



ELSEVIER

Journal of Chromatography A, 781 (1997) 215–221

JOURNAL OF
CHROMATOGRAPHY A

Separation of unsaturated fatty acids and related isomeric hydroperoxides by micellar electrokinetic chromatography

Oliver Schmitz, Siegmar Gäb*

Department of Analytical Chemistry, Bergische Universität-Gesamthochschule Wuppertal, Gauss-Strasse 20, 42119 Wuppertal, Germany

Abstract

The separation of several unsaturated fatty acids and the isomeric hydroperoxides derived from them was examined by micellar electrokinetic chromatography. Uncharged polyoxyethylene lauryl ether (Brij 35) was used both as a surfactant and for dynamic coating. A mixed micelle of Brij 35 and SDS made it possible to separate oleic, linoleic and linolenic acids and nearly all the isomeric hydroperoxides from these fatty acids in a single run in 15 min. The separation of arachidonic acid and the corresponding hydroperoxides was also possible. © 1997 Elsevier Science B.V.

Keywords: Fatty acids; Hydroperoxides; Brij 35

1. Introduction

The complete characterisation of oils and fats requires the determination of the individual fatty acids, some of which, as a result of autoxidation, may have hydroperoxide groups. Particularly susceptible to autoxidation are oils and fats containing polyunsaturated acids such as linoleic and linolenic acids. Hydroperoxides are important in food chemistry, since their thermal decomposition products are largely responsible for rancid odours [1]. In recent decades hydroperoxides have also attracted medical and biochemical interest. The role of oxidised lipids in atherosclerosis, for example, is widely discussed [2–4], and some hydroperoxides from arachidonic acid are of relevance as intermediates in the biosynthesis of prostaglandins, thromboxanes and leukotrienes [5–7].

Fig. 1 shows the structures of the isomeric hydroperoxides from oleic, linoleic and linolenic acids and the non-oxidised acids themselves; Fig. 2

has the structures of arachidonic acid and its corresponding hydroperoxides.

GC [8–11] or HPLC [12–14] is commonly used to analyse fatty acids and their hydroperoxides, but the polarity and the lability of these compounds usually makes derivatisation necessary for GC. Teng and Smith have achieved excellent separation by normal-phase HPLC of the four hydroperoxides derived from linoleic acid [15]. In recent years CE has also been applied to the analysis [16–18], and a method for both saturated and unsaturated fatty acids was presented by Collet and Gareil [19]. Indirect detection was used in all these studies because saturated fatty acids lack chromophores and fluorophores. The method of Collet and Gareil could not be extended to arachidonic acid, because this acid was insoluble in the electrolyte used.

We recently reported the analysis of unsaturated fatty acid hydroperoxides by MEKC, with Brij 35 being used both for dynamic coating and as the surfactant [20]. Almost all the isomeric hydroperoxides from oleic, linoleic and linolenic acids were separated and detected directly by UV. The use

*Corresponding author.

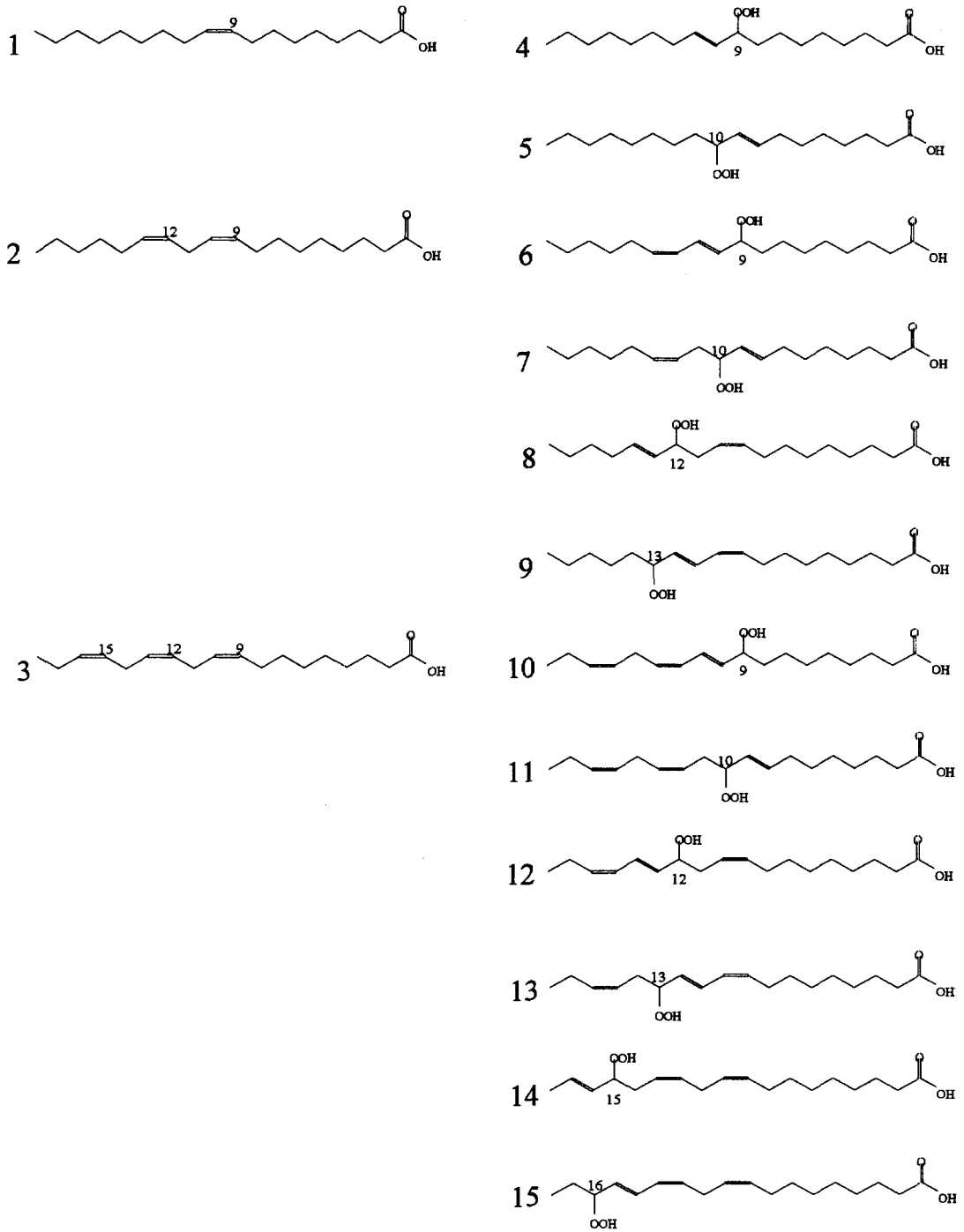


Fig. 1. The isomeric hydroperoxides 4–15 from oleic (1), linoleic (2) and linolenic acids (3).

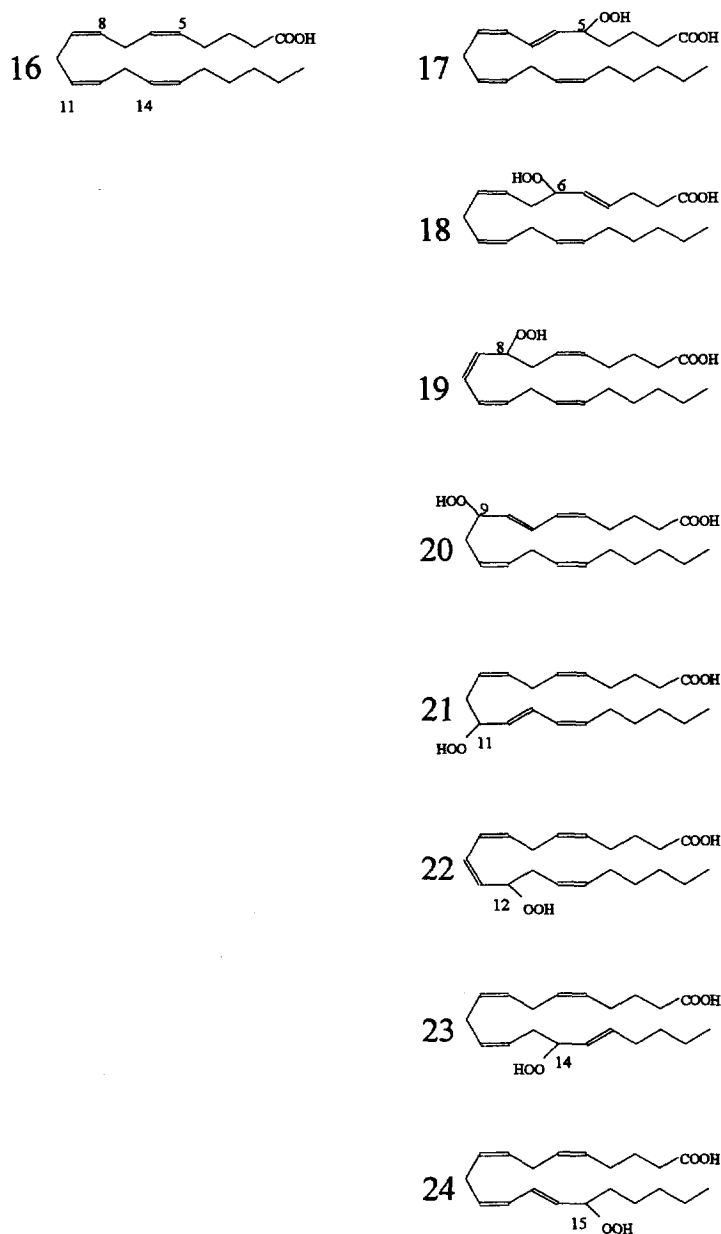


Fig. 2. The isomeric hydroperoxides 17–24 from arachidonic acid (16).

of uncharged micelles led, however, to rather long migration times.

No methods have been reported for the determination of both the isomeric fatty acid hydroperoxides and the fatty acids themselves in a single analysis without derivatization. In the present work the

isomeric hydroperoxide derivatives of oleic, linoleic and linolenic acids and the non-oxidised fatty acids themselves were separated by MEKC in a single run and detected by a UV-photodiode array. Arachidonic acid and the hydroperoxides derived from it were analysed in a separate run.

2. Experimental

2.1. Apparatus

The instrument used for the analyses was a Beckman P/ACE System 5510 with a photodiode-array detector and System GoldV810 software (Munich, Germany). The separations were carried out at 25°C on an untreated fused-silica capillary ($L=40/47$ cm, I.D.=50 μm) also purchased from Beckman; the field strength was 596 V cm^{-1} . Before each run the capillary was rinsed for 1 min with each of the following: 0.1 mM NaOH, tridest. water, 0.1 mM HCl, tridest. water, MeOH, 0.1 mM HCl, and tridest. water and finally for 5 min with buffer. Samples were injected under pressure over 5 s, and the capillary outlet was the anode in all runs.

2.2. Reagents and commercial standards

All chemicals used were of p.a. quality. Arachidonic acid and trilinolenin were purchased from Aldrich (Steinheim, Germany), 5(*S*)-hydroperoxy-eicosa-6E,8Z,11Z,14Z-tetraenoic acid (**17**) and 15(*S*)-hydroperoxy-eicosa-5Z,8Z,11Z,13E-tetraenoic acid (**24**) from Cascade Biochem (Berkshire, United Kingdom). The lipase from *Rhizopus arrhizus* (EC 3.1.1.3; 50000 U ml^{-1}) and the esterase from pork liver (EC 3.1.1.1; 10 mg ml^{-1}) were obtained from Boehringer (Mannheim, Germany). The surfactants with the exception of SDS and Brij 35 were provided by M. Schneider (Wuppertal, Germany). The other chemicals were from Merck (Darmstadt, Germany). 'Tridest' water (de-ionised and doubly distilled) was used for the buffer solution, which was sterilised by filtration through a 0.45- μm cellulose nitrate filter and de-gassed in an ultrasonic bath. Polyoxyethylene lauryl ether (Brij 35) and sodium dodecyl sulphate were used as surfactants and ethanol as organic modifier. In some runs phthalic acid (ca. 200 μM) was used as an internal standard for migration times.

2.3. Oxidation of the fatty acids

As reported earlier [20–22] the hydroperoxides from oleic, linoleic and linolenic acids were prepared by oxidation with singlet oxygen and after purification characterised by NMR spectroscopy.

Linoleic and linolenic acids were oxidised en-

zymatically [23,24] to 13(*S*)-hydroperoxy-octadeca-9Z,11E-dienoic (**9**) and 13(*S*)-hydroperoxy-octadeca-9Z,11E,15Z-trienoic acids (**13**), in order to define more closely two of the peaks observed.

2.4. Enzymatic hydrolysis of triglycerides

Ca. 1 mg of triglyceride was treated with 3 ml of a solution containing 400 mM glycylglycine and 15 mM CaCl_2 at pH 7.5. After the addition of 20 μl of lipase and 100 μl of esterase, the emulsion was allowed to react at 37°C for 10 min [25–28]. It was then diluted with ca. 3 ml of water and extracted several times with ether. The ether was removed on a rotary evaporator and the residue taken into 2 ml of methanol to produce the samples for analysis.

3. Results and discussion

Uncharged polyoxyethylene lauryl ether (Brij 35) was tested as a surfactant and for dynamic coating of the fused-silica capillary [20]. To prevent the resulting long migration times a number of anionic surfactants (SDS, 2-hydroxyalkyl sulphonates, 2-hydroxyalkyl sulphonic acids and N-lauroyl amino acids) were used to form micelles and mixed micelles with Brij 35 at various concentrations, but only mixed micelles of Brij 35 and SDS led to acceptable separation of the fatty acids and the hydroperoxides.

A certain amount of organic modifier was necessary to keep the analytes in solution and to provide for partitioning them between micelles and electrolyte. Several modifiers were studied (methanol, ethanol, 1-propanol, 2-propanol, dimethyl sulphoxide, tetrahydrofuran, acetonitrile, acetone) at 10, 20, 30 and 40%, and their amounts naturally influenced the critical micellar concentrations (CMC). The amount of the buffer was increased so as to maintain constant concentrations after dilution with the modifier. The migration times increased with the organic content of the electrolyte. Of the solvents studied, only methanol gave any separation at a concentration of 40%. At that concentration of the others, either the electrical current was interrupted, no micelles were formed, or the analytes were transported to the cathodic inlet.

Of all the conditions examined, the best separation

was achieved with either 30% ethanol or 30% 1-propanol; migration times of 15 and 45 min, respectively, made 30% ethanol the obvious choice as organic modifier. The conditions for optimal separation and migration times were thus 800 mg l^{-1} Brij 35 and 35 mM SDS in a solution of 70% aqueous buffer (60 mM sodium phosphate, pH 6.3) and 30% ethanol. These concentrations of Brij 35 and SDS are far higher than those necessary to form mixed micelles, which, as determined by the method of Karger et al. [29], are 25 mg l^{-1} and 1.1 mM , respectively.

Figs. 3 and 4 show the electropherograms of oleic (1.3 mM), linoleic (1.3 mM) and linolenic acids (1.2 mM) and the hydroperoxide mixtures from oleic (1.6 mM), linoleic (2.5 mM) and linolenic acids (1.7 mM) obtained at 195 nm and 234 nm, respectively. The latter electropherogram allowed the identification of the signals from conjugated isomers.

The separation of α - and γ -linolenic acids was also examined, since this is of interest in the analysis of various oils and fats. Fig. 5 shows that the buffer system described above gives partial separation of these isomers.

Another useful application of the same back-

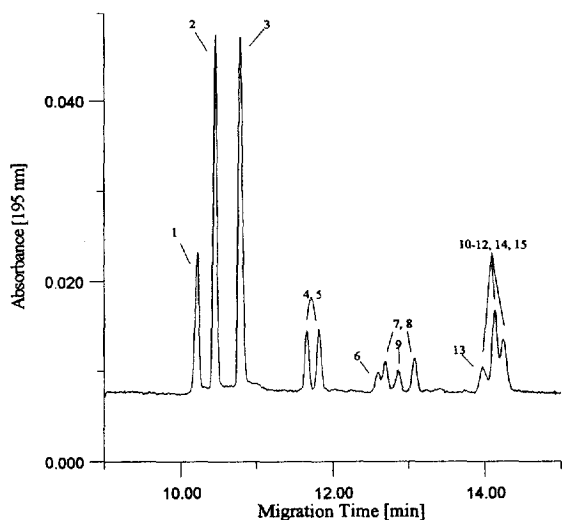


Fig. 3. Electropherogram showing the separation of oleic (1), linoleic (2) and linolenic acid (3) and hydroperoxides 4–15 at 195 nm. Electrolyte: 70% sodium phosphate (60 mM, pH 6.3), 30% ethanol, 800 mg l^{-1} Brij 35 and 35 mM SDS. Capillary: $40/47 \text{ cm} \times 50 \text{ }\mu\text{m}$ uncoated fused-silica.

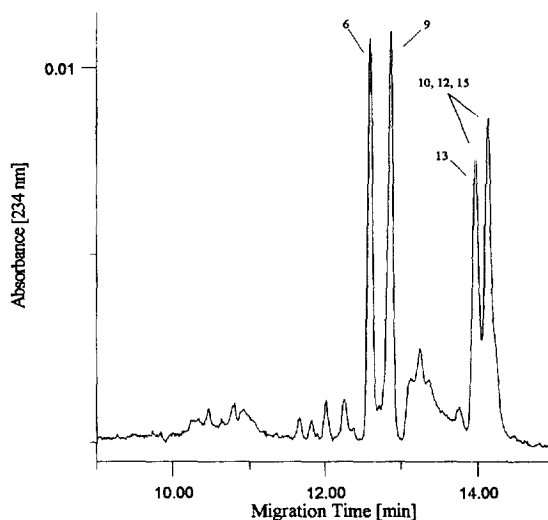


Fig. 4. Electropherogram showing the separation of oleic (1), linoleic (2) and linolenic acid (3) and hydroperoxides 4–15 at 234 nm. Electrolyte: 70% sodium phosphate (60 mM, pH 6.3), 30% ethanol, 800 mg l^{-1} Brij 35 and 35 mM SDS. Capillary: $40/47 \text{ cm} \times 50 \text{ }\mu\text{m}$ uncoated fused-silica.

ground electrolyte is the separation of arachidonic acid and the isomeric hydroperoxides derived from it. Fig. 6 shows the electropherogram of the crude mixture of arachidonic acid and its oxidation products obtained after 4-h treatment of the acid with singlet oxygen (the sensitiser, methylene blue, did not need to be separated before analysis). The commercial availability of two pure hydroperoxides made it possible to assign compounds 17 and 24. The peak at highest migration time was detected with very low sensitivity at 234 nm and must therefore represent one or both of the non-conjugated isomers (18, 23).

The triglycerides of fat samples can be hydrolysed very mildly by enzymes to the free fatty acids needed for analysis. Fig. 7 shows the electropherogram from hydrolysis of trilinolenin by this method.

4. Conclusion

The separation of fatty acids and the isomeric hydroperoxides from these acids by capillary electrophoresis has considerable advantages. Because the method is mild, it is not necessary to derivatise the

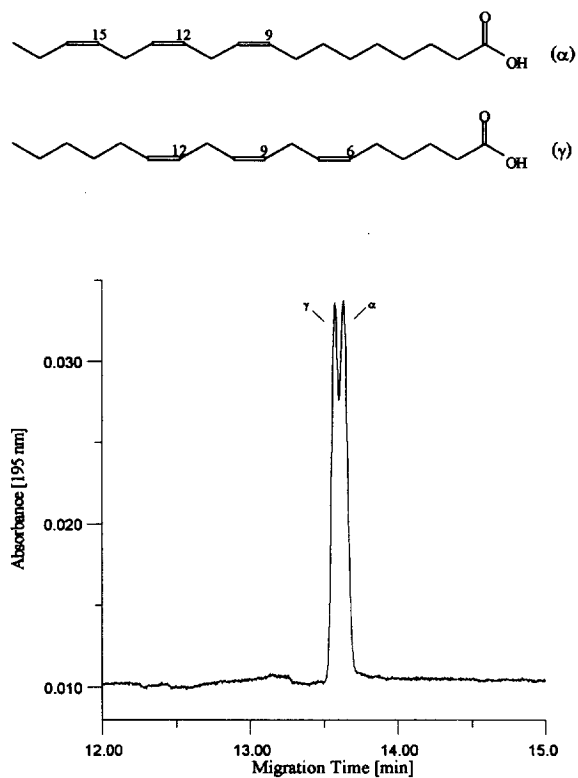


Fig. 5. Electropherogram showing the separation of α - and γ -linolenic acid. Electrolyte: 70% sodium phosphate (60 mM, pH 6.3), 30% ethanol, 800 mg l⁻¹ Brij 35 and 35 mM SDS. Capillary: 40/47 cm \times 50 μ m uncoated fused-silica.

acids before analysis. The use of a mixed micelle of Brij 35 and SDS makes possible the rapid separation of nearly all the isomeric hydroperoxides from oleic, linoleic and linolenic acids as well as the non-oxidised fatty acids themselves in a single run. The separation of arachidonic acid and six of the isomeric hydroperoxides derived from it is achieved with the same buffer in a different run. Further experiments must show whether the combination of enzyme hydrolysis with this CE method can be applied to practical fat samples containing peroxidised triglycerides.

Acknowledgments

Oliver Schmitz is grateful to the Friedrich

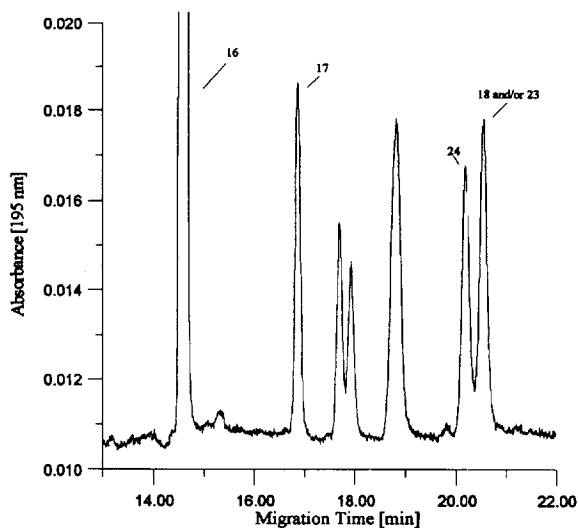


Fig. 6. Electropherogram showing the separation of arachidonic acid (16) and the isomeric hydroperoxides 17–24. Electrolyte: 70% sodium phosphate (60 mM, pH 6.3), 30% ethanol, 800 mg l⁻¹ Brij 35 and 35 mM SDS. Capillary: 40/47 cm \times 50 μ m uncoated fused-silica.

Naumann Foundation for a stipendium. The authors thank Prof. Manfred Schneider for samples of anionic surfactants.

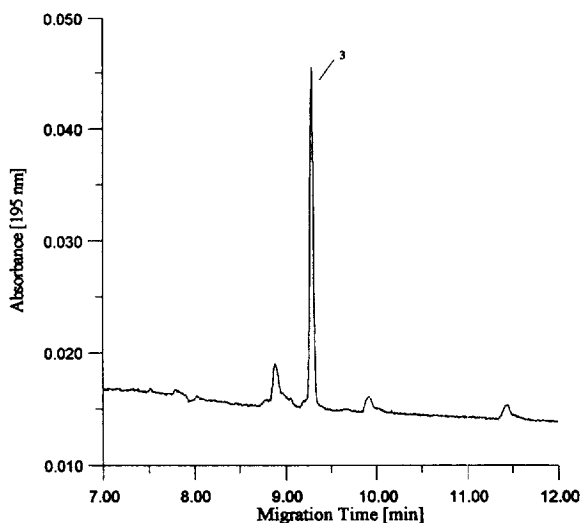


Fig. 7. Electropherogram showing the enzymatic hydrolysed products of trilinolenin. Electrolyte: 70% sodium phosphate (60 mM, pH 6.3), 30% ethanol, 800 mg l⁻¹ Brij 35 and 35 mM SDS. Capillary: 40/47 cm \times 50 μ m uncoated fused-silica.

References

- [1] H.-D. Belitz, W. Grosch, *Lehrbuch der Lebensmittelchemie*, Springer Press, Berlin, 1992.
- [2] S. Parthasarathy, D. Steinberg, J.L. Witztum, *Annu. Rev. Med.* 43 (1992) 219.
- [3] G. Jürgens, Q. Chen, G. Ledinski, A. Hammer, H. Esterbauer, *Acta Med. Austriaca* 20 (1993) 85.
- [4] K.L.H. Carpenter, S.E. Taylor, C. van der Veen, B.K. Williamson, J.A. Ballantine, M.J. Mitchinson, *Biochim. Biophys. Acta* 1256 (1995) 141.
- [5] W. Bartmann, *Angew. Chem.* 87 (1975) 143.
- [6] B. Samuelsson, *Angew. Chem.* 94 (1982) 881.
- [7] B. Samuelsson, *Angew. Chem.* 95 (1983) 854.
- [8] S.B. Turnipseed, A.J. Allentoff, J.A. Thompson, *Anal. Biochem.* 213 (1993) 218.
- [9] A.N. Frankel, W.E. Neff, T.R. Bessler, *Lipids* 14 (1979) 961.
- [10] E. Schulte, K. Weber, *Fat. Sci. Technol.* 91 (1989) 181.
- [11] K.D. Müller, H. Husmann, H.P. Nalik, G. Schomburg, *Chromatographia* 30 (1990) 245.
- [12] Z. Wu, D.S. Robinson, C. Domoney, R. Casey, *J. Agric. Food Chem.* 43 (1995) 337.
- [13] P. Heinmöller, *Doctoral Dissertation*, Technical University, Munich, 1997.
- [14] B. Frei, Y. Yamamoto, D. Niclas, B.N. Ames, *Anal. Biochem.* 175 (1988) 120.
- [15] J.I. Teng, L.L. Smith, *J. Chromatogr. A* 350 (1985) 445.
- [16] F.B. Erim, X. Xu, J.C. Kraak, *J. Chromatogr. A* 694 (1995) 471.
- [17] R. Roldan-Assaf, P. Gareil, *J. Chromatogr. A* 708 (1995) 339.
- [18] G. Gutnikov, W. Beck, H. Engelhardt, *J. Microcol. Sep.* 6 (1994) 565.
- [19] J. Collet, P. Gareil, *J. Cap. Elec.* 3 (1996) 77.
- [20] O. Schmitz, S. Gäb, *J. Chromatogr. A* 767 (1997) 249.
- [21] E.N. Frankel, *Prog. Lipid Res.* 19 (1980) 1.
- [22] E.N. Frankel, *Prog. Lipid Res.* 23 (1985) 197.
- [23] M.O. Funk, J.C. Andre, T. Otsuki, *Biochem.* 26 (1987) 6880.
- [24] H.W. Gardner, *Biochim. Biophys. Acta* 1001 (1989) 274.
- [25] P. Laboureur, M. Labrousse, *Bull. Soc. Chim. Biol.* 48 (1966) 747.
- [26] P. Laboureur, M. Labrousse, *Bull. Soc. Chim. Biol.* 50 (1968) 2579.
- [27] M. Semeriva, G. Benzonana, P. Desnuelle, *Bull. Soc. Chim. Biol.* 49 (1967) 71.
- [28] M. Sémériva, C. Dufour, *Biochim. Biophys. Acta* 260 (1972) 393.
- [29] K.F. Greve, W. Nashabeth, B.L. Karger, *J. Chromatogr. A* 680 (1994) 15.