ПЛОВДИВСКИ УНИВЕРСИТЕТ „ПАИСИЙ ХИЛЕНДАРСКИ“ – БЪЛГАРИЯ
НАУЧНИ ТРУДОВЕ, ТОМ 34, КН. 5, 2006 – ХИМИЯ
UNIVERSITY OF PLOVDIV „PAISII HILENDARSKI“ – BULGARIA
SCIENTIFIC PAPERS, VOL. 34, BOOK 5, 2006 – CHEMISTRY

GLYCEROLYSIS OF OLEIC ACID BY *CANDIDA RUGOSA* LIPASE IN ORGANIC SOLVENTS

Yesim Yesiloglu[1], Ismail Kilic[2]
[1] Department of Chemistry, Trakya University, 22030 Edirne-TURKEY
[2] Education Faculty, Trakya University, 22030 Edirne-TURKEY

ABSTRACT

Glycerolysis or selective hydrolysis of fats and oils, esterification of fatty acids or esters with glycerol, and reactions employing protected glycerols are presented. Monoglycerides (MG) and diglycerides (DG) are the most widely used emulsifiers in food and pharmaceutical industries. In the enzymatic synthesis of glycerides from glycerol and oleic acid in organic solvent was studied, and the optimal conditions for glyceride synthesis by *Candida rugosa* lipase were established. The water content, glycerol content, and temperature were determined as 5%, 2 g, and 30°C, respectively. Isooctane and hexane were particularly useful organic solvents in glyceride synthesis.

Keywords: Candida rugosa lipase, glycerolysis, organic solvent.

INTRODUCTION

Monoglycerides (MG) and diglycerides (DG) are the most widely used emulsifiers in food and pharmaceutical industries [1-4]. Current processes for MG and DG production consist on the inter-esterification of triglycerides (TG) with glycerol (glycerolysis) in the presence of nonselective inorganic catalysts at high temperatures (200-250°C) [5].

The replacement of inorganic catalysts by lipases (E.C. 3.1.1.3), in the synthesis of partial glycerides, avoids side product formation and is less polluting and energy consuming because of the mild conditions used. For the bioconversion of various lipophilic or water-insoluble compounds, it is essential to introduce organic solvents into reaction systems to improve the solubility of these reactants. Furthermore, use of organic solvents is beneficial for construction of homogeneous reaction systems and to facilitate continuous reactor processes. The use of lipolytic enzymes to catalyze the esterification reaction has been investigated by many workers [6,7].

In this study, the synthesis of glycerides from glycerol and oleic acid in organic solvent by *Candida rugosa* lipase (CRL) was studied, and the effects of process parameters, such as organic solvents, water content, glycerol content, and temperature were investigated.

EXPERIMENTAL PROCEDURES

Esterification reaction

Reaction mixtures for glyceride synthesis from glycerol and oleic acid consisted of: glycerol, 2 g (about 20 mM); oleic acid, 0.5 g (about 2 mM); water 150 μL; and *n*-hexane, 3 mL. Reaction mixtures were placed in 50 mL The reaction was started by the addition of 0.1 g lipase in the form of dry powder. The suspension that resulted was agitated on an orbital shaker at 200 rpm at 30°C. At various times during incubation 0.2 mL of the reaction mixture was withdrawn and analyzed by TLC and GC.

Estimation of the degree of synthesis

The reaction was stopped by addition of 20 mL of an acetone/ethanol mixture (1:1, vol/vol), and FFA was titrated with 0.1 N NaOH. The degree of synthesis (%) represents the percentage of initial FA consumed in the reaction mixture.

Identification of reaction products

Reaction products were extracted from each reaction mixture with diethyl ether and identified by TLC. A silica gel plate was prepared and developed in chloroform/acetone/methanol (95:4.5:0.5, by vol). Spots of each lipid were visualized by spraying the plate with a 12.5% (wt/vol) ethanol solution of phosphomolybdic acid (Sigma Chemical Co.) and heating it. Fractions corresponding to each lipid type were scraped from the plates and derivatized for GC analysis.

GC analysis

Each lipid class separated by TLC was methylated. The methyl esters of FA were dissolved in hexane for analysis. Analysis was performed in a gas chromatography (Shimadzu model 14A:Shimadzu, Tokyo, Japan).

RESULTS AND DISCUSSION

Effect of solvent type

The glycerolysis of oleic acid by CRL was carried out in acetone, chloroform, benzene, decane, isooctane, heptane and hexane. The results are shown in Table 1. It was found that isooctane gave higher yield than benzene and chloroform. The use of organic solvents can improve the poor solubility in water of substrates or other reaction components of a hydrophobic nature. Organic solvents produce various physicochemical effects on enzyme molecules, and the effects differ depending on the kinds of organic solvents and enzymes used. With CRL, high activities (75.3-85.7 % synthesis) were observed in heptane, hexane, and isooctane. More polar solvents, such as benzene, chloroform, and acetone, were found to be unsuitable for the synthetic reaction.

Effect of water content

In the esterification reaction, the content of water in the reaction mixture affects the reaction because water is one of the reaction products. A small amount of water is needed to maintain enzyme activity. Initial water content in the range 0-20% (w/w) of glycerol was investigated for glycerides production. The highest yield of glyceride (81%) was obtained when the water in glycerol was 5%. The conversion reached 92% by the addition of 1.0 g of molecular sieve. However, at higher than 10% water in glycerol, the yield of glyceride dropped gradually. Such drop might be due to hydrolysis occur at high water content. The time courses of the glyceride synthesis by CRL in hexane containing different initial moisture contents are illustrated in Figure 1. As expected, the percentage of conversion decreased as the initial water content increased.

Effect of glycerol content

In order to select an efficient glycerol concentration for glycerolysis, the effect of glycerol concentration in organic solvents on glyceride production was investigated. The results are shown in Table 2. The glyceride yield increased with increasing glycerol content. For *Candida rugosa* lipase, glycerol content of 2 g was optimal and the reaction resulted in a glyceride synthesis of 85.5%.

Effect of temperature

The effect of temperature on glyceride production was studied. The temperature was controlled at 0-40°C for glyceride production. When the temperature was controlled in the range 0-40°C the glyceride production increased with increasing temperature. This result is a consequence of the increase in the reaction rate as the temperature increases. In contrast, when increasing the temperature from 40 to 50°C the yield of glyceride was decreased (Table 3). The optimal temperature of enzyme was 40°C (Table 3).

Production of glycerides under reaction conditions

Changes in the composition of lipid in the *n*-hexane mixture during the course of the esterification reaction by lipase are detailed in Figure 2. Initially, almost equimolar MG and DG were produced as FA decreased. The formation of TG was much lower than that of MG and DG. At 24 h, the concentration of MG, DG, and TG reached 22.3, 39.6 and 17.1%, respectively, for *C. rugosa* lipase.

From the above results, it can be concluded that glycerides from glycerol and oleic acid may be synthesized easily. No lipase is available with as broad a range of specificity as attributed to *C. rugosa* lipase.

Table 1. *Effect of organic solvents on glyceride production by C. rugosa lipase.*

Organic Solvent	Degree of Synthesis (%)
	Candida rugosa lipase
Hexane	82.5
Heptane	75.3
Isooctane	85.7
Decane	60.4
Benzene	20.1
Chloroform	22.8
Acetone	31.9

Table 2. *Effects of Glycerol Content on glyceride production by C. rugosa lipase.*

Glycerol (g)	Degree of Synthesis (%)
	Candida rugosa lipase
0.25	13.5
0.50	56.2
1.0	80.3
2.0	85.5
5.0	85.0

Table 3. *Effects of temperature on glyceride production by C. rugosa lipase.*

Temperature(°C)	Degree of Synthesis (%)
	Candida rugosa lipase
0	9.3
10	18.1
20	59.7
30	85.8
40	88.2
50	41

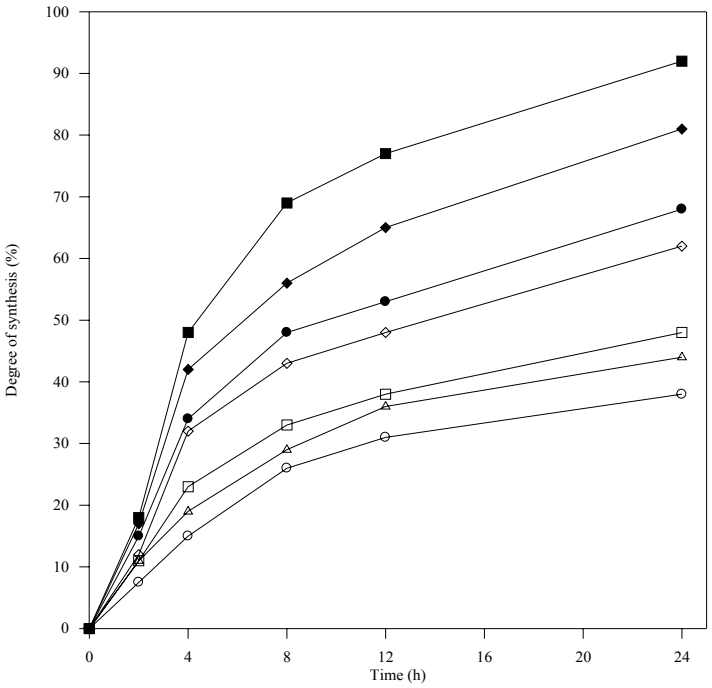

Figure 1. *Effects of water content on glyceride synthesis by C. rugosa lipase. The amount of enzyme used was 0.1 g. The reaction was carried out at 30°C. (◊) 0% H_2O; (○): 1% H_2O; (●): 2.5% H_2O; (♦): 5% H_2O; (■): 5%H_2O+molecular sieve; (□): 10% H_2O; (Δ): 20% H_2O.*

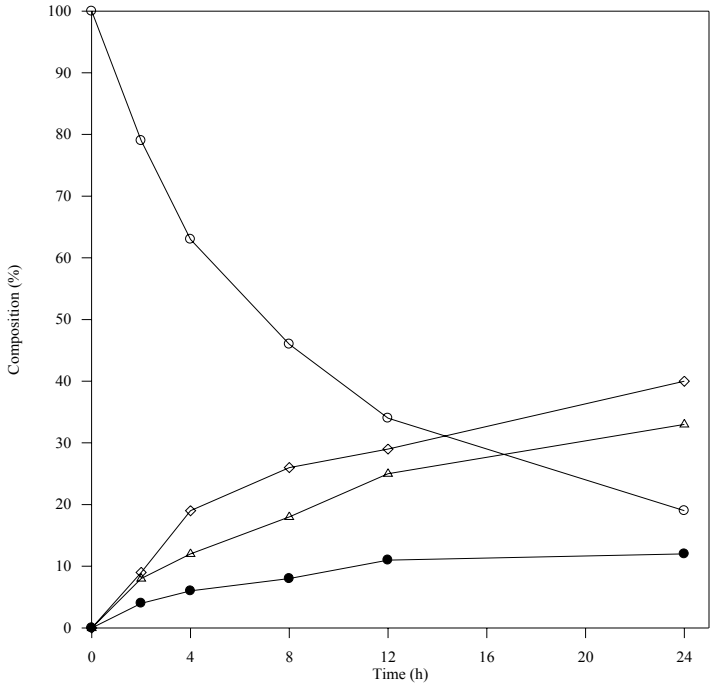

Figure 2. *Composition of glycerides synthesized by C. rugosa lipase. The reaction mixture contained 2.0 g glycerol, 0.5 g of oleic acid, 3.0 mL n-hexane and various amounts of water. The amount of enzyme used was 0.1 g. The reaction was carried out at 30°C. (Δ): MG; (◊): DG; (●): TG; (○): oleic acid.*

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