Supplementary Information

An enzymatic tandem reaction to produce odor-active fatty aldehydes

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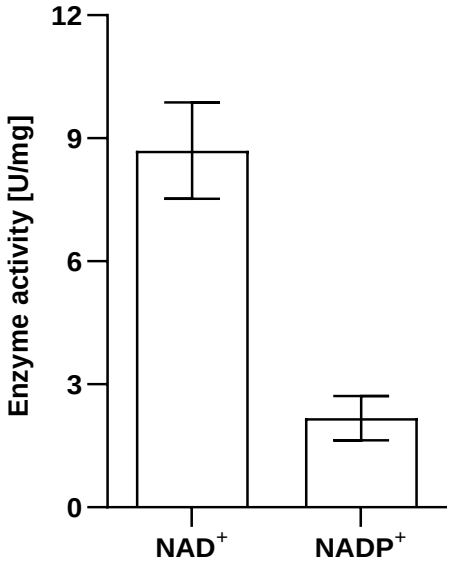

Fig. S1 Comparison of enzyme activities of *Vh*FALDH with cofactors NAD^+ *vs.* $NADP^+$. Error bars indicate standard deviations (GraphPad Prism 8)

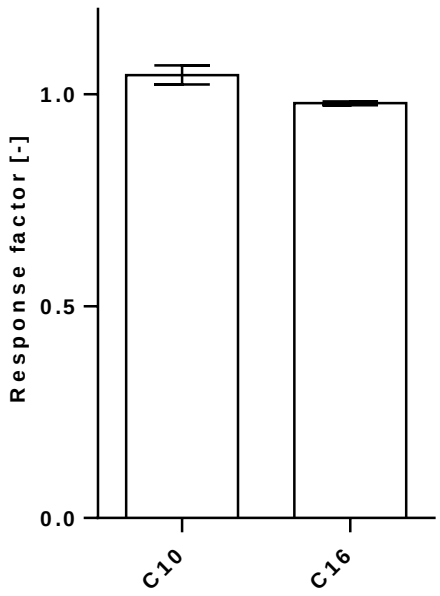

Fig. S2 Relative response factors of two commercially available unsaturated fatty aldehydes and their corresponding saturated counterparts. C10 depicts (*Z*)-7-decenal and decanal, C16 (*Z*)-11-hexadecenal and hexadecanal. Error bars indicate standard deviations. Results showed response factors of ~ 1.0 which indicated a marginal difference of mass spectrometric response of saturated and *Z*-unsaturated aldehydes of the same carbon chain lengths. Thus, saturated counterparts were considered as suitable for approximate quantitation of *Z*-unsaturated aldehydes (GraphPad Prism 8)

Table S1 Fatty acid composition of sea buckthorn pulp oil was determined by means of fatty acid methyl ester derivatization and subsequent instrumental analysis in terms of GC–MS according to the method described by Hammer et al. (2021)

no.	fatty acid (as fatty acid methyl ester)	relative share [%]
1	14:0	0.3
2	14:1(9*Z*)[a]	< 0.1
3	14:1(11*Z*)[a]	< 0.1
4	15:0	< 0.1
5	16:0	32.3
6	16:1(9*Z*)	25.9
7	16:1(11*Z*)	0.3
8	16:2(9*Z*,12*Z*)	< 0.1
9	17:0	< 0.1
10	17:1(9*Z*)	0.1
11	18:0	1.2
12	18:1(9*Z*)	25.0
13	18:1(11*Z*)	9.9
14	18:2(9*Z*,12*Z*)	3.2
15	18:3(9*Z*,12*Z*,15*Z*)	1.0
16	20:0	0.39
17	20:1(11*Z*)	0.2
18	22:0[a]	< 0.1

[a] identity not unambiguously clarified due to limited validity of mass spectrometric fragmentation pattern.

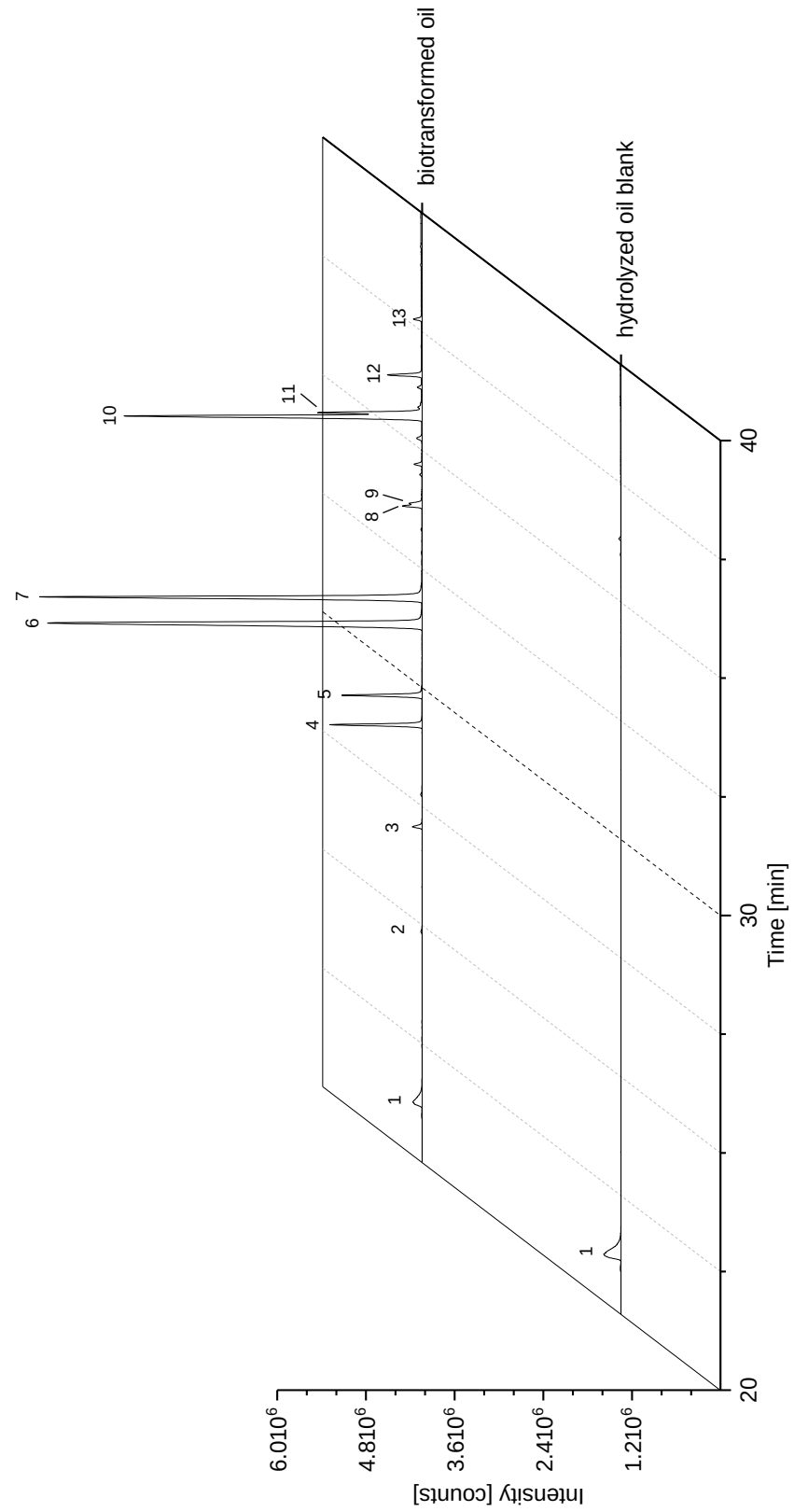

Fig. S3 GC–MS chromatograms of sea buckthorn pulp oil biotransformed with *Csα*-DOX and *Vh*FALDH (top) and corresponding non-biotransformed oil (lipase treated). **1** internal standard (*Z*)-7-decenal; **2** dodecanal; **3** tridecanal; **4** tetradecanal; **5** (*Z*)-5-tetradecenal + (*Z*)-7-tetradecenal; **6** pentadecanal; **7** (*Z*)-5-pentadecenal + (*Z*)-8-pentadecenal; **8** (*Z*)-7-hexadecenal; **9** (*Z*)-9-hexadecenal; **10** (*Z*)-8-heptadecenal; **11** (*Z*)-10-heptadecenal; **12** (*Z*,*Z*)-8,11-heptadecadienal; **13** (*Z*,*Z*,*Z*)-8,11,14-heptadecatrienal (OriginPro 2022)

Table S2 Identified Paternó-Büchi (PB) oxetane adducts along with their deduced diagnostic fragments (α- and ω-ions) during nanoESI–online–PB–MS/MS–analysis of the reaction mixture of oleic acid (see chapter "Identification"). All detected *m/z* signals are assigned in **bold** and showed a maximum deviation of ±5 ppm from theoretical values

compound	*m/z* [PB adduct]	*m/z* [α-ion]	*m/z* [ω-ion]
octadec-9-enoic acid	**404.3159**	262.1802	**232.2060**
heptadec-8-enoic acid	**390.3003**	248.1645	**232.2060**
hexadec-7-enoic acid	**376.2846**	234.1489	**232.2060**
pentadec-6-enoic acid	**362.2690**	220.1332	**232.2060**
tetradec-5-enoic acid	**348.2533**	206.1176	**232.2060**
tridec-4-enoic acid	**334.2377**	192.1019	**232.2060**
heptadec-8-enal	**374.3054**	**232.1696**	**232.2060**
hexadec-7-enal	**360.2897**	218.1539	**232.2060**
pentadec-6-enal	**346.2741**	204.1383	**232.2060**
tetradec-5-enal	**332.2584**	190.1226	**232.2060**
tridec-4-enal	**318.2428**	176.1070	**232.2060**

Table S3 Identified PB oxetane adducts along with their deduced diagnostic fragments (α- and ω-ions) during nanoESI–online–PB–MS/MS–analysis of the reaction mixture of linoleic acid (see chapter "Identification"). All detected *m/z* signals are assigned in **bold** and showed a maximum deviation of ± 5 ppm from theoretical values

compound	*m/z* [PB adduct]	*m/z* [α-ion 1]	*m/z* [α-ion 2]	*m/z* [ω-ion 1]	*m/z* [ω-ion 2]
octadeca-9,12-dienoic acid	**402.3003**	302.2115	262.1802	**190.1590**	**230.1903**
heptadeca-8,11-dienoic acid	**388.2846**	288.1958	248.1645	**190.1590**	**230.1903**
hexadeca-7,10-dienoic acid	**374.2690**	274.1802	234.1489	**190.1590**	**230.1903**
pentadeca-6,9-dienoic acid	**360.2533**	260.1645	220.1332	**190.1590**	**230.1903**
tetradeca-5,8-dienoic acid	**346.2377**	246.1489	206.1176	**190.1590**	**230.1903**
heptadeca-8,11-dienal	**372.2897**	272.2009	232.1696	**190.1590**	**230.1903**
hexadeca-7,10-dienal	**358.2741**	258.1852	218.1539	**190.1590**	**230.1903**
pentadeca-6,9-dienal	**344.2584**	244.1696	204.1383	**190.1590**	**2301903**
tetradeca-5,8-dienal	**330.2428**	230.1539	190.1226	**190.1590**	**230.1903**

Table S4 Identified PB oxetane adducts along with their deduced diagnostic fragments (α- and ω-ions) during nanoESI–online–PB–MS/MS–analysis of the reaction mixture of palmitoleic acid (see chapter "Identification"). All detected *m/z* signals are assigned in **bold** and showed a maximum deviation of ± 5 ppm from theoretical values

compound	*m/z* [PB adduct]	*m/z* [α-ion]	*m/z* [ω-ion]
hexadec-9-enoic acid	**376.2846**	262.1802	**204.1747**
pentadec-8-enoic acid	**362.2690**	**248.1645**	**204.1747**
tetradec-7-enoic acid	**348.2533**	234.1489	**204.1747**
tridec-6-enoic acid	**334.2377**	220.1332	**204.1747**
dodec-5-enoic acid	**320.2220**	206.1176	**204.1747**
pentadec-8-enal	**346.2741**	232.1696	**204.1747**
tetradec-7-enal	**332.2584**	218.1539	**204.1747**
tridec-6-enal	**318.2428**	204.1383	**204.1747**
dodec-5-enal	**304.2271**	190.1226	**204.1747**

References

Hammer AK, Emrich NO, Ott J, Birk F, Fraatz MA, Ley JP, Geissler T, Bornscheuer UT, Zorn H (2021) Biotechnological production and sensory evaluation of ω1-unsaturated aldehydes. J. Agric. Food Chem. 69:345–353