Carica papaya Latex-Catalyzed Synthesis of Structured Triacylglycerols[1]

ABSTRACT: One impediment to the industrial use of enzymes in fat and oil transformations is the higher cost often associated with an enzymatic process compared with the corresponding chemical process. Processes that utilize plant enzymes, however, may have advantages because of their lower cost and ready availability. One example of such a plant-derived enzyme is *Carica papaya* latex (CPL), the principal source of the protease papain. Recently, it has been shown that this latex also catalyzes the lipolysis of triacylglycerols and that this latex lipase has a selectivity for short-chain acyl groups as well as a 1,3-glycerol selectivity. These selectivities can be used in the synthesis of structured triacylglycerols. In this paper we describe the utility of CPL in lipase-catalyzed reactions, specifically the synthesis of low-calorie triacylglycerol analogs.
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KEY WORDS: Hydrogenated soy oil, low-calorie fats, short-chain triglycerides, triacylglycerols, tributyrin.

Interesterification is one of the major reactions used by industry for the modification of natural fats and oils. In its simplest form, interesterification corresponds to an exchange of acyl residues between two triacylglycerols (TAG), resulting in the formation of new TAG that have chemical and physical properties deemed superior to the starting TAG (1). Presently, there are two ways of catalyzing interesterification reactions, namely chemically and enzymatically. Chemical interesterification reactions are generally catalyzed by metal alkoxides, which are relatively inexpensive, readily available, and easy to use. Chemical interesterification, however, produces TAG that have a random distribution of fatty acyl groups on the glycerol backbone, whereas in lipase-catalyzed reactions, the unique specificities of lipases allow for the design of TAG with a predetermined composition and distribution of fatty acyl groups (2). Examples of successful lipase-catalyzed interesterification (or transesterification) reactions abound in the literature (3–6). However, the development of enzymatic interesterification reactions on an industrial scale is limited by the high cost associated with the lipases used. Consequently, the use of plant biocatalysts may have advantages owing to their low cost and availability in comparison with their microbial or animal counterparts (7).

Carica papaya latex (CPL) is well known for containing proteases, among which is papain, a thiol protease with many industrial applications, e.g., as a meat tenderizer, contact lens cleaner, digestive aid, or bloodstain remover in detergents. This plant exudate, however, has not only proteolytic activity but also lipase activity (8). Recently, CPL lipase was characterized by several research groups (9–11), who showed that CPL lipase had *sn*-3 stereoselectivity. More recently, we studied CPL-catalyzed interesterification reactions and found that this lipase also has a strong short-chain fatty acyl selectivity (12). This latter observation led us to consider that CPL might be a useful inexpensive biocatalyst for the synthesis of low-calorie structured TAG.

Structured TAG are defined as triglycerides whose fatty acid composition and location on the glycerol backbone have been predetermined by unequivocal synthetic routes. Presently, such triglycerides are mainly synthesized by enzymatic methods and are designed for use in selected nutritional applications (13). Another area of interest in this field is the synthesis of low-calorie TAG that are characterized by the combination of short-chain and long-chain acyl residues into a single triglyceride structure. The most familiar class of low-calorie fats is the Salatrim™ (14) family of TAG, which are produced by incorporating either acetoyl (C_2), propionoyl (C_3), or butyroyl (C_4) residues into long-chain hydrogenated vegetable oils (canola or soybean). Interest in these types of structured lipids stems from the fact that they contain only 5 cal/g compared with the 9 cal/g of natural fats and oils because of the lower caloric content of short-chain acyl residues compared to their long-chain (C_{16} and C_{18}) counterparts. Low-calorie TAG are intended for use in baking chips, coatings, dips, baked products, or as cocoa butter substitutes. Currently, such restructured lipids are synthesized by chemical interesterification. In this paper, we describe a synthesis of these structured TAG based on a CPL lipase-catalyzed interesterification reaction. The TAG obtained were similar to the chemically produced Salatrim™ family of triglycerides as judged by comparative gas chromatography of both product types (15).

MATERIALS AND METHODS

Materials. Crude CPL and tributyrin (TB) were obtained from Sigma Chemical (St. Louis, MO). The latex was ground in a mortar to a fine powder before use. Lipozyme IM was a gift from Novo Nordisk (Franklinton, NC). Hexane was purchased from Burdick and Jackson (Muskegon, MI) and was high-performance liquid chromatography-grade. Hydrogenated soybean oil (HSO) (12% C_{16}; 88% C_{18}; iodine value >5) was a gift from Nabisco Brands (Indianapolis, IN).

Methods. Esterification reactions were conducted as follows: Into a screw-top vial were placed 1.25 g TB (4.1 mmole) and 2.20 g HSO (2.5 mmole). The mixture was placed in an 80°C water bath to melt the hydrogenated oil. The vial was then placed into a water-jacketed beaker, and 65°C water was circulated through the jacket from a constant-temperature bath. CPL (10% w/w of lipid) was added to the vial, and the mixture was stirred magnetically at 200 rpm throughout the reaction. Over the time course of the reactions, samples that contained approximately 5 mg lipid were removed periodically and diluted with 4 mL hexane. The samples were analyzed by gas–liquid chromatography (GLC) as follows: cold on-column capillary injection onto a DB1-HT (J&W Scientific, Folsom, CA) methyl-silicone capillary column (15 m × 0.32 mm i.d., film thickness 0.1 μm). The chromatographic conditions were: on-column injector, flame ionization detector at 370°C, He carrier gas at 5.5 mL min^{-1}. Separations were made with the following oven temperature profile: initial temperature 70 to 350°C at 20°C min^{-1}, final time 4 min.

CPL lipase recycling. Into a screw-top beaker, 28.6 g TB was mixed with 50.5 g HSO. Crude CPL (10% w/w) was added, and the mixture was magnetically stirred at 200 rpm in a 65°C water bath. After 24 h, the reaction was stopped, and a sample of approximately 5 mg lipid was removed for GLC analysis. The reaction was filtered, and the CPL was recovered by filtration and washed several times with hexane until no traces of lipid appeared by thin-layer chromatography in the hexane wash. The enzyme was dried under a nitrogen flow and reused in successive reactions.

CPL conditioning. Dried CPL was prepared by placing 5 g of crude CPL into a desiccator over anhydrous calcium sulfate. The latex was left for several days until constant weight was obtained (6% wt loss of H_2O). Water-washed CPL (ww-CPL) was prepared by suspending 20 g of CPL in 150 mL water and centrifuging at 10,000 rpm for 10 min. The water was decanted, the pellet was resuspended in water, and the process was repeated four times. The insoluble latex pellet was stored in a desiccator over anhydrous calcium sulfate. Water activity (a_w) of the crude, dried, and ww-CPL powder was determined with an Aqualab CX-2 (Decagon Devices Inc., Pullman, WA) instrument. The a_w of the dried CPL was 0.10. The ww-CPL was removed from the desiccator when its a_w equaled the a_w of the crude CPL ($a_w = 0.56$).

RESULTS AND DISCUSSION

Presently, low-calorie structured TAG are obtained by random chemical interesterification between a short-chain TAG and long-chain TAG, typically a hydrogenated vegetable oil, such as soybean or canola oil (14). The interesterified product is composed of a mixture of two TAG structures; the first type contains two short-chain residues and one long-chain acyl residue (SLS-TAG), and the second contains one short-chain and two long-chain acyl residues (SLL-TAG). By predetermining the fatty acid composition and controlling the ratio of SLS- and SLL-TAG, it is possible to produce products with selected properties that are useful in several applications, such as cocoa butter substitutes, bakery and dairy products (14).

Our interest in the low-calorie class of restructured lipids was in developing enzymatic routes for their synthesis. Because of its *sn*-3 and short-chain acyl selectivity in interesterification reactions (12), CPL has potential as a prospective biocatalyst in the enzyme-catalyzed synthesis of low-calorie TAG and more specifically Salatrim™ analogs. Accordingly, in a solvent-free system, an equimolar mixture of TB and HSO was reacted in the presence of 10% (w/w) CPL. The appearance of newly formed mixed short- and long-chain TAG was followed by GLC (Fig. 1). This chromatogram showed that two new types of TAG were produced, which confirmed the formation of SLS- and SLL-TAG. Both the SLS- and SLL- TAG consisted of a smaller peak, representing incorporation of a palmitoyl group, and a larger peak, which con-

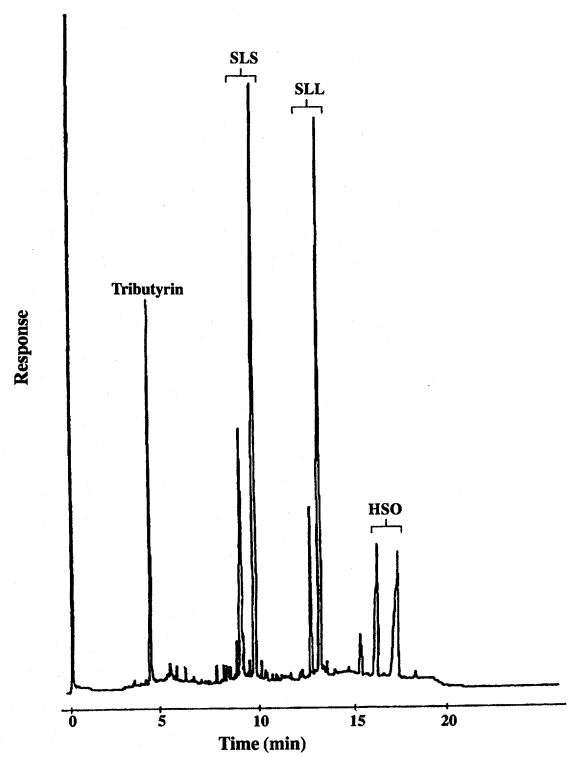

FIG. 1. Gas–liquid chromatography chromatogram of the product from the *Carica papaya* latex-catalyzed interesterification between tributyrin and hydrogenated soy oil (HSO). SLS and SLL represent triacylglycerols that contain one or two butyroyl (S) and one or two palmitoyl (L) or stearoyl (L) residues on the glycerol backbone.

tained a stearoyl group. The ratio of the two peaks for each class of TAG reflected the initial C_{16}:C_{18} ratio (12:88) of the starting HSO. It previously was reported (11) that the lipase associated with CPL has 1,3-selectivity in lipolysis reactions. This apparent selectivity was reflected in the preceding interesterification reaction. Because the interchange of acyl groups occurs primarily at the 1,3-positions of the TAG molecules, the interesterified products obtained have a more defined distribution of acyl residues on the glycerol backbone, unlike the chemical process where acyl residues are randomized. Moreover, repeating this reaction with Lipozyme™, a highly conserved 1,3-regioselective lipase (16), as biocatalyst gave a similar GLC distribution of interesterified TAG products.

Figure 2 shows the time course of the CPL-catalyzed interesterification reaction between TB and HSO. Newly formed TAG represented 65–70 wt% of the product mixture after 24 h reaction, with the SLS-TAG predominating (40%). Extending the reaction time up to 96 h did not significantly alter product composition. In restructured lipids, the SLS/SLL ratio is important because it governs the physical properties of the mixture and consequently the functionality of the product. This ratio also is important to the final caloric content of the mixture (17). As shown in Figure 3, by varying the reaction time of the interesterification reaction, it is possible to alter the SLS-/SLL-TAG ratio. For example, after 24 h reaction, a 3:2 ratio of SLS-/SLL-TAG was obtained, a ratio that was comparable to the product obtained by chemical interesterification (14). Shorter reaction times gave higher amounts of SLS-TAG, whereas longer reaction times resulted in comparable amounts of the two types of TAG.

The effect of CPL catalyst amount used also was studied. When crude CPL was used as the catalyst, best conversions of interesterified TAG (SLS + SLL) were obtained with 10 and 15 wt% of CPL, 68 and 78%, respectively. A lower formation of these TAG was noted when the reaction was carried out with 20 wt% crude CPL. The latter result was caused by the amount of water brought into the reaction medium by the crude CPL (6 wt%, $a_w = 0.56$), which resulted in increased TAG hydrolysis. Hydrolysis could be avoided by using 20% dried CPL ($a_w = 0.10$) as the catalyst, but yields of interesterified TAG were not significantly changed from the reactions with the crude CPL. We previously reported that crude CPL, dried CPL, and ww-CPL ($a_w = 0.56$) had different catalytic activities in transesterification reactions (18). We compared the activity of these CPL (10 wt%) as catalysts for the interesterification of TB and HSO by measuring the amounts of new TAG formed after 24-h reaction. We found that ww-CPL gave slightly higher conversions of new TAG (80%) compared to the crude and dried CPL catalysts (67 and 70% new TAG, respectively). The better result obtained with ww-CPL was mainly due to increased SLL-TAG formation.

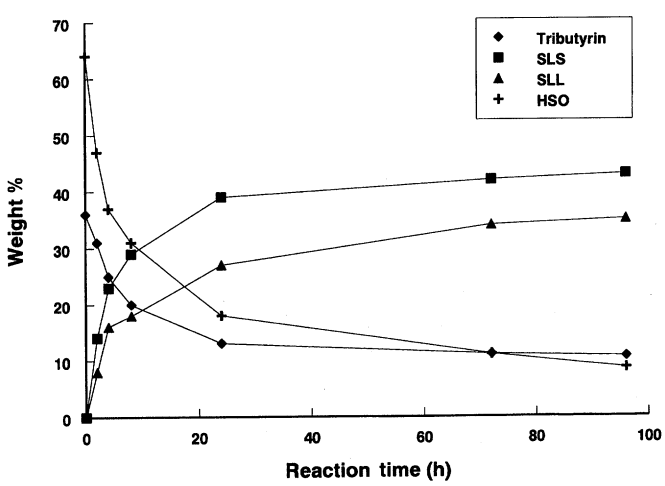

FIG. 2. Time course of the *Carica papaya* latex-catalyzed interesterification reaction between tributyrin and HSO. For abbreviations, see Figure 1.

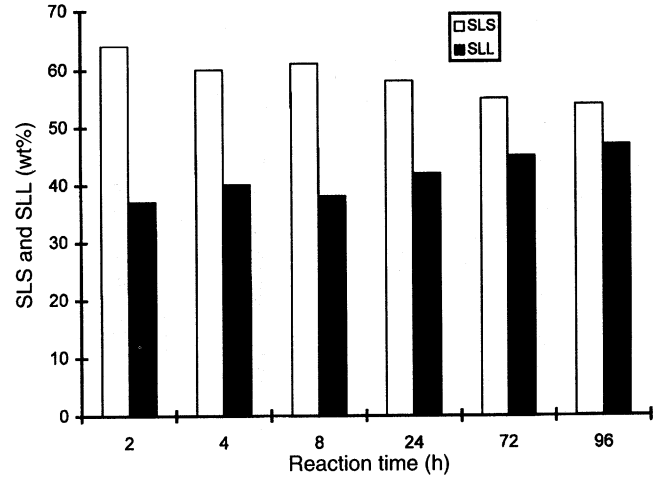

FIG. 3. Distribution of SLS and SLL triacylglycerols formed as a function of time in the *Carica papaya* latex-catalyzed interesterification reaction between tributyrin and HSO. For abbreviations, see Figure 1.

We also evaluated the effect of recycling on the catalytic activity of CPL in larger-scale interesterification reactions between TB and HSO (Fig. 4). This was done by conducting reactions for 24 h, filtering the CPL catalyst, washing with hexane to remove lipid material, and then repeating the interesterification with fresh TB and HSO. The results of these recycle experiments showed a linear decrease in CPL activity so that, after eight consecutive runs, the CPL catalyst retained about 50% of its initial activity for interesterification of the TB and HSO.

In conclusion, we have demonstrated in this paper that CPL, known for its proteolytic activity, also should be viewed as a prospective biocatalyst for oil and fat modifications. Its interesting lipolytic activity and specificity, as well as its relatively inexpensive cost ($75-80/kg, *Chemical Marketing Reporter*, 1996) make CPL a potential biocatalyst of choice for various enzymatic processes involving oils and fats. As one example, we have shown that CPL would be useful for the lipase-catalyzed synthesis of low-calorie structured triglycerides, particularly for producing analogs of currently avail-

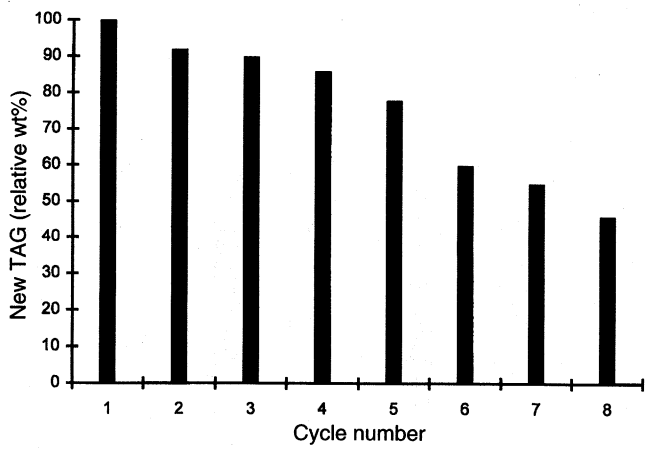

FIG. 4. Effect of recycling on the catalytic activity of *Carica papaya* latex in the interestèrification reaction between tributyrin and HSO. Triacylglycerols (TAG) are SLS- and SLL-TAG formed in the reaction after 24 h. For abbreviations, see Figure 1.

able products, which are presently obtained by chemical interesterification.

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