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Determination of primary oxidation products of linoleic acid and triacylglycerols[1]

N. Gladovič[a,*], L. Zupančič-Kralj[b], J. Plavec[c]

[a]*Droga d.d., Quality Assurance, Seča 112, 6320 Portorož, Slovenia*

[b]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Aškerčeva 5, 1001 Ljubljana, Slovenia*

[c]*National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia*

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Abstract

The primary oxidation products of linoleic acid were analysed using normal-phase high-performance liquid chromatography with UV absorbance detection at 234 nm. The presence of primary oxidation products in fractions containing hydroperoxides and hydroxides was confirmed by fast atom bombardment (FAB)-MS and/or NMR spectroscopy. Hydroperoxides and hydroxides in peanuts exposed to autoxidation after sample pretreatment were analysed using the same method. The determination of peroxide value (PV) of the samples was performed and a correlation between PV and the hydroperoxides peak/non-oxidised triacylglycerols peak ratio was found. By using the HPLC method, it was possible to determine the hydroperoxides above PV 10 meq/kg, i.e., above the critical levels for the organoleptic evaluation of peanut samples. Below PV 10 meq/kg no noticeable rancidity odour and taste was recognised by tasters.

Keywords: Food analysis; Linoleic acid; Triacylglycerols; Monohydroperoxides; Monohydroxides

1. Introduction

Monohydroperoxides are the major initial reaction products of fatty acids with oxygen. Although these compounds are tasteless and odourless, they decompose to volatile carbonyl compounds, which contribute to the sensation of rancidity. The initial rate of formation of hydroperoxides exceeds the rate of decomposition during the oxidation of fats and oils. The relative rates of these reactions are reversed during the later stages of lipid degradation [1,2].

It is well known that the autoxidation of fatty acids [3] and edible oils [1] proceeds by a free-radical chain reaction mechanism, the result of which is a large number of positional and geometrical isomers [3,4]. High-performance liquid chromatography (HPLC) is one of the techniques used for the determination of such compounds [1–7] and has the advantages of making possible the separation of hydroperoxides without derivatization, with good sensitivity, rapidity and small sample consumption. The identification of the components in a chromatogram is, however, not simple, and requires specific techniques. In addition, quantitative evaluation of the chromatograms and the HPLC determination of the oxidative deterioration of lipids in foods is still not possible in the absence of authentic standard compounds, and thus the peroxide value (PV) determi-

*Corresponding author.

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nation by volumetric method is still necessary. Correlation between PV and organoleptic properties has been studied for various materials [9–12].

From the point of view of quality control we are attempting to establish a rapid and simple technique, which enables us to evaluate acceptability, to distinguish between the different quality of incoming material and which would be useful for studying the resistance of material to oxidation.

2. Experimental

2.1. Materials

Linoleic acid (LA) and trilinolein (TL) were purchased from Sigma (St. Louis, MO, USA). To obtain oxidised samples, standards were autoxidised for 24 h at 40°C in the dark in an oxygen atmosphere. Oxidised samples were then diluted in the mobile phase (10 mg/2 ml), sonicated and refrigerated prior to analysis by HPLC.

Samples of peanuts were unshelled, salted and roasted. Oxidation was carried out by incubating unpacked peanuts at 37°C and 44°C in the dark for up to 17 weeks. Lipids were cold-pressed from the samples using a purpose built model press. To avoid contact between the samples and metal surfaces, a polyamide material was used to make the cylinder and the mallet. The faintly turbid oil was centrifuged (3500 rpm, 1800 *g*, 10 min) before being diluted in hexane (60 mg/4 ml).

Reduction of the monohydroperoxides in the oxidised samples to hydroxy derivatives was done using $NaBH_4$ [13].

HPLC grade hexane was purchased from J.T. Baker (Deventer, Netherlands) and 2-propanol from Fluka (Buchs, Switzerland).

2.2. High-performance liquid chromatography

HPLC analyses were carried out using a pump type 6400 and variable UV wavelength detector set at 234 nm (both Knauer, Berlin, Germany). The columns used were Nucleosil 100 Si 5 μm (250×4 mm I.D. or 100×4 mm I.D., Macherey Nagel, Düren, Germany). The solvent systems were 0.9% or 2% 2-propanol in hexane at a flow-rate of 1.0 ml/min.

2.3. Determination of the peroxide value (PV)

The determination of the PV was made following the standard IUPAC method 2.501 for analysis of oils, fats and derivatives.

2.4. FAB ionisation mass spectrometry

FAB mass spectra of negative ions of linoleic acid, monohydroperoxide and monohydroxide of linoleic acid were obtained using an AutoSpecQ mass spectrometer (Fisons, VG Analytical, Manchester, UK) with Cs^+ ion bombardment (30 keV) and glycerol as the matrix.

2.5. NMR spectrometry

NMR spectra were recorded on a Varian Unity plus 300 MHz instrument (^1H at 299.982 MHz, ^{13}C at 75.439 MHz) with tetramethylsilane as the internal standard. $CDCl_3$ (99.9% deuterium) was used as the solvent. The sample temperature was set at 298 K and controlled to approximately ±0.5 K. Sample concentration was ca. 20 mg/0.6 ml. All ^1H NMR measurements were performed under identical spectral and processing conditions: 4000 Hz sweep width, 32 K time domain, zero filling to 64 K, and slight apodization to give enhanced resolution. ^{13}C NMR spectra were acquired under the following conditions: 16.5 kHz sweep width, 16 K time domain, line broadening of 1 Hz before Fourier transformation, complete ^1H decoupling and 20 000 transients were typically collected.

3. Results and discussion

Fig. 1 shows the normal-phase HPLC separation of linoleic acid monohydroperoxides (LA MHP) and the corresponding monohydroxides (LA MH). Fig. 2 shows the normal-phase HPLC separation of trilinolein monohydroperoxides (TL MHP), triacylglycerol monohydroperoxides (TG MHP) and the corresponding triacylglycerol monohydroxides (TG MH). The elution order is the same in all chromatograms. The first to be eluted are the nonpolar nonoxidised components, followed by the monohydroperoxides or monohydroxides, which are detected

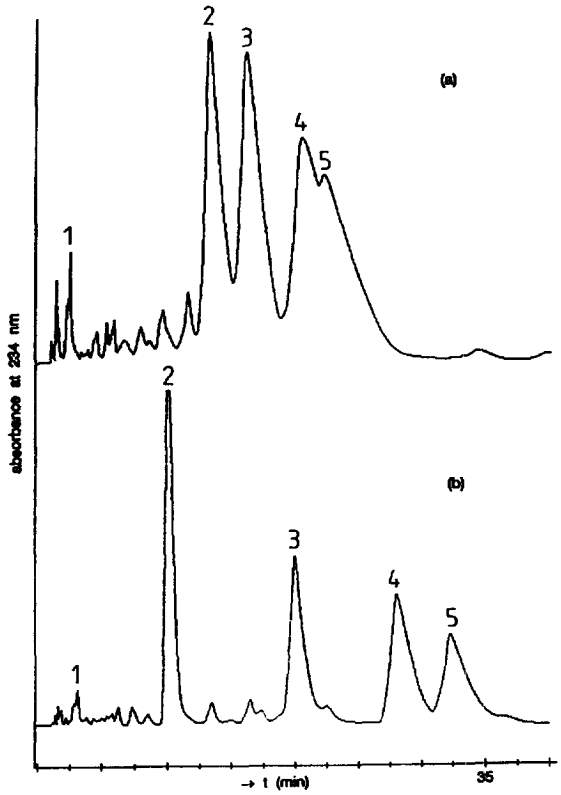

Fig. 1. HPLC chromatograms of oxidised linoleic acid (a) and oxidised linoleic acid after reduction with $NaBH_4$ (b) using UV detection at 234 nm. HPLC conditions: the column used was the 5 μm Nucleosil 100 Si (100×4 mm I.D.), the solvent system was 0.9% 2-propanol in hexane at a flow-rate 1.0 ml/min. Peaks were identified to be as follows: 1=non-oxidised linoleic acid (LA); a-2,3,4,5=linoleic acid monohydroperoxides (LA MHP); b-2,3,4,5=linoleic acid monohydroxides (LA MH).

at 234 nm because of the good absorption coefficient of conjugated double bonds.

After the reaction with $NaBH_4$, monohydroperoxides were partly reduced into more stabile hydroxy compounds. It seems that this improves the HPLC separation (Fig. 1b Fig. 2d).

By varying the polarity of the mobile phase (from 0.9% to 2% 2-propanol in hexane) in the case of oil samples, it is possible to combine the isomers to form a single monohydroperoxides fraction peak (Fig. 2b,c). This fast and simple method of separation between nonoxidised fraction and oxidation products was used for study of the correlation between HPLC triacylglycerol monohydroperoxides

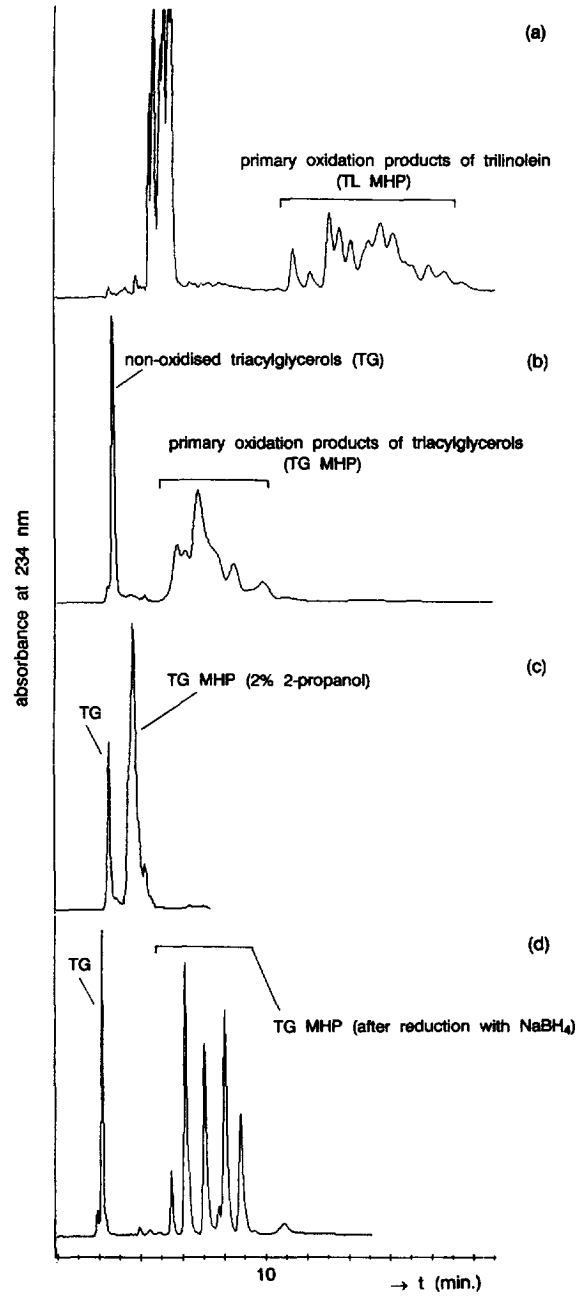

Fig. 2. HPLC chromatograms of oxidised trilinolein (a), peanut oil (b,c) and peanut oil (PV=22 meq/kg) after reduction with $NaBH_4$ (d) using UV detection at 234 nm. Chromatographic conditions for chromatograms (a), (b) and (d) are the same as those described in Fig. 1 except the column length (250 mm), in the chromatogram (c) the percentage of 2-propanol in the eluting solvent was increased from 0.9 to 2%.

(TG-MHP)/non-oxidised triacylglycerols (TG) peak area ratio and peroxide value of peanut oil (see further text).

In the peanut samples, incubated for more than 2 months at 44°C (PV more than approximate 40 meq/kg), an additional broad peak was also observed at higher retention times, thought to be due to a significant amount of conjugated dihydroperoxit-riacylglycerols.

Due to the absence of standards some identifications of monohydroperoxides were performed by: (a) identification using fast atom bombardment (FAB) MS and (b) identification using NMR spectroscopy.

(a) In the mass spectra of negative ions obtained using FAB with the oxidised linoleic acid dissolved in a glycerol matrix, the peaks at *m/z* 279, *m/z* 295 and *m/z* 311 were observed. These three peaks correspond either to the deprotonated molecules of linoleic acid $[LA-H]^-$, or to the deprotonated molecules of monohydroxilinoleic acid or monohydroperoxilinoleic acid, i.e., $[(LA+O)-H]^-$ or $[(LA+OO)-H]^-$. Even though monohydroperoxide and monohydroxide in minimal concentrations were detected in the original standard of linoleic acid, 20-fold increase in concentration was obtained for both compounds after 24 h of oxidation.

In the FAB mass spectra of the collected chromatographic fractions two characteristic peaks at *m/z* 311 and *m/z* 295 were observed. The base peak at *m/z* 311 in the mass spectra of the monohydroperoxide chromatographic fraction corresponds to the deprotonated molecules of the monohydroperoxilinoleic acid $[(LA+OO)-H]^-$. The base peak at *m/z* 295 in the mass spectra of the monohydroxide fraction corresponds to the deprotonated molecules of the monohydroxilinoleic acid $[(LA+O)-H]^-$.

In the FAB MS spectra of oxidised triglycerides the peaks at the same characteristic masses have been observed.

(b) In the ^1H NMR spectra of peroxidated products of linoleic acid signals from δ 4.25–4.43 ppm, which are characteristic of C*H*–OO fragments, were present. The respective ^{13}C NMR resonances for peroxidated methine carbon atoms were observed at δ 86.8 ppm. Several multiplets between δ 5.42 and 6.85 ppm are characteristic of the C*H*=C*H*–CH peroxidated fragments. Their basic shape was doublet of doublet with proton–proton coupling constants from 15.3 to 15.8 Hz and from 6.3 to 11.0 Hz. Integration of the peroxidated methine protons relative to the methine protons of the parent linoleic acid shows that ca. 15% of starting material has reacted after 24 h. The respective carbon atoms of the peroxidated double bond fragments resonate between δ 123.7 to 136.8 ppm. After a prolonged oxidation the ^{13}C NMR signals at δ 193.8–203.3 ppm confirms the formation of aldehydes as subsequent products of the reaction.

Oils pressed from peanuts at various levels of oxidation were analysed by HPLC and triacylglycerol monohydroperoxides (TG-MHP)/non-oxidised triacylglycerols (TG) peak-area ratio to PV plotted. There was no difference in results, when the peak area of partially separated peaks (see chromatograms and chromatographic conditions of Fig. 2b) was measured, or when the percentage of 2-propanol in the eluting solvent was increased to 2% and the MHP appeared as a single peak (Fig. 2c). Fig. 3 shows the correlation between PV and the TG-MHP/non-oxidised TG peak-area ratio on HPLC. In the investigation of peanut samples a prediction was made, that because of the compositional characteris-

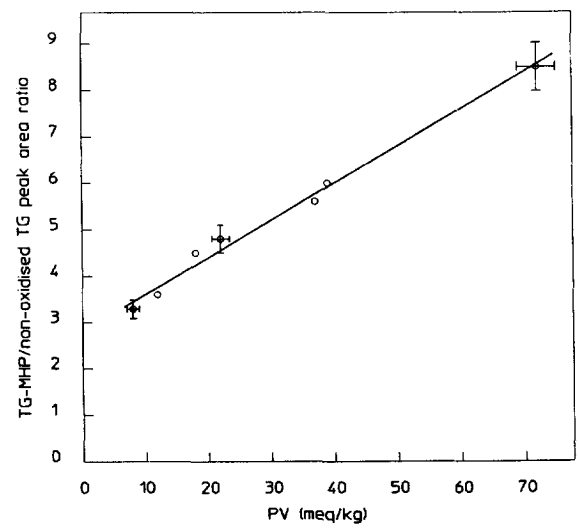

Fig. 3. Relationship between HPLC triacylglycerol monohydroperoxides (TG MHP)/non-oxidised triacylglycerols (TG) peak-area ratio (y) and peroxide value (PV, x) of cold pressed oil from oxidised peanut samples. Peak ratios calculated from chromatograms like those shown in Fig. 2c were used to study this correlation. The relationship above PV 8 meq/kg can be expressed by the equation $y=2.8+0.08x$, $r=0.994$.

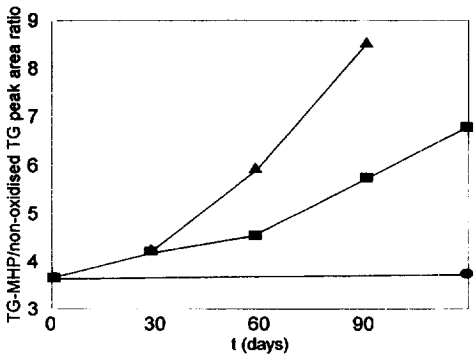

Fig. 4. Oxidative deterioration (y), expressed by HPLC triacylglycerol monohydroperoxides (TG MHP)/non-oxidised triacylglycerols (TG) peak-area ratio, of peanut samples (initial PV 10 meq/kg) incubated unpacked in the dark at 37°C (■) and 44°C (▲) or kept refrigerated at −18°C (●) for up to 120 days.

tics of peanut lipids (app. 30% of linoleic acid, 50% of oleic acid and only traces of linolenic acid present in triacylglycerol moieties) and due to much faster oxidation of trilinolein compared to triolein [8], the main primary oxidation products of peanut oil triacylglycerols will be conjugated MHP derived from linoleic acid as a part of TG. The good correlation between these two methods confirms this prediction. This correlation is expected for lipid samples, in which conjugated monohydroperoxides are the predominant species among the primary oxidation products [13].

Fig. 4 shows the increasing rates of oxidative deterioration of peanut samples (initial PV 10 meq/kg) incubated at 37°C and 44°C. The stability of vegetable oils towards oxidation is affected significantly by their fatty acid composition, tocopherol or other antioxidant contents and peroxide contents [8]. If one considers the same variety of peanuts (in our experiments the sort Runner was used), only the initial peroxide content is a significant variable.

4. Conclusions

Determination of monohydroperoxides by HPLC is not an absolute method for measuring the oxidative deterioration of food and must be performed in parallel with the organoleptic evaluation of the samples. Alternatively, carbonyl compounds which contribute to rancidity of the samples must be determinated using other sensitive techniques. Namely, low concentrations of monohydroperoxides can be found even in rancid samples because of the reversed relative rates of formation and decomposition of monohydroperoxides in the later stages of oxidation.

Normal-phase HPLC is suitable for these separations, and no pre-treatment of a column before running an analysis is needed. Sample consumption is less than 100 mg per analysis. Results are obtained in 20 min including the time needed for sample pretreatment. Running the chromatography after the reduction of peroxides to the more stable hydroxy-compounds is more time consuming than a rapid direct analysis, but it offers better selectivity towards triacylglycerol monohydroperoxides and their isomers.

The presence of monohydroperoxides in various chromatographic fractions was confirmed using FAB MS and NMR spectroscopy. In the absence of authentic standard compounds, the comparison of retention times provides a sufficient base for determining monohydroperoxides. A correlation between PV and the HPLC monohydroperoxi-triacylglycerols/non-oxidised triacylglycerols peak area ratio was found for peanut samples with PV above 8. Because of the lack of the samples with low peroxide values, the correlation under PV 8 meq/kg has not been studied yet.

Acknowledgments

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