Supplementary file

Synthesis of linoleic acid 13-hydroperoxides from safflower oil utilizing lipoxygenase in a coupled enzyme system with *in situ* oxygen generation

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Figure S1

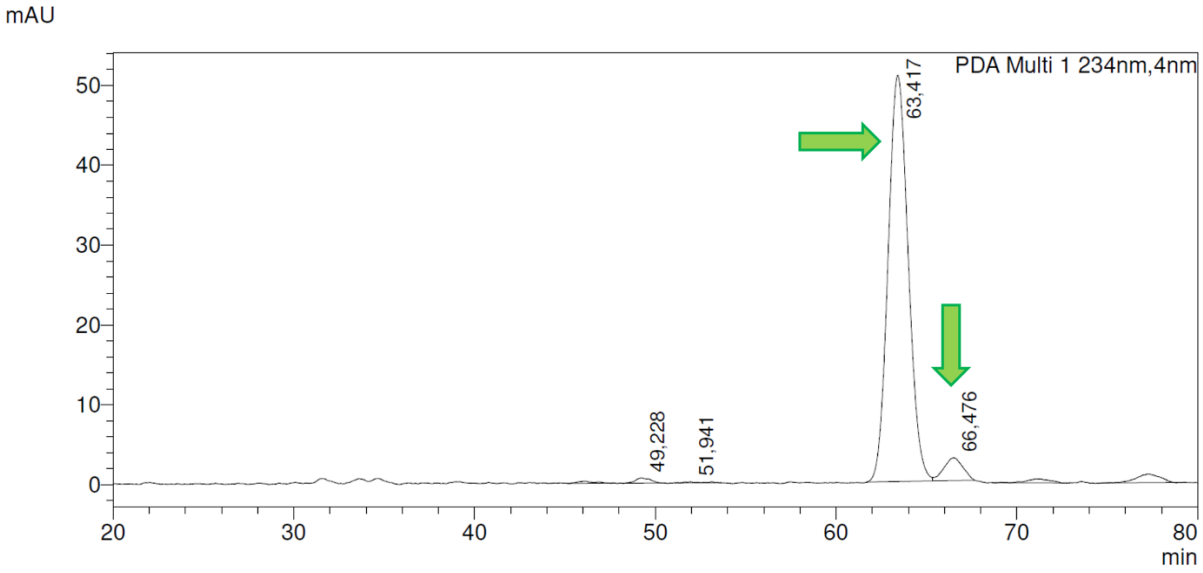
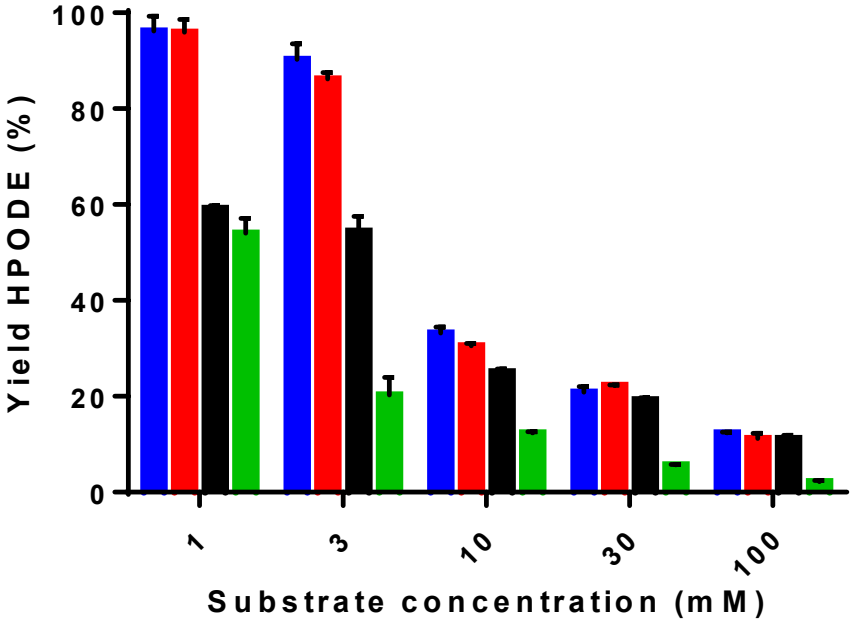


Figure S1. Exemplary chromatogram of the HPLC separation of regioisomers**,** peak assignment according to Larodan reference standards are 13-hydroxyoctadecadienoic acid (13-HODE) at 49.2 min, 9-hydroxyoctadecadienoic acid (9-HODE) at 51.9 min, 13-hydroperoxyoctadecadienoic acid (13-HPODE) at 63.4 min and 9-hydroperoxyoctadecadienoic acid (9-HPODE) at 66.4 min.

Figure S2

A)



B)

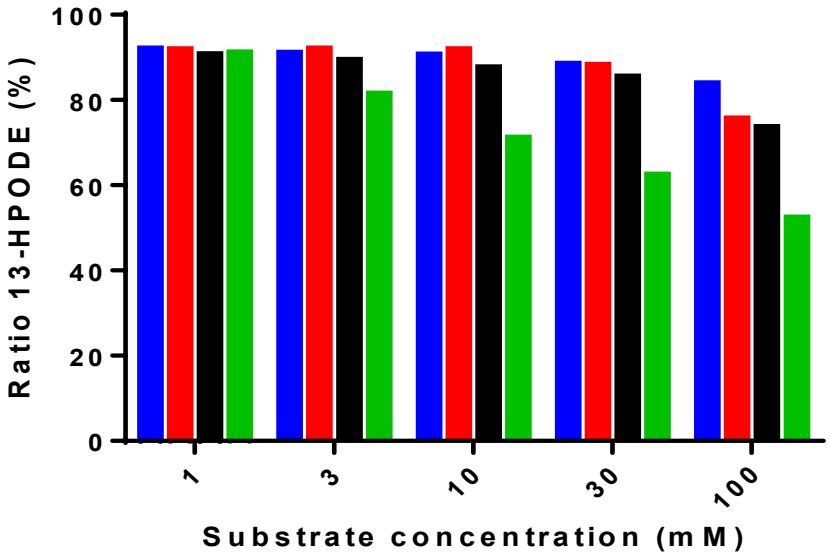


Figure S2. Effects of the linoleic acid concentration and oxygen flowrate on **A)** product formation and **B)** isomeric ratio of 13-HPODE by LOX-1. Influence of 100 ml/min oxygen flowrate (■), 50 ml/min oxygen flowrate (■), 20 ml/min oxygen flowrate (■) and without any oxygen supply (■) The reactions were carried out in 50 mM sodium borate buffer pH 9.0 containing 20,000 U/mmol substrate at different linoleic acid concentrations for 2 h.

Figure S3

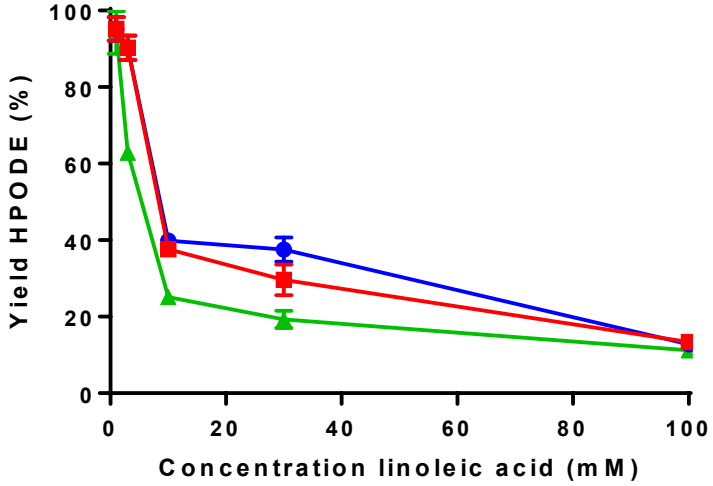
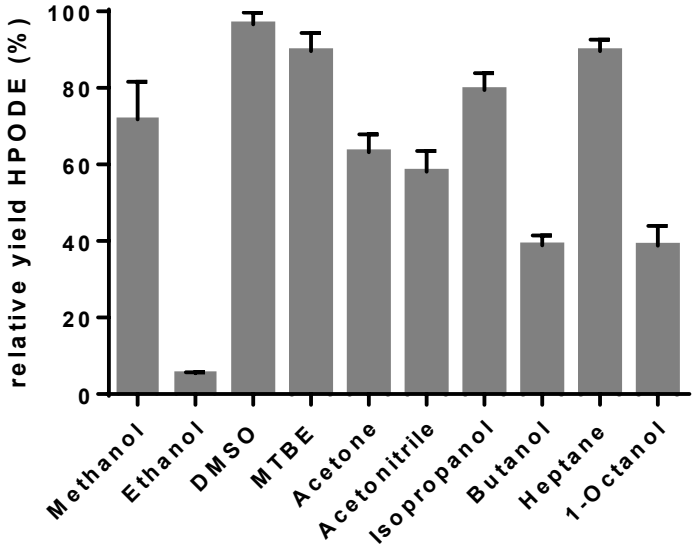


Figure S3. Influence of ultra-turrax (●) and ultrasonic (■) treatment in comparison to stirring (▲). The reactions were carried out in 50 mM sodium borate buffer pH 9.0 containing 20,000 U/mmol of substrate at different linoleic acid concentrations for 2 h.

Figure S4

A)



B)

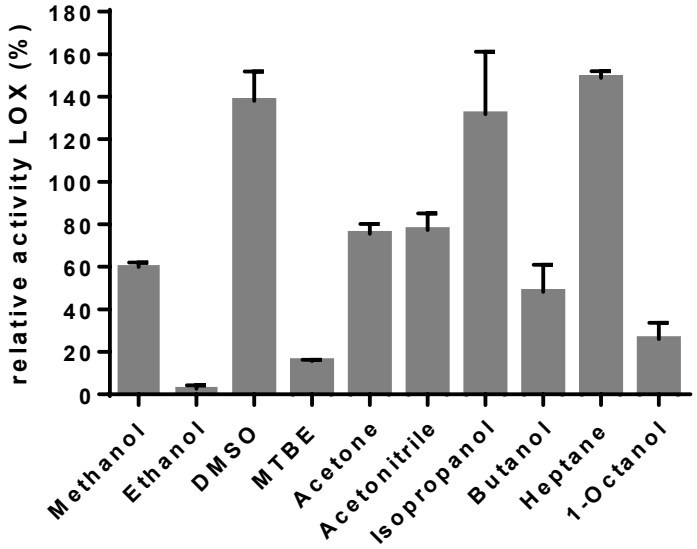


Figure S4. Overview of relative yield and relative initial activity when adding solvents in comparison to a system without solvent; **A)** Relative yield after 2 h reaction time in comparison to reference (35 % yield). **B)** Initial enzyme activity of LOX after addition of different solvents in a concentration of 5 %. (v/v). Addition of DMSO, MTBE and Heptane led to the highest overall yield in comparison to the addition of other solvents. When adding 5 % (v/v) Isopropanol, heptane oder DMSO, LOX shows an increased initial activity in comparison to reference.

Figure S5

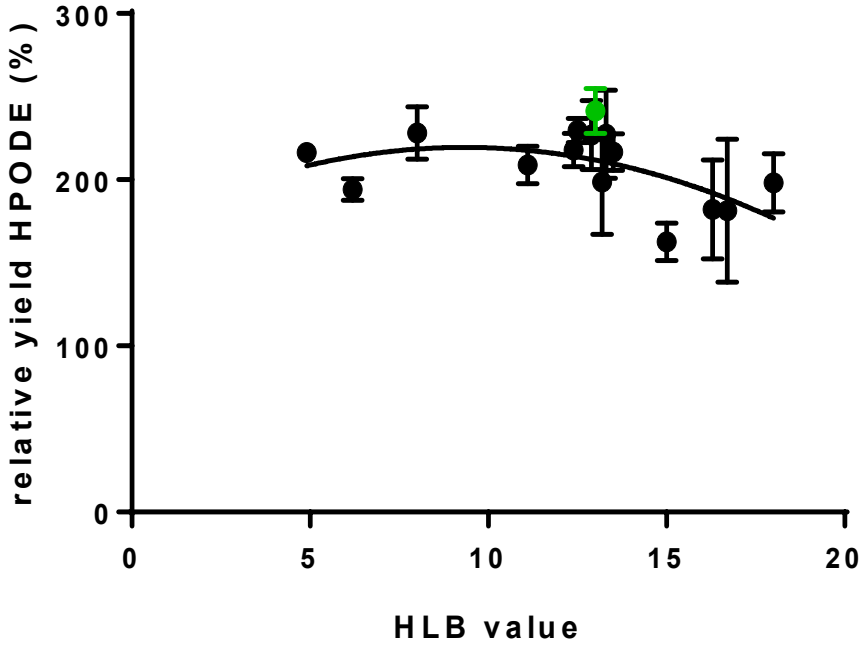
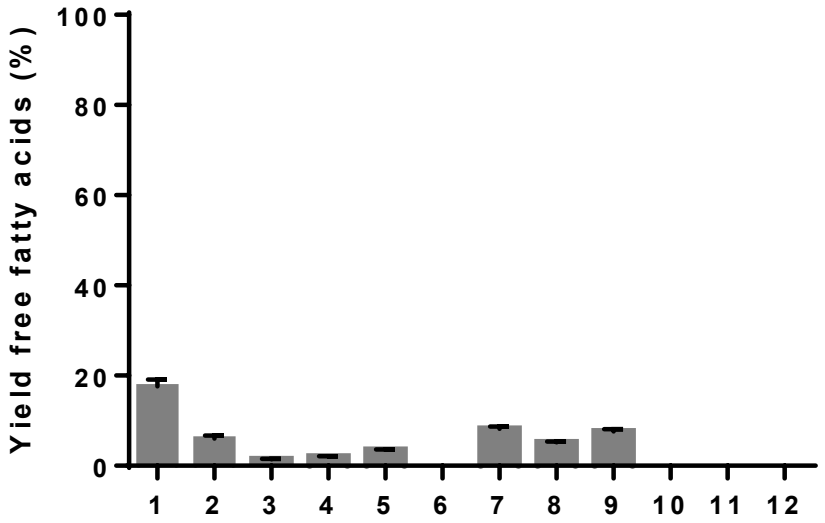


Figure S5. Influence of surfactant addition on HPODE yield; (a) HPODE yields in dependence of HLB-values of surfactants added to linoleic acid transformation at 100 mM substrate concentration. Relative yield of HPODE in the presence of Triton-CG 110 (●). Relative Yields were calculated against the reaction without surfactant. The detail results for each surfactant are given in Table 1.

Figure S6

A)



B)

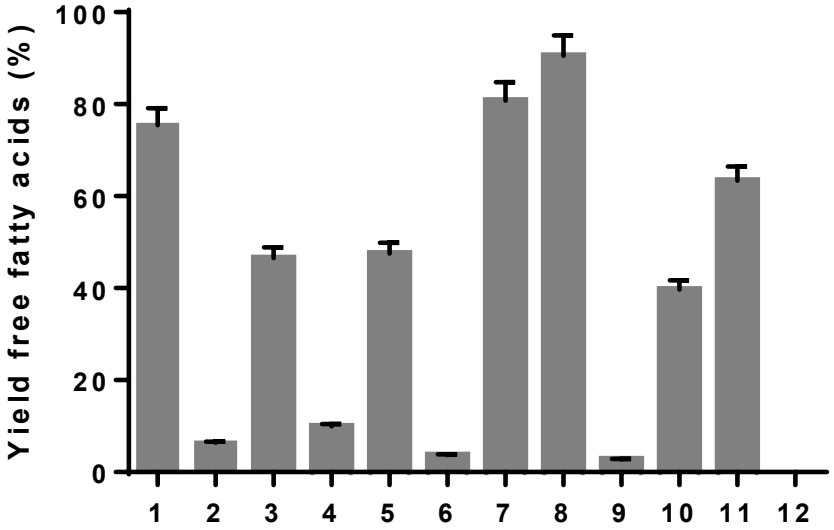


Figure S6. Lipase screening with safflower oil equivalent to 30 mM linoleic acid in the presence of 3 % Triton CG-110 (v/v substrate) and 5 % lipase (w/v substrate) in **A)** borate buffer pH 9 and **B)** Tris HCl buffer pH 8 with 1) Amano (*P. fluorescens*). 2) Amano G (*P. camemberti*). 3) Amano PS (*B. cepacia*). 4) Novozymes Lipolase 100 L (*T. lanugenosis*). 5) Novozymes Eversa Transform 2.0. 6) Novozymes CalB L (*P. antarctica*). 7) Amano AY 30 (*C. rugosa*). 8) Lipase CCL (*C. rugosa*). 9) Palatase 20000 L. 10) Amano F-AP 15. 11) Amano M (*M. javanicus*). 12) Amano A (*A. niger*).

Figure S7

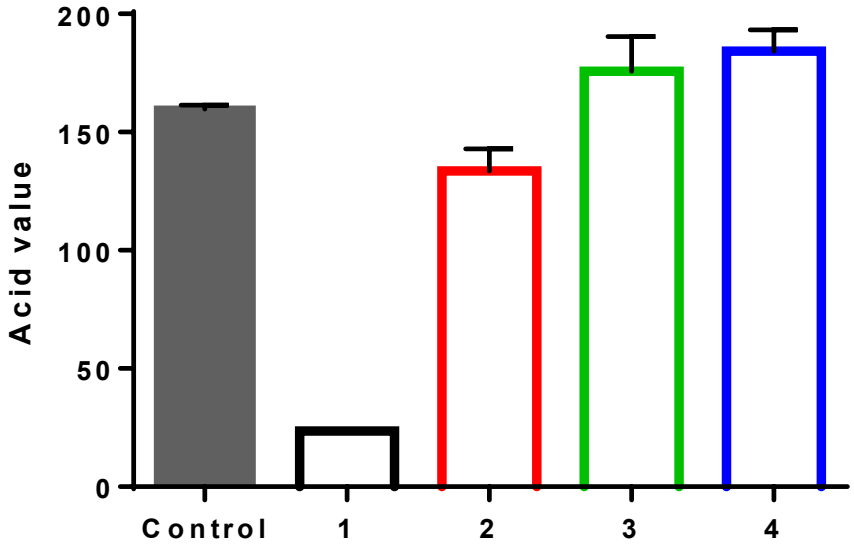


Figure S7. Acid values after termination of the reactions shown in Figure 4 (a), same color scheme was used ■ = safflower oil hydrolysis control reaction without addition of LOX-1.

Figure S8

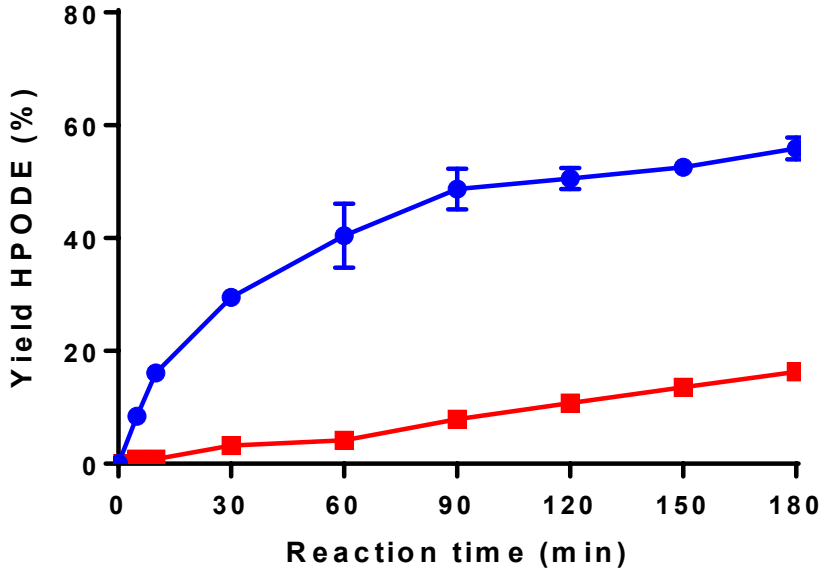


Figure S8. Comparison of linoleic acid (●) and monolinolein (■) transformation with 100,000 U LOX-1 / mmol of substrate.

Figure S9

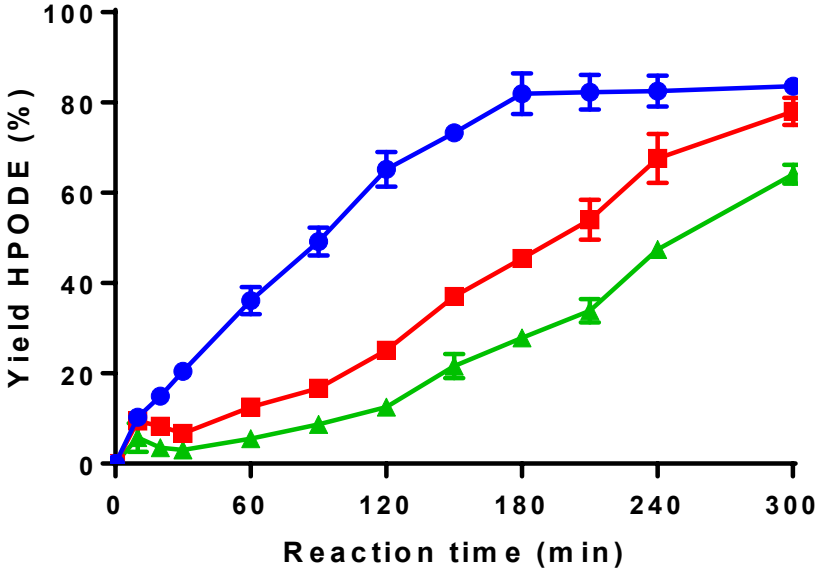


Figure S9. Comparison of HPODE yield with (▲) 2,000, (■) 5,000 and (●) 10,000 U LOX/mmol substrate dosage per h. Reaction was performed with safflower oil equivalent to 30 mM linoleic acid in the presence of 3 % Triton CG-110 (w/v substrate) at pH 8 with 10 % (w/v) *P. fluorecens* lipase and 50 ml/min O_2 flow rate.

Figure S10

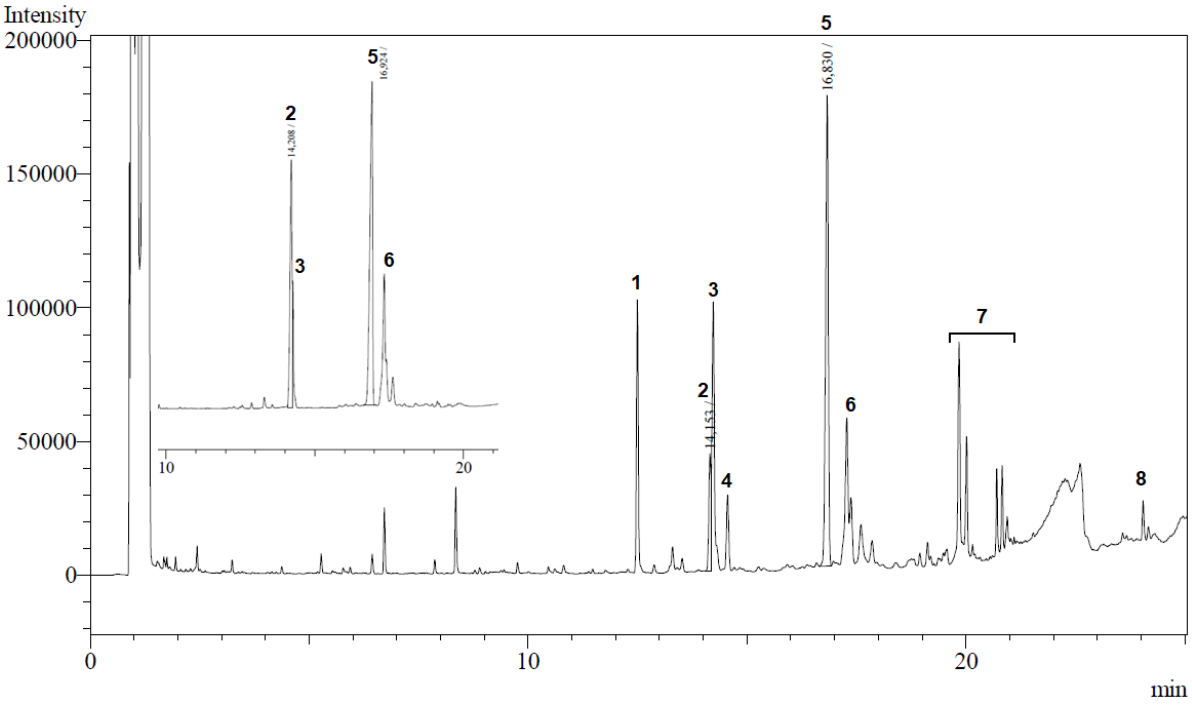


Figure S10. Comparative high-temperature GC analysis of the hydroperoxidation products after hydrogenation and silylation starting with safflower oil and linoleic acid (small cutout) – conditions with LOX-1 dosage as shown in Figure 4 a. Peak assignment was done with reference compounds: **(1)** palmitic acid, **(2)** linoleic acid, **(3)** oleic acid, **(4)** stearic acid, **(5)** hydroxyoctadecadienoic acid (HODE), **(6)** oxygenated byproducts, **(7)** monoglycerides, **(8)** diglycerides.

Figure S11

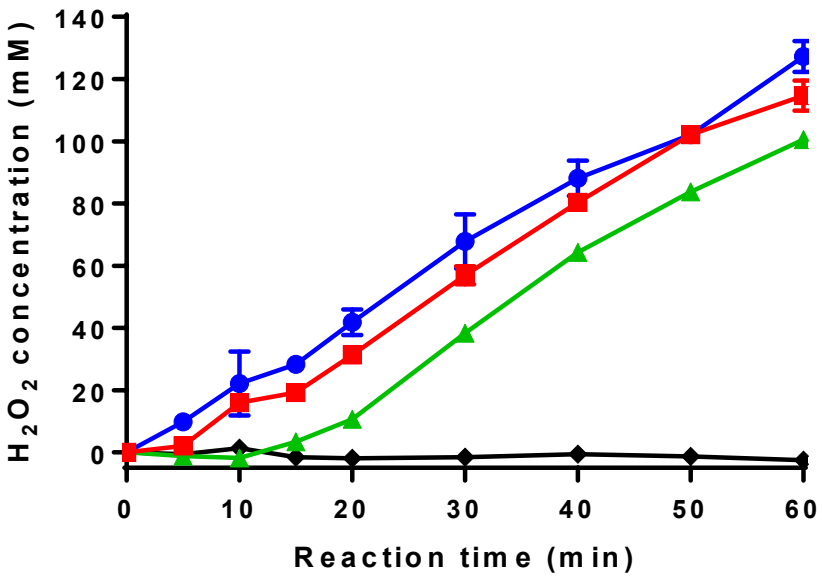


Figure S11. Evaluation of minimum amounts of catalase from *M. lysodeikticus* necessary to continuously remove H_2O_2 at a flowrate equivalent to 50 ml/min oxygen bubbling over 60 min with ● = no catalase added, ■ = 100 U/µmol, ▲ = 300 U/µmol and ◆ = 1000 U/µmol

Figure S12

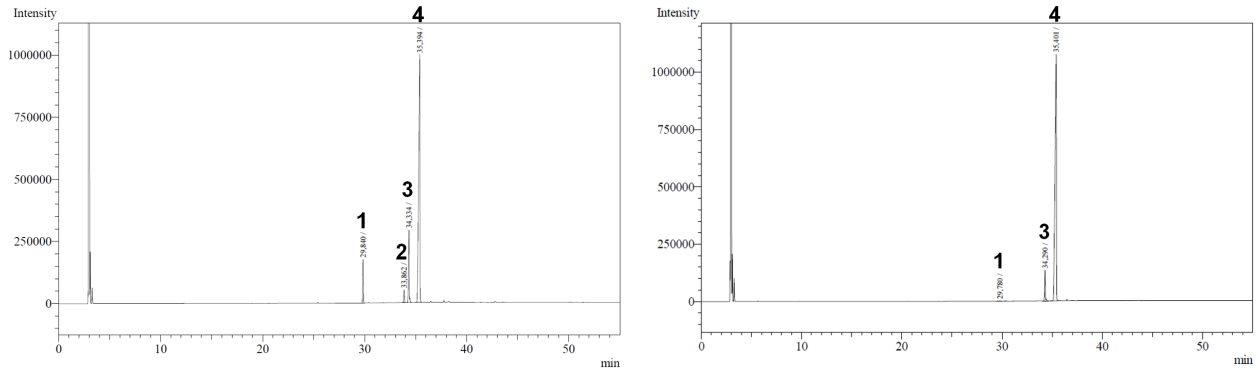


Figure S12. Comparative GC analysis of hydroperoxidation substrates after methylation with safflower oil (left) and urea-enriched linoleic acid (right). Peak assignment was done with reference compounds: **(1)** palmitic acid, **(2)** stearic acid, **(3)** oleic acid, **(4)** linoleic acid.