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2 **Epoxy and hydroxy fatty acids as non-volatile lipid oxidation products in oat**

3

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8 **Abstract**

9 The aim of the study was to investigate the occurrence and formation of peroxygenase catalysed
10 non-volatile oxidised fatty acids (NVOFAs), especially epoxy and hydroxy fatty acids, which
11 potentially provide off-flavours in oat. A method based on extraction of NVOFAs using accelerated
12 solvent extraction and analysis by UHPLC-ELSD/MS was developed. Hydroxy and epoxy fatty
13 acids from oleic and linoleic acids were present as major NVOFAs in non-heat treated (NHT) oat
14 products, and the contents increased markedly during storage. In a controlled storage experiment,
15 NVOFA contents in NHT oat flours increased to 1700–2000 µg/g, whereas in heat-treated samples,
16 only to 200–400 µg/g. Epoxy fatty acids seemed to be the first products that occurred, followed by
17 hydroxy fatty acids and minor NVOFAs. The formation of NVOFAs was related to lipase catalysed
18 lipid hydrolysis and the formation of volatile lipid oxidation products. Inactivation of lipid
19 degrading enzymes is crucial to producing stable oat products.

20

21 **Keywords:** Oat; peroxygenase activity; epoxy and hydroxy fatty acids; lipid oxidation; lipid
22 hydrolysis

23 1. Introduction

24 Oat (*Avena sativa*) is a valuable food source that provides humans and animals many nutrients,
25 including proteins and soluble dietary fibres (Decker, Rose, & Stewart, 2014). Oat have a higher
26 content of lipids than most other cereal grains. Together with the activity of lipid-modifying
27 enzymes, especially that of lipase, this may lead to off-flavour problems during processing and
28 storing oat (Lehtinen & Kaukovirta-Norja, 2011; Yang, Piironen, & Lampi, 2017). When lipase
29 comes in contact with the substrates, especially in milled oat, it begins to extensively hydrolyse acyl
30 esters and release free fatty acids (FFAs). Generally, the FFAs are more prone to oxidation than the
31 acyl esters, through both chemical and enzymatic pathways. However, in oat, the activity of
32 lipoyxygenase (LOX), which is responsible for the oxidation of FFAs to hydroperoxides, is low, and
33 fatty acids are primarily subjected to autooxidation, which results in the formation of hydroperoxides
34 (Yang et al., 2017). Oat also possess high peroxygenase (POX, EC 1.11.2.1) activity, which
35 catalyses hydroperoxide-dependent conversion of unsaturated fatty acids into epoxy and hydroxy
36 fatty acids (Hamberg & Hamberg, 1996). To maintain acceptable quality in oat products, all these
37 enzyme activities must be controlled. Oat enzymes are commonly inactivated using hydrothermal
38 treatments, including kilning or steaming. However, some producers prefer not to use any heat
39 treatments, e.g., to avoid changes in protein functionality.

40
41 Epoxy and hydroxy fatty acids as well as epoxy hydroxy and di- and trihydroxy fatty acids are all
42 produced in oat using multi-step oxidation processes, with POX as the final enzyme (Hamberg &
43 Hamberg, 1996; Doehlert, Angelikousis, & Vick, 2010). The formation of POX products should be
44 avoided because some of them are thought to cause bitter off-flavours in oat products (Biermann,
45 Wittmann, & Grosch, 1980). In the present study, all potentially bitter POX products are called non-
46 volatile oxidised fatty acids (NVOFA). Oat POX catalyses intermolecular oxygen transfer from
47 hydroperoxides to unsaturated fatty acids, which forms epoxy and hydroxy fatty acids, as well as

48 intramolecular oxygen transfer from unsaturated fatty acid hydroperoxides to hydroxy-epoxy fatty
49 acids (Hamberg & Hamberg, 1996; Piazza, Foglia, & Nuñez, 1999; Hanano et al., 2006). The POX
50 pathway also includes epoxide hydrolase activity that produces trihydroxy fatty acids (Heimann &
51 Klaiber, 1977; Hamberg & Hamberg, 1996; Blée 1998). Recently, two new POX activities have
52 been found in oat (Benaragama, Meesapyodsuk, Beattie, & Qiu, 2017).

53
54 Because they are secondary metabolites in plants, several epoxy and hydroxy fatty acids have been
55 found in freshly milled oat seeds, including the mono-epoxides of oleic and linoleic acids, which
56 include 9,10-epoxy-octadecanoic acid, 12,13-epoxy-octadec-9-enoic acid and 9,10-epoxy-octadec-
57 12-enoic acid, which together account for about 0.4% of the FFAs, according to Leonova, Shelenga,
58 Hamberg, Konarev, Loskutov and Carlsson (2008). These authors also detected hydroxy fatty acids,
59 including 15-hydroxy-octadeca-9,12-dienoic acid. In addition, Doehlert et al. (2010) identified 13-
60 hydroxy-octadeca-9,11-dienoic acid and 9-hydroxy-octadeca-10,12-dienoic acid in oat. Although
61 NVOFAs have been suggested as being responsible for an undesirable bitter taste and have been
62 associated with off-flavours (Baur, Grosch, Wieser, & Jugel, 1977; Biermann et al., 1980), their
63 formation in oat has been monitored in only one study (Doehlert et al., 2010), where flour from raw
64 oat groats was stored at 37 °C for 22 weeks. At the end of storage, 5.0% of the total fatty acids were
65 found to be epoxy acids and 1.1% were found to be hydroxy acids. The corresponding amounts in
66 flour from steamed oat groats were less being 1.9% and 0.9%, respectively, indicating that
67 hydrothermal treatment retarded their formation to some extent. However, little is known about the
68 formation and significance of epoxy and hydroxy fatty acids in oat products.

69
70 Finding an efficient extraction method with which to isolate NVOFAs from oat matrix without
71 subjecting them to further reactions is challenging because lipids may be entrapped by and bound
72 with other components, including starch and proteins. Previously, solvent extraction has been used

73 to extract epoxy and hydroxy fatty acids from food matrices (Doehlert et al., 2010; Leonova et al.,
74 2008). Accelerated solvent extraction (ASE) is a technique that provides an alternative way to
75 extract lipids from complex food matrices using high pressure to accelerate penetration of the
76 solvents into the samples. A gentle ASE method has been developed to extract the lipids from dry
77 extruded pet food by limiting the extraction temperature to 40 °C (Yao and Schaich, 2014). Both
78 gas chromatography (GC) (Doehlert et al., 2010; Jenske & Vetter, 2007; Simsek & Doehlert, 2014)
79 and liquid chromatography (LC) have been used to analyse hydroxy and epoxy fatty acids (Levandi,
80 Püssa, Vaher, Toomik, & Kaljurand, 2009; Yang, Yang, & Li, 2013). LC methods are becoming
81 more commonly used to analyse epoxides (Orellana-Coca, Adlercreutz, Andersson, Mattiasson, &
82 Hatti-Kaul, 2005; Yang et al., 2017) and hydroxides (Yang et al., 2013; Levandi et al., 2009)
83 because they require less-complex sample preparation.

84

85 The aim of the present study was to investigate the formation of NVOFAs in oat, focusing mainly
86 on epoxy and hydroxy fatty acids. The study's first aim was to develop a method of analysing
87 epoxy and hydroxy fatty acids in oat samples using ultra-high performance liquid chromatography
88 coupled with evaporative light scattering detection or mass spectrometry (UHPLC-ELSD/MS). The
89 second aim was to study the occurrence and formation of NVOFAs in selected heat-treated (HT)
90 and non-heat-treated (NHT) oat samples; both included in the study to determine whether the
91 NVOFAs were mainly formed enzymatically or chemically. The third aim was to study the
92 formation of NVOFAs and to relate their formation to those of FFAs and volatile lipid oxidation
93 products in a controlled storage experiment using NHT and HT oat.

94

2. Materials and Methods

2.1. Chemicals and standards

Oleic and linoleic acid (purity > 99%) were purchased from Nu-Check Prep (Elysian, MN, USA). Cumene hydroperoxide (80%), triglyceride palmitate and 12-hydroxyoctadecanoic acid (99%) were obtained from Sigma-Aldrich (St. Louis, MO, USA), and 9,10-epoxyoctadecanoic acid was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The 12,13-epoxy-octadec-9-enoic acid and the 9,10-epoxy-octadec-12-enoic acid were produced from linoleic acid as a substrate using the method developed by Yang et al. (2017). The solid phase extraction (SPE) cartridges Strata SI-1 Silica (55 µm, 70 Å, 500 mg/3ml) were obtained from Phenomenex (Torrance, CA, USA). All other reagents were purchased from Sigma-Aldrich and Merck (Darmstadt, Germany). The chemicals were of pro analysis grade, and the solvents were of high performance liquid chromatography (HPLC) grade. The water used in the analyses was purified using a Milli-Q system (0.22 µm, ≥ 18.2 MΩ cm, Millipore Corp.; Bedford, MA, USA).

2.2. Oat samples and storage experiment

A NHT oat flour sample provided by Raisio Group (Raisio, Finland) was stored at 35 °C for 10 weeks to allow the formation of the NVOFAs, particularly of epoxides and hydroxides, and then used as an in-house reference to verify the analytical level of the measurements. It was also used in developing the NVOFA analysis method.

The occurrence of NVOFAs was studied in five commercial oat samples that had various processing and storage histories. They included three old samples: oat flour from HT grains stored in a freezer for several years (O-HT-F1), oat flakes from HT grains stored at room temperature for more than one year (O-HT-F2) and oat powder milled from NHT grains and stored at room temperature for one year (O-NHT-P). The two other NHT oat samples included freshly milled oat

120 grains that had been stored at 4 °C for several years before milling (F-NHT-F1) and freshly
121 purchased oat flour (F-NHT-F2). The oat flakes were milled using a Knife Mill GM200 (Retsch;
122 Haan, Germany), and the oat grains were milled using a Centrifugal Mill ZM200 (Retsch; Haan,
123 Germany) fitted with a 0.5-mm sieve. All the flour samples were stored in a freezer at 20 °C before
124 analysis. To investigate the formation of NVOFAs in the two freshly milled samples, F-NHT-F1
125 and F-NHT-F2, a storage experiment was conducted. These samples were stored at 35 °C for 0, 6,
126 12, 18 and 24 weeks before being analysed for NVOFAs.

127
128 To further study the effect of HT of the oat grains on the formation of epoxy and hydroxy fatty
129 acids, a controlled storage experiment was conducted using freshly milled flours made from the
130 NHT and HT oat grains of two cultivars (NHT/HT-CV1 and CV2, Raisio Group; Raisio, Finland)
131 and of NHT and HT oat grains from a mixture of cultivars (NHT-IS and HT-IS, Fazer Mills;
132 Vantaa, Finland). All HT samples were subjected to kilning (a hydrothermal treatment) at industrial
133 scale. The grains were milled using the method mentioned above. Subsamples of ca. 10 g of each of
134 the NHT/HT-CV1 and CV2 and the NHT-IS and HT-IS materials were divided into glass bottles
135 and stored at 40 °C for 0, 1, 2, 3 and 4 months. One bottle was prepared for each storage point. The
136 NVOFAs contents were measured at each storage point.

137
138 To relate the formation of NVOFAs to lipid hydrolysis and oxidation, FFAs and volatile oxidation
139 products were measured in selected oat samples at certain storage points. In the NHT/HT-CV1 and
140 CV2 oat samples, lipid hydrolysis was measured at 0, 1, 3 and 4 months and volatile analysis at 0, 1
141 and 3 months. In the NHT-IS and HT-IS samples, both lipid hydrolysis and oxidation were
142 measured at 0, 1, 2 and 4 months. FFAs were extracted from the selected subsamples as the
143 NVOFAs. To analyse volatile lipid oxidation products, 1 g of each sample was weighed into 20-ml,

144 dark brown vials that were tightly closed and stored at 40 °C. Then, at each storage point,
145 incubations were conducted in triplicate for volatile lipid oxidation products.

146

147 *2.3. Development of method for measuring NVOFA*

148 *2.3.1. Extraction of lipids using ASE*

149 A gentle method for the extraction of NVOFAs was developed based on a study by Yao and
150 Schaich (2014). Lipids were extracted using selected solvents and solvent mixtures in an ASE
151 instrument (Dionex ASE-200, Dionex Corporation; Sunnyvale, CA, USA). The solvents included
152 acetone, heptane:ethanol (2:1, vol%:vol%) and ethanol. About 1 g of each sample was weighed into
153 11-ml extraction cells and mixed with an equal amount of Ottawa sand (Fisher P/N S23-3), which
154 was also used to fill up the cells. The extraction was performed using 1500 psi pressure, a cell
155 temperature of 40 °C, a cell preheating period of 5 min and 2 static extraction cycles of 10 min.
156 Then, the extracts were transferred from the collection tubes to round-bottom flasks for solvent
157 evaporation in a rotary evaporator at 35 °C. The lipid extraction efficiencies of the three solvents
158 were compared using GC to analyse fatty acid yields as fatty acid methyl esters ($n = 6$) (Yang et al.,
159 2017). Because the heptane:ethanol (2:1, vol%:vol%) solvent mixture gave the highest fatty acid
160 yields, it was chosen for use in further analyses. To analyse the NVOFAs, the lipid residues were
161 dissolved in a total volume of 2 ml of dichloromethane:heptane (1:9, vol%:vol%) prior to the
162 removal of triacylglycerides (TAGs) using SPE purification. To analyse NVOFA, each oat sample was
163 extracted and analysed in triplicate.

164

165 *2.3.2. Purification of NVOFAs using SPE*

166 The extracted lipids contained a large quantity of neutral lipids, especially of TAGs, which had to
167 be removed before subjecting the extract to reversed-phase UHPLC analysis. The cartridges were
168 activated using 5 ml (2.5 ml $\times$ 2 times) of heptane, after which 1 ml of the lipid extract was applied.

169 Heptane:diethyl ether (9:1, vol%:vol%) eluent was used to remove neutral lipids from the cartridge.
170 After testing at several volumes, a total amount of 10 ml ($2.5 \text{ ml} \times 4 \text{ times}$) was selected. Fractions
171 after the first 5 ml of the eluent (Fr 1a) and the second 5 ml (Fr 1b) were collected separately, after
172 which the solutions were dried with nitrogen and the residues were redissolved into 1 ml of heptane
173 for neutral lipid analysis (in Section 2.3.3.) to show the removal of TAGs. The NVOFAs were
174 eluted from the cartridge using heptane:diethyl ether (1:4, vol%:vol%) that contained 1% acetic
175 acid. After testing several volumes, 5 ml ($2.5 \text{ ml} \times 2$) was chosen as the amount to elute the
176 NVOFAs. To analyse the NVOFAs, the collected solution was evaporated with nitrogen at 35°C ,
177 and the residue was dissolved into 0.5 ml of 2-propanol and 0.5 ml of acetonitrile.

178

179 Recoveries of epoxy and hydroxy fatty acids using the ASE extraction and SPE purification steps
180 were studied by analysing the recovery of the 12,13-epoxy-octadec-9-enoic acid, 9,10-epoxy-
181 octadec-12-enoic acid and 12-hydroxyoctadecanoic acid that had been added to the oat flour before
182 extraction. A solution containing about 2 mg of the epoxides and 1 mg of 12-hydroxyoctadecanoic
183 acid was evaporated with nitrogen at 35°C , after which the residue was redissolved into 1 ml of
184 ethanol, transferred to the cells and mixed with the 1-g oat-flour sample. In addition, oat-flour
185 samples mixed only with 1 ml of ethanol were used as references in each extraction batch. After the
186 ASE and SPE processes, the recovery test samples were analysed using UHPLC-ELSD. The
187 recovery tests were conducted in triplicate twice ($n = 6$).

188

189 2.3.3. Analysing neutral lipid classes using NP-HPLC-ELSD

190 Neutral lipid classes were determined using a normal-phase (NP)-HPLC method (Agilent 1200
191 HPLC system, Agilent Technologies; Santa Clara, CA, USA) and ELSD detection (Waters 2420
192 ELSD, Waters; Milford, MA, USA) as developed by Lampi et al. (2015) with slight modifications.
193 The separation of lipids was achieved using a LiChrosorb diol column ($5 \mu\text{m}$, $3 \times 100 \text{ mm}$, VDS

Optilab Chromatographie Technik GmbH; Berlin, Germany) and gradient elution consisting of a mixture of heptane and 0.1% acetic acid (solvent A) and of heptane with 0.1% acetic acid and 3% isopropanol (solvent B). The flow rate was set at 0.5 ml/min and the temperature to 25 °C. The total elution time was 50 min, and the gradient, consisting of solvents A and B (vol%:vol%), was as follows: 0–3 min (97:3); 3–10 min (97:3 to 0:100); 10–35 min (0:100); 35–40 min (0:100 to 97:3); and 40–50 min (97:3). Using this method, it was possible to measure the contents of TAGs and FFAs from SPE fractions (Section 2.3.2.) and diluted ASE lipid extracts (Section 2.4.) as individual lipid classes. The injection volumes of Fr 1a and Fr 1b were 10 µl and those of ASE extracts were 10 µl and 50 µl. A standard mixture of triglyceride palmitate and oleic acid was used to make second-order standard curves for quantification of TAGs and FFAs (100–5000 ng/injection). Lipid contents were given as mg/g of the oat samples.

205

2.3.4. Analysis NVOFAs using UHPLC-ELSD and UHPLC-Q-TOF

A reversed-phase UHPLC-ELSD method for the separation and quantification of the NVOFAs was developed. Briefly, the separation of the NVOFAs was conducted using a reversed-phase Acquity UPLC® HSS T3 column (1.8 µm, 2.1 × 150 mm, Waters; Dublin, Ireland) using a gradient elution that consisted of a mixture of Milli-Q H₂O and 0.05% of acetic acid (solvent A) and of acetonitrile with 0.05% of acetic acid (solvent B) at a column temperature of 30 °C and a flow rate of 0.3 ml/min. The 28-min gradient with the two solvents (A and B, vol%:vol%) was as follows: 0–6 min (50:50 to 38.5:61.5); 6–7 min (38.5:61.5); 7–14 min (38.5:61.5 to 35.5:64.5); 14–20 min (35.5:64.5 to 10:90); 20–25 min (10:90 to 0:100); 25–25.5 min (0:100); 25.5–26 min (0:100 to 50:50); and 26–28 min (50:50). A final 4 µl of the sample solution obtained from Section 2.3.2 was injected into UHPLC-ELSD and UHPLC quadrupole-time-of-flight (Q-TOF) equipment for analysis. The contents of the epoxides were calculated based on the standard curve determined using the 9,10-epoxyoctadecanoic acid, while those of the hydroxy and epoxy-hydroxy compounds were

219 calculated based on the standard curve determined using 12-hydroxyoctadecanoic acid. The
220 NVOFAs were quantified using second-order standard curves (range 80–2000 ng/injection), and
221 results were given as $\mu\text{g/g}$ of the oat samples.
222

223 The identification of the NVOFAs was conducted in a negative ion mode using an UHPLC-Q-TOF
224 instrument (Acquity I class, Waters, Milford, MA, USA) that had an electrospray ionisation (ESI)
225 interface coupled with a SYNAPT G2-Si Mass Spectrometer, as previously described (Yang et al.,
226 2017) but with modifications. The column, eluents and elution gradients were the same as those
227 used in the UHPLC-ELSD method described above. In addition, the instrumental settings of the MS
228 were as follows: mass scanning range m/z : 50–1200; capillary voltage: 2.5 kV; nebuliser gas flow:
229 6.2 bar; cone gas flow: 100 l/h; source temperature: 100 °C; desolvation gas flow: 1000 l/h;
230 desolvation temperature: 500 °C; trap collision energy: 4 eV; ramp of MS/MS trap collision energy:
231 10–70 eV; trap gas flow: 2 ml/min; and scan time: 0.3 s.
232

233 *2.4. Measurement of lipid hydrolysis and volatile oxidation products*

234 To study the lipid hydrolysis in the oat samples, the FFAs were measured using normal phase (NP)-
235 HPLC-ELSD after extraction of the lipids using ASE (Section 2.3.1.) and a method developed
236 earlier (Lampi et al., 2015), except that the flour samples were mixed with 1 ml of ethanol before
237 ASE extraction. Briefly, the lipids were extracted using acetone as the solvent at a pressure of 1000
238 psi and a cell temperature of 100 °C. The cells were preheated for 5 min, and the lipids were
239 extracted using 2 static cycles of 10 min each. After extraction, the extracts were transferred to
240 round-bottom flasks and evaporated to dryness using a rotary evaporator at 35 °C. The lipid residues
241 were first dissolved in 10 ml of heptane, further diluted 1:10 with heptane and then filtered through
242 0.45- μm filters into vials before being injected into the HPLC. The TAGs and FFAs were measured
243 using the NP-HPLC-ELSD method described in Section 2.3.3.

244

245 To study the lipid oxidation, volatile degradation products of hydroperoxides, especially hexanal
246 and 2-pentylfuran, were measured using a head-space solid-phase micro-extraction GC-MS (HS-
247 SPME-GC-MS) method developed by Damerau, Kamlang-ek, Moisisio, Lampi and Piironen (2014).
248 In brief, sample vials were incubated at 60 °C for 20 min, and volatile compounds were extracted
249 using a divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) fibre (50/30-µm) at 60
250 °C for 30 min. The compounds were separated using a SPB-624 column (30 m x 0.25 mm i.d., 1.4
251 µm) and identified and quantified by MS using total ion chromatograms (m/z from 50–300). A
252 porridge mixture of oat flakes and raspberry (Elovena, Raisio Group; Raisio, Finland) was milled
253 and used as a reference sample to monitor the performance of the HS-SPME-GC-MS in each
254 sample set. The volatile compound results were given as peak areas (counts* s* 10^6). The in-house
255 reference gave repeatable peak area values for hexanal (7.2 ± 0.6 counts* s* 10^6 , $n = 6$).

256

257 2.5. Statistical analysis

258 All results were given as mean values $\pm$ standard deviations (SD) of triplicate samples. Figures were
259 drawn using OriginPro 2018 (OriginLab Corporation; Northampton, MA, USA).

260

261 3. Results and Discussion

262 3.1. Analysis of the NVOFAs from oat lipid extracts

263 Of the three solvent mixtures tested using ASE extraction, heptane:ethanol (2:1) yielded the highest
264 lipid amounts, 39.5 ± 0.9 mg/g ($n = 6$), calculated as the sum of fatty acids, and this mixture was
265 used to extract the NVOFAs. The removal of the TAGs and a large portion of the FFAs from the
266 lipid extracts was achieved using SPE fractionation (data not shown). Using the heptane:diethyl
267 ether mixture (9:1, vol%:vol%), TAG residues were no longer detected in the second 5-ml elution
268 of the silica cartridge. NVOFAs were completely eluted from the cartridge using 10 ml of the

269 heptane:diethyl ether mixture (1:4, vol%:vol%) containing 1% of acetic acid, which was shown by
270 the high rates of recovery of the epoxy and hydroxy fatty acids that had been added to the oat
271 sample prior to ASE extraction. The recovery values ($n = 6$) for the 12,13-epoxy-octadec-9-enoic
272 acid and the 9,10-epoxy-octadec-12-enoic acid were 89% and 90%, respectively, and 96% ($n = 6$)
273 for the 12-hydroxyoctadecanoic acid. In addition, the method developed was repeatable and stable
274 during the entire experiment, because the oat in-house reference sample analysed in every batch of
275 samples provided repeatable total quantities of NOVAFs of $1964 \pm 185 \mu\text{g/g}$ of flour ($n = 9$) after
276 being subjected to UHPLC-ELSD analysis, as presented below.

277

278 All the NVOFAs extracted from the in-house reference oat sample and collected after SPE
279 fractionation were separated within the elution time of 28 min using UHPLC-ELSD (Fig. 1), with
280 two compounds apparently co-eluting at a retention time of 9.2 min. To identify the products,
281 UHPLC-Q-TOF with ESI in the negative mode and the relative retention times ($R_r = t_{\text{compound}}/t_{\text{epoxy}}$
282 standard) were used, and quantitative analysis using ELSD as the detector was used. All the
283 compounds produced deprotonated molecular precursor ions $[\text{M}-\text{H}]^-$, and together with their
284 fragmentations, each NVOFA (C1–C7, Fig. 1) produced a characteristic spectrum (a–g, Fig. 2). The
285 major characteristic fragments of $m/z > 150$ were used in the structural identification of the
286 compounds. Each compound had a fragment of m/z 18 less than the precursor ion, indicating that a
287 molecule of water was always lost during fragmentation, forming ion $[\text{M}-\text{H}-\text{H}_2\text{O}]^-$. In all the
288 spectra, either the $[\text{M}-\text{H}]^-$ ion or the $[\text{M}-\text{H}-\text{H}_2\text{O}]^-$ ion had the highest intensity, that is, the base
289 peak.

290

291 The earliest eluted NVOFAs (C1 and C2, Fig. 1) possessed the biggest m/z deprotonated molecular
292 ions, which were m/z 313.2 and 311.2 (Figs. 2a, b), respectively, indicating that they had more
293 oxygen, such as in hydroxy, epoxy or hydroperoxy groups, in their structures than did other

compounds. C1 produced a fragment m/z 183.1 (Fig. 2a) that had an intensity of > 90% relative to the base peak, and C2 produced fragments m/z 155.1, 171.1, 199.1 and 211.1, which had intensities of 10–40% (Fig. 2b). Based on the deprotonated molecular ion and its fragmentation, C1 was identified as 12,13-dihydroxy-octadec-9-enoic acid (C1, R_f = 0.301, Fig. 1), as presented by Newman, Watanabe and Hammock (2002) and Levandi et al. (2009). In line with the results of their studies, the present study tentatively identified a minor peak eluting close after C1 as 9,10-dihydroxy-octadec-12-enoic acid, based on its deprotonated molecular ion (m/z 313.2) and its major fragment (m/z 201.1). Because it was present in such a low quantity, it was not included in the study. Hamberg and Hamberg (1996) suggested that 12,13-dihydroxy-octadec-9-enoic acid was hydrolysed from 12,13-epoxy-octadec-9-enoic acid as a minor product by soluble epoxide hydrolase and that the hydrolase preferred the 12,13-epoxy fatty acid isomer as the substrate.

C2 was tentatively identified as a hydroxy-epoxy fatty acid and apparently contained a mixture of 13-hydroxy-9,10-epoxy-octadec-11-enoic acid and 9-hydroxy-12,13-epoxy-octadec-10-enoic acid (C2, R_f = 0.347, Fig. 1). The MS contained typical fragments for each of the isomers: the m/z 155.1 ion can form after scission of the carbon-carbon bond of the epoxide group at position 9,10 and the m/z 193.1 ion after dehydration of the scission product of the carbon-carbon bond of the epoxide group at position 12,13, as suggested by Huang and Schwab (2012) and Yuan, Majchrzak-Hong, Keyes, Iadarola, Mannes and Ramsden (2018). Epoxy-hydroxy fatty acids are POX-catalysed intramolecular epoxidation products of hydroperoxides that contain a double bond and that have been suggested as forming in oat seeds (Piazza et al., 1999; Hanano et al., 2006). The identification of the structures of C1 and C2 was further supported by their retention order in reversed phase (RP)-UHPLC (Yuan et al., 2018).

318 The largest peak of the NVOFA chromatogram that eluted at 9.2 min consisted of two partly co-
319 eluting compounds (C3 and C4, Fig. 1). Two different mass spectra were obtained (Figs. 2c, d). The
320 two compounds had the same molecular ion m/z 295.2 and a fragment ion m/z 195.1, but the
321 intensity of the $[M-H-H_2O]^-$ at m/z 277.2 was the highest in C4 (Fig. 2d) and much lower in C3
322 (Fig. 2c). Another difference was fragment ion m/z 171.1, which occurred in the spectrum of C4 and
323 was only minor in C3. The compounds were identified as hydroxy fatty acid regio-isomers: the first
324 was 13-hydroxy-octadeca-9,11-dienoic acid (C3, R_f = 0.521, Fig. 1) and the second was 9-hydroxy-
325 octadeca-10,12-dienoic acid (C4, R_f = 0.526, Fig. 1). The spectra were typical for unsaturated
326 hydroxyl fatty acids showing the presence of the hydroxyl group (Murphy, 2015). The conjugated
327 mono-hydroxy compounds C3 and C4 are typical POX-catalysed oxidation products of linoleic acid
328 hydroperoxides, as presented by Hamberg and Hamberg (1996). Co-elution of the two isomers has
329 also been observed when using RP-HPLC (Schneider, Schreier, & Herderich, 1997).

330
331 The next 3 NVOFAs (C5, R_f = 0.779; C6, R_f = 0.797; C7, R_f = 1.000; Fig. 1) were identified as
332 mono-epoxy fatty acids. C5 and C6 had deprotonated molecular ions $[M-H]^-$ m/z 295.2, and C7 had
333 $[M-H]^-$ m/z 297.2 (Figs. 2e, f, g), indicating that C5 and C6 were monounsaturated epoxy fatty
334 acids and that C7 was a saturated one. The fragmentation presented in the spectra was comparable
335 to that presented earlier (Orellana-Coca et al., 2005; Murphy, 2015; Yang et al., 2017). Thus, C5,
336 C6 and C7 were identified as 12,13-epoxy-octadec-9-enoic acid, 9,10-epoxy-octadec-12-enoic acid
337 and 9,10-epoxyoctadecanoic acid, respectively. C5 and C6 are typical POX catalysed oxidation
338 products of linoleic acid and C7 of oleic acid, which all are typical epoxy fatty acids produced by
339 POX catalysis (Hamberg & Hamberg, 1996; Yang et al., 2017).

340
341 Overall, the method developed in the present study was able to quantitatively analyse several
342 NVOFAs from the oat matrix, which had not yet been done. This method enables following their

343 formation and degradation and understanding of the reaction routes that POX activity can catalyse
344 in oat during storage.

345

346 *3.2. Occurrence of the NVOFAs in flours from the NHT and HT oat grains*

347 The method developed was first used to study the occurrence of NVOFAs in selected commercial
348 oat products. There were NVOFAs in the old NHT oat powder (O-NHT-P), the freshly milled flour
349 from old NHT oat grains (F-NHT-F1) and the freshly purchased NHT oat flour (F-NHT-F2) (Table
350 1a). Although the amounts of NVOFAs differed among these NHT samples, all contained hydroxy
351 fatty acids from linoleic acid (C3 and C4) and epoxy fatty acids from linoleic and oleic acids (C5–
352 C7). The same epoxy fatty acids had been found at similar levels in 33 oat accessions, although the
353 hydroxy fatty acids measured in that study were different (Leonova et al., 2008). Doehlert et al.
354 (2010) also found 0.25 mole% of the epoxy fatty acids of total fatty acids, which was also
355 comparable to the level found in the present study. In the two HT oat samples (Table 1a), no
356 NVOFAs were detected. This clearly indicates that the NVOFAs are likely formed via the POX
357 enzyme catalysed lipid oxidation pathway in NHT oat and that formation may already have begun
358 in whole grains to some extent.

359

360 The formation of NVOFAs was further studied in two samples (F-NHT-F1 and F-NHT-F2) during
361 a 24-week storage experiment at 35 °C (Table 1a). Although the F-NHT-F1 and F-NHT-F2 had
362 originally had comparable amounts of NVOFAs (350 ± 11 µg/g and 284 ± 7 µg/g, respectively) and
363 the amounts increased during storage, the distribution of products differed greatly between the two
364 materials. In both, during the first 6 weeks, the total amount of NVOFAs increased almost 4-fold,
365 and the peak values were reached after 18 weeks. Thereafter, the amounts of NVOFAs dropped
366 sharply to about 1100–1200 µg/g of flour by the end of the storage period. The original quality and
367 the storage history of the two flours (F-NHT-F1 and F-NHT-F2) differed, and their profiles of

NVOFAs changed in different ways. In the F-NHT-F1, both hydroxy fatty acids and epoxy fatty acids dominated until 18 weeks of storage, after which the amount of epoxy fatty acids decreased. In the F-NHT-F2, after 12 weeks of storage, the amounts of hydroxy and epoxy fatty acids and of hydroxy-epoxy-octadecenoic acid increased markedly, and the latter was the major product at the end of the storage period. In both flours, dihydroxy-octadecenoic acid formed to a small extent ($< 100 \mu\text{g/g}$). Its formation and that of hydroxy-epoxy-octadecenoic acid have not previously been detected in stored oat flour, but only in experiments using enzyme incubation. The decreasing amounts of NVOFAs indicated that either the POX activity had decreased, the products had reacted further and/or the availability of unsaturated fatty acids as substrates had decreased. To better understand the formation of NVOFAs and to relate it to other degradation reactions in lipids, a controlled study using both NHT and HT oat flours was conducted.

3.3. Formation of NVOFAs in the flours from the NHT and HT oat grains

In the present study, all materials were freshly milled and subjected to controlled storage conditions, and NVOFAs, the indicators of lipid hydrolysis and the formation of volatile oxidation products all were measured at regular intervals. NVOFAs formed in all oat samples during storage at 40°C , but the amounts differed greatly between flours made from NHT (Table 1b) and HT oat grains (Table 1c). Two flours made from NHT oat grains contained small amounts of the primary POX products, that is, epoxy and hydroxy fatty acids ($57 \pm 2 \mu\text{g/g}$ and $112 \pm 1 \mu\text{g/g}$, respectively), at the beginning of the storage experiment, indicating that NVOFAs may form soon after milling. In contrast, in one flour, no NVOFAs were measured. In all three NHT samples, the amounts of NVOFAs increased to their peak values of $1700\text{--}2000 \mu\text{g/g}$ at two months of storage, after which the levels began to decrease to $700\text{--}1100 \mu\text{g/g}$ by the end of the four months of storage. It is worth noting that in the first experiment, the two NHT oat flours (F-NHT-F1 and F-NHT-F2) reached their peak amounts of NVOFAs much later (at 18 weeks) than in the controlled experiment, which might indicate that the

POX activities in the first experiment had already begun to decrease during their unknown storage history. In the controlled experiment, much smaller amounts of NVOFAs were formed in the flours from HT oat grains than in those from NHT oat grains. The NHT-CV1 sample, which contained no NVOFAs at the beginning, contained the highest amount after four months of storage. In the HT-IS sample, the total amount of NVOFAs remained stable at 165–178 $\mu\text{g/g}$ after one month of storage, and in the HT-CV1 and HT-CV2 samples, the levels increased to $253 \pm 10 \mu\text{g/g}$ and $398 \pm 37 \mu\text{g/g}$, respectively, at three months of storage, after which they decreased to $98 \pm 1 \mu\text{g/g}$ at four months of storage. The present study clearly showed that using HT to inactivate lipid-modifying enzymes, particularly POX, is an efficient way to inhibit the formation of NVOFAs in oat. During the storage experiment, their contents in the HT oat samples were always $< 20\%$ of the amounts in the corresponding NHT oat samples.

404

The NHT oat samples always contained more epoxy fatty acids than hydroxy fatty acids, but after the highest NVOFA values were reached, the proportion of hydroxy fatty acids increased. The amount of epoxy-octadecanoic acid was always greater than the sum of epoxy-octadecenoic acids, indicating that oleic acid was as effective as linoleic acid in accepting oxygen from hydroperoxides through POX catalysis. Because only hydroxy-octadecadienoic acids were found and not hydroxy-octadecenoic acids, it was apparent that linoleic acid hydroperoxides were the co-oxidating species in the POX-catalysed reaction. Only modest amounts of hydrolysis products of epoxides, including dihydroxy-octadecenoic acids, and the intramolecular oxygen transfer reaction products of linoleic acid hydroperoxides, including hydroxy-epoxy-octadecenoic acids, were formed. These minor products were detected in NHT-CV1 samples only at the end of storage, whereas in the other samples, they were measured after one or two months of storage. In the flours made from HT grains, epoxy fatty acids were the only products detected until three months of storage, after which small amounts of hydroxy-octadecadienoic acids were present in the HT-CV1 and HT-CV2

418 samples. This, along with the small total amounts of NVOFAs, indicates that peroxygenation was
419 present in much smaller quantities in the HT materials than in the NHT ones.

420

421 In the present study, we showed a clear trend in the formation and degradation of NVOFAs in oat
422 flours during storage. To date, only one study has reported increase of epoxy and hydroxyl fatty
423 acids in oat grains (Doehlert et al., 2010). In that study, the epoxy fatty acid content increased 20-
424 fold in raw oat during storage for 22 weeks at 37 °C, and the hydroxy acids content was only 20%
425 of that of the final epoxy fatty acid content. However, the results of that study did not show what
426 happened during storage because it analysed NVOFAs only at the end of storage, and, as the present
427 study showed, the profile of NVOFAs changes markedly over time. Doehlert et al. (2010) also
428 showed that steaming before storing retarded increases in levels of epoxy and hydroxy fatty acids
429 by 60% and 17%, respectively, which was much less than the effect of HT in the present study.
430 Apparently, the HT the present study was more effective than that in their study at inhibiting POX.
431 To better evaluate the bitterness caused by NVOFAs, it would be interesting to know their threshold
432 values, but unfortunately, these not available. However, it has been found that hexane extract of HT
433 oat exhibits less bitterness (Günther-Jordanland, Dawid, Dietz, & Hofmann, 2016), which could
434 have derived from the NVOFA levels. Unfortunately, that study did not include NHT oat, which
435 could have supported the importance of NVOFAs as a cause of bitterness.

436

437 *3.4. Relating the formation and degradation of NVOFAs in flours made from NHT and HT oat* 438 *grains to lipid hydrolysis and volatile lipid oxidation products*

439 Since oat POX prefers free fatty acids over esterified fatty acids as substrates (Yang et al., 2017),
440 the occurrence of free fatty acids was studied to relate their formation to that of NVOFAs. In
441 addition, free fatty acids enhance lipid oxidation, including both LOX-catalysed oxidation and
442 autoxidation, which is a prerequisite for NVOFA formation. The neutral lipids of freshly milled

443 flours contained 30–45 mg/g of TAGs and 0–7 mg/g of FFAs (Fig. 3). The TAG contents were
444 higher in the HT samples, which did not contain any FFAs, whereas the NHT samples already had
445 1–7 mg/g of FFAs at the beginning of storage, indicating that lipid hydrolysis had begun during
446 milling. HT of oat grains efficiently inhibited lipid hydrolysis, as the content of FFAs increased
447 only to 2 mg/g during storage, while in the NHT samples, the FFA levels had already begun to
448 increase during the first month of storage, and the highest level reached was 42 mg/g in NHT-CV2
449 after three months of storage. Of the three NHT materials, NHT-CV1 was the most resistant to lipid
450 hydrolysis, as indicated by the fact that it had the lowest FFA content and the highest TAG content,
451 results that are in line with the slower formation of NVOFAs. Only after four months had the TAGs
452 degraded to very low levels in NHT-CV1, while this occurred one and two months earlier in NHT-
453 CV2 and NHT-IS, respectively.

454

455 Lipid oxidation, measured as the contents of hexanal and 2-pentylfuran, was intensive in the NHT
456 materials and only minimal in the HT materials during the four months of storage (Fig. 4). The
457 formation of volatile oxidation products showed that the NHT-IS sample oxidised more than the
458 others, and their amounts in this sample reached the highest values after two months of storage,
459 after which they levelled off and began to decrease. This occurred at the same time that the
460 sample's TAGs were completely hydrolysed, and that the NVOFAs reached their highest amounts.
461 After this, the amount of the NVOFAs, particularly of epoxy-fatty acids, began to decrease.
462 Although the lipids in the NHT-CV2 sample hydrolysed more rapidly than in the NHT-CV1
463 sample, hexanal formation, which indicated lipid oxidation, occurred in the NHT-CV1 sample
464 before the NHT-CV2 sample. However, 2-pentylfuran formation, which is considered to be an
465 indicator of enzymatic lipid oxidation and a product of linoleic acid 9-hydroperoxide (Ho & Chen,
466 1994; Lampi et al., 2015), was a little greater in the NHT-CV2 sample than in the NHT-CV1 one,
467 possibly indicating that the NHT-CV2 material had more enzymatic activity than the NHT-CV1

material. In accordance, there were already more NVOFAs in the NHT-CV2 sample at the beginning of the storage experiment. In addition, the NHT-CV2 sample formed more dihydroxy octadecenoic acid and hydroxy-epoxy-octadecenoic acids, and its total amount of NVOFAs remained higher than the total in the NHT-CV1 sample as long as substrates were present. All the NHT samples always had higher 2-pentylfuran content than hexanal content, indicating the importance of enzymatic oxidation. The inhibition of enzymes during heat treatment played a major role in the lipid oxidation of the flours made from the HT oat grains, as measured as the levels of hexanal and 2-pentylfuran. The volatiles in the freshly milled samples likely contributed to the natural flavour of the oat (Molteberg, Magnus, Bjorge, & Nilsson, 1996; Cognat, Shepherd, Verrall, & Stewart, 2012), and the amounts of hexanal and 2-pentylfuran increased only slightly during storage, reaching 8–18% of the values in the NHT oat samples after three months.

479

4. Conclusions

This study showed that accelerated solvent extraction at 40 °C was able to extract lipids, including epoxy and hydroxy fatty acids, from oat flours with good repeatability. The NVOFAs were separated from the neutral lipids using SPE and were further analysed using UHPLC-ELSD/MS. Epoxy and hydroxy fatty acids occurred in flours from NHT oat samples, and the quantities were higher in materials with unknown storage histories than in freshly milled ones. The quantities were insignificant in oat products that had been produced from HT grains, indicating the importance of POX activity to producing NVOFAs, which formed rapidly during storage in all NHT oat materials. Minor amounts of hydroxy and epoxy fatty acids could be detected soon after milling, and the amounts increased markedly during storage. In a controlled storage experiment, the highest total NVOFA amounts (1700–2000 µg/g) were reached in two months and then levelled off. In addition, there was a clear trend in the NVOFA profiles: at the beginning, the major products were epoxy fatty acids, after which the contents of hydroxy fatty acids began to increase. Epoxy fatty acids and

493 hydroxy fatty acids were the primary products of POX formed by intermolecular oxygen transfer.
494 The quantities of both these products and of the hydrolysis products of epoxides, i.e. dihydroxy
495 fatty acids, began to increase only after extensive oxidation and remained at low levels. It could be
496 that off-flavours are related not only to the total amount of NVOFAs but also to the distribution of
497 the products. The importance of lipid-degrading enzymes was further supported by the fact that
498 formation of NVOFAs in line with FFAs, which are produced by lipase, and to that of
499 hydroperoxides, which are produced either by LOX or autooxidation. Finally, inactivating the
500 enzymes using HT significantly retarded all lipid degradation reactions.

501

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590 **Figure legends**

591 Fig. 1. UHPLC-ELSD chromatogram of oxidised fatty acids from oat lipid extracts after SPE
592 fractionation. C1 = 12,13-dihydroxy-octadec-9-enoic acid; C2 = a mixture of 13-hydroxy-9,10-
593 epoxy-octadec-11-enoic acid and 9-hydroxy-12,13-epoxy-octadec-10-enoic acid; C3 = 13-hydroxy-
594 octadeca-9,11-dienoic acid; C4 = 9-hydroxy-octadeca-10,12-dienoic acid; C5 = 12,13-epoxy-
595 octadec-9-enoic acid; C6 = 9,10-epoxy-octadec-12-enoic acid; and C7 = 9,10-epoxyoctadecanoic
596 acid.

597

598 Fig. 2. Mass spectra (a–g) of the compounds C1–C7 as presented in Figure 1, produced using
599 UHPLC-Q-TOF with ESI ionisation in the negative mode.

600

601 Fig. 3. Contents of a) free fatty acids (mg/g flour) and b) TAGs (mg/g flour) in the flours made
602 from NHT and HT oat grains during storage at 40 °C.

603

604 Fig. 4. Peak areas of a) hexanal and b) 2-pentylfuran in counts per second of the flours made from
605 NHT and HT oat grains during storage at 40 °C.

606 **Table legends**

607 **Table 1. Contents ($\mu\text{g/g}$ flour) of non-volatile lipid oxidation products***

608 * a) products from NHT oat grains stored at 35 °C, b) flours from NHT oat grains stored at 40 °C
609 and c) flours from HT oat grains stored at 40 °C ($n = 3$, mean $\pm$ standard deviation). Samples in a)
610 were O-HT-F1, O-HT-F2, O-NHT-P, F-NHT-F1 and F-NHT-F2. Samples in b) were NHT-IS,
611 NHT-CV1 and NHT-CV2. Samples in c) were HT-IS, HT-CV1 and HT-CV2. Compounds analysed
612 were C1: 12,13-dihydroxy-octadec-9-enoic acid; C2: a mixture of 13-hydroxy-9,10-epoxy-octadec-
613 11-enoic acid and 9-hydroxy-12,13-epoxy-octadec-10-enoic acid; C3: 13-hydroxy-octadeca-9,11-
614 dienoic acid; C4: 9-hydroxy-octadeca-10,12-dienoic acid; C5: 12,13-epoxy-octadec-9-enoic acid;
615 C6: 9,10-epoxy-octadec-12-enoic acid; and C7: 9,10-epoxyoctadecanoic acid.

Fig. 1.

Fig. 2.

Fig. 2. (Continued).

Fig. 2. (Continued).

Fig. 2. (Continued).

Fig. 3.

Fig. 4.

Table 1.

a)

Oat samples		Non-volatile lipid oxidation products in oat ($\mu\text{g} / \text{g}$ flour)						
		C1	C2	C3 + C4	C5	C6	C7	Total
O-HT-F1		nd	nd	nd	nd	nd	nd	nd
O-HT-F2		nd	nd	nd	nd	nd	nd	nd
O-NHT-P		nd	54 ± 4	565 ± 23	58 ± 2	54 ± 1	69 ± 2	800 ± 32
Oat samples	Sampling time (35 °C storage)							
F-NHT-F1	0 weeks	nd	nd	119 ± 3	66 ± 2	61 ± 2	104 ± 4	350 ± 11
	6 weeks	58 ± 0.4	57 ± 0.3	667 ± 8	134 ± 5	116 ± 5	298 ± 10	1330 ± 29
	12 weeks	53 ± 2	64 ± 1	665 ± 5	139 ± 4	115 ± 4	331 ± 11	1367 ± 27
	18 weeks	51 ± 1	73 ± 2	732 ± 23	347 ± 3	349 ± 2	881 ± 37	2433 ± 68
	24 weeks	55 ± 0.2	81 ± 4	756 ± 7	61 ± 1	53 ± 1	64 ± 1	1070 ± 14
F-NHT-F2	0 weeks	nd	nd	90 ± 2	55 ± 1	55 ± 3	84 ± 1	284 ± 7
	6 weeks	73 ± 1	97 ± 5	540 ± 8	92 ± 1	79 ± 1	142 ± 3	1023 ± 19
	12 weeks	70 ± 0.4	152 ± 9	475 ± 23	183 ± 3	160 ± 6	404 ± 7	1444 ± 48
	18 weeks	95 ± 5	523 ± 16	257 ± 9	169 ± 3	177 ± 2	505 ± 8	1726 ± 43
	24 weeks	95 ± 7	495 ± 4	223 ± 4	114 ± 2	105 ± 2	196 ± 1	1228 ± 20

b)

Oat samples	Sampling time (40 °C storage)	Non-volatile lipid oxidation products in NHT oat ($\mu\text{g} / \text{g}$ flour)						
		C1	C2	C3 + C4	C5	C6	C7	Total
NHT-IS	Freshly milled	nd	nd	nd	bdl	bdl	57 ± 2	57 ± 2
	1 month	nd	nd	328 ± 8	311 ± 5	303 ± 5	699 ± 7	1641 ± 25
	2 months	60 ± 2	69 ± 1	466 ± 3	243 ± 17	219 ± 13	631 ± 19	1688 ± 55
	3 months	76 ± 1	97 ± 3	564 ± 7	132 ± 17	108 ± 12	426 ± 58	1403 ± 98
	4 months	46 ± 2	67 ± 4	409 ± 27	50 ± 1	49 ± 1	71 ± 1	692 ± 36
NHT-CV1	Freshly milled	bdl	bdl	bdl	bdl	bdl	bdl	bdl
	1 month	bdl	bdl	424 ± 3	167 ± 12	141 ± 11	382 ± 0.07	1114 ± 26
	2 months	bdl	bdl	348 ± 10	352 ± 25	387 ± 14	833 ± 59	1920 ± 108
	3 months	bdl	bdl	601 ± 14	252 ± 16	187 ± 13	471 ± 16	1511 ± 59
	4 months	65 ± 1	121 ± 5	687 ± 14	68 ± 1	61 ± 0.3	107 ± 6	1109 ± 27
NHT-CV2	Freshly milled	nd	nd	49 ± 0.2	nd	nd	63 ± 0.4	112 ± 1
	1 month	47 ± 1	52 ± 0.4	441 ± 3	106 ± 3	97 ± 2	285 ± 11	1028 ± 20
	2 months	51 ± 6	63 ± 9	491 ± 17	297 ± 8	295 ± 26	789 ± 34	1986 ± 100
	3 months	80 ± 5	113 ± 8	716 ± 14	200 ± 7	159 ± 9	533 ± 29	1801 ± 72
	4 months	66 ± 2	99 ± 1	637 ± 7	55 ± 1	53 ± 0.2	76 ± 0.4	986 ± 12

c)

Oat samples	Sampling time (40 °C storage)	Non-volatile lipid oxidation products in HT oat ($\mu\text{g} / \text{g}$ flour)						
		C1	C2	C3 + C4	C5	C6	C7	Total
HT-IS	Freshly milled	nd	nd	nd	nd	nd	nd	nd
	1 month	nd	nd	nd	61 ± 2	55 ± 1	62 ± 3	178 ± 6
	2 months	nd	nd	nd	53 ± 1	51 ± 1	61 ± 2	165 ± 4
	3 months	nd	nd	nd	58 ± 1	52 ± 1	61 ± 3	171 ± 5
	4 months	nd	nd	bdl	54 ± 0.3	52 ± 0.2	59 ± 2	165 ± 3
HT-CV1	Freshly milled	nd	nd	nd	nd	nd	nd	nd
	1 month	nd	nd	bdl	bdl	bdl	bdl	bdl
	2 months	nd	nd	bdl	56 ± 1	53 ± 1	70 ± 3	179 ± 5
	3 months	nd	nd	48 ± 2	67 ± 2	60 ± 1	78 ± 5	253 ± 10
	4 months	nd	nd	44 ± 1	bdl	bdl	54 ± 1	98 ± 2
HT-CV2	Freshly milled	nd	nd	nd	nd	nd	nd	nd
	1 month	nd	nd	bdl	bdl	bdl	60 ± 2	60 ± 2
	2 months	nd	nd	bdl	60 ± 1	57.6 ± 0.1	93.4 ± 0.2	210 ± 1
	3 months	nd	nd	65 ± 6	93 ± 9	76 ± 4	164 ± 18	398 ± 37
	4 months	nd	nd	44 ± 1	bdl	bdl	54 ± 0.4	98 ± 1

Where, nd: not detected; bdl: below detection limit.