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1 **Lipid-modifying enzymes in oat and faba bean**

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10 **Abstract**

11

12 The aim was to study lipase, lipoxygenase (LOX) and peroxygenase (POX) activities in oat and
13 faba bean samples to be able to evaluate their potential in formation of lipid-derived off-flavours.
14 Lipase and LOX activities were measured by spectroscopy, and POX activities via the formation of
15 epoxides. An ultra-high performance liquid chromatography method was developed to study the
16 formation of fatty acid epoxides. The epoxides of esters were measured by gas chromatography.
17 Mass spectroscopy was used to verify the identity of the epoxides. Both oat and faba bean
18 possessed high lipase activities. In faba bean, LOX catalysed the formation of hydroperoxides,
19 whose break-down products are the likely cause of off-flavours. Since oat had low LOX activity,
20 autoxidation is needed to initiate lipid oxidation. Oat had high POX activity, which is able to
21 convert hydroperoxides to epoxy and hydroxy fatty acids that could contribute significantly to off-
22 flavours. POX activity in the faba bean was low. Thus, in faba bean volatile lipid oxidation products
23 could rapidly be formed by LOX, whereas in oat reactions are slower due to the need of
24 autoxidation prior to further reactions.

25

26 **Keywords:** Oat; Faba bean; Lipase; Lipoxygenase; Peroxygenase; Off-flavour; Analysis of fatty
27 acid epoxides

28 **1. Introduction**

29 Oat (*Avena sativa*) and faba bean (*Vicia faba L.*) are good candidates when new sources of plant
30 proteins and contributors to the sustainable development in agricultural and food systems are looked
31 for. Oat has long been recognised as a valuable source of many nutrients for humans and animals
32 (Decker, Rose, & Stewart, 2014). The faba bean is one of the leading grain legumes in the world,
33 and it is mainly used as a rich source of protein, but it also contains lots of starch (Crépon et al.,
34 2010; Lizarazo et al., 2015). One problem associated with using plant proteins in foods is formation
35 of lipid-derived off-flavours, which diminish the consumer acceptance of these products. Some of
36 the off-flavours develop immediately through the action of enzymes, whereas some occur only after
37 long-term storage. To control enzymatic reactions, the inactivation of lipid-modifying enzymes by
38 heat treatment is essential (Decker et al., 2014; Jiang et al., 2016; Lehtinen, Kiiliäinen, Lehtomäki,
39 & Laakso, 2003; Roland, Pouvreau, Curran, van de Velde, & de Kok, 2017). However, overly
40 severe treatments should be avoided because they may cause problems in the sensory and
41 technological properties of the products.

42
43 One lipid-modifying enzyme is lipase, which liberates free fatty acids (FFAs) from their esters. The
44 liberated fatty acids may be oxidised chemically or enzymatically into odourless and tasteless
45 hydroperoxides. The endogenous lipases will start hydrolysing acyl lipids as soon as the seed
46 structure breaks down and the enzyme comes into contact with its substrates. The function of
47 lipases during the processing and storage of oat has been well characterised (Decker et al., 2014;
48 Lehtinen et al., 2003). A lipase has also been isolated from small faba bean (Dundas, Henderson, &
49 Eskin, 1978) and a sequential hydrolytic pathway has been proposed (Henderson, Shambrock, &
50 Eskin, 1981). However, little is known of lipase activity and its impact in faba bean.

51

52 Lipoxygenase (LOX) is the enzyme responsible for the formation of hydroperoxides, which in turn
53 can further react to form volatile off-flavours, such as hexanal, or non-volatile products, such as
54 oxoacids and dimers (Gardner, 2003) through several chemical and enzymatic pathways. LOX
55 activity in legumes is regarded as responsible for the undesirable “beany flavour” through the
56 break-down of its hydroperoxide products (Gardner, 2003; Roland et al., 2017). The faba bean has
57 been categorised as possessing medium-level LOX activity among legumes (Chang & McCurdy,
58 1985). Two LOX isoenzymes have been characterised from the faba bean (Clemente, Olías, &
59 Olías, 2000). Much less is known about LOX activities in cereal grains. In a review paper, Lehtinen
60 and Kaukovirta-Norja (2011) reported that LOX activity in oat was lower than in, for example,
61 wheat and barley.

62

63 Peroxygenase (POX) catalyses the hydroperoxide-dependent conversion of unsaturated fatty acids
64 into non-volatile flavour compounds, namely epoxy and hydroxy fatty acids (Hamberg & Hamberg,
65 1996). The POX pathway begins when one of the oxygens of a fatty acid hydroperoxide is
66 transferred to an unsaturated fatty acid, yielding a hydroxy fatty acid and an epoxy fatty acid. These
67 compounds have been suggested to be responsible for the formation of the bitter taste in aged oat
68 products (Hamberg & Hamberg, 1996; Doeblert, Angelikousis, & Vick, 2010). POX has been
69 isolated from oat seeds (Hamberg & Hamberg, 1996). Currently, oat POX has important potential in
70 the production of fatty acid epoxides in the chemical industry (Piazza, Nuñez, & Foglia, 2003).
71 Hamberg and Fahlstadius (1992) have also observed hydroperoxide-dependent epoxidation of
72 unsaturated fatty acids in faba bean. However, little research has been done on the POX activity in
73 either oat or faba bean foods.

74

75 Although detrimental effects of lipase on oat lipids and of LOX on faba bean lipids are well
76 described (Piazza, Bilyk, Brower, & Haas, 1992; Clemente et al., 2000; Lehtinen & Kaukovirta-

77 Norja, 2011), there is very little knowledge on the overall effects of the lipid-modifying enzymes on
78 oat and faba bean products, and the levels and variation of enzyme activities present in the seeds.
79 Understanding and controlling the lipid-modifying enzymes is essential to prolong the shelf life of
80 the products and raise consumer acceptance towards them.

81

82 The aim of the study was to understand the potential of lipase, LOX and POX activities present in
83 oat and faba bean seeds and to evaluate their possible contribution to the formation of compounds
84 related to lipid-derived off-flavours. The enzyme activities were studied in seeds from several
85 cultivars and growing seasons. Lipase and LOX activities were measured using spectrophotometric
86 methods, whereas methods to study POX activities in various substrates were developed.

87

88 **2. Materials and methods**

89 *2.1. Chemicals and standards*

90 Substrates for the enzyme activity analyses, including methyl oleate (purity > 99%), methyl
91 linoleate (purity > 99%), methyl linolenate (purity > 99%), triolein (purity > 99%), oleic acid
92 (purity > 99%), linoleic acid (purity > 99%), linolenic acid (purity > 99%), as well as the internal
93 standards, nonadecanoic acid (purity > 99%) and its methyl ester (purity > 99%), were purchased
94 from Nu-Check Prep, Elysian, MN, USA. Cumene hydroperoxide (80%) and para-nitrophenyl
95 butyrate (purity $\geq$ 98%) were obtained from Sigma-Aldrich (St. Louis, USA). The epoxy stearic
96 acid used for the quantification of epoxy fatty acids was obtained from Santa Cruz Biotechnology®
97 (Texas, USA). All other reagents were purchased from Sigma-Aldrich and Merck (Darmstadt,
98 Germany). Water was purified using the Milli-Q system (Millipore Corp., Bedford, MA, USA).

99

100 *2.2. Oat and faba bean samples*

Commercial oat flour from the Raisio Group (Nokia, Finland) and faba bean flour milled from the cultivar “Kontu”, grown in the year 2011 at the Viikki Experimental Farm (Lizarazo et al., 2015), were used as in-house reference samples to verify the analytical level of the measurements. The oat flour was used for POX activity measurements, and the faba bean flour for lipase and LOX activity measurements. Oat grain samples (cultivars Akseli, Alku, Meeri and Steinar) were provided by Boreal Plant Breeding Ltd. (Jokioinen, Finland). They were grown in the cultivation years 2012, 2013 and 2014 in the same area in southern Finland and were stored at 10–15 °C. The faba bean samples (cultivars Kontu, Alexia, Fatima and SSNS-1) were obtained from the Viikki Experimental Farm of the University of Helsinki in southern Finland from three cultivation years. Samples from the years 2011 and 2015 were stored at 5 °C, while the samples from the year 2010 were stored at room temperature (RT). Detailed information on the faba bean samples and weather conditions was given by Lizarazo et al. (2015). All seed samples were milled using a Centrifugal Mill ZM200 (Retsch, Haan, Germany) fitted with a 0.5-mm sieve, after which the samples were immediately transferred to a freezer and stored at –20 °C before analysis.

115

116 *2.3. Enzyme extraction from oat and faba bean flour*

Enzymes were extracted from the milled oat and faba bean samples. The flours (2.5 g) were mixed with 23 ml of cold 0.1 M potassium phosphate buffer (pH 6.7) in centrifugation tubes, vortexed (4 x 10 s) and kept on ice for 1 h. The slurries were centrifuged at 4 °C for 10 min at 9000 x g (Sorvall RC5C, SLA-1500 rotor), the sediments were discarded and the supernatants were centrifuged for another 10 min. The supernatants were kept on ice before being used for enzyme activity analyses. Each sample was extracted in duplicate and one in-house reference sample was included in each extraction batch. All enzyme activity measurements were done in triplicate from each extract.

124

125 *2.4. Lipase activity measurement*

126 Lipase activity was measured using a spectrophotometric method with para-nitrophenyl butyrate as
127 a substrate (Brunschwiler, Heine, Kappeler, Conde-Petit, & Nyström, 2013). The reaction was
128 initiated by adding sample extracts into fresh substrate solutions of 2 mM para-nitrophenyl butyrate
129 in 50 mM potassium phosphate buffer containing 0.1% Triton X-100 (pH 8.0). To achieve a
130 minimal slope (dA/min of 0.03), 20 μ l of faba bean extract and 80 μ l of oat extract were mixed with
131 the substrate solution (total volume of 1.00 ml). The increase in the absorption at 405 nm during
132 150 s was measured with the ultraviolet spectrometer (Lambda 25 UV/Vis, Perkin Elmer Inc.,
133 USA). The molar extinction coefficient value of 16.05 mM⁻¹ cm⁻¹ for hydrolysed para-nitrophenol
134 was used to calculate the results. Lipase activity was given as μ mol min⁻¹ g⁻¹ flour.

135

136 2.5. LOX activity measurement

137 LOX activity was measured by a spectrophotometric method using linoleic acid as the substrate
138 (Jiang et al., 2016). For the LOX assays, 200 μ l of the substrate solution with 10 mM of linoleic
139 acid, 200 μ l of the sample extracts and 2.6 ml of 0.1 M potassium phosphate buffer (pH 6) were
140 mixed and incubated for 3 min at 30 °C in a water bath. The reaction was stopped by adding 3 ml of
141 0.1 N KOH solution and the absorbances were measured at 234 nm (Lambda 25 UV/Vis, Perkin
142 Elmer Inc., USA). The results were calculated using the molar absorptivity of conjugated dienes
143 (ϵ = 26 000 l/ mol cm). The LOX activity was expressed as mmol min⁻¹ g⁻¹ flour.

144

145 2.6. Method development for POX activity measurement

146 The POX activity was studied by measuring epoxide formation. FFAs, their methyl esters and
147 triolein were used as substrates to observe the specificities of the enzyme in the oat and faba bean
148 samples. Two methods were developed, because epoxides of esters could be analyzed by GC
149 whereas it was not applicable to epoxides of free fatty acids, for which an UHPLC method was
150 developed.

151

152 *2.6.1. GC-FID/MS method for studying fatty acid methyl esters and triolein as substrates*

153 Methyl oleate, methyl linoleate and methyl linolenate were used as methyl ester substrates for POX.

154 Aliquots of 435 µl of mixtures containing one of the substrates (ca. 3 mg/ml) and the internal

155 standard, methyl nonadecanoate (1.4 mg/ml) in heptane, were first evaporated under N₂ at 35 °C,

156 and then the residues were dissolved in 350 µl of 1 % Tween 20 in Milli-Q water by vortexing. To

157 the substrate solutions 1.75 ml of 100 mM HEPES at pH 7.0, 900 µl of Milli-Q water and 500 µl of

158 the sample extracts were added. To start the enzymatic reaction, 7 µl of 8 % cumene hydroperoxide

159 diluted in ethanol was added and the tubes were mixed by inverting with a rotator for 1 h at RT,

160 after which the reaction was stopped by adding 5 ml of methanol. The lipids were extracted twice

161 with 10 ml of diethyl ether and the extracts were washed with 5 ml of saturated NaCl in Milli-Q

162 water. Aliquots of 10 ml were dried under N₂ evaporation at 35 °C. The residues were immediately

163 re-dissolved into 1 ml of heptane. To study triolein as the substrate, the assay was carried out

164 following the same steps as mentioned above, except that the final dried residues were re-dissolved

165 into 1.5 ml of heptane, after which the substrates and the products were methylated by alkaline

166 transesterification (Christie, 1993).

167

168 Finally, fatty acid methyl esters and their epoxides were analysed by GC. A GC-FID (Agilent

169 6890N, USA) equipped with a fused silica capillary column OmegawaxTM 1250 (30 m × 0.25 mm ×

170 0.25 µm, SUPELCO[®], Bellefonte, USA) was used for quantitative analysis, and a GC (HP 6890

171 series, Agilent Technologies Inc., Wilmington, DE, USA) with an MS detector (Agilent 5973

172 Network, Agilent Technologies Inc., Wilmington, DE, USA) equipped with the same capillary

173 column was used for the identification of the epoxides. In the GC-FID analysis, helium was used as

174 a carrier gas at a flow rate of 1.1 ml/min and 1-µl samples were injected in a split mode (1:15) at

175 240 °C. The oven temperature was programmed to increase from 160 °C (with a 1-min initial hold)

176 with a 4 °C/min to 240 °C (with a 15-min final hold). The GC-MS analysis was conducted
177 similarly, except that the split ratio was 1:20, and the oven temperature was programmed to increase
178 from 150 °C (with a 2-min initial hold) with a 4 °C/min to 240 °C (with a 20-min final hold). The
179 ionisation energy of MS was 70 eV, the ion source temperature was 230 °C and the mass range of
180 m/z 40–450 was scanned. The epoxides and substrates were quantified using an internal standard
181 method and assuming equal FID responses for all compounds. Finally, the results were expressed as
182 a % of the epoxide(s) formed and as a % of the substrate residues left from each substrate.

183

184 2.6.2. UHPLC-ELSD and UHPLC-Q-TOF methods for studying free fatty acids as substrates

185 FFAs at three unsaturation levels – oleic acid, linoleic acid and linolenic acids – were used as
186 substrates (ca. 3 mg/ml) for POX. Nonadecanoic acid used as an internal standard (3.16 mg/ml) was
187 included in the substrate mixtures. The mixtures were incubated, and thereafter the lipids were
188 extracted with diethyl ether, as described in Section 2.6.1. After evaporation, the residues were
189 immediately re-dissolved into 1 ml of isopropanol and 1 ml of methanol. Finally, the fatty acids and
190 their epoxides were analysed by UHPLC with a method developed in this study.

191

192 For the quantification of the lipids, a UHPLC-ELSD instrument was used, and for the identification
193 of the epoxides, a UHPLC-Q-TOF instrument was used. The UHPLC-ELSD instrument consisted
194 of a UHPLC (Waters Acquity, Milford MA, USA) coupled with an ELSD detector (Waters
195 Acquity, Milford, MA, USA). The FFAs and epoxides were separated with a reversed-phase
196 Acquity UPLC[®] HSS T3 column (1.8 µm, 2.1 × 150 mm, Waters, Ireland) using gradient elution
197 consisting of a mixture of Milli-Q H₂O and 0.05% acetic acid (solvent A) and of methanol with
198 0.05% acetic acid (solvent B) at a flow rate of 0.3 ml/min at 30 °C. The 15-min elution program
199 with the two solvents (solvent A:solvent B, vol%:vol%) was as follows: 0–0.5 min (25:75); 0.5–9
200 min (25:75 to 2:98); 9–12 min (2:98); 12–12.5 min (2:98 to 25:75); and 12.5–15 min (25:75). The

201 ELSD drift tube temperature was set to 50 °C and the gain to 500. Nebulisation was performed with
202 filtered air at 40.0 psi, and the cone and desolvation gas flows were 100 and 1000 l/Hr, respectively.
203 The epoxide contents were calculated based on the standard curves of the second-order equations
204 made from the 9,10-epoxystearic acid standard and internal standard nonadecanoic acid. The results
205 were expressed as a % of the epoxide(s) formed and as a % of the substrate residues left from each
206 substrate.

207

208 The identification of the fatty acid epoxides was carried out in a negative ion mode on a UHPLC-Q-
209 TOF (Acquity I class, Milford, MA, USA) with an electrospray ionisation (ESI) interface, coupled
210 with a SYNAPT G2-Si Mass Spectrometer. The column, eluents and elution program were the same
211 as in the UHPLC-ELSD instrument. The scanning was carried out in the mass range of m/z 50–
212 1200. The instrumental settings were as follows: capillary voltage 2.5 kV, sampling cone voltage 40
213 V, source offset 80V, source temperature 100 °C, desolvation temperature 500 °C, desolvation gas
214 flow 1000 l/h, nebuliser gas flow 6.2 bar, cone gas flow 100 l/h, trap collision energy 4 eV, ramp
215 for MS/MS trap collision energy 10–70 eV, trap gas flow 2 ml/min and a scan time of 0.3 s.

216

217 2.7. Statistical analysis

218 All results were expressed as mean values $\pm$ standard deviations of six replicate samples. Statistical
219 analysis was performed using SPSS version 22 (IBM SPSS Statistics, USA). A value of $p < 0.05$
220 was considered statistically significant. To study the effects of cultivars and sample years and their
221 interactions on the enzyme activities, the results were subjected to a two-way analysis of variance
222 (ANOVA), and thereafter to Tukey tests to identify homogenous groups of samples. Figures were
223 drawn using OriginPro 8.6 (OriginLab Corporation, USA).

224

225 3. Results and discussion

226 3.1. Lipase and lipoxygenase activities in oat and faba bean

227 Both the oat and faba bean samples had significant lipase activities when measured using para-
228 nitrophenyl butyrate as the substrate. In oat, the activities ranged between 0.41 ± 0.02 and $0.77 \pm$
229 $0.03 \mu\text{mol min}^{-1} \text{g}^{-1}$ flour (Fig. 1a), while in faba bean, they ranged between 4.44 ± 0.34 and $7.51 \pm$
230 $0.44 \mu\text{mol min}^{-1} \text{g}^{-1}$ flour (Fig. 1b). In both species, the activities varied almost two-fold among
231 cultivars and samples from different years. The activity in the faba bean samples was ten-fold that
232 of the oat samples. Lipase activity and its impact on oat have been well characterised (Decker et al.,
233 2014; Lehtinen et al., 2003; Piazza et al., 1992), while only the presence and the basic
234 characteristics of the enzyme in faba bean have been identified (Dundas et al., 1978; Henderson et
235 al., 1981). The faba bean in-house reference sample gave a stable activity value of $6.72 \pm 0.12 \mu\text{mol}$
236 $\text{min}^{-1} \text{g}^{-1}$ flour ($n = 13$), showing that the level of analysis was stable and that the results could be
237 compared throughout the study.

238
239 In the oat samples, there were differences in lipase activity among the cultivars (ANOVA, $F_{3,60} =$
240 125.8 , $p < 0.05$) and the cultivars were divided into three homogenous groups (Tukey HSD, $p <$
241 0.05). The lowest lipase values were found in cultivar Alku, with an average of $0.54 \pm 0.11 \mu\text{mol}$
242 $\text{min}^{-1} \text{g}^{-1}$ flour, while the highest occurred in cultivar Akseli, with an average of $0.71 \pm 0.08 \mu\text{mol}$
243 $\text{min}^{-1} \text{g}^{-1}$ flour. Ekstrand et al. (1992) found a comparable variation in lipase activity among three
244 cultivars, which ranged 220–268 U/g, but also greater variations have been found (Miller, Fulcher,
245 & Altosaar, 1989; Hu, Wei, Ren, & Zhao, 2009). The lipase activities from the oat samples from
246 2012–2014 were also different (Anova, $F_{2,60} = 553.6$, $p < 0.05$) and samples from each year differed
247 from the others (Tukey HSD, $p < 0.05$). Lipase activities increased from 2012 to 2014, resulting in
248 average values of 0.48 ± 0.08 , 0.64 ± 0.10 and $0.72 \pm 0.05 \mu\text{mol min}^{-1} \text{g}^{-1}$ flour, respectively. It may
249 indicate that long-term storage could have negatively influenced the activity. In addition, the
250 growing and harvesting conditions in different years might have had an effect. However, the trend

among the sample years was not always similar and there was a statistically significant interaction between the cultivars and sample years (Anova, $F_{6.60} = 28.7$, $p < 0.05$). For instance, the activity in cultivar Akseli was higher in samples from year 2013 than from year 2014. In summary, the cultivar and the sample year were observed to have an effect on the oat lipase activity.

For faba bean lipase activities, there were significant differences among the cultivars (Anova, $F_{3.60} = 259.1$, $p < 0.05$) (Fig. 1b) and all the cultivars differed from each other (Tukey HSD, $p < 0.05$). The highest activities were found in cultivar Kontu, with an average of $7.18 \pm 0.45 \mu\text{mol min}^{-1} \text{g}^{-1}$ flour, and the lowest in cultivar Alexia, with an average of $4.54 \pm 0.28 \mu\text{mol min}^{-1} \text{g}^{-1}$ flour. The lipase activities for cultivars Fatima and SSNS-1 were 6.85 ± 0.23 and $6.22 \pm 0.29 \mu\text{mol min}^{-1} \text{g}^{-1}$ flour, respectively. There was more variation in lipase activities among the faba bean cultivars than among the oat cultivars. The activities in the faba bean samples were not statistically significantly different among different cultivation years (Anova, $F_{2.60} = 2.4$, $p > 0.05$), unlike they varied in oat. The average lipase activity values for the faba bean samples were 6.16 ± 1.10 , 6.28 ± 1.11 and $6.09 \pm 0.94 \mu\text{mol min}^{-1} \text{g}^{-1}$ flour for cultivation years 2010, 2011 and 2015, respectively. Large variations within the years further support that the main effect in the variation among the faba bean samples was from the cultivar. The very high lipase activities and the high variation among the faba bean cultivars found in this study are to be taken into account when processing faba bean, because without inactivating the lipases, TAGs and other acyl lipids are prone to lipolysis as soon as the intact seed structures are broken. It should be recognised that the actual lipase activities towards faba bean lipids might be lower, as the faba bean lipase was earlier shown to be more active towards esters of short-chain fatty acids than of vegetable oils (Dundas et al., 1978), and in this study, the substrate was a butyric ester. Oat lipase, on the other hand, has substrate specificity to long chain fatty acids such as oleic, linoleic and linolenic acids (Piazza et al., 1992).

276 LOX activities were observed only in the faba bean samples (Fig. 1c), whereas in the oat samples,
 277 the activity could not be measured. The presence of LOX in faba bean, as in other legumes, is
 278 generally acknowledged (Gökmen, Bahceci, & Acar, 2002; Hildebrand & Kito, 1984), and minor
 279 LOX activities have been found in oat seeds (Lehtinen & Kaukovirta-Norja, 2011). LOX activities
 280 among the faba bean samples ranged from 0.219 ± 0.009 to 0.330 ± 0.004 mmol min⁻¹ g⁻¹ flour
 281 (Alexia 2011 and 2010). The levels of LOX activity found in this study were comparable to the
 282 level (0.44 mmol min⁻¹ g⁻¹ flour) that was found previously in Finland (Jiang et al., 2016). The faba
 283 bean in-house reference sample, gave a LOX activity value of 0.33 ± 0.01 mmol min⁻¹ g⁻¹ flour ($n =$
 284 9). Therefore, the analysis level during this study was stable and the results could be compared.
 285
 286 There were significant differences in the LOX activities of faba bean cultivars (Anova, $F_{3,60} = 50.2$,
 287 $p < 0.05$) and three homogenous groups among the cultivars could be determined (Tukey HSD, $p <$
 288 0.05). LOX values for cultivars Kontu and Fatima were the highest, with average values of $0.309 \pm$
 289 0.027 and 0.295 ± 0.008 mmol min⁻¹ g⁻¹ flour, respectively, while the lowest average value was for
 290 cultivar Alexia at 0.274 ± 0.058 mmol min⁻¹ g⁻¹ flour. Although statistically significant differences
 291 among the cultivars were observed, the value for Alexia was only 11% lower than those of Kontu
 292 and Fatima. Comparably, the variation in LOX activities among three faba bean cultivars was only
 293 $< 10\%$ (Chang & McCurdy, 1985). The sample year had a significant effect on the LOX activity of
 294 the faba bean samples (Anova, $F_{2,60} = 34.2$, $p < 0.05$) and the samples from the year 2011 produced
 295 lower values of 0.278 ± 0.040 mmol min⁻¹ g⁻¹ flour than those from the years 2010 and 2015 at
 296 0.299 ± 0.032 and 0.300 ± 0.029 mmol min⁻¹ g⁻¹ flour, respectively (Tukey HSD, $p < 0.05$). Yet the
 297 average difference among the years was only 7%. However, the interaction between the cultivar and
 298 the year was statistically significant (Anova, $F_{6,60} = 72.8$, $p < 0.05$), with cultivar Alexia having the
 299 greatest variation and the cultivars Kontu and Fatima being the most stable ones. The highest LOX
 300 activity in Alexia was 50% greater than the lowest activity that was found, whereas in Kontu and

301 Fatima, the differences were only 15%. In conclusion, there is LOX activity in faba bean, and the
302 activity is affected both by the cultivar and the sample year as well as their interactions.

303

304 3.2. Analysis of epoxides from fatty acid methyl esters

305 All the fatty acid methyl esters studied could be converted to epoxides by oat POX, and these
306 epoxides were used to identify epoxides isolated from esterified fatty acids. Methyl oleate, linoleate
307 and linolenate and their epoxides were well separated by the GC-FID method (Fig. 2, combined
308 from the chromatograms of the three substrates). To identify the epoxides, relative retention times
309 (R_r) and selected fragments were obtained by the GC-MS method (Table 1). The R_r values of the
310 epoxides were 1.332–1.423. Thus, the later eluted epoxides were clearly separated from the earlier
311 eluted fatty acids with R_r values of 0.913–1.038.

312

313 The epoxides of the fatty acid methyl esters decomposed during ionisation to numerous fragments
314 and the molecular ions (M^+) could hardly be detected. The only epoxide that was built up from
315 methyl oleate was identified as methyl 9,10-epoxyoctadecanoate with two major fragments built up
316 after α -scissions to the epoxide group (Table 1; Fig. 2), as also presented by Christie (2014). Most
317 of the fragments of epoxides from methyl linoleate and linolenate were built up after cleavage of the
318 methanol group and α -scission to the epoxide group followed by rearrangements. Two epoxides
319 were formed from methyl linoleate (Fig. 2). The first eluting epoxide was identified as methyl
320 12,13-epoxy-octadec-9-enoate and the second as methyl 9,10-epoxy-octadec-12-enoate (Table 1;
321 Fig 2). Both of them produced the fragment of m/z 279 characteristic for the cleavage of the
322 methanol group, and more specific fragments of, e.g. m/z 207 and 168 which indicated the position
323 of the epoxide group (Table 1). The mass spectra obtained had similar fingerprints that have been
324 found earlier (Christie, 2014; Meesapyodsuk & Qiu, 2011). Epoxides produced from methyl
325 linolenate could not be fully separated from each other by GC and the mixture of epoxides resulted

326 in a wide GC peak producing lots of fragment ions (Table 1; Fig. 2). No di-epoxy fatty acid methyl
327 esters were observed. Using the R_f values from the GC/MS analyses, the epoxides from different
328 fatty acid methyl esters could be identified via GC/FID analysis and used for the quantification of
329 epoxide formation in POX assays.

330

331 3.3. Analysis of epoxides from free fatty acids

332 All the FFAs and their epoxidation products could be separated from each other and from the
333 internal standard nonadecanoic acid within 15 min using a reversed-phase UHPLC method, except
334 for the isomeric products from linolenic acid that co-eluted (Fig. 3). With the UHPLC-Q-TOF
335 instrument, the R_f values of the epoxides were 0.532–0.649 and for the FFAs 0.732–0.869 (Table
336 1). In both cases, the more unsaturated an epoxide or a FFA was, the earlier it eluted. The
337 epoxidation products were identified by UHPLC-Q-TOF with ESI in the negative mode, which
338 produced deprotonated molecular ions $[M - H]^-$ as precursor ions. Characteristic fragments of $m/z >$
339 150 were selected to structural identification of regio-isomers (Table 1). Each epoxide produced a
340 fragment that was 18 mass units less than its precursor ion $[M - H - H_2O]^-$, verifying a loss of
341 water from all the epoxide precursor ions during fragmentation. Only mono-epoxy fatty acids were
342 formed during incubation.

343

344 One epoxide was found from oleic acid, and it was identified as 9,10-epoxystearic acid based on
345 precursor ions and fragments formed after scission of the carbon-carbon bond of the epoxide group
346 (Table 1; Fig. 3). Similar mass spectra were obtained from the standard used in this study and also
347 in an earlier study using HPLC-ESI-MS (Orellana-Coca, Adlercreutz, Andersson, Mattiasson, &
348 Hatti-Kaul, 2005). Two epoxides were found to be built up from linoleic acid and were identified
349 as 12,13-mono-epoxy-9-octadecenoic acid and 9,10-mono-epoxy-12-octadecenoic acid (Table 1,
350 Fig. 3). The position of the epoxy group was deduced based on the scission of the carbon-carbon of

the epoxide and the position of the double bond on the cleavage of the carbon-carbon bond on the side of the double bond (Orellana-Coca et al., 2005; Murphy, 2015).

Only one linolenic acid epoxide peak with three poorly separated isomers was present when using UHPLC-Q-TOF. There was one single precursor ion from the mono-epoxide octadecadienoic acid isomers, while it decomposed into several negative fragment ions (Table 1, Fig. 3). After studying the fragments as was done with epoxides from linoleic acid, the first one was identified as 9,10-mono-epoxy-12,15-octadecadienoic acid epoxide and the second as 15,16-mono-epoxy-9,12-octadecadienoic acid. The third was tentatively identified as 12,13-mono-epoxy-9,15-octadecadienoic acid. The elution order of the three epoxides presented here is in line with the elution order presented by Orellana-Coca et al. (2005).

In summary, as the newly developed UHPLC method could reliably separate the epoxides from the fatty acids (Fig. 3), the UHPLC-ELSD method could be used to quantify the formation of the epoxides of fatty acids and used to study POX activity in the extracts.

3.4. Peroxygenase activity and substrate specificities in oat and faba bean

POX activity measurements in oat and faba bean using cumene hydroperoxide as the oxygen donor on different lipid substrates and analysed via the formation of epoxides showed great differences between the species. The oat samples had high activity whereas no activity was found in the faba bean samples. In earlier studies, POX or epoxigenase activity has been found in both oat (e.g. Hamberg & Hamberg, 1996) and faba bean preparates (Hamberg & Fahlstadius, 1992), but its products have only been studied in oat seeds and flours (Doehlert et al., 2010). The oat in-house reference sample gave repeatable results using methyl oleate when studying fatty acid esters as substrates, and using oleic acid when studying FFAs as substrates. The methyl-9,10-

376 epoxyoctadecanoate production was $14.2\% \pm 1.2\%$ ($n = 18$) and 9,10-epoxyoctadecanoic acid
 377 production was $54.9\% \pm 3.0\%$ ($n = 18$), which means that the analysis levels using both classes of
 378 substrates and analytical methods were stable and the results within the classes could be compared.
 379 To our knowledge, this study is the first one to compare POX activities in different oat cultivars and
 380 sample years, as well as to use formation of epoxides as the indicator of POX activity.
 381
 382 The POX in the oat samples could use fatty acid methyl esters and FFAs as substrates whereas no
 383 epoxides were built up from triolein (Tables 2 and 3), which means that the TAGs were not suitable
 384 substrates for oat POX. In line with our results, FFAs and methyl esters have been reported as the
 385 more preferred substrates for oat POX than phospholipids (Meesapyodsuk et al., 2011). However,
 386 as oat contains lipase activity, unsaturated fatty acids from acylglycerols may be subjected to
 387 peroxygenation after being hydrolysed. The proportion of epoxides measured after incubation
 388 varied from 19.1%–42.0% in methyl esters and from 34.6%–70.7% in fatty acids, indicating that oat
 389 POX preferred FFAs as substrates over fatty acid methyl esters. When comparing the level of
 390 unsaturation of the substrate on the formation of epoxides, there were statistically significant
 391 differences both among fatty acid methyl esters ($F_{2,213} = 43.3$, $p < 0.05$) and fatty acids ($F_{2,213} =$
 392 11.9 , $p < 0.05$). With both classes of substrates, the mono- and diunsaturated substrates formed a
 393 homogenous group, from which the triunsaturated substrates differed (Tukey HSD, $p > 0.05$). The
 394 average proportions for epoxide formation for the methyl esters were $32.5\% \pm 6.2\%$, $33.0\% \pm 5.2\%$
 395 and $23.6\% \pm 1.7\%$, and for the fatty acids, $59.9\% \pm 5.4\%$, $58.0\% \pm 6.4\%$ and $41.4\% \pm 3.6\%$ in order
 396 of increasing unsaturation. One possible reason for the lower epoxide values with methyl linolenate
 397 and linolenic acid could be that the epoxides might have reacted further or that the substrate was
 398 oxidised via some other mechanism. When summing up the proportions of epoxides and residual
 399 substrates (Tables 2 and 3), it was obvious that the sums were close to 100% with the mono- and
 400 diunsaturated substrates (ranging from 85.0%–98.8% with methyl esters and 97.6%–122.0% with

401 fatty acids), but that they were much lower with triunsaturated substrates (ranging from 66.7%–
402 80.9% with methyl esters and 74.1%–90.8% with fatty acids). Therefore, it may be that oat POX is
403 not selective in terms of the unsaturation level of the substrate. Earlier, an oat POX gene transferred
404 and expressed in *Pichia pastoris* preferred oleic acid over other unsaturated fatty acids as the
405 substrate, whereas the hydroperoxides of polyunsaturated fatty acids were better oxygen donors to
406 the fatty acids than oleic acid hydroperoxide was (Meesapyodsuk et al., 2011), which might
407 influence the reactions with natural oat lipids.

408

409 Observations regarding the selectivity of the products were undertaken for methyl linoleate and
410 linoleic acid. Of the two epoxides built up from methyl linoleate and linoleic acid, there were more
411 12,13-epoxy compounds than 9,10-epoxy compounds, with values of $17.9\% \pm 2.8\%$ and $15.1\% \pm$
412 2.4% for methyl linoleate, and $29.9\% \pm 3.5\%$ and $28.1\% \pm 3.1\%$ for linoleic acid. The differences
413 were not big, although they were statistically significant ($t_{71} = 41.1$, $p < 0.05$ and $t_{71} = 9.7$, $p < 0.05$).
414 This indicates that both epoxides are likely to be observed in comparable amounts in oat. Similarly,
415 Piazza et al., (2003) reported the formation of an equal amount of mono 9,10-epoxy and 12,13-
416 epoxy octadecenoic acids, thus indicating that the oat POX cannot distinguish the double bonds in
417 the substrate.

418

419 No epoxide formation could be found in any faba bean samples using methyl oleate, oleic acid or
420 triolein as the substrate. Therefore, it was concluded that there was no POX activity or that the
421 activity was very low in the faba bean samples.

422

423 3.5. Differences in peroxxygenase activity among oat cultivars and sample years

424 There was a statistically significant difference in POX activities among oat cultivars ($F_{3,420} = 6.2$, p
425 < 0.05) and the values could be clearly divided to produce two homogenous groups (Tukey HSD, p

426 < 0.05). Cultivars Akseli and Meeri belonged to the low POX activity group, with average values of
 427 $36.9\% \pm 11.8\%$ and $40.8\% \pm 14.5\%$, respectively. The cultivar Meeri also belonged to the high
 428 POX group, together with cultivars Alku and Steiner, with values of $43.8\% \pm 14.6\%$ and $44.1\% \pm$
 429 15.2% , respectively. The variation in these average values was large due to the many substrates, but
 430 the differences among the cultivars were clear. Epoxidation was always the lowest in cultivar
 431 Akseli with all substrates and also in every sample year except for one. Usually, cultivars Steinar
 432 and Meeri gave the highest amounts of epoxides and also the lowest residual substrate values. The
 433 average activity in cultivar Steiner was 20% greater than in Akseli. The sample year did not have a
 434 statistically significant effect on POX activities in the oat samples ($F_{2,420} = 0.98$, $p > 0.05$) and nor
 435 was there an interaction between the cultivar and the sample year ($F_{6,420} = 0.72$, $p > 0.05$). Yet a
 436 variation in the POX activities among sample years with individual substrates could be observed.

437

438 *3.6. Evaluation of the potential of lipid-modifying enzymes to cause off-flavours*

439 Based on the enzyme activities of three different lipid-modifying enzymes in oat and faba bean, a
 440 scheme on off-flavour formation was created (Fig. 4). This study showed that enzymatic activity to
 441 hydrolyse lipids occurs both in oat and in faba bean, which may start the lipid oxidation cascade. In
 442 oat, due to very low LOX activities, fatty acids are primarily subjected to autoxidation, yielding the
 443 formation of hydroperoxides and their further reactions. Anyhow, oxidation of oat lipids occurred
 444 much faster when its lipids were being hydrolysed by lipase (Lampi, Damerau, Moio, Partanen,
 445 Forsell & Piironen, 2015). Hydroperoxides of fatty acids are good substrates for oat POX, which
 446 could result in the production of epoxy and hydroxy fatty acids, and possible bitter off-flavours in
 447 oat products. Since lipid oxidation is initiated by autoxidation, the formation of epoxy and hydroxy
 448 fatty acids is not rapid and is more likely to occur when oat or oat products with enzyme activities
 449 are stored for longer periods. The high content of lipids and the degree of unsaturation in oat
 450 enhances the risk of the formation of epoxy and hydroxy compounds. The very high lipid-

451 hydrolysing activity present in faba bean, subjects not only the lipids of the seeds, but also of other
452 lipids in the products, to the formation of FFAs. This should be taken into account when e.g. faba
453 bean emulsions are prepared. As the polyunsaturated fatty acids are substrates for faba bean LOX,
454 fatty acid hydroperoxides are built up rapidly and may decompose into volatile oxidation products
455 causing off-flavours. Thus, the so-called beany flavour may be observed rapidly after faba bean
456 flour is suspended in aqueous systems. It is likely that epoxy and hydroxy fatty acids are not as
457 important in terms of producing off-flavours in faba bean as they are in oat products due to the low
458 POX activity in faba bean.

459

460 In both seeds, controlling lipid-modifying enzyme activities is crucial in the production of foods of
461 good and stable quality. Differences in the enzyme activities among oat and faba bean cultivars and
462 sample years were observed, but the differences were only modest and would probably not have
463 major effects on off-flavour formation in oat and faba bean.

464

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470

471 **References**

- 472 Brunschwiler, C., Heine, D., Kappeler, S., Conde-Petit, B., & Nyström, L. (2013). Direct
473 measurement of rice bran lipase activity for inactivation kinetics and storage stability prediction.
474 *Journal of Cereal Science*, 58, 272-277.
- 475 Chang, P. R. Q., & McCurdy, A. R. (1985). Lipoxygenase activity in fourteen legumes. *Canadian*
476 *Institute of Food Science and Technology Journal*, 18, 94-96.
- 477 Christie, W. W. (1993). Preparation of ester derivatives of fatty acids for chromatographic analysis.
478 In W. W. Christie (Ed.), *Advances in lipid methodology – two* (pp. 69–111). Dundee: Oily Press.
- 479 Christie, W. W. (2014). AOCS Lipid Library.
480 <http://lipidlibrary.aocs.org/content.cfm?ItemNumber=39512>. Updated March 11, 2014.
- 481 Clemente, A., Olías, R., & Olías, J. M. (2000). Purification and characterization of broad bean
482 lipoxygenase isoenzymes. *Journal of Agriculture and Food Chemistry*, 48, 1070-1075.
- 483 Crépon, K., Marget, P., Peyronnet, C., Carrouee, B., Arese, P., & Duc, G. (2010). Nutritional value
484 of faba bean (*Vicia faba* L.) seeds for feed and food. *Field Crops Research*, 115, 329-339.
- 485 Decker, E. A., Rose, D. J., & Stewart, D. (2014). Processing of oats and the impact of processing
486 operations on nutrition and health benefits. *British Journal of Nutrition*, 112, S58-S64.
- 487 Doehlert, D. C., Angelikousis, S., & Vick, B. (2010). Accumulation of oxygenated fatty acids in oat
488 lipids during storage. *Cereal Chemistry*, 87, 532-537.
- 489 Dundas, D. G. A., Henderson, H. M., & Eskin, N. A. M. (1978). Lipase from *Vicia faba minor*.
490 *Food Chemistry*, 3, 171-178.
- 491 Ekstrand, B., Gangby, I., & Åkesson, G. (1992). Lipase activity in oats — distribution, pH
492 dependence, and heat inactivation. *Cereal Chemistry*, 69(4), 379-381.
- 493 Gardner, H. W. (2003). Lipoxygenase and associated enzymes. In J. R. Whitaker, A. G. J. Voragen,
494 & D. W. S. Wong (Eds.), *Handbook of Food Enzymology* (pp. 557-567). New York: Marcel
495 Dekker, Inc.

496 Gökmen, V., Bahceci, S., & Acar, J. (2002). Characterization of crude lipoxygenase extract from
 497 green pea using a modified spectrophotometric method. *European Food Research and Technology*,
 498 215, 42-45.

499 Hamberg, M., & Fahlstadius, P. (1992). On the specificity of a fatty acid epoxigenase in broad bean
 500 (*Vicia faba* L.). *Plant Physiology*, 99, 987-995.

501 Hamberg, M., & Hamberg, G. (1996). Peroxygenase-Catalyzed Fatty Acid Epoxidation in Cereal
 502 Seeds (Sequential Oxidation of Linoleic Acid into 9(S),12(S),13(S)-Trihydroxy-10(E)-
 503 Octadecenoic Acid). *Plant Physiology*, 110, 807-815.

504 Henderson, H. M., Shambrock, D. L., & Eskin, N. A. M. (1981). The enzymic deacylation of *p*-
 505 nitrophenyl esters and phosphatidylcholine in *vicia faba minor*. *Food Chemistry*, 7(4), 249-256.

506 Hildebrand, D. F., & Kito, M. (1984). Role of lipoxygenases in soybean seed protein quality.
 507 *Journal of Agriculture and Food Chemistry*, 32, 815-819.

508 Hu, X. Z., Wei, Y. M., Ren, C. Z., & Zhao, J. (2009). Relationship between kernel size and shape
 509 and lipase activity of naked oat before and after pearling treatment. *Journal of the Science of Food*
 510 *and Agriculture*, 89, 1424-1427.

511 Jiang, Z. Q., Pulkkinen, M., Wang, Y. J., Lampi, A. M., Stoddard, F. L., Salovaara, H., Piironen,
 512 V., & Sontag-Strohm, T. (2016). Faba bean flavour and technological property improvement by
 513 thermal pre-treatments. *LWT - Food Science and Technology*, 68, 295-305.

514 Lampi, A.-M., Damerau, A., Li, J., Moisio, T., Partanen, R., Forssell, P., & Piironen, V. (2015).
 515 Changes in lipids and volatile compounds of oat flours and extrudates during processing and
 516 storage. *Journal of Cereal Science*, 62, 102-109.

517 Lehtinen, P., Kaukovirta-Norja, A. (2011). Oat lipids, enzymes, and quality. F. H. Webster, & P. J.
 518 Wood (Eds.), *Oats: Chemistry and Technology, Second Edition* (pp. 143-156). St. Paul: AACC
 519 International, Int.

520 Lehtinen, P., Kiiliäinen, K., Lehtomäki, I., & Laakso, S. (2003). Effect of Heat Treatment on Lipid
 521 Stability in Processed Oats. *Journal of Cereal Science*, 37(2), 215-221.

522 Lizarazo, C. I., Lampi, A. M., Liu, J., Sontag-Strohm, T., Piironen, V., & Stoddard, F. L. (2015).
 523 Nutritive quality and protein production from grain legumes in a boreal climate. *Journal of the*
 524 *Science of Food and Agriculture*, 95(10), 2053-2064.

525 Meesapyodsuk, D., Qiu, X. (2011). A peroxygenase pathway involved in the biosynthesis of epoxy
 526 fatty acids in oat. *Plant Physiology*, 157, 454-463.

527 Miller, S. S., Fulcher, R. G., & Altosaar, I. (1989). Evaluation of 4-methylumbelliferyl heptanoate
 528 as a substrate for oat lipase. *Journal of Cereal Science*, 10, 61-68.

529 Murphy, R. C. (2015). *Tandem Mass Spectrometry of Lipids: Molecular Analysis of Complex*
 530 *Lipids*. Cambridge: Royal Society of Chemistry, (Chapter 1).

531 Orellana-Coca, C., Adlercreutz, D., Andersson, M. M., Mattiasson, B., & Hatti-Kaul, R. (2005).
 532 Analysis of fatty acid epoxidation by high performance liquid chromatography coupled with
 533 evaporative light scattering detection and mass spectrometry. *Chemistry and Physics of Lipids*, 135,
 534 189-199.

535 Piazza, G. J., Bilyk, A., Brower, D. P., & Haas, M. J. (1992). The positional and fatty acid
 536 selectivity of oat seed lipase in aqueous emulsions. *Journal of the American Oil Chemists' Society*,
 537 69, 978-981.

538 Piazza, G. J., Nuñez, A., & Foglia, T. A. (2003). Epoxidation of fatty acids, fatty methyl esters, and
 539 alkenes by immobilized oat seed peroxygenase. *Journal of Molecular Catalysis B: Enzymatic*, 21,
 540 143-151.

541 Roland, W. S. U., Pouvreau, L., Curran, J., van de Velde, F., & de Kok, P. M. T. (2017). Flavor
 542 aspects of pulse ingredients. *Cereal Chemistry*, 94, 58-65.

543

544 **Figure Legends**

545 **Fig. 1.** Lipase and LOX activities in oat and faba bean samples from four cultivars in different
546 cultivation years (2 extracts x 3 measurements; $n = 6$): (a) lipase activity in oat; (b) lipase activity in
547 faba bean; and (c) LOX activity in faba bean.

548

549 **Fig. 2.** Combined GC-FID chromatograms of methyl oleate, methyl linoleate, and methyl linolenate
550 after incubation with oat extracts containing POX and formation of epoxides. Where, 1 = methyl
551 oleate, 2 = methyl linoleate, 3 = methyl nonadecanoic acid (internal standard), 4 = methyl
552 linolenate, 5 = methyl 9,10-epoxyoctadecanoate, 6 = methyl 12,13-epoxy-octadec-9-enoate, 7 =
553 methyl 9,10-epoxy-octadec-12-enoate and 8 = epoxides produced from methyl linolenate.

554

555 **Fig. 3.** Combined UHPLC-ELSD chromatograms of oleic acid, linoleic acid, and linolenic acid
556 after incubation with oat extracts containing POX and formation of epoxides. Where, 1 = epoxides
557 produced from linolenic acid, 2 = 12,13-epoxy-9-octadecenoic acid, 3 = 9,10-epoxy-12-
558 octadecenoic acid, 4 = (*trans*-) 9,10-epoxystearic acid, 5 = linolenic acid, 6 = linoleic acid, 7 = oleic
559 acid and 8 = nonadecanoic acid (internal standard).

560

561 **Fig. 4.** The most probable enzymatic and chemical reactions to cause potential off-flavour
562 compounds from lipids in a) oat and b) faba bean.

563 **Table legends**

564 **Table 1.** Identification of the epoxides formed from a) fatty acid methyl esters and b) fatty acids,
565 during incubation with oat extracts containing POX from using GC-MS analysis for fatty acid
566 methyl esters, and UHPLC-Q-TOF for free fatty acids.

567

568 **Table 2.** Proportions of epoxidation products[§] and substrate residues[#] of the three unsaturated fatty
569 acid methyl esters incubated with oat extracts containing POX from four oat cultivar samples from
570 different years ($n = 6$).

571 [§] Proportions (%) of epoxides from methyl oleate: methyl 9,10-epoxyoctadecanoate;
572 from methyl linoleate: 1= methyl 12,13-epoxy-octadec-9-enoate and 2: methyl 9,10-
573 epoxy-octadec-12-enoate; and methyl linolenate: a mixture of methyl mono-epoxy-
574 octadecadienoates.

575 [#] Proportions (%) of fatty acid methyl esters left after incubation.

576

577 **Table 3.** Proportions of epoxidation products[§] and substrate residues[#] of the three unsaturated fatty
578 acids incubated with oat extracts containing POX from four oat cultivar samples from different
579 years ($n = 6$).

580 [§] Proportions (%) of epoxides from oleic acid: 9,10-epoxyoctadecanoic acid; from
581 linoleic acid: 1= 12,13-epoxy-octadec-9-enoic acid and 2: 9,10-epoxy-octadec-12-
582 enoic acid; and linolenic acid: a mixture of mono-epoxy-octadecadienoic acids.

583 [#] Proportions (%) of fatty acids left after incubation.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Table 1.

Table 1a)

Fatty acid methyl ester epoxide	R _r : t _{epoxide} / t _{19:0me}	Molecular ion <i>M</i> ⁺ (<i>m/z</i>)	Fragment ions (<i>m/z</i>) formed from cleavage of			Other fragment ions (<i>m/z</i>)
			the methanol group	α C-C bond next to the epoxide		
me-9,10-epoxy- octadecanoate	1.332	312		155; [M-157] •(CH ₂) ₇ COOCH ₃	199; [M-113] •(CH ₂) ₇ CH ₃	171
me-12,13-epoxy- octadec-9-enoate	1.354	310	279; [M-31] •OCH ₃	164; [M-146] C ₇ OH ₁₅ and •OCH ₃	207; [M-103] CH ₃ (CH ₂) ₃ CH ₃ and •OCH ₃	121, 136, 149, 166
me-9,10-epoxy- octadec-12-enoate	1.361	310	279; [M-31] •OCH ₃		168; [M-142] C ₈ H ₁₅ and •OCH ₃	121, 133, 150, 155, 185
me-epoxy- octadecadienoate	1.423	308	277; [M-31] •OCH ₃			121, 133, 135, 155, 207, 236

Table 1b)

Fatty acid epoxide	R_f : $t_{\text{epoxide}} / t_{19:0}$	Precursor ion [$M-H$] ⁻ , (m/z)	Fragment ions (m/z) formed from			
			Loss of H_2O	Homolytic cleavage of the epoxidic C-O bond followed by scission of the C-C bond		
				of the epoxide on the side of fatty acid		on the side of the γ -double bond after hydrogen atom rearrangement
				-COO ⁻	-CH ₃	
9,10 epoxy-octadecanoic acid	0.649	297.2	279.2	155.1	171.1	
12,13-epoxy-octadec-9-enoic acid	0.575	295.2	277.2	195.1		183.1
9,10-epoxy-octadec-12-enoic acid	0.581	295.2	277.2		171.1	183.1
9,10-epoxy- 12,15-octadeca-dienoic acid	0.532	293.2	275.2		171.1	183.1
15,16-epoxy-9,12-octadeca-dienoic acid	0.537	293.2	275.2	235.2		223.2
12,13-epoxy-9,15-octadeca-dienoic acid	0.549	293.2	275.2	195.1	211.1	223.1; 183.1

Table 2.

Oat Samples	Fatty acid methyl ester substrates						
	me-oleate (%)		me-linoleate (%)			me-linolenate (%)	
Year 2012	Epoxide [§]	Residue [#]	Epoxide 1 [§]	Epoxide 2 [§]	Residue [#]	Epoxides [§]	Residue [#]
Alku	36.2 ± 0.5	59.4 ± 0.3	18.9 ± 0.1	16.3 ± 0.1	50.1 ± 0.3	25.7 ± 0.4	44.8 ± 2.0
Akseli	19.5 ± 0.3	78.2 ± 0.7	11.9 ± 0.3	10.4 ± 0.2	67.0 ± 0.7	24.1 ± 0.2	49.1 ± 0.5
Steinar	38.3 ± 0.4	56.1 ± 0.2	20.1 ± 0.0	17.1 ± 0.1	49.7 ± 0.1	25.5 ± 0.3	46.6 ± 0.5
Meeri	38.2 ± 1.2	57.6 ± 1.7	20.9 ± 0.0	17.7 ± 0.1	48.0 ± 0.4	26.5 ± 0.3	44.3 ± 0.7
Year 2013	Epoxide [§]	Residue [#]	Epoxide 1 [§]	Epoxide 2 [§]	Residue [#]	Epoxides [§]	Residue [#]
Alku	35.0 ± 1.7	60.6 ± 2.4	19.0 ± 0.3	15.8 ± 0.3	53.0 ± 0.8	20.6 ± 0.3	50.4 ± 1.1
Akseli	27.9 ± 1.1	69.2 ± 0.9	15.2 ± 0.2	12.7 ± 0.2	62.3 ± 0.2	22.2 ± 0.6	51.6 ± 0.5
Steinar	41.6 ± 0.4	54.0 ± 0.3	21.8 ± 0.5	18.7 ± 0.5	46.7 ± 0.4	22.6 ± 0.6	44.7 ± 1.0
Meeri	29.6 ± 0.7	67.3 ± 0.3	17.1 ± 0.2	14.8 ± 0.2	59.1 ± 1.1	23.0 ± 0.5	49.4 ± 1.0
Year 2014	Epoxide [§]	Residue [#]	Epoxide 1 [§]	Epoxide 2 [§]	Residue [#]	Epoxides [§]	Residue [#]
Alku	30.1 ± 0.4	67.8 ± 0.8	16.0 ± 0.3	13.3 ± 0.2	61.6 ± 0.8	23.5 ± 0.6	54.0 ± 1.4
Akseli	24.2 ± 0.3	74.2 ± 0.7	15.0 ± 0.2	12.4 ± 0.2	63.8 ± 0.7	22.1 ± 0.8	58.0 ± 0.4
Steinar	33.3 ± 0.4	60.9 ± 0.2	19.6 ± 0.2	15.9 ± 0.1	54.2 ± 0.7	23.2 ± 0.7	51.2 ± 0.8
Meeri	35.8 ± 0.4	58.3 ± 0.2	19.7 ± 0.3	16.1 ± 0.2	52.5 ± 0.2	24.4 ± 1.0	51.0 ± 1.3
Total	32.5 ± 6.2	63.6 ± 7.4	17.9 ± 2.8	15.1 ± 2.4	55.7 ± 6.6	23.6 ± 1.7	49.6 ± 4.1

Table 3.

Oat Samples	Free fatty acid substrates						
	Oleic acid (%)		Linoleic acid (%)			Linolenic acid (%)	
Year 2012	Epoxide ^s	Residue [#]	Epoxide 1 ^s	Epoxide 2 ^s	Residue [#]	Epoxides ^s	Residue [#]
Alku	67.5 ± 1.3	44.3 ± 1.1	34.1 ± 1.0	32.0 ± 1.2	37.5 ± 0.7	45.8 ± 1.4	37.4 ± 1.8
Akseli	56.8 ± 2.2	59.3 ± 2.5	26.6 ± 0.2	23.7 ± 0.7	51.7 ± 1.8	38.9 ± 0.8	48.2 ± 1.5
Steinar	63.5 ± 1.2	50.7 ± 0.8	32.9 ± 0.7	30.0 ± 1.0	43.7 ± 0.9	42.9 ± 1.2	43.2 ± 0.6
Meeri	64.0 ± 1.5	50.3 ± 0.6	30.4 ± 0.9	29.0 ± 0.7	42.5 ± 1.0	43.0 ± 0.7	42.5 ± 0.7
Year 2013	Epoxide ^s	Residue [#]	Epoxide 1 ^s	Epoxide 2 ^s	Residue [#]	Epoxides ^s	Residue [#]
Alku	60.4 ± 1.6	48.3 ± 1.2	31.2 ± 1.2	30.3 ± 0.4	43.5 ± 0.8	43.3 ± 0.6	37.8 ± 0.8
Akseli	51.9 ± 2.3	59.8 ± 1.1	24.4 ± 1.4	23.9 ± 0.5	53.1 ± 0.5	35.8 ± 0.9	47.4 ± 1.5
Steinar	67.4 ± 0.6	42.1 ± 1.4	35.2 ± 0.7	32.3 ± 1.5	37.7 ± 1.0	47.1 ± 1.6	37.5 ± 0.6
Meeri	56.2 ± 1.2	55.8 ± 0.9	26.7 ± 0.2	26.4 ± 0.7	50.6 ± 0.9	39.9 ± 1.8	47.1 ± 1.2
Year 2014	Epoxide ^s	Residue [#]	Epoxide 1 ^s	Epoxide 2 ^s	Residue [#]	Epoxides ^s	Residue [#]
Alku	58.2 ± 1.3	49.4 ± 3.1	30.5 ± 1.3	28.3 ± 0.9	44.0 ± 1.1	43.2 ± 1.7	43.3 ± 1.5
Akseli	50.7 ± 2.9	59.4 ± 2.2	24.9 ± 1.1	23.8 ± 1.1	53.8 ± 0.9	35.8 ± 1.2	48.6 ± 1.1
Steinar	60.9 ± 1.1	46.8 ± 1.0	30.3 ± 1.0	28.1 ± 0.8	43.0 ± 0.4	39.7 ± 1.1	35.8 ± 0.6
Meeri	61.1 ± 1.1	43.4 ± 1.4	31.8 ± 0.9	29.2 ± 1.0	39.0 ± 1.0	41.0 ± 1.0	36.1 ± 1.1
Total	59.9 ± 5.4	50.8 ± 6.3	29.9 ± 3.5	28.1 ± 3.1	45.0 ± 5.7	41.4 ± 3.6	42.1 ± 4.9