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ARTICLE

Lipase-Catalyzed Acidolysis of Olive Oil with Capric Acid: Effect of Water Activity on Incorporation and Acyl Migration

Ji-Eun Oh,[†,‡] Kwang-Won Lee,[‡] Hye-Kyung Park,[§] Jee-Young Kim,[§] Kwang-Il Kwon,[§] Jong-Wook Kim,[§] Hak-Ryul Kim,[∥] and In-Hwan Kim*[,†]

[†]Department of Food & Nutrition, Korea University, Seoul, 136-703, Republic of Korea, [‡]Division of Food Bioscience, Korea University, Seoul, 136-701, Republic of Korea, [§]Korea Food & Drug Administration (KFDA), Seoul, 122-704, Republic of Korea, and [∥]Department of Animal Science and Biotechnology, Kyungpook National University, 702-701 Daegu, Republic of Korea

Structured lipids were synthesized by acidolysis of olive oil and capric acid with an immobilized lipase (Lipozyme TL IM) from *Thermomyces lanuginosus*. The acidolysis reaction was carried out by incubating a 1:3 molar ratio of olive oil and capric acid under solvent-free reaction systems at 50 °C. The effect of water activity on the incorporation of capric acid was investigated, and the tested water activity range was between 0.22 and 0.80. Capric acid incorporation into triacylglycerols of the olive oil increased as the water activity increased, but the degree of acyl migration also increased. Also, the degree of acyl migration of modified olive oils with a similar degree of incorporation was investigated. High degrees of acyl migration occurred at water activities of 0.22 and 0.32 for the degree of incorporation of ca. 50 mol %. Only 8 h of reaction time was required to achieve incorporation of ca. 50 mol % at a water activity of 0.80, and the lowest acyl migration occurred at the same water activity. These results suggest that acyl migration can be efficiently minimized by a shorter reaction time at higher water activity.

KEYWORDS: Acidolysis; acyl migration; capric acid; lipase; olive oil; structured lipids; water activity

INTRODUCTION

Olive oil is a fruit oil obtained from olive (*Olea europaea*), a traditional tree crop of the Mediterranean basin. In Mediterranean countries, olive oil is a major constituent of the diet. The consumption of olive oil is considered important for preserving a healthy and relatively disease-free population. Epidemiologic data show that the Mediterranean diet has significant protective effects against cancer and coronary heart disease (*1*). A high consumption of olive oil can afford considerable protection against cancer (colon, breast, and skin), coronary heart disease, and aging by inhibiting oxidative stress (*2*−*4*). Bourre et al. (*5*) also concluded that oleic acid in the diet can lower low-density lipoprotein as well as total cholesterol.

Medium-chain triacylglycerols (MCTs) also offer numerous health benefits and have been widely studied for medical, nutritional, and food applications. MCTs have been used to treat fat absorption abnormalities that occur in premature infants and in patients with cystic fibrosis (*6*). MCTs are burned quickly for energy and are not deposited in the adipose tissue (*7*). However, physical mixtures of MCTs and long-chain triacylglycerols (LCTs) retain their original individual absorption rates. Structured lipids (SLs) containing medium-chain fatty acids at *sn*-1,3 positions and long-chain fatty acids at *sn*-2 positions of triacylglycerols are more readily absorbed and oxidized for energy as compared to LCTs (*8*).

*To whom correspondence should be addressed. Tel: 822-940-2855. Fax: 822-941-7825. E-mail: k610in@korea.ac.kr.

Lipase-catalyzed esterification has been used in fat modification to improve absorption properties and the nutritional value of lipids (*9*, *10*). Acidolysis is one of the most commonly used methods for the production of MLM type (M, medium-chain fatty acid; L, long-chain fatty acid) SLs using regiospecific lipase to incorporate the medium-chain fatty acids into the primary positions of triacylglycerols (TAGs) (*11*, *12*).

Acyl migration is one of the major problems that occurs during a lipase-catalyzed acidolysis reaction, even when highly 1,3-regiospecific lipases are used (*13*). Acyl migration is affected by factors such as water content, reaction time, reaction temperature, enzyme loading, and the reaction medium (*14*). Bloomer et al. (*15*) reported that a higher enzyme load, lower temperature, and the use of ethyl ester as the acyl donor will favor the reduction of acyl migration. In fact, however, acyl migration cannot be totally avoided, only decreased to a lower scale. Water is essential for enzymatic reactions in nonaqueous media since it is associated with the formation and maintenance of an enzyme's active conformation or the "loosening up" of the rigid structure of an enzyme (*16*, *17*). There is a critical lower limit on the water content below which enzymes will not be able to maintain their catalytic activities. This critical water content is referred to as the "essential water". If the water content is too high, the hydrolysis reaction will be favored and yields will decrease. However, if it is too low, the activity of the enzyme will decline.

In this study, the influence of water activity on acyl migration in lipase-catalyzed acidolysis was investigated. Olive oil and capric acid were used as substrates for the synthesis of MLM

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type SLs by lipase-catalyzed acidolysis using 1,3-regiospecific lipase. *sn*-2 positional analysis by pancreatic lipase was performed to determine the influence of water activity on the degree of acyl migration.

MATERIALS AND METHODS

Materials. The olive oil was supplied by Lotte Samgang (Cheonan-Shi, Korea). Lipozyme TL IM (immobilized on silica gel) from *Thermomyces lanuginosus* was donated by Novo Nordisk Bioindustry Ltd. (Seoul, Korea). Capric acid (99% >) and 2,7-dichlorofluorescein were purchased from Sigma (St. Louis, MO).

Water Activity Pre-equilibration of Enzyme. Enzymes were pre-equilibrated with the water vapor phase of saturated salt solutions in sealed containers. The equilibration process was carried out at 25 °C for 1 day. The saturated salt solutions used were prepared with KCH_3CO_2 (a_w = 0.22), $MgCl_2$ (a_w = 0.33), K_2CO_3 (a_w = 0.43), $NaNO_2$ (a_w = 0.65), and $(NH_4)_2SO_4$ (a_w = 0.80).

Enzymatic Acidolysis. Olive oil (1.89 g, average MW 874.85) was mixed with 1.11 g of capric acid (MW 172.27) at a molar ratio of 1:3 in a 25 mL Erlenmeyer flask with silicon stopper. Immobilized lipase (0.3 g of Lipozyme TL IM, 10% of the total weight of substrates) was then loaded in the flask. The mixture was stirred in an orbital shaking water bath (model G76; New Brunswick Scientific Co. Inc., New Brunswick, NJ) at 300 rpm and 50 °C. Individual samples were removed at selected times and analyzed. All reactions were performed in triplicate.

Analysis of Products. When the reaction was complete, the enzymes were removed by filtration. The modified TAGs were isolated using thin-layer chromatography (TLC), developed with petroleum ether/ethyl ether/acetic acid (80:20:0.5, by volume), and detected with 0.2% 2,7-dichlorofluorescein in methanol solution under UV light. The band corresponding to the TAGs was scraped off the TLC plate and methylated according to AOCS standard method Ce 2-66 (*18*). The fatty acid methyl esters (FAMEs) were extracted with 3 mL of *n*-hexane, dried over sodium sulfate, and concentrated under nitrogen. A gas chromatograph (Varian 3900, Varian Inc., Walnut Creek, CA) equipped with a SUPELCOWAX 10 fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Supelco, Bellefonte, PA) and FID was used in the analysis. The column was programmed from 130 to 220 °C at 3.5 °C/min. It was then held at 220 °C for 5 min. The carrier gas was helium, and the total gas flow rate was 50 mL/min. The FAME was identified by comparison with the retention times of the standards. The injector and detector temperatures were 240 and 250 °C, respectively. The sample analyses were performed in triplicate.

Hydrolysis by Pancreatic Lipase. Determination of the *sn*-2 positional distribution of fatty acids in TAGs species obtained after TLC was conducted by the method of Luddy et al. (*19*). Ten milligrams of TAGs was mixed with 2 mL of 1 M Tris-HCl buffer (pH 7.6), 0.5 mL of 0.05% bile salts, 0.2 mL of 2.2% $CaCl_2$, and 18 mg of pancreatic lipase. The mixture was incubated in a water bath at 37 °C for 2 min, vortexed vigorously, extracted with 15 mL of diethyl ether, and dried by anhydrous sodium sulfate. TLC analysis was on silica gel G (Merck Co., Darmstadt, Germany), and the developing solvent system was hexane/diethyl ether/acetic acid (50:50:1, by volume). The band corresponding to 2-monoacylglycerols was scraped and extracted with diethyl ether, methylated, and analyzed by gas chromatography. The sample analyses were performed at least in triplicate.

Statistical Analysis. If not otherwise specified, experiments were carried out at least in triplicate. The Statistical Analysis Systems was used to analyze data (*20*). Significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

Lipase-catalyzed acidolysis by *sn*-1,3-specific Lipozyme TL IM lipase was carried out with a 1:3 molar ratio mixture of olive oil and capric acid in various water activity ranges for 8 h. As expected, the fatty acid composition of olive oil was significantly changed after modification (**Table 1**). Originally, oleic acid was the predominant fatty acid in olive oil, constituting over 76.4 mol %. After acidolysis, however, capric and oleic acids became the major fatty acid in the modified olive oil. After 8 h of reaction, incorporated capric acid was 51.0, 40.7, and 14.1 mol % when catalyzed at water activities of 0.80, 0.43, and 0.22, respectively. Undoubtedly, these results demonstrate that water activity influences significantly lipase-catalyzed acidolysis.

Table 1. Fatty Acid Compositions (Mol %) of Olive Oil before and after Lipase-Catalyzed Acidolysis with Capric Acid[a]

		water activities[b]		
fatty acid	unmodified olive oil	a_w = 0.80	a_w = 0.62	a_w = 0.22
C10:0		51.0 ± 0.9	45.9 ± 1.2	14.1 ± 0.3
C16:0	12.1 ± 0.1	4.5 ± 0.0	4.8 ± 0.3	9.9 ± 0.0
C16:1 (n-7)	0.9 ± 0.0	0.1 ± 0.0	0.5 ± 0.0	0.8 ± 0.0
C18:0	3.7 ± 0.0	1.5 ± 0.2	1.6 ± 0.1	3.2 ± 0.4
C18:1 (n-9)	76.4 ± 0.8	37.8 ± 0.4	41.6 ± 0.2	63.7 ± 0.7
C18:1 (n-7)	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	1.6 ± 0.1
C18:2 (n-6)	6.1 ± 0.3	4.4 ± 0.2	4.7 ± 0.0	6.8 ± 0.0

[a] Mean values of three replicate determinations ± standard deviations. The reaction mixture contained 1.89 g of olive oil, 1.11 g of capric acid, and 0.3 g of enzyme. The reaction mixture was incubated for 8 h in an orbital shaking water bath at 300 rpm and 50 °C. [b] To control the water activities of the enzymes, they were incubated in the desired water activity chamber for at least 16 h.

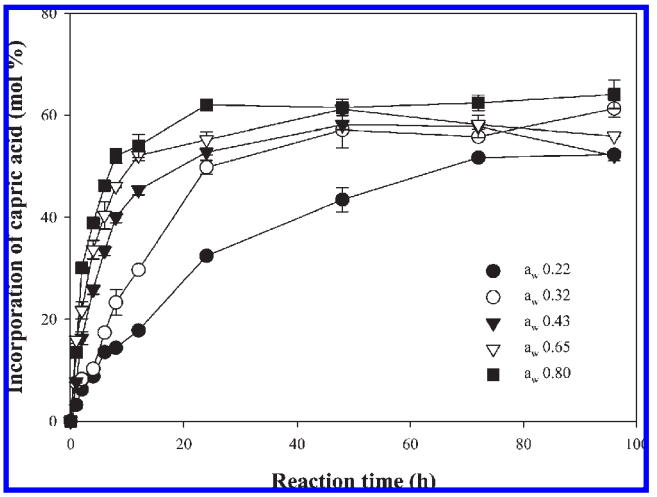

Figure 1. Effect of water activity on the lipase-catalyzed acidolysis between olive oil and capric acid in a solvent-free system. The reaction mixture contained 1.89 g of olive oil, 1.11 g of capric acid, and 0.3 g of enzyme. The reaction mixture was incubated in an orbital shaking water bath at 300 rpm and 50 °C. To control the water activities of the enzymes, they were incubated in the desired water activity chamber for at least 16 h.

The effect of water activity on the incorporation of capric acid into the olive oil as a function of reaction time was investigated (**Figure 1**). Incorporation was defined as the mol % of capric acid into the TAG of olive oil. The water activity range tested in this study was between 0.22 and 0.80. During the first 24 h of reaction, the degree of incorporation of capric acid increased significantly as the water activity increased up to 0.43. However, additional incorporation of capric acid was minimal when the water activity was further increased from 0.43 to 0.80 during the same period. Furthermore, at water activities of 0.22 and 0.32, the degree of incorporation of capric acid was small as compared to water activities higher than 0.32, probably due to the lack of essential water for the catalytic activity of the enzyme. The incorporation of capric acid at water activity of 0.80 increased significantly to ca. 61 mol % after 24 h and remained constant thereafter, whereas the incorporations of capric acid at water activity of 0.22 gradually increased up to 96 h of reaction.

Acyl migration occurs during lipase-catalyzed acidolysis, even though there was a difference in the extent of acyl migration

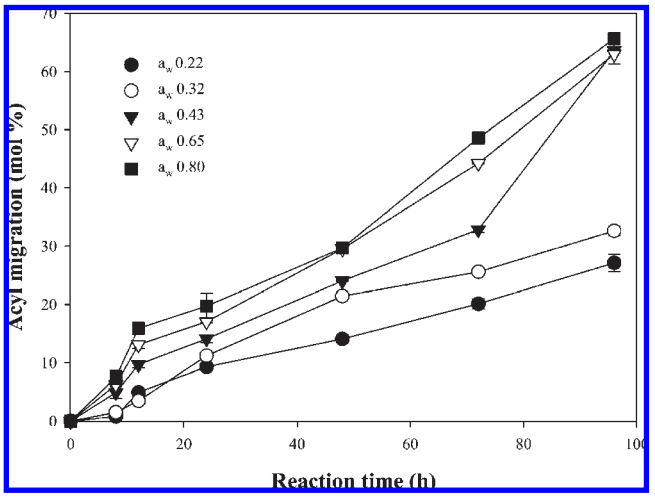

Figure 2. Effect of water activity on the acyl migration in the lipase-catalyzed acidolysis between olive oil and capric acid. See **Figure 1** for reaction conditions.

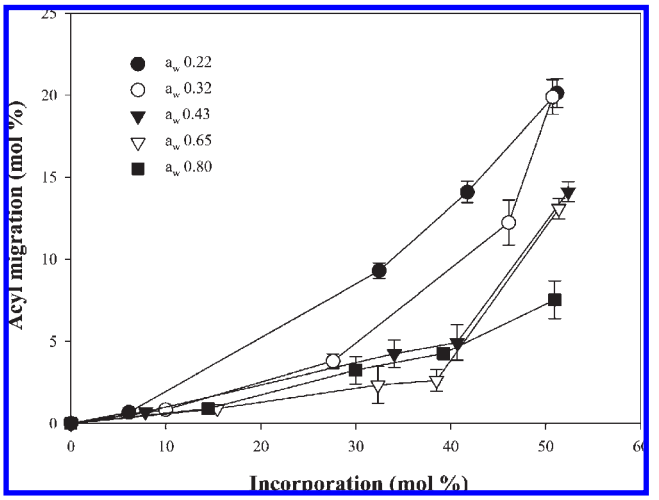

Figure 3. Relationships between similar degrees of incorporation and acyl migration for the acidolysis catalyzed by Lipozyme TL IM. See **Figure 1** for reaction conditions.

depending on the reaction conditions. Hence, acyl migration must be kept as low as possible during lipase-catalyzed acidolysis, because the acyl migration results in decreased purity of the symmetrical SLs. It is well-known that nonaqueous enzymatic reactions critically depend on the level of water in the reaction system. Some researchers (*21*, *22*) have reported that water has a profound influence on both acyl migration and rate of reactions (both hydrolysis and acidolysis). To investigate the effect of water activity on the time course of the acyl migration, incorporation of capric acid (mol %) at *sn*-2 of TAG was studied (**Figure 2**). Acyl migration was defined as the mol % of capric acid in the *sn*-2 position of TAG after acidolysis. The degree of acyl migration increased for all water activity protocols tested, when the reaction time was increased. Significant increases were also observed in the acyl migration, when water activity was increased from 0.22 to 0.65 up to 74 h. However, there was no significant difference in the acyl migration, when water activity was increased from 0.43 to 0.80 at a reaction time of 96 h. These results suggest that acyl migration in lipase-catalyzed acidolysis may be affected by reaction time as well as water activity. The similar results were obtained by Xu et al. (*14*) for the enzymatic transesterification of rapeseed oil and capric acid in solvent-free media.

Acyl migration was investigated in modified olive oils with similar degrees of incorporation (**Figure 3**). Significantly different reaction times were required to synthesize modified olive oils with a similar degree of incorporation at different water activities. For modified olive oils with the degrees of incorporation between 5 and 15 mol %, there were no significant differences in the degree of acyl migration. For modified olive oils, with the degree of incorporation between 25 and 35 mol %, there were also no significant differences in the degree of acyl migration, but there was a significant difference in the modified olive oil synthesized at a water activity of 0.22. Similar trends were observed in modified olive oils with the degrees of incorporation between 35 and 45 mol %. To reach a degree of incorporation of ca. 50 mol % at different water activities, significantly different reaction times were required. For example, the reaction times needed to obtain incorporation of ca. 50 mol % at water activities of 0.80, 0.65, 0.43, and 0.22 were 8, 12, 24, and 72 h, respectively. In addition, we observed different tendencies in acyl migration for the degree of incorporation of ca. 50 mol %. High degrees of acyl migration at water activities of 0.22 and 0.32 occurred, but there were no significant differences in the degree of acyl migration between these water activities. A low water activity required a long reaction time to reach a degree of incorporation of ca. 50 mol %, and this consequently resulted in high degrees of acyl migration. Meanwhile, a significant decrease in acyl migration was observed when the water activity was increased from 0.32 to 0.43. However, there were no significant differences in the degree of acyl migration when water activity was increased from 0.43 to 0.65. Only 8 h of reaction time was required to achieve incorporation of ca. 50 mol % at water activity of 0.80, and the lowest acyl migration occurred at the same water activity. These results may be due to shorter reaction time because of the efficient contact between substrates and lipase. This result is also consistent with that of Mu et al. (*23*), who reported that when SLs were synthesized by acidolysis of sunflower oil with capric acid, systems mediated by shorter reaction time exhibited less acyl migration than systems mediated by longer reaction time.

In conclusion, undoubtedly, acyl migration and incorporation increased significantly when the water activity and reaction time were increased. In this study, interesting results were obtained when degrees of acyl migration were compared between modified olive oils with similar degrees of incorporation, which were synthesized at different water activities and reaction times. Overall, these results imply that acyl migration can be efficiently minimized by appropriate control of reaction times at higher water activities. In addition, reaction conditions and different substrates may affect the extent of acyl migration. Hence, different results might be attained in other lipase applications.

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