The analysis of evening primrose oil

William W. Christie

Scottish Crop Research Institute, Invergowrie, Dundee (DD2 5DA), Scotland

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Abstract

Because of its use in pharmaceutical preparations, the oil of evening primrose (*Oenothera biennis* L.) must be subjected to rigorous analyses as part of any quality control programme. Gas chromatography can be applied for the determination of fatty acid content (especially that of γ-linolenic acid), but more complicated methodology may be necessary for structural analysis of triacylglycerols, for example. These and other methods for analysis of evening primrose oil are reviewed here.

*Key words: E*vening primrose oil; *Oenothera biennis* L.; Fatty acids, Triacylglycerols; γ-Linolenic acid; Gas chromatography; High-performance liquid chromatography.

1. Introduction

Evening primrose oil (*Oenothera biennis*) is being used in increasing amounts in in nutritional and pharmaceutical preparations, and there are claims that it may alleviate various chronic disease states (Fan and Chapkin, 1998; Horrobin, 1992; Huang and Mills, 1995). Discussion of these claims is outwith the scope of this review, and support for them is not implied. To satisfy regulatory bodies, commercial evening primrose oil samples must be subjected to more rigorous analysis than is applied to more conventional oils for food applications. The content of γ-linolenic acid (*cis*-6,*cis*-9,*cis*-12-octadecatrienoic acid) is the single most important parameter to be determined, and this is a rather straight-forward task for gas chromatography nowadays. However, many other factors are involved in the assessing the quality of the oil, and its biological potency. For example, such factors as levels of free acids, unsaponifiables, hydroperoxides and oxidative stability are important indicators of quality. The latter are not merely inherent features of the oil produced by a specific batch of seeds, but are dependent on how the oil is extracted and processed during refining. Biological potency is less easy to ascertain and cannot be discussed here, but there is evidence that the triacylglycerol structure, especially the proportions of specific molecular species may be important. In addition, many minor lipid components may be present in the oil, *e.g*. sterols and tocopherols, that may have an influence on quality. Methods are available to study all of these factors.

A great deal is known about the effects of industrial processing on vegetable oils in general, but little has been published concerning evening primrose oil specifically. It is generally acknowledged that the cold pressing process causes least modification to native oils, and solvent extraction is similarly mild. The latter process is used by one of the larger suppliers of evening primrose oil, Efamol Ltd, and they do not refine the oil further. On the other hand,

vegetable oils in general commerce are subjected to a number of refining processes, including bleaching, de-gumming, neutralisation and deodorization, in order to obtain a colourless, odourless material for various food applications. During these processes, many minor components are lost and there may be some alteration to major components. Modern physical-refining practise includes a deodorisation step in which the oil is heated in an atmosphere of steam at up to 240°C. Volatile materials responsible for off-flavours and free fatty acids are thereby removed. However, tocopherols, natural promoters of oxidative stability, are also lost, and some stereomutation (*i.e*. *cis* to *trans* conversion) of double bonds may occur. If the γ-linolenic acid of evening primrose oil were to be affected in this way, its biological potency would be reduced. It is evident that different commercial suppliers of evening primrose oil, use different processing technologies, and details of these are shrouded in business confidentiality. There is no evidence that there is any cause for concern, although undesirable effects have been demonstrated on a laboratory scale (Wolff and Sebedio, 1994). It is worth noting that the clearest and blandest sample of evening primrose oil may not be the best for pharmaceutical purposes.

Commercial oils may also contain additives, especially antioxidants, to improve the oxidative stability.

Because of the high degree of unsaturation of evening primrose oil and its constituents, care must be taken at all times during analysis to minimise the effects of autoxidation, by excluding air with a blanket of nitrogen and adding antioxidants whenever practicable (Christie, 1982, 1989).

2. Extraction of Seeds - Oil Content and Composition

As discussed above, seeds of evening primrose oil may be extracted and the oil refined in commerce by a variety of methods. However, if seeds *per se* are supplied to the analyst or if the oil content has to be determined, the oil must be recovered first by quantitative extraction with solvents. Usually, the recommended methods involve grinding to a fine meal in a mechanical grinder followed by soxhlet extraction with refluxing hexane for up to 3 hours (IUPAC Method 1.122). Court *et al*. (1993) have suggested the use of a Polytron™ homogeniser to speed up the process. These methods are appropriate for most practical purposes, but if there is a requirement for levels of free fatty acids and other enzymatic by-products to be minimised, extraction with isopropanol may be desirable (Christie, 1993b).

The oil content of evening primrose seeds varies with such factors as the age of the seed, cultivar and growth conditions, and typically varies between 18 and 25%, with occasional out-liers to 12 or 28% (Hulan *et al*., 1987).

The oil consists of about 98% triacylglycerols, with small amounts of other lipids (free acids, diacylglycerols, phospholipids) and about 1-2% unsaponifiable matter, of which sterols and tocopherols are of some importance. The sterol fraction, as determined by gas chromatography (GC), is comprised of 90% β-sitosterol, with the remainder being 4-methyl sterols (citrastadienol, 5%; obtusifoliol, 1%; gramisterol, 1.5%) (Hudson, 1984). Artaud (1992), however, reported 11.8% campesterol. γ-Tocopherol (187 μg/g of oil) and α-tocopherol (76 μg/g of oil) are the natural tocopherol components (Hudson, 1984), and δ-tocopherol (15 μg/g of oil) may also be present (Uzzan *et al*., 1992). Tocopherol acetate may be added to commercial oils as an antioxidant. Hudson (1984) used a GC method to determine tocopherols, but high-performance liquid chromatography procedures would now be preferred (Coors, 1993).

Phospholipids comprised only 0.05% of the oil, with the composition: phosphatidylcholine (31.9%),

phosphatidylinositol (27.1%), phosphatidylethanolamine (17.6%), phosphatidylglycerol (16.7%), phosphatidic acid (6.7%) (Lotti and Quartacci, 1990).

Detailed analysis of the essential oil derived from the evening primrose plant revealed the presence of 79 distinct constituents, including terpenes, aliphatic alcohols and aldehydes and aromatic compounds; the most abundant component was furfural (Miyazawa *et al*., 1978).

3. Fatty Acid Analysis

The main fatty acid components of evening primrose oil are palmitic (16:0), stearic (18:0), oleic (18:1(*n*-9)), linoleic (18:2(*n*-6)) and γ-linolenic (18:3(*n*-6)) acids. They are normally present in the relative proportions listed in Table 1, although there may be occasional samples that fall out with the ranges cited (Artaud, 1992; Hudson, 1984; Hulan *et al*., 1987; Prasad & Azeemoddin, 1997; Uzzan *et al*., 1992). As with oil content, the relative composition varies with such factors as the age of the seed, cultivar and growth conditions, and possibly with processing conditions. A number of minor components are present at low levels, including myristic acid (14:0), palmitoleic acid (16:1), vaccenic acid (18:1(*n*-7)), linolenic acid (18:3(*n*-3)), eicosanoic acid (20:0) and eicosenoic acid (20:1). Court *et al*. (1993) have performed an especially comprehensive analysis, and data for a single oil sample in which minor fatty acids were identified and quantified are listed in Table 2.

Table 1
The fatty acid composition (wt% of the total) of evening primrose oil (from Hudson, 1984).

Fatty acid	Normal range (wt%)
16:0	7-10
18:0	1.5-3.5
18:1(*n*-9)	6-11
18:2(*n*-6)	65-80
18:3(*n*-6)	8-14

Table 2
The detailed fatty acid composition (wt% of the total) of a sample of evening primrose oil (from Court *et al*., 1993).

Fatty acid	wt%	Fatty acid	wt%
12:0	0.04	18:2(*n*-6)	71.20
14:0	0.07	18:3(*n*-6)	8.72
15:0	0.03	18:3(*n*-3)	0.18
16:0	6.44	20:0	0.31
16:1(*n*-9)	0.04	20:1(*n*-9)	0.23
16:1(*n*-7)	0.07	20:2(*n*-6)	0.03
17:0	0.06	22:0	0.12
17:1(*n*-8)	0.04	24:0	0.04
18:0	1.81		
18:1(*n*-9)	9.76		
18:1(*n*-7)	0.84		

The minor components, diacylglycerols and free acids, contained similar amounts of γ-linolenic to the triacylglycerols, but the individual phospholipids contained somewhat less (0.4-4.3%) (Lotti and Quartacci, 1990).

Gas chromatography (GC)is by far the most appropriate method for analysis of fatty acids, generally in the form of the more volatile methyl ester derivatives. For quality control purposes, GC analysis on packed or wide-bore capillary columns with a stationary phase of moderate polarity (Carbowax type) gives satisfactory results. However, more detailed information, especially on the composition of positional isomers, is obtainable with narrow-bore columns coated with a phase of the Carbowax type (more polar phases can be used but confer no advantage). Separation of methyl esters of fatty acids from evening primrose oil on a capillary column is illustrated in Figure 1. The major components are clearly separated, but many of the minor ones would only be seen if the sensitivity were increased.

Fatty acid methyl esters can be identified by their retention times relative to authentic standards or by determining equivalent chain length values as reviewed by Christie (1989). In the analysis cited in Table 2, silver ion chromatography, which separates by degree of unsaturation, was used as a further aid to identification (Court *et al*. 1993).

Transmethylation in the presence of an acidic or basic catalyst must first be employed to convert the fatty acid constituents of lipids to methyl esters. Very many methods are available for the purpose, as reviewed by Christie (1993a). It can be argued that the formally approved procedures (*e.g*. AOCS Official Method Ce 1b-89, British Standard Method BS 684: section 2.34) are needlessly complex; lipids are first hydrolysed with alkali, then the free acids are transesterified with boron trifluoride/methanol reagent (a reagent that has many well-documented drawbacks (Christie, 1994)). Direct transesterification catalysed by sodium methoxide is a mild rapid procedure that can be recommended, especially when the free fatty acid content of the oil is low (Christie, 1989). Alternative, acid-catalysed procedures for direct transesterification are also available.

High-performance liquid chromatography in the reversed-phase mode has been employed to analyse the fatty acid components of evening primrose oil, but the method lacks the precision and convenience of capillary GC (Manku, 1983).

4. Molecular Species of Triacylglycerols

Each lipid class tends to exist in nature as a complex mixture of related components in which the composition of the aliphatic residues varies from one molecule to the next. In triacylglycerols, each of the three positions in the molecules may contain a different fatty acid, and for a complete structural

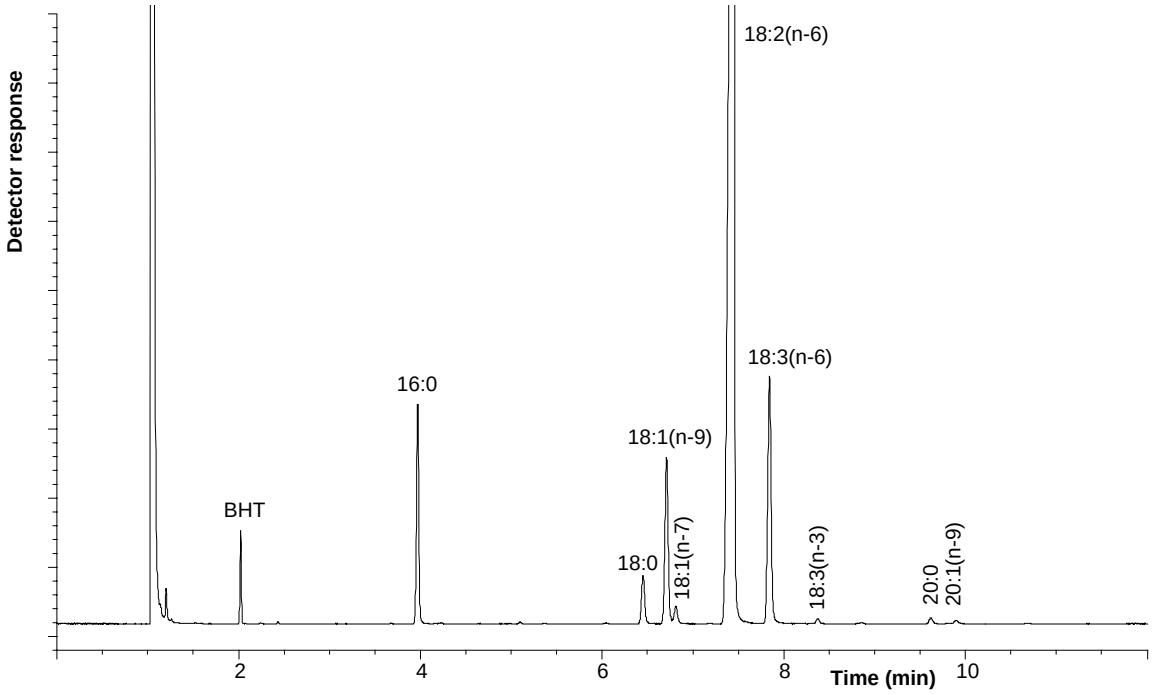

Fig. 1. GC separation of methyl esters of fatty acids from evening primrose oil. A Hewlett Packard Model 5890 Series II gas chromatograph (Hewlett-Packard Ltd., Stockport, Cheshire, U.K.) with a split/splitless injection system, was equipped with a capillary column (25 m x 0.22 mm i.d.) of fused silica coated with Carbowax 20M. The initial temperature of 170^{o}C was held for 3 min and then programmed up to 210^{o}C at 4^{o}C/min, with a final hold for 10 min. Hydrogen was the carrier gas.

analysis, it is necessary to determine the proportions of as many of the molecular species as is feasible technically. This is not merely an academic challenge, as there are claims (still to be fully substantiated) that part of the biological potency of evening primrose oil may be dependent on the nature of the molecular species.

Silver-ion and reversed-phase chromatography, often used in conjunction with thin-layer chromatography (TLC) but more often nowadays with HPLC, are the preferred procedures. The separations achieved depend on the combined physical properties of all the aliphatic residues in each molecule. Thus, with triacylglycerols, reversed-phase HPLC will separate species according to the combined chain-lengths of the fatty acids, with the retention times being reduced by the equivalent of approximately two carbon atoms for each double bond in the three fatty acid constituents. Silver ion chromatography separates only by degree of unsaturation, *i.e*. it will separate those molecules containing three saturated fatty acids from those with one monoenoic and two saturated fatty acids, and these are in turn separated from further fractions with an increasing degree of unsaturation. Often neither method will give the required degree of fractionation, but if the two separation modes are used in sequence, a high degree of molecular simplification may be possible. Procedures for the separation of molecular species of lipids in general have been comprehensively reviewed elsewhere (Christie, 1987a; Nikolova-Damyanova, 1992; Nikolova-Damyanova, 1997).

One of the more comprehensive single analyses of the triacylglycerols of evening primrose oil was a reversed-phase HPLC separation described by Redden *et al*. (1995a). An octadecylsilyl (ODS) stationary phase was employed with acetonitrile-propan-2-ol (65:35, v/v) as mobile phase as illustrated in Figure 2. More than 20 fractions were detected, with most of the species containing γ-linolenic acid eluting among the early components. Fractions were collected and transesterified (in the presence of an internal standard) for identification and quantification by GC. Brief details of similar separations have been published (Aitzetmüller and Grönheim, 1992; Zeitoun *et al*., 1991). Reversed-phase HPLC has also been linked to mass spectrometry for detection and identification purposes with evening primrose oil (Hori *et al*., 1991; Laakso, 1997; Mottram *et al*., 1997).

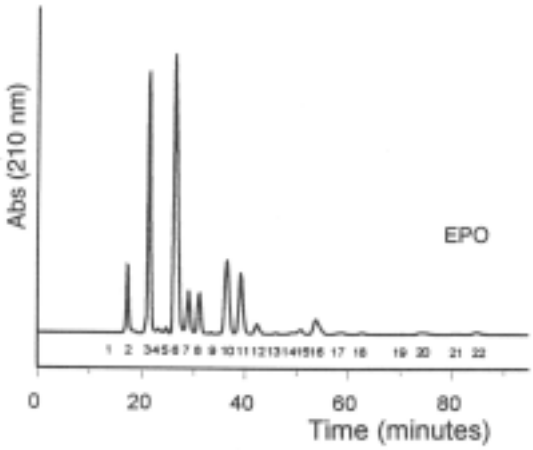

Fig. 2. Reversed-phase HPLC separation of triacylglycerols of evening primrose oil (from Redden *et al*., 1995a; reproduced by kind permission of the authors and of the *Journal of Chromatography*). A column (250 x 4.6 mm) of Supelcosil™ LC-18 (5 micron particles) was eluted with acetonitrile-propan-2-ol (65:35, v/v) at a flow rate of 1 mL/min with UV detection at 210 nm.

Silver ion TLC followed by reversed-phase HPLC of each fraction permitted determination of even more molecular species (Ratnayake *et al*., 1989). More recently, silver ion chromatography has been adapted to HPLC, which gives cleaner fractions than TLC. It does not give as many fractions as reversed-phase chromatography, but chromatograms are easier to understand intuitively, as separation is on the basis of a single molecular property. An application to evening primrose triacylglycerols is illustrated in Figure 3 (Christie, 1991), and the compositions of individual fractions are listed in Table 3. Trilinolein makes up half the total, and the biologically important dilinoleoyllinolenin is 14.5%.

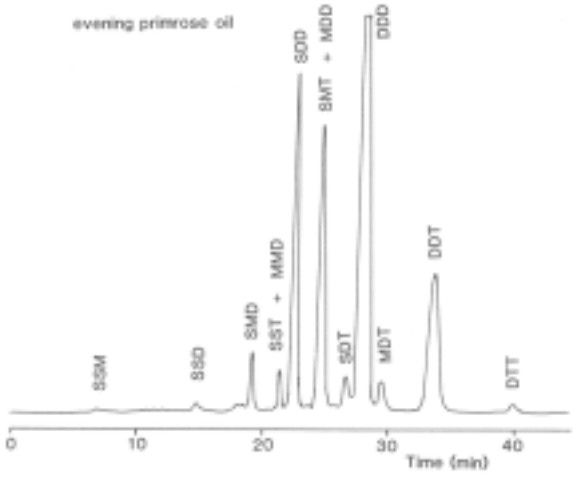

Fig. 3. Separation of triacylglycerols from evening primrose oil by HPLC with a silver ion column and evaporative light-scattering detection (from Christie, 1991; reproduced by kind permission of *Fat Science and Technology*). The HPLC column (250 x 4.6 mm) consisted of Nucleosil™ 5SA in the silver ion form (Christie, 1987b). A ternary gradient elution system was used with: A, 1,2-dichloroethane-dichloromethane (1:1, v/v); B, acetone; C, acetone-acetonitrile (4:1, v/v). A linear gradient of A to 50% A - 50% B was generated over 10 min, then this was changed to 70% B - 30% C over 20 more min, and finally to 100% C over a further 30 min, at a flow-rate of 1 mL/min.

Mass spectrometric detection has been employed with a similar HPLC fractionation (Laakso and Voutilainen, 1996). In addition, tandem mass spectrometry without prior chromatographic separation has been utilized to determine molecular species of evening primrose oil (Kallio *et al*., 1997).

Triacylglycerols with only five different fatty acids can exist in 5^3 molecular species, including positional isomers. Therefore, the above separations are still far from complete, especially if minor components are considered. However, they do give a good representation of the main components. One major task still to be attempted is to combine fractionation of molecular species with positional analyses (see next section).

5. Positional Distributions of Fatty Acids in the Triacylglycerols

Although glycerol itself has a plane of symmetry, a triacylglycerol is asymmetric when the two primary hydroxyl groups are esterified with different fatty acids. The various positions are designated by a "stereospecific numbering" (*sn*) system. The recognised pathway for triacylglycerol biosynthesis is *via sn*-glycerol-3-phosphate, which is acylated sequentially by specific acyl-transferases. As the precursor is of defined stereochemistry, and each of the enzymes catalysing the various steps in the process can have preferences for particular fatty acids or combinations of these in the partially acylated intermediates, it is not surprising that natural triacylglycerols exist in enantiomeric forms with each position of the *sn*-glycerol moiety having a distinctive

Table 3
Fatty acid compositions (mol%) of the triacylglycerols of evening primrose oil and of fractions of this obtained by silver ion HPLC (Christie, 1991).

Fatty acid	Total	Fraction										
		SSM	SMM	SSD	SMD	SST* + MMD	SMT+ SDD	SDT+ MDD	DDD	MDT	DDT	DTT
16:0	6.7	50.5	30.0	48.3	23.8	4.5	23.0	0.8	2.2			
18:0	1.6	14.4	6.7	17.2	8.5	2.3	6.9	0.2	0.6			
18:1	7.8	35.1	63.2		31.6	56.7		30.5	33.4			
18:2	74.9			34.5	36.1	33.7	70.1	67.5	94.2	33.0	66.8	34.5
18:3	8.9					2.8		1.0	3.0	33.6	33.2	65.5
Amount (mol%)		0.1	0.1	0.8	2.5	2.0	13.8	12.3	49.8	2.9	14.5	1.2

* Abbreviations: S, M, D and T refer to saturated, monoenoic, dienoic and trienoic (γ-linolenoyl) acyl residues, respectively.

fatty acid composition. Seed oil triacylglycerols in general tend to exhibit limited asymmetry, *i.e*. the difference between the compositions of positions *sn*-1 and 3 is not marked. However, the composition of position sn-2 is usually appreciably different from those of the primary positions.

Differences in the positional distributions of fatty acids on the glycerol moiety of natural triacylglycerols were first demonstrated systematically by means of hydrolysis with the enzyme pancreatic lipase, which cleaves the fatty acids from the primary positions and permits the determination of the composition of position *sn*-2. A suitable method has been described by Luddy *et al*. (1964). However, it is of limited value for evening primrose oil as the double bond in position 6 of γ-linolenic acid reduces the rate of hydrolysis of molecules containing this fatty acid in the primary positions. This may also influence the availability of dietary γ-linolenic acid *in vivo* (Huang *et al*., 1995). In spite of the known drawbacks to hydrolyses with pancreatic lipase, the error in actual analyses seems to be small (Muderhwa *et al*., 1987).

A useful alternative for regiospecific analysis is ^{13}C-nuclear magnetic resonance spectroscopy, and semi-quantitative data for the content of γ-linolenic acid in position *sn*-2 has been obtained by this means (Gunstone, 1990). The value of this procedure was confirmed by Redden *et al*. (1996), who compared it with a chemical hydrolysis procedure utilizing a Grignard reagent, and by Bergana and Lee (1996).

As no lipase capable of distinguishing between positions 1 and 3 of a triacyl-*sn*-glycerol is known, it has been necessary instead to develop ingenious stereospecific analysis procedures which make use of the specificity of other enzymes (reviewed in more detail elsewhere (Christie, 1995; Kuksis, 1996)). In most such methods, an equimolar mixture of 1,2- and 2,3-*sn*-diacylglycerols is prepared via partial hydrolysis by chemical or enzymatic means for conversion synthetically to phospholipid derivatives. These were hydrolysed by stereospecific lipases, which react only with the "natural" 1,2-diacyl-*sn*-glycerophosphatide. In one such method (Brockerhoff, 1965), the phospholipids were hydrol-ysed by the stereospecific phospholipase A of snake venom, which reacts only with the "natural" 1,2-diacyl-*sn*-glycerophosphatide. The products were a lysophosphatide which contained the fatty acids originally present in position *sn*-1, unesterified fatty acids released from position *sn*-2 and the unchanged "unnatural" 2,3-diacyl-*sn*-phosphatide. After isolation and trans-methylation of each product, their fatty acid compositions were determined by GC. In a second procedure, phospholipase C hydrolysis was utilised to generate 1,2-*sn*-diacylglycerols for analysis (Myher and Kuksis, 1979). Both of these methods have been used for stereospecific analysis of evening primrose oil, and some results are listed in Table 4 (Lawson and Hughes, 1988). In addition, new methods involving resolution of chiral diacylglycerol derivatives by HPLC have been developed (and are likely to used much more in future), and these have also been applied to the triacylglycerols of evening primrose oil (Laakso and Christie, 1990; Redden *et al*., 1995b). Again a representative result is listed in Table 4.

Table 4
Positional distribution of fatty acids in the triacyl-*sn*-glycerols of evening primrose oil.[a]

	Position[b]			Position[c]		
	sn-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3
16:0	9.6	1.8	6.8	10.7	2.6	8.2
18:0	4.4	1.5	0.3	2.8	0.3	2.6
18:1	8.0	7.6	7.1	9.3	7.0	11.1
18:2	73.3	78.9	74.2	70.0	79.4	67.2
18:3	3.6	10.7	13.5	7.2	10.7	10.9

[a] Results are expressed as mol% of the total fatty acids in each position.
[b] Lawson and Hughes (1988)
[c] Laakso and Christie (1990)

The results from the two types of analysis are in reasonable agreement. Position *sn*-2 tends to contain somewhat more of the unsaturated fatty acids, especially linoleate, than the primary positions, and correspondingly less of the saturated components. Position *sn*-1 tends to be more saturated than position *sn*-3. Positions *sn*-2 and *sn*-3 tend to have approximately equal amounts of γ-linol-enic acid, and more than is present in position *sn*-1.

As cautioned in the previous section, a complete analysis of an oil requires that individual molecular species be subjected to stereospecific analysis. Olive oil is as yet the only vegetable oil for which this has been attempted (Santinelli *et al*., 1992). Rather unexpectedly, marked asymmetry was observed in some fractions.

6. Quality Factors

There are many factors that influence the perceived quality of an oil, including peroxide value, acid value, oxidative stability and so forth. Methods are available to determine all of these, although published data are scarce on applications to evening primrose oil. Much of the information supplied below comes from commercial sources who prefer to remain anonymous.

6.1. Acid value

The free acid content of evening primrose oil can be determined by standard methods (BS 684 Section 2.10; AOCS Official Method Cd 3d-63). All involve titration of an oil in ethanol solution against a potassium hydroxide solution. The typical range is 2-6 mg KOH per Kg oil, although values between 0.5 and 12 can be seen on occasion.

6.2. Peroxide value

The approved procedures measure the amount of peroxide in terms of milli-equivalents of active oxygen per Kg oil, which oxidise potassium iodide under standard conditions; iodine liberated is titrated against sodium thiosulphate solution (BS 684 Section 2.14; AOCS Official Method Cd 8-53). Typical values for evening primrose oil are 1.5 to 6 $mEqO_2$, with an absolute range of <1 to 12, although very high values may be present in some encapsulated products.

6.3. Anisidine value

This parameter is a measure of secondary oxidation products and varies with the age and quality of the seed, and increases in oils exposed to air. The amount of aldehydes (2-alkenals and 2,4-dienals) is determined spectrophotometrically after reaction with an anisidine reagent (AOCS Official Method Cd 18-90). Typical values for evening primrose oil are 4 to 8, with an absolute range of 2 to 48.

6.4. Oxidative stability

Natural oils have some in-built resistance to oxidation that is dependent on the degree of unsaturation, the presence of antioxidants and prooxidants, and any prior abuse. Added antioxidants will increase this resistance. Oxidation can proceed only slowly until this initial resistance is overcome, and then it can accelerate and continue rapidly. The *Oil Stability Index* (OSI) is defined as the point of maximum change of the rate of oxidation. In practise, the value is usually measured using a commercial instrument such as the Rancimat™ (Brinkmann Instruments Inc., Westbury, NY, USA) according to AOCS Official

Method Cd 12b-92. An unrefined evening primrose oil tends to give an OSI of 4.5-5.5 hours. Refined oils (without added antioxidants) tend to have lower values, around 2.5 hours, as phospholipids and natural antioxidant levels are lower.

6.5. Unsaponifiable matter

This represents the total material extractable into solvents after hydrolysis with alkali (BS 684 Section 2.7; AOCS Official Method Ca 6a-40). It includes such natural lipid components as sterols, alcohols, pigments, and hydrocarbons, together potentially with foreign matter. Typical values for evening primrose oil are 1.3 to 2%, with an absolute range of 1 to 3%.

6.6. Phosphorus

Phosphorus is best determined by atomic absorption spectrometry (graphite furnace) (AOCS Official Method Ca 12b-92). In unrefined oils, the usual range is 20 to 40 ppm, although values from 5 to 80 have been noted. In refined oils, values are 0 - 5 ppm. It is noteworthy that the phosphorus is present mainly in phospholipids, which contribute to the oxidative stability of evening primrose oil. For example, phospholipids have been shown to have a synergistic activity with tocopherols (Dziedzic and Hudson, 1984; Hudson and Ghavami, 1984).

6.7. Trans Fatty acid content

There is no reliable method of measuring the *trans* content of oils at the low levels likely to be encountered in evening primrose oil, although improved procedures are under development that might be relevant (Ratnayake, 1998).

6.8. Carotene

β-Carotene is a pro-vitamin A and gives a characteristic colour to evening primrose oil. The approved method involves measuring the absorbance of a standard solution of the sample in cyclohexane (1%) at 445 nm (British Standard BS686: Section 220: 1977). Observed values generally fall in the range 1 to 3 ppm.

6.9. Heavy metals

Lead, arsenic, iron and copper may be determined routinely in quality control laboratories, but it is unheard of for samples of evening primrose oil to fail, *i.e*. to be above the limits of <5 ppm copper, <2 ppm lead and <1 ppm arsenic.

6.10. Processing factors

Moisture levels may be determined as a guide to the efficiency of solvent removal. Using a Karl Fischer analyser, the normal range for non-refined oils is 500-900 ppm, while in refined oils values of less than 500 ppm are typical. The level of extraction solvent is regulated by an EU directive, which limits hexane to below 5 ppm; usually, the value is below 2 ppm.

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