Food Research International 60 (2014) 117–122

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

An improved rapid stigmastadiene test to detect addition of refined oil to extra virgin olive oil

Colin Crews *, Celine Pye, Roy Macarthur

The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, United Kingdom

ARTICLE INFO

Article history:
Received 13 June 2013
Received in revised form 5 November 2013
Accepted 16 November 2013
Available online 22 November 2013

Keywords:
Olive oil
Stigmastadiene
Authenticity

ABSTRACT

Extra virgin olive oil is a premium food product that is likely to be the target of adulteration with refined olive oils or seed oils. Refining produces steroidal alkenes (sterenes) including stigmastadiene. This paper describes a rapid GC–MS method for the determination of stigmastadiene which is faster and more sensitive than the current official procedure based on GC-FID. The method does not require a saponification procedure for cold pressed oils, uses a stigmastadiene standard for quantification, has a low limit of quantification (0.015 mg kg^{-1}) and gives excellent confirmation of peak identity at the current regulatory limit of 0.5 mg kg^{-1}.

Crown Copyright © 2013 Published by Elsevier Ltd. All rights reserved.

1. Introduction

Extra virgin olive oil (EVOO) is a premium food product commanding a relatively high price. There is therefore an incentive for its adulteration of EVOO with lower grades of olive oil or with seed oils.

The quality of olive oils of various grades is defined by the European Commission under Regulation (EC) No. 2568/91 (EC, 1991) amended by Commission Regulation (EC) No. 1989/2003 (EC, 2003). The Regulation also lays down specifications for their composition, and analysis. Eight grades of olive oil are defined, the most important being "extra virgin", "virgin", "olive" and "olive-pomace". Virgin olive oil is olive oil obtained directly from olives and solely by mechanical means, and EVOO is virgin oil of a 'superior category'. Ordinary olive oil is a mixture of refined olive oils and virgin olive oils, and olive-pomace oil is that obtained from the residue remaining after the extraction of olive oil from the olives.

Regulation (EC) No. 2568/91 describes several analytical tests to be used to check the quality of these oils. Among these is the gas chromatographic determination of stigmastadiene (ethylcholestadiene) which is an important indicator of the presence of refined oils in virgin oils. Many vegetable oils are refined by steps including bleaching and deodorization, which include treatment with acid bleaching earths and steam treatment at high temperature. These processes dehydrate the sterols present in the oil to form a series of steroidal hydrocarbons or sterenes (Cert, Lanzón, Carelli, Albi, & Amelotti, 1994). The major plant sterol is the β-sitosterol from principally 24-ethylcholesta-3,5-diene (stigmasta-3,5-diene; Fig. 1) and lesser quantities of positional isomers. Other sterols form similar compounds, 24-methylcholesta-3,5-diene (campesta-3,5-diene) is formed from campesterol and 24-ethylcholesta-3,5,22-triene (stigmasta-3,5,22-triene) is formed from stigmasterol.

The Regulation defines EVOO as having less than 0.15 mg kg^{-1} of 'stigmastadiene', which comprises the sum of stigmasta-3,5-diene and an un-named isomer that elutes close to the -3,5-diene using the specified chromatographic conditions.

In the analytical methods for stigmastadiene used by regulatory bodies a non-polar fraction is separated from the acylglycerols by column chromatography on silica gel. 1 g of refined oil is applied to the column and aliquots of solvent (hexane) are collected. The first eluted portion contains aliphatic hydrocarbons and squalene. The second eluted portion contains the sterenes. For virgin olive oils of low stigmastadiene content a 20 g sample is taken and a preliminary saponification is required in order to remove the acylglycerol bulk of the sample prior to the column chromatography.

The eluate is concentrated and analyzed by gas chromatography with flame ionization detection (GC-FID) using a non-polar column. A check must be made to verify that the stigmastadiene is well resolved from aliphatic hydrocarbons and squalene. Stigmasta-3,5-diene is eluted with an isomer appearing as a shoulder and the sum of the peak areas is measured. Stigmastadiene is quantified by comparison of the combined peak area with that of a single point addition of an internal standard of cholestadiene, the sterene derived from cholesterol, which is present in only negligible quantities in olive oil.

The method has been adopted by the International Olive Council (IOC, 2001) who has analyzed its performance by an international collaborative trial, the results of which are discussed later.

The Regulation GC-FID method is relatively antiquated, involving saponification, column chromatography, and gas chromatography with flame ionization detection. Stigmastadiene is measured against cholesta-3,5-diene, a related compound. Long silica columns with slow

* Corresponding author. Tel.: +44 1904 462549.
E-mail address: colin.crews@fera.gsi.gov.uk (C. Crews).

0963-9969/$ – see front matter. Crown Copyright © 2013 Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2013.11.023

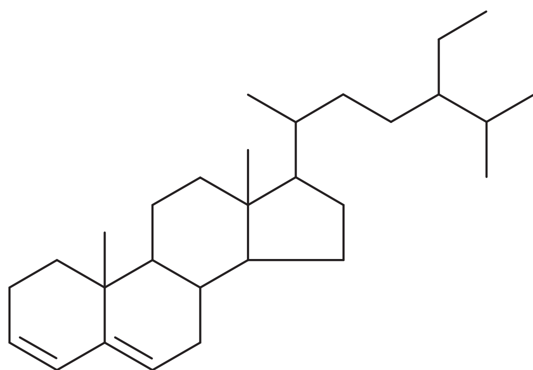

Fig. 1. Structure of stigmasta-3,5-diene.

elution, and careful fraction collection are required for the systematic separation and identification of steroidal hydrocarbons for the authenticity testing of extra virgin olive oils (Bortolomeazzi, Berno, Pizzale, & Conte, 2001). We therefore aimed to update the procedure making use of the higher specificity and sensitivity of the mass spectrometer to enable direct analysis of unsaponified oils and the use of commercially available stigmastadiene standard to allow a direct quantification step.

The saponification step of the Regulation method could be avoided as the mass spectrometric detector is far more sensitive than the flame ionization detector. The column chromatography step of the Regulation method is used to separate stigmasta-3,5-diene from interferences due to squalene and hydrocarbons, but these interferences were not manifested in the mass spectrometric method, thus a shorter and quicker column separation step was possible. No interference was seen from late eluting compounds derived from earlier injections.

2. Materials and methods

2.1. Materials

An authentic EVOO sample was provided by a reputable producer. Six retail samples labeled as EVOO were obtained from local suppliers. Silica gel 60, 0.063–0.2 mm was obtained from Sigma-Aldrich, Gillingham, Dorset, UK. Empty polyfiltration columns/SPE tubes of about 70 ml capacity with 20 μm polyethylene frits (Isolute 120-1028-F) were obtained from Biotage GB Limited, Hengoed, UK.

A solution (0.2 mg ml^{-1}) of 24-ethylcholesta-3,5-diene (stigmasta-3,5-diene, 72.6%) and 24-methylcholesta-3,5-diene (campesta-3,5-diene, 22.3%) was obtained from Chiron, Norway (http://www.chiron.no). Cholesta-3,5-diene was obtained from Sigma-Aldrich, Gillingham, Dorset, UK. Hexane (HPLC fluorescence grade) and iso-octane (HPLC grade) were obtained from Fisher Scientific, Loughborough, UK.

2.2. Gas chromatography/mass spectrometry

Gas chromatography/mass spectrometry was carried out using an Agilent Technologies 6890N instrument fitted with a 7683 series autosampler. The column was a Crossbond® Carbowax® polyethylene glycol (30 m × 0.25 mm i.d., with a film thickness of 0.25 μm). The carrier gas was helium at a constant flow of 1.7 ml·min^{-1}. The split/splitless injector port and mass spectrometer interface were heated to 250 and 280 °C, respectively. The oven temperature was programmed from 60 °C to 260 °C at 20 °C/min^{-1} and held for 15 min. Injections were made in splitless mode with a 1 min hold time.

The mass spectrometer was operated in electron ionization mode with a source temperature of 200 °C and an emission current of 150 mA. Sterenes were detected by selected ion monitoring of the molecular ions at m/z 368 (cholesta-3,5-diene), m/z 396 (stigmasta-3,5-diene), m/z 382 (campesta-3,5-diene) and confirmed by monitoring of the ions of the ring fragment at m/z 255.

2.3. Preparation of standard solutions

Stigmasta-3,5-diene calibration and spiking solutions were prepared in iso-octane from the 0.02 mg ml^{-1} stock supplied by dilution to final concentrations of approximately 0, 0.01, 0.02, 0.1, 0.2, 0.5 and 1.0 μg ml^{-1}. The exact concentration of stigmasta-3,5-diene was calculated from the purity information provided by the supplier.

Cholesta-3,5-diene internal standard solution (1.0 μml^{-1}) was prepared at a concentration of 1 μg ml^{-1} in iso-octane.

2.4. Method development and in-house validation

Method development was based on the method reported previously for confectionery fats (Crews, Calvet-Sarret, & Brereton, 1999). In this method a non-polar fraction was isolated by short column chromatography, analyzed by GC using a polar phase and mass spectrometric detection. Stigmasta-3,5-diene and related sterenes were quantified by means of a calibration curves prepared from authentic standards with cholestadiene being used as an internal standard in the usual sense to make correction for losses.

The elution profile of sterenes and lipid from the silica column was tested by adding 1 g of EVOO containing stigmasta-3,5-diene and cholesta-3,5-diene and eluting with hexane, collecting 8 × 10 ml aliquots in 20 ml vials that had been weighed to 5 decimal places. The eluted portions were evaporated under nitrogen and the vials reweighed to indicate any major elution of the lipids. The weighed residues were dissolved in 0.2 ml iso-octane and analyzed by GC–MS. The peak areas of the eluted stigmasta-3,5-diene and cholesta-3,5-diene were compared with those of standards that had not passed through the column.

The results showed that no quantifiable mass of lipid was eluted from the columns in the first 80 ml of eluting solvent. No stigmasta-3,5-diene or cholesta-3,5-diene was eluted in the first 10 ml of eluting solvent, and over 99% was recovered in the 20–40 ml fraction.

As it was clear that the method could detect much less than 0.1 mg kg^{-1} of stigmastadiene, and provide conformation of identity, it was in-house validated in detail by the repeated analysis (on five separate days) of a blank EVOO, a blank EVOO spiked with stigmasta-3,5-diene at the Regulation limit (0.15 mg kg^{-1}), and a high level reference material made by mixing EVOO with 10% refined grapeseed oil. The samples were analyzed in duplicate, with a set of standards and solvent blanks.

2.5. Final procedure

15 ± 0.5 g silica gel was weighed and transferred to an empty extraction column and a frit placed on top. The silica gel was gently compacted by shaking the column with a vibrating laboratory mixer while pushing down gently on the frit with a glass rod. Portions of 1 ± 0.1 g of sample oil were weighed and 0.5 ml of internal standard solution was added followed by 1 ml hexane. The silica gel column was pre-wetted with 50 ml hexane and when the hexane flow had practically ceased the sample solution was applied to it. The beaker was washed with about 0.5 ml hexane which was added to the column.

A 150 ml round bottomed flask was placed under the column and 50 ml hexane added to the top. The hexane was allowed to flow through the column unrestricted to elute the non-polar fraction. The extract was evaporated to dryness on a rotary evaporator at 40 °C and reduced pressure and the residue was reconstituted in 1.5 ml hexane. This solution was evaporated just to dryness under nitrogen at room temperature and immediately redissolved in 0.2 ml of iso-octane.

2.6. Quantification

Calibration graphs were prepared for each analyte by plotting the ratio of the area of protonated molecular ion peak by the area of the

Download English Version:

https://daneshyari.com/en/article/6396480

Download Persian Version:

https://daneshyari.com/article/6396480

Daneshyari.com