

# Directing the oil composition of oleaginous oil: a multidisciplinary approach

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## Biobased Oil

Oil produced by oleaginous yeasts can be used in the biobased economy as an alternative source of energy and chemicals. Crucial for the application of this biobased oil as a fine chemical is its fatty acid composition, since specific applications ask for a specific composition. Furthermore, the oleaginous yeasts need to be able to grow on biomass to make the oil a true biobased product.

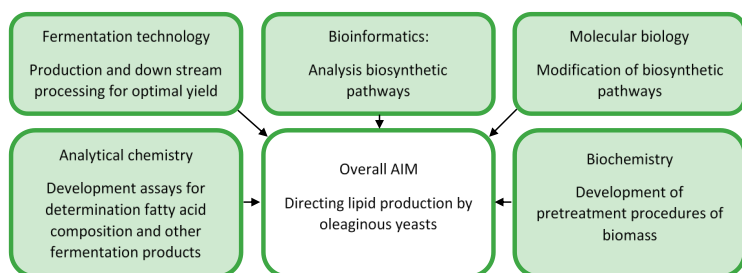


Figure 1 Multidisciplinary project approach

The overall aim of this project is to understand the biosynthesis route of lipids in oleaginous yeasts and to develop a toolbox by which oil with any desired composition can be produced. In order to achieve this, a multidisciplinary approach is needed (Figure 1).

## Analysis of lipids in various organelles of the cell

Various lipid classes are present in the different organelles of the cells (Figure 2). To be able to differentiate between the fatty acid composition of cell membranes and lipid bodies, this project aimed at the development of a method to separate free fatty acids (FFA), phospholipids (PL), sterolesters (SE) and triacylglycerols (TAG) in yeast cells. This method will be used to analyze the changes in lipid metabolism as a result of metabolic engineering and optimization of growth protocols.

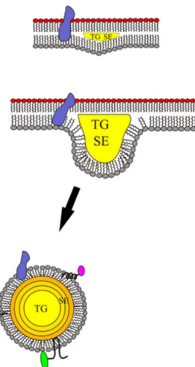


Figure 2 Distribution of lipids in cells

(figure derived from Koch et al., 2014, FEMS Microbiol. Rev., vol 38, 982-915)

## Separation of lipid classes by solid phase extraction

The SPE method as described by Kaluzny *et al.* (1985, J. Lipid Res., vol 26, 135-140) was used and modified (see figure 3). NH<sub>2</sub>-SPE cartridges of Waters were used. The volumes were adjusted and heptane was used to elute the steryl esters (SE) since it was found to elute SE more specifically than hexane. Fatty acid composition of the lipid classes were determined by GC after transesterification.

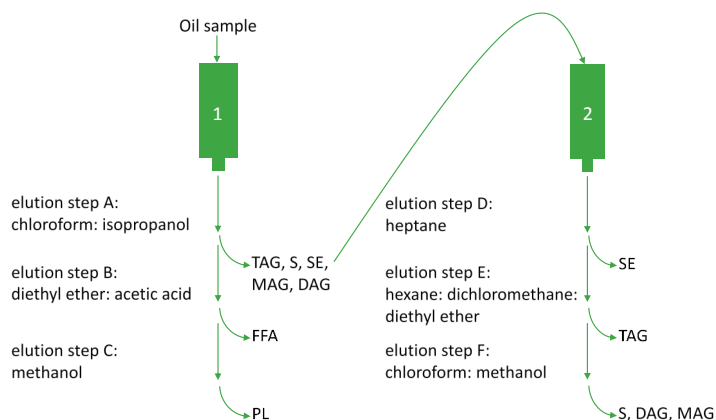


Figure 3 Schematic representation of the SPE method to separate various lipid classes

## Results

In figure 4 the results are shown of the determination of the lipid composition of the oleaginous yeasts. Over 90% of the fatty acids were found to be present as TAG's. About 3.5% of the fatty acids were incorporated in phospholipids (PL). Further analysis of the data showed that the PL contained more poly unsaturated fatty acid compared to the TAG's. Also higher amounts of shorter chain fatty acids (C12:0 and C14:0) were found in the PL. Since PL play an important role in membrane fluidity, it is not remarkable that the fatty acid composition of PL is more complex.

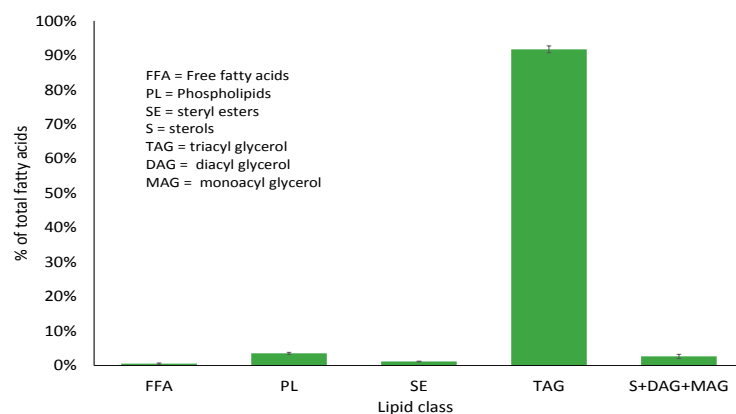


Figure 4 Lipid composition of oleaginous yeast

## Conclusion

Various lipid classes (FFA, PL, SE, TAG) can be separated with the developed method and their fatty acid composition can be determined. This method can now be used to evaluate the results of the optimization and modification processes and help to obtain a biobased oil with desired properties.

### About HAN BioCentre

The central theme of the HAN BioCentre is Biodiscovery; the discovery, analysis, production and application of biomolecules. We are in search for new bioactive compounds and screenings assays which can be applied in agriculture, food and health. All our projects are bringing together multiple disciplines ranging from microbiology and molecular biology to chemical analysis and bio-informatics. For more information: HAN BioCentre, HAN University of Applied Sciences, Laan van Scheut 2, Nijmegen, The Netherlands, e-mail: info@hanbiocentre.nl

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