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ORIGINAL PAPER

Brewer's Yeast as a New Source of Palmitoleic Acid—Analysis of Triacylglycerols by LC–MS

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Abstract We identified both fatty acids and TAG in waste yeast from seven breweries employing both top and bottom fermentation. Brewer′s yeast was found to contain FA exhibiting a broad range of acyl chain lengths, with a high proportion of the nutritionally and biotechnologically important palmitoleic acid. Analysis of FA by GC–MS and of TAG by LC–MS with APCI provided information on the type of brewer′s yeast and technology of fermentation. The composition of TAG containing major FA such as palmitic, palmitoleic and oleic acid was found to depend not only on the type of yeast strain, i.e. *Saccharomyces pastorianus* or *S. cerevisiae*, but also on brewing fermentation process, i.e. top versus bottom fermentation. Top and bottom yeast can readily be distinguished by the ratios of some regioisomers of TAG since, e.g., the ratio of regioisomers PoOPo and PoPoO is always >1 in top fermenting yeast and <1 in bottom yeast. The ratios can thus be used as markers for determining the yeast type and beer-producing technology in a given brewery.

Keywords *Saccharomyces cerevisiae* · *Saccharomyces pastorianus* · RP-HPLC/MS-APCI · Brewer′s yeast · Triacylglycerols · Brewing · Palmitoleic acid

Abbreviations

ACN	Acyl carbon number
APCI	Atmospheric pressure chemical ionization
DAG	Diacyl glycerols
DB	Number of double bond(s)
ECN	Equivalent carbon number
ESI	Electrospray ionization
HPLC/MS-APCI	High performance liquid chromatography-mass spectrometry with APCI
MAG	Monoacyl glycerols
MS/MS	Tandem mass spectrometry
RP-HPLC	Reverse phase high performance liquid chromatography

Introduction

Yeast serves as a very convenient model organism in studies of molecular organization and regulation of the network of metabolic pathways that involve hundreds to thousands of individual lipid species [1]. The variability of lipids is primarily given by three enzyme types, i.e. fatty acid synthases, desaturases and elongases. These enzymes produce both saturated and unsaturated fatty acids (FA) with acyl chain lengths of 8–26 carbon atoms [2]. Previous studies aimed mostly at analyzing the overall FA composition and the composition of FA in individual lipid classes [3] and showed variability in both qualitative and quantitative FA composition. This can be caused, e.g., by the

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flexibility of yeast metabolism, and an intrinsic ability to respond to changes of cultivation conditions by adjusting lipid metabolism.

According to the literature the content of FA in yeasts, especially in the genus *Saccharomyces*, is variable. However, whatever the variability, four FA, i.e. palmitic (16:0), palmitoleic (9–16:1), stearic (18:0) and oleic (9–18:1) are major FA, the content of the palmitoleic acid reaching up to 50 % total FA. The spectrum of FA also includes minor constituents such as medium-length FA, e.g. caprylic (8:0), capric (10:0), lauric (12:0) and myristic (14:0) acid, unsaturated acids such as linoleic (9,12–18:2) and α-linolenic acid (9,12,15–18:3) and/or very-long chain FA i.e. behenic (22:0), lignoceric (24:0), cerotic (26:0) acid and many others, as well as odd-numbered chain FA—pentadecanoic (15:0) or pentadecenoic (15:1) acids [3–7].

Cell lipids of brewer′s yeast *Saccharomyces cerevisiae* and *S. pastorianus* occur largely in specialized structures—lipid droplets, dynamic organelles that play important roles in the production of intracellular neutral lipids—triacylglycerols (TAG) [8]. Production of TAG begins at the beginning of the exponential phase of growth and ends when the cells enter the stationary phase of growth [9]. At this moment (approximately the end of primary fermentation) yeast cells are separated from beer. In the course of further fermentation TAG in yeast cells are degraded by lipases to diacylglycerols (DAG) and free fatty acids, which can serve as sources of energy when fatty acids are present as the only carbon sources in the medium, i.e. during starvation [10].

Characterization and quantification of TAG that have no electrostatic charge in solution can nevertheless be carried out with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) but requires the addition of an electrolyte such as ammonium or metal ions (e.g. Li^+ or Na^+, etc.), to produce adduct ions, $[M+NH_4$, or Li or Na, etc.$]^+$. APCI, on the other hand, produces substantial diacylglycerol-like fragment ions, i.e. $[DAG]^+$, which are ions corresponding to the loss of fatty acyl groups esterified to the glycerol backbone as a neutral carboxylic acid $[M+H–RxCOOH]^+$. Weak $[M+H]^+$ ions are observed, but polyunsaturated TAG produce an $[M+H]^+$ base peak. Tandem mass spectrometry (MS/MS) has yielded further structural information on the regioisomers, i.e. those TAG in which the acyl chains are at different positions (i.e. AAB, ABA) or the double bond(s) in one or more of the acyl chains are at different positions. Identification and quantification of these molecular species in TAG analysis is difficult [11–14]. ESI generates abundant $[M+NH_4]^+$ or $[M+metal]^+$ for TAG species when ammonium ions or alkali metal ions are present in the solvent system being sprayed.

Identification of TAG is an important part of the studies of the yeast lipidome (see Table 1S, i.e. Supplements). Some authors [15, 16] identified only the sum of carbon atoms and the sum of double bonds in all three acyl chains (e.g. 48:3), while others [8, 17] succeeded in determining the structure of individual acyl chains but not of the TAG regioisomers. This has been achieved only by Jaakola et al. [5] who identified only a single pair of regioisomers—PoOPo and PoPoO in a 1.25:1 ratio.

An important component of yeast lipids, and especially TAG, is palmitoleic acid, which has many positive effects on human health and contributes, e.g., to reduced inflammation, protection of the cardiovascular system, and inhibition of oncogenesis. It has even been recognized as a lipid hormone (lipokine) derived from adipose tissue, which is able to stimulate the action of insulin at the muscle level and to decrease the accumulation of lipids in the liver (hepatosteatosis) [18].

Methyl palmitoleate has a low melting point (about −34 °C) and high oxidation stability, which is an asset in its use for the production of foods and nutraceuticals for medical purposes. It can be also used for the production of an industrially important compound, 1-octene used for the production of linear low density polyethylene or biodiesel; methyl palmitoleate has a cetane number of 54. Plant oils containing more than 10 % of 9–16:1 acid are usually obtained from exotic plant sources, see e.g. Table 2 in Wu et al. [19]. Moreover, low yields and poor agronomic characteristics of these plants prevent a large-scale production of these oils. To overcome these problems, attempts were made to express, e.g., Δ^9 desaturase from yeast in higher plants but the outcome has not been satisfactory, as documented in the statement of Wu et al. [19] "However, expression of Δ^9 desaturase alone only results in moderate accumulation of 9–16:1 acid in the transgenic plants". Since the 9–16:1 acid thus cannot be produced on a large scale and at a reasonable price, it is important to find sources that would enable large-scale production. One of these sources is brewer′s yeast. For instance, a brewery with an annual beer output of one million hectoliters also produces, as waste, 1,000 tons of brewer′s yeast that contains up to 50 % of 9–16:1 acid in its lipids (see below).

Based on our previous experience with the analysis of uncommon TAG by RP-HPLC/MS-APCI [11–13, 20] we determined the acyl composition of the molecular species of yeast TAG and characterized regioisomeric TAG containing palmitic, palmitoleic and oleic acids. The proportion of these isomers was found to depend on the type of beer production.

Experimental

Standards and Instrumentation

Acetonitrile, tetrahydrofurane, hexane, dichloromethane, tripalmitolein and tripalmitin were purchased from Sigma-Aldrich (Prague, CR). 1,2-Palmitolein-3-olein was purchased from Larodan Fine Chemicals AB (Sweden).

Yeast

Top brewer′s yeast *S. cerevisiae* and bottom brewer′s yeast *S. pastorianus* from selected breweries (see Table 1).

Isolation of TAG

A 10-ml volume of yeast suspension of 15–17 % dry weight was mixed with 2 ml 0.1 M Na_2CO_3 and the mixture was briefly ground with Ballotini glass beads (diameter 0.2 mm) in a mortar, overlaid with liquid nitrogen and ground again. This process was repeated three times and 50 ml 0.1 M Na_2CO_3 was finally added. The crushed yeast was extracted with a chloroform–methanol mixture according to Bligh and Dyer [32]. The sample was centrifuged and the lower phase was evaporated to dryness.

A total lipid extracts were applied to Sep-Pak Vac Silica cartridge 35 cc (Waters; with 10 g of silica normal-phase), and TAG were subsequently eluted from the cartridge with 40 ml of chloroform–methanol (9:1). The eluate was evaporated and the oil samples were dissolved in an acetonitrile-2-propanol–hexane mixture (1:1:1, v/v/v), which was injected for HPLC analysis.

FAME Analysis

The total TAG (~5 mg) were saponified overnight in 10 % KOH–MeOH at room temperature. A fatty acid fraction obtained from saponification was partitioned between alkali solution (pH 9) and Et_2O to remove basic and neutral components. The aqueous phase containing fatty acids was acidified to pH 2 and extracted with hexane. The fatty acid fraction was methylated using BF_3/MeOH.

Gas chromatography–mass spectrometry of FAME was done on a GC–MS system consisting of Varian 450-GC, Varian 240-MS ion trap detector with electron impact ionization, and CombiPal autosampler (CTC, USA). Splitless injection was at 100 °C, and a fused silica capillary column (Supelcowax 10; 60 m × 0.25 mm i.d., 0.25 μm film thickness; Supelco, Prague) was used. The temperature program was as follows: 100 °C for 1 min, subsequently increasing at 20 °C/min to 180 °C and at 2 °C/min to 280 °C, which was maintained for 1 min. The carrier gas was helium at a linear velocity of 60 cm/s. The structures of FAME were confirmed by comparison of retention times and fragmentation patterns with those of the standard FAME (Supelco, Prague).

Table 1 List of breweries and their basic characteristics

Brewery	Yearly beer output (hl)	No. of pitchings (yeast generations)	Waste yeast (t dry weight/year)
A[a]	>200,000	1×	16.0
B[b]	<200,000	4×	672.0
C[b]	<200,000	7×	1,360.0
D[b]	>200,000	6×	0.2
E[b]	>200,000	5×	25.6
F[b]	>200,000	4×	11.2
G[b]	<200,000	2×	192.0

[a] Top fermentation with *S. cerevisiae*

[b] Bottom fermentation with *S. pastorianus*

RP-HLPC/MS-APCI

HPLC equipment consisted of a 1090 Win system, PV5 ternary pump and automatic injector (HP 1090 series, Agilent, USA) and two Hichrom columns HIRPB-250AM (C8/C18 multi-alkyl phase) 250 × 2.1 mm ID, 5 μm particle size, in series. This setup provided us with a high-efficiency column—~26,000 plates/250 mm. The performance was measured by triheptadecanoin as an internal standard under the conditions given above. A quadrupole mass spectrometer system Navigator (Finnigan MAT, San Jose, CA, USA) was used for the analysis. The instrument was fitted with an atmospheric pressure chemical ionization source (interface temperature 390 °C, capillary heater temperature 260 °C, corona current 7 μA, sheath gas-high-purity nitrogen, pressure 0.45 MPa, and auxiliary gas (also nitrogen) flow rate 15 ml/min. Positively charged ions with m/z 200–1,000 were scanned with a scan time of 0.5 s. Fragmentation of triacylglycerols was carried out by the collision induced dissociation (CID). Helium was used as a collision gas for CID experiments. The normalized collision energy was set at 10–35 %. Isolation width of the parent ion was set at 0.5 Da. The spray voltage in the positive mode was set at 3,500 V. The mass spectra of the daughter ions were acquired in the positive mode. The whole HPLC flow (0.35 ml/min) was introduced into the APCI source without any splitting. TAG were separated using a gradient solvent program with acetonitrile (ACN) and 2-propanol (iPrOH) as follows: initial ACN/iPrOH (99:1 vol/vol); linear from 5 to 120 min ACN/iPrOH 30:70 vol/vol); held until 30 min; the composition was returned to the initial conditions over 10 min. A peak threshold of 0.07 % intensity was applied to the mass spectra. Data acquisition and analyses were performed using PC with MassLab 2.0 for Windows XP applications/operating software.

Results and Discussion

GC–MS analysis of the methyl esters obtained after hydrolysis of TAG confirmed the presence of 17 fatty acids, four of which, 16:0, 9–16:1, 18:0, and 9–18:1, were present in high proportions. Apart from containing always these four major acids, *Saccharomyces* yeast is characterized by a broad range of FA chain lengths, which begins at eight carbon atoms [4] and ends at more than 30 carbon atoms [21].

The content of the requisite palmitoleic acid varied between 25 and 50 % of total FA. It depended on two factors, the yeast strain used for beer production and the type of fermentation process. List of breweries, including beer outputs and type of produced beer is given in Table 1. Two breweries, (E) and (F), use the same yeast strain and the same fermentation technology, bottom fermentation, and their yearly beer output is similar. As seen in Table 2, the contents of all fatty acids in the yeast from these two breweries (E and F) are also similar. Three large breweries, (B, C, and G), form another group. Their yearly beer output is higher than 200,000 hl and they use also bottom fermentation with *S. pastorianus*. The content of fatty acids in the yeast is again similar. The yeast from brewery A, which used a different strain and top fermentation, showed in comparison with other breweries the lowest percentage of 16:0, the second lowest amounts of 12:0 and 14:0 (for the lowest amount of short-chain FA, 8:0–14:0), as well as the highest percentages of 9–18:1 and 20:1. The data for the small brewery (D) show the second greatest range of the FA acyl chain lengths from the yeast and a more remote relationship to the production strains used in breweries (B, C, and G). The fatty acid composition in the waste brewer′s yeast is thus mostly determined by the production strain and the fermentation type.

The production of total FA in yeast lipids or TAG has been widely studied. By contrast, the composition of TAG, i.e. identification of molecular species of TAG in yeast, has received less attention. As opposed to tens of plant oils or some animal oils, in which such identification of TAG has been performed [11, 22], only a few papers have so far dealt with the identification of molecular species of yeast TAG.

The yeast lipids from the seven breweries under study represent a very complex mixture of TAG, which are characterized by a broad range of FA chain lengths that has not been found in any plant or animal oil or fat. Our previous studies [12, 13, 20] identified odd-numbered and branched FA in bacterial TAG and polyenoic FA in algae and cyanobacteria, all of them in a relatively narrow range of acyl chain lengths from C14 to C20 [23]. By contrast, yeast exhibits a broad range of FA chain lengths.

Table 2 Fatty acids content from seven brewer's yeast strains including their abbreviations, carbon numbers (CN), and double bonds (DB)

Fatty acid	Abbreviation of FA	CN:DB	Brewery						
			A	B	C	D	E	F	G
Total lipids[a]			8.9	12.4	13.1	9.8	16.5	17.2	23.1
Caprylic	Cy	8:0	0.0	0.1 ± 0.1[b]	0.0	0.6 ± 0.2	0.0	0.0	0.2 ± 0.1
Capric	C	10:0	1.8 ± 0.2	1.1 ± 0.2	0.0	4.0 ± 0.5	0.0	0.0	1.3 ± 0.4
Lauric	La	12:0	1.7 ± 0.1	2.6 ± 0.3	3.5 ± 0.4	1.3 ± 0.2	3.8 ± 0.5	4.2 ± 0.7	3.1 ± 0.5
Myristic	M	14:0	0.8 ± 0.1	0.5 ± 0.1	1.4 ± 0.2	2.0 ± 0.2	1.7 ± 0.3	1.6 ± 0.4	1.7 ± 0.3
Palmitic	P	16:0	9.2 ± 0.4	16.9 ± 1.3	23.8 ± 1.5	23.1 ± 1.3	16.8 ± 1.4	17.2 ± 1.6	22.0 ± 2.1
Palmitoleic	Po	9–16:1	41.2 ± 1.2	49.2 ± 2.1	39.1 ± 2.3	25.8 ± 1.7	50.6 ± 2.8	54.1 ± 3.1	40.8 ± 2.3
Stearic	S	18:0	4.1 ± 0.5	5.6 ± 0.5	13.9 ± 1.8	9.7 ± 0.8	16.7 ± 1.8	13.4 ± 0.7	12.3 ± 1.4
Oleic	O	9–18:1	33.2 ± 1.3	22.4 ± 1.7	10.3 ± 1.2	21.9 ± 1.9	6.4 ± 0.7	4.2 ± 0.4	11.6 ± 1.2
Linoleic	L	9,12–18:2	2.7 ± 0.2	1.2 ± 0.2	8.0 ± 0.9	7.0 ± 0.8	2.6 ± 0.5	3.3 ± 0.5	6.0 ± 0.9
α-Linolenic	Ln	9,12,15–18:3	0.6 ± 0.1	0.2 ± 0.1	0.0	1.3 ± 0.2	0.4 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
Arachidic	A	20:0	0.2 ± 0.1	0.1 ± 0.1	0.0	0.3 ± 0.1	1.0 ± 0.3	1.3 ± 0.3	0.1 ± 0.1
Gadoleic	G	9–20:1	1.2 ± 0.2	0.0	0.0	0.5 ± 0.1	0.0	0.0	0.0
Behenic	B	22:0	0.7 ± 0.1	0.0	0.0	0.8 ± 0.2	0.0	0.0	0.0
Erucic	E	13–22:1	0.6 ± 0.1	0.0	0.0	0.6 ± 0.1	0.0	0.0	0.0
Lignoceric	Lg	24:0	0.5 ± 0.1	0.0	0.0	0.7 ± 0.1	0.0	0.0	0.0
Nervonic	N	15–24:1	0.8 ± 0.2	0.0	0.0	0.4 ± 0.1	0.0	0.0	0.0
Cerotic	Ce	26:0	0.7 ± 0.1	0.1 ± 0.1	0.0	0.0	0.0	0.0	0.2 ± 0.1

[a] In mg/g of dry weight

[b] Arithmetic means from three analyses ± standard deviations

Bottom and top brewer′s yeast differ in their flocculation properties; in the final phase of fermentation bottom yeast aggregates into flocks and sediments at the bottom of the fermentation vessel whereas top yeast rises to the surface to form thick foam. Bottom fermentation takes place at 8–15 °C. *S. cerevisiae* can stand higher temperatures than *S. pastorianus* and top fermentation usually takes place at 18–22 °C. The yeast strains used in the seven breweries, which include top-fermenting yeast in brewery A and bottom-fermenting strains in breweries B–G, show differences in both FA and the contents of TAG. Altogether, 181 TAG were identified, see Figs. 1 and 2, though none of the strains produced all of them. The separation of TAG by RP-HPLC enabled us to identify in yeast 3–4 times more TAG (see Table 3) than previously reported by the authors who analyzed lipids or specifically TAG from yeasts [8, 15–17] by ESI and who have determined, and some of them also quantified, individual molecular species of TAG, see the symbol + in Table 1S (supplements) and "discussion".

Two papers on yeast lipids show the quantitative proportion of TAG in a graphical form [16, 17]. The figures imply that major TAG are 50:2 and 52:3, which could correspond to POPo and PoOO. These TAG were also found to be among the most abundant in our study. TAG 52:2 and 54:2 were the most abundant in *S. cerevisiae* BY4741 [17]. The separation of TAG in study [16] was unsatisfactory (compare Fig. 1 of this study and our Fig. 2) The paper by Grillitsch et al. [17] does not present the chromatogram. The relative abundances of PoPoPo, PPoPo, and PoPoO were found to be around 10 %. The most abundant TAG reported by Klose et al. [15] were 50:3, 50:2 and 52:2, which could again correspond to the molecular species PoPoO, PPoO, and POO. The authors of two studies [8, 15] did not use LC–MS and the number of identified TAG was thus lower, see Table 1S. Jaakola et al. [5] identified PoOPo, PoOP, and PoPoO as representing about 10 % of the total TAG; the first and last TAG were regioisomers that were quantified by tandem MS in a ratio of 1.25:1.

Molecular species of TAG are most frequently separated by using a nonaqueous mobile phase with octadecylsilane columns, i.e. RP-HPLC [11, 12]. In this separation mode the elution order depends on the length of the acyl chains and the number of double bonds [23]. The method permits the identification of tens to hundreds of molecular species of TAG from various plant and/or animal oils and fats [11, 22].

Figure 1 shows the RP-HPLC with APCI detection of the total ion current (from 200 to 1,000 Da) of TAG from brewery G with a yearly output of more than 200,000 hl beer per year, which is produced by the bottom-fermenting yeast strain *S. pastorianus.* We identified a total of 123 molecular species of TAG having up to 65 carbon atoms in the molecule. The major peaks were sufficiently separated while a number of other peaks defied separation. By using

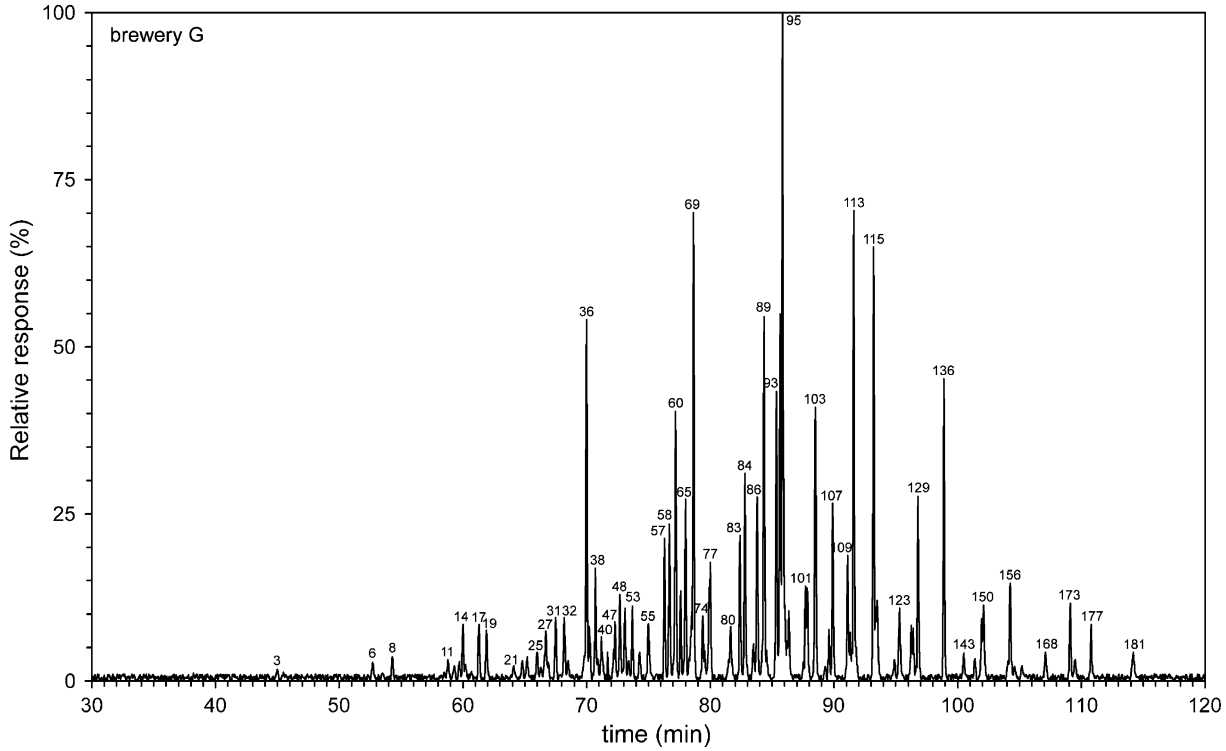

Fig. 1 RP-HPLC/MS-APCI chromatogram of the TAG from yeast from brewery G

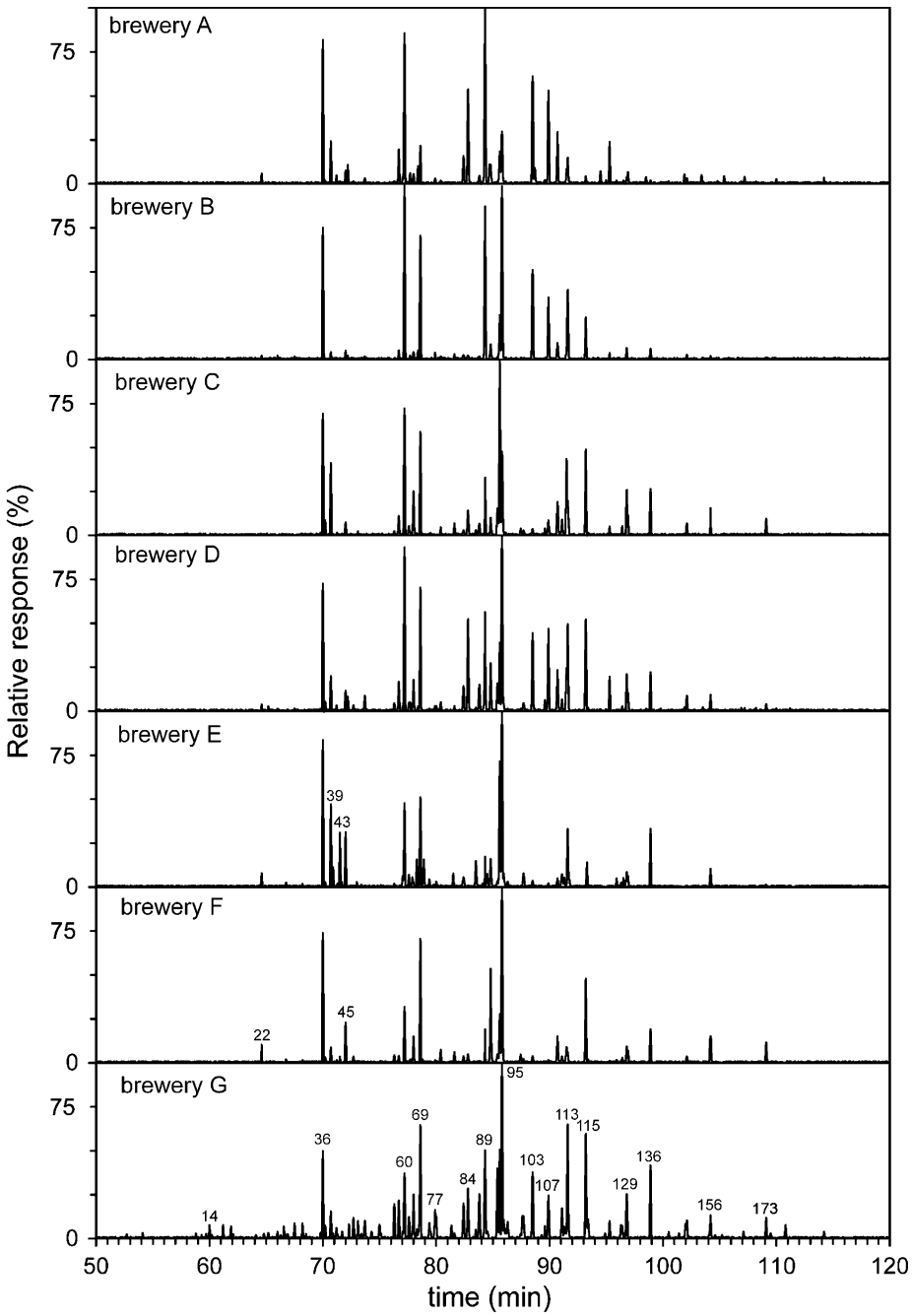

Fig. 2 RP-HPLC/MS-APCI chromatogram of the TAG from yeast from the seven breweries

tandem MS (see below) we then separated molecular species with abundances slightly above 0.01 % of the total TAG.

Preliminary identification, which was later confirmed by tandem MS, was based on the elution order of TAG, which best expresses the ECN (equivalent carbon number), ECN = ACN−2 × DB (ACN = acyl carbon number; DB = number of double bond(s)) [24, 25]. TAG having the same ECN value can be separated on a high-efficiency column such as a combination of two 25-cm columns in series [12, 13, 20], which provides an efficiency of about 52,000 theoretical plates. The combination effectively separated most of the TAG and, in addition, also regioisomers of TAG, as illustrated in Fig. 3.

Positive ion APCI mass spectra of TAG typically exhibited $[M + H]^+$ ion and fragment ions of type $[M + H\text{-RCOOH}]^+$ ($[DAG]^+$), resulting from the loss of an acyl chain from TAG. Monoacyl glycerol ($[MAG]^+$) is equivalent to $[RCO + 74]^+$ ion. The positive ion APCI mass spectrum of PoPPo (for abbreviations of FA, see Table 2) shows $[M + H]^+$ ion at *m/z* 803 and the fragment ions $[M + H\text{-RCOOH}]^+$ at *m/z* 549 $[PPo]^+$ and at *m/z* 547

Table 3 Relative quantities (%) of total acyl groups identified in yeast from the seven breweries, their retention times (tR), acyl carbon number (ACN), number of double bonds (DB), and equivalent chain number (ECN)

Peak no	ACN:DB	ECN	tR	TAG[a]	Brewery						
					A	B	C	D	E	F	G
1	30:0	30	31.0	LaCCy	0	0	0	0	0	0	0.06 ± 0.02[b]
2	32:0	32	38.2	LaLaCy	0	0	0	0	0	0	0.08 ± 0.03
3	32:0	32	45.0	LaLCy	0	0	0	0.01 ± 0.01	0	0	0.12 ± 0.04
4	34:0	34	45.5	LaLaC	0	0	0	0	0	0	0.09 ± 0.03
5	34:0	34	46.1	MLaCy	0	0	0	0	0	0	0.06 ± 0.02
6	38:1	36	52.7	LaOCy	0	0.05 ± 0.01	0	0.02 ± 0.01	0	0	0.19 ± 0.07
7	36:0	36	53.5	MLaC	0	0	0	0.01 ± 0.01	0	0	0.08 ± 0.03
8	36:0	36	54.1	PLaCy	0	0.04 ± 0.01	0	0.02 ± 0.01	0	0	0.25 ± 0.09
9	36:0	36	54.1	MMCy	0	0	0	0	0	0	0.05 ± 0.01
10	54:8	38	58.5	LnLLn	0	0	0	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.09 ± 0.03
11	44:3	38	58.8	OLCy	0	0.04 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0	0	0.22 ± 0.08
12	42:2	38	59.3	LaLLa	0.02 ± 0.01	0	0.04 ± 0.02	0.01 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.16 ± 0.09
13	40:1	38	59.7	LaOC	0.08 ± 0.02	0.05 ± 0.02	0	0.05 ± 0.02	0	0	0.20 ± 0.07
14	42:2	38	60.0	PLCy	0	0.02 ± 0.01	0	0.05 ± 0.02	0	0	0.59 ± 0.16
15	40:1	38	60.2	MOCy	0	0.02 ± 0.01	0	0.02 ± 0.01	0	0	0.17 ± 0.07
16	38:0	38	60.7	MLaLa	0	0	0.01 ± 0.01	0	0.03 ± 0.01	0.03 ± 0.01	0.10 ± 0.03
17	38:0	38	61.2	PLaC	0.03 ± 0.01	0.04 ± 0.02	0	0.06 ± 0.02	0	0.03 ± 0.01	0.59 ± 0.19
18	38:0	38	61.2	MMC	0	0	0	0.01 ± 0.01	0	0	0.06 ± 0.02
19	38:0	38	61.9	PMCy	0	0.02 ± 0.01	0	0.02 ± 0.01	0	0	0.53 ± 0.21
20	38:0	38	61.9	SLaCy	0	0.02 ± 0.01	0	0.01 ± 0.01	0	0	0.19 ± 0.08
21	54:7	40	64.1	LLLn	0	0	0	0.03 ± 0.01	0	0	0.16 ± 0.07
22	50:5	40	64.6	PoLnPo	0.68 ± 0.14	0.31 ± 0.07	0	0.34 ± 0.11	0.94 ± 0.14	1.50 ± 0.21	0
23	48:4	40	64.8	LLLa	0.02 ± 0.10	0	0.10 ± 0.04	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.21 ± 0.08
24	46:3	40	65.2	OLC	0.12 ± 0.03	0.04 ± 0.01	0	0.24 ± 0.08	0	0	0.25 ± 0.10
25	44:2	40	66.0	OOCy	0	0.31 ± 0.06	0	0.12 ± 0.04	0	0	0.30 ± 0.14
26	44:2	40	66.3	MLLa	0	0	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.14 ± 0.07
27	52:6	40	66.6	LnLnP	0.08 ± 0.02	0.09 ± 0.03	0	0.02 ± 0.01	0.09 ± 0.03	0.06 ± 0.02	0.52 ± 0.20
28	42:1	40	66.6	LaOLa	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.24 ± 0.10
29	42:1	40	66.7	LaMPo	0.07 ± 0.03	0.05 ± 0.02	0.10 ± 0.03	0.04 ± 0.02	0.31 ± 0.12	0.29 ± 0.15	0
30	42:1	40	66.9	MOC	0.05 ± 0.02	0.02 ± 0.01	0	0.08 ± 0.04	0	0	0.19 ± 0.09
31	42:1	40	67.5	POCy	0	0.24 ± 0.09	0	0.13 ± 0.07	0	0	0.66 ± 0.28
32	40:0	40	68.2	PLaLa	0.02 ± 0.01	0.07 ± 0.02	0.12 ± 0.04	0.02 ± 0.01	0.22 ± 0.09	0.23 ± 0.12	0.66 ± 0.29
33	40:0	40	68.2	MMLa	0	0	0	0	0.02 ± 0.01	0	0.08 ± 0.03
34	40:0	40	68.5	SLaC	0.02 ± 0.01	0.02 ± 0.01	0	0.02 ± 0.01	0	0	0.21 ± 0.10

Table 3 continued

Peak no	ACN:DB	ECN	tR	TAG[a]	Brewery						
					A	B	C	D	E	F	G
35	54:6	42	69.8	LLL	0.02 ± 0.01	0	0.20 ± 0.08	0.13 ± 0.05	0.02 ± 0.01	0.03 ± 0.01	0.26 ± 0.13
36	48:3	42	70.0	PoPoPo	9.57 ± 1.24	10.14 ± 1.87	8.24 ± 1.39	6.39 ± 1.52	10.01 ± 2.16	10.75 ± 1.62	3.79 ± 0.97
37	52:5	42	70.2	LLPo	0.21 ± 0.08	0.07 ± 0.03	1.02 ± 0.21	0.48 ± 0.14	0.34 ± 0.13	0.47 ± 0.20	0.57 ± 0.24
38	50:4	42	70.7	PoLPo	2.83 ± 0.46	0.58 ± 0.17	4.91 ± 1.47	1.75 ± 0.26	5.63 ± 1.42	1.29 ± 0.31	1.18 ± 0.36
39	54:6	42	70.9	OLLn	0.05 ± 0.02	0.02 ± 0.01	0	0.08 ± 0.02	1.34 ± 0.39	0	0.23 ± 0.11
40	52:5	42	71.2	LnOPo	0.55 ± 0.13	0.15 ± 0.05	0	0.29 ± 0.11	0.02 ± 0.01	0.14 ± 0.08	0.46 ± 0.18
41	48:3	42	71.2	PoLM	0.08 ± 0.03	0.04 ± 0.02	0.19 ± 0.06	0.15 ± 0.07	0.15 ± 0.08	0.23 ± 0.11	0
42	50:4	42	71.3	LLM	0	0	0.04 ± 0.02	0.04 ± 0.02	0.24 ± 0.14	0.03 ± 0.01	0.18 ± 0.09
43	50:4	42	71.5	PLnPo	0.17 ± 0.06	0.13 ± 0.06	0	0	3.72 ± 0.84	0.52 ± 0.16	0
44	48:3	42	71.7	OLLa	0.12 ± 0.05	0.05 ± 0.02	0.12 ± 0.04	0.08 ± 0.03	0.34 ± 0.12	0.06 ± 0.03	0.30 ± 0.14
45	46:2	42	72.0	PoPoM	0.88 ± 0.17	0.69 ± 0.19	0.88 ± 0.18	1.02 ± 0.30	3.74 ± 0.91	3.35 ± 0.57	0
46	46:2	42	72.2	OOC	1.26 ± 0.34	0.31 ± 0.13	0	0.73 ± 0.28	0	0	0.01 ± 0.01
47	52:5	42	72.3	LnLP	0.02 ± 0.01	0.02 ± 0.01	0	0.09 ± 0.03	0.02 ± 0.01	0.03 ± 0.01	0.62 ± 0.27
48	50:4	42	72.7	PLnPo	0.17 ± 0.06	0.13 ± 0.08	0	0.31 ± 0.11	0.03 ± 0.01	0.52 ± 0.19	0.90 ± 0.32
49	54:6	42	73.0	SLnLn	0	0	0	0.01 ± 0.01	0.34 ± 0.16	0	0.17 ± 0.09
50	46:2	42	73.0	MLM	0	0	0	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.12 ± 0.05
51	46:2	42	73.1	PLLa	0.03 ± 0.01	0.04 ± 0.02	0.28 ± 0.12	0.09 ± 0.03	0.02 ± 0.01	0.18 ± 0.09	0.76 ± 0.34
52	44:1	42	73.4	MOLa	0.05 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.16 ± 0.09	0.03 ± 0.02	0.21 ± 0.09
53	44:1	42	73.7	POC	0.36 ± 0.15	0.24 ± 0.10	0	0.77 ± 0.26	0	0	0.78 ± 0.28
54	44:1	42	74.3	SOCy	0	0.09 ± 0.03	0	0.06 ± 0.02	0	0	0.30 ± 0.14
55	42:0	42	75.0	PMLa	0.02 ± 0.01	0.02 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.09 ± 0.04	0.60 ± 0.26
56	42:0	42	75.1	SLaLa	0.02 ± 0.01	0.02 ± 0.01	0.07 ± 0.02	0.01 ± 0.01	0.12 ± 0.05	0.18 ± 0.09	0.25 ± 0.13
57	54:5	44	76.3	OLL	0.17 ± 0.05	0.04 ± 0.02	0.28 ± 0.13	0.41 ± 0.16	0.22 ± 0.10	0.63 ± 0.31	1.49 ± 0.53
58	52:4	44	76.7	OLPo	2.30 ± 0.67	0.73 ± 0.18	1.31 ± 0.64	1.48 ± 0.37	0.03 ± 0.01	0.58 ± 0.24	1.65 ± 0.61
59	54:5	44	77.1	OOLn	0.45 ± 0.16	0.07 ± 0.03	0	0.25 ± 0.09	0.75 ± 0.29	0.03 ± 0.01	0.31 ± 0.13
60	50:3	44	77.2	PoOPo	7.51 ± 1.51	13.69 ± 2.19	6.89 ± 1.08	5.42 ± 1.03	5.07 ± 1.04	4.33 ± 0.86	2.26 ± 0.81
61	50:3	44	77.3	PoPoO	2.51 ± 0.98	2.28 ± 0.85	1.72 ± 0.67	2.78 ± 0.49	0.64 ± 0.28	0.31 ± 0.12	0.57 ± 0.24
62	52:4	44	77.6	LLP	0.05 ± 0.02	0.02 ± 0.01	0.64 ± 0.31	0.43 ± 0.27	0.84 ± 0.36	0.14 ± 0.07	0.94 ± 0.36
63	48:2	44	77.7	PoOM	0.71 ± 0.31	0.33 ± 0.13	0.25 ± 0.14	0.44 ± 0.24	0.12 ± 0.06	0.29 ± 0.11	0
64	50:3	44	77.9	OLM	0.07 ± 0.03	0.02 ± 0.01	0.06 ± 0.03	0.12 ± 0.06	0.66 ± 0.24	0.03 ± 0.01	0.26 ± 0.10
65	50:3	44	78.0	PLPo	0.66 ± 0.28	0.56 ± 0.26	3.00 ± 0.73	1.57 ± 0.51	0.33 ± 0.17	2.20 ± 0.78	1.91 ± 0.74
66	48:2	44	78.2	OOLa	0.02 ± 0.01	0	0.16 ± 0.08	0.04 ± 0.02	1.89 ± 0.62	0.03 ± 0.01	0.41 ± 0.20
67	54:5	44	78.3	SLLn	1.19 ± 0.49	0.71 ± 0.32	0	0.25 ± 0.11	0.03 ± 0.01	0.06 ± 0.02	0.25 ± 0.11
68	52:4	44	78.3	LnOP	0.13 ± 0.08	0.05 ± 0.02	0	0.26 ± 0.12	0.15 ± 0.07	0.06 ± 0.02	0.72 ± 0.39
69	48:2	44	78.5	PoPPo	0.51 ± 0.24	7.23 ± 1.37	4.67 ± 0.86	4.10 ± 0.73	4.58 ± 1.14	7.71 ± 1.43	3.28 ± 0.99

Table 3 continued

Peak no	ACN:DB	ECN	tR	TAG[a]	Brewery						
					A	B	C	D	E	F	G
70	48:2	44	78.5	PPoPo	2.03 ± 0.62	2.42 ± 0.96	2.34 ± 0.64	2.09 ± 0.42	1.53 ± 0.56	2.57 ± 0.68	1.64 ± 0.45
71	50:3	44	78.6	PLPo	0	0	0	0	0.14 ± 0.07	0	0
72	56:6	44	78.7	OPoM	0	0	0	0	0	0.29 ± 0.15	0
73	48:2	44	78.8	ALnLn	0	0	0	0	1.89 ± 0.43	0	0.02 ± 0.01
74	48:2	44	79.4	PLM	0.02 ± 0.01	0.02 ± 0.01	0.12 ± 0.06	0.13 ± 0.07	0.52 ± 0.19	0.09 ± 0.03	0.68 ± 0.24
75	48:2	44	79.5	SLLa	0.02 ± 0.01	0.02 ± 0.01	0.17 ± 0.08	0.04 ± 0.02	0.07 ± 0.02	0.14 ± 0.07	0.31 ± 0.16
76	46:1	44	79.8	MOM	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.04 ± 0.02	0.16 ± 0.08	0	0.19 ± 0.09
77	50:3	44	79.9	PLnP	0.35 ± 0.14	0.55 ± 0.24	0	0.26 ± 0.12	0.02 ± 0.01	0.23 ± 0.12	1.24 ± 0.53
78	46:1	44	79.9	POLa	0.05 ± 0.02	0.05 ± 0.02	0.01 ± 0.01	0.27 ± 0.13	0.37 ± 0.16	0.18 ± 0.09	0.97 ± 0.29
79	46:1	44	80.4	PPoM	0.21 ± 0.08	0.25 ± 0.12	0.55 ± 0.27	0.46 ± 0.20	0.13 ± 0.07	1.10 ± 0.61	0
80	44:0	44	81.5	PMM	0	0	0.02 ± 0.01	0.01 ± 0.01	0.12 ± 0.06	0.09 ± 0.04	0.57 ± 0.21
81	44:0	44	81.5	SMLa	0.10 ± 0.03	0.42 ± 0.19	0.82 ± 0.32	0.27 ± 0.14	0	0.90 ± 0.44	0.22 ± 0.10
82	44:0	44	81.5	LaPP	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.92 ± 0.45	0.06 ± 0.03	0
83	54:4	46	82.4	OLO	1.85 ± 0.69	0.35 ± 0.17	0.35 ± 0.17	1.26 ± 0.48	0.66 ± 0.32	0.56 ± 0.31	1.52 ± 0.67
84	52:3	46	82.8	OOPo	6.28 ± 1.18	0.31 ± 0.14	1.68 ± 0.57	4.61 ± 0.86	0.12 ± 0.05	0.72 ± 0.39	2.18 ± 0.78
85	54:4	46	83.5	SLL	0.02 ± 0.01	0	0.36 ± 0.15	0.18 ± 0.09	1.79 ± 0.71	0.11 ± 0.05	0.39 ± 0.14
86	52:3	46	83.8	OLP	0.53 ± 0.23	0.25 ± 0.12	0.79 ± 0.33	1.33 ± 0.51	0.12 ± 0.06	0.18 ± 0.08	1.93 ± 0.84
87	56:5	46	84.1	ALLn	0	0	0	0	0.25 ± 0.11	0	0.09 ± 0.03
88	50:2	46	84.2	OOM	0	0.16 ± 0.08	0.07 ± 0.02	0.37 ± 0.13	0	0.03 ± 0.01	0.35 ± 0.16
89	50:2	46	84.3	POPo	11.68 ± 2.17	11.79 ± 1.87	3.86 ± 0.94	4.96 ± 1.19	2.07 ± 0.71	2.80 ± 0.74	3.82 ± 0.96
90	54:4	46	84.5	SOLn	0.07 ± 0.03	0.04 ± 0.02	0	0.11 ± 0.04	0.90 ± 0.31	0.03 ± 0.01	0.32 ± 0.15
91	52:3	46	84.7	GPoPo	1.29 ± 0.67	0	0	0.14 ± 0.05	0	0	0
92	52:2	46	84.8	SPoPo	1.28 ± 0.42	1.20 ± 0.37	1.20 ± 0.38	2.41 ± 0.81	1.93 ± 0.67	7.79 ± 1.18	0
93	50:2	46	85.4	PLP	0.15 ± 0.08	0.20 ± 0.09	1.83 ± 0.64	1.40 ± 0.65	0.40 ± 0.18	0.72 ± 0.31	3.04 ± 0.64
94	50:2	46	85.4	SLM	0.02 ± 0.01	0	0.07 ± 0.03	0.06 ± 0.03	0.65 ± 0.31	0.06 ± 0.03	0.27 ± 0.13
95	48:1	46	85.6	PPoP	2.97 ± 0.69	11.52 ± 1.28	11.90 ± 1.37	7.80 ± 1.42	12.96 ± 1.84	14.55 ± 1.67	7.00 ± 1.08
96	48:1	46	85.7	PPPo	2.66 ± 0.58	3.48 ± 0.68	5.68 ± 0.98	4.44 ± 0.87	7.56 ± 1.19	4.04 ± 0.78	3.85 ± 0.49
97	48:1	46	85.8	POM	0.18 ± 0.09	0.13 ± 0.07	0.16 ± 0.08	0.39 ± 0.18	0.92 ± 0.39	0.18 ± 0.09	0.82 ± 0.31
98	48:1	46	85.8	SOLa	0.17 ± 0.08	0.18 ± 0.09	0.20 ± 0.09	0.11 ± 0.05	0.16 ± 0.08	0.09 ± 0.03	0.42 ± 0.19
99	52:3	46	85.9	SLnP	0.02 ± 0.01	0.02 ± 0.01	0	0.12 ± 0.06	0.37 ± 0.15	0.14 ± 0.07	0.73 ± 0.25
100	46:0	46	87.6	SMM	0.05 ± 0.02	0.15 ± 0.07	0.47 ± 0.21	0.12 ± 0.06	0.06 ± 0.02	0.69 ± 0.31	0.20 ± 0.09
101	46:0	46	87.6	SPLa	0	0	0.01 ± 0.01	0.02 ± 0.01	0.13 ± 0.05	0.03 ± 0.02	0.99 ± 0.54
102	46:0	46	87.7	PPM	0.05 ± 0.02	0.09 ± 0.03	0.34 ± 0.14	0.41 ± 0.19	0.92 ± 0.37	0.34 ± 0.18	0.97 ± 0.3
103	54:3	48	88.5	OOO	7.15 ± 1.17	6.99 ± 1.26	0.45 ± 0.21	3.91 ± 0.82	0.43 ± 0.21	0.56 ± 0.26	2.87 ± 0.84
104	54:3	48	88.7	GOPo	1.04 ± 0.39	0	0	0.12 ± 0.06	0	0	0

Table 3 continued

Peak no	ACN:DB	ECN	tR	TAG[a]	Brewery						
					A	B	C	D	E	F	G
105	56:4	48	89.3	ALL	0	0	0	0.01 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.15 ± 0.07
106	54:3	48	89.6	SLO	0.25 ± 0.12	0.09 ± 0.03	0.47 ± 0.22	0.56 ± 0.27	0.02 ± 0.01	0.14 ± 0.07	0.54 ± 0.21
107	52:2	48	89.9	OOP	6.20 ± 1.09	4.85 ± 0.98	1.02 ± 0.34	4.13 ± 0.89	0.25 ± 0.12	0.23 ± 0.12	1.87 ± 0.48
108	52:2	48	90.7	SOPo	3.44 ± 0.85	1.30 ± 0.41	2.26 ± 0.81	2.05 ± 0.54	0.59 ± 0.27	2.20 ± 0.67	0
109	52:2	48	91.1	SLP	0.07 ± 0.03	0.07 ± 0.03	1.07 ± 0.52	0.60 ± 0.28	0.88 ± 0.41	0.54 ± 0.22	1.31 ± 0.41
110	54:3	48	91.3	ALnP	0	0.02 ± 0.01	0	0.01 ± 0.01	0.65 ± 0.31	0.03 ± 0.02	0.51 ± 0.22
111	54:3	48	91.4	SLnS	0.02 ± 0.01	0.02 ± 0.01	0	0.05 ± 0.02	0.02 ± 0.01	0.11 ± 0.06	0.33 ± 0.13
112	50:1	48	91.4	PPoS	0.98 ± 0.41	0.49 ± 0.22	5.19 ± 0.87	2.16 ± 0.47	0.13 ± 0.08	1.31 ± 0.42	0
113	50:1	48	91.5	POP	1.74 ± 0.53	5.43 ± 0.95	2.35 ± 0.61	4.35 ± 0.84	3.96 ± 0.91	0.90 ± 0.31	4.94 ± 0.46
114	50:1	48	91.5	SOM	0.08 ± 0.04	0.05 ± 0.03	0.08 ± 0.04	0.17 ± 0.08	0.55 ± 0.23	0.09 ± 0.05	0.36 ± 0.11
115	48:0	48	93.2	PPP	0.50 ± 0.24	3.28 ± 0.86	5.82 ± 0.91	4.59 ± 0.82	0.16 ± 0.08	6.96 ± 0.98	4.55 ± 0.84
116	48:0	48	93.3	APLa	0.02 ± 0.01	0.02 ± 0.01	0	0.01 ± 0.01	1.69 ± 0.67	0.09 ± 0.03	0.54 ± 0.25
117	48:0	48	93.4	SPM	0.03 ± 0.01	0.04 ± 0.02	0.19 ± 0.09	0.18 ± 0.09	0.07 ± 0.03	0.29 ± 0.12	0.84 ± 0.37
118	56:3	50	94.5	GOO	0.84 ± 0.24	0	0	0.11 ± 0.05	0	0	0
119	56:3	50	94.6	ELP	0.02 ± 0.01	0	0	0.05 ± 0.03	0	0	0
120	56:3	50	94.7	GSL	0.02 ± 0.01	0	0	0.02 ± 0.01	0	0	0
121	56:3	50	94.9	ALO	0.03 ± 0.02	0.02 ± 0.01	0	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.22 ± 0.09
122	54:2	50	95.0	GOP	0.25 ± 0.14	0	0	0.11 ± 0.05	0	0	0
123	54:2	50	95.3	SOO	2.78 ± 0.68	0.51 ± 0.21	0.60 ± 0.27	1.74 ± 0.61	0.02 ± 0.01	0.18 ± 0.09	0.76 ± 0.34
124	54:2	50	95.9	AOPo	0.21 ± 0.10	0.09 ± 0.03	0	0.08 ± 0.03	0.59 ± 0.24	0.26 ± 0.12	0
125	54:2	50	96.3	ALP	0.02 ± 0.01	0.02 ± 0.01	0	0.03 ± 0.01	0.31 ± 0.13	0.06 ± 0.03	0.58 ± 0.21
126	54:2	50	96.4	SLS	0.03 ± 0.01	0.02 ± 0.01	0.64 ± 0.29	0.25 ± 0.11	0.06 ± 0.03	0.43 ± 0.20	0.55 ± 0.24
127	54:2	50	96.5	AOPo	0.21 ± 0.13	0.09 ± 0.03	0	0.08 ± 0.04	0.62 ± 0.32	0	0
128	56:3	50	96.7	ALnS	0	0	0	0.01 ± 0.01	0.31 ± 0.14	0.03 ± 0.01	0.16 ± 0.08
129	52:1	50	96.8	SOP	0.45 ± 0.19	0.90 ± 0.24	3.08 ± 1.08	1.84 ± 0.72	1.02 ± 0.47	1.36 ± 0.49	1.94 ± 0.61
130	52:1	50	96.8	PoSS	0.79 ± 0.34	0.15 ± 0.08	1.38 ± 0.42	0.92 ± 0.35	0.77 ± 0.28	1.03 ± 0.37	0.01 ± 0.01
131	60:4	52	97.6	ELG	0	0	0	0	0	0	0
132	62:5	52	97.7	EELn	0	0	0	0	0	0	0
133	60:4	52	97.8	NLO	0.07 ± 0.03	0	0	0.03 ± 0.01	0	0	0
134	58:3	52	98.4	GOG	0.05 ± 0.02	0	0	0.01 ± 0.01	0	0	0
135	58:3	52	98.5	EOO	0.45 ± 0.21	0	0	0.12 ± 0.05	0	0	0
136	50:0	50	98.9	SPP	0.23 ± 0.09	0.86 ± 0.37	3.15 ± 1.19	1.94 ± 0.64	3.97 ± 1.01	2.80 ± 1.12	3.17 ± 1.14
137	58:3	52	99.1	NLP	0.02 ± 0.01	0	0	0.04 ± 0.02	0	0	0
138	60:4	52	99.4	LgLL	0	0	0	0.02 ± 0.01	0	0	0
139	56:2	52	99.8	EOP	0.13 ± 0.06	0	0	0.13 ± 0.07	0	0	0

Table 3 continued

Peak no	ACN:DB	ECN	tR	TAG[a]	Brewery						
					A	B	C	D	E	F	G
140	58:3	52	100.1	BLO	0.07 ± 0.03	0	0	0.06 ± 0.03	0	0	0
141	56:2	52	100.3	GOS	0.12 ± 0.05	0	0	0.05 ± 0.02	0	0	0
142	60:4	52	100.4	LgOLn	0.03 ± 0.01	0	0	0.02 ± 0.01	0	0	0
143	56:2	52	100.5	AOO	0.18 ± 0.09	0.05 ± 0.02	0	0.07 ± 0.03	0	0.03 ± 0.01	0.29 ± 0.15
144	56:2	52	101.3	BLP	0.02 ± 0.01	0	0	0.06 ± 0.03	0	0	0
145	56:2	52	101.4	ALS	0	0	0	0.01 ± 0.01	0.02 ± 0.01	0.06 ± 0.02	0.23 ± 0.13
146	58:3	52	101.6	LgLnP	0	0	0	0.02 ± 0.01	0	0	0
147	58:3	52	101.7	BLnS	0	0	0	0.01 ± 0.01	0	0	0
148	56:2	52	101.9	BOPo	0.63 ± 0.30	0	0	0.19 ± 0.09	0	0	0
149	54:1	52	102.0	AOP	0.07 ± 0.02	0.04 ± 0.02	0	0.07 ± 0.03	0.06 ± 0.03	0.09 ± 0.03	0.65 ± 0.28
150	54:1	52	102.1	SOS	0.36 ± 0.18	0.38 ± 0.18	0.82 ± 0.41	0.75 ± 0.41	0.12 ± 0.05	0.54 ± 0.28	0.79 ± 0.35
151	62:4	54	102.3	EEL	0	0	0	0	0	0	0
152	60:3	54	103.2	EOG	0.03 ± 0.01	0	0	0.01 ± 0.01	0	0	0
153	60:3	54	103.4	NOO	0.58 ± 0.24	0	0	0.09 ± 0.03	0	0	0
154	54:1	52	103.5	BPoP	0.20 ± 0.01	0	0	0.19 ± 0.08	0	0	0
155	52:0	52	104.1	APP	0.02 ± 0.01	0.04 ± 0.02	0	0.08 ± 0.04	0.06 ± 0.02	0.29 ± 0.14	0.21 ± 0.10
156	52:0	52	104.2	SSP	0.10 ± 0.03	0.29 ± 0.14	1.86 ± 0.31	0.82 ± 0.25	1.25 ± 0.34	2.20 ± 0.49	1.02 ± 0.38
157	60:3	54	104.4	NLS	0.02 ± 0.01	0	0	0.02 ± 0.01	0	0	0
158	62:4	54	104.6	CeLL	0	0	0	0	0	0	0.15 ± 0.07
159	58:2	54	104.8	NOP	0.18 ± 0.07	0	0	0.09 ± 0.03	0	0	0
160	58:2	54	105.0	EOS	0.07 ± 0.04	0	0	0.06 ± 0.02	0	0	0
161	60:3	54	105.1	LgLO	0.05 ± 0.03	0	0	0.05 ± 0.02	0	0	0
162	62:4	54	105.2	CeOLn	0.03 ± 0.01	0	0	0	0	0	0.16 ± 0.09
163	58:2	54	105.4	BOO	0.51 ± 0.22	0	0	0	0	0	0
164	60:3	54	106.3	LgLnS	0	0	0	0.01 ± 0.01	0	0	0
165	58:2	54	106.4	LgLP	0.02 ± 0.01	0	0	0.05 ± 0.01	0	0	0
166	58:2	54	106.5	BLS	0.02 ± 0.01	0	0	0.03 ± 0.01	0	0	0
167	56:1	54	106.9	BOP	0.15 ± 0.08	0	0	0.17 ± 0.09	0	0	0
168	56:1	54	107.1	AOS	0.03 ± 0.01	0.02 ± 0.01	0	0.04 ± 0.02	0.02 ± 0.01	0.06 ± 0.03	0.30 ± 0.14
169	58:2	54	107.2	LgOPo	0.46 ± 0.21	0	0	0.16 ± 0.08	0	0	0
170	62:3	56	107.5	EEO	0.03 ± 0.01	0	0	0.01 ± 0.01	0	0	0
171	62:3	56	107.7	NOG	0.03 ± 0.01	0	0	0.01 ± 0.01	0	0	0
172	58:1	56	108.2	LgPoP	0.15 ± 0.06	0	0	0.17 ± 0.08	0	0	0
173	54:0	54	109.1	SSS	0.05 ± 0.02	0.16 ± 0.07	1.13 ± 0.38	0.36 ± 0.14	0.16 ± 0.09	1.71 ± 0.53	0.91 ± 0.39
174	60:2	56	109.4	NOS	0.08 ± 0.04	0	0	0.04 ± 0.02	0	0	0

Table 3 continued

Peak no	ACN:DB	ECN	tR	TAG[a]	Brewery						
					A	B	C	D	E	F	G
175	62:3	56	109.5	CeLO	0.07 ± 0.04	0.02 ± 0.01	0	0	0	0	0.23 ± 0.16
176	60:2	56	110.0	LgOO	0.32 ± 0.11	0	0	0.14 ± 0.07	0	0	0
177	60:2	56	110.8	CeLP	0.02 ± 0.01	0.02 ± 0.01	0	0	0	0	0.59 ± 0.24
178	60:2	56	111.0	LgLS	0	0	0	0.02 ± 0.01	0	0	0
179	58:1	56	111.2	LgOP	0.12 ± 0.06	0	0	0.15 ± 0.08	0	0	0
180	58:1	56	111.4	BOS	0.08 ± 0.04	0	0	0.07 ± 0.03	0	0	0
181	62:2	58	114.2	CeOO	0.40 ± 0.19	0.05 ± 0.02	0	0	0	0	0.30 ± 0.13

[a] The TAG abbreviations do not indicate any regiospecificity

[b] Arithmetic means from three analyses ± standard deviations

$[PoPo]^+$, $[RCO + 74]^+$ at *m/z* 313 $[P + 74]^+$, or at *m/z* 311 $[Po + 74]^+$ and $[RCO]^+$ at *m/z* 239 [palmitoyl] and at *m/z* 237 [palmitoleyl]. These ions were observed with sufficient intensity to analyze the mass spectrum.

From the APCI mass spectrum of the peak at tR 77.2 min, see Fig. 4, it is obvious that this is a PoOPo, as indicated by the following ions: $[M + H]^+$ at *m/z* 829, $[M + H\text{-}RCOOH]^+$ at *m/z* 575 $[PoO]^+$, and *m/z* 547 $[PoPo]^+$, $[RCO + 74]^+$ at *m/z* 339 $[O + 74]^+$ and *m/z* 311 $[Po + 74]^+$, $[RCO]^+$ at *m/z* 265 $[O]^+$ and *m/z* 237 $[Po]^+$.

Further TAG were identified in a similar manner; the identification was often complicated by the coelution of several TAG. For instance two TAG, PLP, and SLM, were coeluted to form the peak at retention time 85.4 min. They, as well as other coeluted peaks, were identified by tandem MS, which clearly identified peaks of the $[DAG]^+$ type, i.e. $[PL]^+$, $[PP]^+$ at *m/z* 575 and *m/z* 551, or $[SL]^+$, $[LM]^+$, and $[SM]^+$ at *m/z* 603, *m/z* 547, and *m/z* 551.

TAG often occur as regioisomers, i.e. positional isomers on the glycerol backbone—see e.g. Mottram et al. [26]. Symmetrical TAG of the POP type were found to elute before the corresponding asymmetrical TAG such as PPO. For instance, Lin et al. [27] have described the separation of POP with a retention time of 33.12 min and PPO with 33.16 min. Similar results were obtained by Momchilova et al. [24], who obtained a nearly complete separation of PPO from POP with resolution 0.9. As described by Řezanka et al. [13, 20] and Schreiberova et al. [28], the separation of regioisomeric TAG as intact species by RP-HPLC is possible. As already noted [14, 29–31], the ratios of intensities of $[DAG]^+$ ions, i.e. $[M–RCOO]^+$ ions, make it possible to identify FA in the *sn*-2 position because the loss of FA in the *sn*-2 position is assumed to be energetically less favorable than the loss of FA from the *sn*-1 or *sn*-3 positions. We used this effect for identifying the three pairs of regioisomers above. The rule about the energetically less favorable loss of FA from position *sn*-2 and formation of $[M–RCOO]^+$ i.e. 1,3-$[DAG]^+$ ions was also applied to further bifunctional TAG such as other regioisomer pairs, see above. The cleavage of these bifunctional TAG gives rise to an isomeric pair of the same $[DAG]^+$ ions, i.e., $[AA]^+$ and $[AB]^+$. The ratio of $[AA]^+$:$[AB]^+$ was found to be lower for the ABA isomer, since formation of the 1,2-isomer of the $[AB]^+$ ion is energetically more favorable than generating the analogous 1,3-$[AB]^+$ ion from the AAB isomer. If these two fragments were energetically equivalent, then the intensity of ions $[AA]^+$ and $[AB]^+$ should be in a 1:2 ratio irrespective of the position of the A or B acyl substitution on the glycerol backbone; however, this was not found.

In the present study we examined three pairs of regioisomers of the most abundant TAG, i.e. *sn*-PoOPo (Fig. 4) and *sn*-PoPoO (Fig. 5), *sn*-PoPPo (Fig. 1S) and *sn*-PoPoP

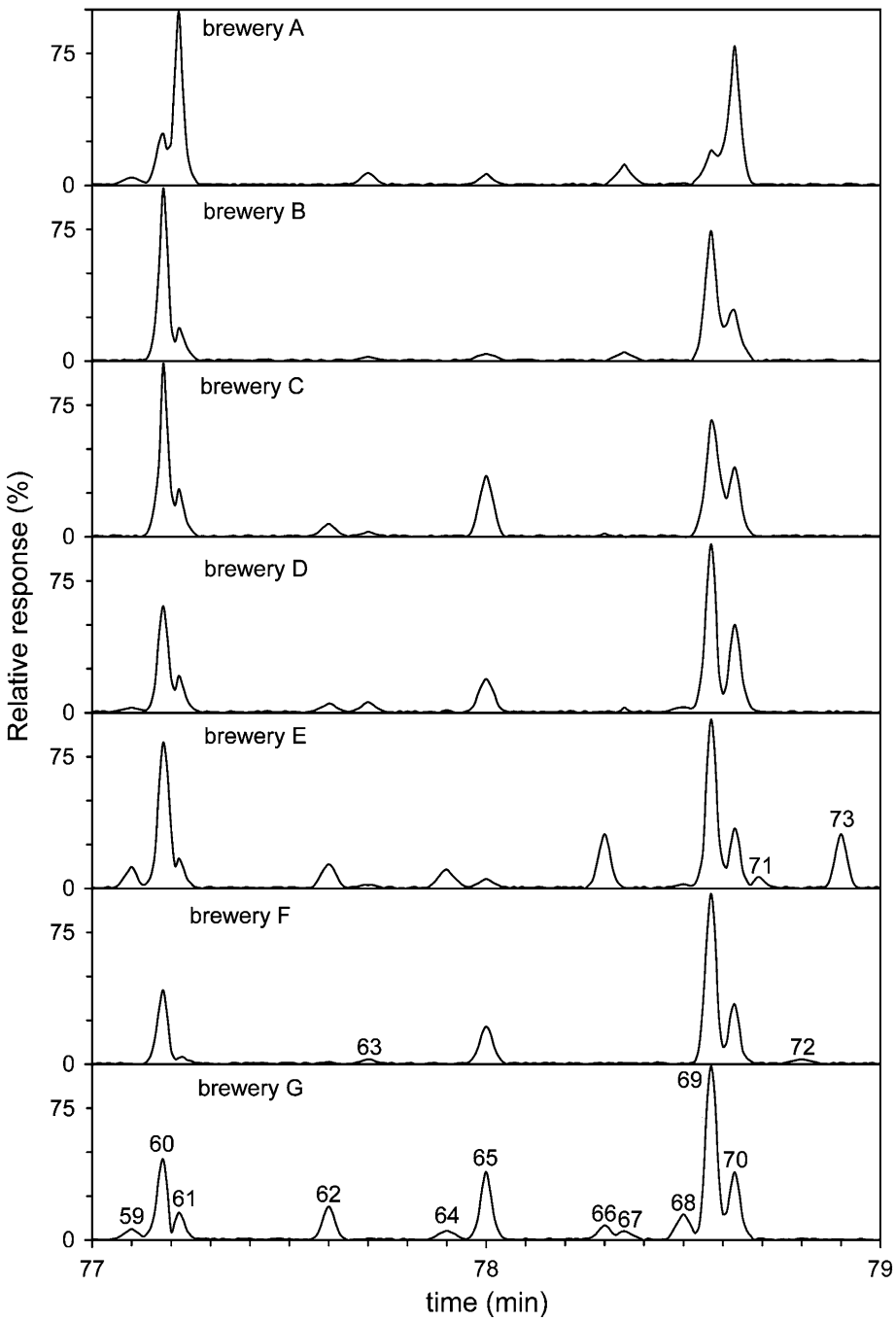

Fig. 3 Total ion monitoring in the interval from 77 to 79 min of TAG in yeast from the seven breweries

(Fig. 2S), *sn*-PPoP and *sn*-PPPo, see also Table 4. The least abundant $[M–RCOO]^+$ ion ($[DAG]^+$ ion, i.e. $[PoPo]^+$ at *m*/*z* 547), is formed by the loss of oleate from the *sn*-2 position. The two more abundant $[DAG]^+$ ions are due to the loss of palmitoleate from the *sn*-1 or *sn*-3 positions giving $[PoO]^+$ ion. The $[PoPo]^+$:$[PPo]^+$ ratio observed for PoPPo (Fig. 1S) is only 32:100. Consequently, the two regioisomers show two different spectra (Figs. 1S and 2S).

A similar situation was found in another pair of regioisomers. Two compounds, i.e. PPoP and PPPo, were separated with elution times 85.7 and 85.8 min, respectively. The mass spectra also demonstrate the structure of separated TAG. Different abundances of $[PP]^+$ and $[PPo]^+$ ions were again found, their ratio for PPoP being 35:100 and for PPPo 78:100.

Our results are in agreement with all these data. It should be however noted that in these studies the quantification was performed by tandem MS with electrospray ionization, usually without the use of standards. As already published, the length of the acyl chains of lipids including

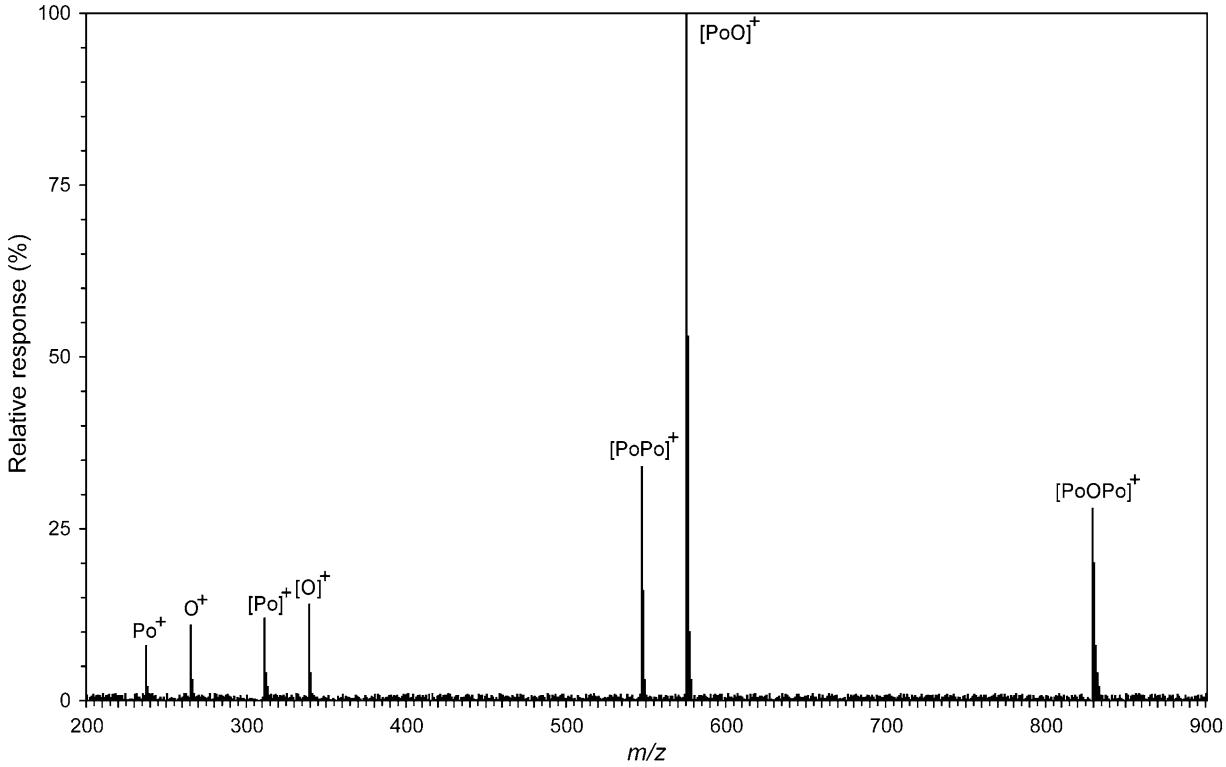

Fig. 4 Mass spectrum (positive APCI) of natural PoOPo

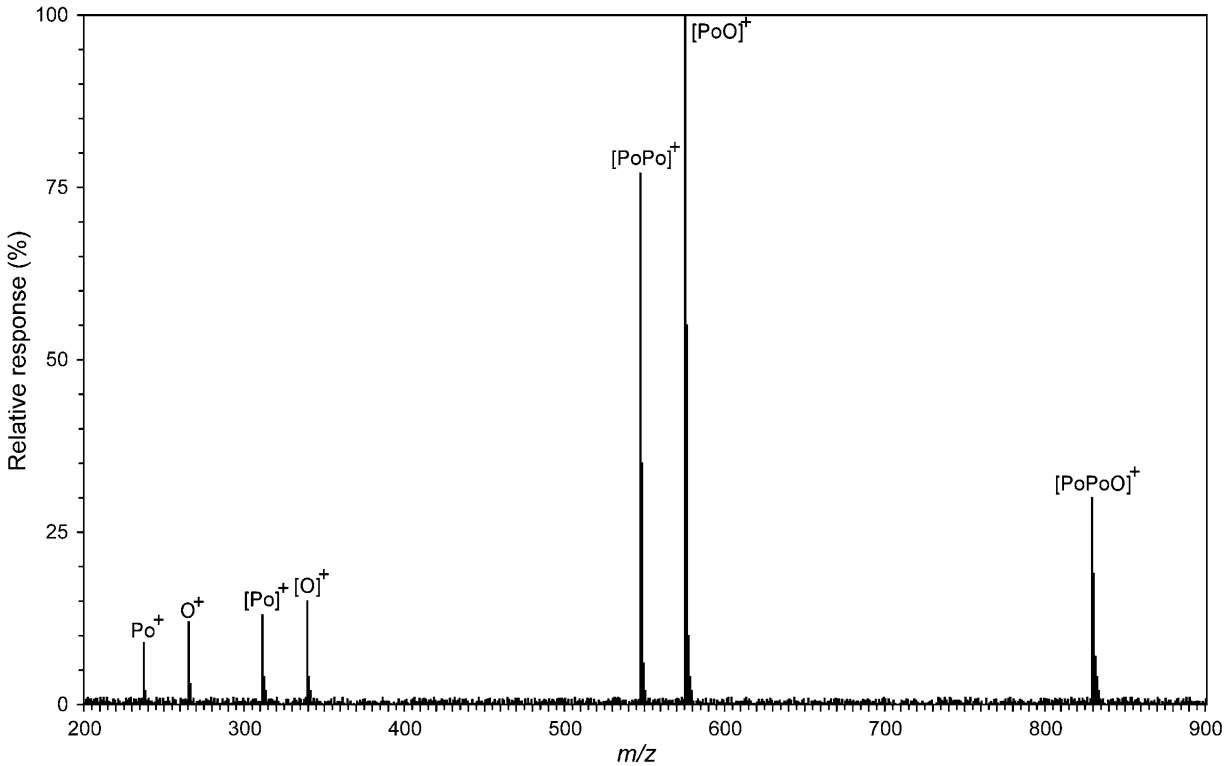

Fig. 5 Mass spectrum (positive APCI) of natural PoPoO

Table 4 The ratios of three TAG, i.e. *sn*-PoOPo/*sn*-PoPoO, *sn*-PoPPo/*sn*-PPoPo, and *sn*-PPoP/*sn*-PPPo in yeast from the seven breweries

Ratios of regioisomers	Brewery						
	A	B	C	D	E	F	G
PoOPo/PoPoO (sym/asym)	1:3	6:1	4:1	2:1	8:1	14:1	4:1
PoPPo/PPoPo (sym/asym)	1:3	3:1	2:1	2:1	3:1	3:1	2:1
PPoP/PPPo (sym/asym)	1:1	3:1	2:1	2:1	2:1	4:1	2:1

TAG strongly affects the response in mass spectrometer [11] and the results of the analyses have to be regarded with caution. The ratios given in Table 4 are likely influenced by beer production technology, i.e. top and bottom fermentation, fermentation temperature, and brewer's yeast generation. In addition, yeast strains in breweries have usually been maintained for a long time under physiological conditions widely different from those used in laboratory trials.

Despite these uncertainties the top and bottom yeasts can readily be distinguished from the ratios of some regioisomers of TAG. For instance, the ratio of the two regioisomers PoOPo and PoPoO is always <1 in top fermenting yeast and >1 in bottom yeast. The ratios are seen to closely copy the results of FA analysis. In the medium-sized breweries (E and F) the ratio of regioisomers PoOPo to PoPoO is nearly 10:1. Breweries (B, C, and G), all of which have a yearly beer output in excess of 200,000 hl, display a ratio of the regioisomers of 1:1–6:1. Minor differences may be caused by different technological processes during beer production, the quality of the raw materials such as malt, water, and hops, etc. The small brewery (D) shows the lowest ratio of the regioisomers whereas brewery (A) producing beer by top fermentation with *S. cerevisiae* instead of *S. pastorianus* has an inverse PoOPo to PoPoO ratio of 1:3. These ratios, and the ratios of other regioisomers, see Table 4, can thus be used as markers for determining the yeast type and beer-producing technology in a given brewery.

Conclusion

We identified both fatty acids and TAG in waste yeast from seven breweries. In these breweries, beer is produced by top (brewery A) and bottom (breweries B–G) fermentation. Based on the analysis of FA by GC–MS and analysis of TAG by LC–MS with APCI we can determine the type of brewing fermentation process, or the type of yeast used for fermentation. In agreement with the literature data, brewer′s yeast was found to contain FA with a broad range of acyl chain lengths. Analysis of intact TAG containing major FA such as palmitic, palmitoleic and oleic acid revealed that their composition depends not only on the type of yeast strain, i.e. *S. pastorianus* or *S. cerevisiae*, but also on the technology of beer production, i.e. top versus bottom fermentation. The different ratios of the above regioisomers of TAG offer a view of the biosynthesis of corresponding TAG and their participation in beer production.

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References

1. Yetukuri L, Ekroos K, Vidal-Puig A, Orešič M (2008) Informatics and computational strategies for the study of lipids. Mol BioSys 4:121–127
2. Daum G, Lees ND, Bard M, Dickson R (1998) Biochemistry, cell biology and molecular biology of lipids of *Saccharomyces cerevisiae*. Yeast 14:1471–1510
3. Hunter K, Rose AH (1972) Lipid composition of *Saccharomyces cerevisiae* as influenced by growth temperature. Biochim Biophys Acta 260:639–653
4. Bravi E, Perretti G, Buzzini P, Sera RD, Fantozzi P (2009) Technological steps and yeast biomass as factors affecting the lipid content of beer during the brewing a process. J Agric Food Chem 57:6279–6284
5. Jaakola S, Vahvaselkä M, Laakso S (2006) Positional distribution of conjugated linoleic acid in triacylglycerol of *Saccharomyces cerevisiae*. J Agric Food Chem 54:5611–5616
6. Athenstaedt K, Daum G (2003) YMR313c/TGL3 encodes a novel triacylglycerol lipase located in lipid particles of *Saccharomyces cerevisiae*. J Biol Chem 278:23317–23323
7. Oosthuizen A, Kock JLF, Botes PJ, Lategan PM (1987) The long-chain fatty acid composition of yeasts used in the brewing industry. Appl Microbiol Biotechnol 26:55–60
8. Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, Klemm RW, Simons K, Shevchenko A (2009) Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc Natl Acad Sci USA 106:2136–2141
9. Müllner H, Daum G (2004) Dynamics of neutral lipid storage in yeast. Acta Biochim Pol 51:323–347
10. Rajakumari S, Grillitsch K, Daum G (2008) Synthesis and turnover of non-polar lipids in yeast. Prog Lipid Res 47:157–171
11. Dembitsky VM, Rezanka T, Bychek IA, Shustov MV (1991) Identification of fatty-acids from *Cladonia* lichens. Phytochemistry 30:4015–4018
12. Rezanka T, Podojil M (1984) The very long-chain fatty-acids of the green-alga, *Chlorella kessleri*. Lipids 19:472–473
13. Rezanka T, Lukavsky J, Nedbalova L, Sigler K (2011) Effect of nitrogen and phosphorus starvation on the polyunsaturated triacylglycerol composition, including positional isomer distribution, in the alga *Trachydiscus minutus*. Phytochemistry 72:2342–2351
14. Byrdwell WC (2005) Qualitative and quantitative analysis of triacylglycerols by atmospheric pressure ionization (APCI and ESI) mass spectrometry techniques. In: Byrdwell WC (ed) Modern methods for lipid analysis by liquid chromatography/mass spectrometry and related techniques. AOCS Press, Champaign, pp 298–412

15. Klose C, Surma MA, Gerl MJ, Meyenhofer F, Shevchenko A, Simons K (2012) Flexibility of a eukaryotic lipidome—insights from yeast lipidomics. PLoS ONE 7:e35063
16. Shui G, Guan XL, Low CP, Chua GH, Goh JSY, Yang H, Wenk MR (2010) Toward one step analysis of cellular lipidomes using liquid chromatography coupled with mass spectrometry: application to *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* lipidomics. Mol BioSyst 6:1008–1017
17. Grillitsch K, Connerth M, Köfeler H, Arrey TN, Rietschel B, Wagner B, Karas M, Daum G (2011) Lipid particles/droplets of the yeast *Saccharomyces cerevisiae* revisited: lipidome meets Proteome. Bioch Biophys Acta 1811:1165–1175
18. Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS (2008) Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell 134:933–944
19. Wu Y, Li R, Hildebrand DF (2012) Biosynthesis and metabolic engineering of palmitoleate production, an important contributor to human health and sustainable industry. Prog Lipid Res 51:340–349
20. Rezanka T, Schreiberova O, Krulikovska T, Masak J, Sigler K (2010) RP-HPLC/MS-APCI analysis of odd-chain TAGs from *Rhodococcus erythropolis* including some regioisomers. Chem Phys Lipids 163:373–380
21. Welch JW, Burlingame AL (1973) Very long chain fatty acids in yeast. J Bacteriol 115:464–466
22. Lisa M, Netusilova K, Franek L, Dvorakova H, Vrkoslav V, Holcapek M (2011) Characterization of fatty acid and triacylglycerol composition in animal fats using silver-ion and non-aqueous reversed-phase high-performance liquid chromatography/mass spectrometry and gas chromatography/flame ionization detection. J Chromatogr A 1218:7499–7510
23. Perona JS, Ruiz-Gutierrez V (1999) Characterization of the triacylglycerol molecular species of fish oil by reversed-phase high performance liquid chromatography. J Liq Chromatogr Relat Technol 22:1699–1714
24. Momchilova S, Tsuji K, Itabashi Y, Nikolova-Damyanova B, Kuskis A (2004) Resolution of triacylglycerol positional isomers by reversed-phase high-performance liquid chromatography. J Sep Sci 27:1033–1036
25. Mu H, Hoy E (2000) Application of atmospheric pressure chemical ionization liquid chromatography-mass spectrometry in identification of lymph triacylglycerols. J Chromatogr B 748: 425–437
26. Mottram HR, Crossman ZM, Evershed RP (2001) Regiospecific characterisation of the triacylglycerols in animal fats using high performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry. Analyst 126:1018–1024
27. Lin J-T, Woodruff CL, McKeon TA (1997) Non-aqueous reversed-phase high-performance liquid chromatography of synthetic triacylglycerols and diacylglycerols. J Chromatogr A 782:41–48
28. Schreiberova O, Krulikovska T, Sigler K, Cejkova A, Rezanka T (2010) RP-HPLC/MS-APCI analysis of branched chain TAG prepared by precursor-directed biosynthesis with *Rhodococcus erythropolis*. Lipids 45:743–756
29. Mottram HR (2005) Regiospecific analysis of triacylglycerols using high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. In: Byrdwell WC (ed) Modern Methods for Lipid Analysis by Liquid Chromatography/Mass Spectrometry and Related Techniques. AOCS Press, Champaign, pp 276–297
30. Fauconnot L, Hau J, Aeschlimann JM, Fay LB, Dionisi F (2004) Quantitative analysis of triacylglycerol regioisomers in fats and oils using reversed-phase high-performance liquid chromatography and atmospheric pressure chemical ionization mass spectrometry. Rapid Commun Mass Spectrom 18:218–224
31. Leskinen H, Suomela J-P, Kallio H (2007) Quantification of triacylglycerol regioisomers in oils and fat using different mass spectrometric and liquid chromatographic methods. Rapid Commun Mass Spectrom 21:2361–2373
32. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917