PREPARATION, CHARACTERISATION AND APPLICATIONS OF LIPASE-CATALYSED INTERESTERIFIED FATS AND OILS

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Introduction

Interesterification (interchange of the fatty acid molecules of oils and fats) reactions can be catalysed either by chemical/metal catalysts or enzymes (lipases). The latter has the added advantages of being, natural, biodegradable, renewable and more specific, and performs catalysis under mild conditions. Lipases catalyse a more directed reaction, forming more specific end products. Also, interesterification does not lead to the synthesis of *trans* fatty acids, usually formed during the hydrogenation (hardening) of oils. These have been implicated in adversely affecting the levels of high-density lipoproteins in the blood. Some products that can be produced using lipase-aided interesterification are margarine, shortenings, and replacement fats for processed cheese and ice cream. The objective of the project was to evaluate the interesterification performance of several lipases in the production of hard fat feedstock, low-temperature stable oils, and replacement fats from palm oil and its fractions. Studies of cell-bound lipases were also carried out.

Materials and Methods

Lipases from several microbial sources were immobilised onto Celite, lyophilised and then added to reaction mixtures composed of oil/fat substrates. The mixtures were incubated at 60^{o}C at 200 rpm for up to 6 h. At the end of reaction, the enzyme was removed and the reaction mixture were analysed to obtain the free fatty acid content, triglyceride profile, melting point, melting and cooling, and solid fat content. Except for the lformer, all other analyses were done after removal of the acids. Products like margarine, and processed cheese were formulated using blends of transesterified fats as the feedstock. These were evaluated in terms chemically, physically and sensorily when freshly prepared and after storage. A cell-bound lipase is a naturally immobilised lipase, and its application would reduce cost of immobilisation. *Aspergillus flavus* isolated locally was found to produce cell-bound lipase. A dried preparation of the lipase was used in acidolysis reaction between an oil and an exogenous fatty acid. Incorportion of the fatty acid is monitored using GC, and the properties of the altered oils were determined as before including texture analysis.

Results and Discussion

Results when palm stearin was reacted with palm kernel olein, sunflower oil or milk fat show that two lipase sources - a *Pseudomonas* sp. and *Rhizomucor miehei* - were superior to the other microbial lipases in causing changes to the stearin. Of the two, the *Pseudomonas* lipase was better, where the products of reaction were usually softer, and β' crystals were dominant. Being GRAS, the *Rhizomucor miehei* lipase (Lipozyme IM60) was used in the preparation of transesterified palm stearin: sunflower oil (40:60) mixture. The mixture was then used in a pilot plant production of margarine. Storage studies indicated that the margarine was relatively stable to oxidation and change in colour. Storage also resulted in the margarine becoming slightly firmer, making speadability less. Lower concentrations of palm stearin are recommended to obtain a softer margarine. Lipozyme was also used to produce a fat substitute for the production of processed cheese. The substrates of reaction were palm kernel olein and anhydrous milk fat. Processed cheese was prepared by incorporating known quantities of the transesterified fat into mixtures containing mature cheddar cheese and plastien casein. Sensory evaluation of the product indicated that while texture and colour were acceptable (compared to commercial processed cheese which acted as the control), the taste was found to be slightly rancid. This was due to the presence of fatty acids, a by-product of the transesterification reaction, and should be removed before the development of the cheese. The production conditions, properties and applications of the cell-bound lipase of a locally isolated strain of *Aspergillus flavus* was studied, and was the first to be reported in the literature. The enzyme is an inducible enzyme, produced only when oil was included in the growth medium. The bound enzyme could be released from the cell wall through agitation in an aqueous solution. The presence of EDTA in the extraction medium lowers losses due to proteolytic action. The soluble enzyme was found to be 1,3-specific. In the dry form, the bound lipase was sufficiently stable to allow catalysis in organic solvents. Acidolysis between coconut oil (contains mainly lauric and myristic acids and negligible quantities of stearic acid) for example, and stearic acid in the presence of the dry enzyme resulted in the formation of `coconut oil' containing significant quantities of stearic acid. The slip melting point of this product which was higher than coconut oil, and the triglyceride and melting profiles obtained from HPLC and DSC analyses reflected the incorporation of stearic acid into coconut oil. Similar `novel' products were obtained when different oils and fatty acids were used as the substrates. Transesterification reaction was also successfully carried out.

Conclusions

Lipases are capable of transforming the physical and chemical characteristics of fats and oils, and through proper selection of fats/oils mixtures, feedstocks could be prepared that are suitable for margarine and processed cheese production. Naturally immobilised cell-bound lipase can successfully be used in fats and oils transformation reactions.

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