

IN SITU REACTIVE EXTRACTION OF PALM KERNEL CATALYZED BY LIPASE ENZYME

T. Herawan¹, M. Rüschen. Klaas², and S. Warwel³

ABSTRACT

A lipase-catalyzed in situ reactive extraction method has been developed to find environmentally friendly process for producing alkyl esters. In this method, dialkyl carbonates such as dimethyl and diethyl carbonate were used as solvent and substrate. In situ reactive extraction was done with solvent/seed ratio about 7.5 ml/g and 60 °C for 24 hours. Various amount of water was added to the solvent to study its effect on the extraction yield and ester formation. The rate of ester formation reaches the highest value when water is not added to the system. However, by addition of 0.2% water, after 24 hour reactive extraction yielded 59% (w/w of seed) and about 90% ester thereof.

Key word: in situ reactive extraction, dialkyl carbonate, lipase, alkyl esters

ABSTRAK

Suatu metoda ekstraksi sekaligus reaksi secara in situ menggunakan katalis lipase telah dikembangkan untuk mendapatkan proses pembuatan alkil ester yang ramah lingkungan. Pada metoda ini, dialkil karbonat seperti metil dan etil karbonat digunakan sebagai pelarut sekaligus juga sebagai substrat. Ekstraksi sekaligus reaksi secara in situ ini dilakukan dengan menggunakan perbandingan pelarut/biji sekitar 7,5ml/g dan suhu 60 °C selama 24 jam. Sejumlah tertentu air ditambahkan kedalam pelarut untuk untuk mempelajari pengaruhnya terhadap hasil ekstraksi dan ester yang terbentuk. Laju pembentukan ester tertinggi terjadi pada saat tidak adanya penambahan air ke dalam sistem, namun demikian, dengan penambahan 0,2% air ke dalam pelarut, setelah 24 jam ekstraksi sekaligus reaksi, diperoleh hasil ekstraksi sebanyak 59% (b/b biji) dan sekitar 90% kandungannya adalah ester.

Kata kunci: in situ reactive extraction, dialkil karbonat, lipase, alkil ester

¹ Indonesian Oil Palm Research Institute (IOPRI), Medan 20158 – Indonesia

² Department of Technology, Neubrandenburg University of Applied Science, 17033 Neubrandenburg – Germany

³ Institute for Lipid Research (BAGKF), 40147 Münster – Germany

INTRODUCTION

Among the various chemicals derived from vegetable or plant oils are alkyl esters. Production of long-chain alkyl esters is industrially important because these are quite hydrophobic compounds and intermediate raw materials that are used in vary many chemical products, including textiles, cosmetics, pharmaceuticals, plastics, and recently as diesel fuel and lubricants (4). Alkyl esters are used rather than fatty acids to produce a number of fatty acids derivatives because alkyl esters are more stable, less corrosive and are more easily fractionated (10).

The feedstock for the most important alkyl esters (such as methyl and ethyl esters) are the most common vegetable or plant oils available in a country or location. The most common source of alkyl esters in Europe is rapeseed oil (low-erucic acid variety), in the United States it is soybean oil, in Malaysia and others countries with a tropical climate it is palm oil, and in some countries coconut oil, sunflower oil and used frying oils are also used (12).

The processes to produce alkyl esters have been used for many years in the oleo chemical industry. Transesterification of vegetable oils or animal fats is the most effective process for the transformation of triglyceride molecules into smaller, straight chain molecules of alkyl esters (13). The most common method to produce alkyl esters is the transesterification of vegetable oils or animal fats with an alcohol in the presence of a chemical catalyst, usually a base such as sodium or potassium

hydroxide, or alcoholysis of vegetable oils or animal fats using enzymes, normally lipases, as biocatalysts (2,3,5). The fat and oil that used as raw material are normally recovered from oil-bearing seeds, fruits, and fatty animal tissues by solvent extraction, wet processing or dry processing (mechanical pressing) (9).

Another approach to produce alkyl esters from oil seed or oily sources is by reactive extraction method. Reactive extraction is simultaneous oil extraction and - in case of alkyl esters producing is - transesterification. In this process, an organic solvent such as alcohol acts as an extraction solvent for oil component and as reagent to esterify this component. By using this process the production cost may be reduced since the oil extraction step in conventional process will be omitted as well.

In situ esterification was first performed by transesterification of sunflower seed oil with acidified methanol, and it resulted that this process produce fatty acid methyl esters in yields significantly greater than those obtained from conventional reaction with pre-extracted seed oil. Yield improvement of over 20% was achieved and could be related to the moisture content of the seed. The presence of moisture in the oilseed reduces the yield of methyl esters (6). By using acid catalyst, several researchers were also reported *in situ* transesterification of different oily sources, such as rice brand (14,15,16), soybean (11), cod-liver and micro algae (1,17). Haas *et al.* (15) used alkaline catalyst for *in situ* transesterification of soybean.

Different method was performed by Torres *et al.* that investigated one pot triacylglycerides extraction and methyl ester formation using fungal resting cells and oilseeds (rapeseed) at moderate temperature with or without solvent. However the yield was only 25 % after 24 hours and rose to 65% after 120 hours (18).

A lipase-catalyzed *in situ* reactive extraction method has been developed to find environmentally friendly process for producing alkyl esters. In this method, dialkyl carbonates such as dimethyl and diethyl carbonate were used as solvent and substrate.

MATERIALS AND METHOD

Material

Oilseed material.

Palm Kernel, rubber seed and saga seed were obtained from Indonesian Oil Palm Research Institute (IOPRI), Medan – Indonesia, while candlenut, fenugreek, and mustard were obtained from local Asian market Germany. The seed was milled using a mechanical grinder before use.

Chemicals.

Dimethyl carbonate, diethyl carbonate, methanol, ethanol and isohexane were for analysis grade and purchased from Merck (Germany). All solvents used were percolated prior by Puralox SCCa 150/145 N before used. N-Methyl-N-Trimethylsilylheptafluor (o) butyramide (MSHFBA) was used for silylation and obtained from Macherey-Nagel (Germany).

Method

To the 250 ml three neck round bottom glass (completed with condenser) containing 10 gram of ground palm kernel was added 75 ml of dimethyl or diethyl carbonate (containing various amount of water) and 500 mg of Novozym 435. The mixture was stirred for 24 hours at 60 °C and normal pressure. Samples were withdrawn at specified interval during the 24 hours *in situ* reactive extraction period. Samples were filtered to remove the enzyme and ground seed, and the excess of dimethyl or diethyl carbonate was evaporated. Filtrates were stored at -20 °C before analyzed further. After 24 hours, the reaction was stopped, enzyme and ground seed residue were filtered by filter paper and excess of solvent was evaporated at reduce pressure. Lipid was then weighed accurately and analyzed. The ground seed residue was reextracted in a Soxhlet apparatus with isohexane for 8 hours, to yield a further quantity of lipid product.

Analysis

Lipid composition was analyzed by Gas Chromatography instrument (HP6890) equipped with a FID Detector and a high temperature HT5 AQ (SGE), 12 m x 0.22 mm i.d column. The film thickness was 0.1 µm. Hydrogen flow was 40.0 ml/min, airflow was 450 ml/min and make up flow (Nitrogen) was 45.0 ml/min. The injector temperature was 400 °C, injector splitting was 10:1 and detector temperature was 420 °C. The oven temperature was programmed from 70 °C (2 minute) to 420 °C at 10

°C/minute and was held at 420 °C for 6 minute. All sample were syllilated prior by N-methyl-N-trimethylsilylheptaFluor-(o)Butyramide (MSHFBA) containing methyl-imidazole (50 µl / ml MSHFBA) for about 30 minute and after the excess of MSHFBA evaporated, sample was dissolved in dichloromethane and directly inject to GC. Heptadecanoic methyl ester was used as internal standard.

RESULTS AND DISCUSSION

One of the most important variables that affect on the esters formation is the molar ratio of dialkyl carbonates to palm

kernel oil. Our previous results found that the highest ester formation was obtained when molar ratio of dialkyl carbonates/palm kernel oil was 20:1 mol/mol. The ester formation was decrease when the molar ratio of substrate was increased more than 100 mol/mol. However previous result on the soxhlet reactive extraction showed that the best yield and ester formation was obtained with solvent/seed ratio about 7.5 ml/g and 60 °C (7). This condition was also used as a condition for *in situ* reactive extraction.

Figure 1 and 2 shows the typical time course of *in situ* reactive extraction of palm kernel with dimethyl and diethyl carbonate. As shown in these figures, the

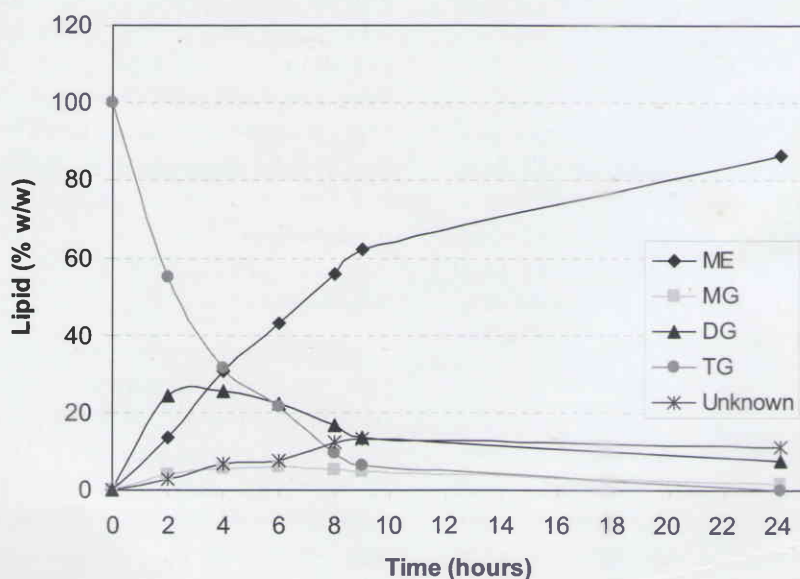


Figure 1. Time Course of *In situ* Reactive Extraction of Palm Kernel Oil with Dimethyl Carbonate.

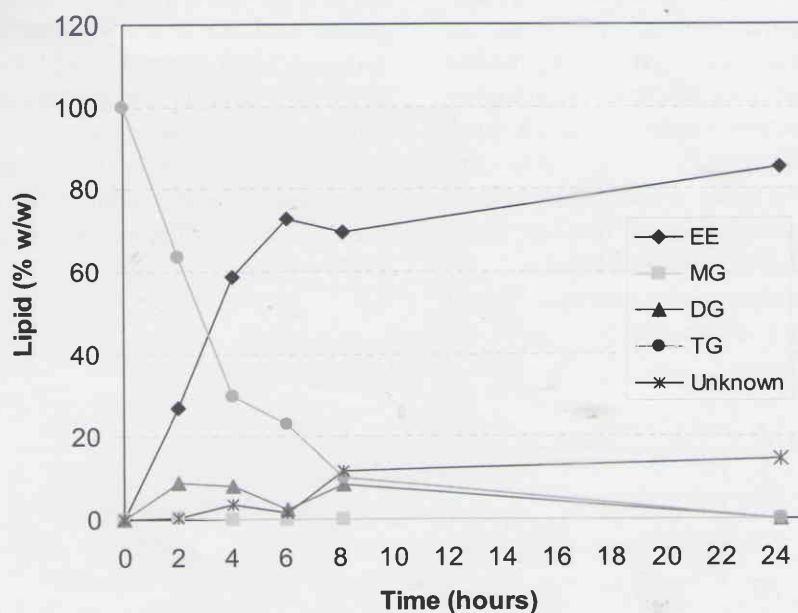


Figure 2. Time Course of *In situ* Reactive Extraction of Palm Kernel Oil with Diethyl Carbonate.

highest production rate was performed when diethyl carbonate was used as solvent and substrate. This could be because the oil more soluble in diethyl carbonate compare to that of dimethyl carbonate.

Our previous result on the lipase-catalyzed transesterification of palm kernel oil with dialkyl carbonates was also found that amount of water present in the media is one of critical parameter. Water is not just necessary for enzyme activity, but it is also needed as a substrate (8). Therefore, various amount of water was added to the solvent to study its effect on the yield of lipid and

ester formation on the *in situ* reactive extraction of palm kernel oil. As shown in Table 1, water content of dimethyl carbonate has an effect both to the yield of lipid and ester formation. The yield of lipid was increase when water content of dimethyl carbonate was increased up to 0.2 % (v/v of solvent), and slightly decreases when water was added up to 0.4 % (v/v of solvent). Water addition to the system was also giving an effect on the rate of ester formation. Additions of water more than 0.1 % (v/v of solvent) reduce the rate of ester formation. As shown in Figure 3, the rate of ester formation reached the highest value

when water was not added to the system. Differ with our previous result on the reactive extraction at low-pressure condition (soxhlet reactive extraction) , at insitu reactive extraction which work at normal pressure water was not removed from the system. Therefore, water that contain in seed was suspected quite enough for activities of lipase.

The similar result was also found when diethyl carbonate was used as

solvent instead of dimethyl carbonate. As shown in Table 2, the yield of lipid was increase when water was added up to 0.2 % (v/v of solvent), and slightly decreased when water was added up to 0.4 % (v/v of solvent). The rate of ester formation was also decrease with increasing the water content in the system, as shown in Figure 4.

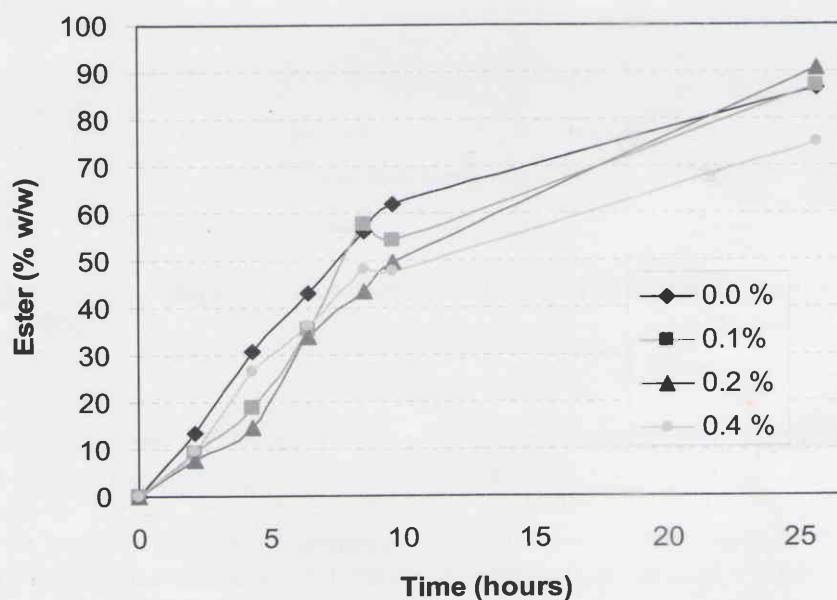


Figure 3. Time Course of *In situ* Reactive Extraction of Palm Kernel with Dimethyl Carbonate Containing Various Amount of Water at 60 °C and Normal Pressure (1013 mbar)

Table 1. Effect of Water Addition on the In situ Reactive Extraction of Palm Kernel with Dimethyl Carbonate

No	Water add. (%) a)	Lipid ext. (%) b)	Lipid ext. (%) c)	Time	Lipid Composition (% w/w)				
					Ester	MG	DG	TG	Other
1	0.00	88.34	55.30	8h	55.98	5.31	16.77	9.71	12.24
				24h	86.68	1.40	7.56	0.00	11.21
2	0.10	90.32	58.59	8h	57.69	0.99	11.22	23.56	6.54
				24h	86.80	1.46	0.89	0.00	10.85
3	0.20	93.47	59.41	8h	43.28	5.57	21.65	19.91	9.59
				24h	90.83	0.00	0.00	0.00	9.17
4	0.40	85.61	57.50	8h	48.13	3.75	18.03	21.39	8.71
				24h	74.89	1.92	1.49	0.00	20.33

a) Water addition (% v/v of DMC)

b) Lipid extracted after 24 hours (% w/w of total lipid)

c) Lipid extracted after 24 hours (% w/w of seed)

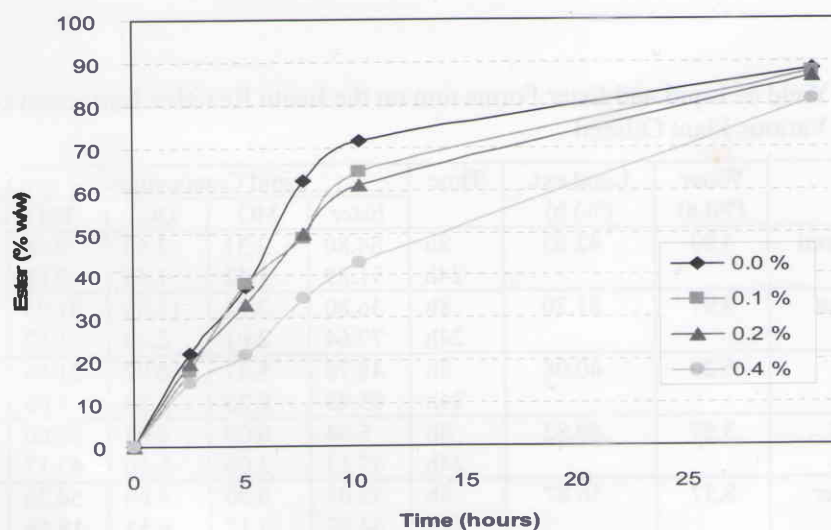


Figure 4. Time Course of Insitu Reactive Extraction of Palm Kernel with Diethyl Carbonate Containing Various Amount of Water at 60 C and Normal Pressure (1013 mbar).

Table 2. Effect of Water Addition on the Insitu Reactive Extraction of Palm kernel with Diethyl Carbonate

No	Water add. (%) a)	Lipid ext. (%) b)	Lipid ext. (%) c)	Time	Lipid Composition (% w/w)				
					Ester	MG	DG	TG	Other
1	0.00	89.00	57.95	8h	71.60	1.41	7.24	7.86	11.89
				24h	88.26	0.00	0.00	0.00	11.83
2	0.10	88.99	57.28	8h	64.47	0.77	8.92	17.48	8.36
				24h	87.66	0.55	0.50	0.00	11.29
3	0.20	89.51	59.39	8h	61.36	1.62	11.22	15.68	10.11
				24h	86.36	0.00	0.00	0.00	13.64
4	0.40	81.05	58.15	8h	42.75	1.68	14.65	36.53	4.40
				24h	80.95	0.99	3.33	3.32	11.42

- a) Water addition (% v/v of DEC)
b) Lipid extracted after 24 hours (% w/w of total lipid)
c) Lipid extracted after 24 hours (% w/w of seed)

Table 3. Yield of Lipid and Ester Formation on the Insitu Reactive Extraction of Various Plant Oilseed

No.	Water (%) a)	Lipid ext. (%) b)	Time	Lipid Composition (% w/w)				
				Ester	MG	DG	TG	Other
1. Rubber seed	4.93	42.83	8h	84.80	2.31	2.53	2.46	7.90
			24h	91.89	1.32	1.13	0.00	5.66
2. Candle nut	4.97	81.20	8h	36.80	3.24	13.49	31.23	15.24
			24h	77.64	2.01	2.40	1.67	16.01
3. Mustard	5.20	40.08	8h	48.75	5.87	16.92	21.56	6.90
			24h	85.48	0.25	2.32	1.06	11.77
4. Rapeseed	5.57	40.82	8h	5.34	0.00	4.05	90.60	0.01
			24h	47.13	2.06	5.50	43.17	2.13
5. Sun flower	5.37	36.87	8h	32.05	0.00	5.66	54.26	8.03
			24h	64.87	3.17	6.55	18.58	6.83
6. Saga seed	5.32	11.04	24h	18.22	0.75	5.20	70.51	5.33
7. Fenugreek	4.70	5.72	24h	10.01	0.00	0.00	84.37	5.62

- a) Water content of seed (% w/w of seed)
b) Lipid extracted after 24 hours (% w/w of seed)

In situ reactive extraction was also applied using several plant oilseed and diethyl carbonate as solvent. As shown in Table 3, the ester formation of each plant oilseed was quite different. With the same *in situ* reactive extraction condition, rubber seed has a highest ester formation compare to that of another oil. This could be happen because in *in situ* reactive extraction with diethyl carbonate several minor contain was also extracted and might be have an effect on the activity of enzyme.

CONCLUSION

Lipase-catalyzed *in situ* reactive extraction can be used as an alternative to produce alkyl esters from oil seed or oily sources. Here, the rate of ester formation reaches the highest value when water is not added to the system because the seed itself contains enough water both for the reaction and the activity of the enzyme. However by addition of 0.2% water, after 24 hour reactive extraction yielded 59% (w/w of seed) and about 90% ester thereof.

ACKNOWLEDGMENT

Authors acknowledge Institute for Lipid Research (BFEL) in Münster – Germany for Research facilities. T. Herawan acknowledge Indonesian Oil Palm Research Institute for the leave of absence and scholarship provided for him.

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