Industrial-Scale Application of Enzymes to the Fats and Oil Industry

L.H. POSORSKE, Novo Laboratories, Inc., P.O. Box D, Wilton, CT 06897

ABSTRACT

Enzymes have advantages for industrial processing. These are: 1) specificity which permits control of the products produced and also can increase yield by reducing the amount of side products; 2) mild conditions which can decrease the cost in terms of energy and capital equipment as well as reducing the amount of unwanted side products by reducing the rate at which they are formed by virtue of the lower temperature, and 3) lowered waste treatment costs. Enzymes function well in an industrial setting, since enzymatic modification of carbohydrate or protein is an integral part of the process in a number of major industries. Enzymatic modification of lipids currently is being used commercially in the area of flavor development as well as degreasing for leather processing. Recent investigations have shown that enzymes have potential in large-scale processing of lipid material, particularly in the areas of fat splitting, synthesis by reversal of hydrolysis and interesterification.

INDUSTRIAL BENEFITS FROM ENZYMES

Enzymes are the catalysts that allow chemical reactions to occur in living things at ambient conditions. On the other hand, the same reaction in a chemical manufacturing plant might require complex reactor systems to contain the extreme conditions required. Use of industrial enzymes allows the technologist to develop processes that more closely approach the gentle, efficient processes in nature.

Three particular benefits offered by enzymes are specificity, mild conditions and reduced waste. First specificity: enzymes work to modify specific chemical bonds, usually at specific sites on a molecule, in contrast to ordinary chemical reactions that occur randomly in response to the laws of thermodynamics. It may be possible, by choosing the right enzyme, to control which products are produced. The enzyme is not catalyzing the production of other products, so side reactions are minimized. A second benefit is that enzymes react under mild conditions. As effective catalysts, however, they provide reasonable reaction rates under these conditions, in contrast to chemical reactions that may require high temperature or pressure in order to achieve satisfactory rates in a chemical process plant. Thus the plant using enzymatic reactions can be built and operated at much lower capital and energy cost.

Finally, enzyme-based processes tend to have lower waste treatment costs. Enzymes, of course, are biodegradable, and since they usually are dosed at 0.1-1.0% of the substrate, the contribution of the enzyme to the BOD in the waste stream is negligible. Due to the specificity of the enzymes, unwanted side products that normally appear in the waste stream are reduced or eliminated.

The values of these benefits have been realized by a number of industries which are currently using enzyme-based processes including the corn-based sweetener industry, the detergent industry, the fruit juice industry and the cheese industry. The corn sweetener industry uses a 3-enzyme process to convert some 8 billion pounds of corn starch into fructose sweetener every year. The process begins with solubilization of the corn starch using an amylase enzyme that functions optimally at 105 C. The next enzyme converts this solubilized starch to glucose without the product losses due to side reactions that occurred in the previously used acid hydrolysis process. Finally, glucose is converted to fructose by an immobilized glucose isomerase enzyme that functions in a packed-bed column with a catalyst life on the order of 10 mos. The entire process functions continuously (1,2).

The detergent industry relies on the specificity of enzymes to degrade the protein matrices that anchor stains to cloth (3). The enzymes perform this function in the normal 10-min wash cycle at temperatures ranging from 20-55 C, in spite of the pH as high as 11 and the high concentration of surfactant that is present in these systems.

The fruit juice industry has found that enzymes can be used in conjunction with their normal mechanical processing techniques to modify the structure of the plant cell walls and increase the release of high quality fruit juice by as much as 10% (4). This reaction occurs between 5-10 C on a time scale of 20-40 min and is used not only for apple or cranberry juice, but also in the production of fine quality wines.

The cheese industry from antiquity has relied on the specificity of enzymes to attack a single bond in the casein molecule in order to modify its functionality from a colloidial suspension to a coagulum (5). The resulting, insoluble curds can easily be separated from the whey by filtration to permit further processing into cheese.

These and many other industries have found enzymes to be a gentle and specific tool for modifying proteins and carbohydrates. Why then are enzymes not routinely used in the processing of lipid material? In the past enzymes were discounted because lipid processing systems were nonaqueous, they involved multiphasic systems and that high temperature was required just to liquify solid substrates. The previous examples illustrate that enzymes can be used in spite of these factors. The most telling reason that lipase applications have not yet been developed may simply be that carbohydrases and proteases were better understood and therefore easier to apply. But with more and more focus on lipases, the potential for application of enzymes in the fats and oils industry is increasing.

CURRENT LIPASE APPLICATIONS

In fact, enzymes have been used for some time, in specific applications for the modification of lipids. Certain fatty acids released from milk fat are crucial to the development of cheese flavors (6). The specificity of animal and microbial lipases present in the ripening cheese is responsible for the release of these fatty acids leading to the development of a good cheese flavor (5,6). Partial hydrolysis of beef tallow has been shown to improve the palatability of dog food. In a process patented by General Foods, lipases are added during the processing of dog food to modify a specific component, beef tallow, without affecting other components in the mixture (7). The result is increased attractiveness of the product for the pet. In leather processing, residual flesh and fat are removed from the hides by the process of bating. In fact, the degreasing effect is due to the presence of microbial lipases which come from microbial contamination of the bate material (8). In all of these processes the lipases originally used were provided as contaminants in some of the other materials added. However, more recently specific enzymes have been developed for enhancement of these processes.

NEW LIPASE APPLICATIONS

An exciting development is the new focus on enzymes that can be used in the mainstream of oleochemical processing. Three key areas with potential for improvement by enzymology are fat splitting for fatty acid production, lipid synthesis via reversal of hydrolysis and lipid modification by ester interchange or interesterification. While none of

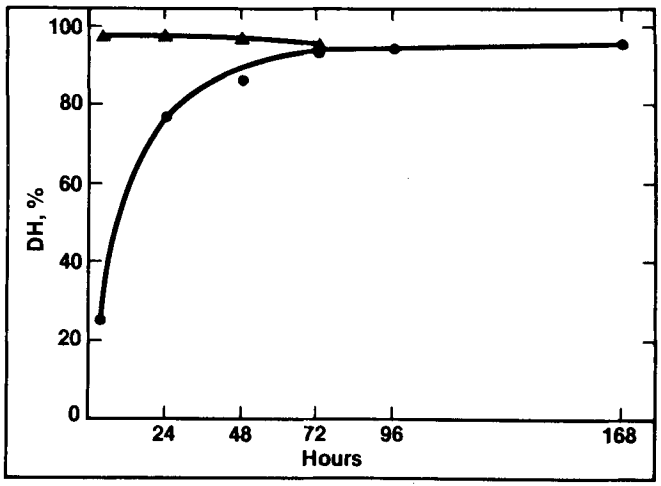

FIG. 1. Enzymatic Hydrolysis of Olive Oil. Lipases were dosed at equal activity (100 NLU/g) into a 1:1 mixture by weight of water (buffer) and olive oil. (▲) Lipase from *Candida cylindracea*, 37 C, pH = 6.0, no buffer; (●) Lipase from *Mucor miehei*, 45 C, pH = 7.5, 0.2M phosphate buffer (13).

TABLE I

Ester Synthesis by Lipase from *Rhizopus arrhizus*

Alcohol	Ester Yield (% of Theoretical Based on Acid Concentration)* Acid				
	2:0	3:0	4:0	8:0	16:0
n-Propanol	0	0	0	29.3	40.0
n-Butanol	0	0	4.9	35.6	52.6
Isopentanol	0	15.0	29.6	81.8	85.0
Iso-octanol	0	26.5	57.4	52.6	78.9

*0.1M of indicated acid dissolved in 2.5 ml indicated alcohol, shaken overnight at 28 C with 50 mg *R. arrhizus* mycelia, and analyzed by titration of free acid.

these applications is yet commercial, each currently is the focus of intensive investigation.

The standard technology for fatty acid production is high temperature and pressure countercurrent steam splitting (9). Alternative chemical technologies involve ambient pressure saponification or chemically catalyzed hydrolysis. Recent patents and publications (10-13) have demonstrated that this reaction can be accomplished by means of a lipolytic enzyme. In this case, the reaction goes at ambient pressure and a relatively low temperature on the order of 40-60 C resulting in much lower energy costs. Fatty acids are much less corrosive under the conditions of enzymatic fat-splitting than under the conditions of the traditional processes (9). Therefore, the reaction vessels are much less expensive. In addition, because of both the mild conditions and the high specificity of the enzyme, the enzymatic system can be used to split fats containing much more reactive fatty acids, such as those high in unsaturation or with attached hydroxyl groups. The enzymes used for this process optimally should show no specificity, either for the fatty acid chain or for the position on the glycerol molecule to which the fatty acid is attached.

Figure 1 shows a comparison of enzymes from 2 different microbial sources in a model system: olive oil splitting (13). This system used an equal weight mixture of olive oil and water containing enzyme. The lipase from *Candida cylindracea* shows no specificity with respect to position or chain length (14). The lipase from *Mucor miehei* is specific for the 1,3 positions or the primary hydroxyls of the glycerol molecule (13). While both enzymes can achieve comparable hydrolysis over a long time period, it is obvious that the *Candida* enzyme is capable of a much more rapid hydrolysis of the triglyceride. In fact, within 4 hrs it was able to accomplish 98% hydrolysis. In this model system, enzyme hydrolysis achieved results approaching those seen in chemical hydrolysis systems, but under much, much milder reaction conditions.

Another area of potential application of enzymes is the reversal of the hydrolysis reaction, in order to synthesize esters (15). In this case, by decreasing the water activity it is possible to pull the reaction in the direction of the esterification. While ester synthesis certainly can be done chemically with acid or base catalysis, there are definite advantages to the use of enzyme technology. The reaction can be run at ambient temperature and pressure with the use of a natural catalyst. The specificity of the enzyme under

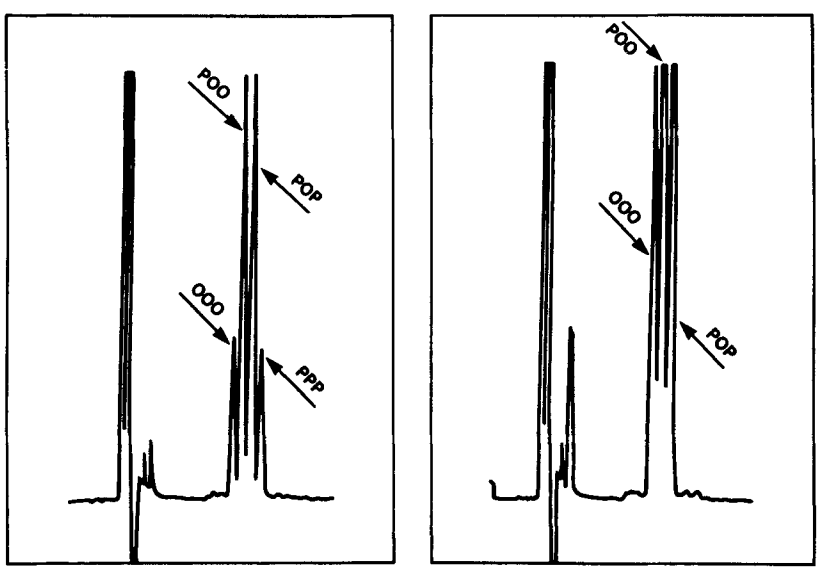

FIG. 2. High Pressure Liquid Chromatography (HPLC) of triglycerides interesterified by lipases with different specificities. 400 mg triolein, 400 mg palmitic acid and 12 ml petroleum ether incubated for 3 hr with 250 mg of immobilized lipase from a) *Candida cylindracea* or b) *Mucor miehei*. After 3 hr at 40 C, triglycerides were analyzed by HPLC on a reversed phase (RP 18) column (13).

these mild conditions will limit side reactions, and by choice of enzyme and raw materials it is possible to produce very specific products. In addition, recent reports indicate that high yields are possible.

For example, an enzyme from *Rhizopus arrhizus* was used to esterify acids of various chain lengths with various alcohols which also were used as the solvent (Table I) (15). The *Rhizopus* enzyme is specific for long chain fatty acids, and in the case of 8 to 16 carbon fatty acids it was possible to obtain over 80% yield of ester in this non-aqueous system.

Another area for potential enzyme application described in numerous patents and publications (16-18) is interesterification. In this case the aim is to exchange one of the fatty acids on the triglyceride molecule for another fatty acid which is present in the reaction medium in order to produce a new triglyceride with properties of greater commercial interest. While ester interchange can be catalyzed chemically, the result of chemical interesterification is simply to randomize the entire fatty acid pool at each position on the triglyceride (9). In contrast, enzymatic interesterification may be done using enzymes specific for either fatty acid chain length or position, resulting in much more control over the final triglyceride product (18). The reaction can be performed under moderate reaction conditions, but because of the efficiency of the enzyme catalyst, it can still proceed at reaction rates that are acceptably rapid with regard to industrial processing time scales. The use of moderate reaction conditions should decrease any problems with side reactions of the fatty acid chains. One other advantage of the enzyme catalized process is the potential for immobilized enzymes which allow continuous processing rather than the batch processing used with chemical interesterification (18).

Figure 2 shows the benefit of using enzymes of different specificity (13). The reaction is the interesterification of triolein with palmitic acid. The enzyme catalyst is the lipase from *Candida cylindracea* in one case and from *Mucor miehei* in the other case. HPLC analysis of the reaction mix after 3 hrs from the *C. cylindracea*-catalyzed system shows the reactant triglyceride plus 3 different product triglycerides. In the case of the *M. miehei* lipase, which is specific for the primary positions, HPLC of the reaction mix shows the reactant triglyceride, triolein, and only 2 peaks for product triglycerides. Since the enzyme will not react with the secondary hydroxyl group the tripalmitin product is not produced.

The success of the research work described above, coupled with a history of proven benefits of enzyme processing in other industries, clearly demonstrates a bright future for enzymes in the fats and oils industry.

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