Enzymatic Modification of Canola/Palm Oil Mixtures: Effects on the Fluidity of the Mixture

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The bioreactor system to interesterify edible oils and fats at an ultra-micro aqueous phase of 100 ppm and less was investigated. The adsorption of lecithin, together with lipase onto a carrier, was effective for conducting the interesterifying reaction efficiently for oils and fats in micro aqueous phase.

To improve the handling properties of palm oil at rather low temperature, palm oil was blended with canola or soybean oil, and then these blended oils were modified by enzymatic selective interesterification in a solvent-free, ultra-micro aqueous bioreactor system with an immobilized lipase that had 1,3-positional specificity. The effects of enzymatic interesterification were confirmed by triglyceride determination, by solid fat content profiles and by cloud point profiles, which were also compared to products of chemical interesterification. The improvement in the fluidity of blended oils with canola oil by the enzymatic reaction was bigger than with soybean oil, and chemical interesterification had no effects on the fluidity of blended oils.

KEY WORDS: **Canola, chemical interesterification, fluidity, interesterification, lecithin, lipase, palm oil, soybean oil, ultra-micro aqua bioreactor system.**

Palm oil is now produced at more than 10 million tons annually throughout the world. This is the second largest amount of oil produced, soybean oil being the first. According to a forecast by *Oil World* (1), the production of palm oil is estimated to reach 20 million tons in the year 2003. This means that palm oil will be the most abundant and economical edible oil in the near future. One of the current technological concerns of the edible oil industry is how to expand the multiple usages of palm oil.

The high content of diglycerides (DG) in palm oil (2) is one of the main problems blocking the potential usages of this oil. To solve this problem, we have developed a new bioreactor system, called the ultra-micro aqua bioreactor system, which works at less than 30 ppm of water concentration to esterify DG to triglycerides (TG) in crude palm oil (3).

Because palm oil is rich in palmitic acid, oleic acid and antioxidants, it is very stable against heating damage or oxidation. However, because of its low fluidity, palm oil is rather difficult to handle at lower temperatures, which limits its usage as a frying or a dipping oil, especially in countries with a cool climate.

Enzymatic interesterification between solid fat and liquid oil has been studied in order to obtain oil with better melting properties (4–8). Some studies on enzymatic interesterification without organic solvents have been published (4–8). The effect of water on reaction kinetics is important because water promotes hydrolysis of lipids and affects the quality of the products (8,9). Although considerable research has been conducted, the benefits of enzymatic modification of edible fats and oils has not yet been realized by industry, especially in such fields as frying oils. As the water concentration becomes higher, the activity of lipase usually increases (9) and, at the same time, the degree of hydrolysis becomes higher and deteriorates the quality of modified oils. For industrialization of an enzymatic modification system, the economics and the products' quality must be improved. Specifically, it is necessary to improve the modification activity of lipase at minimal water concentration in the reaction system.

In this work the enzymatic selective interesterification of palm oil blended with canola oil or soybean oil (to improve its fluidity) was studied in a solvent-free and ultra-microaqua system below 100 ppm of water concentration with 1,3-positional specific lipase immobilized with an activator (lecithin).

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MATERIALS AND METHODS

Lipase. Lipase D-200 (*Rhizopus delemar;* Amano Pharmaceutical Co. Ltd., Nagoya, Japan) was used. The activity of the enzyme was 200 units/mg. This lipase has positional specificity at the 1,3-positions of triacylglycerol. A selective interesterification was expected with this enzyme as the 2-position of triacylglycerol is not changed during the reaction. According to the supplier's information, the enzyme in water solution is stable at not higher than 40°C.

Celite. Celite 535 (diatomaceous earth; Manville, Lompoc, CA) was used as a carrier for the lipase.

Lecithin. As the activator of lipase at the ultra-micro aqueous condition of below 100 ppm, refined soya lecithin (Tsuru Corp., Mie Prefecture, Japan) and sugar ester 0-1570 (Mitsubishi Kasei Corp., Tokyo, Japan) was used.

Substrates. Palm oil, palm olein, soybean oil and canola oil (rapeseed oil) were refined, bleached and deodorized through ordinary procedures. Those oils were dried at 80°C under a vacuum of 2–5 Torr before reaction.

Molecular sieves. MS-3A (Union Showa Co. Ltd., Tokyo, Japan) was used as dehydrator in the reaction. MS-3A was dried at 180°C for 18 h before use.

Immobilization. Lipase was dissolved fully in pure water or lecithin solution and then mixed with Celite 535. Unless described otherwise, the weight ratio of enzyme, water and Celite was 1:9:9. This mixture was dried at 40°C under a vacuum of 10 Torr so as to obtain immobilized lipase with a moisture of less than 1%. This immobilized lipase is stable in oil below 60°C for more than 24 h.

TG, DG, monoglycerides (MG) and free fatty acids (FFA). After the oil samples were heated with *bis*(trimethylsilyl)trifluoroacetamide at 80°C for 40 min, compositions of TG, DG, MG and FFA of blended and reacted oils were analyzed with cholesterol acetate as internal standard by gas chromatography (Hitachi 663, Tokyo, Japan) on Dexil 300GC column. Temperature ascended at the rate of 12°C/min from 100 to 300°C, and

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at the rate of 3°C/min (from 300 to 330°C). The flow rate of He gas was set at 60 mL/min.

TG content and yield. TG content and yield are calculated as follows:

$$TG(\%) = 100(\%) - FFA(\%) - MG(\%) - DG(\%)$$

$$TG\ yield(\%) = TG_e/TG_i \times 100 \quad [1]$$

where TG_e = TG content after reaction and TG_i = TG content before reaction.

Enzymatic interesterification reactions. Substrate oil (20 g), 2 g immobilized lipase and 2 g molecular sieves were put into a 200-mL flask with a plug. The flask was shaken at 120 rpm at 60°C for 5 h after the air in the flask was replaced with dried nitrogen gas. The reaction temperature of 60°C was selected to avoid the solidification of the substrate oils and fats and inactivation of the immobilized enzyme during the reaction. To measure the specific activity of the immobilized lipase, 0.3 g of immobilized lipase, 24 h of reaction time and a mixture of canola oil and palm olein (8:2) were selected.

In the bioreactor system the immobilized lipase was packed in a column and substrate oil (water, 50 ppm) was fed into the column at 2 g oil/g immobilized lipase/h.

The interesterification rate (X_t) was calculated as follows:

$$X_t = (MC_t - MC_0)/(MC_e - MC_0) \times 100 \quad [2]$$

where MC = composition of the TG with the index carbon number. This TG usually shows the biggest change during the reaction. TG with the carbon number 55 is used as the index TG, unless described otherwise; MC_t = MC at time t (h); MC_0 = MC at the start of reaction; and MC_e = MC at equilibrium.

The specific activity (SA_t; g/h/g) of immobilized lipase was calculated by:

$$SA_t = \frac{(MC_t - MC_0)}{t} \times 100 \times \frac{W_o}{W_e} \times \frac{100}{LC_i} \quad [3]$$

where W_o, weight (g) of oil used in the reaction, W_e, weight (g) of immobilized lipase in the reaction and LC_i, lipase content (%) of immobilized lipase.

Chemical interesterification. Sodium-methylate (0.2%) was added as catalyst to raw oil and then maintained at 85°C for 30 min. Warm water (10%) was added to the reacted oil and then separated into oil and water fractions. The oil fraction was refined through ordinary decolorization and deodorization processes to become refined modified oil. The TG yield was 96.4%.

Moisture. The moisture levels of lipase and immobilized lipase were determined as weight loss after drying the samples at 105°C for 4 h. The moisture of oil samples was determined by the Karl Fischer method (CA-05; Mitsubishi-Kasei Corp.).

Solid fat content. Solid fat content was measured by nuclear magnetic resonance (SFC-900A; Praxis, San Antonio, TX). Oil samples were first refined through silica-gel treatment.

Cloud point. Cloud points were measured in accordance with the AOCS Method (10).

RESULTS AND DISCUSSION

Effects of lecithin on lipase activity at micro aqueous conditions. The mixture of canola oil and palm olein (8:2) was used as substrate to study the effects of initial water concentration in oil and molecular sieves on the final water concentration in oil, interesterification rate, TG yield and specific activity of immobilized lipase. When the initial water concentration was 930 ppm, the interesterification activity was 590 g/h/g, the interesterification rate at 5 h was 100% and the TG yield was 94.8% (Table 1). The lower the initial water concentration was, the lower the interesterification activity was, although the TG yield was higher. At 560 ppm of initial water, the addition of molecular sieves was effective to improve TG yield, but still not enough to obtain more than 99% TG yield. When initial water was set below 100 ppm and 10% of dried molecular sieve was added to the reaction medium, almost 100% of TG was attained. However, at this micro-aqueous condition, the interesterification activity of the immobilized lipase almost disappeared.

Formerly, when certain kinds of surface-active agents were immobilized together with lipases onto carriers, they activated the esterification power of lipases at ultra-micro aqueous conditions (3). So, the effects of lecithin and sugar ester 0-1570, two of the most effective surface-active agents, were examined on the interesterification activity at ultra-micro aqueous conditions. The results are shown at Table 2. When 10% lipase and 1.5% lecithin were

TABLE 1

The Effects of Initial Water Concentration in Oil and Added Molecular Sieves on End Water Concentration in Oil, Interesterification Rate, TG Yield, and Apparent Specific Activity of Immobilized Lipase[a]

Initial H_2O in oil (ppm)	Molecular sieves (%)	End H_2O in oil (ppm)	Interesterification rate (%)	TG yield (%)	Specific activity (g/h/g)
930	0	320	100	94.8	590
560	0	320	102	96.4	515
560	10	70	101	97.8	192
44	0	130	48	98.7	83
44	10	67	10	99.9	17

[a]Conditions were as described in the Materials and Methods section. The moisture of immobilized lipase (adsorbed lipase, 10%) was 0.8%. TG, triglycerides.

TABLE 2

Effects of Lecithin and Sugar Ester 0-1570 on Specific Activity (SA_t) at Ultra-Micro Aqueous Phase of Immobilized Lipase[a]

Surface-active agent	Amount of surface-active agent (g% of oil)						
	0	0.8	1.5	2.5	3.8	5.0	7.5
Lecithin							
SA_{24} (g/h/g)	21	105	235	219	188	16	3
TG yield (%)	99.9	99.8	99.8	99.9	99.9	99.7	99.6
0-1570							
SA_{24} (g/h/g)	27	14	12	10	8	6	0
TG yield (%)	99.9	100	100.2	100.1	99.9	100	100.2

[a]The moisture of immobilized lipase (adsorbed lipase, 10%) was 0.6%. 2 g of molecular sieves, 0.3 g of immobilized lipase and 20 g of dried oil mixture (canola oil/palm olein, 8:2) were mixed and shaken at 60°C for 24 h. TG, triglyceride.

immobilized onto Celite, the immobilized lipase was restored to 235 g/h/g of interesterification activity at ultra-micro aqueous condition from 21 g/h/g without lecithin. The specific activity was improved 10-fold. Additional lecithin decreased the activity. Sugar ester 0-1570, which was most effective at restoring esterification activity at ultra-micro aqueous conditions (11), had no effect at all, showing less activity than in the case of no addition at all. From these results, it can be concluded that there are some differences between esterification and interesterification in the activating mechanism of lipase at ultra-micro aqueous conditions by surface-active agents. Anyway, the combination of joint immobilization of lipase and lecithin and the ultra-micro aqueous phase reaction was determined to be a basic condition for the interesterification of oil mixtures.

Enzymatic interesterification of palm oil and canola oil. Ultra-micro aqueous phase interesterification by 1,3-positional specific lipase of *R. delemar* was applied to blends of palm oil and canola oil, with ratios of 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100.

This selective interesterification caused the reduction of TG with trisaturated and with disaturated fatty acid compositions (Fig. 1). The altered triglyceride composition was reflected in solid fat content and in cloud point. A significant reduction of solid fat content through enzymatic reaction was observed. At 10°C, solid fat content of enzymatically-modified oil mixtures were measured as 0 at up to 20% mixing ratio of palm oil (Fig. 2). Cloud points were also lowered (Fig. 3). Thus, enzymatic interesterification was useful to improve the fluidity of blends of palm oil and canola oil.

Enzymatic interesterification of palm oil and soybean oil. Blends of palm and soybean oil were also modified through enzymatic interesterification. The changes of

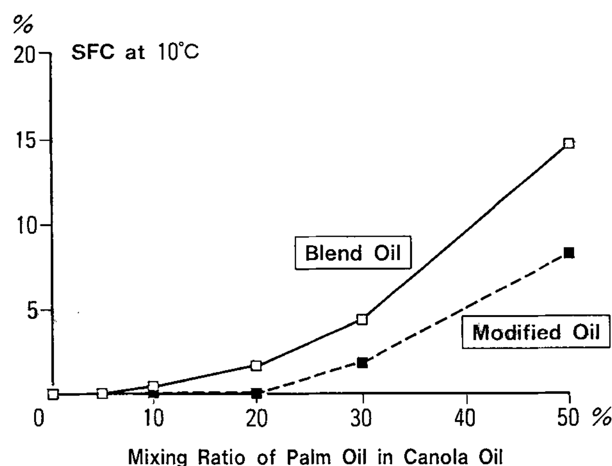

FIG. 2. Change of solid fat contents (SFC) by enzymatic modification through 1,3-regiospecific interesterification.

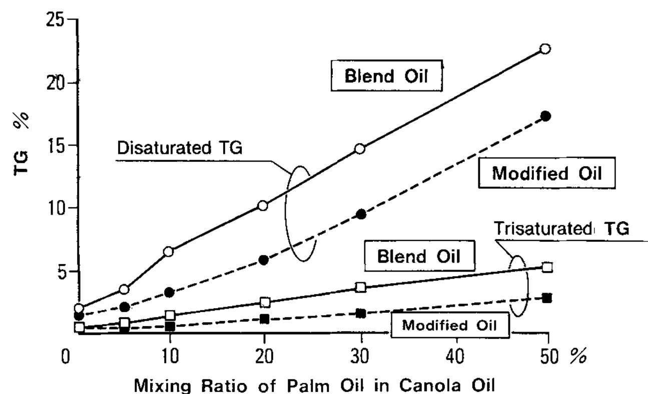

FIG. 1. Change of triglyceride (TG) composition by enzymatic modification through 1,3-regiospecific interesterification.

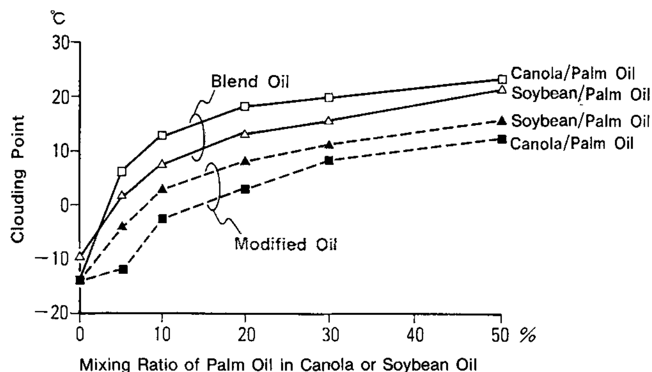

FIG. 3. Change of clouding point by enzymatic modification through 1,3-regiospecific interesterification.

cloud points before and after the reaction are shown in Figure 3. Although the melting point and cloud point of canola oil are lower than those of soybean oil, the blends of palm with canola oil showed higher cloud points than the blends of palm with soybean oil at all mixing ratios. However, after selective interesterification, modified blends of palm and canola oil showed much lower cloud points than the modified blends of palm and soybean oil. The beneficial effect of enzymatic selective interesterification on fluidity was much bigger for the blend of palm and canola oils than for the blend with soybean oil.

Comparison of chemical and enzymatic interesterification. Chemical and enzymatic interesterifications were applied to the blends of palm olein and canola oil, at ratios of 100:0, 75:25, 50:50, 25:75 and 0:100. Solid fat content values at 10°C are compared in Figure 4. Enzymatic interesterification lowered the solid fat contents, although

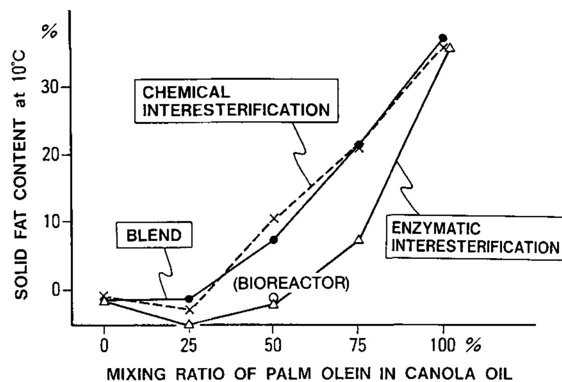

FIG. 4. Comparison of effects of enzymatic and chemical interesterification on solid fat contents at 10°C.

chemical interesterificaton was not effective at all. Figure 4 includes one data point for the bioreactor system. Without changing the advantages of palm oil, such as stability and freedom of hydrogenation odor and *trans* fatty acids, selective enzymatic interesterification improves the fluidity in most mixtures to produce new functional oils for potential use as stable and fluid frying oil.

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