P-011	**Continuous transesterification catalyzed with immobilized lipase.**	

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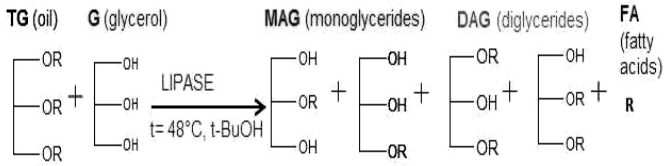
INTRODUCTION AND OBJECTIVES

Among transesterification reactions , glycerolysis of vegetable oils (Scheme 1) takes top position , due to the widespread application of its products (monoacylglycerols, MAG, and less often diacylglycerols, DAG) as emulsifiers in food, cosmetic and pharmaceutical industry. To replace the so far predominant heterogeneous process, several lipases have been tested (e. *Aspergillus niger ,Candida Antarctica, C. rugosa, Rhizomucor miehei, Thermomyces lanuginosa,* and *Pseudomonas* sp.*)*.Their efficiency increased upon encapsulation that ensured also their reusability. In a batch glycerolysis, however, the repeated use of even best commercial catalysts (e.g. Novozym 435) is limited by their abrasion due to essential intense stirring. The improvement of abrasion resistence (Wieman (2009) and a continuous process, (Damstrup (2006) H-Kittikun (2008), Noureddini (2004) and Yang (2005)) might solve this problem.

In the present work, the course of a continuous glycerolysis of several vegetable oils (olive, linseed, rapeseed, soybean and sunflower) catalyzed by *Candida antarctica B* lipase in t-butanol has been studied with the aim to find optimal conditions for the high yield MAG production



Scheme 1

MATERIALS AND METHODS

Chemicals

Olive, linseed, rapseed, sunflower, and soybean oil were commercial products, glycerol (Penta, p.a. > 99%, 0.39 mg H_2O/g), and tert-butanol (Roanal, 99.5 %, 3.1 mg H_2O/g) were used as obtained. *Candida antarctica* B lipase immobilized on polyacrylate (Novozym 435, bulk density 430 kg/m^3) was a commercial product (Aldrich).

HPLC analysis of glycerolysis products

The method proposed by Türkan (2006) was used under the following conditions: the analyses were performed with Watrex chromatograph equipped with Luna C18 column (250x 4.6 mm, 5 µm), C18, 4 x 0.3 mm pre-column, UV and PL-ELS 2100 light scattering detectors. The sample (ca. 100 mg) was diluted with acetone (1 ml) and feeded (5 – 10 µmol) to a mobil phase (acetonitril (AcN) /aceton (Ac) = 70/30 –for 1-8 min, then linear gradient elution to AcN/Ac=10/90 for 8-13 min and isocratic elution for 13-30 min). The evaluation of chromatograms that contained three groups of compounds (FFA+MAG, retention time 3-7min,. DAG r.t. 13-18 min, and TG , r.t. 20-25 min) was carried out with Chrom Guest 4.2 software. Due to low FFA+MAG peak area, the content of FFA was obtained by acidobasic titration

Continuous glycerolysis

The experimental arrangement is shown in Fig.1 A magnetically stirred reaction mixture (for composition see Table 1) was feeded from a storage tank with a peristaltic pump to the reactor equipped with a heated jacket. The collected product mixture was freed of the solvent by its distillation at 100^{o}C (first at atmospheric and then at reduced pressure (4 mm Hg)), and subjected to the HPLC analysis. It was proved that the evaporation of the solvent under these conditions did not lead to the changes in product composition. For comparison of the results obtained with different catalyst loads and feeding rates, the reaction parameter τ (min) = W/(υ_o . ρ), where W is the dry weight of Novozym 435 (g), ρ is its density (g/cm^3) and υ_o is feeding rate of the reaction mixture (cm^3/min), was used as the unified parameter (Damstrup 2006).

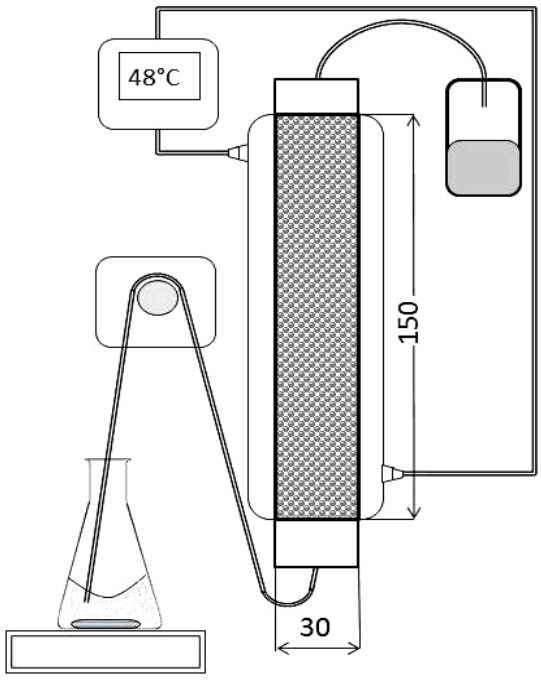


Figure 1: Continuous fixed bed reactor

RESULTS AND DISCUSION

Changes in the product compositon in dependence on the feeding rates are on example of the glycerolysis of olive oil demonstrated in Fig. 2. From the viewpoint of the objective of the present work, it is seen that the high content of MAG at practically quantitative conversion of TG can be achieved at a narrow feeding rate region (up to ca 2 ml/min, corresponding thus to the reaction time of about 15 min)

. The glycerolysis is accompanied by only low FFA formation (cca 4 %) which is independent of the feeding rate. The minor role of hydrolysis as a side reaction results primarily from the known low sensitivity of *Candida antarctica B* lipase (particularly that in immobilized form) to the amount of water in the reaction mixture. On the other hand, the independence of FFA on the feeding rate indicates that hydrolysis is fast and complete in the initial phase of the transesterification process.

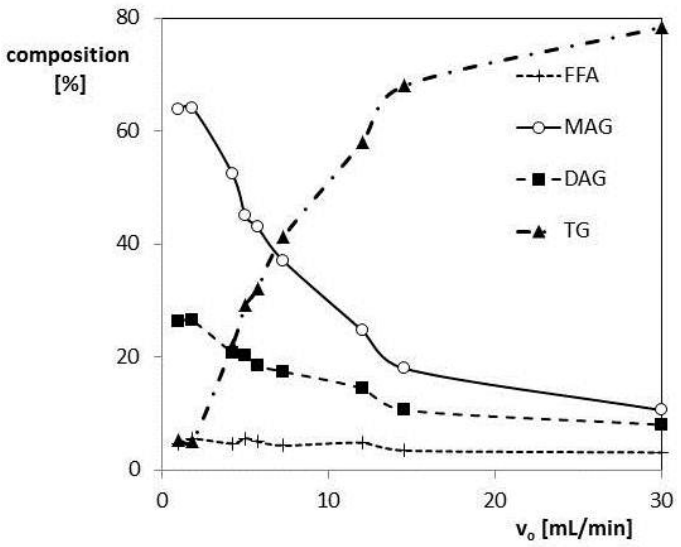


Figure 2: Continuous glycerolysis of olive oil (OO:G:t-BuOH weight ratio = 3.12 : 1 : 4.3 , 48 ^{o}C)

The optimal feeding rates found with olive oil were applied in the glycerolysis of the other oils. To favour MAG formation, the relative amount of G to the oil was increased to the ratio: 2.4 weight parts of oil: 1 part of glycerol and 4. 4 parts of the solvent. The results in Table 1 document that under these conditions MAG contents over 75% can be easily achieved with all the oils tested.

Table 1: Formation of MAG at the oil : glycerol : t-butanol wt. ratio = 2.4 : 1 : 4.4, 48 ^{o}C, feeeding rate = 0.8 ml/min, catalyst load = 17 g)

Oil	MAG	DAG	TG	FFA
Linseed	79	18	0	3
Olive	81	14	0	5
Rapeseed	79	18	0	3
Soybean	76	20	0	4
Sunflower	78	18	0	3

From applicaton viewpoint it is of interest, that similar MAG yields are obtained with the mixtures containing by about 25% lower solvent content (Table 2).

Table 2: Formation of MAG at the oil : glycerol : t-butanol wt. ratio = 2.4 : 1 : 3.4 (the other conditions see Table 1)

Oil	MAG	DAG	TG	FFA
Linseed	82	14	0	4
Olive	76	20	0	4
Rapeseed	74	21	0	5
Soybean	78	17	0	5
Sunflower	77	21	0	2

CONCLUSIONS

The glycerolysis of five vegetable oils (olive, linseed, rapeseed, sunflower, and soybean oil) showed that *Candida Antarctica B* lipase in its imobilized form (Novozym 435) is the catalyst of advantage in a continuous process. In accord with the objective of the work, optimal conditions have been found for the high conversion of the oils to monoacylglycerols, accompanied by the low free fatty acids formation.

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