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## Production of fatty alcohols from non-edible oils by enzymatic cascade reactions†

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A biocatalytic cascade transforming castor oil into (*R,Z*)-octadec-9-en-7-ol is presented by combining a lipase catalysed hydrolysis of castor oil into ricinoleic acid followed by a photoenzymatic decarboxylation into (*R,Z*)-octadec-9-en-7-ol. Conversion of up to 41.7% and overall product concentrations of up to 60 mM, this new bienzymatic and photocatalytic cascade exhibits significant potential for the valorisation of non-edible castor oil. The scope and limitations of the current system are described and discussed.

*En route* to a biobased economy, non-edible plant oils or agricultural waste oils are increasingly getting in focus as renewable feedstock.<sup>1</sup> Here, especially castor oil takes a prominent position and is currently evaluated as starting material for ricinoleate-based biodiesels.<sup>2,3</sup> To the best of our knowledge, however, the methods of the state-of-the-art almost exclusively centre around the transesterification of castor oil or the esterification of ricinoleic acid. All these methods share the intrinsic disadvantage of being reversible reactions thereby necessitating large molar surpluses of the alcohol to attain significant conversion into the desired biodiesel fatty acid methyl ester (FAME) (Scheme 1).

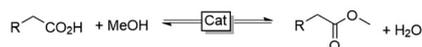
An alternative approach to valorise (waste) vegetable oils into biofuel is to convert them into the corresponding (C1-shortened) alkanes *via* decarboxylation (Scheme 1). First, the irreversible nature of the reaction facilitates process design. Second, the alkanes exhibit, compared to the corresponding FAMES and FAEEs, significantly higher caloric values. Today, a focus is put on thermal decarboxylation methods using homogeneous or heterogeneous transition metal catalysts. Generally, these methods require elevated reaction temperatures (typically >200 °C) and therefore are energy-intensive.<sup>4</sup>

Enzymatic decarboxylation reactions, requiring significantly lower reaction temperatures, today are mostly limited to amino acid decarboxylases<sup>5–8</sup> or malonate decarboxylases.<sup>9</sup> More recently, oxidative decarboxylation of fatty acids to terminal alkenes<sup>10–15</sup> and oxidative decarboxylation of amino acids yielding nitriles<sup>16–18</sup> have received some attention.

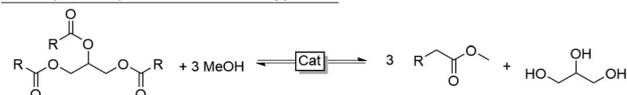
Recently, an alternative route to valorise long-chain fatty acids into value-added products by using a newly identified photodecarboxylase from the microalga *Chlorella variabilis* NC64A (CvFAP)<sup>19</sup> has come up.<sup>20–24</sup> CvFAP catalyses the irreversible decarboxylation of a range of fatty acids into the corresponding (C1-shortened) alkanes. Hence, CvFAP not only opens up an irreversible alternative to the established reversible (trans)esterification methods but also yields products exhibiting an overall higher degree of reduced carbon atoms and therewith also a higher specific caloric value.

For catalytic activity this enzyme requires activation with blue ( $\lambda = 450$  nm) light. Though the catalytic mechanism has not been elucidated fully yet, it is clear that a photoactivated flavin prosthetic group catalyses a single electron transfer from the enzyme-bound, deprotonated carboxylate yielding a carboxyl radical and a flavin semiquinone intermediate. The latter rapidly decarboxylates, assisted by a nearby cysteine serving as H-atom donor for the transient carbon radical

### FAME synthesis by esterification of free fatty acids



### FAME synthesis by transesterification of triglycerides



Scheme 1 Traditional utilisation of fatty acids and fats and oils *via* (trans)esterification.

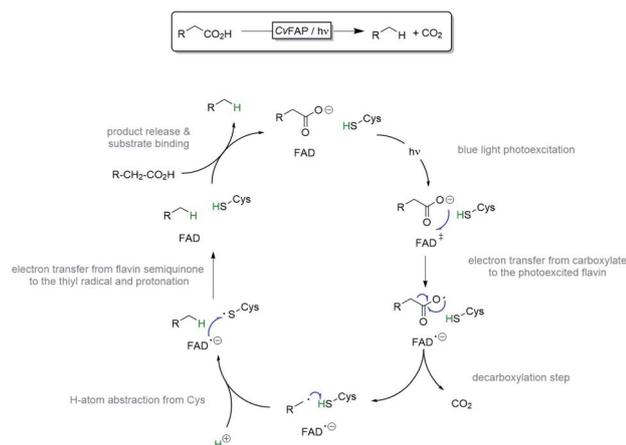
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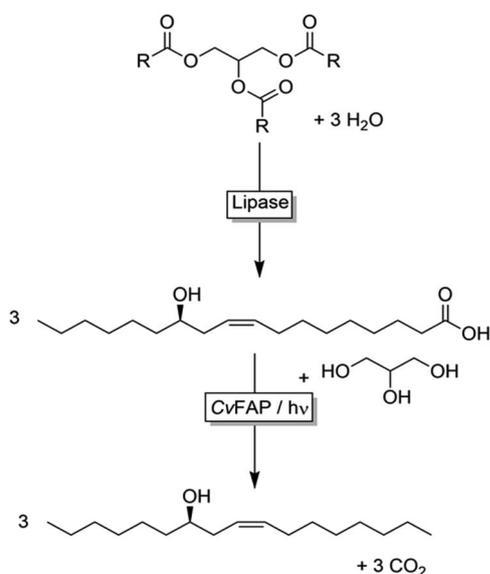


**Scheme 2** Proposed mechanism of the photoenzymatic decarboxylation catalysed by CvFAP.

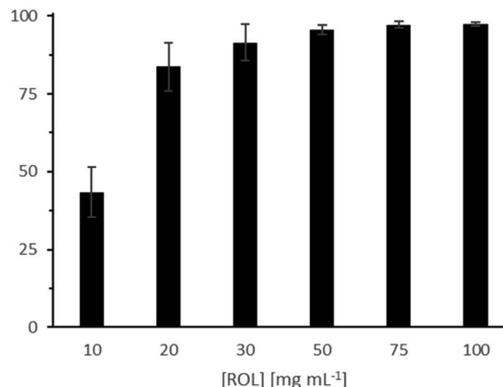
species. Electron transfer from the flavin to the thyl radical and reprotonation closes the catalytic cycle (Scheme 2).<sup>25</sup>

Very recently, we have reported the preparative application of CvFAP for the conversion of some (short and medium chain) carboxylic acids as well as for some natural fatty acids.<sup>20–24</sup> However, only edible oils and fatty acids have been considered so far, which we believe have only a limited potential for the preparation of biofuels. Therefore, we became interested in evaluating non-edible castor oil as possible source for ‘next generation’ biofuels.

Castor oil, containing approximately 90% of ricinoleic acid as fatty acid component, is produced at some 600–800 million pounds every year.<sup>26</sup> Its main applications so far are as lubricant, polymer precursor as well for the synthesis of surfactants.<sup>27–29</sup>



**Scheme 3** Envisioned bienzymatic cascade transforming castor oil into (*R,Z*)-heptadec-9-en-7-ol.

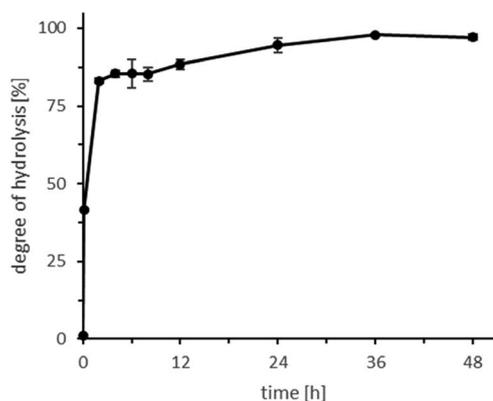


**Fig. 1** Influence of the ROL concentration on the hydrolysis of castor oil. Conditions: 500  $\mu$ L of Tris–HCl buffer (pH 8.5, 100 mM) containing 10–100 mg lipase ROL and 500  $\mu$ L of castor oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [castor oil] = 500  $\mu$ L mL<sup>-1</sup>, 30 °C, 10 W blue LED, 24 h.

Here, we propose using castor oil as starting material for the synthesis of (*R,Z*)-heptadec-9-en-7-ol by a bienzymatic cascade reaction. Castor oil is first hydrolysed using the commercially available (immobilised) lipase from *Rhizopus oryzae* (ROL); the free ricinoleic acid then is decarboxylated to (*R,Z*)-octadec-9-en-7-ol using the aforementioned CvFAP (Scheme 3). Factors influencing the efficiency of the single steps and the combined system have been investigated.

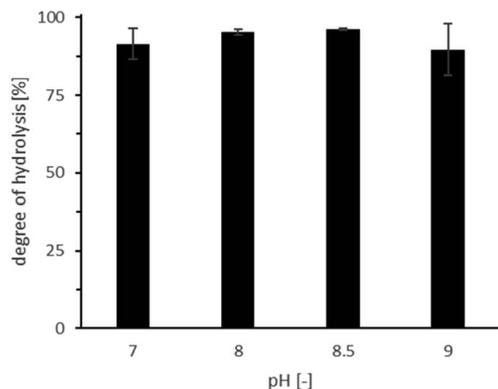
In a first set of experiments, we investigated the first step of the cascade, *i.e.* the ROL-catalysed hydrolysis of castor oil. A ROL concentration of 100 mg mL<sup>-1</sup> was sufficient to reach >95% hydrolysis within 24 h (Fig. 1; Table S2,† entries 1–6).

This preliminary evaluation was confirmed by a more detailed time-course of the reaction (Fig. 2; Table S2,† entries 7–15). Already after 2 h, more than 80% of the initial castor oil was hydrolysed; the reaction was essentially complete (98%) after 36 h.



**Fig. 2** Time course of the ROL-catalysed hydrolysis of castor oil. Conditions: 500  $\mu$ L of Tris–HCl buffer (pH 8.5, 100 mM) containing 50 mg lipase ROL and 500  $\mu$ L of castor oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [ROL] = 50 mg mL<sup>-1</sup>, [castor oil] = 500  $\mu$ L mL<sup>-1</sup>, 30 °C, 10 W blue LED.



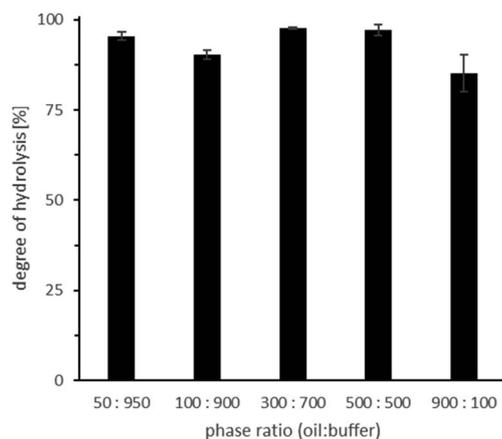


**Fig. 3** Influence of the reaction pH on the ROL-catalysed hydrolysis of castor oil. Conditions: 500  $\mu\text{L}$  of Tris-HCl buffer (100 mM) containing 50 mg lipase ROL and 500  $\mu\text{L}$  of castor oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [ROL] = 50  $\text{mg mL}^{-1}$ , [castor oil] = 500  $\mu\text{L mL}^{-1}$ , 30  $^{\circ}\text{C}$ , 10 W blue LED, 24 h.

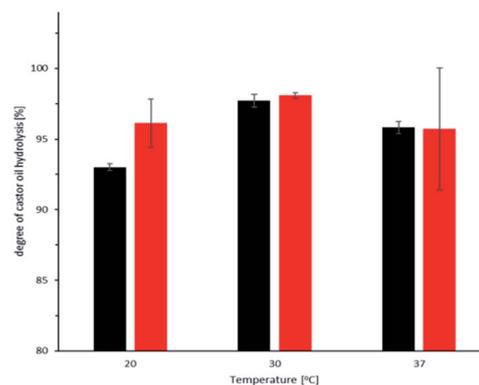
Having the preference of CvFAP for alkaline media in mind, we also evaluated the effect of pH on the ROL-catalysed hydrolysis (Fig. 3; Table S2,† entries 16–19). A very minor reduction at pH 9 may be suspected from these experiments, however, we did not consider this as a major obstacle for the envisioned cascade.

The catalytic potential of ROL is even higher than estimated for these experiments because increasing the substrate amount hardly influenced the overall conversion after 24 h reaction time (Fig. 4; Table S2,† entries 20–24).

Again, having the bienzymatic cascade in mind, we also investigated the influence of blue-light irradiation on the activity of ROL. As shown in Fig. 5 (and in Table S2,† entries 25–27) no significant influence of blue-light illumination was observed. In these experiments we also varied the reaction



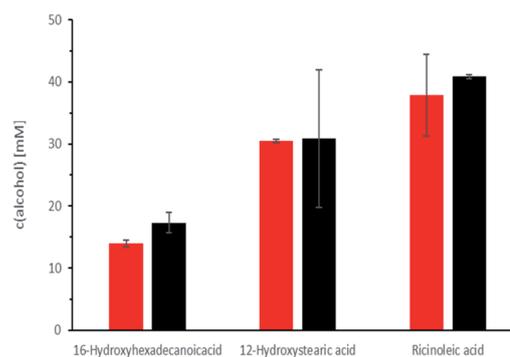
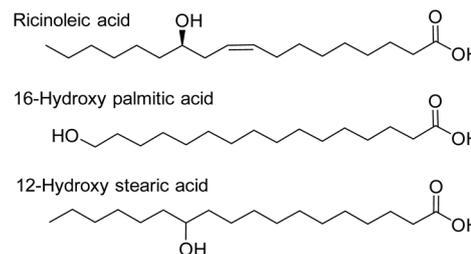
**Fig. 4** Influence of the phase ratio (castor oil : reaction buffer) on the ROL-catalysed hydrolysis. Conditions: 100–950  $\mu\text{L}$  of Tris-HCl buffer (pH 8.5, 100 mM) containing 50 mg lipase ROL and 900–50  $\mu\text{L}$  of castor oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [ROL] = 50  $\text{mg mL}^{-1}$ , 30  $^{\circ}\text{C}$ , 10 W blue LED, 24 h.



**Fig. 5** Influence of reaction temperature and the presence (■) or absence (■) of blue light ( $\lambda = 450 \text{ nm}$ ) on the efficiency of the ROL-catalysed hydrolysis of castor oil. Conditions: 500  $\mu\text{L}$  of Tris-HCl buffer (pH 8.5, 100 mM) containing 50 mg lipase ROL and 500  $\mu\text{L}$  of castor oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [ROL] = 50  $\text{mg mL}^{-1}$ , [castor oil] = 500  $\mu\text{L mL}^{-1}$ , with/without 10 W blue LED, 24 h.

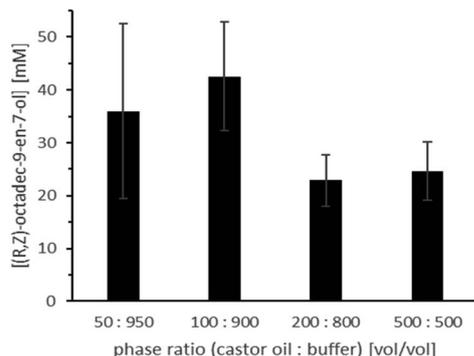
temperature and found only a minor influence on the overall reaction rate.

Therefore, we concluded that ROL is highly compatible with the reaction conditions required for the CvFAP-catalysed photodecarboxylation reaction and drew our attention to the photoenzyme.



**Fig. 6** Activity of CvFAP crude cell extracts (■) or whole cells (■) on several hydroxy fatty acids. Conditions: 700  $\mu\text{L}$  of Tris-HCl buffer (pH 8.5, 100 mM) containing CvFAP either as crude cell extract or within the *E. coli* expression host, in either case the final CvFAP concentration was 8  $\mu\text{M}$ . To this solution, 300  $\mu\text{L}$  of a DMSO stock solution containing the free fatty acid was added (final concentration = 50 mM). Illumination with 10 W blue LEDs at 30  $^{\circ}\text{C}$  for 24 h.



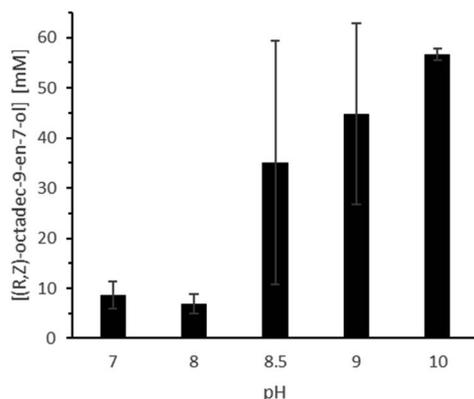


**Fig. 7** Influence of the phase ratio (castor oil : reaction buffer) on the bienzymatic synthesis of (*R,Z*)-octadec-9-en-7-ol. Conditions: 700  $\mu\text{L}$  of Tris-HCl buffer (pH 8.5, 100 mM) containing 0.35 g wet *CvFAP@E. coli*, 50 mg lipase ROL and 50–500  $\mu\text{L}$  of castor oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [Wet *CvFAP@E. coli*] = 0.35 g  $\text{mL}^{-1}$ , [lipase ROL] = 50 mg  $\text{mL}^{-1}$ , 30  $^{\circ}\text{C}$ , 10 W blue LED, 500 rpm, 24 h.

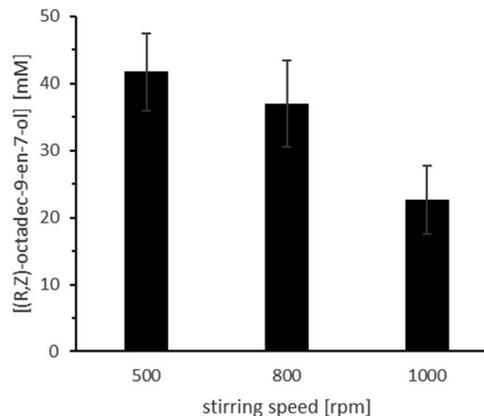
So far, *CvFAP* had been applied mainly for non-functionalised fatty acids. We therefore also investigated whether *CvFAP* also displays catalytic activity on some hydroxylated fatty acids (such as ricinoleic acid) (Fig. 6, Table S3<sup>†</sup>).

Ricinoleic acid (C18) was converted to more than 80% into the corresponding (C1-shortened product), also 12-hydroxy stearic acid (C18) gave very good conversions while 16-hydroxy palmitic acid (C16) was converted to only about 30%. In these experiments we also compared the efficiency of crude cell extracts and whole cells (from which the crude extracts had been prepared) for the photoenzymatic decarboxylation reactions and found no significant difference.

Next, we used both catalysts in a one-pot-one-step reaction cascade and characterised the factors influencing the overall reaction. In contrast to the similar experiment with ROL alone



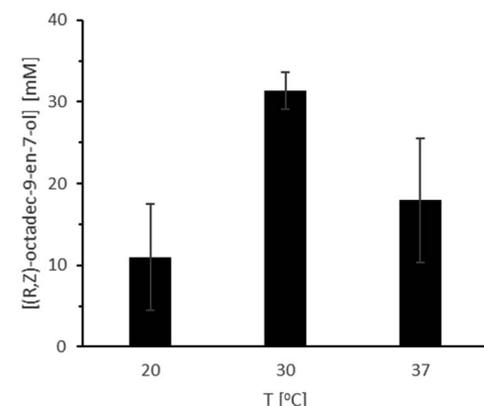
**Fig. 8** Influence of the reaction pH on the bienzymatic synthesis of (*R,Z*)-octadec-9-en-7-ol. Conditions: 700  $\mu\text{L}$  of Tris-HCl buffer (pH 7–10, 100 mM) containing 0.35 g wet *CvFAP@E. coli*, 50 mg lipase ROL and 50  $\mu\text{L}$  of castor oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [Wet *CvFAP@E. coli*] = 0.35 g  $\text{mL}^{-1}$ , [lipase ROL] = 50 mg  $\text{mL}^{-1}$ , [castor oil] = 50 mM, 30  $^{\circ}\text{C}$ , 10 W blue LED, 500 rpm, 24 h.



**Fig. 9** Influence of the stirring speed on the bienzymatic synthesis of (*R,Z*)-octadec-9-en-7-ol. Conditions: 700  $\mu\text{L}$  of Tris-HCl buffer (pH 8.5, 100 mM) containing 0.35 g wet *CvFAP@E. coli*, 50 mg lipase ROL and 50  $\mu\text{L}$  of castor oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [Wet *CvFAP@E. coli*] = 0.35 g  $\text{mL}^{-1}$ , [lipase ROL] = 50 mg  $\text{mL}^{-1}$ , [castor oil] = 50 mM, 30  $^{\circ}\text{C}$ , 10 W blue LED, 500–1000 rpm, 24 h.

(Fig. 4) we found a significant influence of the substrate (castor oil) loading (Fig. 7; Table S4,<sup>†</sup> entries 9–12).

The overall conversion varied significantly in the presence of higher substrate amounts. A possible explanation for this observation is that a higher castor oil amount also provides higher amounts of ricinoleic acid, which can acidify the aqueous reaction medium. Also, increasing the castor oil amount results in a lower absolute amount of buffer and further favours acidification. This hypothesis is supported by the clear preference of the overall reaction system for more alkaline reaction conditions (Fig. 8; Table S4,<sup>†</sup> entries 4–8) and the peculiar influence of the stirring rate (Fig. 9, Table S4,<sup>†</sup> entries 1–3). In the latter case, reduced phase transfer limitations



**Fig. 10** Influence of the reaction temperature on the bienzymatic synthesis of (*R,Z*)-octadec-9-en-7-ol. Conditions: 700  $\mu\text{L}$  of Tris-HCl buffer (pH 8.5, 100 mM) containing 0.35 g wet *CvFAP@E. coli*, 50 mg lipase ROL and 50  $\mu\text{L}$  of castor oil were added to a transparent glass vial (total reaction volume is 1 mL). The final conditions of this reaction were: [Wet *CvFAP@E. coli*] = 0.35 g  $\text{mL}^{-1}$ , [lipase ROL] = 50 mg  $\text{mL}^{-1}$ , [castor oil] = 50 mM, 20–37  $^{\circ}\text{C}$ , 10 W blue LED, 500 rpm, 24 h.



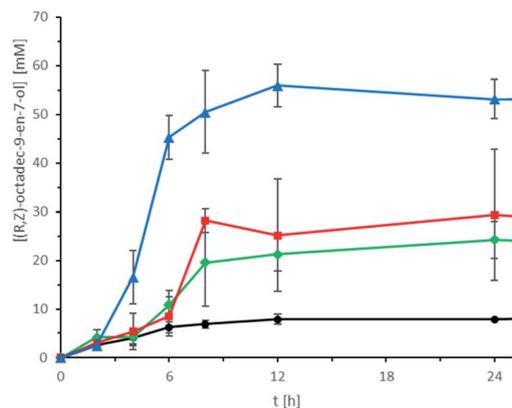


Fig. 11 Influence of the CvFAP concentration on the bienzymatic synthesis of (*R,Z*)-octadec-9-en-7-ol. CvFAP was used as whole cells at ( $0.05 \text{ g L}^{-1}$  (●),  $0.15 \text{ g L}^{-1}$  (◆),  $0.25 \text{ g L}^{-1}$  (■) and  $0.35 \text{ g L}^{-1}$  (▲)). Conditions:  $100\text{--}700 \mu\text{L}$  of Tris-HCl buffer (pH 8.5,  $100 \text{ mM}$ ) containing  $0.05\text{--}0.35 \text{ g wet CvFAP@E. coli}$  and  $50 \mu\text{L}$  of castor oil were added to a transparent glass vial (total reaction volume is  $1 \text{ mL}$ ). The final conditions of this reaction were:  $[\text{Wet CvFAP@E. coli}] = 0.05\text{--}0.35 \text{ g mL}^{-1}$ ,  $[\text{lipase ROL}] = 50 \text{ mg mL}^{-1}$ ,  $[\text{castor oil}] = 50 \text{ mM}$ ,  $30 \text{ }^\circ\text{C}$ ,  $10 \text{ W blue LED}$ ,  $500 \text{ rpm}$ .

probably accelerated hydrolysis rate and thereby the acidification of the aqueous reaction medium.

The temperature dependence of the bienzymatic system differed somewhat on the dependency of the ROL-catalysed hydrolysis (Fig. 5) as a clear optimum at  $30 \text{ }^\circ\text{C}$  was observed (Fig. 10, Table S4,† entries 13–15), which can be rationalised by the comparably poor thermal stability of CvFAP.<sup>23</sup>

This assumption is also supported by the influence of the photodecarboxylase concentration on the overall product concentration. As shown in Fig. 11, a very clear dependency of the overall product concentration on the amount of CvFAP (as whole cells) added to the reaction mixture was observed.

Even though decently high concentrations of the desired (*R,Z*)-octadec-9-en-7-ol have been obtained, one striking observation was that in almost all cases product formation ceased after approx. 8–12 h. This is a clear indication that the robustness of CvFAP soon becomes the overall limitation for the envisaged transformation of castor oil into (*R,Z*)-octadec-9-en-7-ol.<sup>30</sup>

## Conclusions

In the current contribution we have demonstrated the proof-of-concept, scope and limitation of a bienzymatic cascade to transform non-edible castor oil into value added (*R,Z*)-octadec-9-en-7-ol.

Final product concentrations as high as  $60 \text{ mM}$  have been achieved, which may already be sufficient for an economically feasible synthesis of high-value added products. To the best of our knowledge, this contribution represents the first synthesis of (*R,Z*)-octadec-9-en-7-ol, which as chiral, unsaturated alcohol may find various applications ranging from bioactive compound to new lubricant. However, envisioning an application for the synthesis of biofuels, the current system probably

fails in efficiency. Further optimisation of the activity ratios of ROL and CvFAP will be needed to alleviate the acidification issue of the bienzymatic reaction and to eventually increase the product titres. Also the sometimes rather high standard deviations observed in our triplicate experiments necessitate further improvements in the standardisation of the experimental procedure to improve reproducibility. Also the robustness of the photodecarboxylase needs to be improved. Probably, protein engineering represents the most promising approach here.

Overall, provided the aforementioned current limitations have been addressed, we are convinced that the here-proposed valorisation of agricultural by-products may significantly contribute to the development of a more sustainable, bio-based economy.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

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## Notes and references

- M. M. Gui, K. T. Lee and S. Bhatia, *Energy*, 2008, **33**, 1646–1653.
- P. Berman, S. Nizri and Z. Wiesman, *Biomass Bioenergy*, 2011, **35**, 2861–2866.
- N. Sánchez, J. M. Encinar, S. Nogales and J. F. González, *Catalysts*, 2019, **9**, 864.
- G. J. S. Dawes, E. L. Scott, J. Le Notre, J. P. M. Sanders and J. H. Bitter, *Green Chem.*, 2015, **17**, 3231–3250.
- P. M. Könst, M. C. R. Franssen, E. L. Scott and J. P. M. Sanders, *Green Chem.*, 2011, **13**, 1167–1174.
- P. M. Könst, P. M. C. C. D. Turras, M. C. R. Franssen, E. L. Scott and J. P. M. Sanders, *Adv. Synth. Catal.*, 2010, **352**, 1493–1502.
- P. M. Könst, M. C. R. Franssen, E. L. Scott and J. P. M. Sanders, *Green Chem.*, 2009, **11**, 1646–1652.
- Y. Teng, E. L. Scott, A. N. T. van Zeeland and J. P. M. Sanders, *Green Chem.*, 2011, **13**, 624–630.
- R. Kourist, P. Dominguez de Maria and K. Miyamoto, *Green Chem.*, 2011, **13**, 2607–2618.
- C. E. Wise, C. H. Hsieh, N. L. Poplin and T. M. Makris, *ACS Catal.*, 2018, **8**, 9342–9352.
- C. Lu, F. Shen, S. Wang, Y. Wang, J. Liu, W.-J. Bai and X. Wang, *ACS Catal.*, 2018, **8**, 5794–5798.
- J. Wang, R. Lonsdale and M. T. Reetz, *Chem. Commun.*, 2016, **52**, 8131–8133.



- 13 I. Zachos, S. Gassmeyer, D. Bauer, V. Sieber, F. Hollmann and R. Kourist, *Chem. Commun.*, 2015, **51**, 1918–1921.
- 14 M. Pickl, S. Kurakin, F. G. Cantú Reinhard, P. Schmid, A. Pöcheim, C. Winkler, W. Kroutil, S. P. de Visser and K. Faber, *ACS Catal.*, 2018, **9**, 565–577.
- 15 A. Dennig, S. Kurakin, M. Kuhn, A. Dordic, M. Hall and K. Faber, *Eur. J. Org. Chem.*, 2016, 3473–3477, DOI: 10.1002/ejoc.201600358.
- 16 X. M. Xu, A. But, R. Wever and F. Hollmann, *ChemCatChem*, 2020, **12**, 2180–2183.
- 17 A. But, A. van Noord, F. Poletto, J. P. M. Sanders, M. C. R. Franssen and E. L. Scott, *Mol. Catal.*, 2017, **443**, 92–100.
- 18 A. But, J. Le Nôtre, E. L. Scott, R. Wever and J. P. M. Sanders, *ChemSusChem*, 2012, **5**, 1199–1202.
- 19 D. Sorigué, B. Légeret, S. Cuiné, S. Blangy, S. Moulin, E. Billon, P. Richaud, S. Brugière, Y. Couté, D. Nurizzo, P. Müller, K. Brettel, D. Pignol, P. Arnoux, Y. Li-Beisson, G. Peltier and F. Beisson, *Science*, 2017, **357**, 903–907.
- 20 W. Zhang, J.-H. Lee, S. H. H. Younes, F. Tonin, P.-L. Hagedoorn, H. Pichler, Y. Baeg, J.-B. Park, R. Kourist and F. Hollmann, *Nat. Commun.*, 2020, **11**, 2258.
- 21 H. J. Cha, S. Y. Hwang, D. S. Lee, A. R. Kumar, Y. U. Kwon, M. Voss, E. Schuitem, U. T. Bornscheuer, F. Hollmann, D. K. Oh and J. B. Park, *Angew. Chem., Int. Ed.*, 2020, **59**, 7024–7028.
- 22 W. Zhang, M. Ma, M. Huijbers, G. A. Filonenko, E. A. Pidko, M. van Schie, S. de Boer, B. O. Burek, J. Bloh, W. J. H. van Berkel, P. W. A. Smith and F. Hollmann, *J. Am. Chem. Soc.*, 2019, **141**, 3116–3120.
- 23 Y. Ma, X. Zhang, W. Zhang, P. Li, Y. Li, F. Hollmann and Y. Wang, *ChemPhotoChem*, 2020, **4**, 39–44.
- 24 M. Huijbers, W. Zhang and F. Hollmann, *Angew. Chem., Int. Ed.*, 2018, **57**, 13648–13651.
- 25 D. J. Heyes, B. Lakavath, S. J. O. Hardman, M. Sakuma, T. M. Hedison and N. S. Scrutton, *ACS Catal.*, 2020, 6691–6696, DOI: 10.1021/acscatal.0c01684.
- 26 H. Mutlu and M. A. R. Meier, *Eur. J. Lipid Sci. Technol.*, 2010, **112**, 10–30.
- 27 S. Allauddin, R. Narayan and K. V. S. N. Raju, *ACS Sustainable Chem. Eng.*, 2013, **1**, 910–918.
- 28 J. Fiorelli, D. D. Curtolo, N. G. Barrero, H. Savastano, E. M. de Jesus Agnolon Pallone and R. Johnson, *Ind. Crops Prod.*, 2012, **40**, 69–75.
- 29 L. Zhang, M. Zhang, L. Hu and Y. Zhou, *Ind. Crops Prod.*, 2014, **52**, 380–388.
- 30 B. Lakavath, T. M. Hedison, D. J. Heyes, M. Shanmugam, M. Sakuma, R. Hoeven, V. Tilakaratna and N. S. Scrutton, *Anal. Biochem.*, 2020, **600**, 113749.

