning process might relate to the release of fatty acid due to thermal treatment. This increase was in agreement with

heated coconut oil (Pai *et al.*, 1979). These released hydroxy fatty acids and heat treatment facilitate lactonization process via β-oxidation, and yielded an increase of lactones (Nawar, 1989).

Lactones are the most impact compounds in coconut and related products. However, the enatiomeric property of lactones could have an influence on their odor properties. Enatiomeric ratios of δ-octa, -deca- and -dodeca-lactones in the canned coconut milk processed in Thailand were investigated on a Chiramix column. The column composed of mixed derivatives of β-cyclodextrin. The (S)-enatiomeric form of δ-decalactone and δ-dodecalactone was not found. Only the (R)-enatiomeric form of δ-decalactone and δ-dodecalactone was detected. However, the (S)-enatiomeric form of δ-octalactone was found in a small amount of 0.97% (of the total peak area). This result did not conform to the other references as mentioned in the introduction, that both (S)- and (R)-enatiomeric forms of these 3 lactones were identified. Nonetheless, the (S)-form increased in its ratio as the carbon chain length of the lactones increases (Nago and Matsumoto, 1993; Jirovetz *et al*., 2003). The possible reason was the concentrations of lactones in the coconut milk extract were too low to be detected in this chiral column. The different columns might result in different isolation efficiencies. More importantly, coconut milk from different origins might contain different enantiomer ratios.

For caramel/custard-like and creamy of CCM0, furaneol, 2,3-butanedione, phenyl acetaldehyde, β-damascenone and vanillin were considered as potent odorants for these terms. However, these compounds alone did not represent these two terms perfectly. The combination of these compounds with other compounds could give better matches with the notes. These compounds together with lactones, ketones or esters might give a perfect match with the creamy note. The FD values of ketones, including 2-pentanone, 2,3-butanedione, 2-decanone, β-damascenone and 2-aminoacetophenone (Table 11), increased after canning process. The oxidation of unsaturated fatty acid was an important precursor of these ketones (Nawar, 1989). According to Parliament and McGorrin (2000), methyl ketone found

in dairy products is converted from fatty acids via oxidative decarboxylation. It could be hypothesized that the similar formation pathway of ketones occurred in the canned coconut milk.

β-Damascenone was not detected in FCM, but in the canned coconut milks. The FD value of β-damascenone in CCM0 was equal to 14 (Table 11). The formation of β-damascenone was facilitated by heating, such as in heated soy milk (Lozano *et al.*, 2007a) and process grapefruit juice (Lin *et al.*, 2002). The formation pathway of β-damascenone in canned coconut milk could be either the thermal induced of glycoside liberation as found in grapefruit juice (Lin *et al.*, 2002), or the degradation product of carotenoid as found in roasted coffee (Holscher and Steinhart, 1994). The carotenoid content in cold pressed coconut oil is 1.47 mg/kg oil (Senaviratne and Nayomi, 2010). The formation of 2-aminoacetophenone upon heating could be contributed to a degradation product of tryptophan as found in corn tortilla (Buttery and Ling, 1995) In the study of Gunetileke and Laurentius (1974), tryptophan was detected among other amino acids in coconut milk.

Sulfur- and nitrogen-containing aroma compounds that formed after canning process included 2-methyl-3-furanthiol, dimethyl trisulfide, furfuryl methyl disulfide, 2-acetyl-2-thiazoleline, 2-acetyl-5-methylthiophene and 2-aminoacetophenone. Most of them had high FD values, and contributed to meaty, sulfurous and nutty odors in CCM0. Meaty and potato odors also dramatically increased in sensory analysis. According to the odor characteristics of aroma compounds in Table 11 and Table 13, the meaty note was majorly contributed by sulfur containing compounds, whereas the nutty note was highly related to pyrazines and pyrroles. Cooked odor in canned coconut milk after process and storage might relate to the meaty odor. It was noted that the strong meaty note in CCM0 had the same odor characteristic as that of 2-methyl-3-furanthiol perceived by the panelists. This compound has been reported as the aroma impact compound for meaty note in canned tuna and heated meat (Withycombe and Mussinan, 1988; Kerscher and Grosch, 1998). It was suggested that 2-methyl-3-furanthiol could be the aroma impact compound for the meaty note in canned coconut milk.

The detection of dimethyl trisulfide in CCM0 with the FD value of 18 could contribute to meaty odor. The formation pathway of dimethyl trisulfide in canned orange juice has been suggested by Perez-Cacho *et al*. (2007). They have mentioned that methanethiol can be oxidized easily to dimethyl disulfide with the presence of oxygen, which was disproportionate to dimethyl sulfide and dimethyl trisulfide. Moreover, dimethyl trisulfide is also reported as a product of methionine degradation in heated food (Belitz *et al*., 2009). The precursor of dimethyl trisulfide might be methionine which has been reported as amino acid found in coconut milk (Gunetileke and Laurentius, 1974), but the route of formation could be similar to those found in canned orange juice or other heated foods.

The FD values of methional increased from 45 to 729 after canning process (Table 11). It could contribute to the strong potato note in CCM0 (Figure 12). Methional has been identified as Maillard reaction product. Blanda *et al*. (2009) has identified methional as Maillard products in boiled potato, whereas Belitz *et al.* (2009) reported the formation of methional via Strecker degradation of methionine. Evidently, heat treatment facilitated the formation of methional of canned coconut milk.

The FD values of 2AP increased from 2 to 27 after canning process (Table 11). This also indicated the occurrence of Maillard reaction upon canning process. 2AP has been reported as Maillard products in boiled potato (Blanda *et al*., 2010). The odor characteristic of 2AP was popcorn. This compound has a potential of being an aroma impact compound for popcorn odor, due to its odor characteristic and high FD value. The increase of FD value of 2AP after canning process could correlate to the stronger perception of popcorn odor in the sensory analysis (Figure 12).

The FD values of other Maillard compounds also increased after canning process. The FD values of 3-methylbutanal increased from 2 to 81 after canning process. This compound is produced from the Strecker degradation of leucine (Belitz *et al*., 2009). 2,3-Butanedione had been detected only in CCM0. The

formation pathway of 2,3-butanedione has been elucidated in many routes of Maillard reaction. It has been reported as a product of fission reaction of deoxyosones via Amadori rearrangement in model cheese (Bertrand *et al*., 2011), and an intact sugar fragmentation product from Maillard reaction in the model system of glycine and glucose (Totlani and Peterson, 2005).

Noticeably, Maillard reaction plays a crucial role leading to the formation of many aroma compounds. The most interesting one in CCM0 was sulfur-containing aroma compounds. These compounds were focused due to their meaty and off-note aroma. Amino acid that acts as a precursor and gives the sulfur atom either for core structure or functional group should be further studied. Two major sources of sulfur containing compounds in heated food which have been reviewed are amino acids via Maillard reaction and thiamin and its derivatives. According to Seow and Gwee (1997), sulfur-containing amino acids in coconut milk are methionine and cystine. Meanwhile, thiamine is reported as the major vitamin in mature coconut meat (Santoso *et al*., 1996).

The fresh and fruity odors had faded after canning process. As previously mentioned, the fresh note could be related to ethanol content. Under thermal condition of canning process, ethanol might interact with other compounds. The interaction of ethanol and aroma compounds has been reported in wine (Villamor and Ross, 2013). These could result in less perception of fresh note from ethanol in CCM0 and CCM3. The interaction of ethanol and aroma compounds affects the lower perception of fruity note in wine (Villamor and Ross, 2013). It was possible that the interaction of ethanol and aroma compounds in canned coconut milk imparted to the loss of fruity note after canning process (Figure 12). The decrease in FD values of aldehydes, alcohols and ketones had a strong impact on the reduction of the intensity of the fruity note. The decrease could be affected by the thermal-induced reactions or the interaction of ethanol and aroma compounds.

Most of Saturated and unsaturated aldehydes in CCM0 had increased in their FD values. These indicated the occurrence of lipid oxidation. The

increment in FD values of saturated aldehydes was smaller than the unsaturated ones. The high FD value of unsaturated aldehyde in CCM0, like *(Z)*-2-nonenal (FD = 9), *(E)*-2-nonenal (FD = 27) and (*E,Z*)-2,4-decadienal (FD = 27), could contribute to the hay, stale and fatty notes. Moreover, theses unsaturated aldehyde could undergo further reaction and caused the change of canned coconut milk aroma during storage. For example, 2,4-decadienal can undergo further degradation, and produces 2-octenal and hexanal (Shahidi, 2000), which might attribute to green-rancid notes.

The interaction between the Maillard reaction and lipid oxidation products have been studied in model analysis (Adams *et al*., 2011). Maillard reaction products from glycine or lysine, and glucose interact with *(E)*-2-hexanal and *(2E,4E)*-decadienal, and result in the major formation of carbonyl compounds and 2-alkylfurans. For example, 2-pentanone is an elongation product of aldehyde and glycine (Adams *et al*., 2011). As such, the interaction between lipid oxidation and Maillard reaction products should be considerable as an importance in thermally processed foods.

2.2.3 The alteration of aroma compounds in neutral/basic fraction of canned coconut milk during storage for 3 months at ambient temperature and 40 °C analyzed by DB-WAX column

Canned coconut milks were stored for 3 months under 2 temperatures, ambient temperature (23 °C) and 40 °C. At ambient condition, temperature was checked every 2 days, and the average temperature was 23.2±2°C °C. For the accelerated condition, canned coconut milk was stored in a hot air oven at 40 ±1 °C. The temperature was checked every 3 days.

In CCM3-AT (Table 11), the aroma compounds in the neutral/basic fraction with high FD values were δ-decalactone (10,206), δ-octalactone (3,645), methional (729), guaiacol (405), 2-acetyl-5-methylthiophene (54), 2-methyl-3-(methyldithio)furan (45), unknown 4 (42; unripe, metallic, nutty and stale odors), 2-phenylethanol (27), *(E)*-2-nonenal (18), phenylacetaldehyde (15), octanal (15),

dimethyl trisulfide (15), *(E)*-4,5-epoxy-*(E)*-2-decanal (15), δ-dodecalactone (15), lauric acid (15) and 3-methylbutanal (13.5), respectively.

In CCM3-AT, δ-decalactone with peachy and coconut-like odor ranked first in the FD value instead of δ-octalactone. δ-Octalactone had the FD value of 3,645 which was 3 times lower than that of δ-decalactone. In this case, this could contribute to the reduction of the coconut odor in the canned coconut milk after 3 months of storage in both conditions (Figure 12). High FD value of δ-decalactone might associate with higher intensities of the sweet and fruity-like notes instead of the herbaceous and coconut–like notes. The newly found aromas in CCM3-AT and their FD values were unknown 1 (0.5; dark chocolate odor), 2-methylbutanal (1.5), *(Z)*-2-penten-1-ol (0.5), heptanol (1.0), 2-acetylthiazole (1.0), unknown 4 (42.0; unripe, metallic, nutty and stale odors), *(E)*-4,5-epoxy-*(E)*-2-decanal (15.0) and ρ-cresol (1.5).

The most interesting aromas were unknown 4, *(E)*-4,5-epoxy-*(E)*-2-decanal and ρ-cresol. The odor characteristics of unknown 4, *(E)*-4,5-epoxy-*(E)*-2-decanal were metallic and unripe odor. These two aroma compounds had high FD values that could make the noticeable difference in the overall odor. ρ-Cresol was discovered only in stored canned coconut milk in both conditions. Although the FD value of ρ-Cresol in CCM3-AT and CCM340 C was as low as 1.5 (Table 11), but its odor characteristic of dung and stable could lead to the off-odor in canned coconut milk during storage.

On contrary, few aroma compounds that were lost after storage for 3 months in the ambient temperature were 2,3-butanedione, ethylbutanoate, ethyl-3-methylbutanoate, unknown 2, 1-(methylthio)ethanethiol, 3-methyl-1-butanol, 2-methyl-3-furanthiol, unknown 3, 2-decanone, 2-nonanol, 3-mercapto-3-methyl-1-butanol, α-humulene, 2-acetyl-2-thiazoleline, 2-undecanal, unknown 5 and unknown 6. The reduction of sulfur aroma compounds after storage was expected. The decrease in FD values of sulfur-containing compounds during storage might be corresponded

to the decrease in meaty aroma detected in the sensory analysis (Figure 12). Similar phenomenon is observed in the UHT-soy milk (Lozano *et al*., 2007a) and the orange juice (Dreher *et al*., 2003). Even in the chilled storage, the UHT-soy milk had lost in the sulfur note (Lozano *et al*., 2007a).

The most interesting compound for the meaty note in the headspace of canned coconut milk was 2-methyl-3-furanthiol. As mentioned, 2-methyl-3-furanthiol is the degradation product of vitamin B1 (thiamin) or the product from Maillard reaction. The sensory scores of the meaty note in CCM3-AT decreased from 5.75 in CCM0 to 3.0 in CCM3-AT (Figure 12). The loss of 2-methyl-3-furanthiol could be a result of oxidation reaction. 2-Methyl-3-furanthiol is rapidly oxidized to form *bis*(2-methyl-3-furyl)disulfide due to its high antioxidant activity. This activity is related to the abstraction of a hydrogen atom from the thiol group of 2-methyl-3-furanthiol (Hoffmann *et al*., 1996). On the other hands, the loss of 2-methyl-3-furanthiol might be related with the pH of coconut milk. In the model study of products from thiamin degradation, 2-methyl-3-furanthiol was missing at pH 7 and higher. Instead, the acidic pH of 1.5 is preferable for the formation of 2-methyl-3-furanthiol and other furan derivatives (Güntert *et al*., 1992). In canned coconut milk, the pH slightly increased after 3 months of storage (Table 14). At pH 6.32, it might be unfavorable condition for neither being stable compound nor the formation of 2-methyl-3-furanthiol.

The reduction of the potato note after storage was pointed out. Previously, methional was expected to be the major compound responsible for the potato note. Contrary, the FD value of methional in CCM3-AT was equal to those in CCM0. There could be other compounds that were responsible for the potato note. Interestingly, there was no compound with the potato-note characteristic found in the GC-O analysis. Therefore, the masking effect that reduced the detection of the potato note by other aroma compounds could be one of the influential factors. The GC-O analysis is the technique that the panelists sniff the aroma compounds one by one. The sensory analysis is the test that the whole profile of aroma compounds is evaluated at one time.

To ascertain the influence of methional on the potato note, the omission test on aroma model is suggested to investigate. Loss of ketones, aldehydes and alcohols was also found in the UHT soy milk, and had no correlation with the sweet note of the stored soy milk (Lozano *et al*., 2007a). In canned coconut milk, storage did not have a dominant effect on loss of fruity note (Figure 12). The sensory intensity scores of the fruity note in CCM0 and CCM3-AT (Figure 12) slightly decreased from 2.0 to 1.5. Lozano *et al*. (2007b) suggested that the formation of lipid oxidation and hydroxy acid products contributed to the loss of the fresh note of butter during storage. This reaction should be considered if canned coconut milk will be stored for a longer time.

To accelerate the storage condition, canned coconut milks were stored at 40±1 °C in the hot air oven for 3 months. The temperature was checked every 3 days. Previously, the accelerated storage conditions had been pre-studied and the 40±1 °C was chosen over the 35, 45 and 50 °C conditions. The reason was, at 40 °C, the overall odor of the CCM3-40C sample was similar to those of the canned coconut milk sample stored for 6 months in the first experiment. The physical properties of the canned coconut milk sample were similar to those from the supermarket shelf-stored condition. The cans had the regular shape, and no leak was found on the cans at this storage condition. The aroma compound with the highest FD value was δ-decalactone (1,458) which was the same as in CCM3-AT (Table 11). Aroma compounds with high FD values (≥9) in the order of retention indices were 3-methylbutanal (15.0), hexanal (15.0), dimethyl trisulfide (162.0), methional (486.0), *(Z)*-2-nonenal (9.0), *(E)*-2-nonenal (9.0), 2-acetylpyrazine (162.0), 2-methyl-3-(methyldithio)furan (162.0), (*E,Z*)-2,4-decadienal (18.0), 2-acetyl-2-thiazoleline (54.0), unknown 4 (9.0), guaiacol (18.0), 2-phenylethanol (9.0) and δ-octalactone (135.0). The compounds that had been firstly detected in CCM3-40C were 2-acetylpyrazine (162), 3,5-octadienone (4.5), *1H*-indole (1.0) and skatole (5.0).

Pyrazines are the widely distributed compounds in food products, especially the food processed with high temperature and low water content. It is known that 2-acetylpyrazine is an important compound that provides the roasty

characteristic in nuts and related products. High FD value (162) of 2-acetylpyrazine could produce a significant effect on the nutty note in CCM3-40C. However, the sensory score of nutty note had slightly increased from 4.5 to 4.75 in the CCM0 and CCM3-40C (Figure 12). Chen and Ho (1999) have proposed the formation pathway of 2-acetylpyrazine in the serine/threonine/glutamine-ribose/glucose/fructose model system. They have suggested that the retro-aldol condensation products from monosaccharides are important intermediates of 2-acetylpyrazine. 2-Acetylpyrazine then forms α-acetyl-*N*-heterocyclic acetylpyrazine with amino acid(s). From this information, 2-acetylpyrazine should be found in CCM0. This compound, however, was detected only in CCM3-40C but not in CCM0 and CCM3-AT. It suggested that 2-acetylpyrazine might not form during the canning process, or it could be form in very low amount that was not enough for the panelists to be able to detect. In our case, 2-acetylpyrazine formed when the sample was kept at 40 °C. Schieberle (1995) has found that 2-acetylpyrazine in roasted popcorn was stable in the storage condition of 40 °C.

3,5-Octadienone is the lipid oxidation product in corn tortillas (Buttery and Ling, 1995). *1H*-indole and skatole were detected in the GC-O analysis. In dairy products, these two compounds are the metabolites of amino acid tryptophan (Mottram, 1998). They have a potential to be contributors of undesirable flavor in the stored nonfat dry milk (Karagul-Yuceer *et al.*, 2002) and the sweet whey powder (Mahajan *et al.*, 2004). In addition, indole is reported as the Maillard reaction product of tryptophan and a reducing sugar in the model analysis (Yaylayan and Forrage, 1992). The most interesting point of these two aroma compounds is their odor characteristics are undesirable. The characteristics of fecal and mothball might contribute to the off-odor in the canned coconut milk that had been stored for a long time. Even though their odor characteristics were not correlated to the unknown odor in the sensory analysis, these compounds should be paid attention to as they had a potential of being the off-odor compounds in the canned coconut milk.

Apart from the off-note compounds, the sensory attributes of CCM3-40C was not much different from CCM0 and CCM3-AT (Figure 12). Only the

sensory intensity scores of the meaty and the potato notes had reduced after storage for 3 months at 40 °C. And again, methional had a strong potential to be an aroma character compound for the potato note. The FD value of methional reduced from 729 in CCM0 to 486 in CCM3-AT. The similar reduction in the meaty note was also detected.

Storage at 40 °C for 3 months increase the FD values sulfur-containing compounds. Dimethyl trisulfide increased the FD value from 18.0 in CCM0 to 162.0 in CCM3-40C. The other sulfur-containing compounds that dramatically increased in their FD value were 2-methyl-3-(methyldithio)furan and 2-acetyl-2-thiazoleline. According to the result of the increase in the FD values of CCM3-40C, the intensity of the meaty note should be the same as those of CCM0, but the meaty note had decreased in the intensity score from 5.75 to 3.25 (Figure 12). It was possible that, other sulfur containing compounds could play a dominant role on the meaty attribute of CCM3-40C. According to Table 11, other sulfur-containing compounds had decreased in their FD values. Furthermore, the rising of the concentrations of the oxidation products could mask the meaty aroma as was reported in the UHT soy milk (Lozano *et al*. (2007a).

2.2.4 The alteration of aroma compounds in acid fraction of coconut milks analyzed by DB-WAX column

In acid fraction (Table 12), 12 aroma compounds were identified. In fresh coconut milk (FCM), caproic acid with vinegar and sweaty odors had the highest FD value of 18, followed by butyric and 3-methyl butyric acids. Furaneol had the highest FD value in CCM0, whereas sotolon and syringol had the highest FD values in CCM3-AT and CCM3-40C, respectively. Most of aroma compounds in acid fraction had increased in their FD value after canning process, except 3-methyl butyric, caproic and caprylic acids.

Table 12 Aromatic compounds and their FD factors in acid fraction of coconut milk sniffed by 2 panels on DB-WAX column.

RI[1]		Compound[2]	Odor description[3]	FD Values [4]			
WAX	DB-5			FCM	CCM0	CCM3-AT	CCM3-40C
1434	≤700	Acetic Acid	vinegar	1.0	2.0	1.0	6.0
1551	793	Isobutyric acid*	Swiss cheese, cheesy	1.0	n.d	1.0	n.d.
1608	822	Butyric acid*	cheesy, fecal	9.0	18.0	9.0	42.0
1652	870	3-Methyl butyric Acid*	cheesy, fecal	9.0	6.0	54.0	126.0
1833	1035	Caproic acid*	vinegar, acid, sweaty	18.0	15.0	9.0	54.0
2002	1080	Furaneol	burnt sugar	n.d.	135.0	n.d.	54.0
2040	1200	Caprylic aid*	waxy, paraffin	6.0	2.0	3.0	9.0
2165	1090	Sotolon	curry	1.0	2.0	162.0	122.0
2233	1381	Nonanoic acid*	waxy, green	n.d.	1.0	n.d.	n.d.
2315	n.d.	Syringol	phenolic, inky	n.d.	n.d.	45.0	243.0
2498	n.d.	Phenylacetic acid*	rosy	n.d.	n.d.	3.0	2.0
2526	1424	Vanillin	vanilla	6.0	18.0	27.0	54.0

[1] Retention index of DB-WAX column (15 m length, 0.32 mm ID and 0.25 µm film thickness, Restek, Bellefonte, PA), and RTX-5 SLIMS column (15 m length, 0.53 mm ID and 1.5 µm film thickness, Restek, Bellefonte, PA)

[2] Compound; identified by comparing RI, odor characteristic, and * with authentic compounds

Table 12 (Continued)

[3] Odor description; reported by 2 panels on GC-O analysis

[4] FD Values, dilution factor calculated by mean value of 2 panels

n.d. = not detected, FCM = fresh coconut milk, CCM0 = canned coconut milk after canning process, CCM3-AT = canned coconut milk stored for 3 months at ambient temperature, and CCM3-40C = canned coconut milk stored for 3 months at 40 °C

The aroma compounds with the cheesy note had high FD values in CCM0, CCM3-AT and CCM3-40C. Lipolysis could be the major reaction that generated short-chain fatty acids. However, the cheesy note from the short chain fatty acids was not detected in the sensory analysis. The possible reason was these fatty acids could be masked by other aroma compounds that had strong odor intensities, or they lost from the headspace of the sniffing bottle due to their high volatility. However, these cheesy note compounds could be the crucial factor on the retronasal perception in the canned coconut milk that stored for a long period.

Furaneol was detected in CCM0 and CCM3-40C, but not in CCM3-AT. It could be noted that the temperature of canning process and the storage at 40 °C for 3 months affected the presence of furaneol. Furaneol could form at temperatures over 40 °C. Moreover, this compound has been reported as being a pH sensitive. In buffer solution, furaneol had the longest half-life at pH 3.5 for 141 days in the dark and sealed vial. At pH of 5.0 and 6.5, furaneol has half-life values of 28 and 10 days, respectively (Roscher *et al*., 1997). The pH values of CCM3-AT and CCM-40C were 6.32 and 6.15 (Table 14), respectively. On this basis, loss of furaneol in CCM3-AT was related to the pH sensitive properties of furaneol. Furaneol has a very low odor threshold (5.0 ppb in water), which had high impact for overall aroma perception. The precursor of furaneol is the degradation product of 6-deoxy sugar in the presence of amines or amino acids (Schieberle, 1992). The loss of furaneol might contribute to the loss of sweet and fruity notes in the canned coconut milk after storage. Sotolon was detected in all canned coconut milks, and the increase of the FD values had been observed during storage. The characteristic of curry and spice note of sotolon could enhance the off-odor.

Another interesting aroma compounds in the acid fraction were syringol and phenylacetic acid. They were detected only in CCM3-AT and CCM3-40C. Their odor characteristics could cause the difference among the fresh, the processed and the stored canned coconut milk samples.

Vanillin is one of the most of interesting compounds in the acid fraction. The FD value had intensified from 18.0 in CCM0 to 27.0 and 54.0 in CCM3-AT and CCM3-40C (Table 12), respectively. Vanilla odor was assumed to be a pleasant odor in canned coconut milk. It is known that ferulic acid is precursor of vanillin via biogenesis pathway and heating process (Korthou and Verpoorte, 2007; Belitz *et al.*, 2009). The phenolic compound in coconut oil extracting from coconut milk at 100-120 °C was reported by Saneviratne *et al.* (2009). Ferulic acid can undergo the thermal degradation to form vanillin (Belitz *et al.*, 2009). The increase in the FD value of vanillin might correlate to the phase separation of lipid and aqueous phases. Because vanillin is the water soluble compound, the loss of the emulsion stability of the coconut milk system after canning process and during storage could enhance the recovery of vanillin. The loss of emulsion stability of canned coconut milk was confirmed by the separation of cream and coconut skimmed milk layer which the height of cream layer increased as a function of storage time and temperature.

2.2.5 The alteration of aroma compounds focusing on sulfur- and nitrogen-containing compounds in neutral/basic fraction of coconut milks analyzed by RTX-5 SLIMS column

In this study, the sulfur containing compounds gained attention due to the very low odor thresholds and the meaty, sulfurous and cooked odor characteristics. In the case that the meaty note was presumed as the off-odor, the sulfur-containing aroma compounds had to be focused. In this study, sulfur aroma compounds were better separated on the non-polar column (RTX-5 SLIMS) than the polar column (DB-WAX). Hence, the AEDA was conducted on the RTX-5 SLIMS column to focus on sulfur-contain aroma compounds and the aroma compounds not found when the DB-WAX column was used.

Only the neutral/basic fraction containing sulfur aroma compounds was analyzed. Fifty-seven aroma compounds included 4 unknowns (unknown 7-10) were identified (Table 13). Sulfur-containing aroma compounds

detected only in RTX-5 SLIMS column, in the order of retention indices, were 2,4-dimethylthiazole, 2-methyl-4,5-dihydro-3-furanthiol, 2,5-dimethyl-3-furanthiol, formyl methyl thiophene, dimethyl tetrasulfide and 2-methyl-3-furyl 2-oxopropyl disulfide. Nitrogen-containing aroma compounds found only in RTX-5 SLIMS column were 2,6-dimethyl pyrazine, 2-acetylpyridine, 2-acetylpyrrole, 2-isobutyl-3-methoxypyrazine, 2-ethenyl-ethylmethylpyrazine and methyl-o-aminobenzoate. 2-Acetyl-2-thiazoline and formyl methyl thiophene were nitrogen and sulfur aroma compound detected only in RTX-5 SLIMS column. The others were *(2S)*-methyl-ethyl butanoate, ethyl pentanoate, 2-octanol, methyl hexanoate, *(E)*-2-octenal, δ-hexalactone, 3-nonenal, γ-heptalactone, *(E,Z)*2,4-nonadienal, ethyl octenoate, *(E,E)*-2,4-nonadienal, *(E)*-2-undecenal, γ-nonalactone and *(Z)*-6-dodecenyl-γ-lactone. It could be proved that the non-polar column showed the better recovery and separation of sulfur and nitrogen-containing compounds than the DB-WAX column by these results. Nonetheless, 4 sulfur containing aroma compounds were not detected in RTX-5 SLIMS. The identification of aroma compounds in this study needed the retention indices from two columns to confirm. The most interesting result from RTX-5 SLIMS column was the FD values of the sulfur containing compounds in CCM0. FD values of most sulfur-containing aroma compounds analyzed by RTX-5 SLIMS showed the greater values than those in the DB-WAX column. 2-Methyl-3-furanthiol, showed the greater FD value of 18.0 in the RTX-5 SLIMS instead of 9.0 in the DB-WAX column. Interestingly, it was found in CCM3-40C which implied that the high temperature or longer period of storage might cause the formation of 2-methyl-3-furanthiol.

Other important compounds were 2-methyl-3-(methyldithio)furan, dimethyl tetrasulfide and 2-methyl-3-furyl 2-oxopropyl disulfide. The presence of high FD values of these compounds in CCM0 and CCM3-AT was observed (Table 13). The perception of these compounds in CCM3-AT might be responsible for the meaty note. As mentioned above, most sulfur containing aroma compounds found in the DB-WAX column were lost after 3 months of storage, but the meaty note was still detected in the sensory analysis. 2-Methyl-3-(methyldithio)furan, dimethyl tetrasulfide and 2-methyl-3-furyl 2-oxopropyl disulfide could be aroma impact compounds for the meaty note in the canned coconut milk

during storage. Moreover, 2,5-dimethyl-3-furanthiol with a meat-like and sulfurous odors had raised in the FD value from 6 in CCM0 to 243 in CCM3-40C. Therefore, the storage condition might accelerate the formation of this compound. The precursor of 2,5-dimethyl-3-furanthiol in extruded potato snack is furyl alcohol, one of the main Maillard reaction products (Majcher and Jelen, 2009).

Pyrazines, 2-acetylpyridine and 2-acetylpyrrole were detected in CCM3-AT using a non-polar column. According to Mottram and Madruga (1994), pyrazine is formed in the meat model system at the pH higher than 5.5, and the preferable pH condition is in the range of basic system. The pH of canned coconut milk was higher than 5.5, and tended to slightly increase after storage from 5.96 in CCM0 to 6.32 in CCM3-AT (Table 14). At that pH range, pyrazines and other Maillard reaction products was preferred to be formed. Therefore, nutty and meaty notes of pyrazines and Maillard reaction product should be expected in canned coconut milk during storage. However, their FD values were less than 9 that might have a low correlation to the aroma of canned coconut milk after the period of storage in this study. 2-Acetyl-2-thiazoline was detected after the canning process with FD value of 126. It was formed via Maillard reaction in the UHT milk (Colahan-Sederstrom and Peterson, 2005) and the UHT soy milk (Lozano *et al.*, 2007). The meaty note was undesirable in the processed milk and soymilk. According to Lozano *et al.* (2007), this compound has faded after storage which was in agreement with the result in this study. Addition of polyphenol, for example epicatechin, reduces the formation of 2-acetyl-2-thiazoline and the Maillard type aroma compounds in the UHT bovine milk (Colahan-Sederstrom and Peterson, 2005). Thus, natural occurring compounds that be stabilized at high temperature could play a rule in inhibiting Maillard reaction. Polyphenol should be considered as food additive to improve the odor quality of canned coconut milk.

The formation pathways of the interested compounds were as followed. 2-Methyl-3-(methyldithio)furan is reported as the important compound responsible for the meaty note in many food products, especially in meat products. Yang *et al*. (2012) has proposed the generation pathway of 2-methyl-3-

(methyldithio)furan via Maillard reaction. The heterocyclic ring structure of furan is derived from d-glucose sugar, and attaching amino acid is derived from dimethyl disulfide as functional group.

Dimethyl tetrasulfide had a very high FD value of 405, 486 and 378 in CCM0, CCM3-AT and CCM3-40C (Table 13), respectively. It was reported as the degradation product of thiamin in cheddar cheese (Singh *et al.*, 2003) or a degradation product of sulfur-containing amino acids in the baked potato (Duckham *et al.*, 2002). 2-Methyl-3-furyl-2-oxopropyl disulfide was produced via Maillard reaction in the meat model, which cysteine and methanthiol were presumed to be precursors of 2-methyl-3-furyl moiety structure of the compound in meat (Mottram and Madruga, 1994). The formation of 2,4-dimethyl thiazole, 2-acetylpyrrol and 2,6-dimethylpyrazine are through Maillard reaction in the L-cystein/D-glucose model system (Eiserich *et al.*, 1992)

From the results of both columns, sulfur-containing, nitrogen-containing and heterocyclic compounds that mostly were the thermal degradation products of either thiamin or amino acids had faded after 3 month of storage at ambient condition. This phenomenon was similar to that occurred in the processed milk and soy milk. Keeping canned coconut milk at 40 °C for 3 months stimulated the formation of Maillard reaction compounds with S-, N- and heterocyclic-involved. It could be implied that the storage condition at high temperature or longer time induced the formation of these compounds. The presence of these compounds could be related to the off-odor, and ultimately affected the consumer perception.

From the result in Table 13, γ-lactones and unsaturated aldehydes showed better detections in RTX-5 SLIMS column. Their FD values had increased after storage, especially at 40 °C. These compounds were mainly originated from lipid oxidation. The increase of aldehydes and other oxidation products was unexpected in the canned coconut milk that had the low oxygen environment. Aldehydes and other lipid oxidation compounds could have a masking effect on the meaty and nutty odors. In peanut, the occurrence of flavor fading involves to the

Table 13 Aromatic compounds and FD factors in neutral/basic fraction of coconut milk sniffed by 2 panels on RTX-5 SLIMS column.

RI[1] (RTX5)	Compound[2]	Odor description[3]	FD Values[4]			
			FCM	CCM0	CCM3-AT	CCM3-40C
≤ 700	2,3-Butanedione	cheesy, buttery	n.d.	1	1	n.d.
≤700	2-Methylbutanal	dark chocolate	9	n.d.	1	3
≤ 700	3-Methylbutanal	dark chocolate	3	42	54	123
725	3-Methyl-1-butanol	dark chocolate, malty	3	n.d.	2	5
799	Hexanal	green, grass	n.d.	n.d.	3	54
848	*(2S)*-Methyl-ethyl butanoate	fruity	n.d.	2	n.d.	n.d.
893	2-Methyl-3-furanthiol	meaty, canned tuna	n.d.	18	n.d.	2
897	2,6-Dimethyl pyrazine	meaty, nutty	n.d.	n.d.	n.d.	2
900	Ethyl pentanoate	fruity	18	18	14	15
905	Methional	potato	6	243	486	243
923	2-Acetyl-1-pyroline	popcorn, nutty	1	45	6	15
930	2,4-Dimethylthiazole	chicken broth, cabbage	n.d.	n.d.	3	2
938	2-Methyl-4,5-dihydro-3-furanthiol	meaty	n.d.	2	n.d.	n.d.
967	2,5-Dimethyl-3-furanthiol	meaty, sulfurous	n.d.	6	9	243
982	2-Octanol	green, citrus	1	3	n.d.	15

Table 13 (Continued)

RI (RTX5)	Compound	Odor description	FD Values [4]			
			FCM	CCM0	CCM3-AT	CCM3-40C
1000	Methyl hexanoate	floral, sweet	1	15	3	15
1002	Octanal	green, citrus-like	3	15	n.d.	135
1016	2-Acetylpyridine	popcorn	n.d.	n.d.	3	5
1025	2-Acetylthiazole	nutty, popcorn	n.d.	n.d.	27	n.d.
1036	2-Acetylpyrrole	nutty	n.d.	n.d.	3	n.d.
1044	Phenylacetaldehyde	rosy, plastic	1	15	18	6
1060	*(E)*-2-Octenal	melon, cucumber	n.d.	n.d.	1	42
1071	Octanol	pungent, green, citrus	9	18	162	6
1088	δ-Hexalactone	coconut	42	n.d.	135	n.d.
1088	Guaiacol	smoky, stale	n.d.	45	27	243
1096	3-Nonenal	citrus-like	n.d.	n.d.	1	n.d.
1101	2-Isobutyl-3-methoxypyrazine	milky, fresh milk	n.d.	n.d.	14	3
1104	2-Acetyl-2-thiazoline	nutty, popcorn	n.d.	126	2	5
1104	Nonanal	green, citrus	n.d.	n.d.	n.d.	5
1113	2-Phenylethanol	plastic, rosy	42	3	15	2

Table 13 (Continued)

RI (RTX5)	Compound	Odor description	FD Values [4]			
			FCM	CCM0	CCM3-AT	CCM3-40C
1115	Formylmethyl thiophene	meaty, roasted meat	n.d.	n.d.	n.d.	9
1134	γ-Heptalactone	raw nut, raw potato, meaty	n.d.	2	3	n.d.
1147	*(Z)*-2-Nonenal	stale, hay-like	1	n.d.	3	18
1161	*(E)*-2-Nonenal	stale, hay-like	1	n.d.	54	42
1168	2-Methyl-3-(methyldithio)furan	roasted meat, canned tuna	n.d.	162	54	243
1178	2-Ethenyl-ethylmethylpyrazine	earthy, mushroom	n.d.	n.d.	1	5
1189	2-Nonanol	green, metallic, bug-like	n.d.	n.d.	9	15
1198	*(E,Z)*2,4-Nonadienal	cucumber-like	3	n.d.	n.d.	2
1201	Ethyl Octenoate	floral, sweet	n.d.	n.d.	3	1
1215	Dimethyl tetrasulfide	cabbage, garlic	n.d.	405	486	378
1229	*(E,E)*-2,4-Nonadienal	cucumber-like	n.d.	n.d.	3	6
1242	Unknown 7	metallic, sweet, lactone	n.d.	n.d.	2	2
1259	γ-Octalactone	peachy, coconut	27	42	54	122
1278	4-Ethylguaiacol	cloves, sweet	n.d.	n.d.	5	n.d.
1284	δ-Octalactone	herbaceous, coconut	2,187	1,458	3,102	1,215

Table 13 (Continued)

RI (RTX5)	Compound	Odor description	FD Values [4]			
			FCM	CCM0	CCM3-AT	CCM3-40C
1292	*(E,Z)*-2,4-Decadienal	stale	405	729	405	123
1304	*(E,E)*-2,4-Decadienal	stale	n.d.	n.d.	6	3
1316	2-Aminoacetophenone	stale, musky	9	81	126	486
1332	Methyl-o-aminobenzoate	honey-like	9	n.d.	2	1
1353	Unknown 8	meaty, brothy	n.d.	n.d.	5	1
1360	*(E)*-2-Undecanal	cilantro	n.d.	n.d.	1	3
1365	γ-Nonalactone	peachy	9	6	14	42
1372	Capric acid	soapy	n.d.	n.d.	6	6
1381	*(E)*-4,5-Epoxy-*(E)*-2-decenal	metallic, unripe	n.d.	n.d.	9	42
1392	Unknown 9	sulfurous	n.d.	18	27	365
1396	β-Damascenone	apple sauce	n.d.	18	3	3
1448	Unknown 10	spicy, clove	n.d.	n.d.	2	41
1458	2-Methyl-3-furyl 2-oxopropyl disulfide	roasted meat, sulfurous	n.d.	54	15	n.d.
1495	δ-Decalactone	peachy, coconut	2,187	12,822	10,205	4,074

Table 13 (Continued)

RI (RTX5)	Compound	Odor description	FD Values [4]			
			FCM	CCM0	CCM3-AT	CCM3-40C
1519	γ-Decalactone	peachy	n.d.	n.d.	5	n.d.
1699	*(Z)*-6-Dodecenyl-γ-lactone	peachy, cheery, lactone	9	27	41	45

[1] Retention index RTX-5 SLIMS column (15 m length, 0.53 mm ID and 1.5 µm film thickness, Restek, Bellefonte, PA)

[2] Compound; identified by comparing RI, odor characteristic, and with authentic compounds (in some compounds)

[3] Odor description; reported by 2 panels on GC-O analysis

[4] FD Values, dilution factor calculated by mean value of 2 panels

n.d. = not detected, FCM = fresh coconut milk, CCM0 = canned coconut milk after canning process, CCM3-AT = canned coconut milk stored for 3 months at ambient temperature, and CCM3-40C = canned coconut milk stored for 3 months at 40 °C

increment of aldehydes which created the odor masking effect on pyrazine (Reed *et al*., 2002). Moreover, Song *et al.* (2012) has described that, the presence of lipids or fatty acids contributed to the formation of O- and S-heterocyclic structure of compounds, such as thiophenes, formylthiophenes and pyridines with long-chain alkyl substituent. They also mention that alkyl groups of these compounds are usually derived from aliphatic aldehydes from lipid oxidation.

Table 14 The pH of coconut milks.

Sample	pH*
Fresh coconut milk	5.90
Canned coconut milk after canning process	5.96
Canned coconut milk after 3 months of storage at ambient temperature	6.32
Canned coconut milk after 3 months of storage at 40 °C	6.15

* pH was obtained from 3 replications.

2.2.5 The pH of coconut milks

The pH of fresh and canned coconut milk was shown in in Table 14. Based on the results of GC-O analysis, pH played a crucial role in the stability of aroma compound, such as furaneol. Moreover, pH also has influenced on formation pathways of aroma compound, especially Maillard reaction which is one of the main reactions affecting the aroma profile of canned coconut milk. The pH shows a crucial effect on the Maillard reaction when the Amadori compound has been formed (Nursten, 2005). The example of pH influencing the formation of compounds is pyrazine. According to Mottram and Madruga (1994), pyrazine is formed in the meat model system at the pH higher than 5.5, and the preferable pH condition is in the range of basic system. The pH value also has influenced on the conformation structure of protein which is contributed to the interaction of protein and aroma compound. Firstly, the pH of canned coconut milk was expected to be decrease as the function of

released free fatty acid, but pH of canned coconut milk had slightly increase after storage, especially at ambient temperature. However, the released fatty acids in coconut milk are weak acids. They dissociated partially, yielding little amount of H^+ (or H_3O^+). As such, pH of coconut milk did not decrease conflicting to the first expectation. The increase of pH in canned coconut milk after 3 months of storage could be contributed to basic compounds which dissociated to yield OH^- ion or basic condition of solution. It is well known that some compounds contributing to basicity of solution have no OH^- in their structure, such as NH_3 or Na_2CO_3. Moreover, amine groups of amino acid also related to the basicity of the solution. Alkyl group created an effect on pH increasing, as the K_b increase with more number of alkyl group on amine. On the basic of this knowledge, amino acids were majorly suspected affecting the increase of pH in canned coconut milk.

3. GC-MS analysis

3.1 Volatile compounds in the headspace of canned coconut milk

Upon heating, several of original sensory attributes have lost or diminished, and new sensory descriptors eventually appear. The odor perception of food product is retrieved as orthonasal and retronasal olfactions. In this study, headspace analysis was used as a tool to clarify the orthonasal olfaction. The aroma of fresh coconut milk (FCM) is weak and barely detected. In fact, the aroma contributor of coconut milk is readily recognized after heating. Moreover, GC-O headspace analysis using SPME and static headspace technique for the FCM did not exactly represent its aroma profile, because the FCM sample was incubated at temperatures ranged between 35 and 40 °C for at least 20 min during incubation and isolation to liberate the volatile compounds into headspace. Therefore, the aroma perception of FCM was lost and altered during the incubation and isolation. Even though, salt was added to inhibit enzymatic reaction, the acidic odor was still observed in FCM. Hence, from my point of view, the aroma of FCM quickly changed and the SPME-headspace was not suitable for the analysis of FCM.

Therefore, the GC-O headspace analysis was studied only in the canned coconut milk samples. The headspace analysis of canned coconut milk was aimed to monitor the headspace aromas that affected the first perception when the can was opened. The headspace analysis was focused on monitoring the dominant notes and sulfur-containing aroma compounds in air above liquid phase of canned coconut milk. Even though, the GC-O headspace using the static headspace-cryogenic focusing technique to analyze 20 mL of headspace volume showed a good result for identifying headspace aroma compounds in canned coconut milks, this technique could not be used with GC-MS, due to the requirement of the vacuum condition of the MS. Therefore, the SPME technique was employed to quantify the headspace volatile compounds of the canned coconut milk samples.

The result of the headspace GC-MS analysis is shown in Table 15 and 34 volatile compounds were identified. The volatile compounds were classified as 10 esters, 8 acids, 7 alcohols, 6 ketones, 2 lactones and 1 sulfide. Their molecular weights ranged between 46 to 200 Da. The most abundant compound was ethanol (1,006.47±81.2 ppb), followed by caproic acid (604.94±16.0 ppb), acetic acid (445.94±101.8 ppb), pentanoic acid (368.24±20.7 ppb) and δ-octalactone (196.64±7.5 ppb). After integrating results from 2 Tables (Table 10; GC-O result and Table 15; GC-MS result), only dimethyl trisulfide and δ-octalactone were detected in both GC-O and GC-MS headspace analyses. On contrary, the other focused compounds, contributing to meaty note (2-methy-3-furanthiol), popcorn (2-acetyl-1-pyrroline) and coconut-like (γ-octalactone) were not found by GC-MS. The difference in the isolation technique and the sensitivity of mass spectrometer and the human perception played a role on this occurrence.

According to Table 15, most of the detected compounds including esters, acids, ketones, aldehydes and lactones, were parentally from lipid composition. Only few compounds such as, dimethyl trisulfide and alcohols, have been reported to be generated from amino acid (Belitz *et al*., 2009). Due to a small number of detected volatile compounds, especially sulfur and nitrogen containing aroma compounds, the SPME technique was not suitable for isolating aroma compound in the headspace of

coconut milk samples. The SAFE-GC-MS technique, therefore, was used to isolate volatile compounds in the canned coconut milk in this work.

Table 15 Volatile compounds of canned coconut milk after canning process (CCM0) isolated by SPME technique.

#[1]	RI[2]	Compound[3]	Conc.[4] (ng/g; ppb)	Standard diviation
1	849	Ethyl acetate	72.45	3.5
2	862	2-Butanone	130.90	0.8
3	904	Ethanol	1,006.47	81.2
4	937	2-Pentanone	120.00	1.4
5	1006	Propanol	12.53	4.3
6	1052	Methyl pentanoate	22.83	3.2
7	1057	Isobutyl isobutanoate	31.32	4.1
8	1108	Isopropyl pentanoate	27.81	1.3
9	1120	Butanol	133.73	22.6
10	1161	Methyl hexanoate	188.64	0.4
11	1192	Butyl butanoate	36.13	7.5
12	1198	4-Octanone	65.35	3.7
13	1221	Pentanol	8.09	1.8
14	1246	3-Hydroxy-2-butanone	40.24	1.6
15	1280	4-Nonanone	9.14	1.0
16	1303	Hexanol	15.74	1.5
17	1320	Dimethyl trisulfide	0.38	0.0
18	1338	Methyl octanoate	36.37	4.9
19	1372	5-Decanone	4.26	1.1
20	1381	Ethyl octanoate	40.45	0.4
21	1403	Acetic acid	445.94	101.8
22	1435	2-Ethyl-1-hexanol	14.61	0.5
23	1456	Pentyl hexanoate	15.28	0.1
24	1515	2-Methyl-propanoic acid	70.45	0.7
25	1573	Butyric acid	136.08	6.4
26	1580	Ethyl decanoate	8.64	2.3

Table 15 (Continued)

#[1]	RI[2]	Compound[3]	Conc.[4] (ng/g; ppb)	Standard deviation
27	1613	3-Methyl-butyric acid	35.10	1.9
28	1636	γ-Hexalactone	15.42	1.6
29	1679	Pentanoic acid	368.24	20.7
30	1784	Caproic acid	604.94	16.0
31	1893	δ-Octalactone	196.64	7.5
32	1941	Phenol	3.93	0.6
33	1991	Caprylic acid	121.92	14.4
34	>2000	Benzoic acid	34.99	2.0

[1] No.; number of compound is corresponding to Figure C4 (Appendix C).

[2] Retention index Stabilwax (30 m length, 0.25 mm ID and 0.25 µm film thickness, Restek, PA).

[3] Compound; identified by comparing RI, and mass spectrum libraries.

[4] Relative concentration; calculated by comparing with internal standard compounds.

3.2 Volatile compounds of SAFE extracts

The aim of this part of the work was to identify and quantify the aroma compounds found in coconut milk. Moreover, the alteration of volatile profiles after canning process and storage at the ambient temperature of 23 °C and at 40 °C were discussed.

Quantitative analysis of volatile extracts, from fresh, canned coconut milk after process (CCM0), canned coconut milk after 3 months of storage at ambient temperature (CCM3-AT) and canned coconut milk after 3 months of storage at 40 °C (CCM3-40C), were carried out on the GC-MS instrument. Volatile extracts from the neutral/basic and the acid fractions were injected into a polar column and a non-polar column.

In this part, the solvent assisted flavor evaporation (SAFE) technique was used to extract the volatile compounds instead of direct solvent extraction (DSE) that had been used in the early experiment. SAFE provided a better recovery of volatile compounds in coconut milk than that of DSE. In DSE, the polarity of the solvent influenced the types of volatile compounds being extracted. The partition coefficients (or distribution constant) of volatile compounds played a crucial role on the DSE method. Volatile profiles were varied by the selected type of solvents. As previously mentioned, diethyl ether showed the best recovery of volatile compounds in coconut milk. On the other hands, the SAFE technique relies on the volatility of compounds. By applying high pressure to suppressed the evaporation of volatile compounds in the complex food system, more varieties of volatile compounds were extracted. The SAFE technique is, therefore, recommended to extract volatile compounds from an emulsion food system.

Moreover, the stable isotope dilution assay (SIDA) was used in this experiment to provide accurate quantification of the interested compounds. Basically, to achieve a high accurate amount of a volatile compound, the internal standard compound must be a chemical and physical mimic of the analyst. Therefore, isotopically labeled analogues of the analysts are considered to be the best choice (Fay *et al.*, n.d.).

From Table 16, 117 volatiles were discovered in the neutral/basic fraction including 30 alcohols, 19 aldehydes, 18 esters, 14 ketones, 12 lactones, 8 acids, 3 terpenes, 3 furans, 3 thiophene, 2 thiazoles, 2 sulfides, 1 pyrazine, 1 phenol and 1 pyrrole. Lactones had the highest amount among other compounds in all coconut milks, followed by alcohols, ketones, ester, acids, aldehydes and others (Figure 13). From Table 17, 13 volatile compounds were discovered in the acid fraction. The dominant compounds in acid fraction were caproic and caprylic acids.

3.2.1 The volatile compounds in neutral/basic fraction of fresh coconut milk (FCM)

In the neutral/basic fraction of the FCM, 82 volatile compounds were identified (Table 16). δ-Decalactone had the highest amount (1,493.58 ppb), followed by octanol (954.78 ppb), δ-dodecalactone (649.38 ppb), ethyl octanoate (515.91 ppb) and δ-octalactone (467.83 ppb), respectively. Lactones possessed the highest amount among groups of compounds, followed by alcohols and acids (Figure 13). Lactones are important odorants in coconut and related product. The sensory score for coconut odor in FCM, CCM0, CCM3-AT and CCM3-40C were 2.25, 6.50, 5.50 and 5.50, respectively (Figure 12). The amount of lactone in FCM, CCM0, CCM3-AT and CCM3-40C were 2,701.43, 8,825.21, 7,693.09 and 6,235.10, respectively (Table 16). The sensory intensitis of coconut odor relatively contributed to the amount of lactone. This data could be applied when creating the odor model of coconut milk, and could be used to predict the amount of lactone with sensory score.

The aroma compounds in the FCM could be formed from natural occurring and formed during the preparation and extraction of coconut milk that mainly involved enzymatic reactions and lipid oxidation. The detection of acids and aldehydes in FCM could be affected by the enzyme activities. Two most discussed enzymes in coconut are lipase and lipoxygenase. Lipase activity, assayed by the titrimetric method of FCM after extraction, was 2.77% that liberated free fatty acid (Waisundara *et al*., 2007). Lipases hydrolize ester bonds in lipid and produce free fatty acids, especially short- and medium-chain fatty acids that attributed to the flavor of coconut milk. Furthermore, the released unsaturated fatty acids went through further reactions and formed aroma compounds, such as aldehydes, esters and lactones that were found in this study.

Free fatty acids and triacylglycerols are capable of being oxidized via auto-oxidation and by the action of lipoxygenase and peroxidase. Lipoxygenase is inactivated at lower temperatures than peroxidase, and both enzymes contribute to the lipid oxidation. Many researches have reported the peroxide value

rather than the lipoxygenase activity. Waisundara *et al*. (2007) determined the peroxide value of fresh coconut meat before the extraction of coconut milk. The peroxidase activity had beed detected during the preparation and storage. The initial peroxide value of coconut milk extracting from fresh coconut meat was approximately 28 miliequivalent of peroxide oxygen per kg of oil, which was close to the onset of rancidity at 35.5 miliequivalent of peroxide oxygen per kg of oil (Waisundara *et al*., 2007). Aldehydes, such as hexanal, octanal, decanal, *(E)*-2-nonenal and 2-undecenal found in FCM could be formed during preparation and coconut milk extraction by enzymatic oxidation.

Lactones presented wit the highest amount in FCM (Table 16 and Figure 13). The formations of lactones and acids in FCM attributed to lipase activity. The natural occurring hydroxy fatty acids were the precursors of lactones. Interestingly, alcohols presented in high amount, especially octanol (Table 16). There are several pathways of alcohol formation. One or more of these following pathways could be responsible for the formation of alcohols in FCM. The natural occurring alcohols in fruits mostly arise from the degradation of fatty acids during ripening and maturation. Three different oxidative routes are suggested by Christensen *et al.* (2007): (1) β-oxidation of acetyl-CoA, (2) oxidation by the lipoxygenase activity and (3) autoxidation. They also mentioned that lipoxygenase and autoxidation pathways typically produce aldehydes and alcohols responsible for the fresh and green notes. Volatile alcohols might form in plant by reductase activity. The precursors could be the corresponding aldehydes or ketones originated from the metabolism of fatty acids and amino acids (Sucan and Russell, 2002). Alcohols could be formed via another pathway of the carotenoid degradation as reported in tomato (Sucan and Russell, 2002).

Although, fermentation typically is the action of microorganism under anaerobic condition, spontaneous fermentation of coconut meat and milk was suspected to occur during the preparation without heating in this study. As reported by Seow and Gwee (1997), *Saccharomyces* spp. was the predominant yeast in coconut milk. In this study, ethanol which is the major product of fermentation was detected in

the headspace of the fresh and the canned coconut milk samples. *Saccharomyces* spp. is also found in coconut sap (Seow and Gwee, 1997). Spontaneous fermentation was found in the fresh coconut sap with 0.07% (by volume) of ethanol was produced. Additionally, alcohols that had higher molecular weights than ethanol have been reported in coconut sap (Borse *et al.*, 2007). Other alcohols found in the fresh coconut milk sample in this study could be the products of spontaneous fermentation process. Carbohydrtaes are the typical precursors of alcohols on fermentation. Coconut meat contains about 10% of carbohydrates, of which 50% is cellulose, and 75% of cellulose is α-cellulose (Rosenthal *et al.*, 1996). Carbohydrates in coconut milk are in the range of 3.5-8.1%, while sugars and starch are dominant (Seow and Gwee, 1997). These carbohydrates could be precursors of aroma alcohols via Embde-Meyerhof-Parnas pathway. From this pathway, pyruvic acid is formed, and goes further to yield isopropyl alcohol and *n*-propyl alcohol (Reineccius, 2006).

Noticeably, acids, aldehydes, esters and alcohols with carbon numbers of 8 and 10 were dominant within their groups (Table 16). This phenomenon agreed with that found in coconut oil (Pai *et al.*, 1979). The C8 and C10 compounds could be naturally occurred or formed during the preparation and extraction of coconut milk.

It is known that aldehydes, ketones and alcohols are the oxidative products of fats and oils. Volatile compounds are formed in the termination step of oxidation. Alkoxyl radicals ($RO^{\cdot}$) and peroxyl radicals ($ROO^{\cdot}$) and hydroperoxide, the primary products of lipid oxidation, are very unstable due to theirs excited state of radicals. The oxygen-oxygen bonds of hydroperoxide go further to the recombination or scission to yield aldehydes, ketones and alcohols (Shahidi, 2000; Schaich, 2005). The autoxidation could occur in the state of maturation of coconut meat. However, the higher rate of oxidative product formation could occur during the preparation of coconut milk. Once coconut meat was washed and grated, the accelerating factors such as, water and high amount of oxygen, were presented. Moreover, lipoxygenase induced oxidation readily when the cell membrane of coconut meat was broken. The

Table 16 Concentration of volatile compounds in neutral/basic fraction of fresh and canned coconut milks isolated by SAFE technique.

#[1]	RI[2]		Compound	Concentration (ppb; ng/g)							
				FCM		CCM0		CCM3-AT		CCM3-40C	
	WAX	SAC-5		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	≤1000	n.d.	Ethyl Acetate	37.34^{b}	14.8	40.17^{b}	12.9	75.27^{a}	19.8	57.41^{ab}	22.4
2	≤1000	n.d.	2-Methylbutanal	0.00^{ns}	0.0	1.68^{ns}	0.3	3.36^{ns}	2.4	3.03^{ns}	2.2
3	≤1000	n.d.	3-Methylbutanal	0.00^{b}	0.0	2.72^{b}	0.6	10.82^{a}	3.3	6.94^{a}	1.5
4	≤1000	n.d.	2-Pentanone	4.47^{b}	1.3	9.98^{ab}	3.5	18.29^{a}	7.4	23.47^{a}	9.1
5	≤1000	n.d.	Pentanal	0.00^{ns}	0.0	3.11^{ns}	2.1	14.58^{ns}	9.7	5.03^{ns}	7.2
6	≤1000	≤800	2-Hexanone	22.28^{a}	9.8	0.90^{b}	0.6	14.11^{ab}	12.2	3.14^{ab}	0.2
7	1026	n.d.	*(E)*-2-Butenal	6.40^{b}	4.0	25.38^{ab}	2.3	54.85^{a}	37.3	34.40^{ab}	8.9
8	1037	n.d.	*(Z)*-2-Butenal	3.73^{ns}	0.9	7.49^{ns}	1.7	16.25^{ns}	11.2	2.20^{ns}	2.4
9	1069	≤800	Hexanal	88.15^{a}	33.6	27.87^{b}	2.0	74.26^{ab}	28.9	130.01^{a}	41.7
10	1082	n.d.	2-Methyl-1-propanol	21.88^{b}	7.9	95.06^{ab}	11.6	214.85^{b}	142.7	116.96^{ab}	16.0
11	1113	n.d.	*(R)*-2-Pentanol	4.31^{b}	1.6	25.39^{ab}	2.8	45.56^{a}	29.5	25.26^{ab}	1.7
12	1114	n.d.	3-Methyl-2-butanol	0.00^{b}	0.0	0.00^{b}	0.0	0.00^{b}	0.0	4.00^{a}	2.0
13	1139	n.d.	Butanol	93.79^{b}	21.6	464.72^{ab}	56.6	751.61^{a}	81.3	394.40^{ab}	37.2
14	1175	888	2-Heptanone	149.83^{ns}	56.3	231.11^{ns}	33.4	385.38^{ns}	140.9	229.85^{ns}	4.0

Table 16 (Continued)

#[1]	RI[2]		Compound	Concentration (ppb; ng/g)							
				FCM		CCM0		CCM3-AT		CCM3-40C	
	WAX	SAC-5		Mean	SD	Mean	SD	Mean	SD	Mean	SD
15	1176	901	Heptanal	0.00[b]	0.0	0.00[b]	0.0	78.91[a]	35.6	40.60[ab]	15.5
16	n.d.	954	Benzaldehyde*	1.03[b]	0.2	3.54[b]	0.7	71.60[a]	28.7	112.99[a]	45.2
17	1185	1024	Limonene	39.75[a]	18.2	0.00[b]	0.0	0.00[b]	0.0	0.00[b]	0.0
18	1202	n.d.	3-Methyl-1-butanol	115.18[a]	39.3	135.45[a]	15.2	11.78[b]	7.9	144.76[a]	5.7
19	1209	n.d.	*(E)*-2-Hexenal	0.00[b]	0.0	0.00[b]	0.0	0.00[b]	0.0	4.92[a]	1.1
20	1217	n.d.	2-Hexanol	1.60[b]	0.6	1.03[b]	0.4	6.70[a]	2.6	6.15[a]	2.0
21	1219	n.d.	4-Octanone	6.02[a]	2.8	0.00[b]	0.0	0.00[b]	0.0	0.00[b]	0.0
22	1225	989	2-Pentyl-furan	1.26[c]	0.3	11.80[b]	2.1	28.48[a]	9.8	28.01[a]	8.9
23	1228	1000	Ethyl hexanoate	16.85[c]	6.3	21.64[bc]	3.4	48.39[a]	12.5	33.43[b]	9.2
24	1234	n.d.	1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene	0.00[b]	0.0	2.75[a]	0.5	7.97[a]	4.9	4.03[a]	0.3
25	1242	n.d.	3-Methyl-3-buten-1-Ol	3.21[ns]	1.7	1.89[ns]	0.2	4.51[ns]	3.3	2.46[ns]	0.1

Table 16 (Continued)

#[1]	RI[2]		Compound	Concentration (ppb; ng/g)							
				FCM		CCM0		CCM3-AT		CCM3-40C	
	WAX	SAC-5		Mean	SD	Mean	SD	Mean	SD	Mean	SD
26	1245	n.d.	Pentanol	10.14^{ns}	5.5	17.90^{ns}	2.4	31.37^{ns}	10.8	19.70^{ns}	4.2
27	1275	886	Cyclohexanone	0.13^{b}	0.1	10.05^{a}	0.6	34.25^{a}	16.5	11.41^{a}	1.6
28	1279	n.d.	2-Octanone	0.00^{b}	0.0	11.56^{a}	7.0	0.00^{b}	0.0	1.11^{b}	0.7
29	1282	1002	Octanal	17.41^{c}	6.7	10.46^{c}	7.8	96.53^{a}	37.9	58.46^{b}	9.7
30	1316	904	2-Heptanol	246.27^{ns}	85.8	358.11^{ns}	40.7	596.20^{ns}	160.3	360.92^{ns}	24.2
31	1321	n.d.	2,3-Octanedione	0.00^{b}	0.0	5.27^{a}	1.9	0.00^{b}	0.0	0.00^{b}	0.0
32	1330	n.d.	6-Methyl-5-hepten-2-One	1.72^{ns}	0.4	2.27^{ns}	0.5	3.41^{ns}	2.3	2.09^{ns}	0.3
33	1348	874	Hexanol	186.86^{ns}	62.1	257.94^{ns}	24.8	414.97^{ns}	94.5	243.06^{ns}	20.9
34	1366	960	Dimethyl trisulfide	0.00^{ns}	0.0	0.11^{ns}	0.0	0.22^{ns}	0.1	1.48^{ns}	0.9
35	n.d.	1057	*(E)*-2-Octenal*	0.00^{b}	0.0	0.13^{b}	0.1	0.00^{b}	0.0	9.70^{a}	3.0
36	1383	1090	2-Nonanone	340.88^{b}	90.2	574.60^{b}	106.2	975.56^{a}	279.4	626.74^{b}	80.9
37	1386	1101	Nonanal	28.12^{b}	8.4	74.02^{ab}	15.6	152.29^{a}	68.4	94.61^{ab}	8.6
38	1392	915	2-Butoxy-ethanol	5.50^{c}	1.5	110.35^{b}	4.8	303.99^{a}	77.8	134.89^{b}	35.8

Table 16 (Continued)

#[1]	RI[2]		Compound	Concentration (ppb; ng/g)							
				FCM		CCM0		CCM3-AT		CCM3-40C	
	WAX	SAC-5		Mean	SD	Mean	SD	Mean	SD	Mean	SD
39	1396	n.d.	*(E,E)*-2,4-Hexadienal	3.60[b]	1.9	8.28[b]	1.9	16.67[a]	8.1	7.90[b]	1.9
40	1403	n.d.	Butyl hexanoate	2.35[a]	1.7	0.00[b]	0.0	0.00[b]	0.0	0.00[b]	0.0
41	1404	n.d.	3-Ethy-2-methyl-1,3-hexadiene	0.00[b]	0.0	5.25[a]	1.0	0.00[b]	0.0	0.00[b]	0.0
42	n.d	1124	Methyl octanoate*	5.45[b]	1.0	3.76[b]	1.8	27.55[a]	3.1	32.57[a]	8.8
43	1431	1197	Ethyl octanoate	515.91[b]	96.9	311.63[b]	56.8	1,409.92[a]	384.0	945.65[a]	110.7
44	1433	1007	3-Ethyl-2,5-dimethylpyrazine	0.00[b]	0.0	0.00[b]	0.0	0.00[b]	0.0	19.30[a]	7.1
45	1444	987	1-Octen-3-ol	9.37[b]	3.9	10.06[b]	1.8	29.61[a]	12.2	30.48[a]	6.3
46	1447	903	Methional	0.00[b]	0.0	0.41[a]	0.0	0.26[a]	0.0	0.00[b]	0.0
47	1448	n.d.	Heptanol	11.45[c]	3.4	16.77[c]	2.7	50.30[a]	20.9	30.24[b]	4.4
48	1458	831	Furfural	0.00[b]	0.0	12.34[ab]	6.3	30.84[a]	17.3	22.67[ab]	1.7
49	1479	1042	2-Ethyl-1-hexanol	0.00[b]	0.0	0.00[b]	0.0	106.32[a]	71.5	86.44[a]	64.2
50	1484	n.d.	2-Decanone	9.25[a]	5.5	13.22[a]	5.1	1.67[b]	1.0	1.49[b]	0.1

Table 16 (Continued)

#[1]	RI[2]		Compound	Concentration (ppb; ng/g)							
				FCM		CCM0		CCM3-AT		CCM3-40C	
	WAX	SAC-5		Mean	SD	Mean	SD	Mean	SD	Mean	SD
51	1489	1204	Decanal	2.89^{b}	5.0	10.14^{b}	1.9	100.94^{a}	67.5	0.31^{b}	0.1
52	1513	n.d.	2-Nonanol	108.63^{d}	25.5	166.88^{c}	31.5	436.01^{a}	91.3	230.43^{b}	19.0
53	1525	1159	*(E)*-2-Nonenal	2.67^{b}	0.5	4.23^{ab}	0.9	0.00^{b}	0.0	13.77^{a}	7.9
54	1527	1295	Ethyl nonanoate	3.10^{a}	0.8	0.00^{b}	0.0	0.00^{b}	0.0	0.00^{b}	0.0
55	1536	n.d.	*Cis*-β-tepeniol	11.51^{a}	3.3	5.55^{ab}	1.0	7.56^{ab}	5.0	0.00^{b}	0.0
56	1556	1074	Octanol	954.78^{b}	274.2	1,536.01^{b}	304.8	2,358.06^{a}	327.9	1,620.59^{b}	321.2
57	1594	1291	2-Undecanone	368.81^{c}	116.4	1,031.17^{b}	200.3	1,708.92^{a}	388.5	1,121.85^{b}	178.8
58	1604	1346	Butyl octanoate	13.70^{ns}	4.4	15.43^{ns}	2.6	33.24^{ns}	21.7	25.39^{ns}	4.4
59	n.d.	1322	Methyl decanoate*	3.03^{ab}	0.4	1.73^{a}	0.7	12.60^{ab}	1.3	21.80^{a}	15.2
60	1631	1393	Ethyl decanoate	376.35^{c}	78.9	477.53^{c}	77.4	864.05^{a}	189.3	633.92^{b}	14.7
61	1633	1037	Phenylacetaldehyde	0.00^{a}	0.0	19.64^{b}	6.2	13.45^{b}	5.3	17.01^{b}	1.9
62	1636	1012	2-Acetylthiazole	0.00^{b}	0.0	27.96^{ab}	5.0	13.53^{ab}	9.0	57.59^{a}	25.8
63	1652	n.d.	Nonanol	10.68^{a}	2.5	0.00^{b}	0.0	0.00^{b}	0.0	0.00^{b}	0.0
64	1653	862	2-Furanmethanol	0.00^{b}	0.0	31.80^{ab}	8.3	77.89^{a}	52.3	52.60^{ab}	9.3

Table 16 (Continued)

#[1]	RI[2]		Compound	Concentration (ppb; ng/g)							
				FCM		CCM0		CCM3-AT		CCM3-40C	
	WAX	SAC-5		Mean	SD	Mean	SD	Mean	SD	Mean	SD
65	1667	1036	2-Hydroxy-benzaldehyde	0.00[b]	0.0	19.90[a]	2.8	0.00[b]	0.0	0.00[b]	0.0
66	1683	984	2-Formylthiophene	0.00[b]	0.0	3.68[ab]	0.7	46.10[a]	32.6	28.98[ab]	3.3
67	1688	n.d.	5-Ethyldithio-2(3H)-furanone	16.83[ns]	4.0	20.84[ns]	2.5	23.71[ns]	14.7	18.23[ns]	0.8
68	1704	n.d.	3-(Methylthio) propanol	2.59[a]	0.5	0.00[b]	0.0	0.00[b]	0.0	0.00[b]	0.0
69	1710	1318	2-Undecanol	114.11[b]	32.7	140.03[b]	24.3	214.65[a]	34.8	140.28[b]	15.0
70	1711	1073	3-Methyl-2-formylthiophene	0.00[b]	0.0	0.00[b]	0.0	7.35[a]	2.0	13.72[a]	8.0
71	1739	n.d.	2-Undecenal	5.24[b]	4.1	3.45[b]	0.7	19.75[a]	13.3	23.65[a]	9.2
72	1754	n.d.	Decanol	136.71[b]	37.0	164.12[b]	32.4	256.62[a]	54.9	169.12[b]	22.9
73	1779	1090	δ-Hexalactone	0.95[b]	0.3	9.66[a]	2.4	13.12[a]	4.2	7.01[a]	3.3
74	1791	1148	γ-Heptalactone	10.46[ns]	6.5	14.56[ns]	0.4	16.06[ns]	2.5	7.86[ns]	2.6

Table 16 (Continued)

#[1]	RI[2]		Compound	Concentration (ppb; ng/g)							
				FCM		CCM0		CCM3-AT		CCM3-40C	
	WAX	SAC-5		Mean	SD	Mean	SD	Mean	SD	Mean	SD
75	1800	1492	2-Tridecanone	13.35^{d}	6.7	75.63^{c}	10.0	157.50^{a}	45.8	97.43^{b}	36.8
76	1809	n.d.	Butyl decanoate	5.85^{ab}	2.7	0.00^{b}	0.0	10.51^{a}	5.6	7.50^{ab}	1.6
77	1839	1592	Ethyl dodecanoate	311.21^{b}	34.6	359.18^{b}	28.6	690.89^{a}	231.8	380.98^{b}	31.0
78	1840	1365	2-(2-Butoxyethoxyl)-ethanol acetate	0.00^{b}	0.0	97.49^{a}	13.3	0.00^{b}	0.0	0.00^{b}	0.0
79	1850	1088	Guaiacol	64.74^{b}	9.8	73.24^{b}	10.8	268.14^{a}	73.1	172.64^{a}	8.8
80	1869	n.d.	Benzyl alcohol	7.38^{ns}	1.8	6.90^{ns}	2.1	13.76^{ns}	9.4	8.31^{ns}	0.3
81	1903	1128	2-Phenylethanol	2.71^{b}	4.4	76.06^{ab}	66.8	275.88^{a}	184.8	171.55^{ab}	7.3
82	1935	n.d.	2-Thiophenemethanol	0.00^{b}	0.0	9.65^{a}	4.9	15.00^{a}	10.1	9.88^{a}	0.9
83	1938	1216	Benzothiazole	2.51^{ns}	2.5	10.46^{ns}	4.3	12.49^{ns}	8.8	6.69^{ns}	0.6
84	n.d.	1252	γ-Octalactone*	4.88^{b}	0.5	3.03^{b}	1.1	25.46^{a}	2.6	24.94^{a}	11.7
85	1960	1279	δ-Octalactone	467.83^{d}	45.7	1,861.59^{b}	674.1	2,611.33^{a}	710.1	940.74^{c}	394.3
86	1988	1261	5-Hydroxy-2-decenoic acid-δ-lactone	19.67^{b}	8.7	44.62^{b}	15.3	76.66^{a}	33.2	33.17^{b}	11.7

Table 16 (Continued)

#[1]	RI[2]		Compound	Concentration (ppb; ng/g)							
				FCM		CCM0		CCM3-AT		CCM3-40C	
	WAX	SAC-5		Mean	SD	Mean	SD	Mean	SD	Mean	SD
87	1994	1005	Phenol	10.74^{ns}	3.7	21.63^{ns}	2.7	33.28^{ns}	24.0	23.13^{ns}	1.3
88	2002	n.d.	2-Pentadecanone	1.40^{ns}	2.4	5.09^{ns}	0.9	4.56^{ns}	2.3	4.34^{ns}	0.2
89	2004	n.d.	Butyl dodecanoate	6.68^{a}	3.6	0.00^{b}	0.0	0.00^{b}	0.0	0.00^{b}	0.0
90	2011	1357	γ-Nonalactone	9.71^{ns}	0.6	8.91^{ns}	1.2	20.33^{ns}	12.1	13.18^{ns}	0.2
91	2014	n.d.	4-Ethylguaiacol	4.00^{a}	1.5	3.44^{ab}	1.5	1.16^{ab}	0.8	0.75^{b}	0.0
92	2025	n.d.	3-Phenyl-1-propanol	0.00^{b}	0.0	0.00^{b}	0.0	13.05^{a}	8.4	8.92^{a}	1.3
93	2033	1784	Ethyl tetradecanoate	29.40^{ns}	13.4	22.42^{ns}	7.2	41.06^{ns}	24.1	24.78^{ns}	9.3
94	2039	n.d.	9-Oxo-methyl nonanoate	0.00^{b}	0.0	14.04^{a}	1.2	1.08^{b}	1.1	4.52^{ab}	7.2
95	2056	1190	Caprylic acid	107.60^{ns}	77.6	97.16^{ns}	43.0	109.01^{ns}	79.8	74.93^{ns}	51.6
96	2072	n.d.	3,5-Di-tert-butylbenzoic acid	7.99^{a}	3.0	0.00^{b}	0.0	0.00^{b}	0.0	3.08^{b}	0.0
97	2130	1458	γ-Decalactone	0.00^{c}	0.0	0.35^{bc}	0.0	4.10^{a}	2.3	3.08^{ab}	0.0
98	2133	n.d.	9-Heptadecanone	0.00^{b}	0.0	0.00^{b}	0.0	0.00^{b}	0.0	3.30^{a}	1.6

Table 16 (Continued)

#[1]	RI[2]		Compound	Concentration (ppb; ng/g)							
				FCM		CCM0		CCM3-AT		CCM3-40C	
	WAX	SAC-5		Mean	SD	Mean	SD	Mean	SD	Mean	SD
99	2173	1284	Nonanoic acid	5.99^{b}	1.3	3.68^{b}	3.2	13.09^{a}	8.1	9.81^{a}	5.3
100	2184	1487	δ-Decalactone	1,493.58^{b}	168.7	5,818.64^{a}	1,140	3,860.30^{ab}	808	3,212.49^{ab}	587.4
101	2186	1311	4-Vinylguaiacol	0.66^{b}	0.3	8.83^{a}	1.1	3.99^{b}	1.4	8.25^{a}	1.8
102	2202	1918	Methyl hexadecanoate	13.56^{ns}	4.9	9.06^{ns}	1.7	14.98^{ns}	9.1	10.33^{ns}	1.8
103	2241	1987	Ethyl hexadecanoate	7.99^{ns}	6.0	3.08^{ns}	1.0	3.62^{ns}	2.4	3.11^{ns}	0.8
104	2269	1377	Capric acid	307.87^{ns}	195.8	128.76^{ns}	36.1	205.57^{ns}	120.9	206.38^{ns}	126.2
105	2385	n.d.	Hexadecanol	13.88^{a}	1.5	0.00^{b}	0.0	0.00^{b}	0.0	0.00^{b}	0.0
106	2386	1251	2,3-Dihydro benzofuran	0.00^{b}	0.0	6.43^{a}	1.8	3.86^{ab}	2.0	3.49^{ab}	1.6
107	2410	1698	δ-Dodecalactone	649.38^{b}	50.2	1,020.10^{ab}	110.2	923.01^{ab}	669	1,932.94^{a}	667.8
108	n.d.	1699	*(Z)*-6-Dodecenyl-γ-lactone	0.00^{ns}	0.0	0.06^{ns}	0.0	0.02^{ns}	0.0	0.00^{ns}	0.0
109	2431	1297	*1H*-Indole	0.00^{b}	0.0	5.64^{ab}	4.0	13.74^{a}	8.1	7.46^{ab}	0.5

Table 16 (Continued)

#[1]	RI[2]		Compound	Concentration (ppb; ng/g)							
				FCM		CCM0		CCM3-AT		CCM3-40C	
	WAX	SAC-5		Mean	SD	Mean	SD	Mean	SD	Mean	SD
110	2465	1534	*(R)*-3,4-Dihydro-8-hydroxy-3-methyl-*1H*-2-benzopyran-1-one	44.97^{ns}	18.8	43.69^{ns}	2.7	118.84^{ns}	76.8	44.92^{ns}	1.0
111	2482	1569	Lauric acid	457.42^{a}	106.7	167.27^{b}	66.5	213.82^{b}	92.2	236.08^{b}	55.6
112	2514	n.d.	Methyl nonadecanoate	49.10^{a}	5.8	0.00^{b}	0.0	0.00^{b}	0.0	0.00^{b}	0.0
113	2576	n.d.	Octadecanol	0.00^{c}	0.0	3.17^{bc}	1.5	23.14^{a}	3.8	8.62^{b}	5.5
114	2637	1907	δ-Tridecalactone	0.00^{c}	0.0	0.00^{c}	0.0	23.88^{a}	6.2	14.77^{b}	3.5
115	2695	1855	Myristic acid	150.22^{a}	80.9	29.52^{c}	9.1	44.70^{b}	14.6	52.66^{b}	26.4
116	2889	1961	Palmitic acid	303.97^{ns}	62.8	103.57^{ns}	54.6	242.77^{ns}	99.1	161.71^{ns}	57.1
117	>3000	2165	Stearic acid	151.42^{ab}	69.5	65.97^{c}	13.4	86.43^{bc}	90.9	131.31^{ab}	52.8

[1] Number of peaks was matched to the chromatogram on the Figure C5, C6, C7, C8 (Appendix C)

[2] Retention index from 2 columns; DB-WAX column (30 m length, 0.25 mm ID and 0.25 µm film thickness, Restek, Bellefonte, PA) and SAC-5 column (30 m length, 0.25 mm ID and 0.25 µm film thickness, Supelco, PA)

Table 16 (Continued**)**

* Concentrations of compounds were calculated on SAC-5 column; without * = Concentrations of compounds were calculated on DB-WAX column

[a-d] Values with the different letters in row of means were significantly different ($p \leq 0.05$), [ns] = not significant

n.d. = not detected, FCM = fresh coconut milk, CCM0 = canned coconut milk after canning process, CCM3-AT = canned coconut milk stored for 3 months at ambient temperature, and CCM3-40C = canned coconut milk stored for 3 months at 40 °C

contact of substrates and enzymes produced a high rate of oxidation during the preparation before preheating.

Apart from oxidation, lipase catalyzed esterification and transesterification reactions of fatty acids and alcohols were possible pathways to form fatty acid esters. Acid, base and lipase catalyzed esterification are used to produced esters (Sun *et al.*, 2012). Transesterification allows the synthesis of esters directly from coconut oils and alcohols without breaking into free fatty acid. Sun *et al.* (2012) established the conversion of coconut oil to esters using lipase as a catalyst. They reported the formations of many esters and acids, including ethyl octanoate, ethyl decanoate and acids. It is possible that esters in FCM were formed via this route.

In the system of O/W emulsion, lipase catalyzed esterification and transesterification occur at the interface of emulsion. Activity of lipase in aqueous phase pronouncedly increases with increasing interface area of emulsion micelles (Adlercreutz *et al.*, 1997). Moreover, Pai *et al.* (1979) has described that C8- and C10-skeletal compounds are derived from the breakdown of 9-hydroperoxide and 10-hydroperoxide of oleic acid. The hydroxy-fatty acids are indigenous (Schwab, 2000) and form from the oxidation of unsaturated fatty acids (Schwab, 2000; Lozano *et al.*, 2007b).

According to the sensory analysis (Figure 12), the fresh and fruity notes were dominant in the fresh coconut milk sample. Aroma compounds analyzed by GC-O that might contribute to fruity were ethyl-2-methylpropanoate, 2-hexanol, octanol, octanal, nonanal, nonanol, 2-undecanone, ethyl hexanoate, lactones, ketones and esters. Among these compounds, ethyl hexanoate was the only compound that could not be detected in the GC-MS analysis (Table 16).

The fading of the fruity note should accompany with the reduction of the fruity-note compounds after canning process and storage. On contrary, the amount of all compounds with fruity note, except nonanal, increased after canning process and storage. As such, the fading of fruity note was not

corresponded to the amount of the fruity-note compounds. The loss of fruity note was rather contributed to the formation of other strong note compounds that could produce a masking effect. For example, the presence of sulfur-containing aroma compounds could mask the fruity note in the canned coconut milk samples. In the first experiment, ethyl octanoate with the OAV of 30.5 in water media was expected to be the aroma impact compound for fruity note (Figure 12). Moreover, the odor threshold in water of ethyl octanoate is quite low at 70 ppb (van Gemert, 2003). However, ethyl octanoate was not detected in the GC-O analysis. Ethyl decanoate has the similar property as that of ethyl octanoate. It was also not detected by the GC-O. The results suggested that these two compounds were not the main contributors to sweet and fruity note in coconut milk.

3.2.2 Effect of canning process on the volatile compounds in neutral/basic fraction of canned coconut milk

In the neutral/basic fraction of canned coconut milk after process (CCM0), 98 compounds were identified. Among these compounds, δ-decalactone (5,818.64 ppb) had the highest amount, followed by δ-octalactone (1,861.59 ppb), octanol (1,536.01 ppb), 2-undecanone (1,031.17 ppb) and δ-dodecalactone (1,020.18 ppb), respectively. Twenty-five compounds which were newly found after the canning process included 2-methylbutanal, 3-methylbutanal, pentanal, 1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene, 2-octanone, 2,3-octanedione, *(E)*-2-octenal, dimethyl trisulfide, 3-ethy-2-methyl-1,3-hexadiene, methional, furfural, phenylacetaldehyde, 2-acetylthiazole, 2-furanmethanol, 2-hydroxy-benzaldehyde, 2-formylthiophene, 2-(2-butoxyethoxyl)-ethanol acetate, 2-thiophenemethanol, 9-oxo-methyl nonanoate, γ-decalactone, 2,3-dihydro benzofuran, *(Z)*-6-dodecenyl-γ-lactone, indole and octadecanol.

Eleven compounds that significantly increased ($P \leq 0.05$) in their amount after canning process but not correlated to FD values (Table 11) were 2-pentyl-furan, cyclohexanone, 2-butoxy-ethanol, 2-undecanone, δ-hexalactone, 2-tridecanone, and 4-vinylguaiacol. The compounds that significantly increased in their

amount (Table 16) as well as their FD values (Table 11) were 3-methylbutanal, pentanal, 2-nonanol, dimethyl trisulfide, methional, δ-octalactone, *(Z)*-6-dodecenyl-γ-lactone and δ-decalactone. Thus, these compounds that their increases in concentrations correlated with their FD values can be used as odor markers to differentiate the fresh from the canned coconut milk. Beyond that, they could contribute to the off-odor of the canned coconut milk.

Referring to the newly formed and increasing compounds after the canning process, some of them were detected by the GC-O analysis and the formation pathways had already been discussed. From this point forward, only the formation of the new compounds that were not mentioned in the GC-O analysis will be discussed.

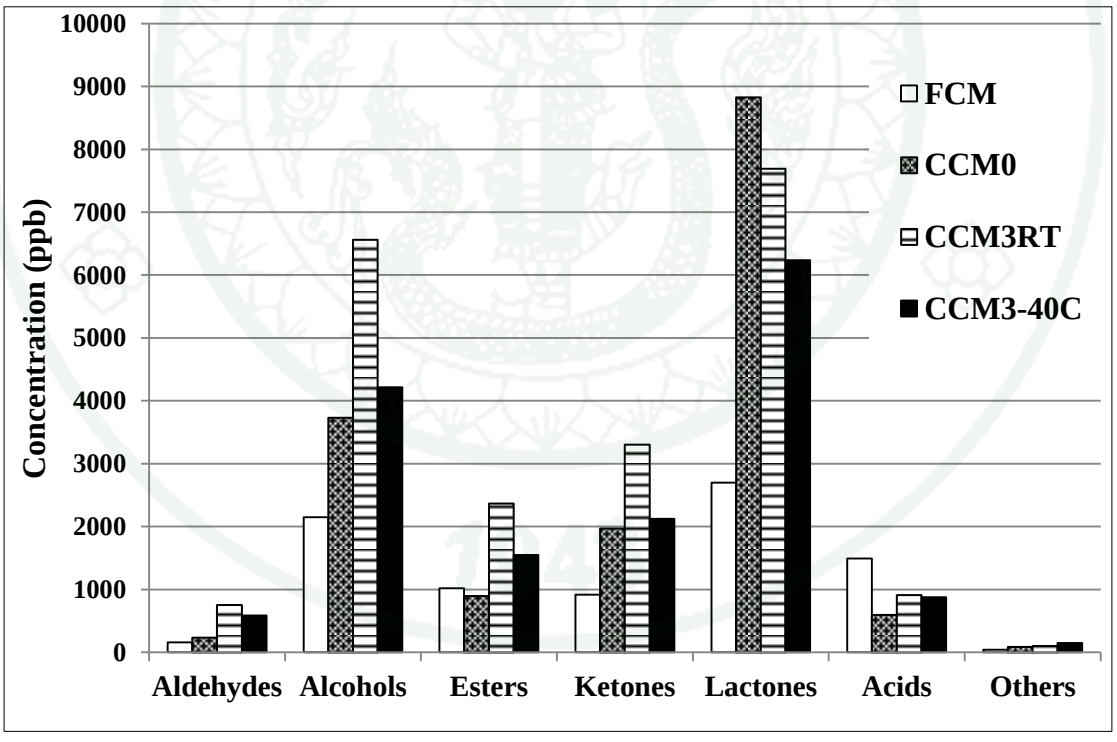


Figure 13 Groups of volatile compounds in neutral/basic fraction of fresh and canned coconut milks isolated by SAFE technique.

Pentanal is the lipid oxidation product (Schaich, 2005). It has been founded in roasted barley (Collins, 1971), Cheddar cheese (Singh *et al.*, 2003) and unsaturated fatty acid/conjugated linoleic acid enriched butter (Mallia *et al.*, 2009).

2-Octanone was not found in FCM, but in canned coconut milks (Table 16). The formation of 2-octanone upon heating in the canned coconut milk is agreed with that reported in the coconut oil, in which 2-octanone was found when coconut oil was heated (Pai *et al.*, 1978). In dairy product, C6-C16 methyl ketones are produced from oxo-fatty acids via decarboxylation reaction (Vazquez-Landaverde *et al.*, 2005). Furthermore, 2-octanone can be generated by the combination of xylose or its fragment and Maillard compounds (Yang *et al.*, 2012). 2-Octanone in CCM0 could be formed via one or more mentioned pathways.

2,3-Octanedione was formed after canning process. It was found in CCM0, but not in the fresh and the 3 month stored-canned coconut milks (Table 16). 2,3-Octanedione is associated with warmed-over flavor in heated beef (Vercellotti *et al.*, 1988). It has been found in the used frying oil (Takeoka *et al.*, 1996) and the fish oil enriched milk (Venkateshwarlu *et al.*, 2004). According to literatures, the formation of 2,3-octanedione is highly correlated to the presence of lipid in food system.

(E)-2-Octenal was newly formed in CCM0 (Table 16). It was reported to be the predominant lipid-derived odorant in the thermally derived soy milk (Lozano *et al.*, 2007a).

Furfural was also the compound found in canned coconut milks, but not in FCM (Table 16). It is one of the Maillard indicators in heated milk. It generally serves as the indicator for the extent of Maillard reaction. Furfural is the intermediate of melanoidin in the most advanced stage of Maillard reaction (Ferrer *et al.*, 2000). As such, the formation of furfural could confirm the presence of Maillard reaction in canned coconut milk upon canning process.

The formation of 2-furanmethanol attributed to Maillard reaction occurring on canning process. 2-Furanmethanol was not found in FCM (Table 16). It is discovered in Maillard model system composed of serine/threonine/ glutamine and ribose/glucose/fructose model system (Chen and Ho, 1999), and the model composed of glucose and dipeptides; glycine-serine, and serine-glycine model system (Lu *et al.*, 2008).

(Z)-6-dodecenyl-γ-lactone was found only in CCM0. The formation route of *(Z)*-6-dodecenyl-γ-lactone is unknown, but it is a potent odorant in Cheddar cheese (Avsar *et al.*, 2004).

Considering the possible formation pathways of volatile compounds in CCM0, it could be concluded that lipid derived- and Maillard-compounds were predominant in CCM0. Heat treatment led to the formation of these compounds mainly via lipid-derived and Maillard reactions. In case that lipid oxidation compounds are unfavorable. The alternative vacuum packaging, de-aeration or inert gas purging before can sealing are recommended to help decreasing the occurrence of lipid oxidation.

Apart from those formations, compounds in CCM0 which had significantly less amount than those of FCM were 2-hexanone, hexanal, lauric acid, myristic acid and stearic acid (Table 16). In addition, the compounds that were completely lost after canning process were limonene, 4-octanone, butyl hexanoate, ethyl nonanoate, nonanol, 3-(methylthio) propanol, butyl dodecanoate, 3,5-di-*tert*-butylbenzoic acid, hexadecanol and methyl nonadecanoate. Among these compounds, the compounds that were detected in GC-O were hexanal and lauric acid. The quantity and FD values of hexanal were not well correlated. The amount of hexanal decreased after canning process (Table 16), but its FD value had changed from not detected to 3.0 (Table 11). The detection of odor in GC-O was analyzed on one sample. Therefore, the result from GC-MS should be more reliable. The alteration of hexanal during storage was more interesting because it is an indicator of lipid oxidation. The

developing of hexanal could be responsible for the off-odor in the canned coconut milk during storage.

In Figure 13, the amount of alcohols, ketones and lactones had extensively increased after the canning process. In the meantime, aldehydes and other groups of compounds including furans, thiophenes, thiazoles, sulfides and pyrrole slightly increased after the canning process. Lactone concentrations had increased approximately 4 times comparing to those found in FCM. They had high FD values (Table 11) that was related to the increment of coconut odor in the sensory analysis. It was obvious that lactones were the most important compounds in coconut milk, in term of concentration and odor perception. The alteration of lactones plays a crucial role on the overall aroma of the fresh and the canned coconut milk.

The increase of alcohols and ketones in CCM0 might relate to the increase in the sweet note in coconut milk (Figure 12). Even though, aldehydes and other groups including furans, thiophenes, thiazoles, sulfides and pyrrole increased only slightly, their odor attributes were important. The identification of strong note aroma compounds relating to coconut, caramel and custard-like, creamy, popcorn, meaty, potato and nutty odors in canned coconut milk were investigated by GC-O. To determine their concentrations, SIDA-GCMS was used. Unfortunately, some aroma compounds detected by GC-O analysis could not be detected by GC-MS. However, the combination of the results from GC-O and GC-MS were very useful for understanding of coconut milk odor.

Furans, thiophenes, thiazoles, sulfides and pyrrole, detected in CCM0 by GC-MS included 2-pentylfuran, 2,3-dihydro benzofuran, 3-methyl-2-formylthiophene, 2-thiophenemethanol, 2-acetylthiazole, benzothiazole, dimethyl trisulfide, methional and indole. The presence of these compounds might attribute to the cooked odor in CCM0.

3.2.3 The alteration of volatile compounds in neutral/basic fraction of canned coconut milk during storage for 3 months at ambient temperature and 40 °C

To identify the volatile compounds after storage, the canned coconut milks were stored for 3 months at ambient temperature (23.2 °C, CCM3-AT) and 40 °C (CCM3-40C). In the first experiment, the alteration profile of the canned coconut milks stored at 32-35 °C for 6 months was investigated, and 28 compounds were identified (Table 7). By SAFE technique, 95 volatiles compounds were identified in the neutral/basic fraction of CCM3-AT (Table 16). The dominant compounds were δ-decalactone (3,860.30 ppb), δ-octalactone (2,611.33 ppb), octanol (2,358.06 ppb), 2-undecanone (1,708.92 ppb) and ethyl octanoate (1,409.92 ppb). Comparing to CCM0, the amount of aldehydes, alcohols, esters, ketones acids, furans, thiophenes and pyrroles in CCM3-AT increased. The results agreed with the data from the early experiment when the canned coconut milks were kept at 32-35 °C for 6 months.

In contrast to the results in the early experiment (Table 7 and Figure 8), the amount of lactone in the canned coconut milks that were kept at 23 and 40 °C decreased comparing to CCM0 (Table 16 and Figure 13). In the first experiment (Table 7 and Figure 8), the amount of lactone had approximately 20 times increased after 3 months of storage at tropical temperature (32-35 °C). On contrary, lactones in the second experiment had slightly decreased from 8,825.21 ppb in CCM0 to 7,963.09 ppb in CCM3-AT and 6,235.10 ppb in CCM3-40C (Table 16 and Figure 13). These could indicate the great influence of raw materials used in canned coconut milk. The coconuts used in the first experiment were originated from Chumphon, Thailand, while coconuts from California were used in the second experiment. Coconut from different origins could be different in nutrient composition that resulted in different volatile profiles.

Moreover, the canning process condition might have an effect on the content of volatile compounds. The canning process was divided into 3; the come up time, the processing time and the cooling time. In the first experiment, the come up

time was 15 min with the F_0 of 0.0015 min. The processing time was 38 min with the F_0 of 5.34 min. The cooling time that the temperature of the canned coconut milk was reduced to 30°C was 44 min (F_0 = 5.39 min). The total time of canning process in the first experiment was 97 min with the F_0 of 10.73 min at 121°C. In the second experiment, the come up time was 3.5 min with the F_0 of 0.0002 min. The processing time was 52 min with the F_0 of 5.22 min. The cooling time was 38 min with the F_0 of 9.43 min. The total time of canning process in the first experiment was 98 min with the F_0 of 14.65 min at 121°C. The most critical time that could make the difference between the first and the second experiment was the cooling time.

The use of different types of the internal standard compound could be another possible reason that made the difference between the amount of lactone in the first and second experiments. In the first experiment, 6-undecanone was used as an internal standard for lactone quantification. On the other hands, the isotope labeled compounds were used for lactone quantification in the second experiment. This phenomenon could influence the relative concentration of lactone. In this circumstance, more accurate amount of lactone should be derived in the second experiment.

The increment of aldehydes, alcohols, esters, ketones and acids in CCM3-AT agreed with those found in the first experiment. Volatile compounds that significantly increased after storage for 3 months at ambient temperature (23 °C) included ethyl acetate, 3-methylbutanal, 2-hexanone, heptanal, benzaldehyde, 2-hexanol, 2-pentyl-furan, ethyl hexanoate, octanal, 2-nonanone, 2-butoxy-ethanol, *(E,E)*-2,4-hexadienal, methyl octanoate, ethyl octanoate, 1-octen-3-ol, heptanol, 2-ethyl-1-hexanol, decanal, 2-nonanol, octanol, 2-undecanone, ethyl decanoate, 2-undecanol, 3-methyl-2-formylthiophene, 2-undecenal, decanol, 2-tridecanone, butyl decanoate, ethyl dodecanoate, guaiacol, γ-octalactone, δ-octalactone, 5-hydroxy-2-decenoic acid-δ-lactone, 3-phenyl-1-propanol, γ-decalactone, nonanoic acid, octadecanol, δ-tridecalactone, and myristic acid.

Lipolysis, lipid oxidation and Maillard reaction could play the important role on the increment of volatile compounds in canned coconut milk during storage. Although the oxygen content of canned coconut milk was low, oxidation of canned coconut milk during storage could occur. Nawar (1996) has stated that oxygen content is one of the factors affecting rate of oxidation. At low oxygen content, rate of oxidation is approximately proportional to the oxygen concentration. Other factors, such as temperatures and surface areas, influenced the rate of oxidation under low oxygen conditions. As such, storing canned coconut milk at higher temperature could increase rate of lipid oxidation.

The amount of acids in CCM3-AT (Table 16 and 17) gained nearly 2 times of those found in CCM0. Lipolysis of the ester bonds in triacylglycerols, as well as lipid oxidation should play a dominant part leading to the increase of free fatty acids during storage. The oxidative compounds, $C_{5,6,8,9,10}$-esters, also dramatically increased after storage at ambient temperature. Moreover, the numbers of ketones and esters were detected in higher amount in CCM3-AT than in FCM, CCM0 and CCM3-40C. It could be pointed out that the keeping canned coconut milk at lower temperature of 23 °C might accelerate the rate of ketone formation rather than the thermal process and keeping at higher storage temperature. It was noted that the ketones that increased in their concentrations in CCM3-AT had high molecular weight. Reineccius (2006) has stated that when the molecular weights of ketones increased, the fruity characteristic would be replaced by the floral note. This occurrence could relate to the loss of the fruity note and more detection of sweet odor founded in the stored canned coconut milks.

Raising of Maillard reaction products in CCM3-AT, such as 2-methylbutanal, 3-methylbutanal, furfural and indole, was observed (Table 16). Of these Maillard compounds, methional, 2-acetylthiazole and indole were the most interesting compounds. They were the strong odorants contributing to the potato, nutty and fecal notes, respectively. The amount of methional in CCM3-AT was not significantly different from that found in CCM0 ($P>0.05$), which agreed with the results of FD values (Table 11). The amount of 2-acetylthiazole in CCM3-AT

decreased, but not significantly different (P>0.05) comparing to CCM0. However, it was detected by GC-O in CCM3-AT, but not in CCM0. The presence of 2-acetylthiazole in CCM3-AT in GC-O result (Table 11) could contribute to the nutty note which was slightly stronger in CCM3-AT than in CCM0 (Figure 12).

Indole is classified as an off-note compound due to its odor characteristic of fecal. The amount of indole increased from 5.64 ppb in CCM0 to 13.74 ppb in CCM3-AT. The detection odor threshold in water of indole is 140 ppb (van Gemert, 2003). Indole might not have an influential role on the aroma of canned coconut milk because the OAV was less than 1. However, the amount of indole could be developed during storage and could be an inherent odorant contributing to off-odor in the long stored canned coconut milk. Urbach *et al*. (1972) added 0.02 ppm of indole and 0.3 ppm of skatole that were lower than the detection thresholds. They founded that the deodorized synthetic butter still had the mothball, hospital and fecal odors described by the panelists.

The amount of dimethyl trisulfide, an odor attributing to the sulfury note, was not significantly different to that in CCM0 (P>0.05). There could be other important sulfur-containing compounds responsible for the loss of meaty and sulfury notes, that were unable to be discovered by GC-MS. Reineccius (2006) has described that oxidative and Maillard compounds formed via thermal treatment differ from those formed at room temperature due to the unique activation energy required. Some compounds may need higher activation energy to form which is highly related to high temperature treatment applying to food products. For example, furanthiol is produced at higher rate than pyrazine and furfural during thermal treatment, due to requirement of higher activation energy. This explained why some compounds had high tendency to form upon heating process rather than the storage period. If the off-odor developed during storage is more focused than those from canning process, the compounds formed by low activation energy should be studied.

In the accelerating condition of storage, the canned coconut milk samples were stored in the hot air oven at 40 °C for 3 months. The reason for

selecting this temperature of storage was based on the preliminary result. Keeping the canned coconut sample at 40 °C was chosen over the temperatures of 35, 45 and 50 °C because the can leaked within 3 months at the storage temperature of 45 and 50 °C. The odor of canned coconut milk kept at 35 °C was not different from those of CCM3-AT determined by the lab scale triangle test. Moreover, the temperature of 40 °C could represent the extreme condition of storage that can occur in the tropical warehouse.

Dominant compounds in CCM3-40C were δ-decalactone (3,212.49 ppb), δ-dodecalactone (1,932.94 ppb), octanol (1,620.59 ppb), 2-undecanone (1,121.85 ppb), ethyl octanoate (945.56 ppb) and δ-octalactone (940.74 ppb). The amount of lactones in CCM3-40C had reduced (Figure 13). The most lost lactone was δ-octalactone, but δ-dodecalactone had increased. The amount of δ-decalactone was not significantly different ($p>0.05$) from that of CCM3-AT (Table 16). If the coconut odor in the sensory analysis was corresponding to the amount of lactones, the most contributed lactone to the coconut odor was δ-decalactone. Other groups of compounds in CCM3-40C, including aldehydes, alcohols, esters, ketones, acids, furans and pyrroles, had lower concentrations than those in CCM3-AT, but the concentrations were higher than those in FCM and CCM0 (Table 16). Storage of the canned coconut milk at higher temperature of 40 °C can cause the loss of these compounds.

The most interesting point for the odor of CCM3-40C was the unknown odor in the sensory analysis. The unknown odor contributed to stale, green, rancid, mettalic or astringency notes, and more importantly it was the off-odor in the canned coconut milk during storage. Because this compound was described as rancid, it could be related to lipid oxidation such as saturated and unsaturated aldehydes. According to Table 16, aliphatic aldehydes found in CCM3-40C were pentanal, *(E)*-2-butenal, *(Z)*-2-butenal, hexanal, heptanal, octanal, *(E)*-2-octenal, nonanal, decanal and *(E)*-2-nonenal. Of these compounds, hexanal, octanal, *(E)*-2-octenal (RTX-5), nonanal and *(E)*-2-nonenal were detected by GC-O analysis (Tables 11 and 13). Most of saturated aldehydes decreased in CCM3-40C. The amount of unsaturated aldehydes

slightly increased, that were corresponded to the high FD values in the GC-O results. Most of their FD values in CCM3-40C were the highest among all treatments. Most of monounsaturated and diunsaturated aldehydes (dienal) that were not detected by GC-MS, but were able to detect with the GC-O, had the highest FD values (Tables 11 and 13). Their odor attribute were stale, hay-like, metallic, green and cucumber-like. From the odor attributes, unsaturated aldehydes could be odorants responsible for green, rancid, and probably the off-note in the canned coconut milk during storage. The amounts of aldehydes in CCM3-40C were slightly lower than those of CCM3-AT, but higher than those of CCM0. Unsaturated aldehydes could not be detected by GC-MS.

The increases in concentrations of dimethyl trisulfide and 2-acetylthiazole could be a result of methionine and cysteine degradation (Belitz *et al*., 2009). Storing canned coconut milk at 40 °C induced more degradation of methionine and cysteine than storing at ambient temperature (Table 16). These amino acid degradation products could be related to the sulfury and roasted nut odors in the canned coconut milk. Even though storing at 40 °C caused reductions of many compounds, Maillard compounds such as methylbutanal, 3-methylbutanal, furfural and indole, were not significantly different from those in CCM3-AT ($P>0.05$). Moreover, the amount of some compounds had increased, such as benzaldehyde, 3-ethyl-2,5-dimethylpyrazine and 2-acetylthiazole. These indicated that Maillard reaction progressed during storage, especially at high temperatures and long storage time.

The behavior of pyrazines detected by GC-MS was similar to those detected by GC-O. The only pyrazine detected by GC-MS was 3-ethyl-2,5-dimethylpyrazine, and it was firstly found in CCM3-40C. As mentioned earlier on the GC-O study, pyrazine might need time to form and could be the odorants responsible for the nutty and the smoky notes in the canned coconut milk during storage.

The Maillard compounds were dominantly found in the canned coconut milk samples during storage. The importance of Maillard compounds is their

potent odor characteristic, especially those that have low odor thresholds. The small amount of these compounds could have an impact on consumer preferences. According to the results, the storage period was necessary for the development of Maillard compounds. Storing at higher temperature (40 °C) might accelerate the formation of Maillard compounds.

The compounds formed through lipid oxidation were also found in canned coconut milk samples during storage. The odor characteristic of aldehydes could be an off-odor. The alternative vacuum packaging, keeping canned coconut milk under lower temperature, the de-aeration or inert gas purging before can sealing are recommended to help decreasing the occurrence of lipid oxidation.

3.2.4 The alteration of volatile compounds in acid fraction of coconut milks

Thirteen volatiles were identified in the acid fraction of coconut milk (Table 17). Caprylic acid (3,175.20 ppb) had the highest amount in FCM followed by caproic acid (1,726.40 ppb), capric acid (466.24 ppb), pentanoic acid (244.82 ppb) and acetic acid (80.47 ppb), respectively. With exception of acetic acid, caprylic acid and furaneol, the amounts of most acids were not significantly different after canning ($P>0.05$). Acetic acid decreased from 80.47 ppb in FCM to 4.26 ppb in CCM0. On the other hands, furaneol was firstly found in CCM0, and caprylic acid increased approximate 2 times. The presence of furaneol had influence on the odor of canned coconut milk, and the formation of furaneol was discussed earlier in the GC-O study. After 3 months of storage in both ambient and 40 °C, all acids, except caprylic acid increased. In this second experiment, acetic acid in CCM3-AT and CCM3-40C increased about 40 and 20 times in comparison to that in CCM0, but it had approximately 300 times increased after 3 months of storage under the tropical ambient temperature (32-35 °C) in the first experiment.

More interestingly, the amount of acetic acid in CCM3-40C was less than that found in CCM3-AT. The difference in the increase rate of acetic acid

Table 17 Concentration of volatile compounds in acid fraction of fresh and canned coconut milks isolated by SAFE technique.

#[1]	RI[2]		Compound	Concentration (ppb; ng/g)							
				FCM		CCM0		CCM3-AT		CCM3-40C	
	WAX	SAC-5		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1461	≤800	Acetic acid	80.47^{c}	13.8	4.26^{d}	0.8	155.67^{a}	16.7	94.81^{bc}	44.6
2	1556	≤800	Propanoic acid	4.91^{b}	0.4	2.81^{b}	1.4	2,437.94^{a}	57.5	2,305.07^{a}	136.5
3	1574	≤800	Isobutyric acid	11.01^{b}	8.4	17.26^{b}	4.3	127.34^{a}	24.7	169.70^{a}	74.1
4	1637	824	Butyric acid	44.33^{b}	34.3	29.89^{b}	11.1	2,393.36^{a}	188.6	2,280.74^{a}	215.9
5	1665	868	3-Methyl butyric acid	15.62^{b}	17.3	14.58^{b}	3.0	80.44^{a}	2.4	49.13^{a}	18.5
6	1734	936	Pentanoic acid	244.82^{b}	44.9	252.75^{b}	18.7	1,738.62^{a}	51.4	1,561.0^{a}	153.1
7	1840	1037	Caproic acid	1,726.40^{b}	455.3	1,471.00^{b}	93.8	12,995.01^{a}	1,462.6	11,288.17^{a}	2,995.5
8	2019	n.d.	Furaneol	0.00^{b}	0.0	6.56^{a}	3.9	0.00^{b}	0.0	0.00^{b}	0.0
9	2053	1224	Caprylic acid	3,175.20^{b}	564.0	5,386.10^{a}	211.7	3,017.90^{b}	180.7	3,302.64^{b}	284.6
10	2197	1276	Nonanoic acid	49.32^{c}	16.4	26.49^{c}	14.6	482.16^{a}	60.8	223.85^{b}	25.4
11	2274	1377	Capric acid	466.24^{b}	299.4	96.29^{b}	41.2	1,804.47^{a}	138.7	1,397.23^{a}	256.6
12	2489	n.d.	Lauric acid	63.44^{b}	24.8	43.08^{b}	5.2	1,508.55^{a}	227.9	67.33^{b}	13.0
13	2556	n.d.	Vanillin	11.21^{c}	2.4	35.69^{b}	3.5	59.25^{a}	14.0	66.30^{a}	6.9

Table 17 (Continued)

[1] Number of peaks was matched to the chromatogram on the Figure C9, C10, C11, C12 (AppendixC).

[2] Retention index from 2 columns; DB-WAX column (30 m length, 0.25 mm ID and 0.25 µm film thickness, Restek, Bellefonte, PA) and SAC-5 column (30 m length, 0.25 mm ID and 0.25 µm film thickness, Supelco, PA).

[a-d] Values with the different letters in row of means were significantly different ($p \leq 0.05$).

Concentrations of compounds were calculated on DB-WAX column.

n.d. = not detected, FCM = fresh coconut milk, CCM0 = canned coconut milk after canning process, CCM3-AT = canned coconut milk stored for 3 months at ambient temperature, and CCM3-40C = canned coconut milk stored for 3 months at 40 °C.

during storage between these two experiments could contribute to the composition property of coconut used as raw material. Since acetic acid was found in a very high amount after 6 months of storage and the acidity note was perceived dominantly by notification in the first experiment, the formation pathway of acetic acid was suggested to be investigated, as well as to monitor the amount of acetic acid that might reach the odor threshold and being an off-odor compound during storage of canned coconut milk.

The odor characteristic of the unknown odor was related to fatty, and a mild acid as described by the Asian panelists. The OAV of volatile compounds found in the acid fraction were calculated based on the odor threshold in water (van Germert, 2003) as followed. The most impact compounds in the acid fraction of CCM3-AT were capric acid (OAV=13.9), caproic acid (OAV=4.3), isobutyric acid (OAV=2.5), butyric acid (OAV=2.4), lauric acid (OAV=1.5), propanoic acid (OAV=1.2) and vanillin (OAV=1.0).

In CCM3-40C the most dominant compounds in the acid fraction were capric acid (OAV=10.7), caproic acid (OAV=3.8), isobutyric acid (OAV=3.4), butyric acid (OAV=2.3), propanoic acid (OAV=1.2), caprylic acid (OAV=1.1) and vanillin (OAV=1.1). Keeping the canned coconut milk at higher temperature during storage resulted in the increase of OAV values. These could attribute to the increment of acidic odor that some panelists were detected in CCM3-40C. The acidic note might be increased after the storage period more than 3 months. This acidic note has a potential to be an off-odor character in canned coconut milks. The next section will discuss on the changes of fatty acid compositions in coconut milk, which could give a better understanding in the behavior of lipid in canned coconut milk after canning process and storage.

4. Fatty acid compositions of fresh and canned coconut milks

In this work, fat contents were determined in both the fresh and the canned coconut milks using the Roese-Gottlieb method (AOAC, 1990). The fresh and the canned coconut milks had the fat content of 8.15±0.2% (w/w).

According to the first and the second experiments, acids had raised in their concentrations during storage. Moreover, unsaturated fatty acids were the precursors of oxidative odorants formed during storage. Thus, this part of the work aimed to investigate fatty acid profiles of coconut milks. Short chain fatty acids (≤C10) can be directly analyzed by gas chromatography because these fatty acids are volatile and stable enough for GC analysis. The results were discussed in chapter 3.2.4. However, the medium and long chain fatty acids have to be derivatized as fatty acid methyl esters to make them volatile enough to be analyzed (Cadwallader *et al.*, 2007). In this work, fatty acid methyl esters were produced via a single stage alkaline transesterification. Boron trifluoride (BF_3) in methanol was used to derivatize the carboxyl groups of fatty acids (Bielawska *et al*., 2010).

The analysis protocol used in this work was adapted from the method used by Cadwallader *et al.* (2007) to analyze the medium and the long chain fatty acids in cheese. To extract lipid from coconut milk system, the solvent extraction was applied. The coconut milk sample was added with 3 mL of 2.5 M sulfuric acid to facilitate the isolation of fatty acids using diethyl ether-heptane (1:1 v/v) as a solvent. The extract of diethyl ether and heptane was loaded into the aminopropyl bonded phase column to separate the individual lipid classes from the lipid mixture. This method of separation has been suggested to have high yield and high purity of lipid classes by Kaluzny *et al*. (1985). The monoacylglycerol, diacylglycerol and triacylglycerol were eluded first by using chloroform:2-propanol (2:1). Free fatty acid was later eluded by using 2% formic acid in diethyl ether (v/v). Free fatty acid was directly derivatized, while acylglycerols were underwent the saponification first and then derivatization prior to GC analysis.

The short chain fatty acids with carbon numbers of 6, 8 and 10 were also recovered by solvent extraction in this part of study. To calculate the concentrations of fatty acids, the AOAC official method 996.06 (AOAC, 2002) was followed (see the details in Appendix A).

According to Tables 17 and 18, the solvent extraction followed by solid phase separation using aminopropyl bonded phase columns showed the better recovery for fatty acids with carbon more than 8 comparing to the SAFE-SIDA-GCMS technique. For example, the amount of caprylic acid in FCM analyzed by SAFE-SIDA-GCMS was 3.17±0.6 ppm, whereas that of solvent extraction was 17.42±3.6 ppm. Thus, the solvent extraction followed by solid phase separation using aminopropyl bonded phase columns combined with the derivatization method was suggested for fatty acid analysis in coconut milk.

The amount of free fatty acids was approximately 20 to 300 times lower than those of acylglycerol fatty acids. Lauric acid was the major fatty acid found in both the free and the acylglycerol forms. The presence of lauric acid in a dominant amount was agreed with other reports on coconut oil (Jayadas and Nair, 2006; Kumar *et al.*, 2006; Kumar, 2011; El-Anany and Ali, 2012). Unsaturated free fatty acids of coconut milks presented in less amounts than those of saturated free fatty acids. The monounsaturated fatty acids found in both free and the acylglycerol forms of coconut milks were myristoleic (C14:1), palmitoleic (C16:1) and oleic (C18:1) acids, whereas linoleic acid (C18:2) was the only polysaturated fatty acid found in coconut milks.

Regarding to free fatty acids in FCM (Table 18), the high abundant fatty acids were lauric (62.26 ppm), myristic (33.59 ppm), olelic (20.22 ppm), myristoleic (17.80 ppm) and caprylic acids (17.42 ppm). In FCM, enzymatic reaction during sample preparation could play the dominant role on the presence of these free fatty acids. However, the free fatty acids in FCM were found in the lowest amounts when compared with the other samples, which indicated their low odor perception in FCM.

The acylglycerol fatty acids were about 99% of total fatty acids in FCM. The most abudant acylglycerol fatty acid was lauric acid (42,593.17 ppm), followed by myristic (5,294.16 ppm), capric (3,722.34 ppm), palmitoic (2,517.77 ppm) and caprylic (1,953.53 ppm), respectively (Table 19). The distribution of acylglycerol fatty acids in coconut oil was metioned by Nawar (1996). He reported that approximately 80% of the triacylglycerol in coconut oil was tri-saturated. Lauric acid was preferably located at the *sn*-2 position, caprylic at the *sn*-3, and myristic and palmitic at the *sn*-1 positions.

After canning process, most of free fatty acids of CCM0 had raised in their amounts. The significant increase ($p \leq 0.05$) was observed in caprylic, capric and palmitic acids (Table 18). The increase could indicate the liberation of fatty acids from the acylglycerol parents, or it could be a result of lipid oxidation that yield the formation of free fatty acids upon thermal process. It is known that unsaturated fatty acids are susceptible to be oxidized. Nawar (1996) has described that the rate of oxidation of the unsaturated fatty acid was influenced by the function of temperature. He mentioned that high temperatures facilitated the oxidation rate. Although, the amount of saturated aldehydes in CCM0 was not significantly different from those in FCM ($p>0.05$; Table 16), the presence of aliphatic unsaturated aldehydes in GC-O and GC-MS analysis could be the evidence of the oxidation reaction.

The unsaturated fatty acid in coconut milk were myristoleic (C14:1), palmitoleic (C16:1), oleic (C18:1) and linoleic acid (C18:2). Caprylic and capric acids have been reported as lipid oxidation product of oleic acnd linoleic acids (Schaich, 2005). The unsaturated fatty acid in both free and acylglycerol forms could be a precusor of shoter chain-saturated fatty acids.

Depending on their relative abundance, caprylic and capric acids as free fatty acids could impart to the sweaty, waxy and racid notes in canned coconut milk. Moreover, the increase of the medium and long chain-free fatty acids (C12-C18) could also impart to the soapy odor and taste as described by Cadwallader *et al.* (2007).

A substancial loss of acylglycerol fatty acids was presented. The loss of acylglycerol-saturated fatty acids could indicated the lipolysis of the ester linkages when fat was heated in the presence of moisture, and free saturated fatty acids were released. The loss of acylglycerol-unsaturated fatty acids could attribute to lipolysis and lipid oxidation. In the study by Gladovic *et al*. (1997), the oxidation of trilinolein at 40 °C was monitored by measuring the peroxide values, and the increase of peroxide value of trilinolein solution was observed. As such, the oxidation of acylglycerol-unsaturated fatty acids in CCM0 could occur during preparation, extraction and retorting and caused the reduction of acylglycerol-unsaturated fatty acids.

For the emulsion system, the thermal induced lipolysis takes place within the oil phase rather than on the water-acylglycerol interface (Nawar, 1969). The loss of acylglycerol fatty acids showed no correlation with the amounts of free fatty acids presented in the canned coconut milk after canning process. The total of acylglycerol fatty acids in CCM0 lost about 10,000 ppm, whereas the free fatty acids increased 100 ppm after canning process (Table 18 and 19). These indicated that the released fatty acids could undergo to further reactions, or were thermally decomposed.

The amounts of free fatty acids in CCM3-AT had slightly increased compared to those of CCM0 (Table 18). Storage at 40 °C caused the extensive increases of free fatty acids The free fatty acid contents of the CCM3-40C was about 2 times higher than those in CCM0 (Table 18). The high amount of free fatty acid in the canned coconut milk stored at higher temperature of 40 °C indicated a remarkable influence of storage temperature on the presence of free fatty acids. Keeping the canned coconut milk at higher temperature could induce lipolysis as well as lipid oxidation, yielding a higher content of free fatty acids.

Although keepping canned coconut milk at ambient temperature of 23 °C for 3 months caused the decrease of acylglycerol fatty acid, the greater decrease was found in CCM3-40C. The decrese of acylglycerol fatty acid in CCM3-AT could reveal the lipolysis and lipid oxidation of canned coconut milk at low temperature of

storage. Moreover, the more substatial loss of acylglycerol fatty acid in CCM3-40C could indicate the higher rate of reaction when canned coconut milks were stored at higher temperature.

Table 18 Free fatty acid compositions of coconut milks.

Fatty acid	Content of free fatty acid (mg/kg of coconut milk; ppm)			
	FCM	**CCM0**	**CCM3-AT**	**CCM3-40C**
C6:0	0.00^{ns}±0.0	0.00^{ns}±0.0	0.00^{ns}±0.0	0.46^{ns}±0.8
C8:0	17.42^{b}±3.6	30.72^{a}±2.8	15.01^{b}±4.3	35.72^{a}±8.6
C10:0	14.80^{c}±3.7	27.83^{ab}±7.4	17.28^{bc}±3.0	35.41^{a}±9.1
C12:0	62.26^{c}±4.8	145.28^{bc}±5.5	197.72^{b} ±26.6	377.59^{a}±8.3
C14:0	33.59^{b}±5.3	37.05^{b}±1.4	41.55^{b}±5.0	74.62^{a}±15.0
C14:1	17.80^{a}±0.6	0.00^{b}±0.0	0.00^{b}±0.0	0.59^{b}±07
C16:0	12.99^{c}±0.2	29.57^{b}±3.7	33.56^{b}±3.4	63.38^{a}±9.4
C16:1	0.00^{b}±0.0	0.00^{b}±0.0	0.00^{b}±0.0	0.47^{a}±0.4
C18:0	5.53^{b}±0.7	6.19^{b}±0.9	8.79^{b}±0.9	24.82^{a}±5.2
C18:1	20.22^{b}±3.0	25.12^{b}±3.7	24.13^{b}±2.7	64.94^{a}±8.6
C18:2	1.07^{b}±0.1	1.51^{b}±0.2	3.19^{a}±0.4	3.19^{a}±0.5

The loss of acylglycerol fatty acid after storage attribute to the presence of free fatty acid. In this case, the increases of C8:0 and C10:0 could contribute to the sweaty and waxy notes of CCM3-AT and CCM3-40C which were the odor characteristic of the unknown odor. Furthermore, the soapy taste charcteristic of C12-18 fatty acids could be perceived and might impart to the undesirable flavor of the canned coconut milk after storage. The high temperature of storage that yielded the greater amount of free fatty acids could contribute to a faster detection of off-flavor in canned coconut milk.

Table 19 Acylglycerol fatty acid compositions in coconut milks.

Fatty acid	Content of acylglycerol fatty acid (mg/kg of coconut milk; ppm)			
	FCM	**CCM0**	**CCM3-AT**	**CCM3-40C**
C6:0	115.86^{a}±41.3	116.92^{a}±16.1	96.62^{a}±20.5	45.47^{b}±1.9
C8:0	$1,953.53^{a}$±911.5	$2,345.27^{a}$±199.3	$2,099.39^{a}$±143.9	$1,036.02^{b}$ ±125.1
C10:0	$3,722.34^{a}$±615.5	$3,137.63^{a}$±261.1	$2,126.14^{b}$±158.9	$1,159.69^{c}$±139.5
C12:0	$42,593.17^{a}$±11,602.9	$30,482.70^{b}$±608.8	$16,869.66^{c}$±1,602.0	$10,266.82^{c}$±1,236.7
C14:0	$5,294.16^{a}$±1,528.9	$4,599.94^{a}$±290.5	$2,477.01^{b}$±252.2	$1,566.39^{b}$±250.7
C14:1	44.12^{a}±14.6	20.67^{b}±2.8	0.71^{c}±0.6	0.00^{c}±0.0
C16:0	$2,517.77^{a}$±1,132.8	$2,347.27^{a}$±111.4	$1,007.74^{b}$±100.3	682.40^{b}±63.8
C16:1	6.69^{ns}±7.2	6.60^{ns}±1.3	0.69^{ns}±0.6	0.69^{ns}±0.7
C18:0	735.51^{a}±218.4	505.89^{b}±56.1	310.14^{bc}±34.2	252.90^{c}±39.0
C18:1	$1,933.93^{a}$±673.5	$1,959.65^{a}$±176.1	499.49^{b}±41.0	470.19^{b}±140.8
C18:2	206.73^{a}±79.8	129.21^{ab}±16.5	94.49^{b}±20.9	87.26^{b}±46.7

CONCLUSION AND RECOMMENDATIONS

Conclusion

In the first experiment, the volatile alteration of the canned coconut milk samples during 6 months of storage under the tropical room temperature (32-35°C) was observed in two stages. The major changes in volatile profiles were in month 2 and month 5. The first alteration was indicated by the increases of alcohols, acids and lactones. The second alteration was observed during month 5 when lactones and acids increased concomitantly with the notification of coconut-like and rancid odors.

The compounds that possibly caused off-odor compounds in canned coconut milk during storage of 6 months under tropical room temperatures (32-35°C) were lactones and acids. Their intensive increases corresponded with the notification of strong coconut and rancid odors. The oxidation reaction and lipolysis could generate lactones and acids during storage of 6 months under the tropical room temperature (32-35°C).

The second experiment was performed to gain a better understanding of the aroma compound of coconut milk. The consensus descriptive analysis revealed 9 odor attributes, including coconut, creamy, caramel/custard-like, popcorn, nutty, potato, meaty, fruity and fresh. There was an unknown odor that had green, stale, metallic and rancid odor. The unknown could contribute to the off-odor in the shelf-stored canned coconut milk. The fruity and the fresh notes were dominant in the fresh coconut milk, while the others attributes were much perceived after canning process and during storage. Meaty and potato notes had a substantial increase after the canning process, but ultimately reduced after 3 months of storage at ambient temperature (23 °C) and at 40 °C.

Eleven compounds were identified as headspace aroma compounds in canned coconut milk immediately after processing (CCM0). 2-Methyl-3-furanthiol was aroma compounds contributed to the dominant meaty note in headspace of CCM0.

The GC-O analysis was investigated to identify the aroma compounds of coconut milk. Sixty-two and twelve aroma compounds were identified in neutral/basic and acid fraction of coconut milk, respectively. With high FD values, δ-decalactone- and δ-octalactone were predominant in all coconut milk, including fresh coconut milk (FCM), canned coconut milk after canning process (CCM0), canned coconut milk stored for 3 months at ambient temperature of 23 °C (CCM3-AT) and 40 °C (CCM3-40C). The FD values of lactones in canned coconut milk samples were higher than that of FCM contributing to an increase of coconut odor. Pyrolines, thiazoles, pyrrole, and pyrazine had the odor characteristic of popcorn and nutty. The increment of these compounds was also observed after canning process and with higher FD values after the storage.

Sulfur-containing compounds were detected after canning process. With their odor characteristics of sulfury and meaty, these compounds could be responsible for the meaty note in CCM0. The reductions in some sulfur containing compounds during storage were correlated with the fading of the meaty note.

Methional was an aroma compound detected by GC-O analysis and possess the odor characteristic of potato. Methional might be the major contributor of the potato note in the canned coconut milk.

Indole and skatole were detected in CCM3-AT and CCM3-40C. These compounds could be undesirable when presented in high amount. In addition, they could be the off-odor compounds in the canned coconut milk after long storage time.

For aroma compounds in the acid fraction, FD values of most acid aromas had increased after canning process, and showed greater increase during storage. Furaneol with burnt sugar note was detected in FCM and CCM0. The pH of the coconut milk samples had highly influence on the presence of furaneol. The FD values of sotolon, syringol and vanillin also increased during storage. These might make the difference in the odor perception of CCM0 and CCM3.

From GC-O analysis, the major abundant compounds in all coconut milk were lactones. The fresh coconut milk had the lowest number of volatile compounds. This could relate to the weak odor characteristic of the fresh coconut milk. Canning extensively increased the amounts of alcohols, ketones and lactones, while aldehydes and other compounds only slightly increased. Most of sulfur containing compounds that were previously detected by GC-O and were responsible for the meaty and the sulfury odors, were undetectable by GC-MS. Only dimethyl trisulfide and methional were detected in the small amount.

After storage at ambient temperature of 23 °C for 3 months, the increment of aldehydes, alcohols, esters, ketones and acids in CCM3-AT agreed with those found in the first experiment. In addition, pyrazine and furfural that were not detected in the first experiment had increased. These indicated the dominant occurrence of Maillard reaction during storage.

However, lactones in CCM3-AT slightly decreased that could contribute to the slightly fading of coconut odor illustrated in sensory odor profile. Most of volatile compounds in CCM3-40C were in significant higher amount than those of CCM0 ($p \leq 0.05$), but not different from CCM3-AT ($p > 0.05$). However, some Maillard compounds in CCM3-40C were found in higher amount ($p \leq 0.05$) than those found in CCM3-AT.

For Asian market, the odor attributes that differentiate between fresh and canned coconut milk could be off-odor. The odor attributes that differentiate fresh and canned coconut milks, in the order of intensity score, included coconut, creamy, nutty, caramel/custard-like, potato, meaty and popcorn odors. However, the European market might response to the odor of canned coconut milk differently. The odor attributes that could have a potential being an off odor for European market could be the ones that differentiate the short and long time stored canned coconut milks. In this case, the unknown odor could be an off-odor attribute in European market. The presence of the unknown odor in CCM3-AT and CCM3-40C could be the result of an individual increase of the unsaturated aldehyde that had higher amount and FD value

than those of other treatments. In addition, the presence of acids could also impart to this unknown odor. The presence of Maillard compounds was observed in CCM3-AT and CCM3-40C, indicating its importance as source of odor formation. In addition to Maillard compounds, the products from the degradation, lipolysis and oxidation were also identified. The consumer test of individual market is highly recommended for the future study to indicate the off-odor attributes, and more importantly the aroma compounds.

Lipid profile analysis showed alteration of fatty acid composition occurring during canning process and also during storage. The relative amount of acylglycerol fatty acids decreased after caning process, and continuous decreased during storage. The contents of free fatty acids released from the acylglycerol parents were not proportional to the loss of acylglycerol fatty acids. These indicated the susceptibility of free fatty acids to further reactions that caused the formation of aroma and volatile compounds in canned coconut milk.

Recommendations

For the future study, the solvent assisted flavor evaporation technique (SAFE) is recommended for the extraction of coconut milk. In addition, a clarification of the unknown odor should be investigated, because it seems to have an influential effect on the odor of the canned coconut milk during storage. A descriptive analysis is recommended to use for sensory analysis instead of the consensus descriptive analysis to get more accurate odor profile. The identification of off-odor and aroma impact compounds in canned coconut milk by the omitted test is also recommended for a further study.

A better understanding of reactions related to aroma formation pathways would help improving the odor quality of canned coconut milk. Further reaction, which recommended be investigated, included Maillard reaction, lipid oxidation and the degradation of other compositions. The isotope labeled compounds would be the best choice to follow the reaction pathways. Model analysis for off-odor study is also

recommended to indicate the compounds responsible for the off-odor characteristics in the canned coconut milk during storage.

LITERATURE CITED

Acree, T. and H. Arn. 2004. **Flavornet Kovats retention indices**. Available Source: http://www.flavornet.org/f_kovats.html. January 25, 2010.

Adams, A., V. Kitryte, R. Venskutonis and N. De Kimpe. 2011. Model studies on the pattern of volatiles generated in mixtures of amino acids, lipid-oxidation-derived aldehydes, and glucose. **J. Agric. Food Chem.** 59: 1449-1456.

Adlercreutz, P., T. Gitlesen. I. Ncube and J.S. Read. 1997. Vernonia lipase: a plant lipase with strong fatty acid selectivity, pp. 220-232. *In* B. Rubin and E.A. Dennis, eds. **Methods in Enzymology, Lipases, Part A: Biotechnology**, volume 284. Academic Press, CA.

Al-Attabi, Z., B.R. D'Arcy and H.C. Deeth. 2009. Volatile sulphur compounds in UHT milk. **Crit. Rev. Food Sci. Nutr.** 49: 28–47.

Ames, J.M. 1998. Applications of Maillard reaction in the food industry. **Food Chem.** 62: 431-439.

Amri, I.N. 2011. The lauric (coconut and palm kernel) oils, pp. 169-197. *In* F.D. Gunstone, ed. **Vegetable Oils in Food Technology: Composition, Properties and Uses**. 2nd ed. Blackwell Publishing Ltd., West Sussex, UK

Ariyaprokai, S., T. Limpachoti and P. Pradipasena. 2013. Interfacial and emulsifying properties of sucrose ester in coconut milk emulsions in comparison with Tween. **Food Hydrocolloids** 30: 358-367.

Asian and Pacific Coconut Community (APCC). 1994. **International Codes and Standard for Aqueous Coconut Products, 2nd Draft, Standards Task Force, Asian and Pacific Coconut Community**. Vol. 10. No. 2.

Asian and Pacific Coconut Community (APCC). 2003. **Coconut growing.** Coconut world's global area. Available Source: http://www.foodmarketexchange.com/datacenter/product/fruit/coconut/dc_pi_ft_coconut.html, May 12, 2009.

Association of Official Analytical Chemistry (AOAC). 1990. **Official Method of Analysis**. 15th ed. The Association of Official Agricultural Chemists, Virginia.

———. 2002. **Official Method of Analysis**. 17th ed. The Association of Official Agricultural Chemists, Virginia.

Avsar, Y.K., Y. Karagul-Yuceer, M. A. Drake,T. K. Singh, Y. Yoon and K. R. Cadwallader. 2004. Characterization of nutty flavor in cheddar cheese. **J. Dairy Sci.** 87: 1999–2010.

Balasubramaniam, K. and K. Sihotang. 1979. Studies of coconut protein and its enzyme activities. **J. Food Sci.** 44(1): 62-65.

Belitz, H.D., W. Grosch and P. Schieberle. 2009. **Food Chemistry**, 4th ed. Springer-Verlag Berlin Heidelberg, Heidelberg, Germany.

Bertrand, E., E.M. Maturana, C. Chevarin, S. Portanguen, F. Mercier, P. Tournayre, S. Abouelkaram and A.S. Guillard. 2011. Heat-induced volatiles and odor-active compounds in a model cheese. **Int. Dairy J.** 21: 806-814.

Bezard, J., M. Bugaut and G. Clement. 1971. Triglyceride composition of coconut oil. **J. Am. Oil Chem. Soc.** 48: 134-139.

Bicchi, C., G. Artuffo, A. D'Amato and G. Pellegrino. 1991. GC separation of enantiomers of γ- and δ-lactones on a mixture of 2,6-dimethyl-3-trifluoroacetyl-γ-cyclodextrin and OV-1170. **J. High Res. Chromatogr.** 14: 701-704.

Bielawska, K., I. Dziakowska and W. Roszkowska-Jakimice. 2010. Chromatographic determination of fatty acids in biological material. **Toxicol. Mech.** 20(9): 526-537.

Blanda, G., L. Cerretani, P. Comandini, T.G. Toschi and G. Lercker. 2010. Investigation of off-odor and off-flavor development in boiled potatoes. **Food Chem.** 118: 283-290.

Blank, I, S. Devaud, W. Matthey-Doret and F. Robert. 2003. Formation of odorants in Maillard model systems based on L-proline as affected by pH. **J. Agric. Food Chem.** 51, 3643−3650.

Borse, B.B., L.J.M. Rao, K. Ramalakshmi and B. Raghavan. 2007. Chemical composition of volatiles from coconut sap (*neera*) and effect of processing. **Food Chem.** 101: 877-880.

Buttery, R.G. and L.C. Ling. 1995. Volatile flavor components of corn tortillas and related products. **J. Agric. Food Chem.** 43: 1878-1882.

———, W.J. Orts, G.R. Takeoka and Y. Nam. 1999. Volatile flavor components of rice cakes. **J. Agric. Food Chem.** 47: 4353-4356.

Cadwallader, K.R., T.K. Singh and J.P. Jerrell. 2007. Streamlined analysis of short-, medium- and long-chain free fatty acids in dairy products, pp. 111-122. *In* K.R. Cadwallader, M.A. Drake and R.J. McGorrin, eds. **Flavor of Dairy Products**. American Chemical Society, USA.

———. 2007. Wood smoke flavor, pp. 201-210. *In* L.M.L. Nollet, ed. **Handbook of Meat, Poultry and Seafood Quality**. Blackwell Publishing, Lancaster, PA.

Cancel, L.F. 1979. Coconut food products and bases, pp. 202-239. *In* J.G. Woodroof, ed. **Coconuts: Production, Processing, Products**, 2nd ed. AVI Publishing Co.,Ltd. Westport, Connecticut.

Chambal, B., B. Bergenstahl and P Dejmek. 2012. Edible proteins from coconut milk press cake; one step alkaline extraction and characterization by electrophoresis and mass spectrometry. **Food Res. Int.** 47: 146-151.

Chen, C.Q. and E. Robbins. 2000. Chocolate Flavor via the Maillard Reaction, pp. 286-292. *In* T.H. Parliament, C.T. Ho and P. Schieberle, eds. **Caffeinated Beverages: Health Benefits, Physiological Effects, and Chemistry.** American Chemical Society, Washington, DC.

Chen, J. and C.T. Ho. 1998. Volatile compounds generated in serine-monosaccharide model systems. **J. Agric. Food Chem.** 46 (4): 1518-1522.

——— and ———. 1999. Comparison of volatile generation in serine/threonine/glutamine-ribose/glucose/fructose model systems. **J. Agric. Food Chem.** 47: 643-647.

Chiewchan, N., C. Phungamngoen and S. Siriwattanayothin. 2006. Effect of homogenizing pressure and sterilizing condition on quality of canned high fat coconut milk. **J. Food Eng.** 73: 38–44.

Christensen, L.P., M. Edelenbos and S. Kreutzmann. 2007. Fruits and vegetables of moderate climate, pp. 135-187. *In* R.G. Berger, ed. **Flavors and Fragrances: Chemistry, Bioprocessing and Sustainability**. Springer-Verlag Berlin Heidelberg, Heidelberg.

Colahan-Sederstrom, P.M. and D.G. Peterson. 2005. Inhibition of key aroma compound generated during ultrahigh-temperature processing of bovine milk via epicatechin addition. **J. Agric. Food Chem.** 53: 398-402.

Collins, E. 1971. Steam volatile components of roasted barley. **J. Agric. Food Chem.** 19(3): 533-535.

Damodaran, S. 1991. Interfaces, protein films, and foams. **Adv. Food Nutr. Res.** 34: 1-81.

Davidek, T., D. Stephanie, R. Fabien and I. Blank. 2006. Sugar fragmentation in the maillard reaction cascade: isotope labeling studies on the formation of acetic acid by a hydrolytic beta-dicarbonyl cleavage mechanism. **J. Agric. Food Chem.** 54(18): 6667-6676.

Dayrit, F.M., O.E.M. Buenafe, E.T. Chainani and I.M.S. de Vera. 2008. Analysis of monoglycerides, diglycerides, sterols, and free fatty acids in coconut (*Cocos nucifera* L.) oil by ^{31}P NMR spectroscopy. **J. Agric. Food Chem.** 56: 5765-5769.

DebMandal, M. and S. Mandal. 2011. Coconut (*Cocos nucifera* L.: Arecaceae): In health promotion and disease prevention. **Asian Pac. J. Trop. Med.**: 241-247.

Deeth, H.C. 2006. Lipoprotein lipase and lipolysis in milk. **Int. Dairy J.** 16: 555-562.

deMan, J.M. 1999. **Principles of Food Chemistry**. 3rd ed. Aspen Publishers, Inc., Maryland, USA.

Drake, M.A., R.E. Miracle and J.M. Right. 2009. Sensory properties of dairy proteins, pp. 429-448. *In* A. Thompson, M. Bo and H. Singh, eds. **Milk Protein from Expression to Food**. Elsevier Inc., London.

Duckham, S.C., A.T. Dodson, J. Bakker and J.M. Ames. 2002. Effect of cultivar and storage time on the volatile flavor components of baked potato. **J. Agric. Food Chem.** 50: 5640-5648.

Eiserich, J.P., C. Macku and T. Shibamoto. 1992. Volatile antioxidants formed from an L-cysteine/D-glucose Maillard model system. **J. Agric. Food Chem.** 40: 1982-1988.

El-Anany, A.M. and R.F.M. Ali. 2012. Studies on the hypolipidemic effects of coconut oil when blended with tiger nut oil and fed to albino rats**. Grasas y Aceites** 63 (3): 303-312.

Engel, W., W. Bahr and P. Schieberle. 1999. Solvent assisted flavor evaporation - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrix. **Eur. Food Res. Technol**. 209: 237-241.

Eskin, N.M. and U. Cogan. 1971. **Biochemistry of Foods**. 2nd ed. Academic Press, CA.

Fay, L.B., S. Metairon, J. Lin and I. Blank. n.d. Stable isotope dilution assay mass spectrometry in flavor research: internal standard and calibration issues. **Frontiers of Flavor Sci.**: 125-130.

Fayle, S.E. and J.A. Gerrard. 2002. **The Maillard Reaction**. The Royal Society of Chemistry, Cambridge.

Fazzalari, F.A. 1978. **Compilation ofOodor and Taste Threshold Values Data**. American Society for Testing Materials, USA.

Ferrer, E., A. Alegría, G. Courtois, and R. Farré. 2000. High-performance liquid chromatographic determination of Maillard compounds in store-brand and name-brand ultra-high-temperature-treated cows' milk. **J. Chromatogr.** 881: 599-606.

Fisher, C. and T.R. Scott. 1997. **Food Flavors : Biology and Chemistry.** Atenaeum Press Ltd., Gateshead, Tyne and Wear, UK.

Gassenmeier, K. and P. Schieberle. 1995. Potent aromatic compounds in the crumb of wheat bread (French-type). Influence of pre-ferments and studies on the formation of key odorants during dough processing. **Z. Lebensm.-Unters. Forsch.** 201: 241-248.

Gervajio, G.C. 2005. Fatty acids and derivatives from coconut oil, pp. 1-56. *In* F. Shahidi, ed. **Bailey's Industrial Oil and Fat Products, volume 6: Industrial and nonedible products from oils and fats.** 6th ed. John Wiley & Sons, Inc., New Jersey.

Gladovic, N., L. Zupancic-Kralj and J. Plavec. 1997. Determination of primary oxidation products of linoleic acid and triacylglycerols. **J. Chromatogr.** 767: 63-68.

Grosch, W. 2001. Evaluation of key odorants of food by dilution experiments, aroma models and omission. **Chem. Sens.** 26: 533-545.

Gundberg, A. 2008. **Microbial Spoilage and Shelf-life Extension of Coconut Milk**. Project in Microbial Biotechnology TVMB04, Linköping University.

Gunetileke, K.G. and S.F. Laurentius. 1974. Conditions for the separation of oil and protein from coconut oil milk emulsion. **J. Food Sci.** 39: 230-233.

Güntert, M., J. Brüning, R. Emberger, R. Hopp, M. Köpsel, H. Surburg and P. Werkhoff. 1992. Thermally degraded thiamin: a potent source of interesting flavor compounds, pp. 140-163. *In* R. Teranishi, G.R. Takeoka and M. Güntert, eds. **Flavor Precursors: Thermal and Enzymatic Conversions**. American Chemical Society, Washington, DC.

Hagenmaier, R., C.M. Cater and K.F. Mattil. 1973. Aqueous processing of fresh coconuts for recovery of oil and coconut skim milk. **J. Food Sci.** 38: 516-518.

Hamilton, R.J. 1994. The Chemistry of Rancidity in Foods, pp 1-21. *In*: J.C. Allen and R.J. Hamolton, eds. **Rancidity in Foods**. 3rd ed. Chapman & Hall, Glasgow.

Hayashi, S., K. Yagi, T. Ishikawa, M. Kawasaki, T. Asai, J. Picone, C. Turnbull, J. Hiratake, K. Sakata, M. Takada, K. Ogawa and N. Watanabe. 2004. Emission of 2-phenylethanol from its β-D-glucopyranoside and the biogenesis of these compounds from [$^{2}H_{8}$] L-phenylalanine in rose flowers. **Tetrahedron** 60: 7005-7013.

———, T. Ohnishi, H. Ishida, K. Tomida, M. Sakai, M. Haru and N. Watanabe. 2012. Functional characterization of aromatic amino acid aminotransferase involved in 2-phenylethanol biosynthesis in isolated rose petal protoplasts. **J. Plant Physiol.** 169: 444-451.

Hoffmann, T., P. Schieberle and W. Grosch. 1996. Model studies on the oxidative stability of odor-active thiols occurring in food flavors. **J. Agric. Food Chem.** 44:251-255.

Holscher, W. and H. Steinhart. 1994. Formation pathways for primary roasted coffee aroma compounds, pp. 206-217. *In* T.H. Parliament, M.J. Morello and R.J. Mcgorrin, eds. **Thermally Generated Flavors: Maillard, Microwave, and Extrusion Processes**. American Chemical Society, Washington, DC.

Jackson, R.S. 2008. **Wine Science**. 3rd ed. Elsevier Inc., Waltham, MA.

Jayadas, N.H. and K.P. Nair. 2006. Coconut oil as base oil for industrial lubricants-evaluation and modification of thermal, oxidative and low temperature properties. **Tribol. Int.** 39: 873-878.

Jayalekshmy, A. and A.G. Mathew. 1990. Changes in the carbohydrates and proteins of coconut during roasting. **Food Chem**. 37: 123-134.

Jirapeangtong, K., S. Siriwatanayothin and N. Chiewchan. 2008. Effects of coconut sugar and stabilizing agents on stability and apparent viscosity of high-fat coconut milk. **J. Food Eng.** 87 (3): 422-427.

Jirovetz, L., G. Buchbauer and M.B. Gassoum. 2003. Solid-phase-microextraction-headspace aroma compounds of coconut (*Cocos nucifera* L.) milk and meat from Cameroon. **Ernahrung Nutr.** 27: 300-303.

Kaluzny, M.A., L.A. Dunncan, M.V. Merritt ad D.E. Epps. 1985. Rapid separation of lipid classes in high yield and purity using bonded phase columns. **J. Lipid Res.** 26: 135-140.

Karagul-Yuceer, Y., M.A. Drake and K.R. Cadwallader. 2001. Aroma-active component of nonfat dry milk. **J. Agric. Food Chem.** 49: 2948-2953.

———, ——— and ———. 2002. Volatile flavor components of stored nonfat dry milk. **J. Agric. Food Chem.** 50:305-312.

Kerscher, R. and W. Grosch. 1998. Quantification of 2-methyl-3-furanthiol, 2-furfurylthiol, 3-mercapto-2-pentanone, and 2-mercapto-3-pentanone in heated meat. **J. Agric. Food Chem.** 46: 1954-1958.

Kinderlerer, J.L. and B. Kellard. 1984. Ketonic rancidity in coconut due to xerophilic fungi. **Phytochem.** 23(12): 2847-2849.

——— and P.V. Hatton. 1991. The effect of temperature, water activity and sorbic acid on ketone rancidity produced by *Penicillium crustosum* Thom in coconut and palm kernel oils. **J. Appl. Microbiol.** 70: 502-506.

Kinsella, J.E., S. Patton and P.S. Dimick. 1967. Chromatographic separation of lactone precursors and tentative identification of the γ-lactones of 4-hydroxy octanoic and 4-hydroxy nonanoic acids in butterfat. **J. Am. Oil Chem. Soc.** 44: 202-205.

Konar, T.A. 2000. Natural flavor production using enzymes, pp. 87-92. *In* S.J. Risch and C.T. Ho, eds. **Flavor Chemistry Industrial and Academic Research.** American Chemical Society, Washington, DC.

Korthou, H. and R. Verpoorte. 2007. Vanilla, pp. 203-217. *In* R.G. Berger, ed. **Flavors and Fragrances: Chemistry, Bioprocessing and Sustainability**. Springer-Verlag Berlin Heidelberg, Germany.

Kuksis, A., M.J. McCarthy and J.M.R. Beveridge. 1964. Triglyceride composition of native and butter and coconut oils. **J. Am. Oil Chem. Soc.** 41: 201-205.

Kumar, S.N., A. Balakrishnan and V. Rajagopal. 2006. Fatty acids in coconut oil. **Indian Coconut J.**: 4-14.

———. 2011. Variability in coconut (*Cocos nucifera* L.) germplasm and hybrids for fatty acid profile of oil. **J. Agric. Food Chem.** 59: 13050-13058.

Kwon, K., K.H. Park and K.C. Rhee. 1996. Fractionation and characterization of proteins from coconut (*Cocos nucifera* L.). **J. Agric. Food Chem.** 44: 1741-1745.

Labuza, T.P., N.D. Heidelbauga, M.Silver and M. Karel. 1971. Oxidation at intermediate moisture contents. **J. Am. Oil Chem. Soc.** 48: 86-90.

Laksanalamai, V. and S. Ilangantileke. 1993. Comparison of aroma compound (2-acetyl-1-pyroline) in leaves from pandan (*Pandanus amaryllifolius*) and Thai fragrant rice (Khao Dawk Mali-105). **Cereal Chem.** 70(4): 381-384.

Langourieux, S. and F.E. Escher. 1998. Sulfurous off-flavor formation and lipid oxidation in heat-sterilized meat in trays. **J. Food Sci.** 63(4): 716-720.

Laureles, L.R., F.M. Rodriguez, C.E. Reano, G.A. Santos, A.C. Laurena and E.M.T. Mendoza. 2002. Variability in fatty acid and triacylglycerol composition of the oil of coconut (*Cocos nucifera* L.) hybrids and their parentals. **J. Agric. Food Chem.** 50: 1581-1586.

Lee, Y.B. and C.V. Morr. 1994. Changes of headspace volatile compounds due to oxidation of milk fat during storage of dried dairy products, pp. 98-107. *In*: C.T. Ho and T.G. Hartman, eds. **Lipids in Food Flavors**. Amer. Chem. Soc., Washington, DC.

Liang, M., M. Qi, C. Zhang, S. Zhou, R. Fu and J. Huang. 2005. Gas chromatography–mass spectrometry analysis of volatile compounds from *Houttuynia cordata Thunb* after extraction by solid-phase microextraction, flash evaporation and steam distillation. **Analytica Chimica Acta** 531: 97-104.

Lin, F.M. and W.F. Wilkens. 1970. Volatile flavor components of coconut meat. **J. Food Sci.** 35:5 38-539.

Lin, J., R.L. Rouseff, S. Barros and M. Naim. 2002. Aroma composition changes in early season grapefruit juice produced from thermal concentration. **J. Agri. Food Chem.** 50: 813-819.

Litman, I. and S. Numrych. 1978. The role lipids play in the positive and negative flavors of food, pp. 1-17. *In*: M.K. Supran, ed. **Lipids as a Source of Flavor**. American Chemical Society, Washington, DC.

Lozano, P.R., M. Drake, D. Benitez and K.R. Cadwallader. 2007a. Instrumental and sensory characterization of heat-induced odorants in aseptically packaged soy milk. **J. Agri. Food Chem.** 55: 3018-3026.

———, E.R. Miracle, A.J. Krause, M. Drake, D. Benitez and K.R. Cadwallader. 2007b. Effect of cold storage and packaging material on the major aroma components of sweet cream butter. **J. Agri. Food Chem.** 55: 7840-7846.

Lu, C.Y., R. Payne, Z. Hao and C.T. Ho. 2008. Maillard volatile generation from reaction of glucose with dipeptides, Gly-Ser, and Ser-Gly, pp. 147-157. *In* H. Tamura, S.E. Ebeler, K. Kubota and G.R. Takeoka, eds. **Food Flavor: Chemistry, Sensory Evaluation, and Biological Activity.** American Chemical Society, USA.

Luan, F., A. Degenhardt, A. Mosandl and M. Wust. 2006. Mechanism of wine lactone formation: demonstration of stereoselective cyclization and 1,3-hydride shift**. J. Agri. Food Chem.** 54: 10245-10252.

Mahakan, S.S., L. Goddik and M.C. Qian. 2004. Aroma compounds in sweet whey powder. **J. Dairy Sci.** 87: 4057-4063.

Majcher, M. and H.H. Jelen. 2009. Comparison of suitability of SPME, SAFE and SDE methods for isolation of flavor compounds from extruded potato snacks. **J. Food Comp. Anal.** 22: 606-612.

Mallia, S., F. Escher and H. Schlichtherle-Cerny. 2008. Aroma-active compounds of butter: a review. **Eur. Food Res. Technol.** 226: 315-325.

Menzel M. and P. Schreier. 2007. Enzymes and flavor biotechnology, pp. 489-505. *In* R.G. Berger, ed. **Flavors and Fragrances: Chemistry, Bioprocessing and Sustainability**. Springer-Verlag Berlin Heidelberg, Germany.

Mosandl, A. 2007. Enantioselective and isotope analysis-key Steps to flavor authentication. 379-407. *In* R.G. Berger, ed. **Flavors and Fragrances: Chemistry, Bioprocessing and Sustainability**. Springer-Verlag Berlin Heidelberg, Germany.

———, C. Askari, U. Hener, D. Juchelka, D. Lehmann, P. Kreis, C. Motz, U. Palm and H.G, Schmarr. 1992. Chirality evaluation of flavor and essential oil analysis. **Chirality** 4: 50-55.

Mottram, D.S. and M.S. Madruga. 1994. Important sulfur-containing aroma volatiles in meat, pp. 180-187. *In* C.J.Mussinan and M.E. Keelen, eds. **Sulfur Compounds in Foods**. American Chemical Society, Washington, DC.

———. 1998. Chemical of tainting of foods. **Int. J. Food Sci Technol.** 33: 19-29.

Menzel, M. and P. Schreier. 2007. Enzymes and flavor biotechnology, 489-505. *In* R.G. Berger, ed. **Flavors and Fragrances Chemistry, Bioprocessing and Sustainability**. Springer-Verlag Berlin Heidelberg, Heidelberg, Germany.

Nago, H. and M. Matsumoto. 1993. Enatiomeric composition of 5-alkanolides contained in coconut and dairy products. **Biosci. Biotechnol. Biochem.** 57(3): 427-432.

Nawar, W.W. 1969. Thermal degradation of lipid: a review. **J. Agric. Food Chem.** 17(1): 18-21.

———. 1989. Thermal decomposition of lipids. 94-104. *In* T.H. Parliament, R.J. McGorrin and C.T. Ho. eds. **Thermal Generation of Aromas**. American Chemical Society, USA.

———. 1996. Lipids, pp. 225-319. *In* O.R. Fenneman, ed. **Food Chemistry**. 3rd ed. Marcel Dekker, Inc., New York.

Nursten, H. 2005. **The Maillard Reaction: Chemistry, Biochemistry and Implications**. The Royal Society of Chemistry, Cambridge.

O'Brien, R.D. 2009. **Fats and Oils: Formulating and Processing for Applications**. 3rd ed. Taylor & Francis, Boca Raton, FL.

Pai, J.S., S.S. Lomano and W.W. Nawar. 1979. Effect of heat treatments on the volatile composition of coconut oil. **J. Am. Oil. Chem. Soc.** 56: 494-497.

Parliament, T.H. and R.J. McGorrin. 2000. Critical flavor compounds in dairy products. 44-71. *In* S.J. Risch and C.T. Ho, eds. **Flavor Chemistry Industrial and Academic Research**. American Chemical Society, USA.

Pereda, J., D.P. Jaramillo, J.M. Quevedo, V. Ferragut, B. Guamis and A.J. Trujillo. 2008. Characterization of volatile compounds in ultra-high-pressure homogenized milk. **Int. Dairy J.** 18(8): 826-834.

Perez-Cacho, P.R., K. Mahattanatawee, J.M. Smoor and R. Rouseff. 2007. Identification of sulfur volatiles in canned orange juices lacking orange flavor. **J. Agric. Food Chem.** 55: 5761-5767.

——— and R. Rouseff. 2008. Processing and storage effects on orange juice aroma: a review. **J. Agric. Food Chem.** 56: 9785-9796.

Peterson, D.G. and G.A. Reineccius. 2003. Determination of aroma impact compounds in heated sweet cream butter. **Flavor and Frag. J.** 18:320-324.

Pham, L.J., E.P Casa, M.A. Gregorio and D.Y. Kwon. 1998. Triacylglycerols and regiospecific fatty acid analyses of Philippine seed oils. **J. Am. Oil Chem. Soc.** 75(7): 807-811.

Rawls, H.R. and P.J. van Santan. 1970. A Possible Role for Singlet Oxygen in the Initiation of Fatty Acid Autoxidation. **J. Am. Oil Chem. Soc.** 47: 121-125.

Reineccius, G. 2006. **Flavor Chemistry and Technology**. 2nd ed. Taylor & Francis, Boca Raton.

Reed, K.A., C.A. Sims, D.W. Gorbet and S.F. O'Keefe. 2002. Storage water activity affects flavor fade in high and normal oleic peanuts. **Food Res. Int.** 35: 769-774.

Rocher, R., W. Schwab and P. Schreier. 1997. Stability of naturally occurring 2,5-dimethyl-4-hydroxy-3[2*H*]-furanone derivatives. **Z. Lebensm.-Unters. Forsch.** 204: 438-441.

Rosenthal, A., D.I. Pyle and K. Niranjan. 1996. Aqueous and enzymatic processes for edible oil extraction. **Enzyme Microb. Technol.** 19: 402-420.

Rychlik, M., P. Schieberle and W. Grosch. 1998. **Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants.** Deutsche Forschungsanstalt fur Lebensmittelchemie and Institut fur Lebensmittelchemie der Technischen Universitat Munchen, Germany

Saittagaroon, S., S. Kawakishi and M. Namiki. 1984. Aroma constituents of roasted coconut. **Agric. Biol. Chem.** 48 (9): 2301-2307.

Salunkhe, D.K., J.Y. Do and J.A. Maga. 1976. Biogenesis of aroma constituents of fruits and vegetables. **Crit Rev Food Sci Nutr.** 8(2): 161-190.

———, J.K. Chavan, R.N. Adsule and S.S. Kadam. 1992. **World Oilseeds: Chemistry, Technology and Utilization.** AVI Publishing Co., Ltd., Van Nostrand Reinhold, New York.

Samson, A.S., R.N. Khaund, C.M. Cater and K.F. Mattil. 1971. Extractability of coconut proteins. **J. Food Sci.** 36: 725-728.

Santoso, U., K. Kubo, T. Ota, T. Tadokoro and A. Maekawa. 1996. Nutrient composition of kopyor coconuts (*Cocos nucifera* L.). **Food Chem.** 57 (2): 299-304.

Schaich, K.M. 2005. Lipid oxidation: theoretical aspects, pp 269-355. *In*: F. Shahidi, ed. **Bailey's Industrial Oil and Fat Products**, **Volume 1: Edible Oil and Fat Products: Chemistry, Properties, and Health Effects**, 6th ed. John Wiley & Sons, Inc. New Jersey.

Schieberle, P . 1991. Primary odorants in popcorn. **J. Agric. Food Chem.** 39: 1141-1144.

———. 1992. Formation of furaneol in heat-processed. 164-173. *In* R.Teranishi, G.R. Takeoka and M. Guntert. eds. **Flavor Precursors: Thermal and Enzymatic Conversions.** American Chemical Society, USA.

———. 1995. Quantitation of important roast-smelling odorants in popcorn by stable isotope dilution assays and model studies on flavor formation during popping. **J. Agric. Food Chem.** 43: 2442-2448.

Schwab, W. 2000. Biosynthesis of plant flavors: analysis and biotechnological approach. 72-86. *In* S.J. Risch and C.T. Ho, eds. **Food Flavor Chemistry Industrial and Academic Research.** American Chemical Society, USA.

Seneviratne, K.N., C.D. HapuarachchI and S. Ekanayake. 2009. Comparison of the phenolic-dependent antioxidant properties of coconut oil extracted under cold and hot conditions. **Food Chem.** 114: 1444-1449.

——— and N.A.T. Nayomi. 2010. HPLC-diode array determination of total carotenoids in coconut oil, pp. 94. *In* H. Goonatilake, ed. **Proceedings of the 66th Annual Sessions**. 6-10 December, 2010, Sri Lanka Association for the Advancement of Science. Colombo, Sri Lanka.

Seow, C.C. and C.N. Gwee. 1997. Coconut milk: chemistry and technology. **Int. J. Food Sci. Technol**. 32: 189-201.

Sessa, D.J. 1979. Biochemical aspects of lipid-derived flavors in legumes. **J. Agric. Food Chem.** 27(2): 234-239.

Shahidi, F. 2000. Lipid in flavor formation, pp. 24-43. *In* S.J. Risch and C.T. Ho, eds. **Flavor Chemistry: Industrial and Academic Research**. American Chemical Society, Washington, DC.

Siek, T.J., I.A. Albin, K.A. Sather and R.C. Lindsay. 1970. Comparison of flavor thresholds of aliphatic lactones with those of fatty acids, esters, aldehydes, alcohols, and ketones. **J. Dairy Sci.** 54(1): 1-4.

Silanoi, S. 2004. **Aroma Active Compounds of Rice Cooked with Coconut Milk**. M.E. Thesis, Kasetsart University.

Simuang, J., N. Chiewchan and A. Tansakul. 2004. Effects of fat and temperature on the apparent viscosity of coconut milk. **J. Food Eng.** 64: 193–197.

Singh, T.K., M.A. Drake and K.R. Cadwallader. 2003. Flavor of cheddar cheese: a chemical and sensory perspective. **Comp. Rev. Food Sci. Food Safety** 2: 166-189.

Song, S., X. Zhang, Z. Xiao, Y. Niu K. Hayat and K. Eric. 2012. Contribution of oxidized tallow to aroma characteristics of beef like process flavor assessed by gas chromatography–mass spectrometry and partial least squares regression. **J. Chromatogr. A** 1254: 115-124.

Sserunjogi, M.L., R.K. Abrahamsen and J. Narvhus. 1998. A review paper: current knowledge of ghee and related products. **Int. Dairy J.** 8: 677-688.

Sucan, M.K. and G.F. Russell. 2002. Effects of processing on tomato bioactive volatile compounds, pp. 155-172. *In* T.C. Lee and C.T. Ho, eds. **Bioactive Compounds in Foods: Effect of Processing and Storage**. ACS Symposium Series; American Chemical Society: Washington, DC.

Sun, J., J.H. Chin, W. Zhou, B. Yu, P. Curran and S.Q. Liu. 2012. Biocatalytic conversion of coconut oil to natural flavor esters optimized with response surface methodology. **J. Am. Oil Chem. Soc.** 89: 1991-1998.

Takeoka, G., C. Perrino, Jr. and B. Buttery. 1996. Volatile constituents of used frying oils. **J. Agric. Food Chem.** 44:654-660.

Tan, H.S.G., B. Yu, P. Curran and S.Q. Liu. 2011. Lipase-catalysed synthesis of natural aroma-active 2-phenylethyl esters in coconut cream. **Food Chem**. 124: 80-84.

Tangsuphoom, N. and J.N. Coupland. 2005. Effect of heating and homogenization on the stability of coconut milk emulsions. **J. Food Sci.** 70(8): 466-470.

——— and ———. 2008a. Effect of pH and Ionic Strength on the Physicochemical Properties of Coconut Milk Emulsions. **J. Food Sci.** 73(6): 274-280.

——— and ———. 2008b. Effect of surface-active stabilizers on the microstructure and stability of coconut milk emulsions. **Food Hydrocolloids** 22: 1233-1242.

——— and ———. 2009. Effect of thermal treatments on the properties of coconut milk emulsions prepared with surface-active stabilizers. **Food Hydrocolloids** 23: 1792-1800.

Tieman, D.M., H.M. Loucas, J.Y. Kim, D.G. Clark and H.J. Klee. 2007. Tomato phenylacetaldehyde reductases catalyze the last step in the synthesis of the aroma volatile 2-phenylethanol. **Phytochem.** 68: 2660-2669.

Totlani, V.M. and D.G. Peterson. 2005. Reactivity of epicatechin in aqueous glycine and glucose Maillard reaction models: quenching of C_2, C_3, and C_4 sugar fragments. **J. Agric. Food Chem.** 53: 4130-4135.

Urbach, G., W. Stark and D.A. Forss. 1972. Volatile compounds in butter oil II. flavor and flavor thresholds of lactones, fatty acids, phenols, indole and skatole in deodorized synthetic butter. **J. Dairy Res.** 39: 35-46.

van Gemert, L.J. 2003. Odour Threshold: Compilation of Odor Threshold Values in **Air, Water and Other Media.** Oliemans Punter & Partners BV, Houten, The Netherlands.

van Boekel, M.A.J.S. 2006. Formation of flavor compounds in the Maillard reaction. **Biotechnol. Adv.** 24: 230-233.

van Ruth, S.M. 2001. Methods for gas chromatography-olfactometry: a review. **Biomolecular Eng.** 17: 121-128.

Varlet, V. and X. Fernandez. 2010. Review: sulfur-containing volatile compounds in seafood: occurrence, odorant properties and mechanisms of formation. **Food Sci. Technol. Int.** 16(6): 463-503.

Vazquez-Landaverde, P.A., G. Velazquez, J.A. Torres and M.C. Qian. 2005. Quantitative determination of thermally derived off-flavor compounds in milk using solid-phase microextraction and gas chromatography. **J. Dairy Sci.** 88: 3764–3772.

Venkateshwarlu, G., M.B. Let, A.S. Meyer and C. Jacobsen. 2004. Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. **J. Agric. Food Chem.** 52: 311-317.

Vercellotti, J.R., A.J. St. Angelo, M.G. Legendre., G. Sumrell, H.P. Dupuy and G.J. Flick, Jr. 1988. Analysis of trace volatiles in food and beverage products involving removal at a mild temperature under vacuum. **J. Food Compos. Anal.** 1: 239-249.

Villamor, R.R. and Ross, C.F. 2013. Wine matrix compounds affect perception of wine aroma. **Annu. Rev. Food Sci. Technol.** 4: 1-20.

Waisundara, V.Y., C.O. Perera and P.J. Barlow. 2007. Effect of different pre-treatments of fresh coconut kernels on some of the quality attributes of the coconut milk extracted. **Food Chem.** 101: 771-777.

Whetstine, M.E.C., K.R. Cadwallader and M.A. Drake. 2005. Characterization of aroma compounds responsible for the rosy/floral flavor in cheddar cheese**. J. Agric. Food Chem.** 53: 3126-3132.

Withycombe, D.A. and C.J. Mussinan. 1988. Identification of 2-methyl-3-furanthiol in the steam distillate from canned tuna fish. **J. Food Sci.** 53(2): 658-660.

Yanagimoto, K., K.G. Lee, H. Ochi and T. Shibamoto. 2002. Antioxidative activity of heterocyclic compounds formed in Maillard reaction products. **Int. Congr. Ser.** 1245: 335-340.

Yang, C., H.L. Song and F. Chen. 2012. Response surface methodology for meat-like odorants from Maillard reaction with glutathione I: the optimization analysis and the general pathway exploration. **J. Food Sci.** 77(9): 966-974.

Yaylayan, V.A. and N.G. Forage. 1992. A kinetic model for the reaction of tryptophan with glucose and mannose-the role of diglycation in the Maillard reaction. **Food Chem.** 44:201-208.

———. 1997. Classification of the Maillard reaction: a conceptual approach. **Trends Food Sci. Technol.** (8): 13-18.

Yoshihashi, T , N.T.T. Huong and H. Inatomi. 2002. Precusors of 2-acetyl-1-pyroline, a potent flavor compound of aromatic rice variety. **J. Agric. Food Chem.** 50: 2001-2004.

APPENDICES

Appendix A

Formula calculation

Calculation of Retention Index

Retention index is a measure of relative retention using normal alkanes as a standard of reference. Retention index can be calculated from data obtained by programmed GC using the formula derived by Van den Dool and Kratz (1963).

$$RI = (n \times 100) + \left[\left(\frac{RT - RT_n}{RT_N - RT_n} \right) \times 100\ (N-n) \right]$$

where; N = carbon number of the higher alkane

n = carbon number of the lower alkane

RI = the retention time of unknown or test compound

RT_n = the retention time of the lower alkane

RT_N= the retention time of the upper alkane

Calculation of Relative Concentration for Volatile Compounds

Relative concentration is a measure of relative concentration of compound to internal standard compound. Retention index can be calculated from data obtained by programmed GC using the formula as followed;

Mass of compound *i* = (area of *i* / area of isd) x mass of isd added x R

Relative concentration = mass of compound *i* / mass of sample

where; compound *i* = unknown or test compound

area of *i* = peak area of compound *i* or ion extracted of compound derived from programmed GC

area of isd = peak area of internal standard or ion extracted of isotope labeled compound derived from programmed GC

R = response factor; in case that internal standard was used to calculate, the response factor was equal to 1

Calculation of Relative Concentration of Fatty Acids

To calculated the amount of free and acylglycerol fatty acids, the AOAC official method 996.06 (AOAC, 2002) were conducted. The amount of acylglycerols that were calculated based on triacylglycerol equivalents. The factors for the conversion of fatty acid methyl esters (FAMEs) to fatty acids and acylglycerols were calculated as followed.

$$R_i = (Ps_i/Ps_{isd}) \times (W_{isd}/W_i)$$

where R_i = response factor for each fatty acid; Ps_i = peak area of individual fatty acid in mixed FAMEs standard solution; Ps_{isd} = peak area of internal standard fatty acid in mixed FAMEs standard solution; W_{isd} = weight of internal standard in mixed FAMEs standard solution; W_i = weight of individual fatty acid in mixed FAMEs standard solution.

To calculate the amount of individual triacylglycerols (W_{TG}) and fatty acids (W_{FA}), these equations were used;

$$W_{FAMEi} = (Pt_i \times Wt_{c17:0} \times 0.9552)/(Pt_{c17:0} \times R_i)$$
$$W_{FAMEi} = (Pt_i \times Wt_{c15:0} \times 1.0053)/(Pt_{c15:0} \times R_i)$$
$$W_{TGi} = W_{FAMEi} \times f_{TGi}$$
$$W_{FAi} = W_{FAMEi} \times f_{Fai}$$

where W_{FAMEi} = the amount of individual FAME, Pt_i = peak area of fatty acid *i* in test portion, $Wt_{c17:0}$ = weight of C17:0 internal standard added to test potion, 0.9552 = conversion factor of internal standard from triacylglycerol to FAME, $Pt_{c17:0}$ = peak area of C17:0 internal standard added to test potion, R_i = response factor for each fatty acid, $Wt_{c15:0}$= peak area of C15:0 internal standard added to test potion, W_{TGi} = the amount of individual triacylglycerol *i* in test portion, f_{TGi} = conversion factor from FAME to triacylglycerol for individual fatty acid *i* (see in the table of AOAC official method **996.06**; AOAC, 2002), W_{Fai} = the amount of individual fatty acid *i* in test

portion, f_{Fai} = conversion factor from FAME to fatty acid for individual fatty acid *i* (see in the table of AOAC official method 996.06; AOAC, 2002).

The amounts of fatty acids and acylglycerols calculated from those equations were the weight per test portion of approximately 10 g of sample. Thus, each amount was further divided by weight of test sample to report as the amount per gram of sample. The amount of acylglycerol was calculated based on triacylglycerol form, due to the fact that triacylglycerols are dominant among the others glycerols in coconut oil (O'Brien, 2009). Heptadecanoin was used as an internal standard.

Appendix B

Synthesis of isotopes

Synthesis of Isotopes

The synthetic methods of isotope labeled compounds were advised by Prof. Keith R. Cadwallader from Agricultural Bioprocess Laboratory, Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign.

1. Synthesis of δ-lactones

Five isotopes of lactones were synthesized, including [$^2H_2$]-δ-octalactone, [$^2H_2$]-δ-nonalactone, [$^2H_2$]-δ-decalactone, [$^2H_2$]-δ-undecalactone, [$^2H_2$]-δ-dodecalactone. For [$^2H_2$]-δ-undecalactone and [$^2H_2$]-δ-dodecalactone, the commercial 1-decen-4-ol and 1-undecen-4-ol were not available. Therefore, 1-alkenyl-4-ols forms of these two compounds had to be synthesized.

1.1 Synthesis of 1-alkenyl-4-ols

1.1.1 Chemicals

Heptanal (Aldrich H212-0, M.W. 114.19, 95% purity), octanal (Aldrich O5608-25 mL, M.W. 128.22, 99% purity, allymagnesium bromide, 1.0 M solution in diethyl ether (Aldrich 225754-200 mL), diethyl ether (HPLC grade).

1.1.2 Methods

The following method was for 1-decen-4-ol synthesis. For 1-undecen-4-ol, octanal was used as the starting material instead of heptanal and the synthesis procedure was conducted as followed.

A dry 100 mL round bottom flask was set in ice-water bath (0 °C) with a magnetic stirrer under nitrogen gas purging. Allymagnesium bromide solution (15 mL) was transfer into the flask following by heptanal 0.5 g in ether. Then, the flask was removed from the ice bath and continued stirring at room temperature for 1

h. After that, the flask was capped by a rubber septum and the solution was continued being stirred overnight. On the next day, the flask was placed in the ice-water bath and 30 mL of saturated NH_4Cl was added dropwisely. The solution was let to stand for 15 min, before 30 mL of double deionized water was added. The solution was allowed to reach the room temperature before the diethyl ether extraction (2 × 25 mL). The diethyl ether extract was evaporate to almost dryness and re-dissolved in 10 mL of pentane. The extract was dried over anhydrous Na_2SO_4 and purified through the flash chromatography of 30 g silica gel. The orders of solvent elutes were 100% pentane, 10:90 pentane:ether, 20:80 pentane:ether, 30:70 pentane:ether and 40:60 pentane:ether, respectively. Each solvent (50 mL) was washed through the column and 30:70 pentane:ether fraction was collected. The fraction was evaporated at room temperature until dryness. The purity and yield of the collected elute were checked by GC-MS and GC-FID.

1.2 Synthesis of 1-alken-4-yl acrylolates

1.2.1 Chemicals

1-Hepten-4-ol (Alfa-Aesar B20591, M.W. 114.19, 98% purity), 1-octen-4-ol (Alfa-Aesar B20707, M.W. 128.21, 98% purity), 1-nonen-4-ol (Alfa-Aesar B20284, M.W. 142.24, 98% purity), 1-decen-4-ol (synthesis, M.W. 156.27), 1-undecen-4-ol (synthesis, M.W. 170.29), Acryloyl chloride (Aldrich 549797-5G, M.W. 90.51, 98 purity) and triethylamine (Aldrich 471283-100 mL, M.W. 101.19), sulfuric acid (H_2SO_4), sodium bicarbonate ($NaHCO_3$) , diethyl ether (HPLC grade), dichloromethane (HPLC grade)

1.2.2 Methods

The following method is for 1-hepten-4-yl acryloylate synthesis. For others 1-alken-4-yl acrylolates, 1-hepten-4-ol was replaced by other alcohols in the chemical list. The synthesis procedure was conducted as followed.

A dry 50-mL test tube was set with a mechanical stirrer and a nitrogen purge line. 1-Hepten-4-ol (approximately 0.5 mg) and 1.0 mL of triethylamine were dissolved in 15 mL of dichloromethane. The solution was cooled in the ice-water bath (0 °C) and was stirred with a magnetic stirrer under the nitrogen gas purging. Then, acrylolyl chloride (0.5 mg in 3 mL of dichloromethane) was slowly added into the solution. The mixture was stirred for 2 h. After that, the 20 mL of doubled deionized water was added. The solution was stirred until all precipitate had been dissolved. The solution was then extracted by diethyl ether (3 x 20 mL). The collected diethyl ether extract was then washed twice by a 10 mL sulfuric acid solution (10% w/w) and saturated sodium bicarbonate solution (2 x 10 mL). The remaining extract was subjected to high vacuum distillation. Then, the solvent extract was dry over anhydrous sodium sulfate and evaporated the solvent to dryness under nitrogen flushing. The purity and yield of collected elute were checked by GC-MS and GC-FID.

1.3 Synthesis of dehydro-δ-lactone

1.3.1 Chemicals

1-Hepten-4-yl acryloylate (synthesis), 1-octen-4-yl acryloylate (synthesis), 1-nonen-4-yl acryloylate (synthesis), 1-decen-4-yl acryloylate (synthesis), 1-undecen-4-yl acryloylate (synthesis), titanium isopropoxide (Aldrich 377996; M.W. = 284.26), Grubbs catalyst, generation I (Aldrich 579726; M.W. = 822.96), Florisil (Aldrich, F9127), silica (Aldrich 381276), diethyl ether (HPLC grade), dichloromethane (HPLC grade).

1.3.2 Methods

The following method is for dehydro-δ-octalactone synthesis. For others dehydro-δ-lactones, 1-hepten-4-yl acryloylate was replaced by other 1-alken-4-yl acrylolates in the chemical list. The synthesis procedure was conducted as followed.

A dry 100 mL-3-neck round bottom flask was set with the mechanical stirrer, a rubber septa (with nitrogen purge line and vent needle) in the 4 °C water bath. The solution of 0.5 g of 1-hepten-4-yl acryloylate dissolved in 50 mL of dichloromethane was added to the flask. Then, 0.25 g of Grubbs catalyst, generation I and 200 µL of titanium isopropoxide were added into the solution. After that, the mixture solution was continuously refluxed for 72 h and the product formation was periodically checked by GC-MS. Once the reaction completed, the reaction medium was passed directly through a column of 10 g Florisil gel. The column was washed by several volume of dichloromethane to extract the products. Dichloromethane extract was dry over anhydrous sodium sulfate and evaporated the excess solvent to yield the yellow-brownish oil. Dehydro-δ-lactone was purified by using the column of 50 g silica gel that was prebaked at 120 °C for 3 h. The fractions of styrene, esters, hydroxyl acids or lactones and the final products of dehydro-δ-lactone were eluted by using 200 mL of pentane:diethyl ether at the ratio of 90:10 (styrene fraction), 70:30 (ester fraction) and 50:50 (lactone fraction), respectively.

1.4 Synthesis of $^{2}H_{2}$-δ-lactones

1.4.1 Chemicals

Dehydro-δ-octalactone (synthesis), dehydro-δ-nonalactone (synthesis), dehydro-δ-decalactone (synthesis), dehydro-δ-undecalactone (synthesis), dehydro-δ-dodecalactone (synthesis), Wilkinson's catalyst (Aldrich 14694952, M.W. = 925.22), deuterated methanol (Aldrich 811983), silica (Aldrich 381276), diethyl ether (HPLC grade), pentane (HPLC grade), deuterium gas.

1.4.2 Methods

The following method is for [$^{2}H_{2}$]-δ-octalactone synthesis. For others [$^{2}H_{2}$]-δ-lactones, dehydro-δ-octalactone was replaced by other dehydro-δ-lactones listed as chemicals. The synthesis procedure was conducted as followed.

Dehydro-δ-octalactone (50 mg) and 7.5 mg (20% w/w) of Wilkinson's catalyst were placed to a pressure reactor tube equipped with a rubber septum. The 2.0 mL of deuterated methanol was added. After adding the stir bar, the pressure reactor tube was sealed and connected with the deuterium line via a needle that was placed in ?? the solution. During stirring, the deuterium pressure was applied in coupling with an inserting vent vacuum line for 1-2 min to completely flush the reactor with deuterium. After that, the vent vacuum line was removed and the solution was pressurized to 40 psi. The product formation was periodically checked using 10 μL syringe to remove 2 μL of solution and injected to a GC-MS. Once the reaction completed, the red/orange color of the catalyst turned to light yellow/white. Then, the deuterium line was carefully removed without turning off the deuterium flow from the reactor tube. The reaction solution was then flushed with nitrogen purging until close to dryness to remove deuterated methanol. Diethyl ether:pentane (50:50; 2-3 mL) was added to the reactor tube. The solution was filtered through anhydrous sodium sulfate column. The collected solution was further rinsed in a 2 g silica gel column, and 5-10 mL of diethyl ether:pentane (50:50) was used to elute the end products. The end product solution was dry over anhydrous sodium sulfate and evaporated the solvent to dryness under nitrogen flushing. The purity and yield of the collected elute were checked by GC-MS and GC-FID.

2. Synthesis of saturated aldehydes

2.1 Deuteration of unsaturated alcohols with deuterium gas

2.1.1 Chemicals

3-Octyn-1-ol (Aldrich, 545430, M.W. = 126.20), 3-nonyn-1-ol (Alfaa-Aasar A12922, M.W. = 140.23), Wilkinson's catalyst (Aldrich 14694952, M.W. = 925.22), deuterated methanol (Aldrich 811983), pyridinium chlorochromate (98%, Aldrich 190144-100G, M.W. = 215.56), deuterium gas, dichloromethane (HPLC grade), diethyl ether (HPLC grade).

2.1.2 Methods

The following method is for [$^2H_4$]-octan-1-ol synthesis. For [$^2H_4$]-decan-1-ol, 3-decyn-1-ol was used as the starting material instead of 3-octyn-1-ol and the synthesis procedure was conducted as followed.

3-Octyn-1-ol (1.0 g) and 0.15 g (20% w/w) of Wilkinson's catalyst were placed to a pressure reactor tube equipped with a rubber septum. Deuterated methanol (2.0 mL) was added. After adding of a stir bar, the pressure reactor tube was sealed and connected with the deuterium line via the needle that was placed in the solution. While stirring, the deuterium pressure was applied in couplings with the inserting vent vacuum line for 1-2 min to completely flush the reactor with deuterium. After that, the vent vacuum line was removed and the solution was pressurized to 20-40 psi. The product formation was periodically checked using a 10 μL syringe to remove 2 μL of the solution and then injected to GC-MS. Once the reaction completed, the red/orange color of the catalyst turned to light yellow/white. Then, the deuterium line was carefully removed from the reactor tube without turning off the deuterium flow. Diethyl ether (5 mL) was added to the reaction solution, and then centrifuged for 2-3 min. The layer of diethyl ether was collected. Diethyl ether (5 mL) was added to re-extract the products, and repeated the centrifugation procedure. The diethyl ether extract was transferred to the pre-weighted beaker, and was allowed to evaporate off in the hood overnight. The purity and yield of the collected elute were checked by GC-MS and GC-FID.

2.2 Synthesis of aldehyde via the oxidation of alcohol

2.2.1 Chemicals

[$^2H_4$]-Octan-1-ol (synthesis), [$^2H_4$]-decan-1-ol (synthesis), pyridinium chlorochromate (98%, Aldrich 190144-100G, M.W. = 215.56), Florisil (Aldrich, F9127), diethyl ether (HPLC grade).

2.2.2 Methods

The following method is for [$^2H_4$]-octanal synthesis. For [$^2H_4$]-decana1, [$^2H_4$]-3-decan-1-ol was used as the starting material instead of [$^2H_4$]-3-octan-1-ol and the synthesis procedure was conducted as followed.

A 10 mL suspension of 1.5 g pyridinium chlorochromate in dichloromethane was placed to a 40-mL vial. [$^2H_4$]-3-Octan-1-ol (0.4 g) was added to the suspension under nitrogen purging. After the addition of alcohol, the nitrogen purging line was removed. The reaction solution continued being stirred for 1.5 h. Then, diethyl ether (20 mL) was added to the extract end products, and the supernatant was collected. The extract residue was re-extracted with 3 x 20 mL of diethyl ether until the black gum became a granular solid. The diethyl ether extracts were combined and filtered through a 10 g bed of Florisil gel. The passing through fraction was collected, and then was subjected to Vigreux distillation to remove the solvent. The extract was purified by the high vacuum distillation. The left-over fraction was purged under nitrogen gas to remove the solvent. The purity and yield of the collected elute were checked by GC-MS and GC-FID.

3. Synthesis of saturated ketones

3.1 Synthesis of [$^2H_4$]-alkan-2-ol

3.1.1 Chemicals

[$^2H_4$]-Octanal (synthesis), [$^2H_4$]-decana1 (synthesis), methylmagnesium iodide (Aldrich 254363), pyridinium chlorochromate (98%, Aldrich 190144-100G, M.W. = 215.56), ammonium chloride, sodium chloride, diethyl ether (HPLC grade), dichloromethane (HPLC grade).

3.1.2 Methods

The following method is for [$^2H_4$]-nonan-2-ol synthesis. For [$^2H_4$]-undecan-2-ol, [$^2H_4$]-decana1 was used as the starting material instead of [$^2H_4$]-octanal and the synthesis procedure was conducted as followed.

A dry 50-mL test tube was set with the magnetic stirrer and the nitrogen purge line. Diethyl ether (10 mL) was added into the test tube. Methylmagnesium iodide (approximately 2.0 mL) was then added into the tube under nitrogen purging. The tube was chilled in a 0 °C ice-water bath. Then, [$^2H_4$]-octana1 solution (0.4 g in 2 mL of dichloromethane) was slowly added into the tube while the solution was being stirred without the nitrogen purge line. The solution was continued being stirred in the ice-water bath for 30 min. Then, the test tube was removed from the ice bath and capped loosely. The solution was continued being stirred overnight at room temperature. After that, the test tube was chilled in the ice-water bath and added dropwisely with 15 mL of saturated ammonium chloride solution. The solution was extracted with 3 x 10 mL of diethyl ether. The diethyl ether extract was washed with saturated sodium chloride solution (3 x 20 mL). The end product solution was dry over anhydrous sodium sulfate and evaporated the solvent to dryness under the nitrogen flushing. The purity and yield of the collected elute were checked by GC-MS and GC-FID.

3.2 Synthesis of [$^2H_4$]-alkan-2-one

3.2.1 Chemicals

[$^2H_4$]-Nonan-2-ol (synthesis), [$^2H_4$]-undecan-2-ol (synthesis), pyridinium chlorochomate (98%, Aldrich 190144-100G, M.W. = 215.56), Florisil (Aldrich, F9127), diethyl ether (HPLC grade).

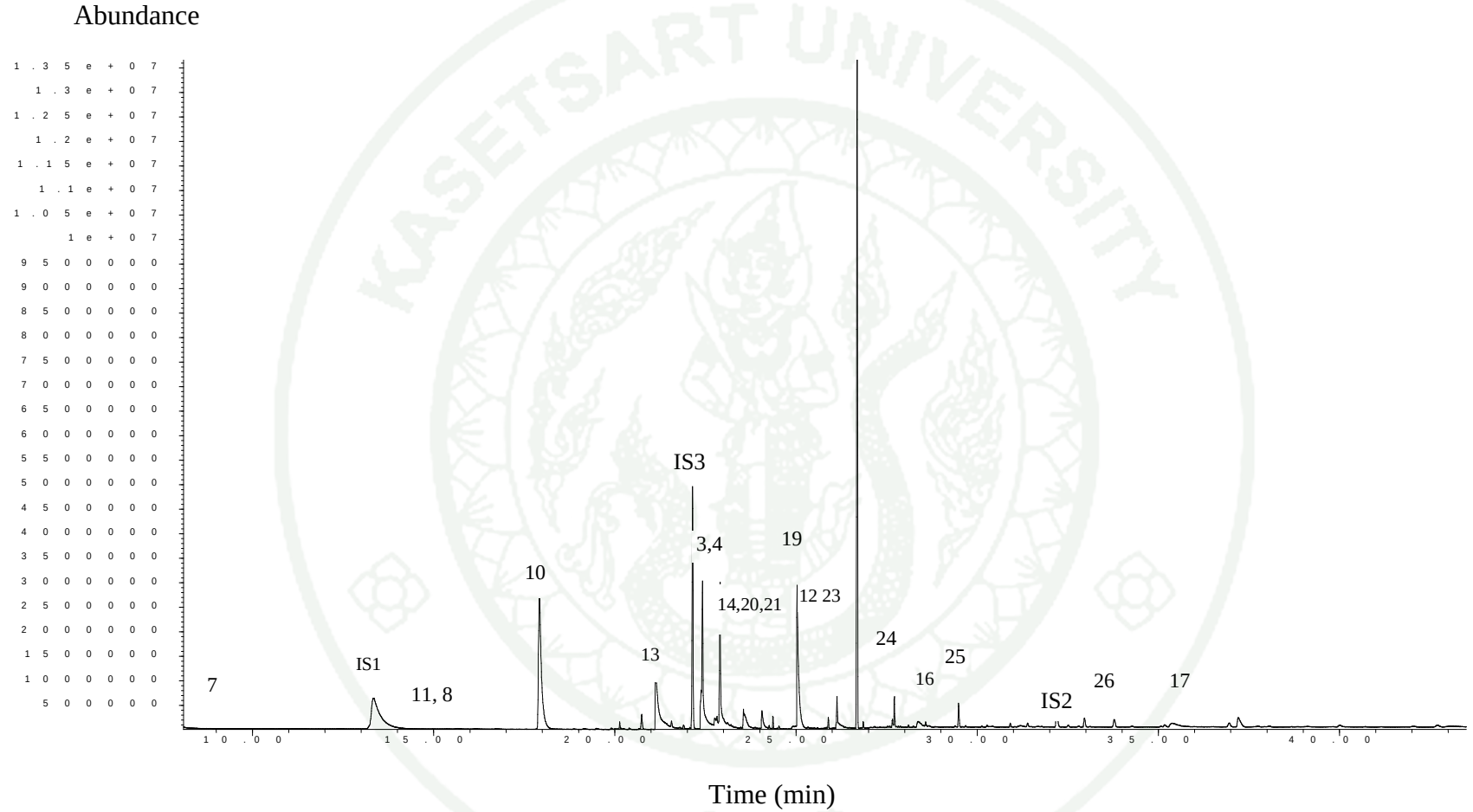
3.2.2 Methods

The following method is for [$^2H_4$]-nonan-2-one synthesis. For [$^2H_4$]-undecan-2-one, [$^2H_4$]-undecan-2-ol was used as the starting material instead of [$^2H_4$]-nonan-2-ol. The synthesis procedure was conducted as followed.

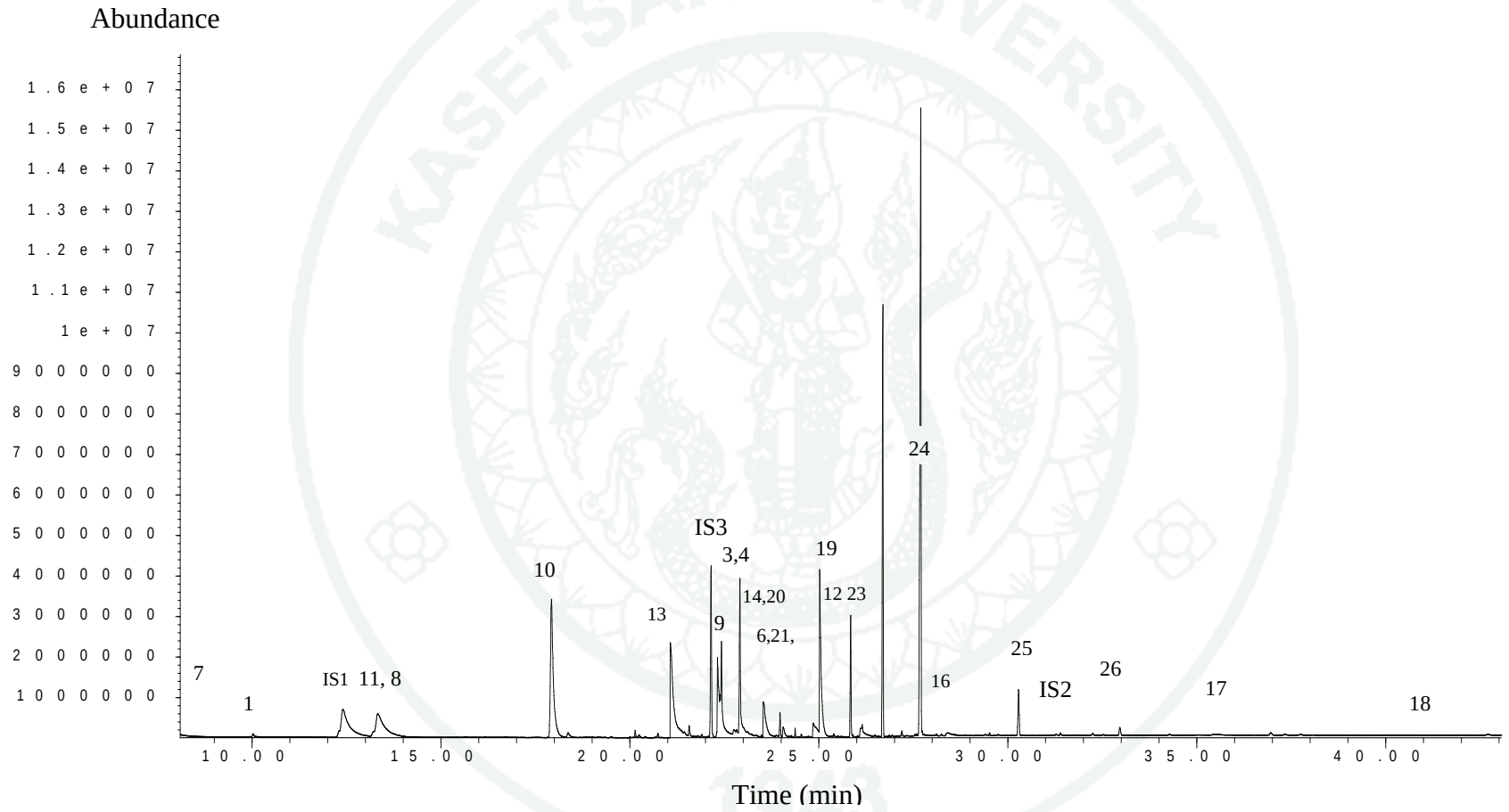
A 10 mL suspension of 0.5 g pyridinium chlorochromate in dichloromethane was placed to a 40-mL vial. [$^2H_4$]-Nonan-2-ol solution (0.18 g in 2 mL of dichloromethane) was added to the suspension under nitrogen purging. After the addition of alcohol, the nitrogen purging line was removed. The reaction solution continued being stirred for 1.5 h. Then, diethyl ether (20 mL) was added to the extract end product, and the supernatant was collected. The extract residue was re-extracted with 3 x 20 mL of diethyl ether until the black gum became a granular solid. The diethyl ether extracts were combined and filtered through a 5 g bed of Florisil gel. The passing through fraction was collected, and then was subjected to Vigreux distillation to remove the solvent. The extract was purified by the high vacuum distillation. The left-over fraction was purged under nitrogen gas to remove solvent. The purity and yield of the collected elute were checked by GC-MS and GC-FID.

Appendix C

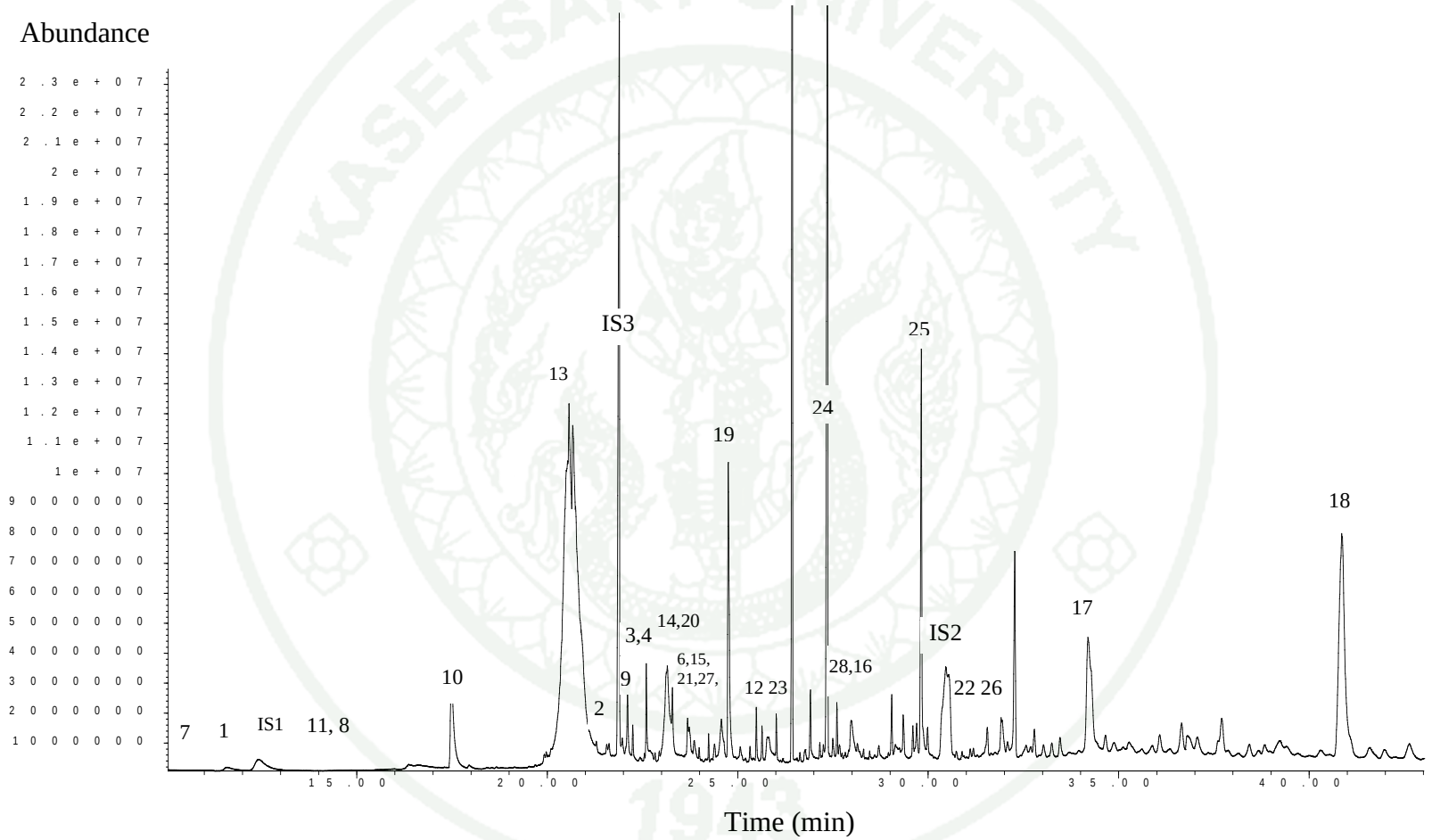
Chromatograms



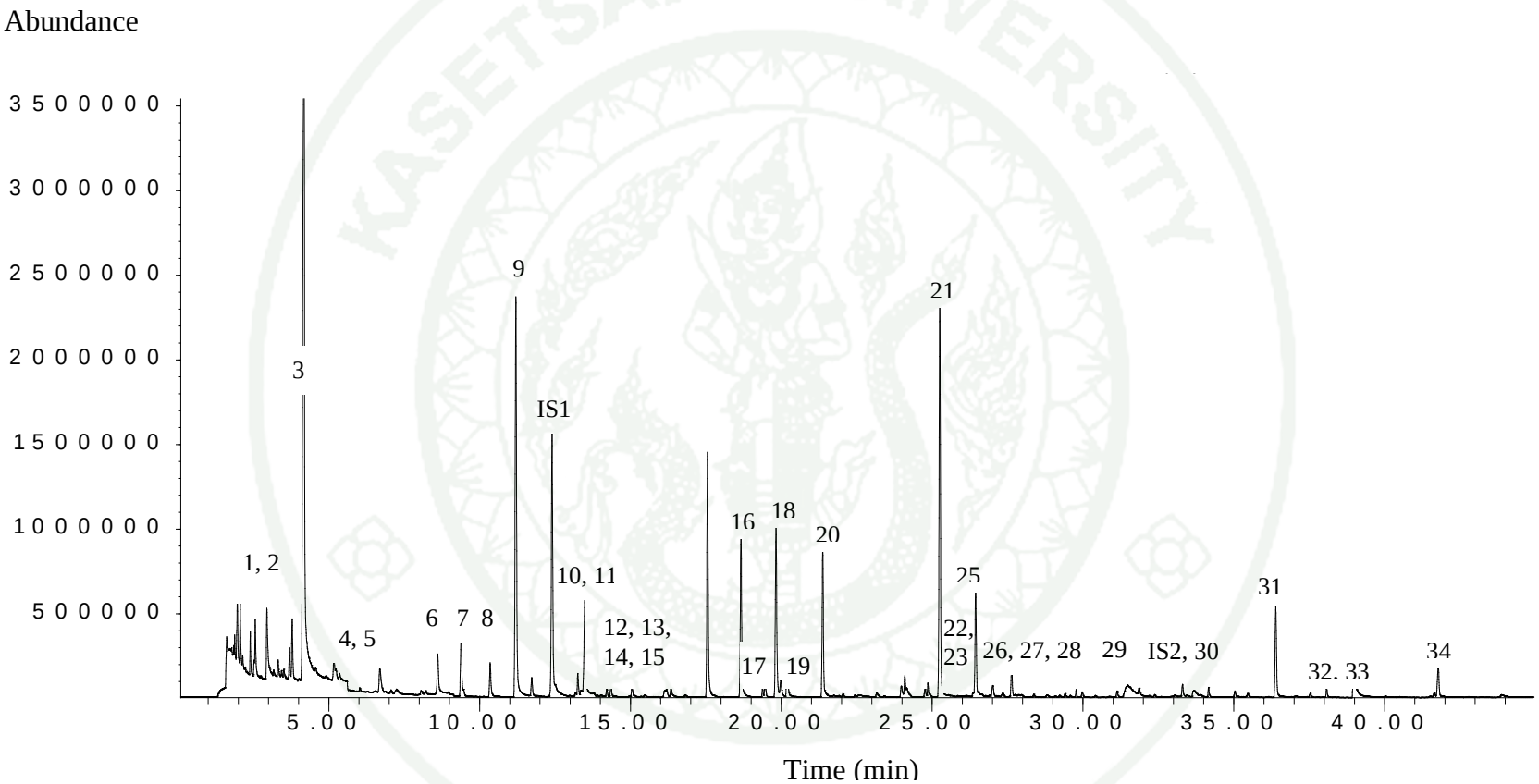
Appendix Figure C1 Chromatogram of volatile compounds extracted from canned coconut milk at month 0 in the first experiment, analyzed on the FFAP column; IS1 = 2-methyl-3-heptanone, IS2 = 2-ethyl butyric acid, IS3 = 6-undecanone.



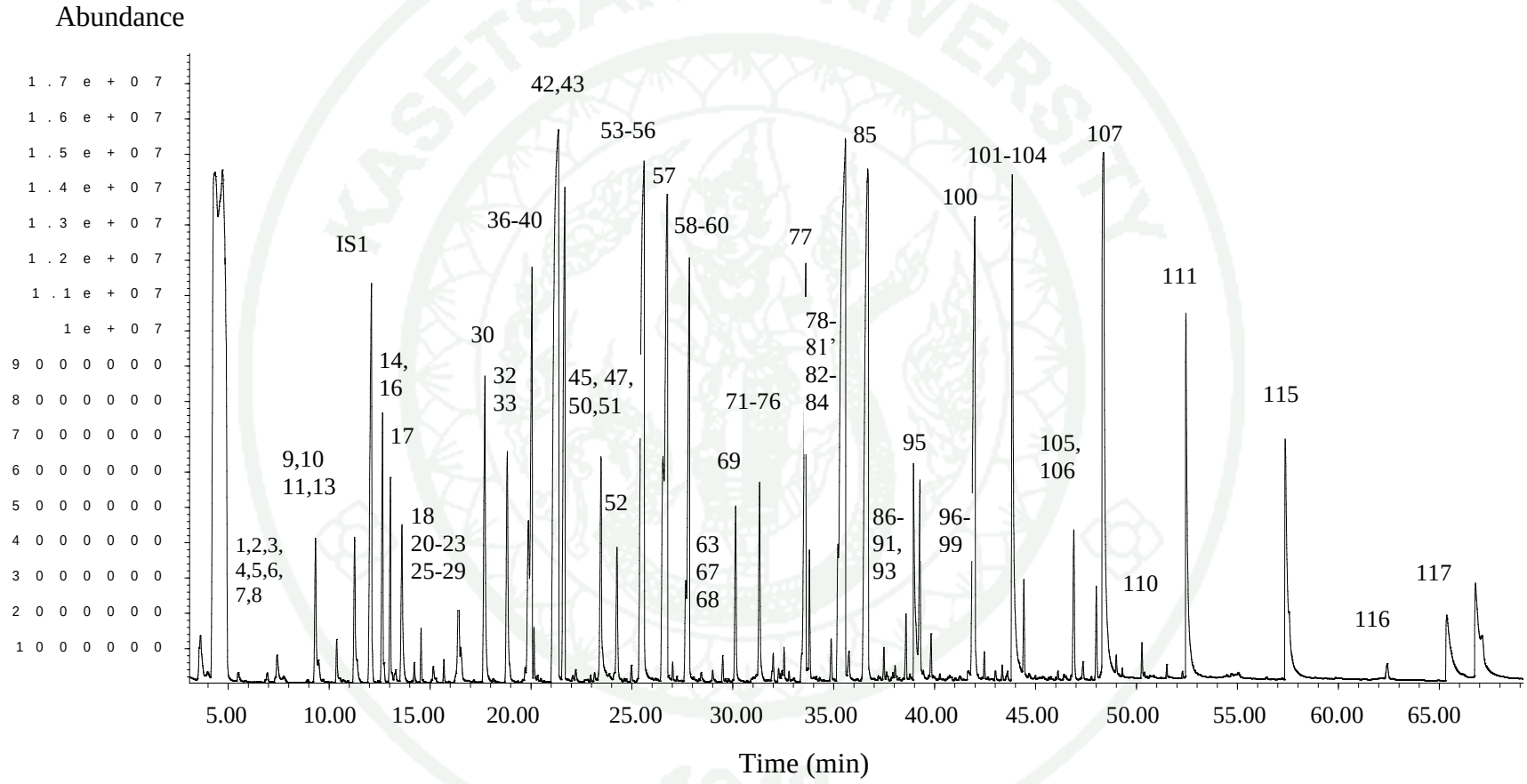
Appendix Figure C2 Chromatogram of volatile compounds extracted from canned coconut milk at month 2 in the first experiment, analyzed on the FFAP column; IS1 = 2-methyl-3-heptanone, IS2 = 2-ethyl butyric acid, IS3 = 6-undecanone.



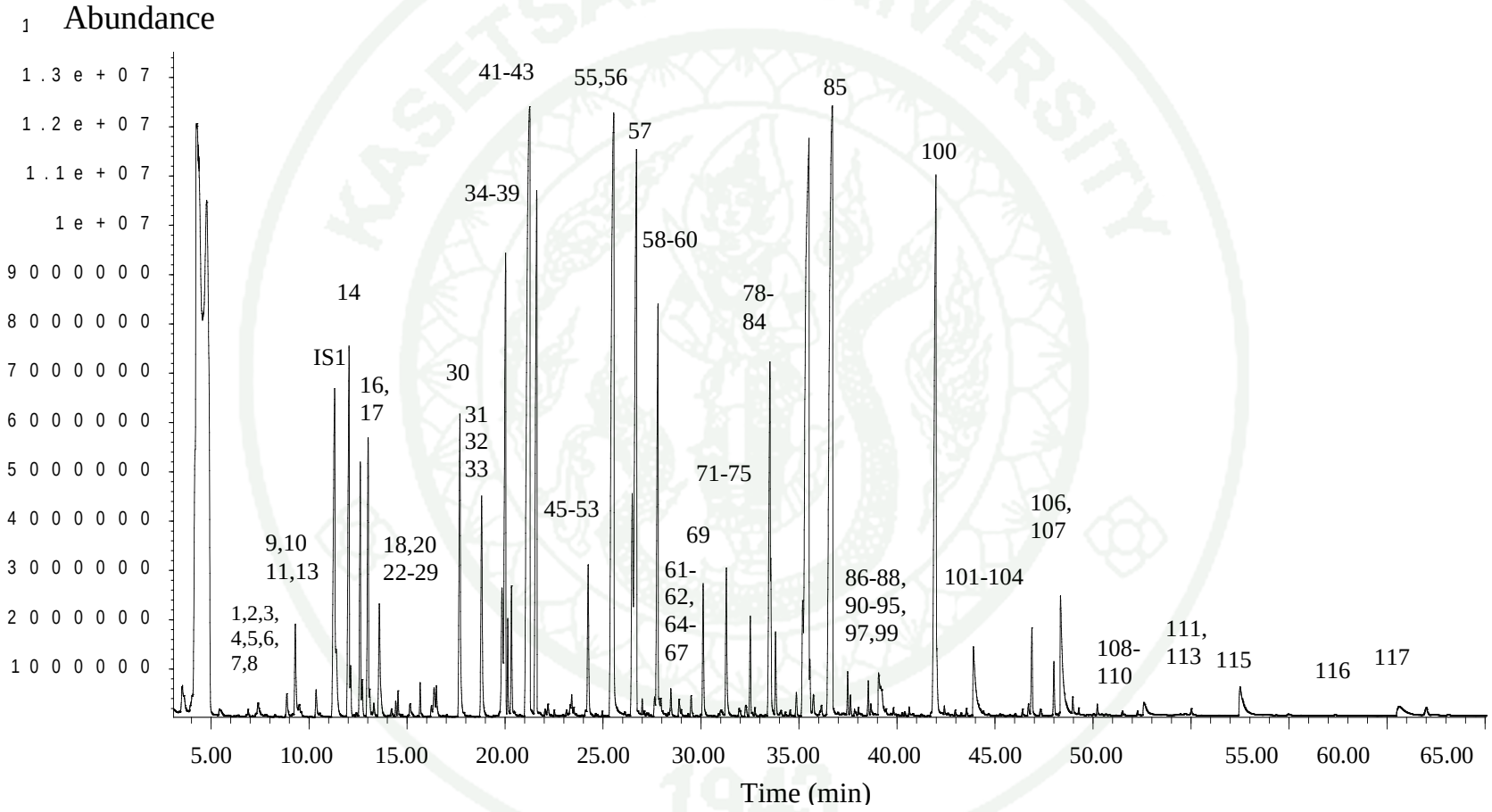
Appendix Figure C3 Chromatogram of volatile compounds extracted from canned coconut milk at month 5 in the first experiment, analyzed on the FFAP column; IS1 = 2-methyl-3-heptanone, IS2 = 2-ethyl butyric acid, IS3 = 6-undecanone.



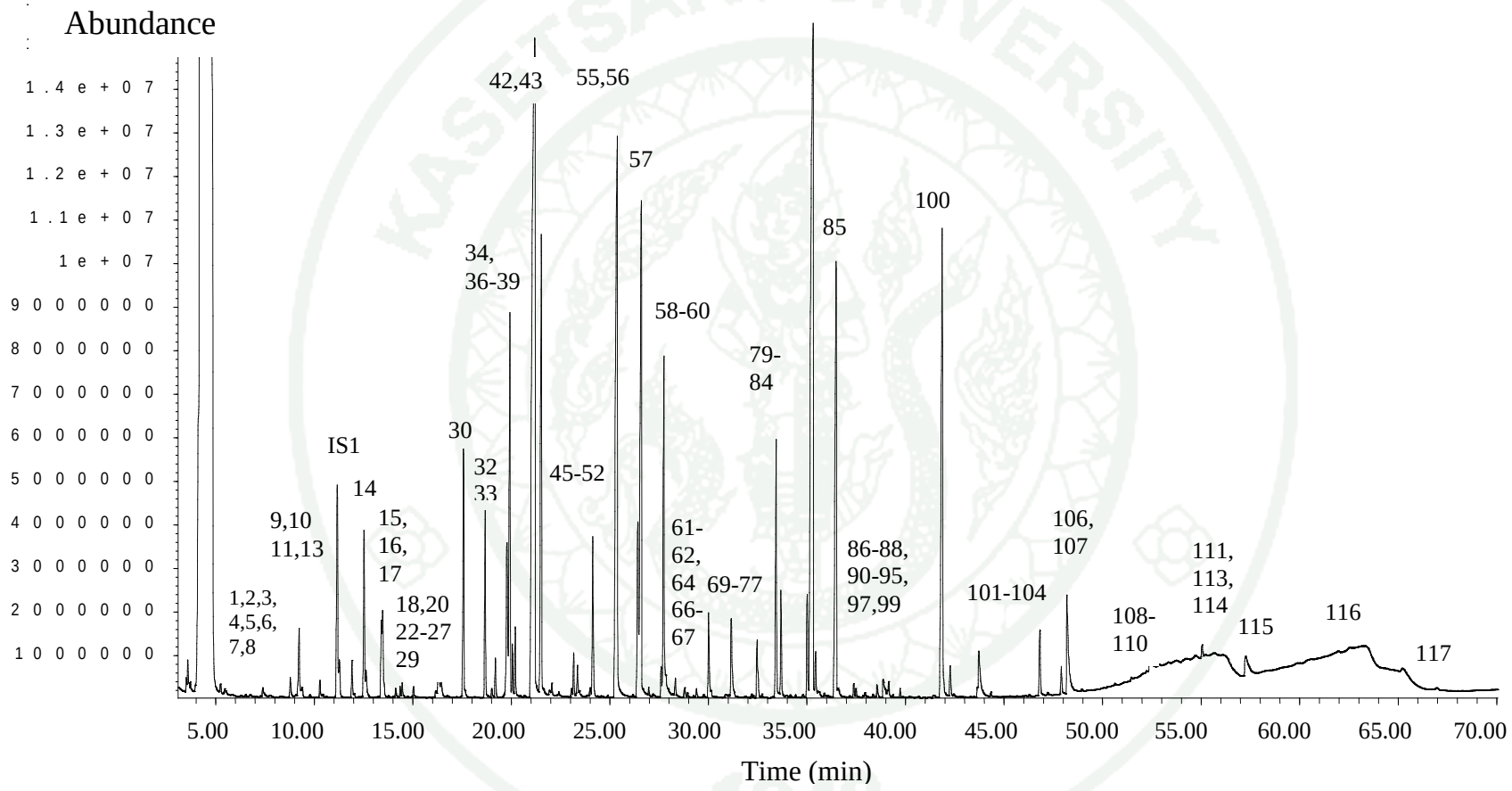
Appendix Figure C4 Chromatogram of headspace volatile compounds of canned coconut milk after canning process (CCM0) in the second experiment, analyzed on the SPME-DB-WAX column; IS 1 =2-methyl-3-heptanone, IS 2 = 2-ethylbutyric acid.



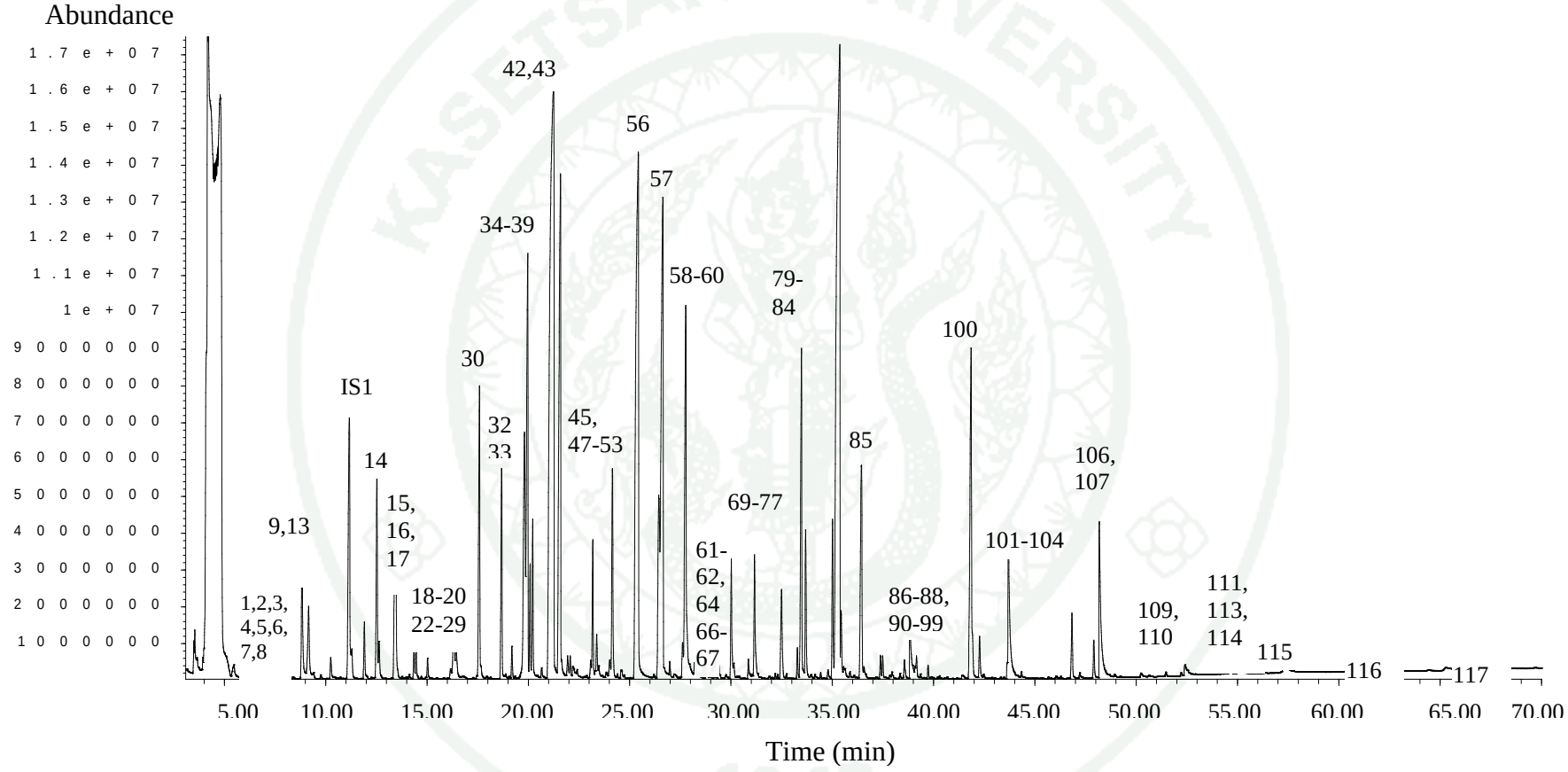
Appendix Figure C5 Chromatogram volatile compounds (N/B fraction) from SAFE extract of fresh coconut milk (FCM) in the second experiment, analyzed on the DB-WAX column; IS1 =2-methyl-3-heptanone.



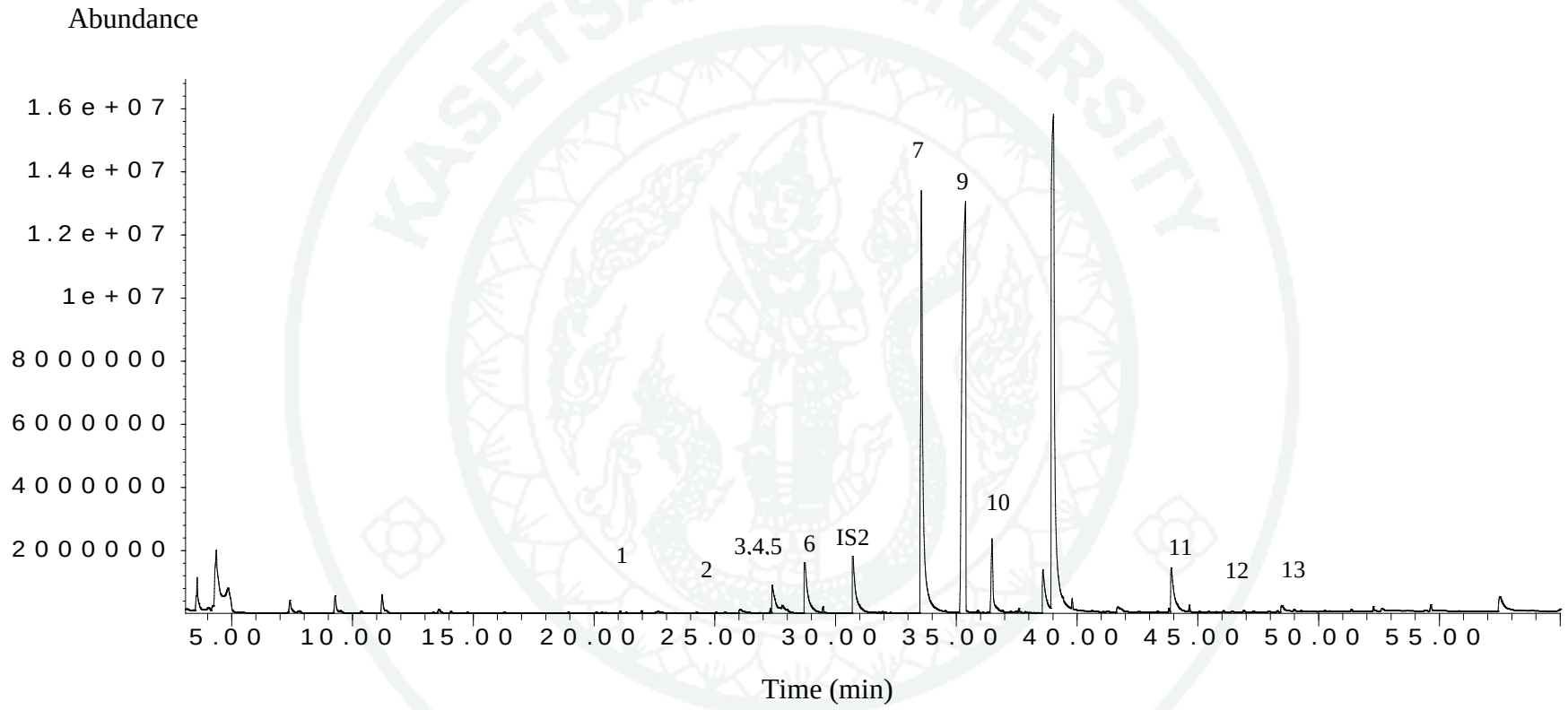
Appendix Figure C6 Chromatogram volatile compounds (N/B fraction) from SAFE extract of canned coconut milk after canning process (CCM0) in the second experiment, analyzed on the DB-WAX column; IS1 =2-methyl-3-heptanone.



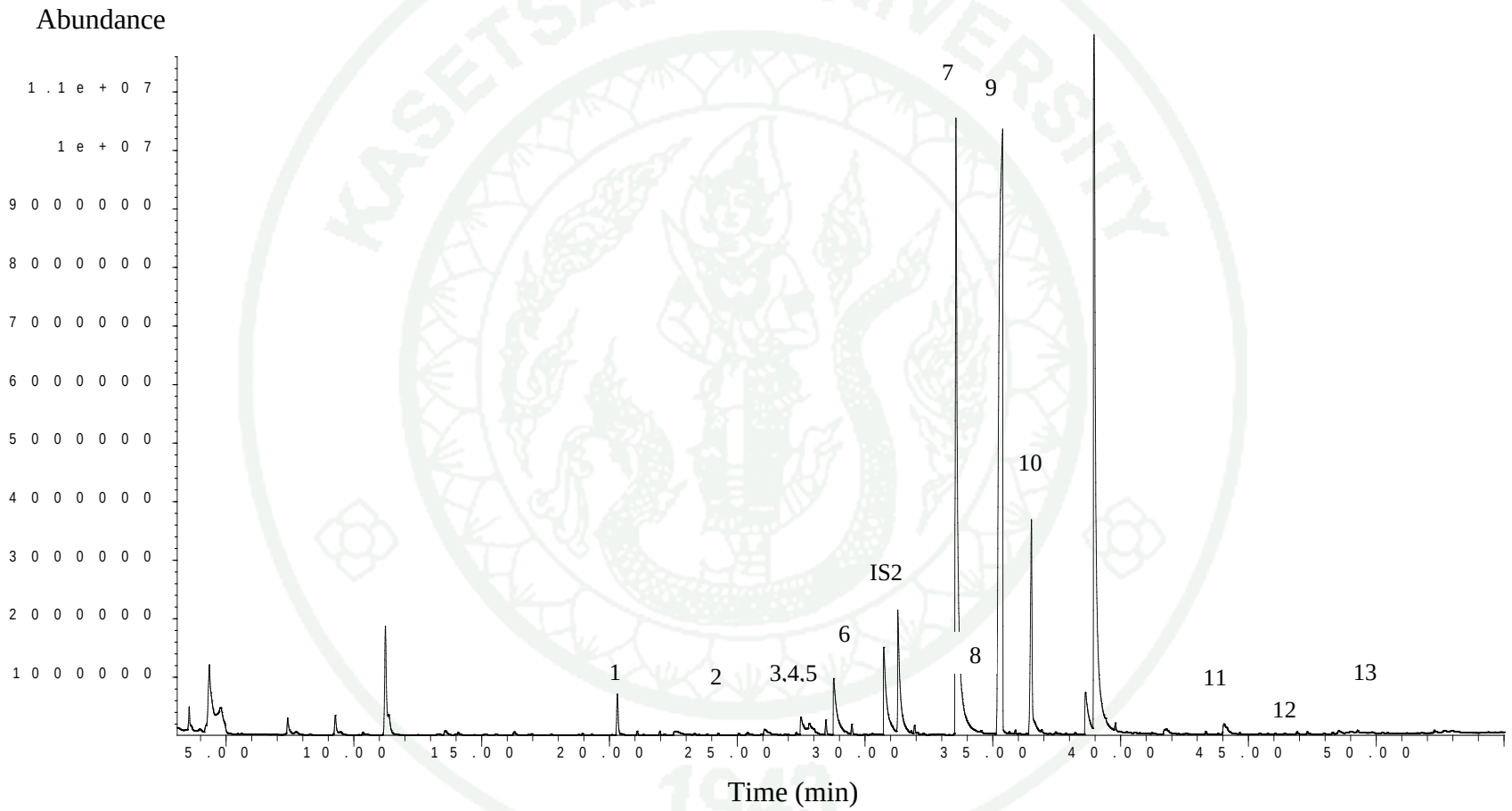
Appendix Figure C7 Chromatogram volatile compounds (N/B fraction) from SAFE extract of canned coconut milk after storage for 3 months at ambient of 23 °C (CCM3-AT) in the second experiment, analyzed on the DB-WAX column; IS1 =2-methyl-3-heptanone.



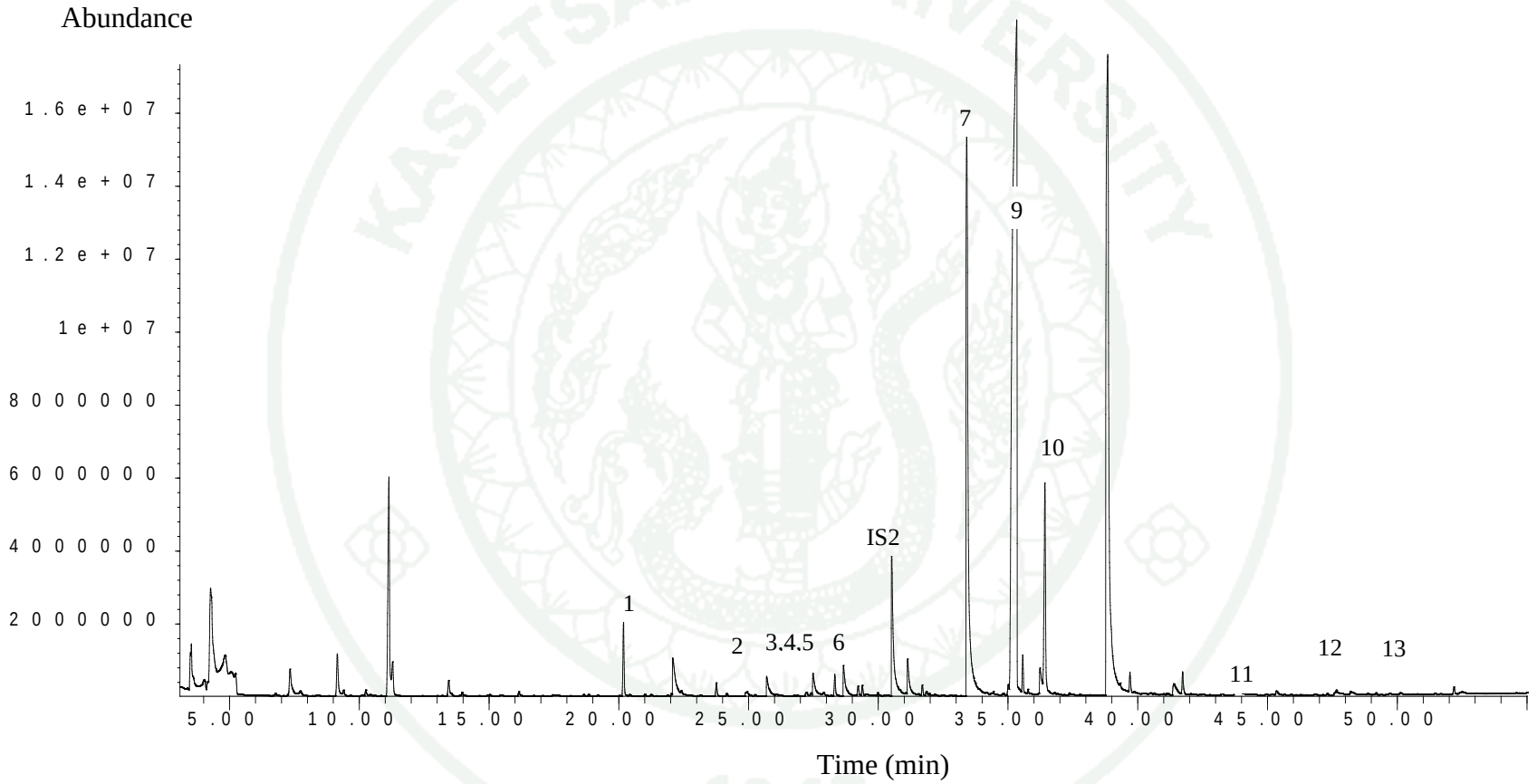
Appendix Figure C8 Chromatogram volatile compounds (N/B fraction) from SAFE extract of canned coconut milk after storage for 3 months at ambient of 40 ° (CCM3-4C) in the second experiment, analyzed on the DB-WAX column; IS1 =2-methyl-3-heptanone.



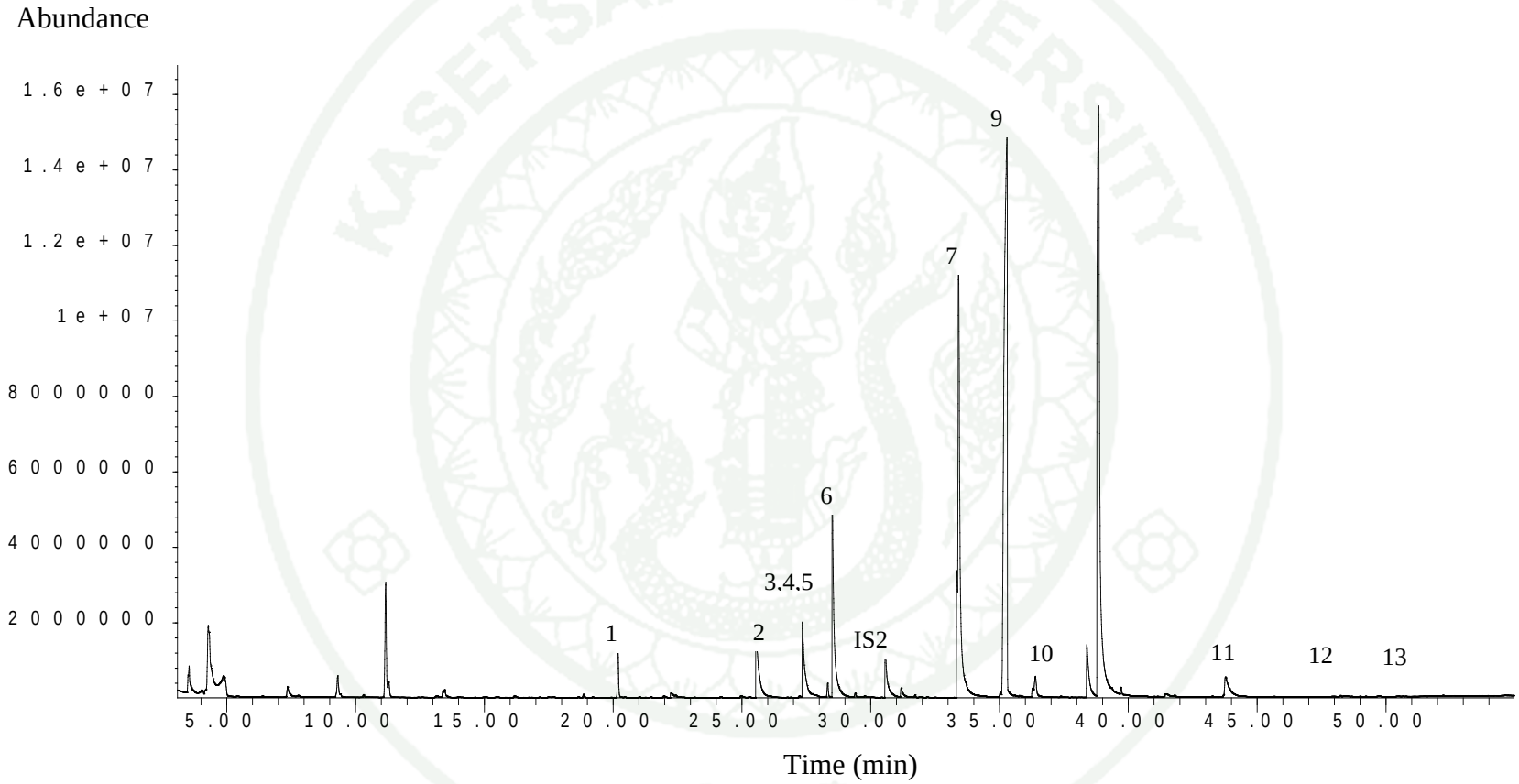
Appendix Figure C9 Chromatogram volatile compounds (acid fraction) from SAFE extract of fresh coconut milk (FCM) in the second experiment, analyzed on the DB-WAX column; IS2 =2-ethyl butyric acid.



Appendix Figure C10 Chromatogram volatile compounds (acid fraction) from SAFE extract of canned coconut milk after canning process (CCM0) in the second experiment, analyzed on the DB-WAX column; IS2 =2-ethyl butyric acid.



Appendix Figure C11 Chromatogram volatile compounds (acid fraction) from SAFE extract of canned coconut milk after storage for 3 months at ambient of 23 °C (CCM3-AT) in the second experiment, analyzed on the DB-WAX column; IS2 =2-ethyl butyric acid.



Appendix Figure C12 Chromatogram volatile compounds (acid fraction) from SAFE extract of canned coconut milk after storage for 3 months at 40 °C (CCM3-40C) in the second experiment, analyzed on the DB-WAX column; IS2 =2-ethylbutyric acid.

Appendix D

Effect of homogenization on emulsion property of coconut milk

Effect of homogenization on the emulsion property of coconut milk

This work was preliminary conducted to study the effect of homogenization on the emulsion property of coconut milk. Three levels of pressure; 11/4 (low), 17/4 (medium) and 23/4 (high) MPa, were used with a two stage homogenizer. The visual property of coconut milks was observed under light and confocal lazer scanning microscopes.

1. Materials and methods

1.1 Sample preparation

Fresh coconut meat was bought from a local market in Bangkok, Thailand. The brown testa of coconut meat was removed. White-fresh coconut meat was grated using a grating machine. The drinkable water was added to grated coconut meat at the ratio of 1:1 (coconut:water). The fresh coconut milk was obtained by using a coconut press and was then heated in hot water at 72 °C for 1 min for pasteurization.

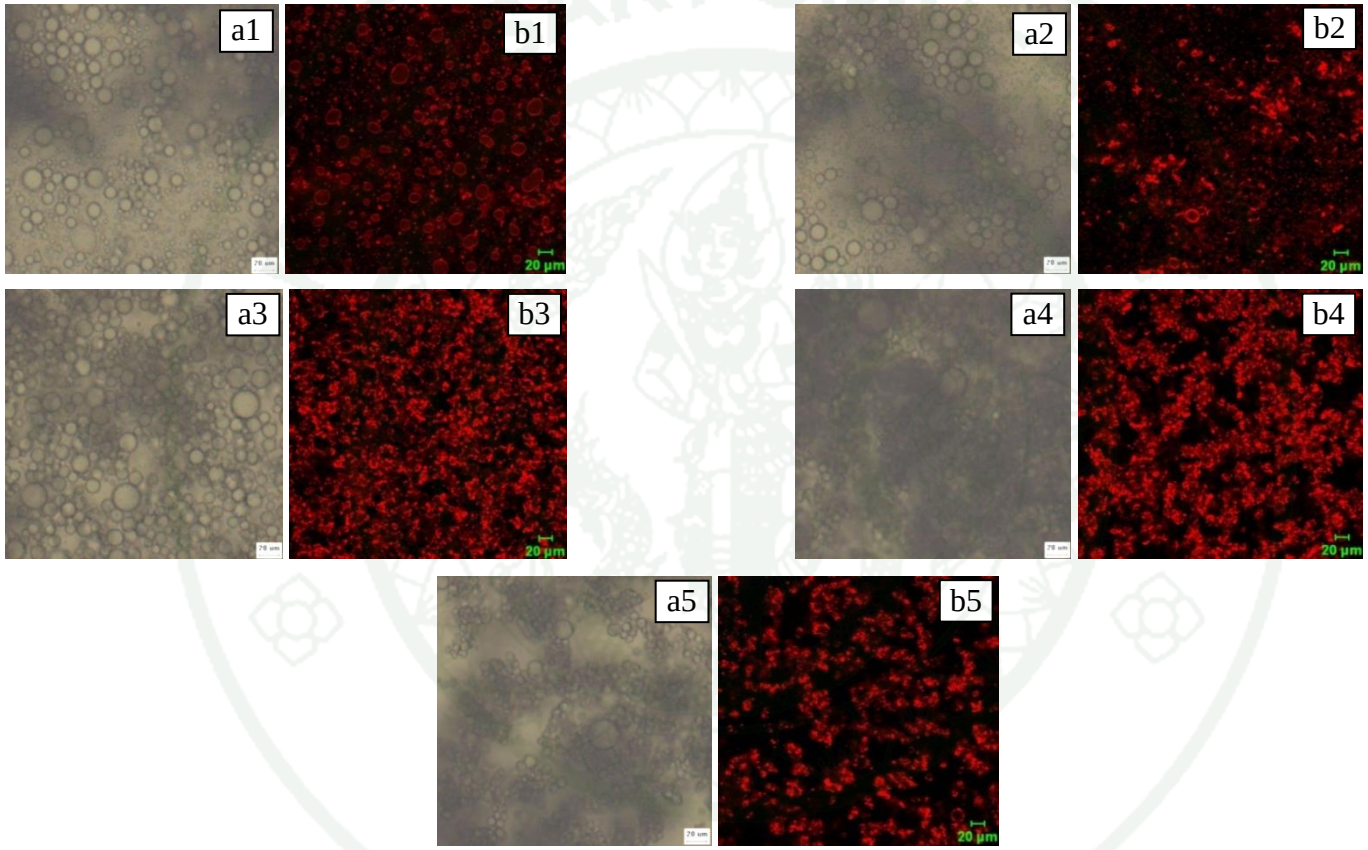
1.2 Homogenization

Heated coconut milks were homogenized under 2 stages homoginizer at three level of pressures, including 11/4 (low), 17/4 (medium) and 23/4 (high) MPa. The visual property of fresh, pasteurized and pasteurized-homogenized coconut milk emulsions was observed under light and confocal lazer scanning microscopes. One milliliter of coconut milk was dyed with 10 μL of 0.2% (w/v) Rhodamin B solution before the confocal lazer scanning microscope observation. The wavelength of 568 nm was used.

2. Results and discussions

The result of this preliminary work was shown in Figure D1. The red layer surrounding oil droplet of coconut milk was the layer of natural proteins. The droplet size of homogenized coconut milk was smaller than those of the fresh and pasteurized coconut milks. However, the small oil droplets tended to flocculate after homogenization because there was low amount of natural proteins to act as emulsifiers. According to Figure D1, the small oil droplets tend to flocculate not to coagulate. Tungsuphoom and Coupland (2008b) had suggested that homogenization increase the surface area of oil droplets, but the amount of natural proteins was not enough to keep the dispersion between fat globules. High shear force combining with the pasteurization temperature might cause the protein denaturation, change of the size and the structure of proteins, followed by the loss of proteins' stabilizing properties. The proteins that previously located at the surface of oil droplets had evidently moved closer and aggregated.

This preliminary had suggested that homogenization without adding stabilizers or emulsifiers was unable to stabilize the emulsion of coconut milk. As such, homogenization was not applied for the preparation of canned coconut milk in this work.



Appendix Figure D1 The emulsion of coconut milk under light (a) and Confocal-laser scanning (b) microscopes; fresh- (a1, b1), pasteurized- (a2 ,b2), pasteurized and homogenized-coconut milk at 11/4 (a3, b3), 17/4 (a4, b4)and 23/4 Mpa (a5, b5).

CURRICULUM VITAE

NAME : Ms. Patcharaporn Tinchan

BIRTH DATE : December 7, 1982

BIRTH PLACE : Phare, Thailand

EDUCATION:

YEAR	**INSTITUTE**	**DEGREE/DIPLOMA**
2000	Kasetsart Univ.	B.Sc. (Food Technology)
2007	Kasetsart Univ.	M.S. (Food Science)

WORK PLACE : Faculty of Natural Resources and Agro Industry, Kasetsart University, Sakon Nakhon Province Campus

SCHOLARSHIP/AWARDS : Royal Golden Jubilee, Ph.D. program. Scholarship 2007-2013