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A direct and fast method to monitor lipid oxidation progress in model fatty acid methyl esters by high-performance size-exclusion chromatography

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Abstract

A new method based on high-performance size-exclusion chromatography (HPSEC) is proposed to quantitate primary and secondary oxidation compounds in model fatty acid methyl esters (FAMEs). The method consists on simply injecting an aliquot sample in HPSEC, without preliminary isolation procedures neither addition of standard internal. Four groups of compounds can be quantified, namely, unoxidised FAME, oxidised FAME monomers including hydroperoxides, FAME dimers and FAME polymers. Results showed high repeatability and sensitivity, and substantial advantages versus determination of residual substrate by gas–liquid chromatography. Applicability of the method is shown through selected data obtained by numerous oxidation experiments on pure FAME, mainly methyl linoleate, at ambient and moderate temperatures.

Keywords: High-performance size-exclusion chromatography; Lipid oxidation; FAME; Methyl linoleate; Oxidation kinetics; Tocopherol

1. Introduction

Pure fatty acid methyl esters (FAMEs) are commercially available substrates widely used as model compounds for studying a broad variety of lipid chemical and biochemical reactions. In the field of lipid oxidation, FAME and particularly methyl linoleate are extensively used to gain insight into oxidation kinetics, to test antioxidants efficiency and to study the influence of a number of variables on oxidative stability. Besides, oxidation of model FAME is often evaluated in reactions involved in organic coating chemistry and in production of conjugated linoleic acid. Just to cite some recent examples, numerous researchers have used FAME to measure the inhibitory properties of pure compounds and plant extracts against lipid oxidation [1–5], to study lipid oxidation kinetics and elucidate reaction mechanisms either in bulk or dispersed systems [6–10] and to evaluate interaction of oxidised model FAME with proteins and aminoacids [11,12].

The literature reflects the main problem encountered in the evaluation of lipid oxidation in general, as well as in model FAME in particular, which is the necessity to use more than one measurement to obtain a complete picture of the oxidation process [13,14]. Hence, it is not strange to find that similar substrates and experimental conditions may lead to opposite results depending on the analytical method used, usually providing only partial information of the oxidation state. Most studies on model FAME oxidation report only data based on residual amounts of intact FAME, as analysed by gas–liquid chromatography with addition of internal standard. Indirect calculation of substrate loss is used as a measurement of the level of oxidation.

High-performance size-exclusion chromatography offers the possibility to quantify groups of oxidation compounds differing in molecular weight or size. In this context, we have developed methodologies based on combination of adsorption chromatography and HPSEC to quantify oxidised triacylglycerol monomers, dimers and polymers (over two molecules), starting from oils [15,16], as well as from transesterified oils [17]. Adsorption chromatography was a necessary step for separation of the abundant unoxidised substrate. The main advantage of these methodologies is the accurate quantification achieved of the total non-volatile oxidation compounds formed, of greatest nutritional significance because they are retained in the food and hence ingested [18]. Furthermore, primary and secondary oxidation products are quantified in a single analysis. These

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methodologies are nowadays broadly used by many researchers in the food research fields of frying and lipid oxidation [19,20].

In this paper, a direct and rapid method is proposed to quantify lipid oxidation products of model FAME by simply injecting an aliquot sample in HPSEC. The method was applied to samples of methyl linoleate, methyl oleate and methyl linolenate, with or without α-tocopherol added, at different surface-to-sample volume ratios, and stored at ambient and moderate temperatures. The method proposed served to monitor lipid oxidation progress throughout storage and data obtained was used for calculation of kinetic parameters. Also, comparative results obtained by determination of residual substrate by gas-liquid chromatography are presented.

2. Experimental

2.1. Samples and chemicals

Methyl esters of oleic acid (MO), 9-*cis*,12-*cis*-linoleic acid (ML), linolenic acid (MLn) and nonadecanoic acid (all 99% of purity) were purchased from Nu-Chek Prep. Inc. (Elysian, MN, USA). α-Tocopherol was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Tetrahydrofuran was HPLC-grade and purchased from Romil (Cambridge, United Kingdom). All other chemicals and reagents were of analytical grade and obtained from local suppliers.

2.2. Oxidation conditions

Samples were placed in open glass beakers of different dimensions as to achieve surface-to-sample volume (S/V) ratios of 1, 10 and 50 cm^{-1}. Samples were oxidised at 25 and 60 °C in an oven in the dark and experiments were repeated at each temperature and S/V ratio tested.

Selected samples were added 100 μL/g FAME of a solution of α-tocopherol (2.5 mg/mL). Samples were homogenised using a magnetic stirrer and purged of solvents at 30 °C with a stream of nitrogen.

2.3. Analytical methods

2.3.1. Quantitation of oxidised monomers, dimers and polymers by HPSEC

About twenty-milligram samples were dissolved in tetrahydrofuran (50 mg/mL) for direct analysis by HPSEC, using a Rheodyne 7725i injector with 10-μL sample loop, a Waters 510 pump and a Waters 2414 refractive index detector (Waters, Milford, MA, USA). The separation was performed on two 100 and 500 Å Ultrastyragel columns (25 cm × 0.77 cm I.D.) packed with porous, highly cross-linked styrene–divinylbenzene copolymers (film thickness: 10 μm) (Hewlett–Packard, Avondale, PA, USA) connected in series, with tetrahydrofuran (1 mL/min) as the mobile phase. The groups of compounds separated and quantified in FAME samples were: unoxidised FAME, oxidised FAME monomers (OxMONs), FAME dimers (DIMs) and FAME polymers (POLs).

2.3.2. Quantitation of α-tocopherol by HPLC

α-Tocopherol levels were quantitated by HPLC and fluorescence detector [21].

2.3.3. Determination of residual substrate by gas–liquid chromatography (GLC)

Samples were analysed in a HP-6890 Series chromatograph (Hewlett–Packard, Avondale, PA, USA) equipped with a J&W DB-Wax fused-silica capillary column, 30 m × 0.25 mm I.D., film thickness 0.25 μm (J&W Scientific, USA) and flame ionization detector. The analyses were run using hydrogen (1 mL/min) as carrier gas and with the following oven temperature programme: 90 °C held for 2 min, 4 °C/min to 240 °C held for 20 min. Two microliter samples (approx. 50 mg/mL containing 20 mg/mL of nonadecanoic acid methyl ester as internal standard) were introduced into a split-splitless inlet operating in mode split with a 40:1 split ratio at 250 °C. Injections were carried out automatically using a 6890 Series injector (Agilent Technologies, Karlsruhe, Germany).

2.3.4. Determination of peroxide value

Peroxide value (PV) was determined by the iodometric method [22] starting from 100 mg-samples.

3. Results and discussion

Fig. 1 shows representative HPSEC chromatograms of ML samples oxidised at 25 °C (S/V ratio: 10 cm^{-1}), illustrating the resolution obtained between the peaks corresponding to the groups of compounds quantified. Sample A is the starting sample containing exclusively unoxidised ML. Sample B was taken during the early stage of oxidation, before rancidity was detected (3 days) and shows an additional peak (2%) corresponding to oxidised monomeric molecules of ML. Unoxidised ML and oxidised ML monomers elute at distinct retention time by virtue of the clear differentiation in molecular weight between the intact compounds and the oxidised monomeric molecules. However, a preliminary separation by adsorption chromatography of the unoxidised fraction is essential when analysing model triacylglycerols [23], oils [15,16] or even methyl esters obtained by oil transesterification [17], since, in such cases, a single peak would comprise oxidised monomers and unoxidised substrate when the total sample were directly analysed by HPSEC. Coelution at similar retention time in such samples is due to insufficient differences in molecular weight between unoxidised molecules and those including an oxygenated function, mainly as a result of the variable fatty acid composition.

Sample C corresponds to the end of the induction period (6 days), as indicated by initiation of polymerisation, rancidity detection and exhaustion of antioxidants if present. This finding was already reported for pure triacylglycerols [23,24] and oils [25]. This sample contained 8% OxMON and 1.1% DIM. Sample D was taken beyond the end of the induction period (11 days) and already comprised polymeric products. Composition of oxidation compounds was 29.5% OxMON, 6.4% DIM and 2.7% POL.