

Tropentag, September 16-18, 2015, Berlin, Germany

"Management of land use systems for enhanced food security: conflicts, controversies and resolutions"

## Optimisation of Enzyme-Assisted Aqueous Extraction of High Oleic Sunflower Oil Using Response Surface Methodology

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## Abstract

The enzyme-assisted aqueous extraction (EAAE) is a highly efficient method for both, the recovery of oil and protein from different crops. It works on a completely solvent free basis by biochemical degradation of cell compounds, the water-based displacement of oleosomes from the cytoplasm as well as the coalescence of these oil-bodies by breaking up of the surrounding protein layer. Aside from the fact that the process is environmentally uncritical, the quality of oil will also be superior, compared to other extraction techniques. No subsequent refining is required. In the present study, high oleic sunflower seeds of variety PR65H22, harvested at a conventional farm near Würzburg, Germany, were used. Whole seeds were ground to a size < 1 mm and treated with either protease or cellulasecomplexes in a prototype continuously stirred tank reactor, specifically designed for EAAE. In previous experiments, the enzyme mass, pH-value, process temperature and the amount of water were found to be the most influencing parameters on extraction efficiency and therefore varied during this study. Response surface methodology with a four factor Box-Behnken design was chosen for optimisation. In the protease experiment, second order terms (TWI and PQ) contributed significantly to the model. Proton concentration (pH-Value), temperature and enzyme concentration were found to have a significant influence on yield. The stationary point of the response surface was situated on a saddle point with an extraction efficiency of  $82.3 \pm 1.3$ %. This was validated in a mass balance, comprising five not significantly different repetitions at an extraction efficiency of  $80.8 \pm 3.1$  %. A canonical analysis estimated a trespass of the 95% extraction efficiency level outside the models boundaries by increase of the enzyme concentration, which is to be proven in an ongoing optimisation attempt. The cellulase based approach only found the first order term to contribute significantly to the model with only the proton concentration having significant influence. Further experiments will comprise a two-phase extraction with cellulase-followed by protease-complex. After successful implementation, the process can be brought forward to other crops such as oil palm, rape or jatropha.

**Keywords:** Enzyme-assisted aqueous extraction, optimisation, response surface methodology, sunflower oil

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