

## Enzymatic Synthesis Of Palm Based Amino Acid Surfactants

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### Introduction

Amino acid surfactants, with their antimicrobial activity, biocompatibility and excellent skinfeel, have immense potential for use as emulsifiers and preservatives in food, cosmetic and personal care products. The ecological disadvantages and non-selectivity of their chemical synthetic method with petroleum based raw materials have in recent years motivated the development of enzymatic processes employing more biodegradable oleochemicals as substrate. One such abundant source of oleochemical in Malaysia is palm oil. This project therefore aims to develop an optimized process for the synthesis of palm based amino acid surfactants with the view of future graduation to industrial scale.

### Materials and Methods

Two palm oil fractions were reacted with the amino acid L-lysine in the presence of the lipase Lipozyme. Reaction mixtures at the end of a specified incubation period were analyzed structurally (using infrared and nuclear magnetic resonance spectroscopy), qualitatively (using thin layer and gas chromatography) and quantitatively (using TLC-photodensitometry). Response surface methodology (RSM) was employed for optimization of reaction parameters using a 5-level, 5-variable experimental design. Statistical analyses were performed using the computer software Design Expert Version 6.0.4.

### Results and Discussion

From preliminary work with a range of amino acids, L-lysine was selected as the model amino acid substrate, and, the fatty acyllysine products from its reaction with palm olein have been purified and structurally characterized (Soo *et al.*, 2000, 2001). Enzyme screening has also revealed the immobilized lipase from *Mucor miehei* (Lipozyme) to be the best biocatalyst for the reaction (Soo *et al.*, 2001). Using this enzyme, the main and interactive effects of several reaction parameters were studied using both conventional one-parameter-at-a-time approach and response surface methodology (RSM). With the one-parameter-at-a-time approach, the effects of temperature, solvent, incubation period, fatty substrate/amino acid molar ratio, enzyme amount, and water removal on the reactions were analyzed and compared to those with free fatty acids and pure triglycerides as fatty substrates. Reactions with all substrates were best carried out at high temperatures (70-80°C) in hexane as solvent. On the other hand, while reactions with free fatty acids proceeded better when slight excesses of the free fatty acids over the amino acid were used, reactions with triglycerides and palm oil fractions were best performed at equimolar ratios. Also, addition of molecular sieves slightly enhanced reactions with free fatty acids but adversely affected reactions with triglycerides and palm oil fractions. Although reactions with palm oil fractions took longer (6 d) to reach equilibrium compared to reactions with free fatty acids (4 d) and pure triglycerides (4 d), better yields were obtained. RSM then showed that reactions of L-lysine with both palm olein and palm kernel olein were best described mathematically using a quadratic model. The results obtained showed that the yields were better in the more hydrophobic solvents and at higher oil concentrations. In most cases, the yields also improved with an increase in the amount of enzyme used while the amount of molecular sieves added for removal of synthetic water was less influential on the reactions. Optimal conditions for predicting maximum fatty acyllysine yields were achieved at a temperature of 50°C with hexane (Log P 3.5) as solvent, a palm olein or palm kernel olein concentration of 100 mM, an enzyme amount of 83 mg for palm olein and 186 mg for palm kernel olein, and, molecular sieves amounts of 154 mg for palm olein and 94 mg for palm kernel olein. Under these conditions, the experimental yields were 496 mM for palm olein based acyllysines and 914 mM for palm kernel olein based acyllysines; these were well correlated with their predictive values of 456 mM and 926 mM, respectively.

### Conclusions

Palm based acyllysine surfactants can be produced by direct transacylation reactions between palm oil and L-lysine. The enzymatic reactions were also successfully modelled and optimized to obtain maximum yields.

### Benefits from the study

The novelty of using palm oil as raw material enables a mixture of medium to longer chain and more saturated amino acid surfactants to be prepared, thus offering the surfactant consumer a wider choice in selecting a surfactant product most well suited to the application required. The conditions of the enzymatic process optimized here are less extreme than those currently employed in the chemical synthetic method and does not use toxic solvents.

**Patent(s), if applicable:**

Nil

**Stage of Commercialization, if applicable:**

Nil

**Project Publications in Refereed Journals**

1. Soo EL, Salleh AB, Basri M, Rahman RNZA and Kamaruddin K. 2003. Response surface methodological studies on lipase-catalyzed synthesis of amino acid surfactants. *Process Biochemistry* (In press).
2. Soo EL, Salleh AB, Basri M, Rahman RNZA and Kamaruddin K. 2003. Optimization of the enzyme-catalyzed synthesis of amino acid surfactants from palm oil fractions. *Journal of Bioscience and Bioengineering* 95(4): 361-367.

**Project Publications in Conference Proceedings**

1. Soo EL, Salleh AB, Basri M, Rahman RNZA and Kamaruddin K. 2002. Enzymatic synthesis of palm-based amino acid surfactants: Process optimisation using response surface methodology. In: Proceedings of the 27<sup>th</sup> Annual Conference of the Malaysian Society for Biochemistry and Molecular Biology, 2002; p27.
2. Soo EL, Salleh AB, Basri M, Razak CNA and Rahman RNZA. 2001. Enzymatic synthesis of palm-based amino acid surfactants. In: Proceedings of the Enzyme Engineering Conference XVI, Potsdam, Germany, 2001; p313-316.
3. Soo EL, Salleh AB, Basri M and Razak CAN. 2000. Enzymatic synthesis of palm-based amino acid surfactants. In: Proceedings of the 12<sup>th</sup> National Biotechnology Conference, Lumut, Perak, 2000; p399-402.

**Graduate Research**

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Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
23. Soo Ee Lin	Enzymatic synthesis of palm-based amino acid esters	Enzymology	PhD	2003

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