Summary

The main goal of the study was to evaluate the usefulness of lipases of different origin and the carriers used for their immobilization in the synthesis of enantiomerically enriched esters of solketal. Solketal (IPG) is a glycerol derivative, obtained as a by-product in biofuels production. Industrial scale synthesis of racemic IPG is facile and efficient, but only enantiomerically enriched esters are valuable substrates in the synthesis of biologically active preparations. Their chemical synthesis appears expensive and time-consuming. A rational way to get such derivatives of solketal could be an enzymatic resolution of racemic mixtures of IPG. Development of a method of solketal esters resolution with high enantiomeric purity and an arrangement of the mathematical model of this process is an aim of the research presented in this thesis. The studies have covered three possible paths of the synthesis of enantiomerically enriched esters, i.e. enantioselective esterification involving acid and alcohol, enantioselective transesterification of vinyl esters and enantioselective hydrolysis of the solketal esters. Potentials of commercially available lipases preparations of a different origin and the whole cells of the mycelium of *Mucor circinelloides* were tested. Mesoporous silica materials and the multi-walled carbon nanotubes were used as carriers for immobilization of lipases.

Enzymatic esterification using carboxylic acids, similarly as the hydrolysis of esters did not gave satisfactory results. In the case of the solketal esterification with acids, most of biocatalysts remained inactive. Catalytical activity revealed only commercial immobilized preparations. Enantiomeric excess of the product in that biotransformation rarely exceeded 40%, and the low efficiency (ca, 10%), of the process was commonly observed. The experiments performed indicate that the structure of acylating agents has a crucial effect on both catalytic activity and optical purity of the product. Carboxylic acids having long alkyl chain, in the reaction catalyzed by Lipozyme RM IM give a product with a higher enantiomeric excess. Opposite tendency was observed when Novozymes 435 was applied. In this case, elongation of the carbon chain in the acid molecule decreased the optical purity of the synthesized esters. A similar trend was observed in case of esters hydrolysis, i.e. higher value ee were obtained in hydrolysis of solketal butyrate than of solketal acetate.

The best results (*ee* 80%, W 16%) were obtained in reaction of trans-esterification of the vinyl butyrate with IPG carried out in the presence of lipase with *Pseudomonas fluorescens*. Numerous experiments were made in view of improving enantioselectivity of the applied biocatalysts; they involved the modification of the reaction environment, i.e. change
of the solvent, temperature, amount of the enzymes and reagents and even by the supplementation with other substances. It was ascertained, that the successive elongation of the carbon chain in the acyl part in a donor molecule increased ee of the synthesized esters. Similarly, the reaction medium has a serious influence of the enzymatic process. The usage of the diisopropyl ether as a solvent in the transesterification reaction allowed to obtain the transesterification products with about 15% higher ee in comparison with reaction run in n-hexane. It has been also showed that the temperature of the process has an impact on the lipase activity and enantioselectivity of the transesterification reaction. Lower temperatures of transesterification were found to have a positive effect on optical purity of the products, most likely by slowing the reaction rate.

The influence of immobilization of the selected enzymatic preparations and of the applied enzyme carriers on enantioselectivity of the esterification were examined. Two types of porous silica were used: mesoporous silica of SBA-15 type and a monolithic material with bimodal macro- and mesoporous structure, and also multi-walled carbon nanotubes. The obtained catalysts were extensively examined due to their stability, selectivity and expressed activity.

Kinetics of the solketal esters synthesis was determined from the experiments carried out in a batch reactor using the native lipase and its immobilized form. It appeared that enzymatic transesterification of vinyl butyrate by solketal could be described by the bi bi ping-pong family equations. More detailed analysis of the results indicated that assumption of the reaction reversibility does not decrease the precision of description, and that the products formed in reaction of transesterification adversely affected (inhibited) activity of the enzymes. The influence of the diffusive kinetic limitation of the biocatalyst has been also observed for the immobilized enzymes.

A test of the continuous synthesis of the solketal esters in monolithic siliceous continuous microreactor has been carried out. These experiments confirmed the high activity of the protein immobilized on the silica monolith and his perfect stability while storage and in the continuous process of the esters synthesis. The increase of the IPG’s enantiomers efficiency in these experiments has not been noticed.