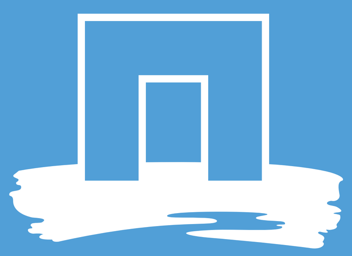

Isolation of gondoic acid from Camelina oil by hydratase-catalysed reactive separation

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Introduction

Medium chain (C10-C14) fatty acids are an important feedstock for the European oleochemical industry. Currently, they are obtained from oils of plants grown in (sub)tropical climates, such as palm kernel or coconut oil. The EU-financed COSMOS project aims to establish an alternative feedstock of medium chain fatty acids based on oils from two plants that are suited to the temperate European climate: *Camelina sativa* and *Crambe abyssinica*. Camelina oil contains significant amounts of the rare fatty acid gondoic acid (C20:1, *cis*-Δ^{11}). The unusual position of the double bond in gondoic acid makes it a potential precursor for the synthesis of the medium chain fatty acid lauric acid (C12:0) via olefin metathesis followed by hydrogenation. To facilitate this process, methods are required by which gondoic acid can easily be separated from other fatty acids present in Camelina oil hydrolysates. These other fatty acids are predominantly the C18 unsaturated fatty acids oleic, linoleic and α-linolenic acid, which all contain a *cis*-Δ^9 double bond.

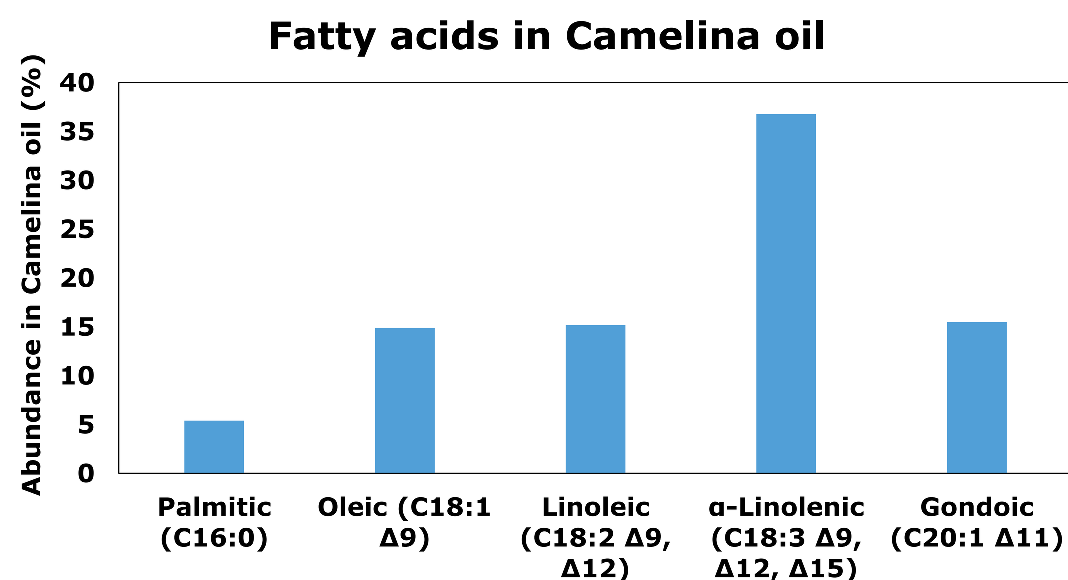

Figure 1. *Camelina sativa* (left) and the fatty acid composition of its seed oil (right). Data for the fatty acid composition of Camelina oil are from Zubr and Matthäus (2002), *Ind. Crop. Prod.*, **15**, 155-162. All fatty acids that make up >5% of the total fatty acid content are shown.

Approach

Here, we present a novel approach for the reactive separation of gondoic acid from Camelina oil. This concept involves hydrolysis of Camelina oil by a lipase, after which the fatty acids containing a *cis*-Δ^9 double bond are hydrated to 10-hydroxy fatty acids using a *cis*-Δ^9 specific oleate hydratase. The formed hydroxy fatty acids can then be separated from the gondoic acid by e.g. cold fractionation. In addition to providing an efficient method for obtaining gondoic acid from Camelina oil, this cascade provides access to 10-hydroxy fatty acids, which have applications in cosmetics or as chemical intermediates.

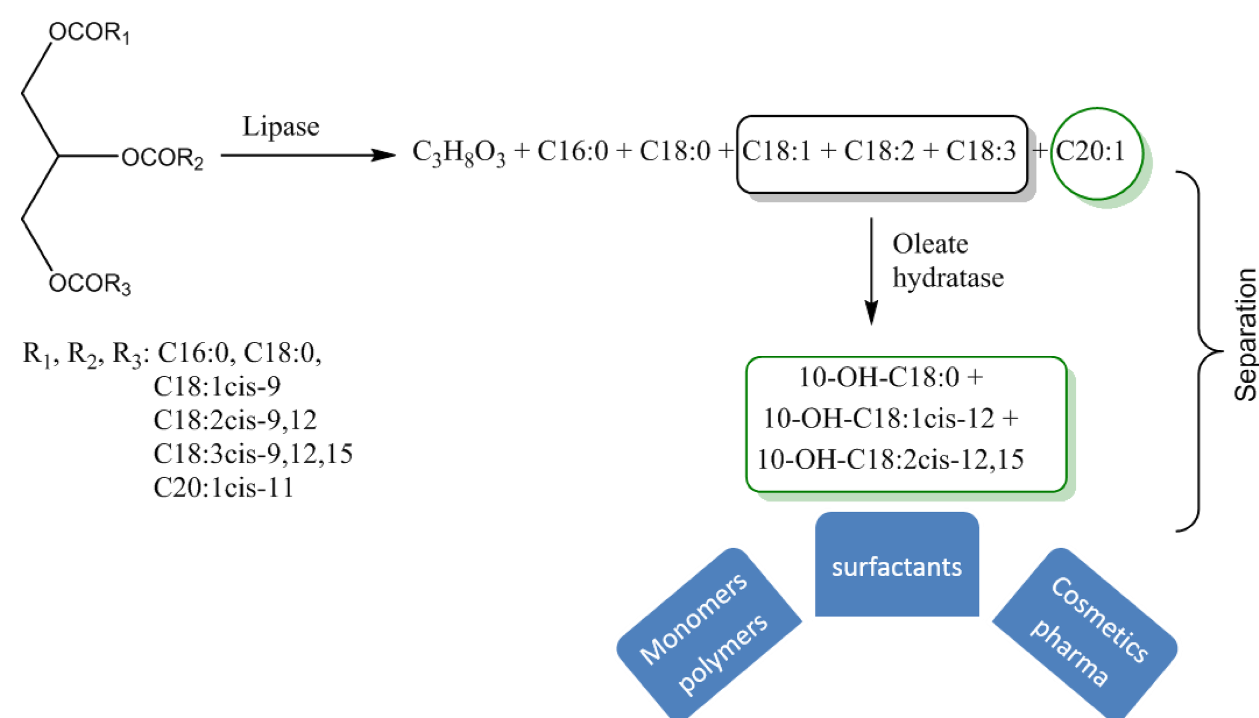

Figure 2. Envisioned two-enzyme process for the reactive separation of gondoic acid from C18, *cis*-Δ^9 unsaturated fatty acids found in Camelina oil.

Results

Hydratase selection

Screening of hydratases reveals that the enzyme from *Stenotrophomonas maltophilia* is most suitable for conversion of C18 unsaturated fatty acids from Camelina oil.

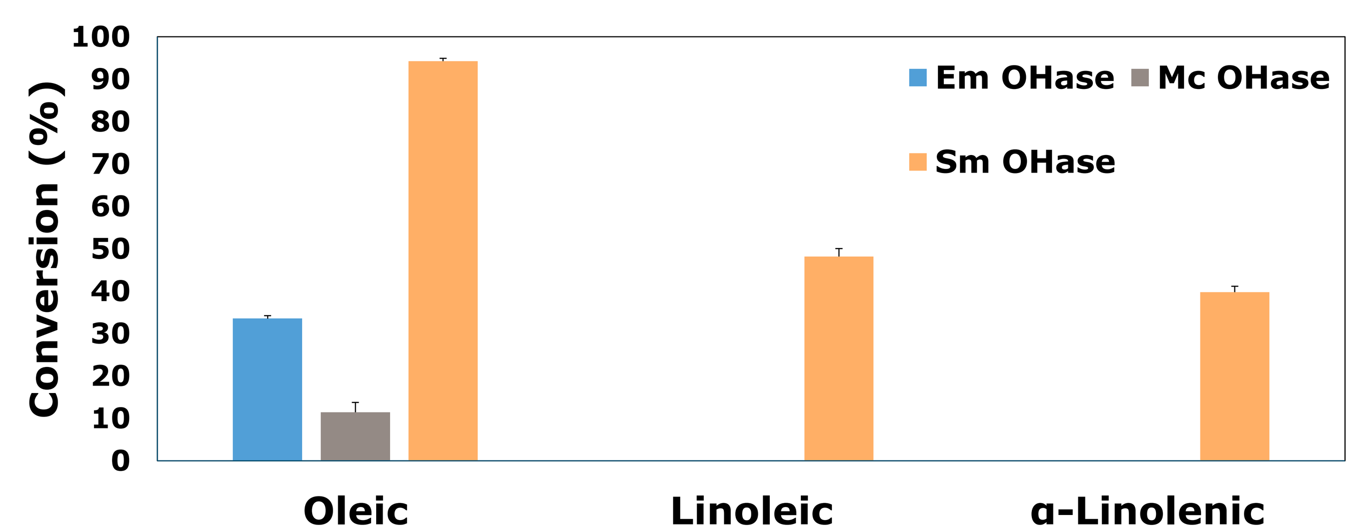

Figure 3. Conversion of C18 unsaturated fatty acids by oleate hydratases (OHases) from *Elizabethkingia meningoseptica* (Em), *Macrococcus caseolyticus* (Mc) and *Stenotrophomonas maltophilia* (Sm). Fatty acid substrates (2 mM) were incubated in 500 µL 50 mM PIPES buffer, pH 6.5, containing 10 µL cell-free extract of *E. coli* cells expressing the desired enzyme (Mc and Sm) or 0.02 mg/mL purified OHase (Em) for 2 h at 30 °C. After this time period, the reactions were stopped and products were extracted and analysed by GC and GC/MS. For all reactions where conversion was observed, a sole product was observed, which was identified as the corresponding 10-hydroxy fatty acid by GC/MS.

Hydration of fatty acid mixtures

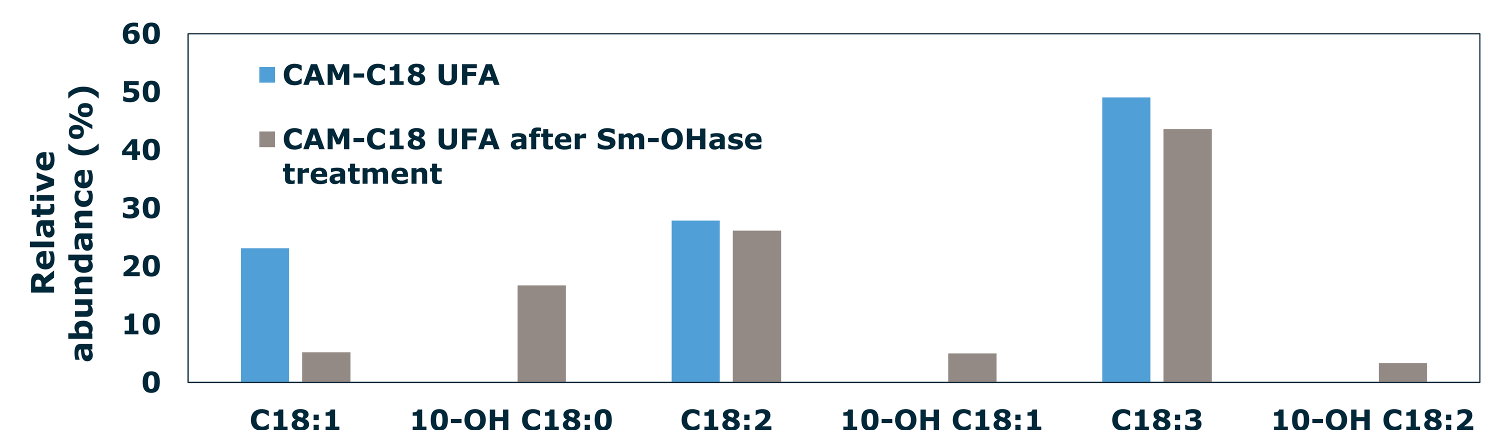

Figure 4. Conversion of fatty acids in mixtures of C18 unsaturated fatty acids obtained from Camelina oil by oleate hydratase (OHase) from *Stenotrophomonas maltophilia* (Sm). 100 mg of a mixture of C18 unsaturated fatty acids derived from Camelina oil and 0.45 mL of *E. coli* cell-free extract containing Sm OHase (10 U/mL, 4.5 U total) were incubated in 10 mL 100 mM citrate/phosphate buffer for 24 h at 30 °C. After this time period, the reactions were stopped and products were extracted and analysed by GC and GC/MS.

Conclusions

- *S. maltophilia* oleate hydratase is suitable for the conversion of Camelina oil derived C18 unsaturated fatty acids, converting oleic, linoleic and α-linolenic acids to their 10-hydroxy derivatives. In mixtures of Camelina oil-derived C18 unsaturated fatty acids, oleic acid is preferentially hydrated.

Future plans

- Analyse hydration of fatty acids in Camelina oil hydrolysates.
- Select a lipase that is suitable for hydrolysis of Camelina oil, preferably under conditions where *S. maltophilia* oleate hydratase is also active and combine lipase-catalysed oil hydrolysis with hydration in a one-pot, two-step reaction.
- Develop methods for the separation of 10-hydroxy fatty acids and gondoic acid, e.g. cold fractionation.

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ADDING VALUE TO CAMELINA AND CRAMBE OIL

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