Composition of Lipids of Beech (*Fagus sylvatica* L.) Seed Oil

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Dedicated to Professor Hildegard Debuch on the occasion of her 70th birthday

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The lipid composition of beech *(Fagus sylvatica)* seeds is reported in the present study. Triacylglycerols (94.8%) were found to be the major component in the oil followed by sterols (0.9%) diacylglycerols (0.8%), phospholipids (0.7%), free fatty acids (0.5%) and monoacylglycerols (0.3%) in minor quantities. The composition of molecular species of triacylglycerols was analyzed by GC on the basis of chain length and C-57 was found to be the major species followed by C-55, C-59, C-53 and C-61. The sterol fraction constituted β-sitosterol and stigmasterol with 89.3 and 10.7% respectively. Phosphatidylinositol and phosphatidylethanolamine were the two phospholipids with 55 and 45% present in the phospholipid fraction of the beech seed oil. Oleic, linoleic and palmitic acids were found to be the major fatty acids in all the acyl lipid classes with small variations in quantities.

Introduction

Fagus sylvatica L., popularly known as Common or European Beech is a common forest tree in Central and South Europe including British Isles, extending to Crimea [1]. These trees grow up to 30 to 45 m and yielding nuts with a size of 12 to 18 mm [2, 3]. Beech nuts have been used as food for man and animals since ancient times. Eventhough the nuts are reported to be containing low molecular weight toxic components [4], the oil was reported to be a good source for edible purpose because of its good taste and stability. The physical and chemical characteristics of the beech seed oil [5, 6] and the fatty acid composition of its triacylglycerols [7] were reported earlier. However, the complete lipid composition of the seed oil has not been found in the literature. Hence the present study describes the total composition of the individual lipid classes namely triacylglycerols (TG), diacylglycerols (DG), monoacylglycerols (MG), free fatty acids (FFA), phospholipids (PL) and sterols (SL) including the fatty acid composition of acyl lipids. These analyses are in continuation with the study of the epicuticular waxes of beech leaves and seeds [8].

Materials and Methods

The beech seeds were harvested in 1986 and obtained from "Staatliches Forstamt Oerrel" in Münster-Oerrel. Moisture content was determined by heating the seeds in an oven at 110 °C for 3 h. The seeds (50 g) were homogenized with an Ultra-Turrax and extracted thrice with a mixture of chloroform: methanol (2:1, 150 ml) for 5 min. The extract was filtered, evaporated, dissolved in diethyl ether and dried over magnesium sulphate.

A part of the lipid extract (5.222 g) was separated into different fractions by silica gel column chromatography [9]. The elution was done with chloroform, acetone and methanol. The chloroform fraction contained TG, acetone eluted DG, MG, FFA and SL and methanol eluted PL.

The acyl lipids and sterols were qualitatively identified by thin-layer chromatography (TLC) after developing with a solvent system (Solvent I) hexane: diethyl ether: acetic acid (60:40:1) along with authentic samples (Sigma, Deisenhofen). The spots were visualized with iodine vapours. The presence of SL was further confirmed by carbazole spray [10] with Solvent II, dichloromethane: ethyl acetate (24:1). The acetone eluate was separated into individual lipid classes by preparative TLC using Solvent I and estimated by GC quantitatively [11] using methyl heptadecanoate (17:0) as an internal standard. The phospholipid fraction (methanol eluate) along with authentic samples (pure phospholipids were gifted by Prof. H. Debuch, Institut für Physiologische Chemie, Universität zu Köln, Köln) was developed with Solvent III [12], chloroform: acetone: methanol: acetic acid: water (5:2:1:1:0.5) and Solvent IV chloroform: methanol: acetic acid

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(65:28:8) separately. The spots were visualized with different spray reagents such as a) molybdenum blue [13] specific for all phospholipids, b) ninhydrin reagent [14] for ethanolamine or serine containing phospholipids, c) Dragendorff reagent [15] for choline containing phospholipids and sodium metaperiodate-benzidine [16] for inositol containing lipids. The individual phospholipids were separated from the methanol fraction by preparative TLC using Solvent III and quantified by phosphorus assay according to Bartlett [17].

Methyl esters of fatty acids from TG, DG, MG, FFA, PI and PE were prepared by refluxing with 2 N HCl/methanol for 3 h.

GC was carried out with a Hewlett-Packard 5710 unit fitted with a FID and an integrator 3380 S. Acylglycerols and sterols were analyzed on a capillary column OV 1 (10 m) and fatty acid methyl esters on FFAP (10 m) column. The column temperature was programmed between 150–340 °C as required. GC-MS detection of sterols was done by Finnigan MAT 4510, 70 eV, EI, with fused silica capillary column 15 m DB-1.

Table I. Composition and yield of lipids of *Fagus sylvatica* seed oil.

Lipid class	[mg]	[% of oil]
Triacylglycerols (TG)	4950	94.8
Diacylglycerols (DG)	42	0.8
Monoacylglycerols (MG)	16	0.3
Free fatty acids (FFA)	26	0.5
Phospholipids (PL)	37	0.7
Sterols (SL)	47	0.9
Unidentified and lost on column	104	2.0
	5222	100.0

Results and Discussion

The total lipid extract obtained was 40.7% of the beech seeds based on dry basis. Because of its high content of oil, these seeds are a good source for edible or industrial uses.

The major component in the oil is TG with 94.8% followed by SL (0.9%), DG (0.8%), PL (0.7%), FFA (0.5%) and MG (0.3%) (Table I).

The TG (94.8% of oil) was fractionated in a pure form with chloroform. The fatty acid composition of TG (Table II) is in good agreement with earlier studies reported by Neubeller and Buchloh in 1971 [7]. The predominant fatty acids present in TG were linoleic (18:2) and oleic (18:1) followed by palmitic (16:0), gadoleic (20:1), linolenic (18:3) and stearic (18:0) acids in small quantities. The molecular species of TG (Table III) were studied by GC on the basis of chain length. The retention times of the molecular species of different TG were determined using authentic samples. The major molecular species of the beech oil TG was C-57 (55.8%) with three C-18 fatty acids. The next major molecular species are C-55 (26.3%) with di- C-18 + mono- C-16 and C-59 (13.7%) with di- C-18 + mono- C-20. The C-53 and C-61 species were found in minor quantities. Similar molecular species composition was found even after the hydrogenation of TG with palladium as catalyst.

Table II. Fatty acid composition (peak area %) of the acyl lipids of *Fagus sylvatica* seed oil.

Carbon No.	TG	DG	MG	FFA	PE	PI
14:0	+ (+)	+	+	+	+	+
16:0	8.7 (5.2)	18.4	21.9	10.8	24.5	25.5
16:1	+ (0.6)	+	+	+	0.5	0.6
18:0	1.8 (3.4)	3.2	6.5	2.8	7.1	+
18:1	37.5 (38.8)	34.2	26.2	32.2	33.5	44.3
18:2	42.3 (41.1)	40.2	39.6	48.6	29.2	24.4
18:3	4.2 (4.6)	0.8	2.3	2.8	3.5	2.2
20:0	+ (−)	+	+	+	+	+
20:1	5.5 (6.3)	3.2	3.5	2.8	1.7	3.0
22:0	+ (−)	+	+	+	+	+
22:1	+ (−)	+	+	+	+	+

Results in parenthesis were reported by Neubeller and Buchloh [7].
+ = Traces < 0.1%.

Table III. Composition of triacylglycerol molecular species of *Fagus sylvatica* seed oil (peak area %).

Carbon No.	Molecular species			% of TG
51		16, 16, 16		+
53	18, 16, 16	+	16, 18, 16	2.4
55	16, 18, 18	+	18, 16, 18	26.3
57		18, 18, 18		55.8
59	20, 18, 18	+	18, 20, 18	13.7
61	18, 20, 20	+	20, 18, 20	1.8
63		20, 20, 20		+

+ = Traces < 0.1.

The acetone eluate (2.5% of the total oil) was found to contain MG, DG, FFA and SL. Normally these lipid classes are eluted with chloroform [9] but in the present investigation all these classes could able to elute selectively with acetone immediately after TG with chloroform. All the lipid classes in acetone eluate were identified by TLC along with authentic samples using Solvent I. The presence of SL (R_f 0.25) was further confirmed with a positive (pinkish-violet) carbazole reaction after developing with Solvent II. MG, DG and FFA were separated into individual classes by preparative TLC from acetone eluate using Solvent I and estimated quantitatively using methyl heptadecanoate as an internal standard and found to be 0.3, 0.8, and 0.5% respectively of the oil (Table I). MG, DG and SL were resolved by GC with OV 1 capillary column and confirmed with the retention times of the authentic samples. Based on the peak area per cent, the SL content was computed as 0.9% of the beech seed oil.

The sterol fraction constituted β-sitosterol and stigmasterol with 89.3 and 10.7% respectively. The presence of sterols was further confirmed by GC-MS data [18].

The fatty acid composition of the MG, DG, and FFA were also analyzed by GC (Table II). Linoleic was found to be the major fatty acid followed by oleic as in TG. But the content of palmitic was considerably increased in MG and DG than in the TG.

The methanol fraction (0.7% of the oil) contained phospholipids. Qualitative analysis based on response to specific spray reagents and comparison of R_f values with authentic samples indicated the presence of phosphatidylethanolamine (PE) (R_f3, 0.45; R_f4, 0.45) and phosphatidylinositol (PI) (R_f3, 0.15; R_f4, 0.25). Phosphatidylcholine (PC) was found only in traces with undetectable amounts. The quantification of phospholipids was carried out on a colorimetric estimation of phosphorus and PI and PE were found to be present in 55.1 and 44.9% respectively. In general, oil seeds contain PC, PE and PI as the major phospholipids [19–23]. Only traces of PC in beech seed oil is an unusual observation. The individual phospholipids PI and PE were isolated in pure form by preparative TLC using Solvent II. The predominant fatty acid in PI and PE was oleic and the content of palmitic was found to be significantly higher than in TG.

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[1] B. Hora, in: The Oxford Encyclopedia of Trees of the World, p. 128, Oxford University Press, Oxford 1981.
[2] T. G. Tutin, Fagus, in: Flora Europaea, **Vol. 1** (T. G. Tutin *et al.*, eds.), p. 61, Cambridge University Press, Cambridge 1984.
[3] G. Krüssmann, Fagus, in: Handbuch der Laubgehölze, **Vol. 2,** p. 68, Verlag Paul Parey 1977.
[4] Ib. Kristensen, P. O. Larsen, and H. Sorensen, Phytochemistry **13,** 2803 (1974).
[5] G. Rankoff, Fette und Seifen **48,** 294 (1941).
[6] I. Cornea and A. Rudenco, Bull. etudes et recherches tech. **1,** 169 (1969).
[7] J. Neubeller and G. Buchloh, Mitt. Klosterneuburg, Rebe und Wein, Obstbau und Früchteverwertung **21,** 469 (1971).
[8] P.-G. Gülz, E. Müller, and R. B. N. Prasad, Z. Naturforsch. **44c,** 731 (1989).
[9] G. Rouser, G. Kritchevsky, and A. Yamamoto, in:

Lipid Chromatographic Analysis, **Vol. 1** (G. Marinetti, ed.), p. 99, Marcel Dekker, New York 1967.
[10] P. Gosh and S. Thakur, J. Chromatography **240,** 515 (1982).
[11] M. Kates, in: Techniques of Lipidology, 2nd Edition, p. 248, Elsevier, Amsterdam 1986.
[12] M. Lepage, Lipids **2,** 244 (1967).
[13] V. E. Vaskovsky and E. Y. Kostetsky, J. Lipid Res. **9,** 396 (1968).
[14] V. P. Skipski, R. F. Peterson, and H. Barclay, J. Lipid Res. **3,** 467 (1962).
[15] H. K. Mangold, J. Am. Oil Chem. Soc. **38,** 708 (1961).
[16] J. A. Cifonelli and F. Smith, Anal. Chem. **26,** 1132 (1954).
[17] G. R. Bartlett, J. Biol. Chem. **234,** 466 (1959).
[18] P.-G. Gülz, R. W. Scora, E. Müller, and F.-J. Marner, J. Agr. Food Chem. **35,** 716 (1987).
[19] P.-G. Gülz and C. Eich, Z. Naturforsch. **37c,** 1286 (1982).
[20] P. Krollmann and P.-G. Gülz, Z. Pflanzenphysiol. **110,** 469 (1983).
[21] Y. Nagender Rao, R. B. N. Prasad, and S. V. Rao, Fette, Seifen, Anstrichmittel **86,** 107 (1984).
[22] R. B. N. Prasad, Y. Nagender Rao, and S. V. Rao, J. Am. Oil Chem. Soc. **64,** 1424 (1987).
[23] T. Galliard, in: Form and Function of Phospholipids (G. B. Ansell *et al.*, eds.), p. 253, Elsevier, Amsterdam 1973.