LETTER TO THE EDITOR

Low-Temperature Chemical Glycerolysis: An Evaluation of Substrates Miscibility on Reaction Rate

Nanjing Zhong · Bing Li · Xuebing Xu ·
Ling-Zhi Cheong · Zhenbo Xu · Lin Li

Dear Sir,

Partial acylglycerols namely monoacylglycerol (MAG) and diacylglycerol (DAG) are widely used in the food, pharmaceutical and cosmetic industries. They can be obtained by hydrolysis of triacylglycerol (TAG), esterification of glycerol and fatty acids (FA) or glycerolysis of TAG [1, 2]. Chemical glycerolysis, usually conducted at high temperature (220–260 °C), is one of the commonly used methods in industrial production of partial acylglycerols. The high temperature in chemical glycerolysis is deteriorative for highly unsaturated fats with respect to oxidation. Thus, the possibility of partial acylglycerols production through low temperature chemical glycerolysis will offer a favorable alternative to the fats and oils industry.

One of the main governing factors of glycerolysis is substrate miscibility. Substrates for glycerolysis, namely TAG and glycerol, are immiscible especially at low temperatures. It is well reported in literature that substrate immiscibility will lead to a lower mass transfer rate and subsequently reaction rate. To improve substrate miscibility, solvents are often introduced into the reaction system.

N. Zhong · B. Li · Z. Xu · L. Li (✉)
College of Light Industry and Food Science,
South China University of Technology, Guangzhou,
People's Republic of China
e-mail: felinli@scut.edu.cn

N. Zhong
School of Food Science, Guangdong Pharmaceutical University,
Zhongshan, China

X. Xu · L.-Z. Cheong
Department of Molecular Biology, Aarhus University,
Gustav Wieds Vej 10, 8000 Aarhus C, Denmark

Solvents with both hydrophilic and hydrophobic structures like *tert*-butanol (TB) or *tert*-pentanol (TP) have been reported to be efficient in improving substrate miscibility in glycerolysis [2–6]. The present study investigates the use of solvents namely isopropanol (IP), TB and TP to improve substrate miscibility in low temperature chemical glycerolysis for partial acylglycerols production.

Refined, bleached and deodorized (RBD) soybean oil was kindly provided by Kerry Oleochemical Industrial Co., Ltd (Shanghai, China). Glycerol with a purity of more than 99.0% was purchased from the Guangzhou Chemical Reagent Factory (Guangzhou, China). Sodium hydroxide (NaOH) from the Chengdu Chemical Reagent Factory (Chengdu, China) was of analytical grades. The standards of 1-monoolein, 1,3-diolein and triolein (>99.0%) were from Sigma (St. Louis, MO). All other solvents and reagents were of analytical or chromatographic grades.

The ability of the three different solvents in improving substrate miscibility was studied by mixing (using a stirrer at 600 rpm) the substrates consisting of soybean oil and glycerol at the same ratio as the reaction blends in a cuvette for 5 min. After mixing, the mixtures were allowed to stand for 24 h at two different temperatures, namely 15 and 50 °C, and analyzed for homogeneity using laser diffraction particle size analysis (Mastersizer 2000, Malvern, UK). For the reaction substrates analyzed, the corresponding pure solvent was first used for baseline correction before the reaction mixtures (after 24 h standing) were analyzed. If the signal remains in the baseline for the reaction mixtures, it indicates that the mixture is in homogenous state, and otherwise the heterogeneous state.

Low temperature chemical glycerolysis was then conducted to examine the effect of substrate miscibility on the reaction rate. First, reaction mixtures consisting of triolein (1 mmol), glycerol (5 mmol), NaOH (0.4 wt%) and solvent

(300 wt% of triolein) were incubated in 50-mL capped flasks at a temperature of 45 °C. Unless otherwise stated, the reaction mixtures were stirred at a rate of 600 rpm. Samples were withdrawn at predetermined time intervals for analysis of the lipid profile. The lipid profile was analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) according to a method described by Holčapek [7]. The chromatographic separation was carried out using a Purospher® STAR RP-18e column (250 × 4.6 mm i.d., particle size 5 μm) at a gradient elution with UV detection at 205 nm. The amounts of monoolein, diolein and triolein were calculated using the calibration curves of the standards. Other chromatographic conditions were as previously described [7]. Double determinations were performed and the absolute standard deviations were calculated. The initial reaction rate v was calculated according to the following equation:

$$v = -\frac{C_t - C_0}{t}$$

Where C_t is the triolein concentration at time t, C_0 is the initial triolein concentration. The t value selection should be within the time when reverse reaction has not proceeded.

Interesting findings were observed in the present study. Of the three different solvents, TP was found to be the best solvent to improve miscibility of TAG and glycerol followed by TB and IP (Table 1). If the reaction rate was controlled by the substrate miscibility and diffusion of glycerol and TAG, the reaction rates should increase with similar order whereby

$$v_{TP} > v_{TB} > v_{IP}$$

Nevertheless, our findings showed otherwise. The time courses of triolein conversion are presented in Fig. 1. The highest initial reaction rate was found in the IP (0.17 mg/mL/min) system, followed by the TB (0.13 mg/mL/min) and TP (0.038 mg/mL/min) solvent systems. This indicates that low temperature chemical glycerolysis was not governed completely by substrate miscibility. Substrate

Table 1 The state of substrates in solvent systems[a]

Solvent	15 °C	50 °C
IP	Phase separated quickly	Phase separated quickly
TB	Phase separated after 15 min duration	Homogeneous state
TP	Homogeneous state	Homogeneous state

[a] The substrates consisted of soybean oil and glycerol at the same ratio as the reaction blends

The homogeneous state was confirmed by laser diffraction particle size analysis; no signals were observed when it was in a homogeneous state
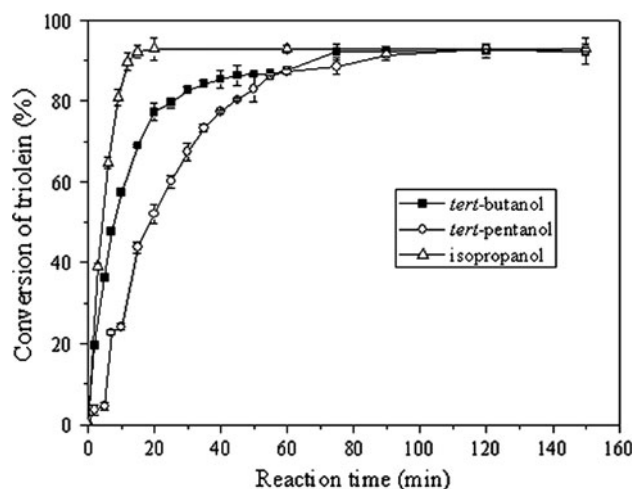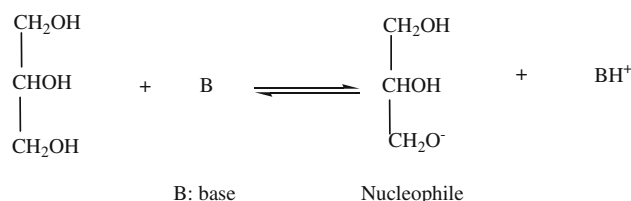
Fig. 1 Time courses of triolein conversion in TB, TP and IP solvent systems. Reaction conditions: triolein 1 mmol, glycerol 5 mmol, NaOH 0.4 wt% of triolein, solvent amount 300 wt% of triolein, agitation speed 600 rpm and reaction temperature 45 °C

miscibility and a homogeneous state were not the critical factors in enhancing the reaction rate. To further support this finding, the effect of agitation speeds (200, 400, 600 and 800 rpm) on the reaction rate was studied. Theoretically, a higher agitation speed will improve substrate miscibility and subsequently the reaction rate. Nevertheless, it was found that reaction rate remained constant and unchanged with increases in agitation speed. Thus, substrate miscibility and homogeneity may not be the key determining factor of the reaction rate in low temperature chemical glycerolysis.

Instead of substrate miscibility, the authors postulate that the solubility of NaOH, which was the reaction catalyst, as being the key factor affecting reaction rate. The solubility of NaOH in the solvents was determined by mixing NaOH with the solvent added drop by drop at 30 °C, according to Chen et al. [8]. Indeed, it was found that NaOH had the highest solubility in IP (0.205 g NaOH/100 g IP) followed by TB (0.0124 g NaOH/100 g TB) and TP (limited solubility). Thus, based on the chemical glycerolysis reaction mechanism [9, 10], the rate-limiting step of the glycerolysis reaction would be the following:

This deduction was partially supported by the fact that methanolysis of the TAG reaction was fast, without any other solvents. Methanol can dissolve NaOH and glycerol

well, though difficult to dissolve TAG because of its high polarity.

In short, the present study found that substrate miscibility is not the only key factor in determining the reaction rate of low temperature chemical glycerolysis. Solubility of the chemical catalysts plays an important role in affecting reaction rate of low temperature chemical glycerolysis. Thus, selection of the appropriate solvent in low temperature chemical glycerolysis should be based on both substrate miscibility and the solubility of the chemical catalysts.

References

1. Bornscheuer UT (1995) Lipase-catalyzed syntheses of monoacylglycerols. Enzyme Microb Technol 17:578–586
2. Damstrup ML, Jensen T, Sparsø FV, Kiil SZ, Jensen AD, Xu X (2005) Solvent optimization for efficient enzymatic monoacylglycerol production based on a glycerolysis reaction. J Am Oil Chem Soc 82:559–564
3. Damstrup ML, Abildskov J, Kiil S, Jensen AD, Sparsø FV, Xu X (2006) Evaluation of binary solvent mixtures for efficient monoacylglycerol production by continuous enzymatic glycerolysis. J Agric Food Chem 54:7113–7119
4. Damstrup ML, Jensen T, Sparsø FV, Kiil SZ, Jensen AD, Xu X (2006) Production of heat-sensitive monoacylglycerols by enzymatic glycerolysis in *tert*-pentanol: process optimization by response surface methodology. J Am Oil Chem Soc 83:27–33
5. Zhong N, Li L, Xu X, Cheong L-Z, Li B, Hu S, Zhao X (2009) An efficient binary solvent mixture for monoacylglycerol synthesis by enzymatic glycerolysis. J Am Oil Chem Soc 86:783–789
6. Tanaka R, Hirose S, Hatakeyama H (2008) Preparation and characterization of polyurethane foams using a palm oil-based polyol. Bioresource Technol 99:3810–3816
7. Holčapek M, Jandera P, Fischer J, Prokeš B (1999) Analytical monitoring of the production of biodiesel by high-performance liquid chromatography with various detection methods. J Chromatogr A 858:13–31
8. Chen B, Guo Z, Tan T, Xu X (2008) Structures of ionic liquids dictate the conversion and selectivity of enzymatic glycerolysis: theoretical characterization by COSMO-RS. Biotechnol Bioeng 99:18–29
9. Corma A, Iborra S, Miquel S, Primo J (1998) Catalysts for the production of fine chemicals: production of food emulsifiers, monoglycerides, by glycerolysis of fats with solid base catalysts. J Catal 173:315–321
10. Stavarache C, Vinatoru M, Maeda Y (2006) Ultrasonic versus silent methylation of vegetable oils. Ultrason Sonochem 13:401–407