Triacylglycerols and Regiospecific Fatty Acid Analyses of Philippine Seed Oils

Laura J. Pham[a], Edmar P. Casa[a], Myla A. Gregorio[a], and Dae Young Kwon[b,*]

[a]National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines, Los Banos College, Laguna, 4031, Philippines, and [b]Korea Food Research Institute, Poondang, Songnam, Kyongki-do, 463-050, Korea

ABSTRACT: Three Philippine seed oils, namely coconut (*Cocos nucifera* Linn.), pilinut (*Canarium ovatum* Engl.), and cashew (*Anacardium occidentale* Linn.), which were selected for their local abundance and availability, were examined for their triacylglycerol profiles and fatty acid compositions. Triacylglycerol molecular species in terms of carbon number and partition number were determined by gas chromatography and liquid chromatography, respectively. The distribution of fatty acids in the primary and secondary positions of the glycerol backbones for the three oils were examined by regiospecific analysis by using pancreatic lipase. Coconut oil had high concentrations of lauric and myristic acids, while the other two oils did not have such fatty acids. Lauric acid in coconut oil and linoleic acid in pilinut oil were distributed mainly in the primary positions (*sn*-1,3) of the glycerol backbone. Trilaurin and dioleylpalmitoylglycerol were the major triglycerides in coconut and pilinut oils, respectively. *JAOCS 75,* 807–811 (1998).

KEY WORDS: Carbon numbers, equivalent carbon numbers (partition numbers), regiospecific analysis, triacylglycerol molecules.

The Philippines has a vast potential of seed oil resources, such as coconut, pilinut, and cashew. Some of their oils could be altered into products with desired characteristics by using advanced technology (1). The biotechnological route of enzymatic interconversion of the triacylglycerols (TG) could result in tailored fats or specialty oils and could represent a revolutionary possibility for upgrading Philippine oils. However, there is little information about structural properties of these oils, such as TG molecule compositions, the stereo- or regiospecific distribution of fatty acids in the glycerol backbone, and even fatty acid compositions, although their structural characteristics should be known before tailoring such oils and fats (2).

The physical properties of oils and fats are determined by their TG structure (molecular species) and by the positional fatty acid structure, in addition to total fatty acid composition (3). Stereospecific analyses on the three different positions (*sn*-1, -2, and -3) of the glycerol backbone give the most valuable information (4). However, owing to the difficulty of stereospecific analysis, regiospecific analysis, which discriminates only between primary positions (outside: *sn*-1 and -3) and secondary positions (center: *sn*-2) of the glycerol backbone, is more commonly used for structural properties of oils and fats (1). For cocoa butter, the stereospecific fatty acid composition within the primary positions (*sn*-1 and -3) is similar, but the regiospecific differences of the fatty acid composition between the primary and secondary positions differ substantially (5). The characteristic physical properties of cocoa butter should be due to this regiospecific difference in the fatty acid composition.

Determining the TG molecular species, such as tripalmitin (PPP), triolein (OOO), and dipalmitoylolein (PPO), is another good way to estimate the structural properties of oils and fats, which are important for oils and fats processing (6). Usually, TG species are analyzed by determining the carbon number (CN = total carbon number of acyl groups) by gas chromatography (GC) (7,8) and the partition number (PN = CN − 2 × total number of double bonds), which is also called the equivalent carbon number, by high-performance liquid chromatography (HPLC) (9,10). Free fatty acids (FFA) could be also analyzed by HPLC to determine PN (11).

This research looked into the characteristics of Philippine seed oils in terms of their TG molecular species and regiospecific fatty acid compositions.

MATERIALS AND METHODS

Materials. Seed oils were used from coconut (*Cocos nucifera* Linn.), pilinut (*Canarium ovatum* Eng.), and cashew (*Anacardium occidentale* Linn.), which were procured from the Horticulture Department, University of the Philippines, Los Banos, and from the Los Banos junction market. The commercial lipase utilized was a Type II pancreatic lipase from Sigma Chemical Co. (St. Louis, MO). TG—tricaprin (CN = PN = 30), trilaurin (CN = PN = 36), trimyristin (CN = PN = 42), tripalmitin (CN = PN = 48), triolein (CN = 54, PN = 48), trilinolein (CN = 54, PN = 42), and trilinolenin (CN = 54, PN = 36)—and other substituted TG mixtures (for example, 1,2-dioleoyl-3-stearoylglycerol; CN = 54, PN = 50), which were used as standards for CN and PN identification, were purchased from Sigma. Standard fatty acids were also from Sigma. All other chemicals were of analytical grade.

Separation of TG and fatty acid composition determinations. Thin-layer chromatography (TLC) separations of seed

*To whom correspondence should be addressed at Korea Food Research Institute, Poondang, P.O. Box 2, Songnam, Kyongki-do, 463-050, Korea. E-mail: dykwon@hanmail.net

oils were done on silica gel impregnated with 5% boric acid. After developing the plate with a mixture of hexane/diethyl ether/formic acid (80:20:2, vol/vol/vol), the amounts of each fractionated monoacylglycerol (MG), diacylglycerol (DG), and TG were estimated with a video densitometer (Zeineh, Biomedical Instrument, Fullerton, CA) (2,12). TG were separated by using modified standard methods for fats and oils on silicic acid columns eluted with hexane/diethyl ether (12). Enzymatic hydrolysis of oils by pancreatic lipase was conducted by a modified Luddy method (13).

Products of hydrolysis, FFA, DG, and MG were isolated by TLC, impregnated with boric acid (2,14), and their esterified fatty acids were hydrolyzed and converted into methyl esters with BF_3-methanol by the conventional AOAC method (15). The composition of fatty acid methyl esters of TG was also determined by GC (Shimadzu 7AG-PRPF, flame-ionization detector, Tokyo, Japan) with a capillary Carbowax 10 column (Supelco, Bellefonte, PA). Fatty acids were identified according to the usual procedure by means of fatty acid methyl ester standards (16).

TG molecular species analysis. TG were fractionated in terms of PN by HPLC (Waters 510; Milford, MA). The column used was a reversed-phase μ-Bondapak C_{18} analytical column (4 mm i.d. × 30 cm, 4 μm), and the mobile phase was acetonitrile/acetone/methanol/chloroform = 3:3:3:1 (by vol) (17). The flow rate was isocratically controlled at 1.2 mL/min. The peaks were detected by refractive index detector (Waters Model 410 Differential Refractometer) and integrated (Waters Data Module, Model 745). After collecting each fraction from HPLC, the TG composition in terms of CN was determined by GC on a 1% SE 30 packed glass column [1% SE 30 on 80/100 Chromosorb W HP (Supelco), 0.5 m × 3 mm i.d.]. The flow rate of nitrogen was 60 mL/min, and the column oven temperature was programmed from 200 to 340°C at the rate of 4°C/min. Identification of each species by HPLC and GC was done directly with standard materials or from calibration curves between PN and CN retention time (8,10,17).

Regiospecific analysis of fatty acids of each oil. After hydrolyzing the TG with pancreatic lipase, which has *sn*-1,3-specific regiospecificity (18), the resulting *sn*-2-MG were separated from FFA by TLC on silica gel impregnated with boric acid, and bands were scraped off separately. The fatty acid composition of *sn*-2-MG was analyzed by GC after esterification (15). The fatty acid composition of the *sn*-1,3-positions (*sn*-1,3) were calculated from the concentrations in the intact TG and in *sn*-2-MG with the following equation (2,20):

$$sn\text{-}1,3 = 1.5 \times \text{TG} - 0.5 \times sn\text{-}2\text{-MG} \qquad [1]$$

RESULTS AND DISCUSSION

TG and fatty acid profiles of seed oils. TLC separation of the seed oils (Table 1) shows that the majority of the lipid molecular species is TG. The FFA and the partial glycerols are low in comparison, which is in agreement with the findings of other researchers (19).

TABLE 1
TLC Separation of Local Seed Oils on Silica Gel Impregnated with 5% Boric Acid for Mono-, Di-, and Triacylglycerol Species[a]

	Composition (%)		
Glycerides	Coconut	Pilinut	Cashew
Monoacylglycerol	3.45	5.20	6.95
Diacylglycerols	6.55	14.03	21.19
1,3-Diacylglycerols	(6.55)	(6.55)	(8.49)
1,2-Diacylglycerols		(7.58)	(7.42)
Triacylglycerols	85.38	75.01	54.24
Complex lipids	4.62	5.66	1.71

[a]Developing solvent for thin-layer chromatography (TLC): hexane/diethylether/formic acid (80:20:2, vol/vol/vol).

The fatty acid compositions of the TG for each oil were determined (Table 2). Fatty acid analysis of the TG shows that the TG of coconut oil has a low degree of unsaturation (9%) and contains high levels of the two saturated fatty acids, lauric ($C_{12:0}$) and myristic ($C_{14:0}$). These two saturated fatty acids make up 51 and 21% of the fatty acid content, respectively. Cashew oil TG contains four major fatty acids and is 87% unsaturated, with 66% of this being oleic acid ($C_{18:1}$). Pilinut is likewise abundant in oleic acid and is 67% unsaturated. Among the local seed oil TG, only pilinut and cashew oils are predominantly unsaturated and do not contain shorter-chain fatty acids (19). The shortest fatty acid detected in these oil TG is palmitic acid ($C_{16:0}$).

For detailed analysis of TG species for each oil, separation of TG was made by HPLC in terms of PN (Table 3). Each PN fraction separated as almost single peaks, but some fractions contain shoulder peaks (not shown). In fact, within the same PN, higher-CN TG appear faster than lower-CN fractions in HPLC (8,17). There were nine PN components in coconut oil, the smallest being PN28, and the highest PN44. The predominant component is PN36, followed by PN38 and PN34. Pilinut TG has five components, with PN48 dominating, followed by PN46; the highest is PN50. Cashew on the other hand has four components, and PN48 dominates, followed by PN50.

TABLE 2
Fatty Acid Profile of Different Local Seed Oil Triacylglycerols

	Fatty acid composition (%, w/w)		
Fatty acid	Coconut	Pilinut	Cashew
C_8	0.91		
$C_{10:0}$ (C)[a]	3.78		
$C_{12:0}$ (La)	50.92		
$C_{14:0}$ (M)	21.09		
$C_{16:0}$ (P)	9.46	26.46	8.11
$C_{18:0}$ (S)	4.88	6.53	5.13
$C_{18:1}$ (O)	8.39	56.55	66.18
$C_{18:2}$ (L)	0.56	10.09	20.58
$C_{18:3}$ (Ln)		0.37	
$C_{20:n}$		trace	
Unsaturated fatty acid (%)	8.95	67.01	88.76

[a]Symbols in parentheses after fatty acid are: C, capric acid; La, lauric acid; M, myristic acid; P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid.

Further separation of TG on a molecular weight basis by high-temperature GC showed that coconut oil had 14 components with different CN, from CN26 to CN52 (Table 4). The predominant TG has a carbon number of CN38, followed by CN36. Compared to other seed oil TG studied, only coconut oil has the low-molecular-weight components, which was expected because it contains short- and medium-chain fatty acids (Table 2). Cashew and pilinut oils, which do not have medium-chain fatty acids (Table 2), showed marked differences in the TG species, with the CN52 and CN54 species dominating (Table 4).

Molecular species analysis of TG. Each TG component, based on PN, is not necessarily a single TG molecule, as confirmed by GC analysis. For example, for coconut TG, PN36 could possibly consist of three CN based on fatty acid composition data, such as CN36, CN38, and CN40 (Table 2). Therefore, in some experiments, three shoulder peaks appeared in the PN36 fraction, which could be separated by GC based on CN. However, the CN36 peak did not show any shoulder peak and appeared just like a single peak. After collecting each PN peak from coconut and pilinut oil, CN was determined by GC. Molecular species of TG for each fraction are shown in Table 5.

For the PN36 fraction of coconut oil, there could be three possible TG molecules in CN36, namely MLaC, PCC, and LaLaLa (M, myristic acid; La, lauric acid; C, capric acid; and P, palmitic acid). The other molecules present are dicaproyloleoylacylglycerol (OCC; O, oleic acid) in CN38 and linoleoylauroylcaproylacylglycerol (LLaC; L, linoleic acid) in CN40. Among the TG species of PN36, the CN36 fraction was

TABLE 3
Triacylglycerol Profiles in Terms of Partition Number (PN) of Different Local Seed Oils as Determined by High-Performance Liquid Chromatography (HPLC)

	Composition of fractions (%)		
PN	Coconut	Cashew	Pilinut
26	—		
28	0.39		
30	2.67		
32	11.82		
34	17.25		
36	22.11		
38	19.08		
40	14.26		
42	8.01		1.70
44	4.37	3.29	8.96
46		15.14	26.37
48		60.22	47.51
50		21.34	15.45
52		trace	—
54		—	—

TABLE 4
Triacylglycerol Profiles in Terms of Carbon Number (CN) of Different Local Seed Oils as Determined by Gas Chromatography (GC)

	Composition of fractions (%)		
CN	Coconut	Cashew	Pilinut
26	0.07		
28	0.44		
30	2.28		
32	10.24		
34	14.71		
36	20.10		
38	20.43		
40	13.74		
42	9.00		
44	4.21		
46	2.12		
48	1.52	trace	5.91
50	0.77	2.88	23.21
52	0.36	27.18	45.15
54		69.24	25.28
56		0.68	0.44

TABLE 5
Triacylglycerol Species Profiles of Coconut and Pilinut Oils as Determined by HPLC and GC[a]

			Composition (%)	
PN	CN	Triacylglycerol species	Coconut	Pilinut
30	30	CCC[a]	1.89	
32	32	LaCC	8.75	
34	34	MCC, LaLaC[b]	15.00	
	38	LCC	0.06	
36	36	MLaC, PCC, LaLaLa	23.16	
	38	OCC	1.65	
	40	LLaC	0.03	
38	38	MLaLa, MMC, PLaC, SCC	18.22	
	40	OLaC	1.11	
	42	LMC, LLaLa	0.22	
	44	LnPC, LnMLa	0.33	
40	40	MMLa, PMC, SLaC, PLaLa	11.25	
	42	OMC, OLaLa	2.34	
	44	LPC, LMLa	0.29	
	46	LOC, LnPLa	0.04	
42	42	MMM, SMC, PPC, PMLa, SLaLa	7.15	
	44	OPC, OMLa	1.84	
	46	OOC	0.19	
	48	SLaLn, LLaO	0.08	
44	44	SPC, SMLa, PMM	2.36	
	46	SOC, OPLa	1.54	
	48	OOLa	0.73	
	50	OLM, SMLn, *PLP*[c]	0.08	
	52	*OLnP, LLP*		0.47
	54	*OLL, SLLn, OOLn*		0.80
46	46	SSC, PPM, SPLa	0.30	—
	48	SOLa	0.54	—
	50	OOM, SML, OPP	0.21	0.72
	52	*OLP, SPLn*		4.10
	54	*OOL, SLL, SOLn*		11.14
48	48	*PPP*, SSLa, SMP	0.38	16.57
	50	SOM		—
	52	*OOP, SPL*		34.99
	54	*OOO, SSLn, SOL*		14.63
50	50	SSM, *SPP*		7.55
	52	*SPO*		8.02
	54	*OOS, SSL*		12.47
52	52	*SSP*		—
	54	*SSO*		0.89

[a]For abbreviations see Tables 2–4.
[b]Major triacylglycerol species are underlined.
[c]The triacylglycerols written in italic characters are exclusive for pilinut oil.

about 23%, but MLaC and PCC must be rare because capric acid was very low (less than 4%, see Table 2). Thus, the major component of this fraction was LaLaLa. From similar analyses of the PN34, PN38, PN40, and PN42 fractions, we can deduce that major molecules in decreasing order were CLaLa, MLaLa, PLaLa, and SLaLa (S, stearic acid). These five molecules made up almost 70% of coconut oil. LaLaLa, which was over 20%, was the most predominant molecular species.

With the same procedures for pilinut oils, the major TG molecule was found to be dioleoylpalmitoylglycerol (OOP), followed by PPP, OOO, OOL, PPP, OOS, and SPO. In CN52 of PN48, the total OOP and SPL was about 35% (Table 5). However, palmitic (26%), stearic (6%), and linoleic (10%) acids were low compared to oleic (56%) (Table 2). Thus, SPL should be rare, and the major component must be OOP in CN52 of the PN48 fraction. Because L (11%) in pilinut was higher than S (6.5%), OLP should be expected to be higher than OSP if L and S were distributed randomly. However, results showed that OLP (4%) was less than OSP (8%) in pilinut TG species. This showed that the fatty acids, especially L and S, were not distributed nonspecifically, and this fact was confirmed by the following regiospecific analysis of fatty acid composition.

Regiospecific analysis of fatty acids. Our preliminary work on the positional distribution of fatty acids on the glycerol backbone was done with pancreatic lipase (2). This lipase is completely specific for the primary ester bonds of glycerides. Although there are other purified preparations of microbial lipases that have been produced industrially and are commercially available (21), pancreatic lipase was selected because it is commercially available at low cost and the impurities do not interfere with structural studies.

Pancreatic lipase hydrolysis of coconut oil (Table 6) showed that the major fatty acid, which is La, is preferentially esterified to the primary positions (*sn*-1 and -3), while M and P were preferentially attached to the second position (*sn*-2). Interestingly, in cashew oil, all fatty acids have about equal distribution between *sn*-2 and -1,3. Pilinut oil, on the other hand, which has O and P as the major fatty acids, has almost equal distributions of O and P between the two positions. Stearic acid, however, was preferentially attached to the second position, while the polyunsaturated fatty acid, linoleic, was specifically esterified to the primary position. This information was consistent with the molecular species determination of OLP and OSP mentioned above (Table 5). This is quite a deviation from Brockerhoff's general rule, which states that saturated fatty acids occupy the primary *sn*-1 and -3 positions and unsaturated fatty acids occupy the second position (4). In fact, many other results have deviated from this rule (20).

This information should be useful because fatty acid positions could be highly important aspects of the biosynthetic process and in the tailoring process (1,21), and because the properties of fats and oils are crucially determined by the positional or stereospecific positions of the fatty acids in addition to the total fatty acid composition (2,3).

REFERENCES

1. Kwon, D.Y., E.P. Casa, and L.J. Pham, Lipase-Catalyzed Interesterification in Non-Aqueous System, *Foods Biotechnol. 5:*349–352 (1996).
2. Pham, L.J., and D.Y. Kwon, Regiospecific Analysis of Triacylglycerols of Some Seed Oils by Pancreatic Lipase, *Ibid. 1:*75–78 (1992).
3. Bhati, A., Fatty Acid Sequence in Triglycerides and Related Compounds, in *Recent Advances in Chemistry and Technology of Fats and Oils,* edited by R.J. Hamilton and A. Bhati, Elsevier Applied Science, London, 1987, pp. 13–22.
4. Brockerhoff, H., A Stereospecific Analysis of Triglycerides, *J. Lipid Research 8:*167–169 (1965).
5. Chacko, G.K., and E.G. Perkins, Glyceride Structure of Cocoa Butter, *J. Am. Oil Chem. Soc. 41:*843–847(1964).
6. Birker, P.J.M.W.L., and F.B. Padley, Physical Properties of Fats and Oils, in *Recent Advances in Chemistry and Technology of Fats and Oils,* edited by R.J. Hamilton and A. Bhati, Elsevier Applied Science, London, 1987, pp. 1-12.
7. Halow, R.D., C. Litchfield, and R. Reiser, Gas-Liquid Chromatography of Triglycerides from Erucic Oils and Fish Oils. *Lipids 1:*216–220 (1965).
8. Plattner, R.D., K. Wade, and R. Kleiman, Direct Analysis of Triglyceride by High-Performance Liquid Chromatography, *J. Am. Oil Chem. Soc. 55:*381–383 (1978).

TABLE 6
Regiospecific Analysis of the Fatty Acid Composition of the Different Local Seed Oils with Pancreatic Lipase

		Mol%								
Oil	Position	C_{10}	C_{12}	C_{14}	C_{16}	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total
Coconut	*sn*-2[a]	1.6	44.1	24.9	17.2	9.4	3.7	trace		100
	sn-1,3[b]	4.9[c]	54.3	19.2	5.6	3.1	9.7			95.7
Pilinut	*sn*-2				25.3	11.1	63.6			100
	sn-1,3				24.0	4.3	53.0	15.1		96.0
Cashew	*sn*-2				9.6	5.3	64.8	20.3		100
	sn-1,3				7.4	5.1	66.2	20.5		99.0

[a]Fatty acid composition in *sn*-2 position obtained from *sn*-2-monoglyceride, which was hydrolyzed by pancreatic lipase and separated by TLC (19).
[b]Fatty acid composition in *sn*-1,3-position, which was calculated from intact triacylglycerol (TG) and *sn*-2-monoacylglycerol (2-MG) by (3[TG] − [2-MG])/2 (20).
[c]This value (4.9%) deviated much from the fatty acid composition determination (2.5%) of free fatty acid hydrolyzed from the *sn*-1,3-glycerol backbone after TLC separation. For abbreviation see Table 1.

9. Litchfield, C., Triglyceride Analysis by Consecutive Liquid-Liquid Partition and Gas–Liquid Chromatography. *Ephedra nevadensis* Seed Fat, *Lipids 3:*170–177 (1967).
10. Wada, S., C. Koizumi, A. Takiguchi, and J. Nonaka, A Study on Triglyceride Composition of Lipid from Commercial Beef by HPLC to Oils and Fat Analysis, *Yukagaku 28:*15–20 (1978).
11. Yoon, S.H., Microbial Lipid Production: Biosynthesis and Accumulation Mechanism of Lipids in Oleaginous Yeast, Ph.D. Thesis, Korea Advanced Institute of Science and Technology, Seoul, 1983.
12. Christie, W.W., *Lipids Analysis*, 2nd edn., Pergamon Press, Oxford, 1982, pp. 25–41.
13. Luddy, F.E., R.A. Barford, S.F. Herb, P. Magidman, and R.W. Riemenscheider, Pancreatic Lipase Hydrolysis of Triglycerides by Semimicrotechnique, *J. Am. Oil Chem. Soc. 41:*693 (1964).
14. Brockerhoff, B., Stereospecific Analysis of Triglycerides, *Lipids 6:*942–956 (1971).
15. Fatty Acids in Oils and Fats, Preparation of Methylester, in *AOAC Official Methods of Analysis,* Association of Official Analytical Chemists, Arlington, 1990, p. 969.33.
16. Paquot, E., *Standard Methods for the Analysis of Oils, Fats and Derivatives,* 6th edn., Pergamon Press, London, 1977, pp. 103–108.
17. Chon, S.J., Molecular Species of Triglycerides in Some Vegetable Oils, Ph.D. Thesis, National Fisheries University of Pusan, Korea, 1986.
18. Macrae, F.E., Microbial Lipases as Catalysts for Interesterification of Oils and Fats, in *Biotechnology for the Oils and Fats Industry,* edited by C. Ratledge, P. Dawson, and J. Rattray, AOCS Press, Champaign, 1984, pp. 189–198.
19. Alexander, B., G.J. Piazza, R.G. Bistline, Jr., and M.J. Haas, Separation of Cholesterol and Fatty Acylglycerols, Acids and Amides by Thin-Layer Chromatography, *Lipids 26:*405–406 (1991).
20. Christie, W.W., The Positional Distributions of Fatty Acids in Triglycerides, in *Analysis of Oils and Fats,* edited by R.J. Hamilton and J.B. Rossell, Elsevier, New York, 1986, pp. 313–340.
21. Kwon, D.Y., H.N. Song, and S.H. Yoon, Synthesis of Medium Chain Glycerides by Lipase in Organic Solvent, *J. Am. Oil Chem. Soc. 73:*1521–1525 (1996).

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