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Preparation of a diacylglycerol-enriched soybean oil by phosphalipase A1 catalyzed hydrolysis

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ABSTRACT

Partial hydrolysis catalyzed by phospholipase A1 (Lecitase Ultra) in a solvent free system was firstly used to produce diacylglycerols (DAGs)-enriched soybean oil. In this study, five reaction parameters namely agitation speed (100–500 rpm), reaction time (2–10 h), water content (10–50 wt% of oil mass), enzyme load (5–40 U/g of oil mass), and reaction temperature (30–70 °C) were investigated. The reaction was up-scaled to 1 kg of soybean oil at 40 °C of reaction temperature, with 300 rpm of agitation speed, 40 wt% of water content, 6 h of reaction time and 22 U/g of enzyme load. Purification by molecular distillation yielded 70% DAG-enriched oil with 42.64 wt% of DAG. The composition of acylglycerols of soybean oil and the DAG-enriched soybean oil was analyzed and identified by high performance liquid chromatography (HPLC) and HPLC/electrospray ionization/mass spectrometer. The released fatty acid from the partial hydrolysis of soybean oil catalyzed by phospholipase A1 showed a higher saturated fatty acid content than that of the raw material. Compared to the lipase catalyzed process, this new phospholipase A1 catalyzed one showed the advantages of low amount production of byproduct, namely, monoacylglycerols.

1. Introduction

Obesity and heart diseases have been more serious in the big cities in developing countries as well as in developed countries due to the diets high in fats and oils [1]. However, fats and oils are also important sources of energy, essential fatty acids and fat-soluble vitamins [2]. The reduced consumption of high fats and oils diets and the intake of healthy oils and a balance of types of fats are recommended by the public health organizations.

Diacylglycerols (DAGs) are esters of glycerol in which two of the hydroxyl group are esterified with free fatty acids (FFAs). They present in there different isomeric forms: 1,2-DAG, 2,3-DAG and 1,3-DAG. It occurs as a natural component of acylglycerols in various fats and oils at levels up to 10% (w/w) [1]. Studies on both animals and humans have shown the beneficial health effects of DAGs. Although DAG has similar digestibility and energy value as triacylglycerol (TAG), it has the ability to decrease postprandial lipids level [3–6]. Consumption of DAGs is also shown to reduce body weight and accumulation of visceral abdominal fat [7,8]. It is suggested that the beneficial health effects of DAGs is due to the differences in digestion and absorption of TAGs and DAGs. The acyl migration from 1,2-DAG to 1,3-DAG occurred in the stomach showed that diacylglycerols isomers had the same function for human health [9]. The commercial DAGs-enriched functional oil was appeared in the market of Japan and the USA in 2003. DAGs are generally regarded as safe (GRAS) food stuff by the FDA of the USA and is approved as a substitution for TAG in the fats and oils in many developed countries.

DAGs can be produced chemically or enzymatically through esterification, glycerolysis and partial hydrolysis processes. The enzymatic processes are preferred because of their mild reaction condition and safe products. In lipase-catalyzed esterification, DAGs are synthesized through esterification of FA and glycerol with simultaneous removal of water [10–12]. Glycerolysis, on the other hand, involved removal of an acyl moiety from the TAGs molecule and by acylation of monoacylglycerols (MAGs) formed during reaction [13,14]. Due to the low activity of lipase in the non-aqueous and high viscosity of glycerol during esterification and glycerolysis, the yield of DAGs was quite low and the byproduct of monoacylglycerols (MAGs) was very high. Partial hydrolysis of TAGs to produce DAGs by lipase is more attractive than the others due to the plentiful output of main feedstock, the vegetable oils and animal fats, and the cheap reactant, the water. However, the high amount of MAGs produced in the partial hydrolysis catalyzed by some commercial lipases was the main disadvantage of these processes [15].

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Phospholipase A1 (PLA1) constitutes a very diverse subgroup of phospholipases with 1-acyl hydrolysis activity. PLA1 displays broad substrate specificity and harbors some lipases activity considering sequence identity similarity of PLA1 to lipases [16]. PLA1 is used to catalyze the hydrolysis of phospholipids to produce the sn1-lysophospholipids which will change to more stable sn2-lysophospholipids by the acyl migration. The commercial PLA1 (Lecitase Ultra, by Novozymes A/S, Bagsvaerd, Denmark) is a preparation obtained from the fusion of the genes of the lipase from *Thermomyces lanuginosa* and the phospholipase from *Fusarium oxysporum* recommended for the degumming of crude edible oil for hydrolysis of non-hydratable phosphatides/gums (NHP) into hydratable phosphatides (HP) and hydrolysis of lecithin, the byproduct of edible oil industry. The hydrolysis of TAG was observed during our previous experiments of hydrolysis of soy lecithin catalyzed by PLA1. So far, PLA1 for partial hydrolysis of TAG to produce DAG-enriched oil has received little attention.

In this study, the partial hydrolysis of soybean oil catalyzed by PLA1 was firstly introduced to produce the DAG-enriched oil. Five reaction conditions namely agitation speed, reaction temperature, reaction time, water content and enzyme load were investigated. The optimal conditions were applied to hydrolysis of 1 kg of soybean oil. The final product was purified by the molecular distillation (MD) to remove the free fatty acid. The HPLC and HPLC/ESI/MS were introduced to analyze and identify the acylglycerol composition of soybean and the final product.

2. Materials and methods

2.1. Materials

Refined soybean oil was purchased from Donghai Cereal and Oil Co. Ltd. (Zhangjiagang, Jiangsu, China). Commercial phospholipase A1 (Lecitase Ultra) was obtained from Novozymes A/S (Bagsvaerd, Denmark). The PLA1 was claimed to have enzyme activity of 10,000 U/mL for hydrolyzing the acyl group.

2.2. Enzymatic activity assay determination

This assay was performed by measuring the free fatty acid released from TAGs in the hydrolysis of 5 g of soybean oil in 100 mL 0.25 mM sodium phosphate buffer with 0.1 mL of Lectitase Ultra at pH 6.8 and 40 °C for 10 min with shaking (approx. 180 rpm), and the amount of free fatty acid was determined by titration with 0.01 M KOH solution. One unit was defined as the amount of enzyme that was necessary to hydrolyze 1 μmol of fatty acid from TAGs per minute under the conditions described above.

2.3. Hydrolysis of soybean oil

Five variables and their levels were specified as agitation speed (100–500 rpm), reaction time (2–10 h), enzyme load (6–40 U/g, of the oil mass), reaction temperature (30–70 °C) and water content (10–50 wt%, of the oil mass). The partial hydrolysis of soybean oil at different reaction conditions were performed in a 250 mL three-necked round bottomed flask. Firstly, the water bath was heated to the desired temperature, and the soybean oil of 50 g was introduced to the flask. Desired amount of enzyme and water content were then added to the flask. The reaction was started by the mechanic stirring for desired time. After reaction, 10 mL of reaction mixture were then withdrawn and centrifuged at 10,000 rpm for 5 min to separate into two layers, the upper oil layer and the lower water layer. The acid value of the upper oil layer was determined by titration with 0.1 M KOH solution. All determinations were performed in duplicate and the mean value is reported.

2.4. Pilot plant production

One kg of soybean was hydrolyzed at agitation speed of 300 rpm, temperature of 40 °C, water content of 40%, reaction time of 6 h and enzyme load of 22 U/g. After reaction, the mixture was settled for 60 min to separate into two layers. The upper oil layer was the mixture of DAG, unreacted TAG and the free fatty acid. The lower layer was the enzyme solution with some remaining enzymatic activity which could be potentially applied for next term hydrolysis. The lower layer was drained, and then the upper layer was collected. The oil layer was transferred to a round bottomed flask and evaporated to remove the residual water under the vacuum.

2.5. Purification of DAGs

To remove FFA from the reaction mixture, the conditions for the distillation process were: evaporator vacuum, 1 Pa; roller speed, 300 rpm; and condenser temperature, 40 °C; evaporator temperature, 130 °C; feed rate, 0.5 l/h; feed temperature, 80 °C. After the MD, the light phase with free fatty acid and the heavy phase with DAG and TAG were collected. The heavy phase was molecular distillated again to remove the residual free fatty acid at the same parameters, because the acid value of final product was not over 0.20 mg KOH/g for the directly edible purpose. The heavy phase was collected as the diacylglycerol-enriched soybean oil (DESO).

2.6. Analysis of acylglycerol by HPLC and HPLC/ESI/MS

The acylglycerol composition of soybean oil and DESO was analyzed by reverse-phase high performance liquid chromatography (HPLC-RP). Samples were dissolved into mobile phase (acetonitrile and isopropanol, 56:44, v/v) with the concentration of 10 mg/mL, followed by filtered through a 0.45 μm nylon membrane filter to remove impurities. Twenty microliters of sample were then injected into a Diamonsil C18 5 μm (150 mm × 4.6 mm) (Dikma, USA) column by HPLC (Aglient 1100, USA) with a refractive index detector. The flow rate was set at 1 mL/min. The area of peaks was calculated as the content of acylglycerols.

The composition of acylglycerol was identified by the HPLC/ESI/MS. Samples were dissolved in the concentration of 1 mg/mL, and then were injected into a PinnadeII 5 μm (150 mm × 2.1 mm) (RESTEK, USA) column by an Aglient 1100 HPLC. The column was connected with a 4000 QTRAP mass spectrometer (Applied Biosystems, USA) with an atmospheric pressure ion source to sample positive ions from the electrospray interface. Formic acid (0.5%) in acetonitrile/isoproanol (55:45, v/v) was added to improve the ionization. The mass spectra, between the m/z of 300 and 1200, were obtained with an ion scan rate of 5500 amu/s.

2.7. Diacylglycerol fraction analysis

Sn-1,3-DAG and sn-1,2(2,3)-DAG was isolated by thin layer chromatography (TLC) on silica gel plates (SIL GF254, 20 cm × 20 cm, 0.25 mm),using benzene: diethyl ether: acetic ether: acetic acid (80:10:10:0.2, v/v/v/v) as developing mixture. The sn-1,3-DAG fraction (RF = 0.87) and sn-1,2(2,3)-DAG fraction (RF = 0.78) were scraped off and extracted with diethyl ether (2 mL × 3).

Samples of DAGs were then filtered through a 0.45 μm nylon membrane filter to remove impurities. Twenty microliters of sample were injected into a LichrosorbSi-60 5 μm (250 mm × 4.6 mm i.d., Alltech, USA) column by HPLC (LC20 AT, Shimadzu, Kyto, Japan) detected at UV 210 nm. The flow rate of mobile phase composed of

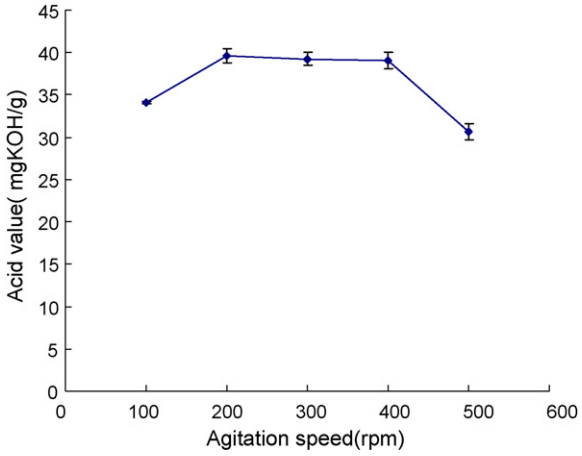

Fig. 1. The influence of agitation speed on acid value of oil layer at the reaction temperature of 40 °C, enzyme load of 20 U/g (of oil mass), water content of 20 wt% (of oil mass) and reaction time of 2 h.

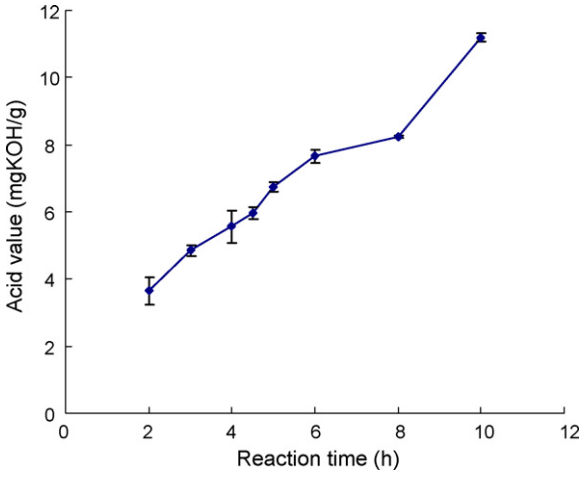

Fig. 2. The influence of reaction time on acid value of oil layer at the reaction temperature of 50 °C, enzyme load of 10 U/g (of oil mass), agitation speed of 300 rpm and water content of 20 wt% (of oil mass).

n-hexane with isoproponol containing 2% H_2O (99.6:0.4, v/v) was set at 1 mL/min. The ratio of sn-1,3 DAG and sn-1,2(2,3)-DAG were calculated by area of peaks.

2.8. Fatty acid composition analysis

The fatty acid composition of the soybean oil, the DESO and the light phase of MD were analyzed by the gas chromatography (GC 900A, Kechuang China) equipped with a capillary column (HP-5, 30 m × 0.32 mm × 0.25 μm) and a flame ionization detector (FID). Nitrogen was used as the carrier gas. The injection was performed in split mode with a split ratio of 80:1. Samples of soybean oil and DESO of 4 g were reacted with 40 mL of methanol and then 0.5 mL of 1 M methanolic KOH was added, after 10 min, *n*-hexane (20 mL) and water (40 mL) were added. The upper organic phase was dried over anhydrous Na_2SO_4 and then concentrated under nitrogen steam for analysis. The lighter phase of MD (free fatty acid) of 4 g was reacted with 50 mL of 1 M methanolic H_2SO_4 for 15 min, and then *n*-hexane (30 mL) and water (100 mL) was added. The upper phase was dried and concentrated for analysis. The FAME (fatty acid methyl ester) solution was injected at the injector temperature of 240 °C, column temperature of 195 °C, FID temperature of 240 °C and carrier gas (N_2) flow of 60 mL/min. The percentage of each fatty acid was based on the area response using FID detector.

3. Results and discussion

3.1. Enzymatic activity assay determination

The enzyme activity of Lecitase Ultra was 771.72 ± 36.80 (n = 3) U/mL under the measurement condition. For the convenient of enzyme load; the claimed activity of Lecitase Ultra was used in this study.

3.2. Partial hydrolysis of soybean oil

The acid value of oil layer varied with agitation speed at a narrow range (from 30.65 ± 0.93 to 39.58 ± 0.80 mg KOH/g) (see Fig. 1). For partial hydrolysis, the interfacial surface area between water and oil had an effect on PLA1 activity. With a higher agitation speed, the interfacial surface area was larger, which would promote the hydrolysis rate by adsorption of more amount of enzyme on the interfacial surface. However, it was reported that the shear effect caused by too high agitation speed decreased the enzyme activity [17]. Therefore, the agitation speed for the following reaction was fixed at 300 rpm. Theoretically, the more fatty acids are released, and the higher DAGs are produced. For the partial hydrolysis of TAG, the optimal conditions were investigated to release the maximum amount of fatty acid, namely the maximum acid value (AV) of oil layer of the reaction mixture.

The influence of reaction time on AV of oil layer in the soybean oil was shown in Fig. 2. The AV increased with the increasing of reaction time. However, it was not over12 mg KOH/g even at reaction time of 10 h, enzyme load of 10 U/g, water content of 20 wt% (oil mass) and reaction temperature of 50 °C. Because of the immiscibility of oil in the water, the area of interfacial surface was depended on the water content in the reaction system at a fix agitation speed. The hydrolysis rate was quite slow at low water content. At a high water content (40 wt%, of oil mass), the acid value reached 18.07 ± 0.15 mg KOH/g at enzyme load of 14 U/g (of oil mass), reaction time of 4 h and reaction temperature of 50 °C (see Fig. 3). However, the AV decreased to 14.38 ± 0.60 mg KOH/g at water content of 50 wt% (of oil mass). The high water content increased the interfacial surface area meanwhile decreased the enzyme concentration, and the low enzyme concentration was negative for the hydrolysis rate of TAGs.

The AV of the oil layer increased very rapidly (from 1.65 ± 0.45 to 40.42 ± 2.02 mg KOH/g) at low value of enzyme load (less than

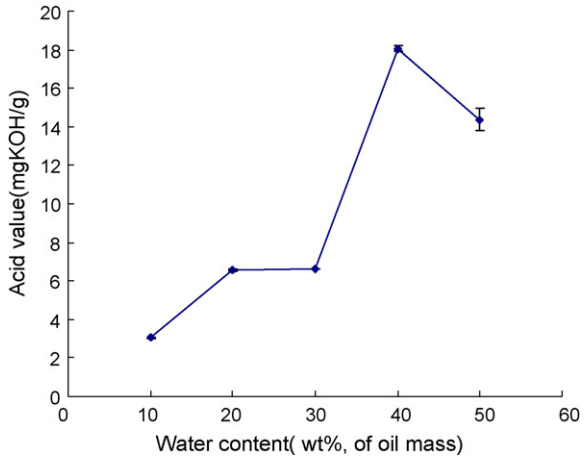

Fig. 3. The influence of water content on acid value of oil layer at the reaction temperature of 50 °C, enzyme load of 14 U/g (of oil mass), agitation speed of 300 rpm and reaction time of 4 h.

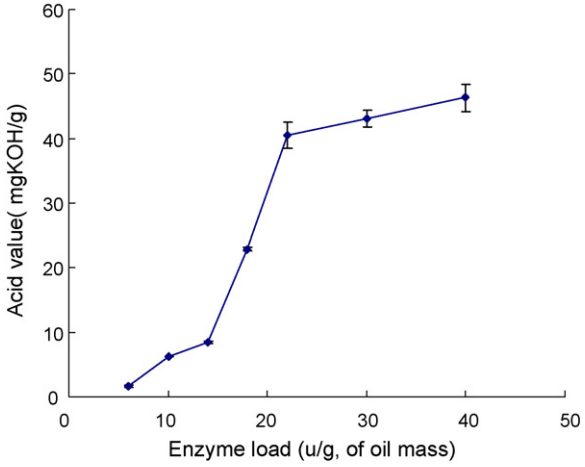

Fig. 4. The influence of enzyme load on acid value of oil layer at the reaction temperature of 50 °C, reaction time of 4 h, agitation speed of 300 rpm and water content of 40 wt% (of oil mass).

20 U/g, of oil mass), but it became flat when the En was over 20 U/g (of the oil mass) (see Fig. 4). Due to the high excess of addition of water than theoretically required for the hydrolysis, the interfacial surface area between the water and oil was quite stable during the reaction when the agitation speed was fixed. At the low enzyme concentration, PLA1 adsorpted to the surface of oil and water was not saturated, so the hydrolysis rate was decided by the enzyme concentration on the surface. However, when the surface was saturated with PLA1 at a high concentration, increasing of enzyme load showed no significant influence on the hydrolysis of TAGs.

The AV of oil layer increased at a low temperature range (less than 40 °C), but decreased at a high temperature (over 60 °C) (see Fig. 5). PLA1 showed even no activity to TAGs at reaction temperature of 70 °C (the AV of oil layer was 2.36 ± 0.03 mg KOH/g). The suitable temperature for hydrolysis by PLA1 was 40 °C which was accordance with the recommended temperature by the producer. Reaction temperature plays an important role in enzyme activation and deactivation. PLA1 become more susceptible to thermal deactivation at a high temperature. No emulsification was observed after the partial hydrolysis, which was accordance with the result that few of monoacylglycerol (MAG) was newly formed in the reaction by the HPLC analysis.

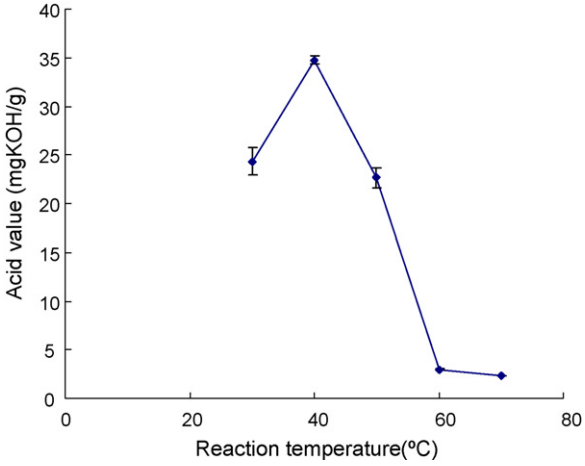

Fig. 5. The influence of reaction time on acid value of oil layer at the enzyme load of 18 U/g (of oil mass), reaction time of 3 h, agitation speed of 300 rpm and water content of 40 wt% (of oil mass).

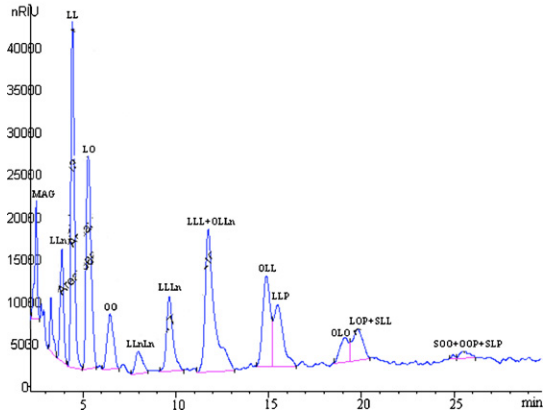

Fig. 6. HPLC chromatogram of diacylglyerol-enriched soybean oil by reversed phase column (C-18 5 μm 150 mm × 4.6 mm) and an RI detector with a mobile phase of acetonitrile/isopropanol (55:45), coupled to a mass spectrometer. L, linoleic; Ln, linolenic; O, oleic; P, palmitic; S, stearic.

3.3. Scaling up of partial hydrolysis

One kilogram of refined soybean oil was hydrolyzed at reaction temperature of 40 °C, with water content of 40 wt% (of the oil mass), reaction time of 6 h and enzyme load of 22 U/g (of the oil mass). After settling for 1 h for separation, the acid value of oil layer of the production was analyzed with the value of 53.80 ± 1.26 mg KOH/g. The oil layer from the partial hydrolysis process consisted of DAGs, free fatty acid (FFA) and TAG. The FFA can be separated from the final product by the MD, but the separation of TAGs from the DAGs was quite difficult due to the small vapor pressure difference between DAGs and TAGs. Therefore, TAG is not removed and remained in the final product. The up-scaled oil layer (500 g) was molecular distillated into two phase. The light phase of 135.4 g was the released free fatty acid by partial hydrolysis with the acid value of 194.62 ± 2.54 mg KOH/g, and the heavy phase of 364.6 g was the DAG and unhydrolyzed TAG with the acid value of 7.42 ± 0.23 mg KOH/g. After the second time distillation, the acid value of the heavy phase with light yellow color which was similar to the soybean oil was 350.2 g with an AV of 0.14 ± 0.02 mg KOH/g. The yield of DESO was 70%.

3.4. Acylglycerol analysis by HPLC and HPLC/ESI/MS

Fig. 6 was the HPLC chromatogram of samples of soybean oil and the DESO where acylglycerols were identified by mass spectrometry. Some TAG did co-elute. A similar chromatographic profile was observed when coca butter TAG was analyzed by HPLC-RP system [18]. The retention time of acylglycerol by the HPLC-RP increases with the equivalent carbon number (ECN) defined as total carbon number (CN) minus two times the number of double bonds (DB), i.e. ECN = CN − 2DB. The acylglycerol with the same ECN did co-elute under some conditions [19]. The percentages of each TAG based on area response using a RI detector are in Table 1. Compared to PDA detector, RI detector is more suitable for determining the content of acylglycerol, since the refractive index of them is very similar, and the area response could be used directly for the calculation of the content of acylglycerol [20]. The DAG content in the DESO was 42.64 wt% determined by HPLC-RP. The composition of TAG in soybean oil was very similar to that reported by other researchers [19].

A variety of chromatographic techniques have been evaluated to determine acylglycerol (MAG, DAG and TAG) composition of many lipid sources such as thin layer chromatography (TLC), GC, GC–MS

Table 1
Composition, mass (M), mass of sodium and potassium adduct molecular ion, the equivalent carbon numbers (ECN) of acylglycerol in the soybean oil and diacylglycerol-enriched soybean oil[a]

Acylglycerol	ECN	*M*	$[M+Na]^+$ (*m*/*z*)	$[M+K]^+$ (*m*/*z*)	Soybean oil composition (%)[b]	Diacylglycerol-enriched soybean oil composition (%)[b]
MAG					1.34	2.12
DAG					2.28	42.64
LLn	26	614	638	654	0.36	5.59
LL	28	616	640	656	0.65	17.17
LO	30	618	642	658	0.97	15.03
OO	32	620	644	660	0.30	4.85
TAG					96.38	55.24
LLnLn	38	874	898	914	1.55	2.04
LLLn	40	876	900	916	8.17	6.69
LLL + OLLn	42	878, 878	902, 902	918, 918	25.57	19.41
OLL	44	880	904	920	17.95	9.99
LLP	44	854	878	894	17.03	8.42
OLO	46	882	906	922	9.66	2.69
LOP + SLL	46	856, 882	880, 916	896, 922	9.84	4.66
SLO + OOP + SLP	48	884, 858, 858	918, 882, 882	924, 898, 898	6.61	1.34
SOO + SOP	50	886, 860	910, 884	926, 900	–	–

[a] The relative acylglycerol composition of the chromatogram peaks was achieved by integration of the peak area obtained by a RI detector. MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol; L, linoleic; Ln, linolenic; O, oleic; P, palmitic; S, stearic.
[b] Results are means of two replications.

and HPLC [20]. The HPLC/ESI/MS seems to be a suitable analytical method to completely elucidate the composition of the acylglycerol present in crude vegetal oils [21]. In contrast to atmospheric pressure chemical ionization (APCI) which produces ion fragments, ESI did not produce these ions. A large amount of structural information was provided by this technique.

The presence of sodium and potassium adducts in the MS spectra collected during lipid analysis has been well documented in the literature [22–24]. When an acylglycerol was dissolved in an acidic solvent such as formic acid, electrospray mass analysis produced only a weak ion current for the protonated molecule. Sodium and potassium adduct ions, which are formed because they are present as a low-level impurity in the sample, were often more abundant than the protonated molecules. When the total ion chromatogram of soybean oil and the DESO was analyzed, sodium and potassium adduct molecular ions $[M+Na]^+$ and $[M+K]^+$ were identified by the tandem mass spectrometry (see Fig. 7).

3.5. Diacylglycerol fraction analysis

Diacylglycerol fraction including sn-1,3-DAG,sn-1,2-DAG and sn-2,3-DAG could be isolated by the TLC firstly, and then after derivatization, these three isomers were separated by HPLC with normal phase silica gel column [25,26]. However, Sn-1,2-DAG and sn-2,3-DAG were not separated by the normal phase column without derivatization [13]. High temperature gas chromatography has been used to obtain the information of profile of DAG fraction, but the peak of sn-1,2-DAG and sn-2,3-DAG was not separated [9]. Sn-1,2-DAG and sn-2,3-DAG have very similar physical and biochemical properties, it is the reason why these two isomers were not separated in this study. After isolated by the TLC, the sn-1,3-DAG and sn-1,2(2,3)-DAG was analyzed by the NP-HPLC. The peak area responded by the UV detector showed that the ratio of sn-1,3-DAG vs. sn-1,2(2,3)-DAG was 1.08 ± 0.05 ($n = 7$). Therefore, sn-1,3-DAG content in the scaled-up DESO was 22.14 wt%, and sn-1,2(2,3)-DAG content was 20.50 wt%. The ratio of sn-1,3-DAG vs. sn-1,2(2,3)-DAG varied with the reaction time (2–72 h) when the 1,3-specific lipases were used to catalyze glycerolsis of fatty acid to produce diacylglycerol. Sn-1,3-specific lipases showed no advantage on increasing the content of sn-1,3-DAG when compared to no-specific lipases. [13]. But for re-esterification of fatty acid ethyl esters with glycerol catalyzed by sn-1,3-regiospecific lipase, more sn-1,3-DAG was produced at a low temperature (12 °C) [26] Due to the short reaction time (2–8 h) in this study, the influence of time on the ratio of sn-1,3-DAG against sn-1,2(2,3)-DAG was not investigated. The digestion research showed that sn-1,2(2,3)-DAG was changed into sn-1,3-DAG by acyl migration in the stomach with acidic environment, which proved that the sn-1,2(2,3)-DAG had the same function as sn-1,3-DAG did in our body [9].

3.6. Fatty acid composition analysis

The main fatty acids composition of soybean oil, DESO and the light phase of MD were shown in Table 2. Compared to the soybean oil, the palmatic acid content of light phase of MD increased from 10.75 to 15.89 wt%. However, the linoleic acid content decreased from 55.40 to 47.36%. The composition of light phase from MD is directly from the released fatty acid by PLA1 catalyzed hydrolysis of soybean oil. In natural vegetable oils, the saturated fatty acids take the priority to distribute in the sn-1 position, and the unsaturated fatty acids, such as the linoleic acid, often take the sn-2 position.

Table 2
The fatty acid composition of soybean oil, light phase of molecular distillation and diacylglycerol-enriched soybean oil

Fatty acid	Composition (%)[a]		
	Soybean oil	Light phase of molecular distillation	Diacylglycerol-enriched soybean oil
Palmtic acid	11.75	15.89	10.35
Oleic acid	28.37	30.73	30.32
Linoleic acid	55.40	47.36	56.13
Linolenic acid	4.48	6.01	3.19

[a] Results are means of two replications.

PLA1 was reported to have the selectivity for sn-1 acyl position of the phospholipids molecular [16]. However, since Lecitase Ultra was obtained from the fusion of the genes of the lipase from *T. lanuginosa* and the phospholipase from *F. oxysporum* [27], we postulated that it might possess some extent of 1,3 specificity to TAGs. The commercial *T. lanuginosa* lipase (Lipozyme TL IM) which showed 1,3 specificity to TAGs was used for catalysis of transesterification and glycerolsis [13–15]. The big difference of partial hydrolysis of TAGs between Lecitase Ultra and lipase (LipozymeRM IM) was the amount of production of byproduct of monoacylglycerols (MAGs) [15]. No large amount (see Table 1) production of MAG was observed in the hydrolysis of TAG by PLA1 in this study.

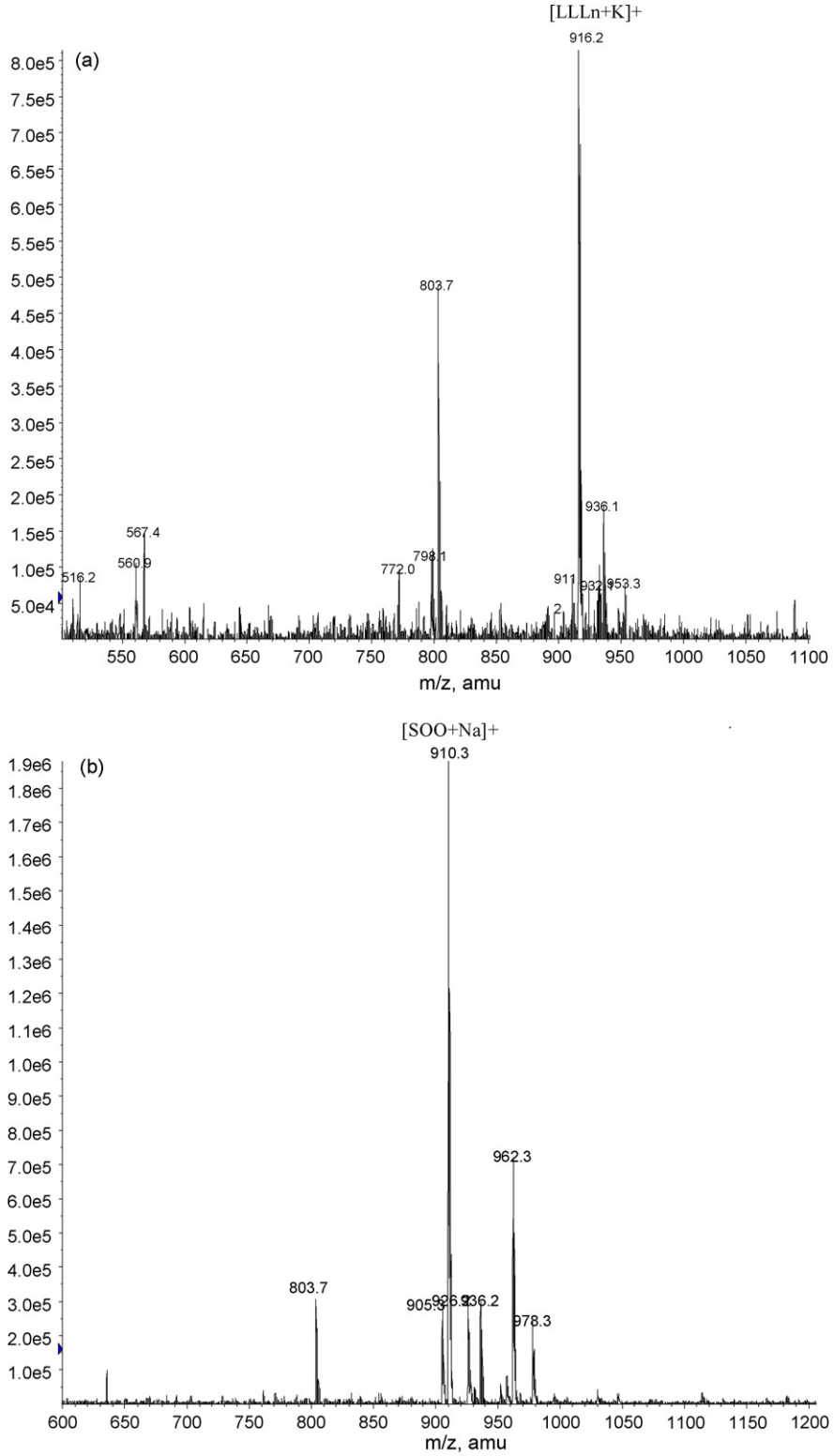

Fig. 7. Sodium and potassium adduct molecular ion $[M+Na]^+$ and $[M+K]^+$of representative TAG and DAG present in soybean oil and DAG-enriched soybean oil. (a) $[LLLn]^+$ (m/z 916.2), (b) $[SOO]^+$ (m/z 910.2), (c) $[LLn]^+$ (m/z 637.8), (d) $[OO]^+$ (m/z 644.0).

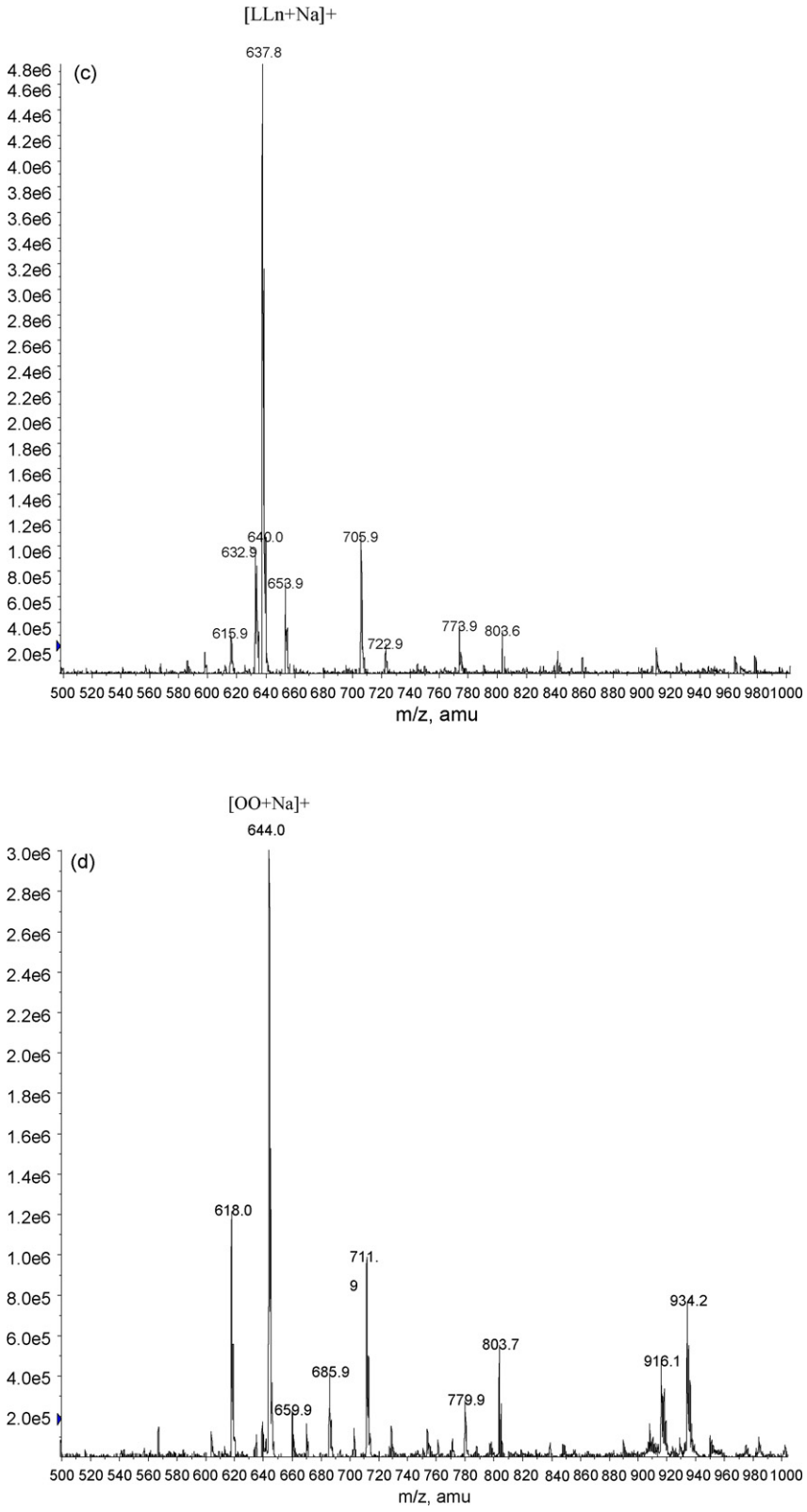

Fig. 7. (*Continued*).

4. Conclusion

PLA1 was successfully applied in this study to the partial hydrolysis of soybean oil to produce DAG-enriched vegetable oil. The five reaction parameters were carefully investigated. The scaled up hydrolysis was conducted at reaction temperature of 40 °C, with water content of 40 wt% (of oil mass), reaction time of 6 h and enzyme load of 22 U/g (of oil mass). The DESO with a yield of 70% was obtained by molecular distillation of scaled up oil layer with the AV of 53.80 ± 1.26 mg KOH/g. The HPLC-RP with a RI detec-

tor and the HPLC/ESI/MS were used to analyze the acylglycerols composition of soybean oil and the DESO. The DESO which has 42.64 wt% DAG has the potential to be applied for cooking oil for healthy diet. The fatty acid released from the PLA1 catalyzed hydrolysis has a 15.89 wt% of palmatic acid other than 11.75 wt% of that in the soybean oil. Compared to the lipase catalyzed process, this new PLA1 one shows the advantages of low production of monoacylglycerols.

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