Production of Cocoa Butter-Like Fat from Interesterification of Vegetable Oils[1]

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Cocoa butter-like fat was prepared from completely hydrogenated cottonseed and olive oils by enzymatic interesterification. The optimum reaction time to produce the major component of cocoa butter, 1(3)-palmitoyl-3(1)-stearoyl-2-monoolein (POS), was 4 hr. The cocoa butter-like fat was isolated from the reaction mixture by two filtration steps. The yield of cocoa butter-like fat was 19%, based on the weight of the original oils. Chromatographic analysis of the product by reverse-phase high-performance liquid chromatography (HPLC) has shown it contains triglyceride components similar to those of cocoa butter, but that it has slightly more diglycerides. The melting point of this product, as measured by a differential scanning calorimeter, is 39°C, which compares well to the 36°C melting point of natural cocoa butter.

KEY WORDS: Cocoa-like fat, cottonseed oil, interesterification, lipase.

Cocoa butter is the premium candy fat, primarily because of its unique physical characteristics. At room temperature (below about 26°C), it is hard and brittle. When eaten, it melts completely in the mouth with a pleasing, cooling sensation. These distinctive physical properties are the result of an unusual glyceride composition. Unlike most fats and oils, cocoa butter has few triglycerides, and three of them comprise 80% of its weight (1). The major components of cocoa butter are 1(3)-palmitoyl-3(1)-stearoyl-2-monoolein (POS) and 1,3-distearoyl-2-monoolein (SOS) which are 52% and 18.4% respectively, of the total.

Interesterification of fats is of commercial interest for the production of margarine, shortenings, and other specialty fats. Preparation of cocoa butter substitutes from lower value oils by chemical interesterification has been studied (2). Recently, the use of lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) to catalyze interesterification reactions has received considerable attention because lipases offer certain advantages over chemical catalysts. These include a product distribution more closely resembling cocoa butter and the ability to run the reaction at lower temperatures. The major triglyceride components of cocoa butter have been obtained enzymatically from olive oil and stearic acid (3,4) and from midfraction of palm oil and stearic acid or tristearin (5), and the methods have been reviewed recently (6,7).

Our study was aimed at developing an enzymatic continuous process for producing cocoa butter-like fat from hydrogenated cottonseed and olive oils. These oils were enzymatically interesterified and then fractionally crystallized from acetone to yield cocoa butter-like fats.

EXPERIMENTAL PROCEDURES

Materials. Hydrogenated cottonseed oil was donated by Anderson Clayton Foods, Inc. (Sherman, TX) and lipozyme was donated by Novo, Inc., Danbury, CT. The triglyceride standards were purchased from Sigma (St. Louis, MO) and the olive oil (Italian Extra Virgin) was bought from a local supermarket.

Enzymatic interesterification. The enzyme used in this study is a 1,3-specific lipase from the fungus, *Mucor miehei,* which had been immobilized on a macroporous resin (8). This immobilized enzyme is available from Novo Laboratory, Inc., under the trade name Lipozyme. An immobilized lipase was chosen because it could be easily removed from mixture and reused. Interesterification reactions were studied at 70°C, with various weight ratios of hydrogenated cottonseed oil to olive oil and a constant weight ratio of Lipozyme to total substrate. To initiate the reactions, Lipozyme equal to one-tenth of the weight of the oil at 70°C was added. Small aliquots were withdrawn from the reaction vessel at various times and subjected to high-performance liquid chromatography (HPLC) analysis. The optimum reaction time was determined by following the yield of POS.

Isolation of cocoa butter-like fat. Cocoa butter-like fat was prepared from completely hydrogenated cottonseed oil and olive oil by enzymatic interesterification followed by acetone fractionation. Acetone fractionation was performed by the procedure published by Feuge and Lovegren (2), with modifications. A typical preparation is as follows: 1.5 g of hydrogenated cottonseed oil and olive oil were interesterified in the presence of 0.3 g of Lipozyme for 4 hr at 70°C. Thirty mL of acetone were added at the end of the reaction. The acetone reaction mixture was quickly filtered through filter paper to remove the enzyme. The filtered solution was then cooled overnight to room temperature, about 25°C, thus forming a precipitate which was filtered out. The filtrate was then cooled to 4°C for about 4 hr and the resulting slurry filtered at 4°C to produce the cocoa butter-like fat. The product was then subjected to HPLC and differential scanning calorimetry (DSC) analysis.

HPLC chromatography and triglycerides identification. Triglycerides were analyzed by reverse-phase HPLC chromatography. Each sample was weighed and dissolved in tetrahydrofuran to make a 10% solution. Five μL of solution were injected onto two Supelco C-18 columns (150 × 4.6 mm), Supelco Inc., Bellefonte, PA. Triglycerides were separated by isocratic elution at a flow rate of 1.5 mL/min; a mixture of acetone and

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TABLE 1

Triglycerides Formed by the Lipozyme-Catalyzed Interesterification of Completely Hydrogenated Cottonseed and Olive Oils

Triglyceride components of cottonseed oil	Triglyceride components of olive oil	Mixture of triglycerides			
PPS	OOL	LPL	POP	OOL	PPP
PSS	POL	LSL	OSO	POL	SSL
SSS	OOO	PPL	POS	OOO	PPS
	OPO	PSL	SOS	SOL	PSS
	OSO	OPO	SSS		

acetonitrile (3:1) was used as the mobile phase. The peaks were detected by a refractive index detector and individual peaks identified by comparing the retention time of the peak to the retention time of reference triglycerides. Those peaks for which commercial standards were not available at the time these experiments were conducted, such as POS, were isolated from cocoa butter and identified by mass spectrometry.

Differential Scanning Calorimeter analysis. Melting characteristics of the product were analyzed by a differential scanning calorimeter (model DSC-2, Perkin-Elmer, Norwalk CT). The data were collected by a Perkin-Elmer data station, model 3000, and analyzed by a standard DSC program.

RESULTS AND DISCUSSION

Enzymatic interesterification. Eighteen chromatographically detectable triglycerides potentially can be produced by interesterifying hydrogenated cottonseed oil and olive oil (Table 1). Lipase, in general, has position specificity, not fatty acid specificity. Many interesterification products will be produced in small quantities in long reaction times. Controlling the reaction time results in the best yield of POS, the major triglyceride component of cocoa butter, in the product fat. The optimal reaction time for producing POS is 4 hr (Fig. 1). The yield of POS reaches equilibrium after 20 hr. If the ratio of enzyme to total oil weight is constant, a 1:1 or higher ratio of hydrogenated cottonseed oil to olive oil is required for the best yield of POS.

Preparation of cocoa butter-like fat. The yield of isolated cocoa butter-like fat was about 19% based on the weight of the original oils. This percentage yield is the mean value of three runs. HPLC chromatography of Hershey's cocoa butter and of our cocoa butter-like fat indicated that our product contains more diglycerides than the cocoa butter (Fig. 2). The two major triglycerides in our cocoa butter-like fat, POS and SOS, are found to be 23% and 28%, respectively. Our product has more SOS than Hershey's cocoa butter, which probably gives our product its higher melting point. An earlier study by Landmann, *et al.* (9) showed that every saturated triglyceride in a fat will contribute to making the melting point higher. A short plastic range is an important characteristic of a cocoa butter substitute. Our product fat melted between 29° and 49°C, cocoa butter melts between 29° and 43°C (Fig. 3).

Based on the data presented here, we are propos-

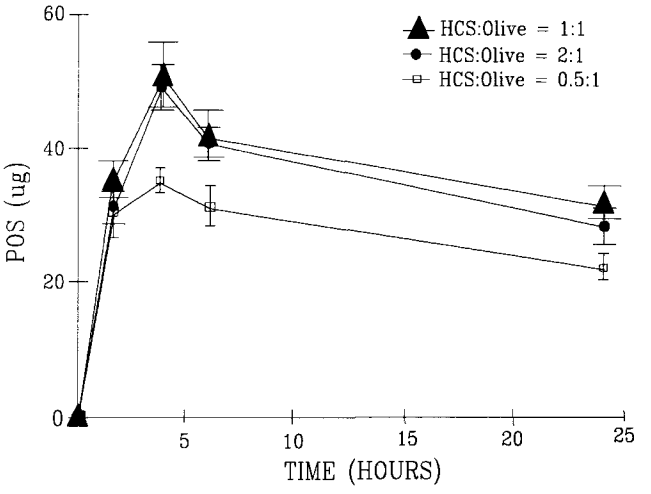

FIG. 1. Determination of optimum reaction time for producing POS. Each data point was the mean value of three runs. Error bar represented the standard deviation. Five μL of 10% reaction mixture in tetrahydrofuran were injected onto two Supelco C-18 columns. (HCS: Hydrogenated Cottonseed Oil).

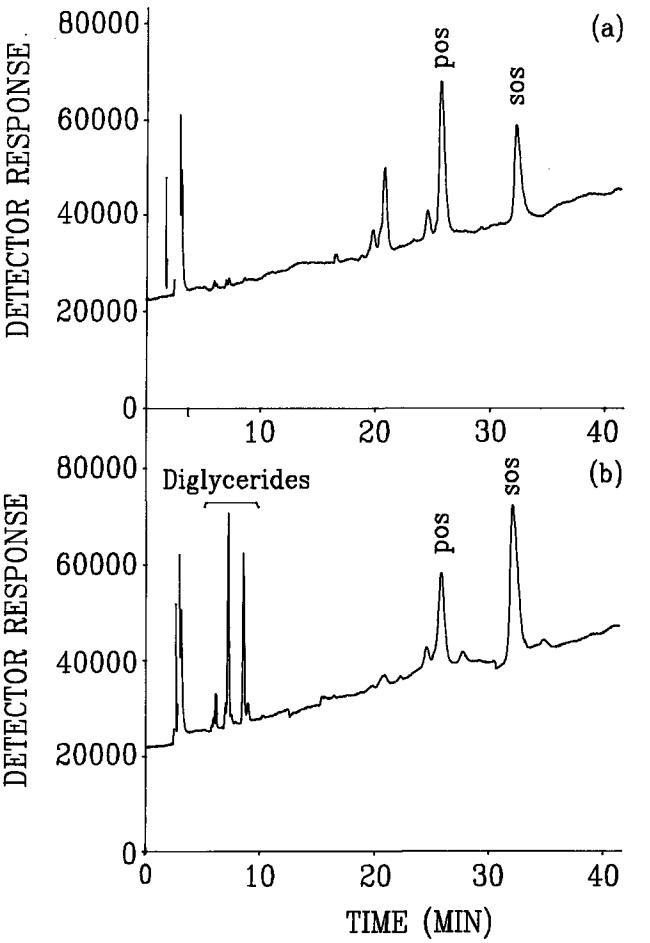

FIG. 2. HPLC chromatography of cocoa butter (a) and cocoa butter-like fat (b).

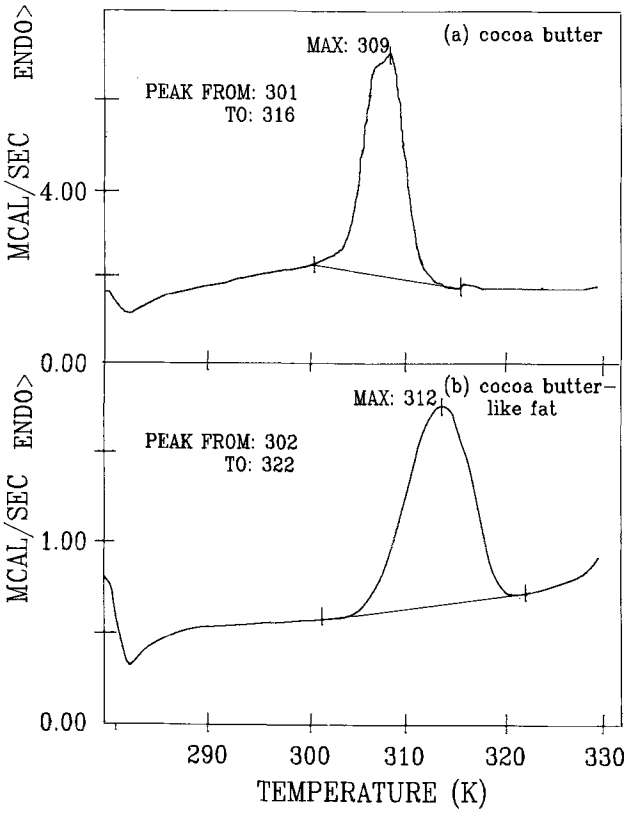

FIG. 3. DSC analysis of cocoa butter (a) and cocoa butter-like fat (b).

ing a continuous process for production of cocoa butter-like fat from enzymatically interesterifying hydrogenated cottonseed and olive oils. In this process, the two fats will be interesterified in the presence of an immobilized lipase at 70°C for 4 hr and then crystallized from acetone at two different temperatures. The first crystallization will be conducted at room temperature to remove a predominantly saturated triglyceride and a second crystallization at 4°C to produce the cocoa butter-like fat. Both unreacted hydrogenated cottonseed oil and olive oil will recycle back to the reactor. The highest temperature needed in this process to ensure the free flow of fat in both the reactor and the pipe lines that connect the first crystallizer and the reactor is 70°C. Further studies will be needed to determine whether residual acetone in the recycle streams denatures the lipase. If it does, then ways of further reducing acetone in recycle stream must be devised.

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