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Journal of Chromatography A, 665 (1994) 191–195

JOURNAL OF CHROMATOGRAPHY A

Lipid analysis of baker's yeast

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Abstract

Commercial baker's yeast was analysed for lipids by TLC and GC. The TLC system of a silica gel plate with *n*-hexane–diethyl ether–acetate acid (70:30:1, v/v/v) allowed the total lipids to be divided into sterol esters (ES), free fatty acids (FFA), triacylglycerols (TAG), diacylglycerols (DAG), free sterols (FS), monoacylglycerols (MAG) and phospholipids (PL). Phospholipids were separated by two-step chromatography with the systems SiO_2 with acetone and SiO_2 with chloroform–methanol–acetic acid–water (25:15:4:2, v/v). The following phospholipids were detected: phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylcholine (PC) and lysophospholipids (LPL). The fatty acid composition of TAG, ES, FFA, DAG, MAG, PC, PE, PS and PI was investigated by using packed column (15% DEGJ + 3% H_3PO_4) and capillary column (SP 2340) GC, Twenty-two individual fatty acids with carbon chain lengths in the range 12–24 were detected by capillary GC. The presence of squalene, zymosterol, ergosterol and lanosterol in non-saponifiable lipid was proved and the compounds were determined by GC (SE-30).

1. Introduction

There are many reports dealing with analyses of microbial lipids [1,2]. The main interest is focused on lipid classes [3], fatty acids [4] or sterol profile analysis [5]. It is clear that analytical data are necessary for further studies of the physiology [6], taxonomy [7] and biotechnology [8] of yeasts.

A variety of solvent systems have been used to separate simple lipids by TLC on silica gel in one dimension. Those used most frequently contain hexane (or heptane or light petroleum) diethyl ether and acetic (or formic) acid in various proportions. For example, Korte and Casey [9] divided neutral lipids into monoacylglycerols, diacylglycerols, free fatty acids, triacylglycerols and cholesteryl esters using TLC with *n*-heptane–diethyl ether–acetic acid (75:25:4, v/v/v). Bergheim *et al.* [10] recommended the preparative separation of neutral lipids and phospholipids by centrifugally accelerated TLC. This method has not been applied to the preparation of phospholipids, although it is commonly used for other natural products. Non-acidic microbial phospholipids are usefully separated on silica gel plates developed with chloroform–methanol–water (25:10.1, v/v/v), although the solvent system may also contain small amounts of other polar compounds. A mixture of phospholipids was excellently separated using SiO_2 with chloroform–ethanol–water–triethylamine (30:34:8:35, v/v) [9].

Lipids in microorganisms contain a variety of fatty acids differing in chain length, degree of unsaturation, position and configuration of dou-

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0021-9673/94/$07.00
SSDI 0021-9673(93)E1217-N

ble bounds and the presence of special functional groups. Augustyn and Kock [11] used capillary columns with different stationary phases spanning a range of polarity for the GC analysis of fatty acids from *Saccharomyces cerevisiae*. Fatty acids were identified by interpretation of picolinyl ester mass spectra without suitable reference standards. Ten fatty acids in the range C_{14}–C_{18} including saturated and monounsaturated acids with precise double-bond localization were found. The fatty acid composition of twenty *Saccharomyces cerevisiae* strains (laboratory and industrial) was investigated by Bendová *et al.* [12]. Methyl esters of fatty acids with carbon chain lengths of 10–28 were separated on SPB-1 by capillary GC.

It is known that the non-saponifiable part of the lipid of *Saccharomyces cerevisiae* contains ergosterol and its biochemical precursors. GC–MS has been used for the identification of $\Delta^{5,7}$-sterols in sterol mixtures [13]. Other methods of sterol analysis by GC were summarized by Itoh *et al.* [14] and Patterson [15].

The aim of this present work was to analyse neutral lipids (by TLC), to identify and determine fatty acids in selected lipid structures (by GC) and to analyse the non-saponifiable lipid isolated from commercial baker's yeast (*Saccharomyces cerevisiae*).

2. Experimental

Commercial baker's yeast (Slovlik Trenčín, Slovak Republic) was extracted by the method of Bligh and Dyer [16].

2.1. TLC of lipids

Precoated silica gel plates (Merck, Darmstadt, Germany) (20 × 20 cm, 0.5-mm layer) were used. A 5-mg amount of the sample [5% lipid solution in chloroform–methanol (1:1)] was applied with a Hamilton syringe as a line of spots 1.5 cm from the lower edge of the silica gel layer. For TLC of neutral lipids a solvent system consisting of *n*-hexane–diethyl ether–acetic acid (70:30:1, v/v/v) was used. Phospholipids were separated by two-step TLC. The plates were first developed in a chamber at ambient temperature with acetone to a height of 18 cm above the origin. After drying, the plates were developed to 17 cm above the origin with chloroform–methanol–acetic acid–water 25:15:4:2 (v/v). The spots were revealed by exposure to iodine vapour.

2.2. GC of fatty acid methyl esters

Lipids containing fatty acids were scraped off from five dry plates (iodine vapour and water traces were removed under reduced pressure), extracted with chloroform–methanol (1:1) and subjected base-catalysed transesterification (sodium methanolate) and acid-catalysed esterification and transesterification (methanolic HCl). Fatty acid methyl esters (FAMEs) were determined by packed or capillary column GC under the conditions outlined below.

Packed column GC

A Chrom 5 chromatograph (Laboratorní přístroje, Prague, Czech Republic) equipped with a flame ionization detector was used; column, 15% DEGJ + 3% H_3PO_4 on Chromaton N AW DMCS (0.125–0.150 mm) (1.8 m × 2 mm I.D) (Lachema, Brno, Czech Republic); column temperature, 180°C; injection port and detection space temperatures, 220°C; carrier gas, nitrogen at 30 ml/min; sample, 1 μl of hexane solution (10 mg FAMEs per ml hexane); integrator, Apex 2.5 (Apex Data, Prague, Czech Republic). The identification of the GC FAME peaks was performed using FAME standards (Supelco, Bellefonte, PA, USA).

Capillary column GC

A Carlo Erba Model 2400 T gas chromatograph equipped with a flame ionization detector was used; column, 78 m × 0.3 mm I.D. glass capillary coated with cyanopropylsiloxane SP 2340 (Supelco); the initial column temperature was 150°C, which was maintained for 3 min, then programmed to 220°C at 3°C/min, the maximum temperature being maintained for a further 9 min before cooling; injection and detection tem-

peratures, 235°C; carrier gas, hydrogen at a linear velocity of 30 cm/s; splitting ratio, 50:1; sample size, 1 μl (solutions in hexane). Peaks were identified by means of known standards (Supelco) and determined with an SP 4000 integrator (Spectra-Physics).

2.3. Saponification of microbial lipids

A 100-mg amount of the lipid from whole cells was hydrolysed with 2 ml of 1 *M* KOH (in 95% ethanol) for 1 h. After cooling, 5 ml water were added and the solution was extracted completely with *n*-hexane (3 × 5 ml). The extract was dried with anhydrous sodium sulphate and the non-saponifiable materials were recovered on removal of the solvent in a rotary evaporator.

2.4. GC of non-saponifiable components

The sample of non-saponifiable lipid was dissolved in 2-propanol (concentration *ca.* 15 mg/ml) and analysed by GC under the following conditions: Chrom 5 chromatograph (Laboratorní přístroje) equipped with a flame ionization detector; column, 10% SE-30 on Chromaton N AW DMCS (0.125–0.150 mm) (1.8 m × 2 mm I.D) (Lachema); column temperature, 260°C; injection port and detection temperatures, 320°C; carrier gas, nitrogen at 30 ml/min; sample, 1 μl of 2-propanol solution: integrator, Apex 2.5 (Apex Data). The identification of the sterol peaks was performed using standards (Sigma, St. Louis, MO, USA).

3. Results and discussion

The total lipid content of *Saccharomyces cerevisiae* has been discussed in many reports [6,17,18]. The wide variation in this content (in the range 3.5–14.7% of lipids, dry mass) depends on various influences including especially growth stage, nutritional aspects and cultivation conditions.

A relatively low content of intracellular lipid in the whole cells of baker's yeast was found (5.3%). The lipid extract from whole cells was separated by TLC using SiO_2 with *n*-hexane–diethyl ether–acetic acid (70:30:1, v/v/v.). Separation in this solvent is carried out according to the polarity of the lipids with the least polar compound migrating furthest. Thus, sterol esters (ES) were near the solvent front (R_F 0.96) followed by triacylglycerol (TAG) (R_F 0.76), free fatty acids (FFA) (R_F 0.65–0.5), free sterols (FS) (R_F 0.25), monoacylglycerols (MAG) (R_F 0.08) and phospholipids (PL) (R_F 0).

Two-step chromatography for polar lipids isolated from whole cells made it possible to separate commonly occurring phospholipid structures such as phosphatidylethanolamine (PE) (R_F 0.51), phosphatidylserine (PS) (R_F 0.43), phosphatidylinositol (PI) (R_F 0.36), phosphatidylcholine (PC) (R_F 0.24) and lysophospholipids (LPL) (R_F 0). Less polar lipids were crowded together at the acetone solvent front.

Myristic (14:0), myristoleic (14:1), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic acid (18:1) and linoleic acid (18:2) were detected in the samples analysed by packed column GC.

Table 1
Fatty acid composition of sterol esters (ES), triacylglycerols (TAG), free fatty acids (FFA), diacylglycerols (DAG) and monoacylglycerols (MAG) isolated from baker's yeast

Lipid structure	Fatty acid (%)						
	14:0	14:1	16:0	16:1	18:0	18:1	18:2
ES	0.4	0.6	10.9	34.2	0.8	50.8	2.1
TAG	0.1	0.3	11.4	39.1	2.2	45.0	1.7
FFA	2.1	1.2	21.4	33.6	7.2	33.2	0.6
DAG	0.2	0.2	8.4	31.5	6.8	48.8	3.8
MAG	Trace	0.2	9.9	35.6	7.7	43.1	3.1

Table 2
Fatty acid composition of neutral lipids (NL), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylcholine (PC) isolated from baker's yeast

Lipid structure	Fatty acid (%)						
	14:0	14:1	16:0	16:1	18:0	18:1	18:2
NL	1.1	0.8	13.4	36.1	5.2	41.2	1.8
PE	0.8	0.4	8.9	42.8	1.4	42.1	1.4
PS + PI	0.3	0.7	18.7	35.6	6.8	34.8	1.7
PC	Trace	0.2	16.5	39.1	6.6	32.2	3.9

Although all lipid structures contained identical compounds, the relative amounts of the individual acids varied (Tables 1 and 2). It is interesting that acylglycerols were relatively rich in monoenes (palmitoleic and oleic acid) whereas the fraction of free fatty acids contained mainly saturated fatty acids (palmitic and stearic). Nurminen *et al.* [19] reported a high content of short-chain fatty acids in TAG of *S. cerevisiae* and a similar profile of fatty acids in FFA and TAG. On the other hand, Ng and Laneelle [20] discussed the differences between the content of monoenes in neutral lipids and FFA. Our results showed that the phospholipids contained a higher concentration of 16:1 and a low concentration of 16:0 in comparison with neutral lipids. This observation is in agreement with a previously published report [5].

Table 3 shows the fatty acid composition of yeast lipid analysed by capillary GC. Particularly the analysis of the fatty acid composition on the polar SP 2340 stationary phase allowed us to detect 22 individual fatty acids including their positional and geometrical isomers. It is interesting that in addition to the usual types of acids, the odd-carbon-numbered fatty acids and *trans* isomers of monoenes were found. The presence

Table 3
Fatty acid composition of baker's yeast analysed by capillary GC on an SP 2340 column

Peak No.	Fatty acid	Concentration (%)
1	12:0	0.6
2	14:0	0.1
3	14:1 ω5 *cis*	0.8
4	15:0 *anteiso*	Trace
5	15:0	0.1
6	16:0	10.5
7	16:1 ω7 *trans*	0.1
8	16:1 ω7 *cis*	32.9
9	17:0	0.5
10	17:1 ω8 *cis*	0.2
11	18:0	7.1
12	18:1 ω9 trans	0.1
13	18:1 ω9 *cis*	43.9
14	18:1 ω7 *cis*	Trace
15	19:0	0.1
16	18:2 ω6 *cis, cis*	1.1
17	20:0	Trace
18	18:3 ω3 all-*cis*	0.8
19	20:1 ω9 *cis*	Trace
20	21:0	Trace
21	22:0	Trace
22	24:0	0.1

Table 4
Analysis of non-saponifiable lipids of baker's yeast

Peak No.	Retention time (min)	Content (%)	Structure
2	3.9	0.1	Unidentified
3	4.4	1.0	Unidentified
4	6.3	15.1	Squalene
5	8.1	0.2	Unidentified
6	10.5	0.3	Unidentified
7	13.8	0.2	Zymosterol
8	17.3	78.9	Egosterol
9	18.6	0.2	Unidentified
10	21.9	3.7	Lanosterol

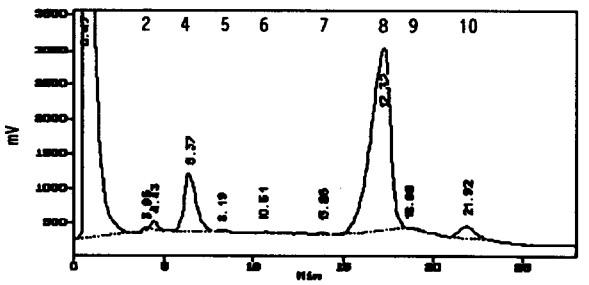

Fig. 1. GC of non-saponifiable lipid from baker's yeasts on an SE-30 packed column. See Table 4 for identification of peaks.

of vaccenic acid in baker's yeast confirmed an earlier observation [21], although its concentration in our sample was extremely low.

Free sterols and steryl esters especially are highly variable components of the yeast cell. Squalene, zymosterol, ergosterol and lanosterol were identified and quantified among nine divided structures from the non-saponifiable lipids of baker's yeast (Table 4, Fig. 1). Considering the earlier reports [6,22], zymosterol is frequently a major component of the steryl ester, but is generally absent in the free sterol component. On the other hand, ergosterol predominates as the free sterol.

4. References

[1] K. Hunter and A.H. Rose, in A.H. Rose and J.S. Harrison (Editors), *The Yeasts*, Academic Press, London, 1971, pp. 211–270.

[2] J.B.M. Rattray, A. Schibeci and D.K. Kidby, *Bacteriol. Rev.*, 39 (1975) 197.

[3] F. Paltauf and J.M. Johnston, *Biochim. Biophys. Acta*, 218 (1970) 424.

[4] L. Kováč, I. Gbelská, M. Poliachová, J. Šubík and V. Kováč, *Eur. J. Biochem.*, 11 (1980) 491.

[5] J.A. Hossack, D.M. Belk and A.H. Rose, *Arch. Microbiol.*, 114 (1977) 137.

[6] J.B.M. Rattray, in C. Ratledge and S.G. Wilkinson (Editors), *Microbial Lipids*, Vol. 1, Academic Press, London, 1988, pp. 555–697.

[7] O.P. Augustyn and J.L.F. Kock, *J. Microbiol. Methods*, 10 (1989) 9.

[8] J. Šajbidor, F. Malík, and H. Kissantalová, *Food Biotechnol.*, 6 (1992) 187.

[9] K. Korte and L.M. Casey, *J. Chromatogr.*, 232 (1982) 47.

[10] S. Bergheim, K.E. Malterud and T. Anthonsen, *J. Lipid Res.*, 32 (1991) 877.

[11] O.P.H. Augustyn, and J.F. Kock, *J. Microbiol. Methods*, 10 (1989) 9.

[12] O. Bendová, V. Richter, B. Janderová and J. Häusler, *Appl. Microbiol. Biotechnol.*, 35 (1991) 810.

[13] S. Popov, I. Stoilov and P. Demirev, *Biomed. Mass. Spectrom.* 11 (1984) 608.

[14] T. Itoh, H. Tani, K. Fukushima, T. Tamura and T. Matsumoto, *J. Chromatogr.*, 234 (1982) 65.

[15] G.W. Patterson, *Anal. Chem.*, 43 (1971) 1165.

[16] E.G. Bligh and W.J. Dyer, *Can. J. Biochem. Physiol.*, 37 (1959) 911.

[17] J. Ahvenainen, *J. Inst. Brew.*, 88 (1982) 367.

[18] A.M. Ramsay and L.J. Douglas, *J. Gen. Microbiol.*, 110 (1979) 185.

[19] T. Nurminen, K. Konttinen and H. Suomalainen, *Chem. Phys. Lipids*, 14 (1975) 15.

[20] K.H. Ng and M.A. Laneelle, *Biochimie*, 59 (1977) 97.

[21] H. Suomalainen and A.J.A. Keränen, *Chem. Phys. Lipids*, 2 (1968) 296.

[22] D.E. Quai and J.M. Haslam, *J. Gen. Microbiol.*, 11 (1979) 343.