Advanced analysis of nutraceuticals

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ABSTRACT

In this article, we present a review work on different nutraceuticals found in natural matrices together with the analytical techniques used to identify and/or quantify them with special emphasis in the period January 2005-May 2010. The work is distributed according to the different families of nutraceuticals (lipids, vitamins, proteins, glycosides, phenolic compounds, etc) discussing the analytical techniques employed for their determination (separation, spectroscopic, hyphenated techniques, etc). Information about the claimed health promoting effects of the different families of nutraceuticals is also provided together with data on the natural matrices in which they can be found (e.g., fruits, vegetables, plants, microalgae, cereals, milk, etc).

Keywords: Nutraceuticals /advanced analytical techniques / health promoting compounds / bioactive ingredients / functional foods.

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List of abbreviations used

APCI: Atmospheric Pressure Chemical Ionization
CE: Capillary Electrophoresis
CID: Collision Induced Dissociation
CLA: Conjugated Linoleic Acids
CZE: Capillary Zone Electrophoresis
DAD: Diode Array Detector
ECD: Electronic Capture Detector
ELSD: Evaporative Light Scattering Detector
ESI: Electrospray Ionization
ESR: Electron Spin Resonance
FAME: fatty acid methyl esters
FASE: fatty acid steryl esters
FID: Flame Ionization Detector
FLD: Fluorescence Detector
FTIR: Fourier Transformed Infrared Spectroscopy
GC: Gas Chromatography
GPC: Gel Permeation Chromatography
HPAEC: High-Performance Anion Exchange Chromatography
HPLC: High Performance Liquid Chromatography
HPSEC: High-Performance Size-Exclusion Chromatography
HPTLC: High Performance Thin Layer Chromatography
HSCCC: High-Speed Countercurrent Chromatography
ICP: Inductively Coupled Plasma
IT: Ion Trap
LCxLC: Bidimensional Liquid Chromatography
LIF: Laser Induced Fluorescence
MAE: Microwave Assisted Extraction
MALDI: Matrix Assisted Laser Desorption Ionization
MEKC: Micella Electrokinetic Chromatography
MS/MS: Tandem Mass Spectrometry
MS: Mass Spectrometry
NMR: Nuclear Magnetic Resonance
PAD: Pulsed Amperometric Detection
PAGE: Polyacrylamide Gel Electrophoresis
PDA: Photo Diode Array
PLE: Pressurized Liquid Extraction
PMF: Polymethoxylated Flavones
prep-HPLC: Preparative High Performance Liquid Chromatography
PUFA: polyunsaturated fatty acids
QQQ: Triple Quadrupole
QTOF: Quadrupole-Time of Flight
RP: Reversed-Phase
RT-PCR: Real Time Polymerase Chain Reaction
SAX-LC: Strong Anion-Exchange Liquid Chromatography
SDS: Sodium Dodecyl Sulfate
TLC: Thin Layer Chromatography
TOF: Time of Flight
UV: Ultraviolet Detector
VWD: Variable Wavelenght Detector

1. INTRODUCTION

For a long time, natural products obtained mainly from plants have been used as a prominent source of prophylactic agents for the prevention and treatment of diseases in humans and animals [1]. Hippocrates (460–370 BC) stated "Let food be your medicine and medicine be your food". Nowadays, the relationship between food and drugs is getting closer. Thus, the term nutraceutical was firstly mentioned 20 years ago to describe a union between nutrition and pharmaceutics, both key contributors to human wellnes[2]. In the last 20 years, many research publications were devoted to so-called "functional foods" and "nutraceuticals". Research into functional ingredients was showing promising prospects for the use of such ingredients in food products, thereby creating added value for manufacturers and benefits for consumer health [3]. The rising interest in this field can be seen in Figure 1 in which the exponential growing of reasearch papers dealing with nutraceuticals and functional foods in the last 20 years is shown. It is also interesting to mention that more than 150 revision works related to nutraceuticals and functional foods have been published in the same period of time. Some of them are focussed onto the beneficial properties of a particular natural matrix as sesame [4], tea [5] or spices [6], other manuscripts paid their attention onto specific natural compounds like phytochemicals [7], proteins and peptides [8] or lipids [9], meanwhile other works showed the benefits of nutraceuticals against several diseases like atherosclerosis [10] and degenerative joint diseases [11]. It must be pointed out that, to our knowledge, there is not a revision work in which the advanced analytical techniques used to analyze nutraceuticals are summarized and discussed.

Nutraceuticals as defined by Zeisel [12] are dietary supplements that deliver a concentrated form of a presumed bioactive agent from a food, presented in a non-food matrix, and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods. No specific regulation exists in Europe to control nutraceuticals, although they are considered under the same laws that regulate medicine and drug. In the USA, the Food and Drug Administration regulates dietary supplements under a different set of guidelines than those covering conventional foods and drug products [13].

Otherwise, functional foods are those that when consumed regularly produce a specific beneficial health effect beyond their nutritional properties. The boundary between nutraceuticals and functional foods is not always clear being the main difference the format in

which they are consumed: nutraceuticals are consumed as capsules, pills, tablets, etc. while functional foods are always consumed as ordinary foods. Thus, when a phytochemical is included in a food formulation is considered a functional food. If the same phytochemical is included in a capsule it will constitute a nutraceutical [13].

The capacity of some plant-derived foods to reduce the risk of chronic diseases has been associated, at least in part, to the occurrence of secondary metabolites (phytochemicals) that have been shown to exert a wide range of biological activities. In general, these metabolites have low potency as bioactive compounds when compared to pharmaceutical drugs, but since they are ingested regularly and in significant amounts as part of the diet, they may have a noticeable long-term physiological effect [13]. There are numerous biological mechanisms by which nutraceuticals might be expected to exert favourable influences on pathophysiological processes. These products are safe and well tolerated, but interpretation of the collective results is hampered by heterogeneity of the studies, inconsistent results, and/or not well designed investigations. On the other hand, nutraceuticals are expected to be substantially safer and with less secondary effects than many drugs routinely prescribed in the treatment of certain symptoms; however, they are often expensive, lack pharmaceutical-level manufacturing standard controls, and may not work [14].

An additional problem related to the production and consume of nutraceuticals is that the composition and contents of active constituents in natural plants (like in any other natural source) vary depending on season, climate, temperature, humidity, soil and several other factors. So the collection, identification and maintenance of uniform quality, quantification and standardization are critical factors to consider.

The development of advanced analytical techniques is, therefore, indispensable in nutraceuticals research. It includes the identification of new nutraceuticals, characterization of their chemical structure and bioactivity, quantification in the natural source, product development, quality control in their dosage forms, etc. Due to the complexity of these natural matrices, the use of advanced analytical techniques (such as MS, NMR, HPLC, GC, CE, HPLC-NMR, HPLC-MS, GC-MS and CE-MS) is mandatory in order to carry out the mentioned studies. Some of these techniques are already applied for quality control of the natural product confirming their composition from lot to lot and assuring the safety of the final product. Also, these techniques are typically used in a combined way for product development at the initial

stages of their discovery, mainly for facing the challenge to analyse multiple components or multiple classes of components. Moreover, the choice of the analytical technique depends also on the target compounds and the matrix in which they can be found. For example, their physico-chemical properties (polarity, size, volatility,...) will have a strong influence onto the sample preparation procedure, separation mechanism and technique (GC, HPLC, CE) and the type of detector to be employed (UV, FLD, FID, MS, etc). Moreover, advanced analytical techniques are also needed to obtain a better understanding of the health promoting effects of the nutraceuticals, and for knowing the body exposure and bioavailability after the intake of these compounds [15,16]. Important aspects during product development should include nutraceuticals bioactivity and bioavailability studies, so, *in vitro*, *in vivo* and clinical trials should ideally be employed. However, the current legislation on these compounds is in many countries not as demanding as for standard drugs, what usually results in minimum studies to confirm their activity.

Considering all these aspects, the aim of the present work is to present and discuss the main analytical techniques used to identify, characterize and/or quantify nutraceuticals found in different natural matrices with special emphasis in the period January 2005-May 2010. The review work is distributed according to the different families of bioactive compounds (lipids, vitamins, proteins, glycosides, phenolic compounds, etc) discussing also for each family their claimed beneficial health effect.

2. LIPIDS

Lipids are a large group of natural compounds which includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, carotenoids and others. Molecules such as fatty acids and their derivatives (including tri-, di-, and monoglycerides and phospholipids), sterol-containing metabolites, such as cholesterol, are also grouped as lipids. The main biological functions of lipids include energy storage, structural components of cell membranes, and important signaling molecules. Although humans and other mammals use various biosynthetic pathways to both break down and synthesize lipids, some essential lipids cannot be made this way and must be obtained from diet. Interestingly, many papers have discussed the health benefits that can be derived from some of these lipids (see **Table 1, 2** and **3**).

Lipids with potential benefits for human health have been identified in several natural sources (cereals, fruits, animals, oils, plants, mushrooms…) using for their chemical identification gas chromatography (GC) coupled to several detectors (FID, ECD, MS…), high performance liquid chromatography (HPLC) with different detection modes (UV, PDA, MS, MS/MS…) or nuclear magnetic resonance (NMR) and mass spectrometry (MS) as stand-alone techniques.

The most important lipids that can be used as nutraceuticals are described below within their specific lipid group, including their potential health benefits and the advanced analytical tools employed for their determination.

2.1. Fatty acids

Fatty acids are carboxylic acids with a variable unbranched aliphatic tail (chain), which is either saturated or unsaturated. They are important as nutritional substances in living organisms. Long- chain polyunsaturated fatty acids (PUFA), especially those of the ω-3 series, such as α-linolenic (18:3 n-3), are essential for human metabolism and have many beneficial effects including the prevention of a number of diseases, such as coronary heart diseases, inflammation, autoimmune disorders, hypertension, hypotriglyceridemic effect, etc. [83, 84]. Regarding to the analytical techniques more used for determining fatty acids, 95% of the works summarized in **Table 1** employed gas chromatography or high temperature gas chromatography (HT-GC) with FID or MS detectors. It must be taken into account that most of the times, before GC analysis, it is necessary to prepare non-reactive derivatives of fatty acids (methyl esters, FAMEs, steryl esters FASEs, or other derivatives) which are also more volatile than the free acid components. There are only few works in which other analytical techniques have been used. As an example, Herrero et al. [51] characterized several free fatty acids in *Spirulina platensis* by using LC-QTOF-MS, while Yin et al. [56] used electron spin resonance (ESR) and spin-label oximetry methods to determine conjugated linoleic acids (CLAs) in soybean and other matrices [56].

Approximately in 40% of the articles devoted to the analysis of fatty acids the matrix was oil (vegetable or animal) [17-32], and all of them employed gas chromatography with FID or MS detectors. The analysis by GC-FID of fatty acids in rice oil have been carried out in several works [27-32], discussing the importance of, this matrix as source of fatty acids.

Fatty acids have also been determined by GC-FID and GC-MS in salmon, catfish or crustaceans [19, 27-35], beef [36], cheese [37] and plants [38, 39]. Fatty acids could also be found in several fruits by using GC-FID [52-56] and in daily dietary intakes [85]. The nutritional and biological properties of mushrooms have also been studied [40-42], identifying several fatty acids that were analyzed by GC-FID previous methylation of these compounds.

According to the published literature in the reviewed period (2005-2010), an important source of fatty acids are algae (see **Table 1**). Fatty acids, mainly as methyl or ethyl esters, were determined by GC-MS and GC-FID in several varieties of alga (e.g., *Chaetoceros Muelleri, Chlorella vulgaris, Spirulina platensis*, etc.) [43-51]. Liquid chromatography coupled to a MS detector has also been employed in the analysis of fatty acid in algae [51]. The separation provided by HPLC combined with the high mass accuracy and MS/MS capability of the QTOF mass analyzer made possible the direct identification of four free fatty acids and nine polar lipids (Glycerolipids) in this complex matrix without any further sample pre-treatment or derivatization, as it could be seen in Figure 2.

2.2. Sterols

Sterols are an important group of organic molecules that can be found in plants, animals, and fungi, being cholesterol the most popular animal sterol. Sterols from plants, which are known as phytosterols, have been shown in clinical trials to block cholesterol absorption sites in the human intestine, and decrease the level of plasma cholesterol associated with low density lipoproteins (LDL), thus helping to reduce cholesterol in humans. According to some studies they have anti-cancer anti-inflammatory and antithrombotic activities. For these reasons, its use has been approved by US-FDA as food additive. After checking the published literature (see **Table 2**), it could be stated that sterols compounds have been usually identified using GC coupled to FID or MS [17, 18, 23, 24, 27, 35, 52, 57-63].

Analysis of sterols has been carried out in several classes of vegetable oils, like: olive [57], sunflower [58], Indian rice bran [27] and other plants [17, 18, 23, 24, 59]. Sterols could be also found in enriched milk and yoghurt by using an optimized GC-MS method [62], meanwhile total, free and esterified phytosterols in tetraploid and hexaploid wheats where determined by GC-FID and GC-MS [63]. GC was also employed in the analysis of the lipid composition of Italian walnut [52] and for the chemical characterisation of lipids from crustacean which can be employed for the skin-care, with potential benefits on burns, inflammations etc. [35]. Sterols have been also analyzed in different varieties of rice together

with γ-oryzanol and other compounds like steryl ferrulates or squalene, showing antioxidant activity and decreasing cholesterol [32, 60, 61]. Chromatographic techniques as GC or HPLC have been used to determine the sterol composition in the different rice samples. Also, on-line coupling between LC and GC (on-line LC-GC-FID) can be used to determine sterols [60]. This coupling is an efficient approach for the analysis of minor constituents in complex matrices, because it avoids laborious off-line purification steps. In that work [60], γ-oryzanol is pre-separated by normal phase HPLC from other rice lipids (Figure 3a) and transferred on-line to GC analysis to separate its major constituents, (Figure 3b). Total γ-oryzanol content was quantified by HPLC-UV and the ratios of each individual steryl ferulate calculated by GC-FID.

2.3. Terpenes

Terpenes, which could be also named as isoprenoids, constitute the largest and most diverse class of natural products. A majority of these compounds are found only in plants, but some of the larger and more complex terpenes occur in animals. Squalene, which is a natural complex terpene produced by all plants and animals, has been proposed to be an important ingredient of the Mediterranean diet as it may be a chemopreventive substance that protects against cancer. Squalene has been analyzed in rice by GC coupled to FID and MS detectors [61]. This compound has also been identified in vegetable oils using HPLC with evaporative light scattering detection (ELSD) [64]. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers [65-67] or cereals [68] and they have shown antibacterial and antioxidant activity among other beneficial effects for human health. They have been usually analyzed by GC-MS or HPLC-UV-MS although sometimes to elucidate the real structure of these compounds NMR is preferred [68].

2.4. Glycerolipids

Glycerolipids are mainly composed of mono-, di- and tri-substituted glycerols, the most well-known being the fatty acid esters of glycerol (triacylglycerols), also known as triglycerides. These compounds posses several functional activities like antimicrobial, antiinflammatory and are beneficial for the skin care [69]. There is not a unique analytical tool to analyze glycerolipids. For example, GC-FID has been used to determine these compounds in seed oils [22, 25], while silver ion thin layer chromatography (TLC) and HPLC were employed for the analysis of triacylglycerols in rice bran oil [27]. GC-MS and HPLC-MS have been also employed to identify the milk lipids that possess antimicrobial or antiinflammatory properties

[69], and LC-MS was used to characterize triacylglycerols in black currant seed oil [18]. HPLC-ELSD has been used to identify those compounds in *Chaetoceros muelleri* microalgae [47], and LC-QTOF-MS has been used to determine substituted glycerols in *Spirulina platensis* microalgae [51].

2.5. Sphingolipids

Gangliosides are molecules composed of a glycosphingolipid with one or more sialic acids linked on the sugar chain. A glycosphingolipid is composed of an oligosaccharide plus a ceramide, the latter composed of sphingosine and a fatty acid. Gangliosides have protective action against enteric pathogens, prebiotic functions and are considered to present some therapeutic effect against neurodegenerative disorders [71]. These compounds have been determined in biological samples, including milk using several techniques. Thus, high performance thin layer chromatography (HPTLC), GC-MS, LC-MS and matrix assisted laser desorption ionisation time of flight MS (MALDI-TOF-MS) have been used to analyse gangliosides as discussed in a recent review work on this topic [70]. In that work [63], it was stated as a conclusion that MALDI-TOF is the technique of choice, due to its specificity, greater sensitivity and capacity to generate structural information.

2.6. Carotenoids

Carotenoids are a class of more than 600 naturally occurring pigments synthesized by plants, algae, yeast, fungi and photosynthetic bacteria. They are prominent for their distribution, structural diversity and various functions. Fruits and vegetables provide most of the carotenoids in the human diet. Carotenoids can be broadly classified into two classes, carotenes (α-carotene, β-carotene or lycopene) and xanthophylls (β-cryptoxanthin, lutein or zeaxanthin). These compounds show antioxidant and inmunomodulation activities, and they can prevent degenerative diseases, such as cardiovascular diseases, diabetes, and several types of cancer especially prostate and digestive-tract tumors.

HPLC with DAD or UV detectors is the analytical technique of choice for determining carotenoids, as it can be deduced from **Table 3** [43, 72-82]. Nevertheless, comprehensive liquid chromatography coupled to photodiode array and mass spectrometry detection (LCxLC-DAD-MS) have been also shown to provide impressive results in carotenoid analysis [65, 66]. Free carotenoids and carotenoid esters from mandarin and orange juice have been identified with this methodology [72, 73]. Some carotenoids, like β-carotene, and lycopene,

which presents antioxidant, inmunomodulation and anti-cancer properties have been determined by HPLC-UV/Vis or DAD in Thai fruits [74], chesnut [75] and tea seed oils [76].

Algae and microalgae are also an important sources of carotenoids as astaxanthin, β-carotene, lutein, cantaxanthin, violaxanthin, lutein and neoxanthin, being HPLC the analytical tool most frequently employed to identify these compounds [43, 77-79]. In some cases, a strongly hydrophobic column (C30) has been used to separate and quantify certain carotenoids isomers from microalgae [78, 79].

Lycopene, a bright red carotene and carotenoid pigment, and β-carotene have been also identified in different tomato products and wastes by using HPLC, NMR or MS [80, 81] but other more easy techniques like HPTLC could be used for the lycopene determination in nutritional supplements [82].

3. VITAMINS

Vitamins are a diverse group of organic compounds essential in trace amounts for the normal growth and maintenance of life. To ensure the adequate intake of vitamins the human diet can be completed with a high range of multivitamin tablets and food products enriched with vitamins, in other words, these compunds are usually administered as nutraceutical or functional ingredient.

They are classified as either water-soluble or fat soluble. In humans there are 13 vitamins: 4 fat-soluble (A, D, E and K) and 9 water-soluble (8 B vitamins and vitamin C)

These compounds have diverse biochemical roles. Some have hormone-like functions as regulators of mineral metabolism (e.g. vitamin D), or regulators of cell and tissue growth and differentiation (e.g. some forms of vitamin A). Others work as antioxidants (e.g. vitamin E and sometimes vitamins B and C). The largest numbers of vitamins (e.g. $B_{complex}$ vitamins) work as precursors of enzyme cofactors. Recently, it has been published one work where vitamins have been usually determined by HPLC with several detectors as UV/Vis, VWD, PDA, FLD, MS… (Table 4) although GC-FID [18, 52] and HPTLC [86] have been also employed.

Some compounds related to vitamin B group have been identified in mushrooms by HPLC-DAD-FLD [91], in more complex food matrices by using HPLC-MS/MS [92] and in energy drinks employing HPTLC [86].

Vitamin C (L-ascorbic acid or L-ascorbate) is an essential nutrient for humans and other animal species. Deficiency in this vitamin causes the disease known as scurvy in humans. This compound is also widely used as a food additive because of its antioxidant activity. In all the works summarized in Table 4 devoted to the analysis of vitamin C in vegetables [95] and fruits [93, 94, 98], HPLC with UV or VWD was employed to analyse this vitamin. This approach was also used by Kim et al. [96] to determine S-methyl-L-methionine (SMM, so-called vitamin U) that may be effective in reducing ulcers of the skin and intestinal tract.

Compared to the other vitamins, the number of works about Vitamin E is by far the highest one, as it can be seen in Table 4. Vitamin E is a generic term for tocopherols and tocotrienols, and it is a fat-soluble antioxidant that block the production of reactive oxygen species formed when lipids undergoes oxidation. The most frequently employed analytical tool for determining vitamin E has also been HPLC coupled to all possible types of detectors as FLD, UV,PDA, VWD, MS…(Table 4). However, in two works [18, 52], based on the relative volatility and thermal stability of vitamin E, GC-FID was employed to detect vitamin E in vegetable and fruit matrix. As can be seen in Table 4, some algae can also be good natural sources of this vitamin as reported in several works in which HPLC with FLD or DAD was used to identify vitamin E [44, 49, 90]. However, it has to be noted that the main natural source of vitamin E are vegetable and vegetable oils, being HPLC the analytical tool more usually employed (see Table 4).

A new HPLC-DAD method was recently developed for the simultaneous detection and quantification of water- and fat-soluble vitamins in different beverages from different natural sources (orange, strawberry, apple, peach pineapple, plum and blackcurrant juices, soybean milk and beers) [97], with the additional advantage that it was not required any previous sample preparation prior to their analysis. This fact was attributed to the use of an endcapped column, which posses an ultralow silanol activity.

4. PROTEINS, PEPTIDES AND AMINOACIDS

According to the information showed in **Table 5**, there are several benefits for the human health that can de derived from the consume of some proteins, peptides and/or aminoacids. They can have antibacterial, antioxidant, immunostimulating, antithrombotic and anti-inflammatory activities; they could be used for prevention and treatment of hypertension, diabetes and hepatitis among other positive effects in the organism. All these health promoting effects make these compounds of great relevance as nutraceuticals.

Proteins, peptides and/or aminoacids are found in a great variety of matrices including animals, fungi, vegetables, cereals, etc., Their identification requires the use of advanced analytical techniques due to the complexity of these compounds. As a result, HPLC, GC, CE, NMR, FTIR, ICP-MS, inmunosensors, etc, have been used to analyze these compounds. Thus, amino acids have been identified and quantified in different natural matrices using MEKC, micro chip electrophoresis or HPLC [95, 103-105].

Marine animals like sardinelle [107], tuna [108, 110], and echiuroid worms [109] are important sources of peptides with antioxidant, anticoagulant and antihypertensive properties. For their identification HPLC coupled to mass spectrometry (QQQ, QTOFMS) have been usually employed. Amino acids and protein hydrolisates have been analyzed in fishes, as herring [106], using gel permeation chromatography (GPC) and HPLC-FLD.

Another important source of proteins, peptides and amino acids is milk and dairy products. An omics-rooted study of milk proteins has been carried out using advanced analytical techniques (HPLC-MS/MS, 2D-PAGE, MALDI-TOFMS) showing the great potential of this modern approach [69]. Analysis of these compounds in milk usually is carried out employing liquid chromatography coupled to mass spectrometry, although other analytical techniques like capillary electrophoresis [99-102] have also been used for this purpose. Also, immunosensors have been applied for the determination of lactoferrin and immunoglobulin G in milk [99].

Polyacrylamide gel electrophoresis (PAGE), sodium dodecyl sulfate PAGE (SDS-PAGE) and 2D-PAGE have been employed to analyze proteins in several matrices like milk, chick, curry leaves, garlic or fungi [69, 111-114]. These more classical techniques do not provide an identification of these biomolecules as accurate as CE or HPLC coupled to mass spectrometry. Thus, mass spectrometry alone or coupled to HPLC has been used to

characterize, identify and analyse proteins, peptides and amino acids in several matrices, for example lisozyme derived peptides with antimicrobial activity were detected in hen eggs [115], while several cyclopeptides with estrogen activity *in vivo* were found in cow cockle seeds [116].

High-performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) has been used to measure the α-amylase inhibitor activity of phaseolamin from kidney bean [117]. Using several advanced analytical techniques, as ICP-MS, MALDI-TOF-MS, nano-HPLC-MS/MS it was possible to obtain the whole selenopeptide map of Brazilian nuts [118]. As an example, Figure 4 shows a size exclusion ICP-MS chromatogram of the extracted proteins of Brazilian nuts together with the MALDI-TOF spectrum of one protein fraction [118].

5. CARBOHYDRATES, GLYCOSIDES AND RELATED COMPOUNDS

Carbohydrates perform numerous essential roles in living beings. Thus, monosaccharides are the major source of energy for metabolism, while polysaccharides serve for the storage of energy and can act as structural components. Moreover, other beneficial health effects have been linked to these compounds, including their prebiotic effect or other less common as antioxidant or antiinflammatory activity. The identification and quantification of these compounds have brought about the development of multiple analytical trategies mainly based on analytical techniques such as HPLC, GC, CE and/or NMR [68, 116, 119-132].

Chondritin sulphate is a mucopolysaccharide, or sulfated glycosaminoglycan, that acts as an important structural component of cartilage providing much of its resistance to compression. Along with glucosamine, chondroitin sulfate has become a widely used dietary supplement for treatment of osteoarthritis. It has been determined in raw materials and formulations by CE-UV [119]. It has also been analysed in dietary supplements by using a specific and sensitive agarose-gel electrophoresis and strong-anion exchange–high performance liquid chromatography method [120], and this latter technique was followed by a high-performance size-exclusion chromatography (HPSEC) to determine the chondritin sulphate molecular mass [121].

Other saccharides have been analysed in different matrices (plant, fungus, etc) with different analytical techniques like NMR, HPLC or CE [122-124]. Two different CE approaches have been developed to determine glucosamine, an important and abundant monosacharide, in nutraceutical preparations [125, 126]. This compound is shown to be effective in treating osteoarthritis pain, rehabilitating cartilage, renewing synovial fluid and repairing joints that have been damaged by osteoarthritis.

Glycosides are compounds containing a carbohydrate molecule (sugar) bound to a non-carbohydrate moiety. These compounds are mainly found in plants, and they can be converted, by hydrolytic cleavage, into a sugar and a non-sugar component (aglycone). They are named specifically by the type of sugar that they containe, as glucosides (glucose), pentosides (pentose), fructosides (fructose), etc. Many plant glycosides have shown activity for cancer prevention, as well as antidiabetic, anti-obese, antibacterial or antineoplastic effect [127-130]. Among the multiple glycosides, several complex glucosides have been determined in plants and cereals using HPLC, MS, NMR or GC as can be seen in **Table 6**.

Saponins, which are amphipathic glycosides, have been also studied in seeds, plants and cereals. Saponins can stimulate muscle growth and raise testosterone levels [131] and they can also show antibacterial, immunological and antidiabetic properties [68, 116, 132]. The analytical methods used to determine saponins have been GC-MS [131] as well as HPLC, NMR, and MS [68, 116, 132].

6. PHENOLIC COMPOUNDS

Under the denomination “phenolic compounds” there are more than 4000 compounds divided in 12 subclasses. Vegetables, fruits, fungi and some bacteria produce, as part of their secondary metabolism, a wide variety of phenolic compounds. Some of them are highly important for their physiological functions, some others are used to defend themselves from stress situations or to attract or repeal other organisms. In the early 1960s, phenolic compounds were widely viewed as metabolic waste products that were stored in the plant vacuole. Whilst there was interest at that time in their function as flower colorants, and in their distribution between plant taxa, the earliest investigations of their biosynthesis had just begun [133]. In foods this kind of compounds acts as pigments, antioxidants, flavor precursors, etc [133, 134] and, nowadays, as part of our diet they have been associated with

485 several health promoting activities such as: decreasing blood sugar levels, reducing body
486 weight [135], anticarcinogenic [136, 137], antiinflamatory, antiaging [138] and
487 antithrombotic activity [139, 140]. However, the major claimed activity of phenolic
488 compounds has been as antioxidants, as can be seen in **Table 7**.
489
490 The main difference between *bioactive phenolic compounds* that can act as nutraceuticals and
491 *other phenolic compounds* without noticeable bioactivity is their metabolic origin. The first
492 ones are derived from two biosynthetic routes: shikimic acid and/or polyacetates routes.
493 Usually phenolic compounds bind sugars or other phenolic compounds. A fast classification
494 of phenolic compounds could be done as follows [133, 134]:
495 -Benzenediols: are simplest structures, based on the hidroxy phenol.
496 -Phenolic acids: derived from benzoic acid (C6-C1) or cinnamic acid (C6-C3), when
497 phenolic acids are associated as long polymers form tannins and lignans.
498 -Coumarins: with a basic structure of 2H-1-benzopiran-2-one.
499 -Flavonoids: with a basic structure of diarylpropane (C6-C3-C6), this group is the widest
500 and is formed by subfamilies like catechins, flavones, flavonols, flavanones, isoflavonoids
501 and anthocyanes [134].
502
503 The classical method to analyze total phenolic compounds is the Folin-Ciocalteau method
504 where the measured colour change is associated with the reduction of a molibdo-tungstate
505 reagent induced by the phenols in the sample. Curently, HPLC has become the analytical
506 method of choice for phenolic compounds. HPLC was first used for the determination of
507 flavonoids in 1976 by Fisher and Wheaton [134]. Since then, many methods have been
508 developed for the detection and quantification of phenolic compounds, being liquid
509 chromatography the most used technique, as seen in Table 7 [23, 42, 55, 75, 76, 89, 93-95, 97,
510 141-201, 218-229]. Besides HPLC, other separation techniques have been used, namely, GC
511 [202-205], TLC [206, 207] or CE [208-211], and also spectrometric techniques [212-217].
512
513 In HPLC, reversed-phase columns (RP) are the most commonly used, mainly C18, ranging
514 from 150 to 250 mm in length with ID ranging from 4.6 mm, and particle sizes of 5 μm. In
515 general terms, endcapped columns provide better separations. Elution mobile phases are
516 usually binary, with an aqueous acidified solvent (solvent A) such as aqueous acetic acid,
517 perchloric acid, or formic acid and an organic solvent such as methanol or acetonitrile,
518 generally acidified (solvent B). Trifluoroacetic acid in both solvents enhances the resolution

519 and eliminates peak tailing of catechins. In terms of detection systems, UV-visible with diode
520 array detection is the standard method used for detection of phenolic compounds [134].
521 Simple phenolic compounds present a single absorption band in the range 240-290 nm, while,
522 more complex phenolic compounds (flavonoids family) present a second absorption band
523 with a maximum in the 300- to 550-nm range, induced by the B ring [133].
524
525 When MS is used the most employed ion source is ESI due to both the polarity and molecular
526 weight of these analytes match well the requirements of this interface. For example Abad-
527 García et al [157] developed an interesting method based in HPLC-DAD-ESI-MS/MS (triple
528 quadrupole mass spectrometer) for the characterization of unknown phenolic compounds in
529 fruit juices. Their HPLC method consisted of using a C18 column with a gradient and acetic
530 acid–water (0.5:99.5, v/v) and methanol as eluents. Their strategy was based in four steps: (i)
531 taking into account its UV–visible spectrum and elution order, assign the unknown
532 polyphenol to a polyphenol class, (ii) identify the quasi-molecular ion using positive and
533 negative MS spectra, being supported by adducts generated with solvent or sodium and
534 molecular complexes, (iii) determinate the pattern of glycosylation in positive mode using
535 ESI(+)-CID MS/MS product ion scan experiments, selecting the quasi-molecular ion as
536 precursor ion, and finally, (iv) study the identity of the aglycone through ESI(+)-CID MS/MS
537 product ion spectra from the protonated aglycone, $[Y_0]^+$. In this work, a triple quadrupole
538 mass spectrometer was used, which is more indicated for the quantification of known
539 analytes. To overcome this limitation, Abad-García et al. studied the elution time, UV spectra
540 and fragmentation patterns of each polyphenol before analyzing real samples. One example of
541 the study of the fragmentation pattern can be seen in Figure 5.
542
543 The previously depicted strategy is highly useful when dealing with known compounds, but
544 for the study of previously undescribed compounds, other kind of mass spectrometers must be
545 used. For example, Fu et al. [160] used LC-DAD coupled to a time-of-flight mass
546 spectrometer (ESI-TOF-MS) and an ion-trap multiple mass spectrometer (ESI-IT-MS^n) to
547 characterize novel phenolic compounds in extra virgin olive oils.
548
549 Despite LC is the most used separation technique to analyze phenolic compounds, some
550 authors have used CE for this purpose [208, 210, 211]. For example, Orlandini et al [210]
551 developed by chemometric optimization a rapid and simple method based on capillary
552 electrophoresis for the quality control of nutraceuticals (effervescent tablets) containing

resveratrol. This compound is thought to be one of the compounds responsible for the cardioprotective action of red wine and is promoted as an antioxidant for the prevention of atherosclerosis. Resveratrol also has mixed agonist/antagonist activity at oestrogen receptors and some anti-inflammatory and antiproliferative activity [210]. In order to set up the method for the quality control of the mentioned nutraceuticals (effervescent tablets), a multivariate strategy based in response surfaces was followed. In this study, the factors considered were buffer concentration, percentage of acetonitrile and voltage. The factors were studied in the following experimental range: X_1, buffer concentration; X_2, percentage of acetonitrile; X_3, voltage. The resulting method, found by Derringer desirability function, made it possible to achieve good resolution and low analysis time (7 min) also in the presence of a complex sample matrix. The optimisation involved the separation of 11 effervescent tablet components detectable at 280 nm, including the active compounds vitamin C, vitamin B_2, flavanones and hydroxycinnamic acids [210].

In terms of detectors, UV and MS are the dominant, but for certain purposes FTIR and NMR have been used [179, 181, 183, 197, 199, 200, 212-217, 223, 224]. A good example of the use of FTIR for the analysis of nutraceutical compounds was done by Manthey in 2006 [214], he analyzed polymethoxylated flavones (PMF) in orange peel oil, which are by-products of juice industry. Polymethoxylated flavones exhibit anticancer and antiinflammation actions, as well as triglyceride and low-density lipoprotein cholesterol-lowering properties. Although this analysis (FTIR) does not provide information on the levels of individual PMFs, it provides a rapid, solvent-free measurement of the total PMF content in orange oil residues. The ethanol extraction of the PMFs from the bulk of the oil residue provides a rapid and easy enrichment and recovery of the PMF from the oil residues. LH20 size exclusion chromatography provides a means of isolating high percentage PMF material, consisting of relative proportions of the individual PMFs similar to those in the original oil residues. As can be seen in Figure 6 the near absence of other non-PMF constituents is evident by the low intensity of the ν(C=O) vibration at 1724 cm^{-1}. The intensities of the FTIR vibrations of the phenyl ring ν(C=C) stretch at 1515 cm^{-1} of the PMFs can be used, relative to the intensity of the carbonyl stretch at 1733 cm^{-1} of the non-PMF orange oil residue components, to measure PMF content. Excellent correlations for the ratios of the intensities of these vibrations and the total PMF content were observed irrespective of the source, viscosity, and presence of particulate material.

7. OTHER BIOACTIVE COMPOUNDS

Sulphur rich compounds in food have shown to reduce significantly the risk of cancer development. Sulforaphane belongs to this group of compounds and it is usually found in cruciferous vegetables such as broccoli, cabbage, Brussels sprouts, etc. As can be sen in **Table 8**, HPLC-UV [230] and GC-MS [231] have been applied to determine sulforophane in cruciferous vegetables.

Phenylpropanoid amides are plant-specific secondary metabolites and represent an important class of nutraceuticals, having strong antioxidant and chemotherapeutic effects. They have been studied by Kang et al [232], in a work where HPLC combined with real time polymerase chain reaction (RT-PCR) have been used to quantify and analyse these compounds in transgenic tomato tissues.

An interesting combination of analytical techniques (all-liquid high-speed countercurrent chromatography technique and 1D- or 2D-NMR) has been used to characterize phaeophitins, a degradation product of chlorophylls, in leaves and stems of plants [233]. Other nutraceuticals which have been also studied by HPLC are monacolins in rice [234], capsaicinoids from peppers [235] and different acids and related compounds in plants [96, 236], whose health promoting effects are shown in Table 8.

8. CONCLUSIONS AND FUTURE TRENDS

In this work, we have presented an overview on nutraceuticals covering the period January 2005-May 2010, discussing the different bioactive compounds (lipids, vitamins, proteins, glycosides, phenolic compounds…), their claimed health promoting effects, the analytical techniques mainly employed for their analysis and the natural matrices in which they are found. The scientific interest of this topic is demonstrated through the number of research papers (> 200) published on this topic in the reviewed period.

It can be concluded that the preferred analytical tools for analysing bioactive compounds are GC and HPLC, probably due to their versatility, generalized availability, low-cost and simplicity. Other techniques such as CE, MS, NMR or FTIR have also given good results, although their use is not as widespread as GC or HPLC.

It must be pointed out that practically all the claimed health promoting effects of the bioactive compounds presented in this work, have not been recognized yet by the pertinent authorities (EFSA, FDA, etc), because in most of the cases there is a lack of long-term studies and/or clinical trials that demonstrate unquestionably their health effects.

In this sense, it is highly required the development and application of advanced analytical approaches as the new Foodomics strategy [227] in order to obtain superior information on nutraceuticals. This new information should make easier the identification in natural matrices of new nutraceuticals, their chemical characterization and the unquestionable confirmation of their health promoting effects when combined with well designed clinical trials.

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Figure captions

Figure 1.- Research articles dealing with nutraceutical or functional foods published in the period 1989-2009 (ISI Web of Knowledge, Copyright 2010 Thomson Reuters).

Figure 2.- Chromatograms obtained from the HPLC-UV-QTOF-MS analysis of a selected ethanolic PLE extract obtained from *Spirulina platensis*, using the UV detector at 280 nm (A), and the ESI-QTOF-MS detector in negative (B) and positive (C) ion modes. Reprinted from [51] with permission from John Wiley and Sons. http://dx.doi.org/10.1002/rcm.3017. Copyright (2007) John Wiley & Sons, Inc.

Figure 3.- On-line LC-GC analysis of γ-oryzanol in a crude lipid extract from brown rice. (a) LC chromatogram; UV detection of γ-oryzanol at 325 nm. The fraction transferred to GC is shown by the indicated time window. (b) GC chromatogram of the γ-oryzanol-containing fraction; separation of γ-oryzanol into campesteryl ferulate (1), campestanyl ferulate (2), β-sitosteryl ferulate (3), cycloartenyl ferulate (4), and 24-methylenecycloartanyl ferulate (5). (I) and (II) were identified as the co-transferred free sterols cycloartenol and 24-methylenecycloartanol. Reprinted from [60] with permission from American Chemical Society. http://dx.doi.org/10.1021/jf061688n Copyright (2006) American Chemical Society.

Figure 4.- (a) Size-exclusion–ICP MS chromatogram of the extracted Brazil nut proteins. Regular line: ^{78}Se; thin line: ^{34}S.The rectangular area indicates the fraction collected for further analyses; (b) MALDI-TOF mass spectrum of the fraction indicated in Fig. 4a. The insets show the zooms of the 6 and 12 kDa peaks (doubly and singly charged ions, respectively); (c) size-exclusion–ICP MS chromatogram of the tryptic digest (5 kDa cutoff filtered) of the fraction indicated in Fig. 4a. The arrows mark the elution volumes of the calibration standards. The rectangular fractions labelled with numbers were collected for nanoHPLC analyses. Reprinted from [118]. Reproduced by permission of The Royal Society of Chemistry. http://dx.doi.org/10.1039/b608041c Copyright (2007) The Royal Society of Chemistry.

Figure 5.- MS/MS product ion spectra of $[M+H]^+$ ion of apigenin-8-*C*-glucoside-4′-*O*-rhamnoside (cone voltage 30 V, collision energy 20 and 40 eV). Reprinted from [157] with permission from Elsevier http://dx.doi.org/10.1016/j.chroma.2009.05.039 Copyright (2009) Elsevier B.V.

Figure 6.- FTIR spectum of polymethoxylated flavones (PMFs) in orange peel oil isolated by 95% ethanol extraction and LH20 column chromatography (A) and spectum of the non-PMF residue recovered after 95% ethanol extraction (B). The spectra are a summation of eight scans taken with the residue thinly applied to a PTFE IR card. Reprinted from [214] with permission from American Chemical Society. http://dx.doi.org/10.1021/jf053134a Not subject to U.S. Copyright.

Table 1.- Natural matrices containing fatty acids and analytical techniques employed for their analysis.

Matrix	Analytical techniques	Reference
Vegetable oils (plant and seed of bittermelon, Kalahari melon, kenaf, pumpkin and roselle, Ethiopian mustard, pumpkin, *apiaceae*, kachnar, black currant, fruits, nut, soybean).	GC-FID	[17-26]
Rice oil	GC and HT-GC (FID, MS) TLC	[27-32]
Aquatic animals (salmon, catfish, crustacean)	GC (FID, MS)	[33-35]
Grass-fed and grain-fed beef	GC (FID, MS)	[36]
Leuconostoc paramesenteroides, isolated from cheddar cheese	GC-MS	[37]
Plants (*Typhonium flagelliforme, Cistus ladanifer*, *Cupressus lusitanica* and *Eucalyptus gunnii)*	GC (FID, MS)	[38, 39]
Wild and commercial mushrooms	GC-FID	[40-42]
Alga (*Porphyridium cruentum, Himanthalia elongata* and *Synechocystis s, Chaetoceros muelleri, Chlorella vulgari, Spirulina platensis)*	GC (FID, MS) HPLC-QTOF-MS	[43-51]
Fruits (Italian walnut, Black mulberry, orange, blackthorn and rose fruits)	GC-FID, ESR, spin-label oximetry methods.	[52-56]

Table 2.- Lipids (except fatty acids) with potential nutraceutical activity found in different natural matrices, potential health effect and analytical techniques employed for their analysis.

Nutraceutical	Matrix	Possible health effect	Analytical techniques	Reference
Plant sterols (Phytosterols)	Vegetable oils (olive, sunflower, rice bran, seeds,…)	Phytosterols decrease cholesterol associated with LDL, have anti-cancer activity and modulate the immune function and inflammation.	GC (FID, MS)	[17, 18, 23, 24, 27, 57-59]
Glycerolipids	Seed oils	Skin care and source of fatty acids	GC-FID	[22, 25]
Phytosterols, γ-oryzanol and steryl ferulates octacosanol, and squalene.	Rice	Antioxidant, decrease cholesterol absorption, protect against atherosclerosis, nerve imbalance and disorders of menopause	HPLC-GC-FID, GC (FID, MS) HPLC-PDA	[32, 60, 61]
Sterols	Mediterranean mussel and *Rapana venosa* (hard-shellclam)	Skin-care	GC-MS	[35]
Glycerolipids	Microalga	Antimicrobial and anti-inflammatory activities	HPLC-ELSD, HPLC-QTOF-MS	[47, 51]
Sterols	Italian walnut	Decrease cholesterol and reduce the risk of coronary heart disease	GC-FID	[52]
Phytosterols and phytostanols	Milk and yoghurt	Decrease cholesterol levels	GC-MS	[62]
Phytosterols	Tetraploid and hexaploid wheats	Decrease cholesterol levels	GC (FID, MS)	[63]
Squalene	Vegetable oil	Decrease cholesterol and anti-cancer activity	HPLC-ELSD, GC-FID	[64]
Terpenes and terpenoids	Essential oils	Antiseptic, carminative, antimicrobial, and antioxidative effects.	GC-MS, HPLC (DAD,MS)	[65-67]
Terpenoids	Quinoa flour (pseudo- cereal)	Antibacterial and antineoplastic properties.	HPLC (UV, MS) NMR	[68]
Milk lipids (triglycerides, diacylglycerides, saturated fatty acids and PUFAs).	Milk	Immuno-suppressive, anti-inflammatory, andantimicrobial properties.	HPLC-MS/MS, GC/LC.	[69]
Gangliosides	Dairy products (milk)	Protect against enteric pathogens, and prebiotic functions.	MALDI-TOF-MS, HPTLC, HPLC-MS	[70, 71]

Table 3.- Carotenoid nutraceuticals found in different matrices, potential health effect and analytical techniques employed for their analysis.

Nutraceutical	Matrix	Possible health effect	Analytical techniques	Reference
β-carotene, β-cryptoxanthin, mutatoxanthin, antheraxanthin, luteoxanthin, epoxycarotenoids esters…	Mandarin, Orange juices	Antioxidant, inmunomodulation and cancer prevention	LCxLC-DAD-MS(APCI-IT-TOF-MS)	[72, 73]
β-carotene, lycopene	Thai fruits	Antioxidant, anti-cancer, prevent degenerative diseases	HPLC-UV/Vis	[74]
β-carotene, lutein, lycopene	Chesnut	Antioxidant, inmunomodulation and cancer prevention	HPLC-PDA	[75]
β-carotene	Tea seed oils	Antioxidant effects	HPLC-DAD	[76]
Astaxanthin, β-carotene, lutein, cantaxanthin, violaxanthin, neoxanthin	Alga	Antioxidant, inmunomodulation and cancer prevention.	HPLC (UV/Vis,DAD)	[43, 77-79]
Lycopene	Tomato products, nutritional supplements	Antioxidant, anti-cancer	HPLC (UV,DAD) NMR, ESI-MS/MS, HPTLC	[80-82]

Table 4.- Vitamins found in different matrices, potential health effect and analytical techniques employed for their analysis.

Nutraceutical	Matrix	Possible health effect	Analytical techniques	Reference
Tocopherols (Vitamin E)	Vegetable and vegetable oils	Antioxidant, antitumor, hypocholesterolemic potential and for the treatment of cardiovascular disease and angiogenic disorders	HPLC (PDA,FLD, VWD, MS), GC-FID	[17, 18, 23, 24, 30, 31, 52, 62, 64, 87-89]
Tocopherols (Vitamin E)	Microalga	Antioxidant and prevents degenerative disorders	HPLC (DAD, FLD)	[44, 49, 90]
Vitamin B_1 and B_2	Mushrooms	Antioxidant	HPLC (DAD,FLD)	[91]
Water-soluble vitamins (B1, B2, two B3 vitamers, B5, five B6 vitamers, B8, B9, B12 and C).	Maize flour, green and golden kiwi and tomato pulp.	Antioxidant and co-enzymes	HPLC-MS/MS	[92]
Vitamins B_2, B_3 and B_6	Energy drinks	Antioxidant and co-enzymes	HPTLC, ESI MS/MS	[86]
Vitamin C (L-ascorbic acid)	Fruits	Antioxidant	HPLC (UV/Vis, VWD)	[89, 93, 94]
L-ascorbic acid_dehydroascorbic acid)	Buckwheats	Antioxidant	HPLC-UV/Vis	[95]
S-methyl-L-methionine (vitamin U)	*Centella asiatica*	Wound healing	HPLC-UV	[96]
Fat and water soluble vitamins	*Beer and bioactive drinks*	Antioxidant and co-enzymes	HPLC-DAD	[97]

Table 5.- Proteins, peptides and aminoacids with potential nutraceutical activity found in different matrices, bioactivity and analytical techniques employed for their analysis.

Nutraceutical	Matrix	Possible health effect	Analytical techniques	Reference
Milk proteins, peptides Lactoferrin and immunoglobulin G.	Milk and derived products	Antihypertensive, antimicrobial, anti-inflammatory and inmunostimulating activities. Important source of amino acids	HPLC-MS/MS, 2D-PAGE, MALDI-TOFMS, Inmunosensors, CE (UV, MS),	[70, 99-102]
Amino acids	Sprouts, alga and sport drinks and tablets	Eeffect on the nervous system, antioxidant, anti-cancer and source of muscle energy	HPLC (UV/Vis,MS) MEKC-LIF, Microchip electrophoresis (MCE)	[95, 103-105]
Peptide	Fishes	Antihypertensive, antioxidant and anticoagulant activities	HPLC-MS/MS,QTOFMS, GPC, HPLC-FLD	[106-110]
Type II collagen	Chick	Can suppress Rheumatoid arthritis (RA) and promote healthy joints.	SDS-PAGE, HPLC-UV/Vis, FTIR	[111]
~35 kDa antioxidant protein	Curry leaves	Antioxidant properties	SDS-PAGE, MALDI-TOF-MS	[112]
Immunomodulatoryproteins	Garlic (*Allium sativum*)	Immunomodulation activity	SDS-PAGE	[113]
Total proteins	*Ganoderma lucidum* (fungi)	Prevention and treatment of hypertension, diabetes, hepatitis,cancers and AIDS	2D-PAGE	[114]
Lysozyme-derivedpeptides	Hen's egg	Antimicrobial activity	HPLC-MS/MS	[115]
Cyclopeptides	Cow cockle seed	Estrogen like activity *in vivo*	HPLC-MS	[116]
Phaseolamin	Kidney bean (*Phaseolus vulg.*)	May reduce calorie absorbance, thereby promoting weight loss.	HPAEC-PAD	[117]
Selenopeptides	Nuts	Antioxidant, anti-cancer, anti-heart disease	nanoLC-Q/TOFMS/MS. ICP-MS	[118]

Table 6.- Glycosides with potential nutraceutical activity found in different matrices, bioactivity and analytical techniques employed for their analysis.

Nutraceutical	Matrix	Possible health effect	Analytical techniques	Reference
Saponins	Vegetables	Stimulate muscle growth and raise testosterone levels. Antidiabetic or anti-obese effects, antibacterial and antineoplastic properties	GC–MS, HPLC (UV/Vis, MS) ESI-MS/MS, NMR-	[68, 116, 131, 132]
Chondroitin sulfate	Raw materials, formulations and dietary supplements.	Treatment of osteoarthritis and some ophthalmologic diseases.	CE-UV, SAX-LC, HPSEC	[119-121]
Polysaccharide (1,3-α-galactan)	*Poria cocos* (fungus)	Anti-inflammatory effects	1D and 2D NMR	[122]
Saccharides	Black currant pomace	Antioxidant properties	HPLC-UV	[123]
Galactooligosaccharides	Dairy-based prebiotic ingredient.	Increased absorption of calcium and magnesium, and improved elimination of toxic compounds	GC-FID	[124]
Glucosamine	Nutraceutical preparations and tablets	Treatment of osteoarthritis	CE (PDA.UV)	[125, 126]
Glycosides (glucosinolates, glycyrrhetic acid, glycyrrzhin, liquiritin, steroidal glycosides…)	Plants	Choleretic, anti inflammatory, anti-cancer, antioxidant, anorexant and diuretic properties	HPLC-UV/Vis, HR-MS, 1D and 2D NMR.	[127-130]

Table 7.- Phenolic compounds with potential nutraceutical activity found in different matrices, bioactivity and analytical techniques employed for their analysis.

Nutraceutical	Matrix	Possible health effect	Analytical techniques	Reference
Phenolic acids	Seed-oil	Antioxidant	HPLC-UV	[23]
Phenolics	Fruits, Mushrooms, legumes	Antioxidant	HPLC-DAD	[42, 55, 75, 76, 89, 97, 141-156]
Phenolics	Fruits, Mushrooms, legumes	Antioxidant	HPLC-MS, HPLC-MS/MS	[157-166]
Anthocyanins	Fruits, Nutraceutical Capsules	Antioxidant	HPLC-DAD	[167-173]
Anthocyanins	Fruits, tubers	Antioxidant	HPLC-MS, HPLC-MS/MS	[174-177]
Phenolics	Marula (*Sclerocarrya birrea*)	Antioxidants and Antiatherogenic	HPLC-DAD	[93]
Catecholamines	Banana peel	Antioxidant	HPLC-DAD	[94]
Rutin	Buckwheats	Antioxidant	HPLC-DAD	[95]
Flavone isomers	lemon juice	Antioxidant	HPLC-DAD-ESI-MS/MS	[178]
Phenolics	Potatoe	Antioxidant	HPLC-DAD, NMR	[179]
Phenolic acids	Cooked meat	Antioxidant	HPLC-DAD	[180]
Flavonol	Bean	Antioxidant	HPLC-DAD, prepHPLC, NMR	[181]
Phenolics	Moscatel sweet wines	Antioxidant	HPLC-DAD, HPLC-FD	[182]
Phenolics	*Carex distachya* roots	Antioxidant	HPLC-DAD-MS, NMR	[183]
Curcuminoids	*Curcuma longa*	Antioxidant	HPLC HPTLC	[184]
Lignans	Flaxseed	Antioxidant	PLE HPLC-UV	[185]
Phenolic acids, proanthocyanidins, and lignans	Triticale	Antioxidant	HPLC-DAD	[186]
Flavonoids	*Ulmus davidiana*	Antioxidant	HPLC-DAD	[187]
Resveratrol	Grape canes	Antioxidant	HPLC-DAD	[188]
Phenolic acids and flavonoids	*Glycin tomentella* Hayata (*Leguminosae* family)	Antioxidant	HPLC-DAD	[189]
Phenolic acids	Mulberry	Antioxidant	HPLC-DAD	[190]
Chicoric acid	seagrass *Syringodium filiforme*	Antioxidant	HPLC-DAD-MS	[191]

Resveratrol	Nutritional suplements	Antioxidant	HPLC-UV	[192]
Flavonoids	Wild rice	Antioxidant	HPLC-DAD-MS/MS	[193]
Phenolic acids	Rice	Antioxidant	HPLC-DAD-MS/MS	[194]
Silymarin	Milk thistle	Antioxidant	HPLC-UV	[195]
Flavonoid aglycones	black currant	Antioxidant	HPLC-MS/MS	[196]
Flavonoids	Cranberry	Antioxidant	HPLC-DAD-MS, NMR	[197]
Demethyloleuropein	Olive fruit	Antioxidant	HPLC-DAD-MS	[198]
Alkil phenols	Anacardum	Antioxidant	HPLC-MS, GC-MS, NMR	[199]
Flavonoids	*Hypericum perforatum*	Antioxidant	HSCCC, prep-HPLC, NMR	[200]
Phenolic acids, isoflavones	Black Soybeans	Antioxidant	HPLC-DAD	[201]
Phenolics	Pepper	Antioxidant	GC, TLC, Voltametry	[202]
Phenolic acids	Malt	Antioxidant	GC-MS	[203]
Catechins and condensed tannins	Green Tea	Antioxidant	GC-MS	[204]
Phenolic acids	Mangosteen	Antioxidant	GC-FID GC-MS	[205]
Phenolics	*Bergenia ciliata*	Antioxidant	HPTLC	[206]
Phenolics	*Vanilla planifolia*	Antioxidant	HPTLC PLE MAE	[207]
Phenolics	Grape	Antioxidant	CE-UV	[208]
Flavonoids and phenolic acids	Chinese herbal tea	Antioxidant	CZE-AD	[209]
Resveratrol	Nutraceutical capsules	Prevention of atherosclerosis	CE	[210]
Phenolics	Grape skin	Antioxidant	CE-Fluo CE-DAD	[211]
Lignans	*Myristica fragrans* (nutmeg)	Anticariogenic	NMR, MS, GPC	[212]
Prunate	Prunus	Anticarcinogenic	MS, NMR	[213]
Polymethoxilated Flavones	Orange Oil	Antioxidant	FTIR	[214]
Carnosic	Rosemary	Antioxidant	NMR FTIR	[215]
Licochalcone A	Glycyrrhiza uralensis	Lipase inhibition	NMR MS	[216]
Phlorotannins	Alga *(Ishige okamurae)*	Cholinesterase inhibition	NMR	[217]
Phytoestrogens	Dietary supplements	Estrogenic activity	HPLC-UV	[218]
Flavonol glycosides	Ginkgo biloba	Memory enhancing	HPLC-UV	[219]

Isoflavones	Soy milk	Estrogenic activity	HPLC-DAD	[220]
Isoflavones	Soy supplements	Antimenopausial sympthoms	HPLC-DAD	[221]
Phenolics	*Tamarix gallica*	Antioxidant and antimicrobial	HPLC-DAD	[222]
Flavonoids	Citrus peel	Antiinflammatory, anticarcinogenic and antiatherogenic	HPLC-ESI-MS NMR	[223]
Resveratrol Oligomers and Flavonoids	*Carex folliculata* Seeds	Antioxidant, cytotoxicity and antibacterial	HPLC-DAD, NMR	[224]
Phenolic acids	Infant cereals	Antioxidant & aroma	HPLC-DAD-MS/MS	[225]
O-glucoside phenolic compounds	Olive by-products	Antioxidant, maturity indicators	HPLC-DAD-MS/MS	[226]
Isoflavones	Soybean seeds	Antimenopausial sympthoms	HPLC-UV	[227]
Isoflavones	Red clover	Antifungal activity	HPLC-DAD	[228]
Isoflavones	Nutritional supplements	Estrogenic activity	HPLC-DAD	[229]

Table 8.- Other potential nutraceuticals found in different natural matrices, their possible health effect and the analytical techniques employed for their analysis.

Nutraceutical	Matrix	Possible health effect	Analytical techniques	Reference
Sulforaphane	Crucifer vegetables (*Brassica* species)	Anticarcinogenic properties	HPLC-UV/Vis, GC-MS,	[230, 231]
Phenylpropanoid amide	Transgenic tomato	Antioxidant and chemotherapeutic effects	HPLC, RT-PCR	[232]
Phaeophytines	*Amaranthus tricolor* (Amaranthaceae)	Antioxidant, cancer prevention	HSCCC, 1D NMR, 2D NMR, MS/MS.	[233]
Monacolins	Rice	Cholesterol lowering and anticancer agent	HPLC-MS	[234]
Capsaicinoids	Peppers	Antioxidants, anti-mutagenic, anti-inflammatory and anti-tumoral properties .	HPLC-FLD	[235]
Acids (bitter acids, asiatic acid and asiaticoside)	Plants (*Centella asiatica*, hop)	Anticarcinogenic properties	HPLC-UV	[96, 236]

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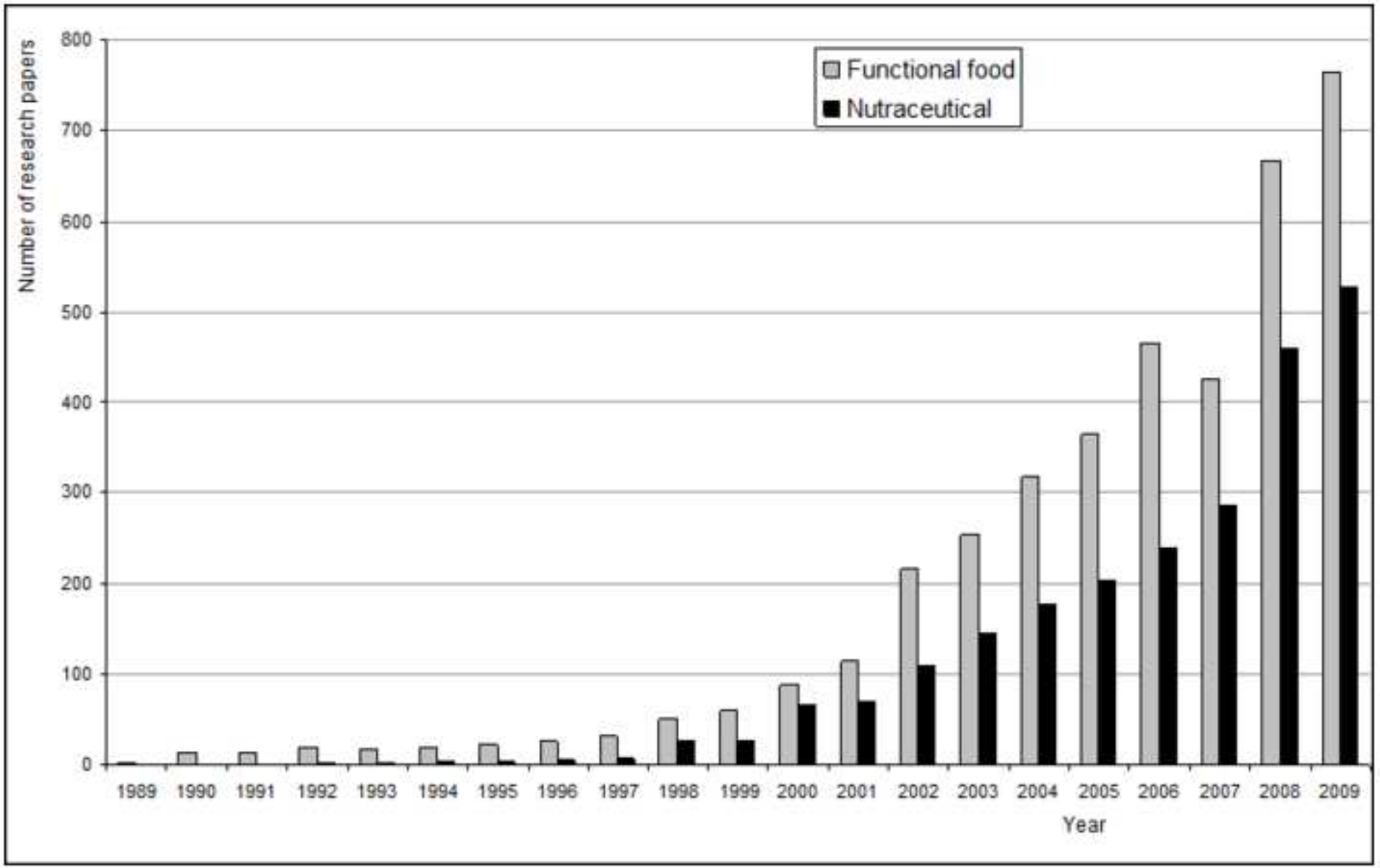

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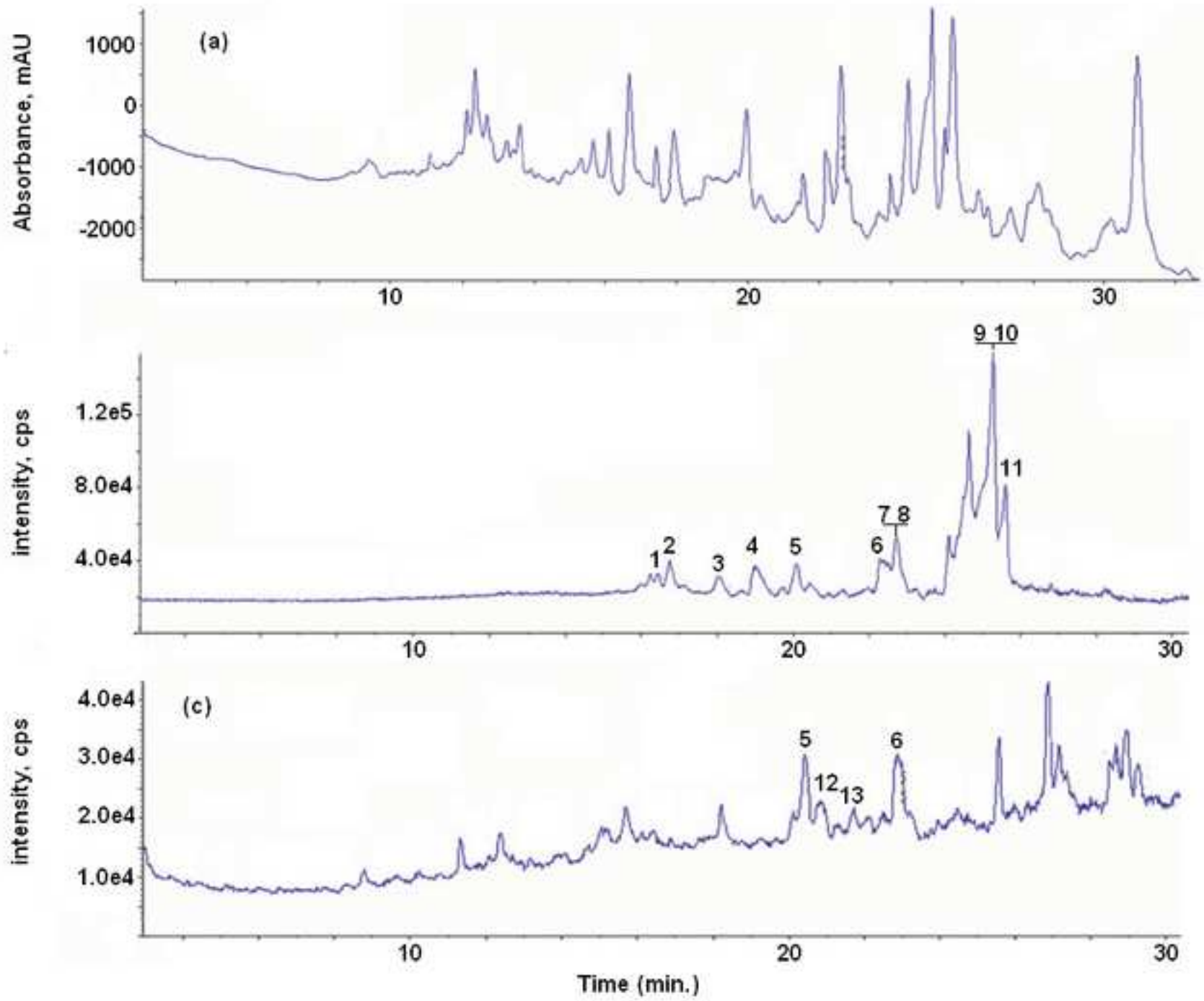

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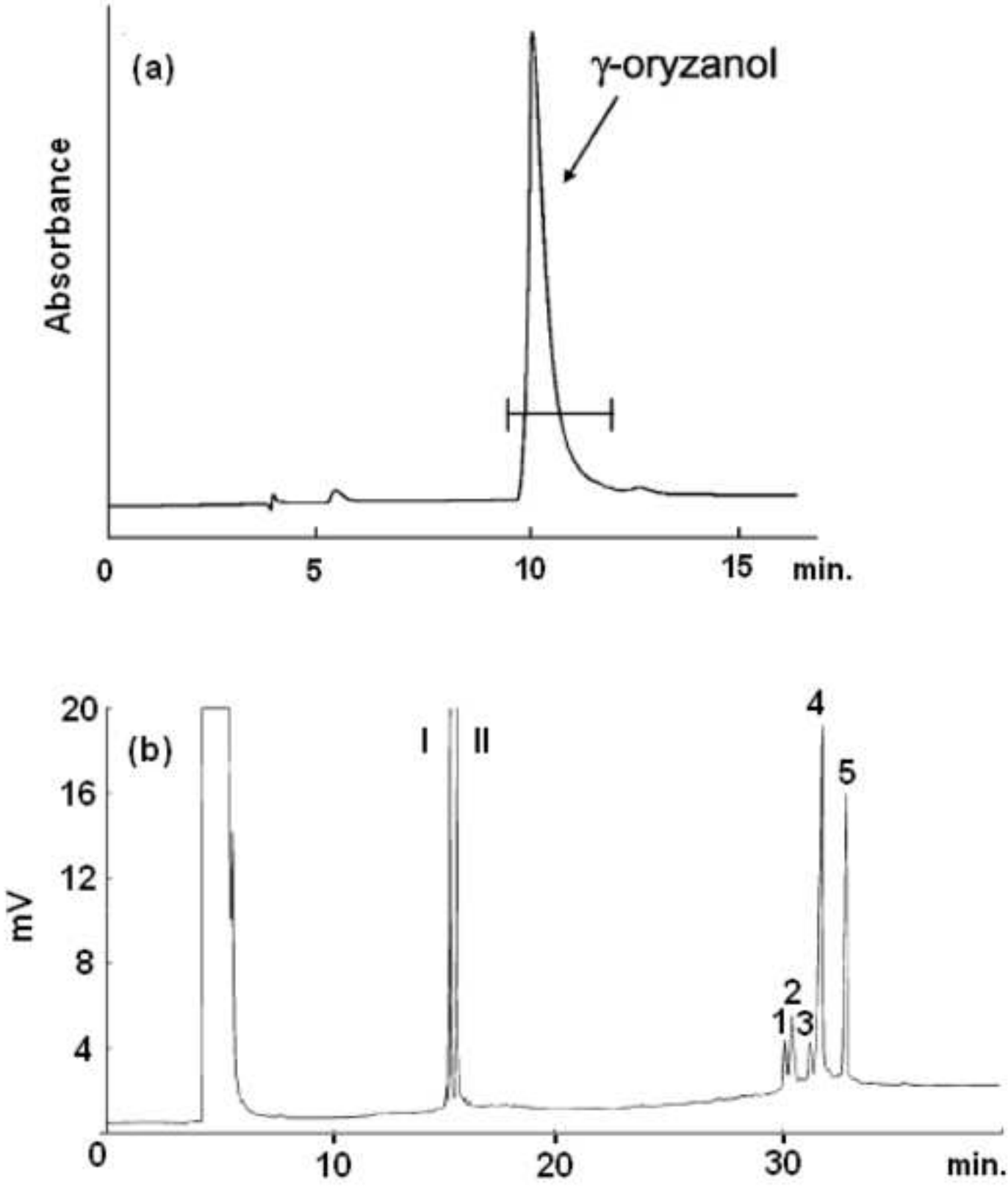

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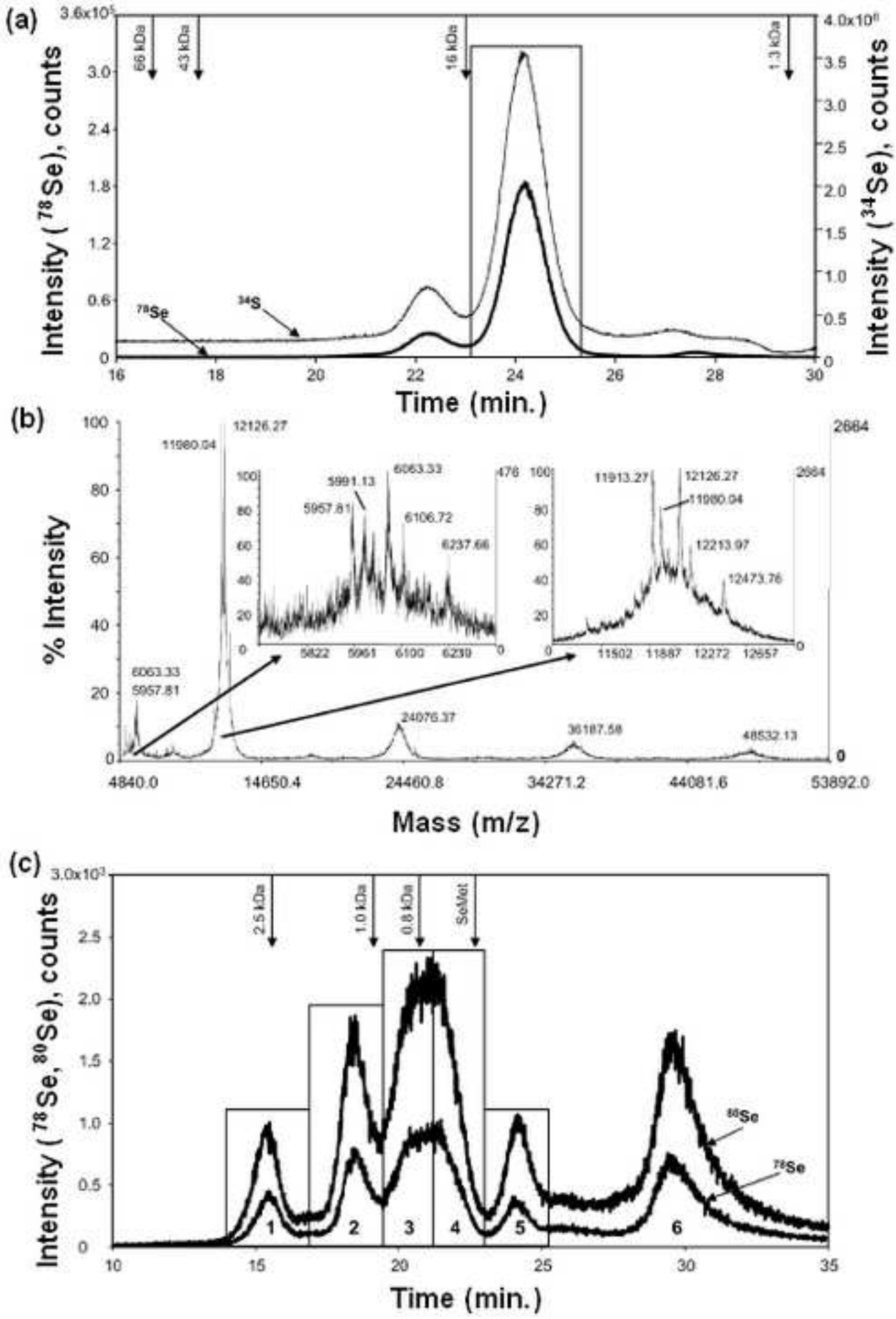

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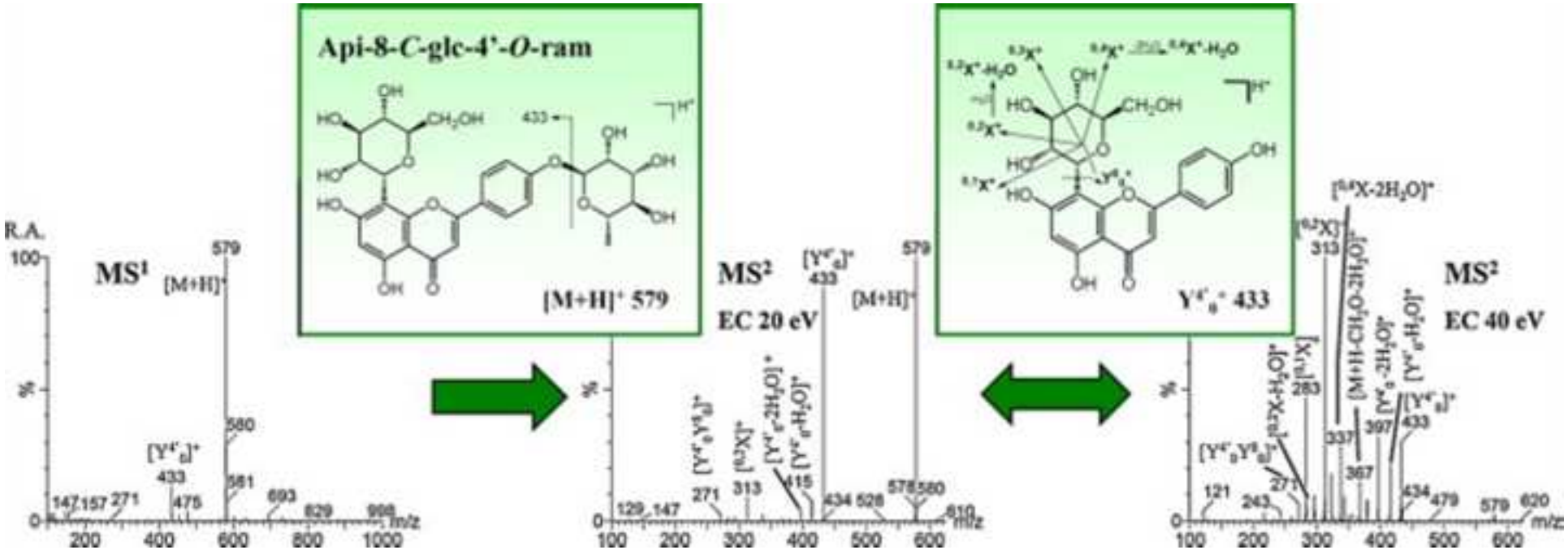

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