Rapid high-performance liquid chromatographic method of determining malondialdehyde for evaluation of rancidity in edible oils

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A simple HPLC method for the determination of malondialdehyde (MDA) in oxidised vegetable oils was developed and the results were compared with those given by a spectrophotometric method. Vegetable oil was steam-distilled in a Kjeldahl distillation apparatus and the MDA was determined in the aqueous distillates by HPLC, using a μ-Bondapak C_{18} column, with a mobile phase of 1% acetic acid–acetonitrile (85 + 15, v/v). A total time of 2 min was necessary to assay each distillate and only MDA was detected. MDA can be determined at a level of 1.5 × 10^{-9} mol l^{-1}.

Keywords: *Malondialdehyde; high-performance liquid chromatographic analysis; thiobarbituric acid analysis; vegetable oil; oxidation*

Autoxidation is mainly responsible for rancidity in edible oils. The primary products of oxidation are hydroperoxides which then undergo further degradation to a variety of secondary decomposition products.[1]

The peroxide value and the thiobarbituric acid (TBA) test are two of the more commonly used methods for the determination of the peroxidative lipids in edible oils. The peroxide value is a valuable measure of the early stages of oxidation.[2]

Malondialdehyde (MDA), one of the well-known secondary products, has been measured by the TBA method. The TBA test involves the reaction of 2-thiobarbituric acid (TBA) with MDA in edible oils to produce a chromogen which can then be determined spectrophotometrically at 532–535 nm. The major problem with the method is a lack of specificity. The TBA reacts with products of lipid peroxidation such as hydroperoxides and conjugated aldehydes to generate substances which absorb at 535 nm, similar to the adduct of MDA and TBA.[3–6] Thus, the analysis of vegetable oils by the spectrophotometric procedure is subject to error.

Most of the MDA present in fatty foods exists bound to other food constituents and very little of it exists in the free form. Thus, acid must be added to the food to be analysed in order to liberate the MDA.[7]

An HPLC method has been developed for determining total MDA in vegetable oils, after conversion of the MDA, released from its precursor, to a dansyl-pyrazole derivative .[8]

Kakuda *et al.*[7] suggested an HPLC method for the quantification of MDA in aqueous distillates from freeze-dried chicken meat. The MDA was determined using a mobile phase of 1% acetic acid–acetonitrile (15 + 85, v/v), with UV detection, at a level of 1.0 × 10^{-6} mol l^{-1}.

The purpose of the present work was to develop a selective, sensitive and simple method involving distillation of MDA from vegetable oils and its determination by HPLC. The advantage of such a method is a better knowledge of the actual oxidation status of the oil.

Experimental

Reference compounds and solvents

The TBA reagent 1,1,3,3-tetramethoxypropane (TMP) was purchased from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade) and acetic acid (analytical-reagent grade) were purchased from Merck (Darmstadt, Germany).

Fresh vegetable oils (sunflower, soybean, olive residue and corn) were purchased from ELAIS (Athens, Greece).

Instrumentation

Chromatographic determinations were performed on a Millipore–Waters (Milford, MA, USA) liquid chromatograph equipped with a 600E pump and a Waters 486 tunable UV absorbance detector. A computer integrator running a Waters Baseline 815 program was employed to record retention times and chromatograms and to evaluate peak areas. A Waters μ-Bondapak C_{18}, reversed-phase column (300 × 3.9 mm id) was used at ambient temperature. Chromatograms were monitored at 254 nm, with a sensitivity of 0.01 aufs. The mobile phase was 1% acetic acid–acetonitrile (85 + 15, v/ v), the flow rate was 2.5 ml min^{-1}, the pressure was 1300–1500 psi and the injection volume was 20 μl. The retention time of MDA was 1.44 min.

Preparation of standards

MDA standards

A 10 μl volume of TMP was accurately diluted to 10 ml with 0.1 M HCl in a screw-capped test-tube and immersed in a boiling-water bath for 5 min, then rapidly cooled with tap water (solution X). A working stock solution of MDA was prepared by pipetting 1.0 ml of the hydrolysed acetal (solution X) into a 100 ml calibrated flask and diluting to volume with water. The working stock solution was 6.07 × 10^{-5} M acetal or 4.37 μg ml^{-1} MDA. A 10-fold dilution of the working stock solution was made before preparing the calibration graphs. These standards were also used for the TBA method.

TBA solution

A 0.02 M solution of TBA in 90% glacial acetic acid was prepared.

HPLC determination

Approximately 10 g of vegetable oil (sunflower, soybean, olive residue or corn) were weighed into a glass petri dish and placed on a water-bath (50 °C) with a UV light (260 nm), 50 cm directly above (Bold UV 260 nm; General Electric, Cincinnati, OH, USA), for 12 h. A 0.5 g portion of the oxidised oil was subsequently accurately weighed into a 250 ml boiling-flask, 80 ml of water were added and the pH was adjusted to 1.5–1.8 with

2 M HCl. The flask was connected to a standard micro-Kjeldahl unit and the contents were distilled. The distillation was conducted as rapidly as possible using the maximum heater setting and terminated when 50 ml of distillate had been collected in a 50 ml calibrated flask (this usually took about 15 min). A 5 ml aliquot of the distillate was used for the TBA test and 20 μl for HPLC analysis. Typical chromatograms for the MDA standard and a sunflower oil sample are shown in Fig. 1.

TBA test

This was performed according to the method of Kakuda *et al.*[7] The sample (5 ml) was mixed in a screw-capped tube with 5 ml of TBA reagent. The tube was heated in a boiling-water bath for 30 min, cooled with tap water and the absorbance measured at 532 nm (Hitachi 3210 spectrophotometer, Hitachi, Tokyo, Japan).

Quantification

Standard calibration graphs for MDA were prepared by plotting peak area measurements at 254 nm *versus* concentration. The recovery of 98.4% was used to calculate the results.

Results and discussion

Method development

The method for MDA determination reported by Kakuda *et al.*[7] proved unsuccessful when applied to the determination of free MDA in vegetable oils because with the suggested mobile phase [1% acetic acid–acetonitrile (15 + 85, v/v)] no peaks were detected. After many trials a more suitable mobile phase was found to be 1% acetic acid–acetonitrile at (85 + 15, v/v). The detection limit of the modified method is 1.5×10^{-9} mol l^{-1}, whereas the method suggested by Kakuda *et al.*[7] could detect only 1×10^{-6} mol l^{-1}. The improved method was subsequently adopted for all HPLC work.

Stability of the MDA standards

Nine series of MDA standards were prepared in triplicate and immediately assayed by HPLC. After analysis, the standards were stored at 5 °C for 8 d and then re-analysed by HPLC. Table 1 shows the concentrations of the standard solutions and their corresponding peak areas on the 1st and 8th day. The plot of the values displayed in Table 1, which represent the concentration of MDA standards against HPLC peak area, was linear with an r^2 value of 0.998 and $y = 1948.9x + 25.172$. Statistical analysis of the data reported in Table 1 revealed that there was no difference between the MDA peak areas for day 1 and day 8 (Student's *t*-test) at the 5% level of significance. Thus, the MDA standards are stable during storage for 8 d at 5 °C.

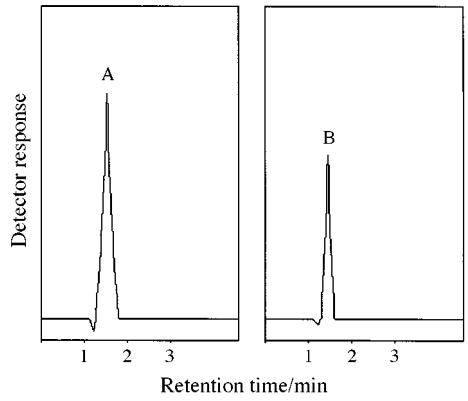

Fig. 1 Chromatograms showing, A, an oil sample and B, an MDA standard.

Recovery test

A working stock solution (100 mg of MDA per 100 ml of H_2O) was prepared using the same method as described previously. Nine standard solutions were prepared using 1–9 ml of the working stock solution to give final concentrations of 1–9 mg per 100 ml, respectively. These solutions were distilled following the procedure described under HPLC determination. The distillates were subsequently assayed by HPLC.

The recoveries (Table 2) are within the range 97.6–99% (mean 98.4%). This represents a significant improvement on the 70% recovery of MDA when determined by the TBA test[9] or 86.7, 78.9 and 88.3% for the single extraction, distillation and reflux heating procedures, respectively,[10] and demonstrates a further benefit of the proposed method.

Comparison of MDA levels in vegetable oils by the HPLC and TBA test method

Samples of oxidised vegetable oil were prepared as described previously. These samples were analysed for their MDA content by the TBA test and HPLC method. The HPLC results were as follows: 2.9×10^{-6}, 5.6×10^{-6}, 5.9×10^{-6} and 7.5×10^{-6} mol l^{-1} for olive residue oil, corn oil, sunflower oil and soybean oil, respectively. An explanation for the different levels of MDA in the above oils is that only peroxides which possess unsaturation β or γ to the peroxy radical are capable of undergoing cyclisation with the ultimate formation of MDA. Such peroxides could only be produced from fatty acids containing three or more double bonds.[3]

The linolenic acid content of the four oils varies as follows: olive oil, 0.3–1.1%; sunflower oil, 0.1–0.2%; corn oil, 0.6–2.6%; and soybean oil, 5–11%.[11] Thus, soybean oil, with

Table 1 Stability of MDA standards during storage at 5 °C for 8 d

Concentration/ 10^{-10} mol l^{-1}	Peak area		Loss (%)
	1st day*	8th day*	
0.3	540 ± 0.07	503 ± 0.18	6.85
0.9	1655 ± 0.50	1634 ± 0.09	1.27
1.8	3427 ± 0.33	3261 ± 0.12	4.84
2.7	5520 ± 0.06	5504 ± 0.04	0.29
3.31	6575 ± 0.03	6510 ± 0.05	0.99
3.92	7981 ± 0.11	7830 ± 0.16	1.89
4.52	8972 ± 0.27	8897 ± 0.08	0.84
5.15	9831 ± 0.08	9751 ± 0.21	0.81
5.72	10 962 ± 0.15	10 697 ± 0.03	2.42

* Average ± standard deviation ($n = 3$).

Table 2 Recovery values for MDA measured by HPLC

No.	MDA standards/mg		Recovery (%)
	Without distillation	After distillation	
1	1.000	0.99	99
2	2.000	1.98	99
3	3.000	2.96	98.6
4	4.000	3.92	98
5	5.000	4.93	98.6
6	6.000	5.87	97.8
7	7.000	6.92	98.9
8	8.000	7.81	97.6
9	9.000	8.83	98.1

the highest content of linolenic acid, showed the highest level of MDA after oxidation.

According to Varela,[12] the greater oxidative stability of olive oil compared with other vegetable oils is related to the high monounsaturated fatty acid content (about 80%). This observation can be used to explain the lower level of MDA formed in the olive residue oil compared with the other vegetable oils tested (*NB*: olive residue oil has a similar monounsaturated fatty acid content to olive oil) .

A correlation of the results from the HPLC method and the TBA test are given in Fig. 2.

The sensitivity of the TBA test was lower than that of the HPLC method: *i.e.*, 5×10^{-6} and 1.5×10^{-9} mol l^{-1} MDA, respectively. In addition, the TBA values for MDA were found to be higher than those given by the HPLC method. An explanation for this is that the TBA reagent can react with a variety of compounds present in oxidised lipids other than MDA and that impurities can also lead to the production of interfering coloured products.[13]

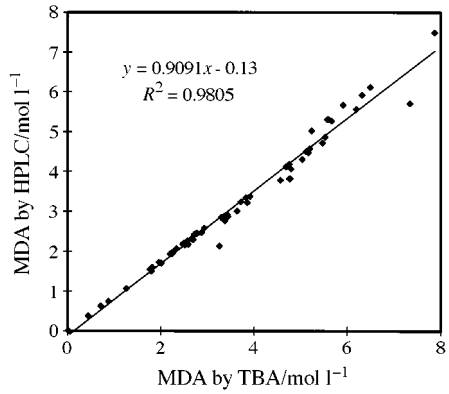

Fig. 2 Correlation of HPLC concentrations (mol l^{-1} MDA) *versus* TBA test concentrations (mol l^{-1} MDA).

The HPLC method is faster since only 2 min per injection is required for routine analysis. The method is also more accurate and specific because the results do not depend on the formation of a coloured complex.

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