

Chromatography in authenticity and traceability tests of vegetable oils and dairy products: a review

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ABSTRACT: The new applications of various chromatographic techniques such as gas–liquid chromatography, high-performance liquid chromatography and electrophoretic methods employed for the analysis in macro- and micro-components in vegetable oils and dairy products are compiled and critically evaluated. The employment of these methods for authenticity tests and traceability is discussed. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: chromatography; vegetable oils; dairy products; authenticity test; traceability

INTRODUCTION

Adulteration is generally motivated by maximizing profit by adding a cheaper ingredient. The growing interest in the authenticity of foods and food products requires reliable verification methods because the properties of foods from different origins can also be different. Furthermore, traceability is required more and more by consumers and government organizations because it is a significant component of overall food safety (Anklam and Battaglia, 2001). The methods used for the verification of food authenticity and traceability have to be suitable for the prevention of deliberate or accidental mislabelling. They are important for commercial reasons and play a considerable role in the assurance of public health. Moreover, these methods have to be able to link a finished product to its ingredients. As the origin is a decisive characteristic of foods, rapid, inexpensive and efficient analytical methods are demanded for the measurement of this criterion. These analytical techniques have to be applied in food manufacturing industry for process performance evaluation, for the detection of faults and to achieve a continually high-quality food product.

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Abbreviations used: ACN, acetonitrile; COP, cholesterol oxidation product; DAG, diacyl-glycerol; LOL, dilinoleoyl-oleyl glycerols; TAG, triacyl-glycerol; TFA, trifluoroacetic acid.

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A considerable number of methods have been developed for the assessment of the authenticity and traceability of foods. These include the successful application of polymerase chain reaction (Ahmed, 2002), various DNA methods (Hold *et al.*, 2001), enzyme immunoassays (Roux *et al.*, 2000), nuclear magnetic resonance spectroscopy (Gil *et al.*, 2000), infrared and near-infrared spectroscopy combined with principal component analysis (PCA; Schulz *et al.*, 2003), mid-infrared and Raman spectroscopies (Marigheto *et al.*, 1998) and headspace-mass spectrometry (Lorenzo *et al.*, 2002).

Various chromatographic techniques offer a unique possibility for the rapid and reliable separation and quantitative determination of macro- and micro-components of highly similar chemical structures in complicated matrices of foods and food products (Forgács and Cserhádi, 2003; Nollet, 2003). Because of their advantageous separation characteristics, numerous chromatographic techniques have been tested, accepted and employed in the analysis of food and food products. Chromatographic methods can be equally employed for the measurement of the component of foods, the amount of legal or illegal additives, environmental pollutants and xenobiotics that are health hazards. Because of the high separation capacity of chromatographic techniques, they can be used for authenticity and traceability tests of foods.

The aim of this review is the collection and concise enumeration of the most decisive new results in the field of the employment of various chromatographic techniques suitable for the authenticity and traceability tests of foods and the critical discussion of the results.

ANALYSIS OF MACRO- AND MICRO-COMPONENTS OF OILS OF VARIOUS ORIGIN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) AND GAS CHROMATOGRAPHY (GC)

Plant oils contain many components, such as triacyl- and diacyl-glycerols (TAGs and DAGs) of various saturated and unsaturated fatty acids as main components, and tocopherols, phytosterols, phospholipids, free fatty acids, waxes as minor ingredients. The composition of the oils (triglyceride profile) is a characteristic of the plant species and to a lesser extent for the origin of the oil. As the macro- and micro-components of oils are semi-volatile, both HPLC and GC techniques can be employed for their measurement. Consequently, a considerable number of HPLC and GC methods have been developed for the determination of the composition of various oils. As the evaluation methods used for the authenticity and traceability tests of oils include a high number of variables (e.g. composition of TAGs) the applications of various multivariate mathematical and statistical methods greatly facilitate the evaluation of the results. The chromatographic techniques applied for the authenticity test of vegetable oils have been previously collected and critically evaluated (Aparicio and Aparicio-Ruiz, 2000).

HPLC METHODS

The potential of the triglyceride profile of Cretan olive oils obtained by HPLC for identification and classification was investigated. Separation of the oil components was performed on an ODS column (250 × 4 mm i.d.; particle size, 5 μm) at 40°C. The isocratic mobile phase consisted of acetone–acetonitrile (ACN) (60:40, v/v). The flow rate was 0.7 mL/min. Analytes were detected with a refractive index detector. The data were evaluated by various multivariate mathematical statistical methods such as PCA, canonical correlation analysis and a cluster dendrogram. It was concluded from the data that triglyceride composition determined by HPLC and combined by statistical methods can be successfully employed for the classification of olive oil samples (Stefanoudaki *et al.*, 1997).

Reversed-phase HPLC and chemometrics (PCA and discriminant analysis) has also been applied for the detection of adulteration of sesame oils with perilla oil. Separation of triglycerides was carried out on an ODS column (150 × 3.9 mm i.d.; particle size, 10 μm) using isocratic elution with ACN–acetone 50:50, v/v). The flow rate was 0.5 mL/min, the column was thermostated at 30°C and analytes were detected using a refractive index detector. Calculations proved that about 5%

perilla oil could be detected in sesame oil using HPLC profile and chemometrics (Lee *et al.*, 2001).

Hyphenated techniques such as HPLC combined with various mass spectrometric detection procedures have also frequently been applied for the analysis of oils. Almond, avocado, corn germ, grape seed, linseed, mustard seed, olive, peanut, pumpkin seed, sesame seed, soybean, sunflower, walnut and wheat germ oils have been analysed by HPLC–atmospheric pressure chemical ionization mass spectrometry (APCI-MS) and matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOFMS). Separations were performed on an ODS column (125 × 4 mm i.d.; particle size, 5 μm). TAGs were separated by gradient elution in mixtures of acetone–ACN. Acetone concentration was increased from 20 to 66% in the first 3 min followed by a 13.5 min hold, then from 66 to 80% acetone in 1 min, with a final hold for 30 min. The column was thermostated at 25°C; the flow rate was 0.6 mL/min. Oil samples were dissolved in acetone–ACN (2:1, v/v) at a concentration of 1% (v/v). Linear discriminant analysis was applied for the classification of the oils according to their TAG composition. This combined technique classified 67 oil samples correctly and only six samples were classified incorrectly. The differences were the same using HPLC-APCI and MALDI-TOFMS (Jakab *et al.*, 2002a).

A similar method was employed for the analysis of plant oils. It was found that HPLC-APCI-MS combined with linear discriminant analysis is a suitable tool for the classification of plant oils according to the chromatographic profile of TAGs (Jakab *et al.*, 2002b).

HPLC combined with APCI-MS and UV detection was employed for the characterization of triacylglycerol and diacylglycerol composition of plant oils (hazelnut, pistachio, poppy-seed, almond, palm, Brazil-nut, rapeseed, macadama, soybean, sunflower, linseed, *Dracocephalum moldavica*, evening primrose, corn, amaranth and *Silybum arianum*). Oils were dissolved in acetonitrile–2-propanol (1:1, v/v) yielding a solution of 3% w/v. This solution was employed for HPLC analysis after appropriate dilution. Separations were carried out on two octadecylsilica (ODS = C₁₈) columns (150 × 3.9 mm i.d.; particle size, 7 μm). Column temperature was 40°C and flow rate was set to 1 mL/min. The gradient elution for the majority of oils was: 0 min, 70% aqueous ACN; 20 min, 100% ACN; 36 min, 100% ACN; 132 min, 60% 2-propanol + 40% ACN; 135 min, 70% aqueous ACN. The gradient elution used for analysis of *Draco-cephalum moldavica*, evening primrose, corn, amaranth and *Silybum arianum* oils was: 0 min, 100% ACN; 56 min, 30% ACN + 70% ethanol using a single column. The high separation capacity of the method makes it suitable for the determination of the composition of different plant oils, and the comparison of the triacylglycerol and diacylglycerol profile

of oils may help their identification (Holcapek *et al.*, 2003).

The ratio of positional isomer dilinoleoyl-oleyl glycerols (LOL) in vegetable oils (grape seed, olive, pumpkin seed, soybean, sunflower and wheat germ oil) has also been measured by a similar HPLC-APCI-MS method. It has been established that the relative LOL content in oils was: 44.2 ± 2.6 (grape seed), 26.8 ± 3.2 (sunflower), 16.7 ± 4.6 (pumpkin seed), 15.9 ± 2.9 (soybean) and 13.9 ± 4.3 (wheat germ oil). It has been further demonstrated that olive oil contained only LLO isomer (Jakab *et al.*, 2003).

Because of the good separation characteristics, monolithic silica column has also found application in the separation of TAGs in various vegetable oils. Cold-pressed peanut, pumpkin seed, sesame, soybean and wheat germ oils were dissolved in acetone-ACN (2:1) at a concentration of 1% and injected into a silica monolithic column (50×4.6 mm i.d.). Mobile phase gradient began with 10% of acetone and 90% of acetonitrile and changed to 66% acetone in 10 min. The flow rate was 5 mL/min and auto-sampler and column temperatures were 20 and 25°C, respectively. APCI capillary, source and block temperatures were 300, 200 and 200°C. Spectra were taken in the range of m/z 500–1000. The data were evaluated by PCA followed by two-dimensional nonlinear mapping of PC loadings and variables. It has been stated that the method can be employed for the differentiation between vegetable oils (Jakab and Forgács, 2002).

It has been established many times that not only the macro-components (TAGs) of oils but also the minor lipid constituents play a considerable role in the evaluation of the quality and authenticity of oils. The oxidation products of cholesterol in processed food are also indicators of the quality of the oil. Because of their considerable importance, an HPLC-APCI method has been developed for the analysis of seven toxicologically important cholesterol oxidation products (COPs; 7α -hydroxycholesterol, 7β -hydroxycholesterol, 25-hydroxycholesterol, 7-ketocholesterol, $5,6\alpha$ -epoxycholesterol, $5,6\beta$ -epoxycholesterol and cholestan- $3\beta,5\alpha,6\beta$ -triol). Butter, butter oil and lard were melted and dissolved in 3 mL hexane. Egg powder (1 g) was extracted with 30 mL of chloroform, the supernatant was dried, evaporated to dryness and redissolved in hexane. COPs were purified on a silica solid phase extraction (SPE) cartridge. The cartridge was pre-conditioned with 3 mL hexane, then loaded with the sample dissolved in hexane. The cartridge was washed successively with 10 mL hexane-diethylether (95:5, v/v), 25 mL hexane-diethylether 90:10, v/v) and 15 mL hexane-diethyl ether (80:20, v/v). COPs were removed using 5 mL acetone, evaporated to dryness and redissolved in the mobile phase. HPLC separations were carried out on an ODS column (250×4.6 mm i.d.;

particle size, 5 μ m) thermostated at 25°C. The mobile phase was ACN-methanol (60:40, v/v); the flow rate was set to 1 mL/min. It was established that the HPLC-APCI method is sensitive, robust and can be employed for the identification and quantitative determination of toxic cholesterol oxides in biological matrices (Razzazi-fazeli *et al.*, 2000).

GAS CHROMATOGRAPHIC TECHNIQUES

A considerable number of oils were analysed by GC measuring the composition of fatty acid TAGs and other constituents. The GC technologies applied for the analysis of essential oils have been reviewed (Marriott *et al.*, 2001). Because of the high number of chemical parameters, the data generally were evaluated by various multivariate mathematical statistical methods.

Fatty acids and TAGs of argania, corn, cotton, grape, olive, soy cocoa, palm, sunflower, and peanut oils were also measured by GC and the data were evaluated by hierarchical cluster analysis. Oils were dissolved in isooctane and TAGs were separated and quantitatively determined on a fused-silica capillary column (25 m \times 0.25 mm i.d.; film thickness 0.1 mm). Oven temperature varied from 320 to 350°C at 1.5°C/min; flame ionization detector (FID) temperature was 375°C. Calculations demonstrated that the method is suitable for the differentiation between argania oils of diverse origin and it can detect adulteration of the oil (Rezanka and Rezanková, 1999).

The separation of triacylglycerols in Umbrian extra-virgin olive oil was carried out by GC. Analyses were performed on a Supelcowax 10 capillary column (30 m \times 0.25 mm i.d.; film thickness 0.25 μ m). Injector and FID temperatures were 270°C. The gradient for oven temperature initiated at 165°C, followed by a 3 min hold, then 3°C/min to 240°C. TAGs were separated by thin-layer chromatography; the isolated fraction was treated with pancreatic lipase to obtain *sn*-monoacylglycerols. TAGs were also partially deacylated by chemical hydrolysis. DAG enantiomers were separated on a boric acid-silica TLC plate. Data were evaluated by linear discriminant analysis. Calculations illustrated that this combined method can be employed for the classification of extra virgin olive oils according to the climatic characteristics (Damiani *et al.*, 1997).

Olive oils have also been characterized by GC coupled to a stable isotope ratio mass spectrophotometer via a combustion interface. In order to increase the reliability of the authenticity test, the fatty acid concentrations and the isotopic data were evaluated by PCA. Samples were hydrolysed by mixing 0.1 mL of oil with 10 mL of 1 M aqueous ethanolic (95 vol%) potassium hydroxide and treating the mixture at 70°C for 3 h. After hydrolysis the sample was neutralized and extracted

with 1×15 and 2×5 mL of hexane. The rest was acidified and the free fatty acids were methylated with BF_3 . Fatty acid methylethers were separated on a capillary column ($50 \text{ m} \times 0.20 \text{ mm}$ i.d.; film thickness, $0.33 \mu\text{m}$). The initial column temperature was 100°C for 2 min then elevated to 220°C at $5^\circ\text{C}/\text{min}$. The final hold was 30 min. The data demonstrated that carbon isotope analysis of the bulk oil and the individual fatty acids can be employed to test the authenticity of oils (Spangenberg *et al.*, 1998).

A similar bulk and molecular carbon isotope analysis was carried out on olive oil and pumpkin seed oil samples. Oil samples of 0.1 mL were hydrolysed with aqueous ethanolic potassium hydroxide at 70°C for 3 h. After hydrolysis the samples were extracted with 1×10 and 2×5 mL of hexane. Free fatty acids were methylated with 0.5 mL 10% methanolic BF_3 ; the methyl esters were extracted with 10 mL of hexane and washed with 2×5 mL saturated aqueous potassium chloride solution. GC-MS measurements were carried out on a fused-silica capillary column ($50 \text{ m} \times 0.20 \text{ mm}$ i.d.; film thickness, $0.33 \mu\text{m}$). The temperature of the electron ionization detector (EID) was 200°C ; it operated at 70 eV in the multiple ion detection mode. After 2 min of initial hold, the oven temperature was increased to 250°C at $5^\circ\text{C}/\text{min}$. GC combined with isotope ratio mass spectrometer was employed for the isotopic analysis of individual fatty acids. It was established that the method is suitable for the detection of thermally induced degradation during deodorization, steam washing and/or bleaching and to test the authenticity of various oils (Spangenberg and Ogrinc, 2001).

High-resolution GC (HR-GC) has been applied for the determination of the TAG profile for the detection and quantification of cocoa butter equivalents in chocolate model systems. Samples were prepared by mixing cocoa butters of different origin with cocoa butter equivalents such as coberine, choclin, shea fat, palm oil mid fraction, illipe fat and 1,3-dipalmitoyl-2-oleoylglycerol in various weight ratios. TAGs were separated on a capillary column ($30 \text{ m} \times 0.25 \text{ mm}$ i.d.; film thickness, $0.15 \mu\text{m}$). The injector was programmed from 100°C to 365°C at $70^\circ\text{C}/\text{min}$. The oven temperature program began at 100°C , held for 0.2 min. It was increased to 115°C at $95^\circ\text{C}/\text{min}$, then to 175°C at $65^\circ\text{C}/\text{min}$, to 300°C at $45^\circ\text{C}/\text{min}$, to 365°C at 35°C , with a final hold for 20 min. Helium was the carrier gas; analytes were detected with FID. It was found that the method is suitable for the detection of the addition of 5% cocoa butter equivalents to cocoa butter. The detection limit of illipe fat was 10% (Simoneau *et al.*, 1999).

Near infrared reflectance (NIR) spectroscopy and GC analysis of the average fatty acid composition of the oil types combined with multivariate mathematical statistical methods has been used for the differentiation of mayonnaise samples containing different oil types

and fatty acid compositions. Mathematical methods included PCA, linear discriminant analysis and quadratic discriminant analysis. The fatty acid composition of soybean oil, sunflower oil, rapeseed oil, olive oil, corn oil and grapeseed oil was measured by GC. TAGs were hydrolysed; the free fatty acids were methylated with methanolic HCl and separated on a capillary column ($25 \text{ m} \times 0.25 \text{ mm}$ i.d.; film thickness, $0.25 \mu\text{m}$).

Analytes were detected by FID. The measurements and calculations demonstrated that the data could be used for the classification of mayonnaises containing various vegetable oils (Indahl *et al.*, 1999). Not only was the fatty acid and TAG composition of oils employed for their classification but also the sugar and polyol components were investigated and used for the characterization of European olive fruit (*Olea europaea* L.) varieties. The varieties *Douro*, *Hojiblanca*, *Cassanese*, *Taggiasca* and *Thasos* were included in the experiments. Sugars and polyols were extracted with a solution of D-arabinose as internal standard. The solution was dried and the analytes were converted to trimethylsilyl ethers using pyridine–hexamethyldisilane–trimethylchlorosilane (2:1:1) at 70°C for 1 h. GC separation of derivatives was performed on a capillary column ($30 \text{ m} \times 0.32 \text{ mm}$ i.d.; film thickness, $0.10 \mu\text{m}$). Column temperature was increased from 70 to 90°C at $20^\circ\text{C}/\text{min}$, and to 300°C at $4^\circ\text{C}/\text{min}$. Analytes were detected by FID. GC-MS analysis was carried out under similar conditions only the film thickness was $0.25 \mu\text{m}$. The fructose, galactose, glucose, sucrose, mannitol and inositol contents of the fruits were quantified. The results were proposed for the optimization of the production process of table oils and for the elucidation of variety and growth differences (Marsilio *et al.*, 2001).

The aroma active compounds in orange essence oil have been identified by applying GC-olfactometry and GC-MS. Before analysis, oil samples were diluted with hexane at a ratio of 1:1. Aroma compounds were separated on a capillary column ($30 \text{ m} \times 0.32 \text{ mm}$ i.d.; film thickness, $0.5 \mu\text{m}$). The initial oven temperature was 40°C , increased to 240°C , with a final hold for 5 min. Injector and FID temperatures were 240 and 250°C , respectively. GC-MS was performed on another column ($60 \text{ m} \times 0.25 \text{ mm}$ i.d.; film thickness, $0.25 \mu\text{m}$). Column temperature varied from 40°C to 250°C at $7^\circ\text{C}/\text{min}$, with an initial and final hold of 0.5 and 10 min, respectively. MS (electron impact ionization) conditions were: ionization energy, 70 eV; mass range, 40–300 u; scan rate, 2 scans/s; electron multiplier voltage, 1050 V. Injector and transfer line temperatures were 200 and 250°C , respectively. The GC-FID method allowed the separation and identification of 55 aroma active constituents while GC-MS detected 95 components. The majority of volatile components were not aroma active according to the olfactometric investigations.

Also, it was not emphasized that the method can be employed for the authenticity and traceability testing of similar essence oils (Högnadóttir and Rouseff, 2003).

As previously stated, the determination of minor lipid components of fats and oils may also contribute to the safe identification of the origin of the product under investigation. The advantageous separation characteristics of HPLC and GC were combined for the measurement of sterol esters in cocoa butter. Samples of cocoa butter were heated at 40°C, and an aliquot of 100 mg was mixed with 50 µL of cholesterol laurate solution (1 mg/mL), with 100 µL of silylating agent [N,O-bis-(trimethylsilyl)trifluoroacetamine = BSTFA + 1% trimethylchlorosilane] and heated for 20 min at 80°C. The solution was diluted with 8 mL hexane and used for LC-GC measurements. HPLC was employed for the pre-separation of silylated sterols and sterol esters from the bulk of TAGs and DAGs. Separation was carried out on a cyano-bonded silica column (150 mm × 2.1 mm i.d.; particle size, 5 µm) using an isocratic mobile phase consisting of 2% of dichloromethane and 0.1% of acetonitrile in *n*-hexane. The column was thermostated at 30°C, the flow-rate was 0.2 mL/min and analytes were detected at 215 nm. GC analyses were carried out on a column of 15 m × 0.25 mm i.d. (film thickness, 0.1 µm) connected in series with a 3 m × 0.25 mm i.d. uncoated phenyldimethyl silylated fused-silica capillary and a coated pre-column of (2 m × 0.25 mm i.d.). Column temperature was 120°C for 5 min, then it increased to 260°C at 30°C/min, then to 340°C at 15°C/min with a final hold of 15 min. GC-MS separations were carried out on a similar capillary column. The temperature program began at 60°C (1 min hold), to 270°C at 30°C/min (5 min hold), to 340°C at 10°C (1 min hold). MS analysis was by chemical ionization mode at 150 eV using ammonia as the ionization gas. The temperatures of the transfer line and source were 300 and 180°C, respectively. Measurements were performed in full scan mode at unit resolution from 100 to 800 Da (scan time, 0.5 s; interscan delay, 0.1 s). The quantity of campesterol palmitate, stigmasteryl palmitate, sitosteryl palmitate, campesterol stearate, oleate, linoleate, stigmasteryl stearate, oleate, linoleate, sitosteryl stearate, oleate and linoleate were determined in commercial cocoa butter samples. The detection limit of the combined LC-GC method was 3 mg sterol ester/kg cocoa butter, and the quantification limit 10 mg/kg. The method was proposed for the authenticity test of cocoa butter (Kamm *et al.*, 2001).

HR-GC and RP-HPLC were applied for the analysis of rice bran oils. The fatty acid and sterol composition were measured by HR-GC while HPLC was used for the TAG analysis. Fatty acid methyl esters were separated on a capillary column (30 m × 0.25 mm i.d.; film thickness, 0.2 µm). Initial column temperature was 50°C

for 2 min, increasing to 250°C at 4°C/min, with a final hold of 15 min. Injector and FID temperatures were 260°C. Sterol fraction was separated from the unsaponified constituents by TLC then derivatized (silylation) and separated on the same column used for fatty acid analysis. Sterol silyl esters were separated isothermally at 265°C, injector and detector temperatures being 280 and 295°C. TAGs were separated on an ODS column (250 × 4.6 mm i.d.; particle size, 5 µm). Analytes were eluted isocratically with a mobile phase of acetone-ACN (55:45, v/v). The column was thermostated at 30°C and the flow rate was set to 0.8 mL/min. Linear discriminant analysis and artificial neural networks were applied for the evaluation of the experimental data. Calculations proved that rice bran oils can be differentiated according to their geographical origin using analytical indices. Linear discriminant analysis required 12 indices to achieve 100% predictive ability, while the artificial neural network needed only nine variables (Marini *et al.*, 2003).

SPE-thin-layer chromatography-GC has been developed and successfully applied for the detection of hazelnut oil in olive oils by the measurement of esterified sterols. Oils were diluted with *n*-hexane before analysis (1:1, v/v). Esterified sterols were separated from the other constituents by traditional column chromatography using 25 g of silica stationary phase. Column was pre-conditioned with 30 mL *n*-hexane-diethyl ether and samples were eluted with 150 mL *n*-hexane-diethyl ether (87:13, v/v). The eluate was evaporated to dryness, and saponified. The unsaponified fraction was dissolved in chloroform and separated on silica TLC plates using *n*-hexane-diethyl ether (65:35, v/v) as mobile phase. The sterol band was scraped off, extracted twice with 5 mL of diethyl ether, and silylated with pyridine-hexamethyldisilazane-trimethylchlorosilane at room temperature for 15–20 min. Esterified sterols were also pre-purified by SPE using silica cartridge washed by *n*-hexane and eluted with mixtures of *n*-hexane-diethyl ether. GC-FID measurements were carried out on a fused-silica capillary column (30 m × 0.25 mm i.d.; film thickness, 0.25 µm). Column, injector and FID temperatures were 270, 290 and 310°C. Identification of sterols was performed by MS using the same column. Oven temperature was programmed from 250 to 320°C at 3°C/min. Injector, transfer line and manifold temperatures were 325, 325 and 220°C, respectively. Filament emission current was 10 µA and electron energy was 70 eV. The GC-FID and GC-MS methods proved the presence of cholesterol, 24-methylcholesterol, campesterol, campestanol, stigmasteryl, 7-campesterol, clerosterol, sitosterol, sitostanol, 5-avenasterol, 5,24-stigma-dienol, 7-stigmasteryl and 7-avenasterol in extra virgin olive oil. It was illustrated that the method is suitable for the measurement of esterified and non-esterified sterol

fractions in olive oils. Because of the good separation capacity, it has been proposed for the detection of adulterations of olive oils (Cercaci *et al.*, 2003).

Because of the high practical and theoretical importance of the authenticity and traceability tests of oils, the chromatographic, spectrophotometric and spectroscopic methods used for this objective were earlier reviewed (Andrikopoulos, 2002). The HPLC and GC methods employed for the measurement of minor constituents in vegetable oils have also been compiled and critically evaluated (Cert *et al.*, 2000).

USE OF CHROMATOGRAPHIC TECHNOLOGIES FOR AUTHENTICITY AND TRACEABILITY TESTS OF DAIRY PRODUCTS

Similarly to vegetable oils, the separation and quantitative determination of the macro- and micro-components of dairy products may facilitate the determination of the origin of the product. Thus, the measurement of *trans* fatty acids isomer distribution by GC was carried out to evaluate the quality of vegetable oils used in infant formula. Fats were extracted with an ammoniacal ethanolic solution followed by extraction with diethyl ether and petroleum ether. The organic phase was separated and evaporated to dryness. *Trans*-esterification of the samples was achieved using 2 M potassium hydroxide in methanol for 2 min. The samples were centrifuged and the upper organic layer was diluted with hexane and applied for GC analysis. Fatty acid methyl esters and 4,4-dimethoxazoline derivatives were analysed by GC using a capillary column (100 m × 0.25 mm i.d.; film thickness, 0.25 µm).

The column temperature programme was: 60°C for 5 min, increase to 165°C at 15°C/min, 1 min hold, to 225°C at 2.0°C/min, with a final hold for 17 min.

It was found that the *trans* fatty acid content of infant formula depends markedly on the deodorization temperature. It was suggested that the method can be used for the detection of vegetable oils in infant formula. Moreover, it was established that *trans* fatty acid content shows considerable geographical and seasonal variation (Dionisi *et al.*, 2002).

Flavour compounds in dairy products, especially in cheeses, are characteristic; consequently, the quantity and quality of flavour constituents analysed by chromatography can be used as an efficient tool for authenticity testing. Besides mineral salts, lactic acid, lactose, amino acids and peptides, the water-soluble extract (WSE) of cheeses contains volatile and non-volatile aroma compounds (Salles *et al.*, 2000). The aroma constituents of WSE have been extensively studied (Engel *et al.*, 2000a,b), and it has been established that the composition of the accompanying matrix considerably influences the taste of aroma compounds (Engel *et al.*,

2001). Furthermore, it has been established that the physicochemical interaction between the components of the WSE may also play a considerable role in the development of taste (Pionier *et al.*, 2002). Ultrafiltration and nanofiltration can also be employed for the separation of the components of dairy products (Jeantet *et al.*, 2000). The volatile constituents and fat components of WSE were separated by TLC-FID and GC-MS methods. Grated cheese samples were mixed with water at a ratio of 1:2 w/w for 4 min at ambient temperature (25°C). The suspension was centrifuged subsequently at 4000 and 100,000 g at 4°C for 30 min. Ultrafiltration of WSE was performed at 10 and 1 kDa cut-off, and nanofiltration at 0.5 kDa cut-off. Volatile components from the permeates and retentates were diluted with water; the pH was adjusted to 2 with 2 M HCl and extracted three times with 10, 3 and 3 mL of dichloromethane. The combined organic phases were dried over anhydrous Na₂SO₄ and concentrated to 0.1 mL. GC-FID separation and GC-MS identification of volatile constituents was performed on a capillary column (30 m × 0.32 mm i.d.; film thickness, 0.25 µm). The oven temperature increased from 40 to 240°C at 3°C/min. Injector and detector temperatures were 240 and 250°C, respectively. Iatroscan TLC-FID was applied for the detection of lipid content of samples (total tri-, di- and mono-glycerides, phospholipids, cholesterol esters, free fatty acids). Free and total amino acids were determined using an amino acid analyser. GC-FID and GC-MS allowed the separation and identification of 43 neutral and acidic components in the samples, the amount of free fatty acids being dominant. Filtration steps resulted in the considerable loss of both neutral and acidic compounds. This effect has been tentatively explained by the adsorption of solutes on the membrane surface (Engel *et al.*, 2002).

Not only have the various lipid fractions and aroma compounds been used for the characterization of dairy products, but also the proteins and protein decomposition products have been extensively investigated by chromatographic techniques.

In order to facilitate the detection of milk adulteration, the major casein fractions have been separated by RP-HPLC and urea-polyacrylamide gel electrophoresis (PAGE). Milk samples were prepared by separation of fat from whole milk by centrifugation at 700 g at 4°C for 10 min. Caseins were separated by precipitation at pH 4.3, 20°C by adding 1 M ammonia-acetate buffer. The suspension was centrifuged for 15 min at 3000 g at 20°C. The procedure was repeated twice. The precipitated casein fraction was washed with acetone and dried at ambient temperature. Protein separation was performed on a 150 mm × 4.6 mm i.d. column filled with polystyrene-divinylbenzene copolymer stationary phase (particle size, 8 µm). Components of the mobile phase were 0.1% aqueous trifluoroacetic acid (TFA)

(A) and 0.1% TFA in acetonitrile–water (95:5, v/v; eluent B). The gradient was initiated at 29% B for 5 min; 5–10 min, 29–37% B; 10–12 min, 37–41% B; 12–14 min, 41–42.5% B; 2 min hold; 16–17 min, 42.5–43% B; 17–19 min, 43% B; 19–21 min, 43–47% B; 21–23 min, 47% B; 23–25 min, 47–54% B; 25–27 min, 54% B; 27–28 min, 54–100% B; 28–30 min, 100–29% B; 30–35 min, 29% B. The column was thermostated at $46 \pm 0.1^\circ\text{C}$. The flow rate was 1 mL/min; the detector wavelength was set to 280 nm. Alpha-, beta- and kappa-caseins were well separated by both RP-HPLC and PAGE. The linear range of detection varied between 0.038 and 1.883 mg/mL depending on the casein fraction. Detection limits were 0.006, 0.019 and 0.15 mg/mL for kappa-, alpha and beta-casein, respectively. The method has been proposed for following casein proteolysis during cheese ripening and for the detection of milk adulteration at a level of 5% (Veloso *et al.*, 2002).

An RP-HPLC method has been developed and successfully applied for the determination of bovine, ovine and caprine milk percentages in protected denomination cheeses. Whey proteins were extracted from cheese with water at a ratio of 5:15 w/v. The mixtures were sonicated, centrifuged and the supernatant was acidified to pH 4.6 using 1 M HCl. The precipitate was removed by centrifugation at 2000 g for 10 min, the supernatant was filtered and used for HPLC analysis. Separation was carried out on the same column used before for the separation of casein fractions (Veloso *et al.*, 2002). Solvents for gradient elution were 0.1% aqueous TFA (A) and 0.1% TFA in 80% aqueous acetonitrile (B). The ratio of B was increased from 36 to 56% in 20 min, from 56 to 60% in 10 min, and from 60 to 36% B in 5 min. The flow rate was 0.5 mL/min, column was thermostated at $45 \pm 0.1^\circ\text{C}$, and protein fractions were detected at 215 nm. The chromatographic profiles of β -lactoglobulins of bovine, ovine and caprine milk showed marked differences, indicating that they can be used for the differentiation between cheeses produced with bovine, ovine or caprine milk. As the method allows the quantification of milk proteins in the concentration range 5–95%, it has been proposed for the authenticity test of cheese and milks (Ferreira and Cacote, 2003).

The importance of the separation, identification and quantitation of volatile aroma constituents of cheese types by application of GC-olfactometry have been reviewed previously (Curioni and Bosset, 2002).

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REFERENCES

- Ahmed FE. Detection of genetically modified organisms in foods. *Trends in Biotechnology* 2002; **20**: 215–223.
- Andrikopoulos NK. Triglyceride species compositions of common edible vegetable oils and methods used for their identification and quantification. *Food Review International* 2002; **18**: 71–102.
- Anklam E and Battaglia R. Food analysis and consumer protection. *Trends in Food Science and Technology* 2001; **12**: 197–202.
- Aparicio R and Aparicio-Ruiz R. Authentication of vegetable oils by chromatographic techniques. *Journal of Chromatography A* 2000; **881**: 93–104.
- Cercaci L, Rodriguez-Estrada MT and Lercker G. Solid-phase extraction thin-layer chromatography–gas chromatography method for the detection of hazelnut oil in olive oils by determination of esterified sterols. *Journal of Chromatography A* 2003; **985**: 211–220.
- Cert A, Moreda W and Pérez-Camino MC. Chromatographic analysis of minor constituents in vegetable oils. *Journal of Chromatography A* 2000; **881**: 131–148.
- Curioni PMG and Bosset JO. Key odorants in various cheese type as determined by gas chromatography-olfactometry. *International Dairy Journal* 2002; **12**: 959–984.
- Daminani P, Cossignani L, Simonetti MS, Campisi B, Favretto L and Favretto LG. Stereospecific analysis of triacylglycerol fraction and linear discriminant analysis in a climatic differentiation of Umbrian extra-virgin olive oils. *Journal of Chromatography A* 1997; **758**: 109–116.
- Dionisi F, Golay PA and Fay LB. Influence of milk fat presence on the determination of trans fatty acids in fats used for infant formulae. *Analytica Chimica Acta* 2002; **465**: 395–407.
- Engel E, Nicklaus S, Garem A, Septier C, Salles C and La Quéré JL. Taste active compounds in a goat cheese water-soluble extract. Part I: development and sensory validation of a model water-soluble extract. *Journal of Agriculture and Food Chemistry* 2000a; **48**: 4252–4259.
- Engel E, Nicklaus S, Garem A, Septier C, Salles C and La Quéré JL. Taste active compounds in a goat cheese water-soluble extract. Part II: Determination of the relative impact of water-soluble extract components on its taste using omission tests. *Journal of Agriculture and Food Chemistry* 2000b; **48**: 4260–4267.
- Engel E, Nicklaus S, Septier C, Salles C and La Quéré JL. Evolution of the taste of a bitter camembert cheese during ripening: Characterisation of a matrix effect. *Journal of Agriculture and Food Chemistry* 2001; **49**: 2930–2939.
- Engel E, Lombardo J-B, Garem A, Leconte N, Septier C, La Quéré JL and Salles C. Fractionation of the water-soluble extract of a cheese made from goats’ milk by filtration methods: behaviour of fat and volatile compounds. *International Dairy Journal* 2002; **12**: 609–619.
- Ferreira IMPLVO and Cacote H. Detection and quantification of bovine, ovine and caprine milk percentages in protected denomination of original cheeses by reversed-phase high-performance liquid chromatography of β -lactoglobulins. *Journal of Chromatography A* 2003; **1015**: 111–118.
- Forgács E and Cserháti T. Gas chromatography. In *Food Authenticity and Traceability*, Lees M (ed.). CRC Press, Boca Raton, FL, 2003; 197–217.
- Gil AM, Duarte IF, Delgadillo I, Colquhoun IJ, Casascelli F, Humpfer E and Spraul M. Study of the compositional changes of mango during ripening by use of nuclear magnetic resonance spectroscopy. *Journal of Agriculture and Food Chemistry* 2000; **48**: 1524–1536.
- Högnadóttir A and Rouseff RL. Identification of aroma active compounds in orange essential oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry. *Journal of Chromatography A* 2003; **998**: 201–211.
- Holcapek M, Jandera P, Zderadicka P and Hrubá L. Characterization of triacylglycerol and diacylglycerol composition of plant oils using high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. *Journal of Chromatography A* 2003; **1010**: 195–215.
- Hold GL, Russel VJ, Pryde SE, Rehbein H, Quinteiro J, Vidal R, Rey-Mendez M, Sotelo CG, Perez-Martin RI, Santos AT and Rosa C. The development of a DNA based method aimed at

- identifying the fish species present in food products. *Journal of Agriculture and Food Chemistry* 2001; **49**: 1175–1179.
- Indahl UG, Narini SS, Kirkhus B and Naes T. Multivariate strategies for classification based on NIR-spectra—with application to mayonnaise. *Chemometrics International Laboratory Systems* 1999; **49**: 19–31.
- Jakab A and Forgács E. Characterization of plant oils on a monolithic silica column by high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. *Chromatographia* 2002; **56**: S-69-S-73.
- Jakab A, Nagy K, Héberger K, Vékey K and Forgács E. Differentiation of vegetable oils by mass spectrometry combined with statistical analysis. *Rapid Communications in Mass Spectrometry* 2002a; **16**: 2291–2297.
- Jakab A, Héberger K and Forgács E. Comparative analysis of different plant oils by high-performance liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry. *Journal of Chromatography A* 2002b; **976**: 255–263.
- Jakab A, Jablonkai I and Forgács E. Quantification of the ratio of positional isomer dilinoleoyl-oleyl glycerols in vegetable oils. *Rapid Communications in Mass Spectrometry* 2003; **17**: 2295–2302.
- Jeanet R, Rodriguez J and Garem A. Nanofiltration of sweet whey by spiral wound organic membranes: Impact of hydrodynamics. *Lait* 2000; **80**: 155–163.
- Kamm W, Dionisi F, Fay L-B, Hischenhuber C, Schmarr H-G and Engel K-H. Analysis of steryl esters in cocoa butter by on-line liquid chromatography–gas chromatography. *Journal of Chromatography A* 2001; **918**: 341–349.
- Lee D-S, Lee E-S, Kim H-J, Kim S-O and Kim K. Reversed phase liquid chromatographic determination of triacylglycerol composition in sesame oils and chemometric detection of adulteration. *Analytica Chimica Acta* 2001; **429**: 321–330.
- Lorenzo IM, Pavón JLP, Laespada MEF, Pinto CG and Cordero BM. Detection of adulterants in olive oil by headspace-mass spectrometry. *Journal of Chromatography A* 2002; **945**: 221–230.
- Marigheto NA, Kemsley EK, Defernez M and Wilson RH. A comparison of mid-infrared and Raman spectroscopies for the authentication of edible oils. *Journal of the AOCS* 1998; **75**: 987–992.
- Marini F, Balestrieri F, Bucci R, Magri AL and Marini D. Supervised pattern recognition to discriminate the geographical origin of rice brain oils: a first study. *Microchemistry Journal* 2003; **74**: 239–248.
- Marriott PJ, Shellie R and Cornwell C. Gas chromatographic technologies for the analysis of essential oils. *Journal of Chromatography A* 2001; **936**: 1–22.
- Marsilio V, Campestre C, Lanza B and De Angelis M. Sugar and polyol compositions of some European olive fruit varieties (*Olea europea* L.) suitable for table oil purposes. *Food Chemistry* 2001; **72**: 485–490.
- Nollet LML. High pressure liquid chromatography (HPLC) in food authentication. In *Food Authenticity and Traceability*, Lees M (ed.). CRC Press, Boca Raton, FL, 2003; 218–238.
- Pionier E, Engel E, Salles C and Le Quééré C. Interactions between non volatile water-soluble molecules and arome compounds in Camembert cheese. *Food Chemistry* 2002; **76**: 13–20.
- Razzazi-Fazeli E, Kleineisen S and Luf W. Determination of cholesterol oxides in processed food using high-performance liquid chromatography–mass spectrometry with atmospheric pressure chemical ionisation. *Journal of Chromatography A* 2000; **896**: 321–334.
- Rezanka T and Rezanková H. Characterization of fatty acids and triacylglycerols in vegetable oils by gas chromatography and statistical analysis. *Analytica Chimica Acta* 1999; **398**: 253–261.
- Roux K, Teuber S and Sathe S. An immunoassay for the detection of almond in foods. *Journal of Allergy and Clinical Immunology* 2000; **105**: 144–151.
- Salles C, Hervé C, Septier C, Demaizières D, Leschaeve I, Issanchou S and Le Quééré JL. Evaluation of taste compounds in water-soluble extract of goat cheeses. *Food Chemistry* 2000; **68**: 429–435.
- Schulz H, Quilitzsch R and Kruger H. Rapid evaluation and quantitative analysis of thyme, origano and chamomile essential oils by ATR-IR and NIR spectroscopy. *Journal of Molecular Structure* 2003; **661–662**: 299–306.
- Simoneau C, Hannaert P and Anklam E. Detection and quantification of cocoa butter equivalents in chocolate model system: analysis of triglyceride profiles by high resolution GC. *Food Chemistry* 1999; 111–116.
- Spangenberg JE and Ogrinc N. Authentication of vegetable oils by bulk and molecular carbon isotope analyses with emphasis on olive oil and pumpkin seed oil. *Journal of Agriculture and Food Chemistry* 2001; **49**: 1534–1540.
- Spangenberg JE, Macko SA and Hunziker J. Characterization of olive oil by carbon isotope analysis of individual fatty acids: implications for authentication. *Journal of Agriculture and Food Chemistry* 1998; **46**: 4179–4184.
- Stefanoudaki E, Kotsifaki F and Koutsafakis A. The potential of HPLC triglyceride profiles for the classification of Cretan olive oils. *Food Chemistry* 1997; **60**: 425–432.
- Veloso ACA, Teixeira N and Ferreira IMPLVO. Separation and quantification of the major casein fractions by reversed-phase high-performance liquid chromatography and urea-polyacrylamide gel electrophoresis. Detection of milk adulterations. *Journal of Chromatography A* 2002; **967**: 209–218.