QUESITI GRUPPO 1

1. Il/La candidato/a descriva la valutazione dei tocoferoli e carotenoidi mediante HPLC per la caratterizzazione degli antiossidanti naturali lipofili in un olio di oliva.
2. Il/La candidato/a descriva l'analisi dello spazio di testa mediante Micro-Estrazione in Fase Solida (HS-SPME) e successiva GC-MS per la determinazione dei composti volatili del vino.
3. La composizione del Consiglio di Dipartimento
4. Il/la candidato/a calcoli la composizione % degli acidi grassi di un campione di lipidi vegetali, sottoposto al trattamento di transesterificazione metanolica ed analisi GC-FID, utilizzando il software Excel.

   I dati sono i seguenti:

	Area
C 16:0	160.6
C 16:1	16.1
C 18:0	32.0
C 18:1	964.3
C 18:2	89.2
C 18:3	9.1
C 20:0	2.8
C 20:1	3.0
C 22:0	2.9

5. Il/La candidato/a legga e traduca una parte dell'articolo scientifico "Journal of Chromatography A, 1547 (2018) 62-70"

Journal of Chromatography A, 1547 (2018) 62–70

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Quantitative determination of major oxidation products in edible oils by direct NP-HPLC-DAD analysis

Joaquín Velasco[a,*], Arturo Morales-Barroso[a], M. Victoria Ruiz-Méndez[a], Gloria Márquez-Ruiz[b]

[a] Instituto de la Grasa, Consejo Superior de Investigaciones Científicas (CSIC), Campus Universidad Pablo de Olavide, Ctra. de Utrera, km 1, E-41013, Sevilla, Spain

[b] Instituto de Ciencia y Tecnología de Alimentos y Nutrición, Consejo Superior de Investigaciones Científicas (CSIC), c/José Antonio Novais, 10, E-28040, Madrid, Spain

ARTICLE INFO

Article history:
Received 9 November 2017
Accepted 10 March 2018
Available online 11 March 2018

Keywords:
Hydroperoxydienes
Ketodienes
Hydroxydienes
Oils
Autoxidation
HPLC

ABSTRACT

The objective of the present study was to explore the possibilities of the direct analysis of vegetable oils by normal-phase HPLC-DAD to evaluate the amounts of the main oxidation products of triacylglycerols containing linoleate, i.e. hydroperoxy-, keto- and hydroxy-dienes. A follow-up of oxidation at 40 °C of trilinolein, used as a simplified model lipid system, high-linoleic sunflower oil and high-oleic sunflower oil was performed to evaluate samples with different fatty acid compositions and different oxidation levels. The results showed that the HPLC-DAD method proposed allows for determining the concentrations of mono-hydroperóxydienes in edible oils without applying any isolation or derivatization step. The method was found to be direct, sensitive (LOQ 0.06 mmol/kg oil), precise (CV ≤ 5%) and also accurate, with 99% of analyte recovery. It also enabled the estimation of the minor amounts of ketodienes, but not those of hydroxydienes, which presented wide chromatographic bands and coeluted with a number of different minor oxidation compounds.

1. Introduction

Autoxidation of food lipids causes a variety of products that eventually impair the food flavor and make the food unacceptable for consumption. Oxidized lipids are also involved in pathophysiological processes of chronic and degenerative diseases [1–6]. These can be incorporated through the diet, be formed during the digestion of lipids or be produced in the body, especially in unhealthy individuals. Oxidized food lipids are therefore of concern because they may be detrimental to health. Numerous nutritional studies evidence the toxicological effects of oxidized oils [2,7–10]. Unfortunately, in most of these studies the characterization of oxidized oils was deficient. This was performed by applying analytical indices such as the peroxide value, anisidine value and others that only provide information about partial aspects of the oxidative degradation. Thereby such indices do not enable to get to know the real oil oxidation state and, more importantly, the chemical nature and amounts of the oxidized lipids that are provided to experimental animals. Despite the known implications of oxidized lipids in a number of degenerative and chronic diseases, the real contribution of those incorporated through the diet is at present unknown. It is not known whether their concentrations are sufficient to cause detrimental nutritional effects [11].

Isolation and structural determination of the main oxidation products of the common dietary unsaturated fatty acids were well established using model lipid systems at the end of the 70's [12]. Complex analytical methods mainly based on the combination of liquid chromatography and mass spectrometry have been reported for the identification of oxidation compounds in a variety of samples, principally those of biological nature [13–15]. Recently, chromatography methods for the analyses of primary [16] and secondary [17] oxidation products of triacylglycerols (TAG) have been reviewed. On the other hand, studies dealing with quantitative analysis of oxidized lipids in foods are rather scarce. The principal limitations of the quantitative determination are due to a large number of compounds formed from numerous TAG, their low contents as individual species and their relatively low stability. Quantitative analytical studies have been concentrated on specific compounds found in biological samples [13]. Because of their potential toxicity, glycerolipid aldehydes, also known as core aldehydes, and small aldehydes such as acrolein, malodialdehyde and hydroxyalquenals have been the most studied [13,18]. The ana-

* Corresponding author.
E-mail address: jvelasco@ig.csic.es (J. Velasco).

https://doi.org/10.1016/j.chroma.2018.03.014
0021-9673/

QUESITI GRUPPO 2

1. Il/La candidato/a descriva la valutazione mediante HPLC delle sostanze fenoliche presenti nel vino.
2. Il/La candidato/a descriva l'analisi dei fenoli totali di un olio di oliva mediante il reattivo di Folin-Ciocalteu impiegando uno spettrofotometro UV-Vis.
3. Il Direttore del Dipartimento: procedure di elezioni e competenze
4. Il/la candidato/a calcoli i valori di: media, deviazione standard e deviazione standard relativa, per i risultati della concentrazione di β-carotene in un campione alimentare, utilizzando il software Excel.

I dati sono i seguenti:

	mg/100 g p.s.
Analisi 1	103.5
Analisi 2	99.8
Analisi 3	106.1
Analisi 4	101.4
Analisi 5	98.6
Analisi 6	105.3

5. Il/La candidato/a legga e traduca una parte dell'articolo scientifico "Food Analytical Methods (2022) 15:761-771"

Food Analytical Methods (2022) 15:761–771
https://doi.org/10.1007/s12161-021-02157-3

A Rapid GC-FID Method for the Determination of Fatty Acids in Walnut Oils and Their Use as Markers in Authenticity Studies

Natasa P. Kalogiouri[1] · Natalia Manousi[1] · Ioannis Mourtzinos[2] · Erwin Rosenberg[3] · George A. Zachariadis[1]

Received: 8 July 2021 / Accepted: 26 October 2021 / Published online: 4 November 2021

Abstract

The composition of walnut oils is affected by multiple factors including their variety, their geographical location, and the environmental factors. In this study, the fatty acid composition of walnut oils from Greece and Bulgaria was assessed by gas chromatography coupled with flame ionization detection (GC-FID). The derivatization process involved the use of 14% BF_3 after alkaline hydrolysis, and was optimized after investigating the effect of temperature (50–100°C) and methylation time (5–30 min). Twenty walnut oils originating from Greece and Bulgaria were analyzed, and the methyl esters of palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid were tentatively identified on the basis of their commercially available standards, and quantitatively determined. The method demonstrated good linearity ($r^2 > 0.999$) and adequate recoveries (RE = 86.2–92.5%, $n = 6$). Precision was expressed as repeatability (intra-day precision, $n = 3$), and as reproducibility (inter-day precision ($n = 9$) and the results ranged between 2.7 and 4.6%RSD for intra-day experiments, and 4.1–5.8%RSD for inter-day experiments. The limits of detection (LODs) and limits of quantification (LOQs) ranged between 0.21–0.32 mg/kg and 0.63–0.97 mg/kg, respectively. The determined fatty acid methyl esters were quantified and the results were analyzed by Student's *t*-test, principal component analysis (PCA), and hierarchical clustering to investigate the differences of the lipid fraction of the walnut oils according to their geographical origin.

Keywords Authenticity · Fatty acids · GC · Walnuts · Chemometrics

Introduction

Walnuts are considered as a valuable source of essential fatty acids, tocopherols, and phenolic compounds while they are associated with a plethora of health benefits including reduced risk of coronary heart disease, inhibition of human plasma oxidation, and inhibition of low-density lipoprotein oxidation (Verardo et al. 2009). Currently, walnuts are commercially cultivated throughout Europe, northern Africa, eastern Asia, USA, and western South America and they are widely consumed due to their high nutritional quality and their desirable organoleptic properties (Poggetti et al. 2018; Kalogiouri et al. 2020). In southeastern Europe, Bulgaria and Greece are walnut producers, and *Juglans regia L.* is the walnut species that is cultivated in these countries (Gandev et al. 2015; Jaćimović et al. 2020).

Walnuts are known to have a high content of oil ranging between 60 and 70% on a mass basis (Savage et al. 1999). Many of the desired components of walnuts are also expected to be present in walnut oils, since the last are obtained from the nuts without a need for destructive refining methods (Crews et al. 2005). Walnut oil is rich in monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs, respectively) such as linoleic acid (46.9–68.6%), oleic acid (10.0–25.1%), and linolenic acid (6.9–17.6%) (Poggetti et al. 2018). There are two families of PUFAs, the omega-3 (n-3) and the omega-6 (n-6) fatty acids, based on the location of the last double bond that is relative to the terminal methyl end of the molecule. These fatty acids exhibit a plethora of health benefits due to their biological functions. PUFAs play significant roles in immune regulation

✉ Natasa P. Kalogiouri
kalogiourin@chem.auth.gr

1 Laboratory of Analytical Chemistry, Department of Chemistry, School of Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

2 Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

3 Institute of Chemical Technologies and Analytics, Vienna University of Technology, 1060 Vienna, Austria

QUESITI GRUPPO 3

1. Il/La candidato/a descriva l'analisi spettrofotometrica nell'ultravioletto di un olio di oliva mediante uno spettrofotometro UV-Vis.
2. Il/La candidato/a descriva la valutazione dei polifenoli mediante UHPLC-LC-MS Q-TOF per la determinazione quali-quantitativa degli antiossidanti naturali idrofili in un olio di oliva.
3. Quali sono gli organi di governo dell'Università
4. Il/la candidato/a calcoli la retta di regressione del composto α-tocoferolo, sottoposto ad analisi HPLC-DAD, utilizzando il software Excel.

   I dati sono i seguenti:

	µg/mL	Area
Standard 1	107	471
Standard 2	54	237
Standard 3	27	118
Standard 4	13	62
Standard 5	6	26
Standard 6	3	12

5. Il/La candidato/a legga e traduca una parte dell'articolo scientifico "Food Chemistry 233 (2017) 385-390"

Food Chemistry 233 (2017) 385–390

Contents lists available at ScienceDirect

Food Chemistry

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

Comparison of HPLC-RI, LC/MS-MS and enzymatic assays for the analysis of residual lactose in lactose-free milk

A. Trani [a,*], G. Gambacorta [a], P. Loizzo [a], A. Cassone [a], C. Fasciano [a], A.V. Zambrini [b], M. Faccia [a]

[a] Department of Soil, Plant and Food Science, University of Bari, Via Amendola 165/A, 70126 Bari, Italy
[b] Department of Quality, Innovation, Safety, Environment, Granarolo S.p.A., Via Cadriano, 27/2, 40127 Bologna, Italy

ARTICLE INFO

Article history:
Received 19 January 2017
Received in revised form 11 April 2017
Accepted 20 April 2017
Available online 22 April 2017

Chemical compounds studied in this article:
α and β-Lactose (PubChem CID:84571 and 6134)
Lactulose (PubChem CID: 11333)

Keywords:
Lactose
Lactulose
Enzymatic assay
Milk
LC-MS
HPLC-RI

ABSTRACT

Lactose intolerance is the decreased ability to digest lactose, and the population involved is rapidly increasing all over the world. Different procedures have been reported in the literature to quantify lactose in dairy products, but the official method of analysis is based on enzymatic assay. In this paper, the effectiveness of two enzymatic kits in detecting residual lactose in lactose-free milk was investigated, and a comparison with two alternative chromatographic methods was done. The investigation used several samples of UHT milk containing different levels of lactose, and the results highlighted the inadequacy of the enzymatic assays and of the HPLC-RI method to analyse lactose-free milk. An LC-MS/MS method using the formate adduct was developed, and it allowed quantitation of lactose and lactulose in all samples at a high level of precision and repeatability.

1. Introduction

Lactose intolerance (LI) is a genetically based disease connected to lactase deficiency. It widely varies among individuals, and an absolute threshold of lactose assumption without effects cannot be determined. LI has become very common, and the percentage of the population involved is increasing (Ingram, Mulcare, Itan, Thomas, & Swallow, 2009). Therefore, the dairy industry continuously develops new types of lactose-free products. According to the European Food Safety Agency, the concentration of lactose in these products must be lower than 1 g/L, nevertheless, several dairy companies indicate on the label a lesser value (0.01%) as a "top quality" feature. In addition to this, some dairy foods are marketed using the claim "reduced-lactose", for which lactose concentration must be lower than 5 g/L. Considering this situation on the market, the reliable assessment of lactose concentration in foods has become a critical task. Currently, the method suggested by the Association of Official Analytical Chemists (AOAC) is the enzymatic assay (Essig & Kleyn, 1983). To our knowledge, it refers to "standard" milk and dairy products, whereas no method has been yet validated for low-lactose or lactose-free products. The enzymatic kits available on the market are based on enzymatic hydrolysis of lactose to glucose and galactose as a first step, then two different biochemical pathways are followed. The "Lactose/D-galactose" kit is based on the action of galactose dehydrogenase on released galactose with the formation of $NADH.H^+$ and galactonic acid. The amount of $NADH.H^+$ formed in the reaction is stoichiometric to the amount of D-galactose present in the media (Fig. 1, pathway 1). The "Lactose/D-glucose" kit is also based on the enzymatic hydrolysis of lactose, but it is followed by the action of a glucose phosphokinase leading to the production of glucose-6-phosphate. Then, this latter reacts with glucose-6-phosphate dehydrogenase and is oxidized by nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to gluconate-6-phosphate, with the formation of reduced nicotinamide-adenine dinucleotide phosphate ($NADPH_2$) (Fig. 1, pathway 2). The amount of $NADPH_2$ formed in the reaction is stoichiometric to the amount of D-glucose present in the media. In both kits, the amount of $NADH.H^+$ or $NADPH_2$ originated from enzyme reactions is measured using light absorbance at 340 nm (Lynch, Barbano, & Fleming, 2007) or using its fluorescence (λ_{ex} 340 nm, λ_{em} 470 nm). Generally, these methods report about 7 mg/L of lactose

* Corresponding author.
E-mail address: antonio.trani@uniba.it (A. Trani).

http://dx.doi.org/10.1016/j.foodchem.2017.04.134
0308-8146/