African Journal of Biotechnology Vol.11(4), pp. 724-731, 12 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.984
ISSN 1684–5315 © 2012 Academic Journals

Review

A review of the methods used in the determination of flaxseed components

Wahid Herchi[1]*, David Arráez-Román[2], Sadok Boukhchina[1], Habib Kallel[1], Antonio Segura-Carretero[2] and Alberto Fernández-Gutierrez[2].

[1]Laboratoire de Biochimie des Lipides, Département de Biologie, Faculté des Sciences de Tunis, 2092 ELmanar-Tunisia.
[2]Department of Analytical Chemistry, Faculty of Sciences, University of Granada, C/Fuentenueva s/n, 18071 Granada, Spain.

Accepted 28 September, 2011

Flaxseed (*Linum usitatissimum* L.) is a multi-purpose crop and its consumption is beneficial for human health. The nutritional components of flaxseed are oil, protein, lignans, fiber and vitamin. The determination of the minor components is of great importance in establishing the flaxseed oil quality and their genuineness. The qualitative and quantitative determination of its constituents has been carried out by using several analytical techniques most of which are based on gas chromatography and some being based on high-performance liquid chromatography. In the present work, the different methods used for the determination of flaxseed components are revised.

Keys words: Methods, chromatography, flaxseed, food, fiber, protein, oil, lignans.

INTRODUCTION

Flaxseed is the seed from the flax plant (*Linum usitatissimum* L.), which is a member of the Linaceae family. The plant is not a new crop and native to West Asia and the Mediterranean (Berglund, 2002). Flaxseed is rich in fat, protein and dietary fibre. Chemical analysis of flaxseed averaged 30 to 40% oil, 20 to 25% protein, 20 to 28% total dietary fibre, 4 to 8% moisture and 3 to 4% ash and the oil contains vitamins A, B, D and E, minerals and amino acids. By virtue of the presence of physiologically active food components that may provide health benefits beyond basic nutrition, flaxseed is often grouped into one of several categories: ‘‘functional food’’, ‘‘bioactive food’’ and an ‘‘endocrine active food’’ (Hasler et al., 2000).

The qualitative and quantitative determination of the constituents is often done by capillary gas chromatography (GC). The use of chromatographic techniques, especially gas chromatography (GC), has become more and more important, because it can be applied to the quality control of many food matrices. In standardized analytical methods, flame ionization detection (FID) is the most widely used together with mass spectrometry (MS) which allows molecular mass data, structural information and identification of compounds. In particular, GC has been useful for detecting the presence of other fat substances in flaxseed oils, even at very low concentration levels. High-performance liquid chromatography (HPLC) is used normally for separating non-volatile, high-molecular-mass constituents, namely normal phase, is widely used to separate classes of constituents according to the nature and number of polar functional groups. Reversed-phase HPLC is used to separate individual components that belong to one constituent class. Several detection methods can be used in conjunction with HPLC, the ultraviolet-visible (UV-Vis) being the most commonly used. Other detection methods, such as refractive index (RI), FID, MS, evaporative light scattering (ELSD), fluorescence (FD) and electrochemical detection are also used (Cert et al., 2000). Recently, there has been a growing interest in the probiotic properties of flaxseed

*Corresponding author. E-mail: wahid1bio@yahoo.fr Tel: (00216) 96 538 999.

and in its beneficial effects on coronary heart disease, some kinds of cancer and neurological and hormonal disorders. This review shows the most commonly used methods in flaxseed in order to assess it nutritional value.

ANALYSIS OF THE DIFFERENT FLAXSEED COMPONENTS

Protein

Flaxseed is a source of raw materials such as protein (35%) with potential application as nutraceuticals and functional foods (Bozan and Temelli, 2008). The content of albumins fraction was higher in flaxseed (40%), whereas in stone pine (*Pinus pinea*) was found in the range of 15% (Sammour et al., 1999).

With the increasing demand for vegetable sources of proteins, there is a potential for utilizing flaxseed proteins as a food source. The identification requires the use of advanced analytical techniques due to the complexity of these compounds. As a result, HPLC, GC, capillary electrophoresis (CE), nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR) have been used to analyse these compounds. Analysis of these compounds were carried out employing HPLC-MS (Oomah et al., 2007), other techniques like differential scanning calorimetry (Li-Chan and Ma, 2002). Sodium dodecyl sulphate PAGE (SDS-PAGE) and 2D-PAGE have been employed to analyse protein in flaxseed (Chung et al., 2005). These more classical techniques do not provide an identification of these biomolecules as accurate as CE or HPLC coupled to mass spectrometry.

Dietary fiber (mucilage or gum)

Flaxseed contains both soluble and insoluble fiber (Bloeden and Szapary, 2004).

The measurement of dietary fibers in flaxseed is a complex issue, associated with the definition of fiber in the analytical method chosen. Methods for the determination of dietary fiber may be divided into three categories: non-enzymatic-gravimetric, enzymatic gravimetric and enzymatic-chemical methods. The later includes enzymatic-colourimetric and enzymatic-chromatographic (GLC/ HPLC) methods. Nowadays, the most commonly used methods for dietary fiber measurement are the enzymatic-gravimetric, the Association of Official Analytical Chemists (AOAC) method (Prosky et al., 1988) and enzymatic-chemical method (Englyst et al., 1994). The most fiber-rich plants include grains such as wheat, barley and oats; legumes such as beans, lentils and soybeans; and vegetables such as garlic, asparagus, broccoli and carrots (Murphy and Hendrich, 2002). Flaxseed is recognized as having about 35 to 50% dietary fiber. It contains 5% viscous fiber (mucilage) (Muir et al., 2000).

Cyanogenic glycosides

A range of 218 to 538 mg/100 g and 73 to 454 mg/100 g of linustatin and neolinustatin, respectively has been reported for 48 flaxseed varieties (Dean, 2003). Methods in the literature related with the detection of cyanogenic glycosides in flaxseed include chemical and enzyme-linked colorimetric tests (Vetter, 2000), thin-layer chromatography (Sherma, 2000), HPLC and GC of trimethylsilyl (TMS) derivatives (Bacala and Barthet, 2007).

Chemical hydrolyses function, by employing strong acids at elevated temperatures to hydrolyze the cyanogenic glycosides, have been used as quantities colorimetric method measuring the liberated cyanide gas. Although, amenable for rapid analysis of large sample sets, this method was found to be much less accurate than enzyme-linked assays (Kobaisy et al., 1996). Enzyme-linked assays function by employing endogenous hydrolytic enzymes to hydrolyze the cyanogenic glycosides. Quantitation is achieved by measuring either the liberated cyanide or glucose using a colorimetric test. Although, one report shows that such a method could be as accurate as HPLC (Bacala and Barthet, 2007), enzymatic methods only provide total cyanogenic glycoside content and no information on identity or levels of individual cyanogenic glycosides in the sample.

Furthermore, two different [beta]-glucosidases are required for liberation of cyanide from linustatin and neolinustatin in flaxseed (Bacala and Barthet, 2007). First, linustatinase converts linustatin and neolinustatin to linamarin and lotaustralin, respectively. Second, linamarase liberates cyanide from the monoglycosides. The abundance and relative activity of each of these enzymes in a seed sample or crude enzyme preparation would affect the observed rate of reaction. This creates additional variables that could adversely affect assay reproducibility, as enzyme levels in different seed samples and enzyme preparations will almost certainly vary over time and from sample to sample. Although, existing TLC methods can resolve linustatin and neolinustatin.

They are not capable of full resolution of diglucosides from their respective monoglucosides (Amarowicz and Shahidi, 1994) and TLC is generally considered a semi quantitative test at best. GC or HPLC methods are typically the preferred method of analysis due to their high resolving power, capability of direct quantitation of analyses and automation.

Flaxseed oil

Flaxseed oil is naturally high in polyunsaturated fatty acids (PUFA), more specifically in ω-3 fatty acids and hence, flaxseed as a component of poultry meal, can provide ω-3 enriched eggs. Rapid drying linseed oil is

used for several purposes in industry, including paint and flooring (linoleum) industries (Jhala and Hall, 2010). Because of its novel oil profile, flaxseed may also be a suitable platform crop for the synthesis of specialized industrial and nutraceutical products.

Fatty acids

Nutraceutical and health virtues of flaxseeds have been attributed to their essential fatty acids (EFAs) which are essentially omega-6 (Linoleic) and omega-3 (Linolenic) fatty acids (Bozan and Temelli, 2008). Fatty acids, the main components of any edible oil, are usually converted to fatty acid methyl esters (FAMEs) for GC analysis using capillary columns (Tuberoso et al., 2007). The use of capillary columns in GC analysis has notably improved the resolution of separations, improving the precision of analysis in terms of quality, quantity, sensitivity and analysis times. American Oil chemistry's Society (AOCS) official methods include various procedures for FAME quantification by GC with capillary columns (Firestone, 1987). The composition of fatty acids has traditionally been used in the food industry as an indicator of purity (Kfapoulas et al., 1981). Palmitic acid is used as an indicator of adulteration of flaxseed oil by palm olein, since flaxseed oil has a palmitic acid content of between 8 and 10%, whereas palm olein contains around 40% palmitic acid. Information regarding linoleic acids is used for the detection of flaxseed adulteration with soybean oil. Soybean oil contains about 50% linoleic acid, whereas flaxseed oil contains 15% (Aparicio and Aparicio-Ruiz, 2000). At present, GC allows not only the percentage of *trans*-fatty acids to be determined, but they can also be identified and the proportion of each one of them to be calculated. The presence of the *trans*-isomers of oleic, linoleic and linolenic acids in flaxseed oils above the maximum levels (Aparicio and Aparicio-Ruiz, 2000), can indicate adulteration with hydrogenated seed oils or mutant (or genetically altered) seed oils desterolised at high temperatures (Paganuzzi, 1997).

Triglycerides

Triglyceride composition has also been established as a measurement of the quality and purity of vegetable oils (Aparicio and Aparicio-Ruiz, 2000). Analysis can be carried out by HPLC or high-temperature GLC, although, HPLC is becoming widely accepted in the analysis of triglycerides (Tuberoso et al., 2007). Different types of sample preparation, stationary and mobile phases and detectors have been studied to separate triglycerides according to the number of carbon atoms by HPLC. The greatest resolution of a triglyceride homologous series was found using an RP-18 (Lisa and Holcapek, 2007).

Depending on the degree of separation demanded and the type of detection used, different mobile phases can be used. The detectors commonly used are UV-Vis which is the most sensitive. An alternative is light-scattering detection. Tuberoso et al. (2007) used the equivalent carbon number (ECN) to cluster triglycerides exhibiting the same behaviour on reversed-phase HPLC columns. From the point of view of authentication, fatty acids are distributed on glycerol molecules according to certain position-specific patterns and hence, triglycerides are considered to be good fingerprints for adulteration purposes. Flaxseed oil is a triglyceride oil consisting mainly of linolenic acid (53%), oleic acid (18%), linoleic acid (15%), palmitic acid (6%) and stearic acid (6%). The major forms of TAG species (LnLnLn, LnLLn, LLLn, LnOLn) (Tuberoso et al., 2007) whereas in corn oil, OLL, OOL, POL and LLL were the major species (Harrabi et al., 2008).

Waxes

The profiles of waxes are of interest as indicators of both quality and purity. Virgin olive oil can be distinguished from refined olive oil and olive-pomace oils, because the first has a higher content of C36 and C38 waxes than of C40, C42, C44 and C46, whilst the other oils have an inverse relation (Aparicio and Aparicio-Ruiz, 2000). The most common methodologies are based on separation by HPLC followed by GC analysis (Herbert et al., 2006). Alternatives such as TLC (Gordon and Miller, 1997), open column chromatography and off-line HPLC methods have all been used for the isolation of the total wax ester fraction. GC analysis is then carried out to quantify each compound (Herbert et al., 2006).

Sterols (4-desmethylsterols)

The analysis of sterols is important for detecting oil adulteration. Chromatographic methods are currently the most widely used for the qualitative and quantitative analysis of this extensive series of compounds clustered in 4-desmethylsterols, 4, 4-dimethylsterols and 4-monomethylsterols or triterpene alcohols. GC for unsaponifiable matter is the prevalent technique in flaxseed (Schwartz et al., 2008). A direct analysis of the silylated unsaponifiable components obtained from different food products was performed on a thermostable polar cGC column (65% phenyl-35% dimethylpolysiloxane); this allowed a fast screening of the components of the lipid fraction that are considered to be the fingerprint of a natural matrix. Using this methodology, it is possible to detect the presence of small quantities of husk oil in the new or rectified olive oils, as well as to establish the quality of the oil with respect to oxidation (Lercker et al., 2000). The unsaponifiable matter is isolated preferably using the diethyl ether procedure that allows the total extraction of

sterols (Schwartz et al, 2008; Herchi et al., 2009b). Various developing liquids can be used to purify this material by TLC on silica gel. Capillary columns give the best performance since they can resolve the sterols almost completely (Herchi et al., 2009b). The sterols fraction, probably due to their relative abundance, is frequently used for tracking commercial frauds. Positional isomers of the double bond in the sterols ring have been recently detected, which can be used as fraud tracers in flaxseed oils. Refining and acidity can modify the sterol composition leading to isomerisation of fucosterol and 5-avenasterol (Lercker et al., 2000). Sitosterol was the most abundant sterol in flaxseed oil (Herchi et al., 2009b). Cholesterol and brassicasterol which were the minor sterols in flaxseed oil, have high contents of butter oil and rapeseed oil, respectively (Schwartz et al., 2008).

Hydrocarbons

Hydrocarbons in flaxseed oil are present in quite small amounts (≤0.2%) (Herchi et al., 2009a). The only exception is virgin olive oil, which contains about 0.5% and is mainly constituted by squalene (Lercker et al., 2000).

They are formed by a homologous series of linear compounds that are mainly saturated chain of 13 to 34 carbon atoms (Cunha and Fernandes, 2001). Silica TLCs of natural lipids using a mobile phase of n-hexane-diethyl ether provide a good separation of hydrocarbons (Cert et al., 2000).

In flaxseed, the hydrocarbons can be determined by GC and GC-MS (Herchi et al., 2009a). The results of Herchi et al. (2009a) provide useful information on the hydrocarbon composition of linseed oil, which contained n-alkanes (C22-C34) and squalene using GC and GC-MS techniques. The high-chain-length n-alkane (C40-C50), characterizing a mineral origin (Neukom et al., 2001) and n-alkenes were absent in the hydrocarbon fraction of three varieties of linseed.

Determination of the aliphatic hydrocarbon profile of vegetable oils has been used as a marker to reveal contamination with mineral oil residues, which could be diffused in vegetable oils. In some unrefined vegetables oils, small quantities of pollutants can be found (pesticides, volatile organic compounds, chlorinated hydrocarbons, aromatic hydrocarbons, etc.) (Lercker et al., 2000).

In the TLC fractionation, the polycyclic aromatic hydrocarbons (PAHs) elute together with the other hydrocarbons. HPLC would be in this case, a suitable analytical choice, since it has proven successfully to separate PAHs from the other hydrocarbons (Moreda et al., 2001).

Aliphatic and triterpenic alcohols

The determination of aliphatic and triterpenic alcohols are also used for ascertaining the genuineness of vegetable oils. Both linear and triterpenic alcohols are more polar than tocopherols and are often badly separated *in silica* TLC and are observed for their determination by GC (Herchi et al., 2009b). The TLC separation of aliphatic and triterpenic alcohols from each other is difficult although procedures have been suggested (Schwartz et al., 2008). In any case, a better TLC separation could be achieved by using a multiple development technique with a slightly different mobile phase for the second development (Cert et al., 2000). The effects of refining and industrial hydrogenation on the structural modifications of linear and triterpenic alcohols have also been studied (Lercker et al., 2000).

Tocopherols and tocotrienols

Flaxseed oil is rich in gamma tocopherol (400 to 500 mg/kg oil) (Herchi et al., 2011d) which have many health effects. The most commonly used method for the analysis of tocopherols and tocotriénols in flaxseed oil is the direct HPLC analysis of the oil sample using normal phase and fluorescence detection (Schwartz et al., 2008; Herchi et al., 2011d).The method with either fluorescence or UV-Vis detection has been standardized by the IUPAC (IUPAC, 1987) and AOCS (AOCS, 1990). The HPLC techniques have been also used with UV-Vis detection and reversed-phase columns. The separation with reversed-phase columns presents the advantage of short equilibrium and analysis time and high reproducibility of retention time, but has the disadvantage of not resolve between β and γ isomers of both tocopherols and tocotrienols (Swigło and Sikorska, 2004). On the other hand, normal phase HPLC allows a good separation of all isomers, but the analysis time is longer and the retention times are more variable (Schwartz et al., 2008). Evaporative light scattering detectors (ELSD) has been compared with fluorescence, being less sensitive than fluorescence needing a very clean sample. In the preparative TLC tocopherols and tocotrienols coelute with epoxy-squalene, the epoxy-squalenes are derive from the oxidation of squalene, which can be of enzymatic or chemical origin, as in the olive husk oil (Lercker et al., 2000). γ-Tocopherol occurred in highest concentrations in linseed (Herchi et al., 2011d), camelina, cold-pressed rapeseed and corn oil (Schwartz et al., 2008). The range of α-tocopherol contents in linseed oil has been reported to be exceptionally wide. For example, in one study, the contents varied between <1 and 12.1 mg/100 g (Bozan et al., 2008) and in another study, between 0 and 9.11 mg/100 g (Schwartz et al., 2008), while the content of the major tocol, γ -tocopherol, was more stable. In contrast, α-tocopherol was the major isomer in wheat germ (192 mg/100 g oil) and γ-tocopherol was found in trace.

Phenolic compounds

A column chromatography method for separation of

phenolic compounds from flaxseed using Sephadex LH-20, RP-8 and silica gel is described (Amarowicz and Shahidi, 1994). TLC analysis proved that separated fractions contained numerous phenolic compounds. The levels of lignans in food vary widely with flaxseed (*L. usitatissimum* L.) being a rich source which contains lignans tens to hundreds times more than most other edible plants (Kraushofer and Sontag, 2002) including minor amounts of phenolic acids as p-coumaric and simple phenols as vanilline (Siger et al., 2008). Extraction methods vary widely depending on the sample and the compound of interest. Following the discovery of SDG by Bakke and Klosterman (1956) and its connection to the mammalian lignans, several methods for the analysis of lignans and other phenolic compounds of flaxseed have been developed. The polymeric powder obtained by ethanol: dioxane extraction of defatted flaxseed flour (DFF) was found to release hydroxymethyl glutaric acid (HMGA), 4-O-β-D-glucopyranosyl coumaric acid and SDG upon alkaline hydrolysis (Bakke and Klosterman, 1956), suggesting that these compounds are bound in ester-linked polymeric structure(s) in flaxseed. The compounds may also be released from the polymeric material as aglycones by enzyme or acid hydrolysis (Mazur and Adlercreutz, 1998). Extractions of flaxseed phenols have usually been carried out with organic solvents (Chimichi et al., 1999) sometimes mixed with water (Sicilia et al., 2003), but the use of supercritical fluid (SCF) extraction has also been reported (Harris and Haggerty, 1993). SDG and cinnamic acids absorb light in the UV-region and have been detected and quantified by column chromatography, HPLC, GC and NMR techniques. Methods for the extraction and purification of SDG in flaxseed have been compiled in Table 1. The colorimetric procedures using the Folin-Ciocalteu reagent have been used for the determination of total phenols (Herchi et al., 2011e; Siger et al., 2008). HPLC-MS detection is one of the most important analytical techniques used for the analysis of phenolic compounds. Herchi et al. (2011c) describe here, a simple HPLC-ESI-TOF (MS) method to analyse phenolic and other polar compounds in oil samples after solid-phase extraction

Pigments

Chlorophylls and carotenoids are the main pigments in vegetables oils, being pheophytin the principal component of the chlorophyll group. Carotenoids are divided into two groups; carotenes and xanthophylls. In flaxseed oil, the main carotenoids are β-carotene (Tuberoso et al., 2007). Both chlorophylls and carotenoids are considered to have an important role in keeping the quality of edible oils, mainly due to their action as photo-sensitizers or singlet oxygen quenchers, respectively (Tuberoso et al., 2007). The analysis of carotenes is usually performed by HPLC, since GC degrades the compounds. For the determination of chlorophylls in flaxseed oil, a spectrophotometric method has been standardized by the IUPAC (Herchi et al., 2011d). Nevertheless, to quantify flaxseed oil, a different chromatographic method was developed using direct injection of the oil onto an HPLC provided with a reversed phase column and UV detection at 408, 430 and 450 nm (Daun and Thorsteinson, 1989). The method allowed the detection of β-carotene, chlorophyll *a* and *b* and pheophytin *a* and *b* simultaneously (Rahmani and Saari, 1991). An improvement of the method was achieved using silica gel columns and UV-Vis diode array detection (Psomiadou and Tsimidou, 1998). The carotenoids were determined after cold saponification of the oil, by means of reversed-phase HPLC (Stancher et al., 1987).

Phospholipids

Phospholipids (PLs) contribute to the stability and quality of edible oils through their antioxidative activity or contribution to the texture (Singleton, 1993). On the other hand, they are responsible for oil discoloration during deodorization and steam distillation, so that their determination is necessary to evaluate the efficiency of degumming (Mounts and Nash, 1990). Recently, a sensitive method has been described for the separation and identification of glycerophospholipids derived from flaxseed samples by negative ion high performance liquid chromatography and tandem mass spectrometry LC-MS/MS (Herchi et al., 2011f). Flaxseed oil contains substantial amounts of phospholipids (Herchi et al., 2011f). The most important members of this class of lipids found in flaxseed oil are phosphatidylethanolamine, phosphatidylinositol and phosphatidylcholine. The purification by SPE was investigated in different stationary phases (Herchi et al., 2011f). The quantitative analysis of the PLs fraction is usually performed by HPLC using silica gel column and UV-Vis detection with both isocratic and programmed elution (Chea Chua et al., 2008). ELSD has been used in conjunction with preparative HPLC to isolate PLs classes from flaxseed oil. In each PLs class, the separation of molecular species differing in the fatty acid composition was achieved using reversed-phase HPLC (Wanasundara et al., 1999).

Future applications

Flaxseed is not a major food plant. Its use as food is limited by its laxative properties and content of cyanogenic glucosides. Nevertheless, given its high levels of biologically active lignans, flaxseed may still be added to foods as a health promoting ingredient. There is a demand for alternative sources of polyunsaturated fatty acids (PUFA) and the possibility of obtaining them from

Table 1. Extraction systems for lignan isolation from flaxseed and flaxseed containing foods.

Extraction	Hydrolysis	Purification	Level SDG (µmol/g seed)	Reference
MeOH-dioxane (1:1) 24 h	Ba methoxide	Cellulose column	3.15	(Bakke and Klosterman, 1956)
In vitro Fermentation	Na methoxide	Silica gel (CHCl3-MeOH-H2O)	0.96 to 3.15	(Thompson et al., 1991)
β-Glucuronidase	β-Glucuronidase	C18 SPE	1.19 to 1.97	(Obermeyer et al., 1995)
β-Glucuronidase	β-Glucuronidase 2 M HCl, 2.5 h, 100°C	Ether extraction/DEAE-Sephadex OH–QAE-SephadexAC–	9.05 to 10.21	(Mazur and Adlercreutz, 1998)
Reflux, 80% MeOH, 2 h	β-Glucuronidase	C18 SPE + Lipophilic gel chromatography	0.22 to 3.41	(Setchel et al., 1999)
70% Aqueous alcohol	NaOH	C18 SPE	5.24 to 15.74	(Westcott and Muir, 1998)
95% EtOH-dioxane (1:1) 8 h	nr	nr	0.001 to 0.004	(Harris et al., 1994)
SCO2 + THF-H2O (1:1)	nr	nr	7.15	(Wilson et al., 1993)
Shaker, 80% MeOH, 4 h, 55°C	1 M HCl, 1 h 100°C	EtOAC-hexane (1:1)	nr	(Meagher et al., 1999)

nr = Not reported. Source: Adapted from (Muir et al., 2000).

higher plants in commercial quantity is particularly attractive. As no oil-seed species produces such products naturally, genetically engineering would be required to synthesize these fatty acids. Because flaxseed already contains the precursor to PUFA and the highest value of alpha-linolenic acid (ALA), it may be a choice platform species. The molecular and gene expression experiments are not widely studied in flaxseed, which may also expand the applications and uses of flaxseed in future (Jhala and Hall, 2010). Apart from the interest on knowing in properties of flaxseed, the determination of its compounds also helps to understand their health benefits that include reduction of risk factors of coronary heart disease, prevention of several varieties of cancer and modification of immune and inflammatory responses. Although, excellent progress has already been made, it is expected that the use of different methodologies of potent techniques coupled with rapid, reliable and sophisticated detectors will become more common in the near future.

Conclusion

The present review shows the different analytical methods used for the analysis of flaxseed components. The qualitative and quantitative determination of the major and minor constituents

of flaxseed are carried out by GC and primarily HPLC, which are the most important two techniques widely applied for the analysis of edible oils and fats. The choice of the method employed depends on the scope of the analytical control, the amount of information that can be acquired and the cost of the overall analytical operation.

ACKNOWLEDGEMENTS

The authors are grateful to the Spanish Ministry of Education and Science for the project (AGL2008-05108-C03-03) and to Andalusian Regional Government Council of Innovation and Science for the project P07-AGR-02619, P09-CTS-4564 and P10-FQM-6563.

REFERENCES

Amarowicz R, Shahidi F (1994). Application of Sephadex LH-20 chromatography for the separation of cyanogenic glycosides and hydrophylic phenolic fraction from flaxseed. J. Liq. Chromatogr. 17: 1291-1299.

AOCS (1990). Official and Tentative Methods of the American Oil Chemists' Society, American Oil Chemist' Society, Cham paign, IL, 4th ed., Official method Ce 8.

Aparicio R, Aparicio-Ruiz R (2000). Authentication of vegetable oils by chromatographic techniques. J. Chromatogr. A. 881: 93–104.

Bacala R, Barthet V (2007). Development of extraction and gas chromatography analytical methodology for cyanogenic glycosides in flaxseed (Linum usitatissimum). J. AOCS. Int. 90 (1): 153-161.

Bakke JE, Klosterman HJ (1956). A new diglucoside from flaxseed. Proceedings of the North Dakota Academy of Science, Grand Forks, North Dak. Volume X: 18–21.

Berglund DR (2002). Flax: New uses and demands. In J. Janick & A. Whipkey (Eds.), Trends in new crops and new uses: pp 358–360.

Bloeden LT, Szapary PO (2004). Flaxseed and cardiovascular risk. Nutr. Rev. 62: 18–27.

Bozan B, Temelli F (2008). Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. Bioressource Technol 99: 6354-6359.

Cert A, Moreda W, Perez-Camino MC (2000). Chromatographic analysis of minor constituents in vegetable oils. J. Chromatogr. A. 881: 131–148.

Chea Chua S, Ping Tan C, Ming Lai O, Long K, Mirhosseini H, Sham Baharin B (2008). Effect of absorbent in solid-phase extraction on quantification of phospholipids in palm-pressed fiber**.** Eur. J. Lipid. Sci. Tech. 110: 334–340.

Chimichi S, Bambagiotti-Alberti M, Coran SA, Giannellini V, Biddau B (1999). Complete assignment of the 1H and ^{13}C NMR spectra of secoisolariciresinol diglucoside, a mammalian lignan precursor isolated from Linum usitatissimum. Magn. Reson. Chem. 37: 860–863.

Chung MWY, Lei B, Li-Chan ECY (2005). Isolation and structural characterization of the major protein fraction from NorMan flaxseed (Linum usitatissimum L.). Food. Chem. 90: 271–279.

Cunha AC, Fernandes FM (2001). Ontogenic variations in n-alkanes during somatic embryogenesis of flax (Linum usitatissimum L.). Plant. Sci. 160: 1137–1143.

Daun JK, Thorsteinson CT (1989). Determination of chlorophyll pigments in crude and degummed canola oils by HPLC and spectrophotometry. J Am Oil Chem Soc. 66: 1124.

Dean JR (2003). Current market trends and economic importance of oilseed flax. In: Muir, A.D. and Westcott, N.D. (eds) Flax-The genus Linum. Taylor & Francis, New York, NY, USA. Pp. 275-291.

Englyst HN, Quigley ME, Hudson GJ (1994). Determination of dietary fiber as non-starch polysaccharides with gas–liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst. 119: 1497–1509.

Firestone D (1987) (Ed.), Official Methods and Recommended Practices, 4th ed, American Oil Chemists' Society, Champaign, IL, Method Ce 1e-91.

Gordon MH, Miller LAD (1997). Development of steryl ester analysis for the detection of admixtures of vegetable oils. J. Am. Oil. Chem. Soc. 74: 505.

Harrabi S, Boukhchina S, Kallel H, Mayer PM (2008). An electrospray-ionization mass spectrometry analysis of triacylglycerols in developing corn kernels, Can. J. Anal. Sci. Spectros. 53(1): 22-27.

Harris RK, Greaves J, Alexander D, Wilson T, Haggerty WJ (1994). Development of stability indicating analytical methods for flaxseed lignans and their precursors. In: Osawa CT, Huang MT, Rosen RT, editors. Food phytochemicals II: teas spices, and herb. Washington, D.C.: Am Chem Soc. p 295–305.

Hasler CM, Kundrat S, Wool D (2000). Functional foods and cardiovascular disease. Curr. Atheroscler. Rep. 2: 467–475.

Herbert MW, Holser R, Akin DE (2006). Cuticular wax from flax processing waste with hexane and super critical carbon dioxide extractions. Ind. Crop. Prod. 24: 119–122.

Herchi W, Harrabi S, Rochut S, Boukhchina S, Kallel H, Pepe C (2009a). Characterization and Quantification of the Aliphatic Hydrocarbon Fraction during Linseed Development (Linum usitatissimum L.). J. Agr. Food. Chem, 57: 5832–5836.

Herchi W, Harrabi S, Sebei K, Rochut S, Boukhchina S, Pepe C, Kallel H (2009b). Phytosterols accumulation in the seeds of Linum usitatissimum L. Plant. Physiol. Biochem, 47: 880–885.

Herchi W, Sawalha S, Arráez-Román D, Boukhchina S, Segura-Carretero A, Kallel H, A Fernández-Gutierrez (2011c). Determination of phenolic and other polar compounds in flaxseed oil using liquid chromatography coupled with time-of-flight mass spectrometry. Food. Chem. 126: 332–338.

Herchi W, Sakouhi F, Boukhchina S, Kallel H, Pepe C (2011d). Changes in Fatty Acids, Tocochromanols, Carotenoids and Chlorophylls Content During Flaxseed Development. J. Am. Oil. Chem. Soc. DOI 10.1007/s11746-010-1750-3.

Herchi W, Sakouhi F, Arraez-Roman D, Segura-Carretero A, Boukhchina S, Kallel H, Fernandez-Gutierrez A (2011e). Changes in the Content of Phenolic Compounds in Flaxseed Oil During Development. J. Am. Oil. Chem. Soc. DOI 10.1007/s11746-011-1783-2.

Herchi W, Sakouhi F, Khaled S, Xiong Y, Boukhchina S, Kallel H, Curtis JM (2011f). Characterisation of the glycerophospholipid fraction in flaxseed oil using liquid chromatography–mass spectrometry. Food Chem doi:10.1016/j.foodchem.2011.04.096.

IUPAC (1987). Standard Methods for the Analysis of Oils, Fats and Derivatives, International Union of Pure and Applied Chemistry, Blackwell, Oxford, 7th ed., Method 2432.

Jhala AJ, Hall LM (2010). Flax (Linum usitatissimum L.): Current Uses and Future Applications**.** Aust. J. Basic Appl. Sci. 4(9): 4304-4312.

Kfapoulas VM, Passaloglou-Emmanouilidou S (1981). Detection of adulteration of olive oil with seed oils by a combination of column and gas liquid chromatography. J. Am. Oil Chem. Soc. 58: 694 – 697.

Kobaisy M, Oomah BD, Mazza G **(**1996). Determination of Cyanogenic Glycosides in Flaxseed by Barbituric Acid–Pyridine, Pyridine–Pyrazolone, and High-Performance Liquid Chromatography Methods. J. Agr. Food. Chem .44 (10): 3178–3181.

Kraushofer T, Sontag G (2002). Determination of matairesinol in flax seed by HPLC with coulometric electrode array detection. J. Chromatogr. B. 777: 61-66.

Lercker G, Rodriguez-Estrada MT (2000). Chromatographic analysis of unsaponifiable compounds of olive oils and fat-containing foods. J. Chromatogr. A. 881: 105–129.

Li-Chan ECY, Ma CY (2002). Thermal analysis of flaxseed (Linum usitatissimum) proteins by differential scanning calorimetry. Food Chem.77: 495–502.

Lisa M, Holcapek M (2008). Triacylglycerols profiling in plant oils important in food industry, dietetics and cosmetics using high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry J. Chromatogr. A. 1198: 115–130.

Mazur WM, Adlercreutz H (1998). Naturally occurring oestrogens in food. Pure. Appl. Chem. 70: 1759–1776.

Meagher LP, Beecher GR, Flanagan VP, Li BW (1999). Isolation and characterization of the lignan, isolariciresinol pinoresinol in flaxseed meal. J. Agr. Food. Chem. 47: 3173–80.
Moreda W, Perez-Camino M.C, Cert A (2001). Gas and liquid chromatography of hydrocarbons in edible vegetable oils. J. Chromatogr. A. 936: 159–171
Mounts TL, Nash AM (1990). HPLC analysis of phospholipids in crude oil for evaluation of soybean deterioration. J. Am. Oil. Chem. Soc. 67: 757.
Muir AD, Westcott ND, Ballantyne K, Northrup S (2000). Flax lignans: recent developments in the analysis of lignans in plant and animal tissues. In Proc. Flax Inst. of the USA, 58. Fargo, N. Dak.: Flax Inst. of the USA. p 23–32.
Murphy PA, Hendrich S (2002). Phytoestrogens in foods. Advanc. Food. Nutr. Res. 44:195–246.
Neukom HP, Grob K, Biederman M, Noti A (2001). Food contamination by C20-C50 mineral parrains from the atmosphere. Atmos.Environ. 36: 4839–4847.
Obermeyer WR, Musser SM, Betz JM, Casey RE, Pohland AE, Page SW (1995). Chemical studies of phytoestrogens and related compounds in dietary supplements: flax and chaparral. Proc. Soc. Exp. Biol. Med. 208: 6–12.
Oomah BD, Berekoff B, Li-Chan ECY, Mazza G, Kenaschuk EO, Duguid SD (2007). Cadmium-binding protein components of flaxseed: Influence of cultivar and location. Food Chem. 100: 318–325.
Paganuzzi V (1997). Current Possible Adulterations of Olive Oil. Riv. Ital. Sostanze Grasse. 74: 49.
Prosky L, Asp NG, Scheweizer TF, DeVries JW, Furda I (1988). Determination of insoluble and soluble, and total dietary fibre in foods and food products: Interlaboratory study. J. Assoc. Off. Agric. Chem. 71: 1017–1023.
Psomiadou E, Tsimidou M (1998). Simultaneous HPLC Determination of Tocopherols, Carotenoids, and Chlorophylls for Monitoring Their Effect on Virgin Olive Oil Oxidation. J. Agr. Food. Chem. 46: 5132.
Rahmani M, Saari CA (1991). Chlorophyll and β-carotene pigments in moroccan virgin olive oils measured by high-performance liquid chromatography. J. Am. Oil. Chem. Soc. 68: 672.
Sammour RH (1999). Proteins of linseed (*Linum usitatissimum L*), extraction and characterization by electrophoresis. Bot.Bull.Acad.Sin.40:121-126.
Schwartz H, Ollilainen V, Piironen V, Lampi AM (2008).Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats. J. Food Compos. Anal. 21: 152–161.
Setchel KDR, Childresse C, Zimmer-Nechemias L, Cai J (1999). Methods of measurement of dietary secoisolariciresinol using HPLC with multichannel electrochemical detection. J. Med. Food. 2:193–8.
Sherma J (2000). Thin-layer chromatography in food and agricultural analysis. J. Chromatogr. A. 880: 129–147.
Sicilia T, Niemeyer HB, Honig DM, Metzler M (2003). Identification and stereochemical characterization of lignans in flaxseed and pumpkin seeds. J. Agr. Food. Chem. 51: 1181–8.
Siger A, Nogala-Kalucka M, Lampart-Szczapa E (2008). The content and antioxidant activity of phenolic compounds in cold-pressed plant oils. J. Food Lipids. 15: 137-149.
Singleton JA (1993). Enrichment of phospholipids from neutral lipids in peanut oil by high-performance liquid chromatography. J. Am. Oil. Chem. Soc. 70: 637.
Stancher B, Zonta F, Bogoni P (1987). Determination of olive oil carotenoids by HPLC. J. Micronutr. Anal. 3: 97.
Swigło AG, Sikorska E (2004). Simple reversed-phase liquid chromatography method for determination of tocopherols in edible plant oils. J. Chromatogr. A. 1048: 195–198.
Thompson LU, Robb P, Serraino M, Cheng F (1991). Mammalian lignans production from various foods. Nutr. Cancer, 16: 43-52.
Tuberoso IGC, Kowalczyk A, Sarritzu E, Cabras P (2007). Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. Food. Chem.103: 1494–1501.
Vetter J (2000). Plant cyanogenic glycosides. Toxicon 38: 11-36
Wanasundara PKJPD, Wanasundara UN, Shahidi F (1999). Changes in flax (Linum usitatissimum L.) Seed Lipids during Germination. J. Am. Oil. Chem. Soc. 76: 41-48.
Westcott ND, Muir NSF (1998). Analysis of flax lignans after enzymatic and chemical extraction. Proceedings of the 57th Flax Institute of United States; Fargo, N.Dak. p 27-30.
Wilson TC, Harris RK, Winters TR, Greaves JG, Haggerty WT (1993). Supercritical fluid extraction of polar materials from animal feeds and plant matrices. 20th ACS Natl. Meeting, Denver, Colo.: American Chemical Society, 33, 391-4.