ISSN:0128-
Pertanika J. Sci. & Technol. 3(2): 383-388 (1995)

COMMUNICATION I
Partial Glycerides Synthesis by Lipase of *Aspergillus niger*

Received 28 July 1993

ABSTRAK

Lipase daripada *Aspergillus niger* telah dikaji untuk sintesis gliserida separa. Darjah sintesis yang bererti melebihi 50% telah dicapai menggunakan laurate sebagai salah satu daripada substrat. Darjah ini dapat diperbaiki lagi dengan mengoptimumkan keadaan tindakbalas. Darjah sintesis yang menghampiri 80% telah diperoleh dengan kehadiran 3.0 g penapis molekul dalam sistem tindakbalas untuk penjerapan air.

ABSTRACT

The lipase of *Aspergillus niger* was examined for partial glycerides synthesis. A significant degree of synthesis (more than 50%) was achieved using laurate as one of the substrates. This can be further improved by optimizing the reaction conditions. A synthesis degree of almost 80% was obtained in the presence of 3.0 g of molecular sieve pellets for water absorption in the reaction system.

INTRODUCTION

In Malaysia, partial glycerides are in great demand for use in food and pharmaceutical industries and also in polymer industries. These glycerides are presently produced using chemical methods under high temperature (170-220°C) and pressure (3-5 x 10^{-3} mmHg) in the presence of a chemical catalyst such as sodium methoxide. Nevertheless, the products normally contain high impurities with low recovery yield. A milder alternative requires the use of lipases, but such a process is still not available in Malaysia. Lipases have been used in partial glycerides and esters synthesis (Ibrahim *et al.* 1987; Ibrahim and Tan 1991), monoglyceride production (Ibrahim *et al.* 1989), fatty acid production (Ibrahim and Nagai 1989) and the production of other value-added products (Ibrahim *et al.* 1988a). With the aim of introducing the application of lipase in the palm oil industry, we have isolated *Aspergillus niger* SBS K020 as a potential producer of exogenous lipase. The production of the enzyme by fermentation processes by both the free and immobilized cells has already been reported (Ibrahim *et al.* 1991; Ibrahim and Noor Izani 1991; Noor Izani and Ibrahim 1992). In this paper, some of the characteristics and important parameters governing the synthesis of partial glycerides by *A. niger* lipase are described.

MATERIALS AND METHODS

The lipase used throughout the experiment was obtained from *A. niger* grown in a palm oil medium as described previously (Ibrahim and Noor

Izani 1991). The crude powder preparation contained 4400 U/g of activity determined by the method of Ibrahim and Noor Izani (1991) using polyvinyl alcohol-refined, bleached, deodorized (RBD) palm oil as the substrate. The enzyme was then used for the partial glycerides synthesis using a basal reaction mixture of glycerol (4 ml, 75%, v/v), fatty acid (0.3 g) and lipase solution (1.0 ml, 4 U) based on the method of Tsujisaka *et al.* (1977). The reaction mixture was placed in a 50-ml conical flask with a silicone cap and incubated for 5 h at 50°C. After the reaction, the degree of synthesis was calculated from the fatty acid consumed in the synthetic reaction by the titration method (Ibrahim *et al.* 1987).

RESULTS AND DISCUSSION

Fig. 1 shows the substrate specificity profiles of *A. niger* lipase for glyceride synthesis. The enzyme exhibited good specificity with laurate (C12) giving a maximum degree of synthesis of 53% after about 20 h. A lower degree of synthesis was observed as the molecular weight of the acid used was increased. Similarly, the degree of synthesis dropped with increasing unsaturation in the fatty acid. Thus the degree of glyceride synthesis by the lipase can be arranged in the order of C12 (laurate) > C14 (myristate) > C18:1 (oleate) > C18:2 (linoleate).

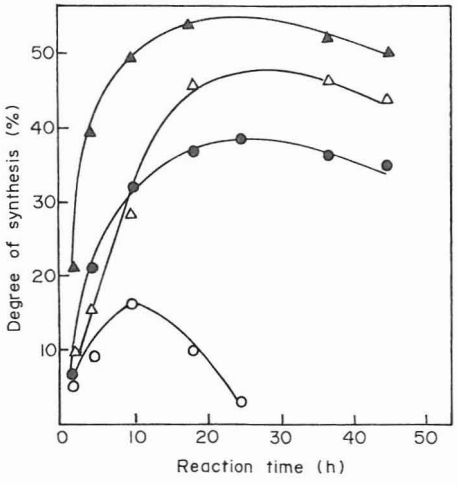

Fig. 1. Substrate specificity profiles of lipase from Aspergillus niger *for glyceride synthesis (▲) laurate; (△) myristate; (●) oleate; (○) linoleate*

Some governing parameters on the formation of glycerides from laurate are shown in *Fig. 2.* The optimum temperature was between 35 to 40°C. Although the optimum temperature of hydrolysis was 50°C (deter-

mined using RBD palm oil), the lower temperature for synthesis indicates that the physical properties of the reactants might play significant roles. In hydrolysis, a high temperature is needed to liquefy the solid lipid substrate. A maximum degree of synthesis (57%) was obtained with a glycerol concentration of 80% in the reaction mixture. Lower synthesis degree was obtained when less than or higher than 80% concentration of the glycerol was used. Yamane *et al.* (1986) demonstrated that glycerol plays two important roles in the reaction system, i.e., as a matrix for lipase and being hydrophilic in nature, acts as an effective agent for water control. These roles, as suggested by Yamane *et al.* (1986), were also observed in the synthesis reaction by *A. niger* lipase (*Fig. 2*). At a high glycerol concentration, the water concentration is reduced, consequently increasing the enzyme synthetic activity. On the other hand, a low glycerol concentration is associated with a high water concentration. Under conditions of high water concentration, the equilibrium of the lipase-catalysed reaction was shifted towards hydrolysis on the formed glycerides. At a glycerol concentration of 80%, an equilibrium between the synthesis and hydrolysis reaction was achieved. In the case of fatty acid, 0.4 g was found to be optimum. Higher acid concentration may be inhibitory to the enzyme (Loh and Ibrahim 1991). In the same figure, an enzyme concentration of 8 U/ml was optimal for maximum synthesis.

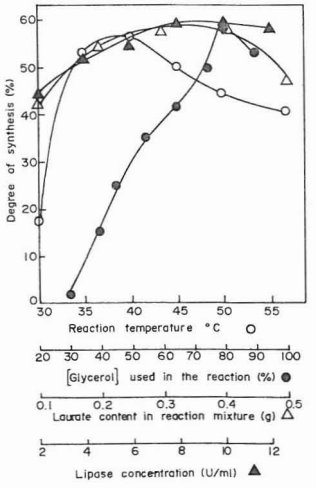

Fig. 2. Governing parameters on the degree of glyceride synthesis

Using the optimized conditions described in *Fig. 2*, the synthesis of partial glycerides in the presence of protective compounds or stabilizers for the enzyme, such as polyvinyl alcohol, albumin, casein and calcium

chloride, was investigated. The results obtained indicated that the catalytic activity of the *A. niger* lipase was not improved in the presence of these compounds (data not shown). Tsujisaka *et al.* (1977) reported that these compounds acted as stabilizers to the lipases from *Rhizopus delemar, Aspergillus niger, Geotrichum candidum* and *Penicillium cyclopium* which enhanced the synthesis of glycerides.

Based on the parameters described above, it is clearly shown that the water concentration is the most crucial factor which determines the equilibrium of a lipase-catalysed reaction. Ibrahim *et al.* (1988b) have demonstrated that molecular sieve pellets can effectively control the water concentration in the reaction mixture for the synthesis of geranyl laurate. The use of molecular sieve pellets as water absorbents (Fluka, Union Carbide, Type 4 Å) for the glyceride synthesis was thus examined. Compared to the system without the addition of molecular sieve pellets, the degree of synthesis increased rapidly with the addition of more pellets.

Water which was produced as a by-product of the reaction was simultaneously absorbed by the pellets, resulting in a shift in the equilibrium towards the synthetic reaction. A maximun of almost 80% degree of synthesis was obtained after 30 h when 3.0 g of molecular sieve pellets were added (*Fig. 3*).

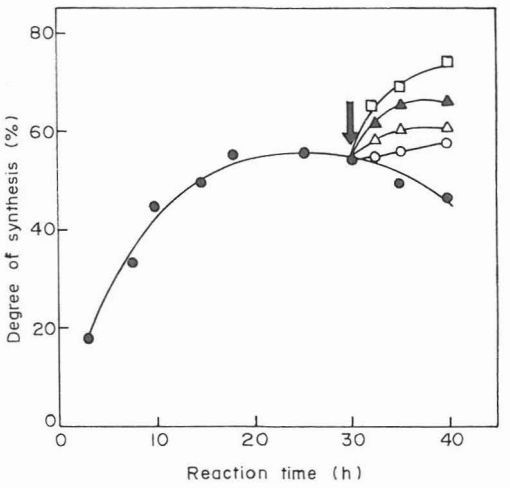

Fig. 3. Effect of molecular sieve pellets as water adsorbents in the reaction system; molecular sieve pellets (g) added at (○) 0.5, (△) 1.0, (▲) 2.0 and (□) 3.0. Arrow indicates the time of addition

Product formations were qualitatively reconfirmed on thin layer chromatography (TLC) using the preparation method and solvent system described previously (Ibrahim *et al.* 1987). From such experiments, synthe-

sis of lauric acid glycerides appeared as spots on the TLC plates (data not shown). The intensity of laurate spot decreased with a corresponding increase in the spot intensity for trilaurin and dilaurin. Nevertheless, monolaurin was not detected, indicating that the rapid synthetic reaction took place without the formation of a monoglyceride intermediate.

CONCLUSION

This short communication demonstrates that the lipase from a newly isolated strain of *A. niger* can be used in the synthesis of glycerides of fatty acids. The degree of glycerides formation can be improved by optimizing several reaction parameters such as the temperature and the concentration of glycerol, acid, and enzyme and also by reducing the water concentration in the reaction mixture.

Although Tsujisaka *et al.* (1977) have reported the synthesis of glycerides by a strain of *A. niger*, comparative studies between the lipase performance of their strain and the new strain that we have isolated is not totally possible. In both cases, reaction conditions with respect to the substrate and enzyme concentration, reaction temperature and stabilizer requirements are different. Nevertheless, under their respective optimum conditions, the performance of the two lipases is almost similar.

H.L. CHIN and IBRAHIM CHE OMAR

School of Biological Sceines
Universiti Sains Malaysia
11800 Minden, Penang, Malaysia

REFERENCES

IBRAHIM, C.O. and S. NAGAI. 1989. Hydrolysis of fats and oil in an immobilised lipase· continuous stirred tank reactor (CSTR). *Sains Malaysiana* **18**: 115-127.

IBRAHIM, C.O., N. NISHIO and S. NAGAI. 1987. Fat hydrolysis and esterification by a lipase from *Humicola lanuginosa. Agricultural Biological Chemistry* **51**: 2153-2159.

IBRAHIM, C.O., N. NISHIO and S. NAGAI. 1988a. Acidolysis reaction by a thermostable lipase from *Humicola lanuginosa. Agricultural Biological Chemistry* **52**: 2923-2925.

IBRAHIM, C.O., N. NISHIO and S. NAGAI. 1988b. The role of water on the equilibrium of esterification by immobilized lipase packed-bed column reactor. *Biotechnology Letters* **10**: 799-804.

IBRAHIM, C.O. and N.J. NOOR IZANI. 1991. Production of an exogenous lipase by *Aspergillus niger* grown on a palm oil medium. *Journal of Bioscience* **1**: 15-28.

IBRAHIM, C.O., N.J. NOOR IZANI and I. DARAH. 1991. Isolation and identification of exogenous lipase producing fungi using palm oil medium. *Journal of Bioscience* **1**: 59-69.

IBRAHIM, C.O., H. SAEKI, N. NISHIO and S. NAGAI. 1989. Synthesis of acetone glycerol acyl esters by immobilized lipase of *Mucor miehei. Biotechnology Letters* **11**: 161-166.

IBRAHIM, C.O. and H.L. TAN. 1991. Esterification by lipase of *Candida cylindracea* with fatty acid distillates (FAD) as acyl donors. *Journal of Malaysian Applied Biology* **20**: 11-23.

LOH, Y.S. and C.O. IBRAHIM. 1991. Hydrolysis of palm oil by calcium-alginate entrapped lipase of *Candida cylindracea*. *Journal of Bioscience* **2**: 47-58.

NOOR IZANI, N.J. and C.O. IBRAHIM. 1992. Lipase production using immobilized *Aspergillus niger*. *Journal of Bioscience* **3**: 14-24.

TSUJISAKA, Y., S. OKUMURA and M. IWAI. 1977. Glyceride synthesis by four kinds of microbial lipases. *Biochimica Biophysica Acta* **489**: 415-422.

YAMANE, T., M.M. HOQ, S. ITOH and S. SHIMIDZU. 1986. Glycerolysis of fat by lipase. *Journal of Japan Oil and Chemical Society* **35**: 45-51.