Fatty Acid Distribution in Triglycerides of Yeasts Grown on Glucose or *n*-Alkanes

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SUMMARY

Lipid contents of yeasts grown on glucose were: *Candida lipolytica*, 5.4 %; *C. tropicalis*, 9.4 %; *C. utilis*, 2.7 %; *Candida* 107, 41 %; *Hansenula anomala*, 12.5 %; *Rhodotorula glutinis*, 2.7 %; and *R. graminis*, 9.1 %. In each yeast about 80 % of the lipid consisted of triglycerides. When the triglycerides from five of the yeasts were analysed in detail, an unsaturated acid was invariably found at the 2-position. With *Candida* 107 and *R. graminis* about 50 % of the total triglyceride fatty acids were saturated, resulting in over 50 % of the triglycerides being of the 1,3-disaturated-2-monounsaturated type. When *Candida* 107 and *C. tropicalis* were grown on individual *n*-alkanes, from C₁₂ to C₁₆, the fatty-acid composition varied according to the chain length of the substrate, although with *n*-tridecane neither yeast produced tridecanoic acid in the triglyceride and with *n*-dodecane only *C. tropicalis* contained an appreciable amount of dodecanoic acid in the triglyceride (32 % of the fatty acids). With both yeasts on each alkane substrate, the lipid contents were not only lower than when grown on glucose but contained a smaller proportion of triglyceride. Saturated acids were now located at the 2-position of the triglycerides: *Candida* 107 grown on *n*-tetradecane produced 46 % of its triglycerides with a saturated acid at the 2-position. The main advantage to be gained by growing yeasts on *n*-alkanes is, as far as lipid formation is concerned, the biosynthesis of specific fatty acids rather than the production of plant-like triglycerides.

INTRODUCTION

The distribution of fatty acids in the triglycerides obtained from plant oils and animal fats is known in some detail (Jurriens, 1968; Fedeli & Jacini, 1971). However, little work has been done on the composition of triglycerides derived from micro-organisms, presumably because microbial fats are not considered to be an economic source of fat (Ratledge, 1970, 1971). The present investigation was undertaken to gain knowledge in this neglected area, being stimulated by earlier work of Harries & Ratledge (1969) who found that the distribution of fatty acids in the triglyceride fraction of a species of *Candida* grown on *n*-alkanes is such that the 2-position of the triglyceride is almost exclusively occupied by unsaturated acids. Although this is typical of most plant oils (Fedeli & Jacini, 1971), the lipid of this yeast had a high proportion of symmetrical disaturated glycerides such as is found in only the commercially expensive cocoa and illipé butters (Coleman, 1963).

METHODS

Organisms and growth. The following yeasts were used: *Candida lipolytica* NCYC153, *C. tropicalis* NCYC4, *C. utilis* NCYC359, *Hansenula anomala* NCYC18, *Rhodotorula glutinis* NCYC59, *R. graminis* NCYC502. Also used was an untyped species of *Candida*, referred to as

Candida 107, which has been used in previous studies related to lipid formation (see Ratledge, 1970).

For growth of yeasts on glucose, a medium was used containing: KH_2PO_4 , 2 g; $(\text{NH}_4)_2\text{SO}_4$, 0.5 g; glucose (sterilized separately), 25 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; yeast extract, 0.5 g; distilled water to 1 l with the pH value adjusted to 5.5 with NaOH. Cultures were grown in 4 l conical flasks containing 1.5 l medium on a gyrotary shaker at 28 °C. *Candida* 107, and *C. tropicalis* were also grown on a medium containing: $(\text{NH}_4)_2\text{SO}_4$, 2 g; K_2HPO_4 , 5.0 g; NaCl, 0.5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.1 g; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.5 g; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.01 g; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 5 mg; yeast extract, 0.5 g; distilled water to 1 l and the pH value adjusted to 5.5. The carbon source was either glucose at 25 g/l or an *n*-alkane (C_{12} to C_{16}) at 10 % (v/v) final concentration. Cultures were grown at 30 °C in 1 l vortex-aerated fermenters similar in concept to that described by Harvey, Fewson & Holms (1969).

Yeasts were harvested by centrifuging at 500 g for 20 min. After washing with water the yeast paste was freeze-dried.

Extraction and fractionation of lipid. The method of Folch, Lees & Sloane-Stanley (1957) was used to extract lipid from the freeze-dried yeast. The chloroform + methanol (2:1, v/v) extract, after filtering from the yeast residue, was evaporated almost to dryness under reduced pressure and then partitioned between equal volumes of chloroform and water. The chloroform layer was evaporated and the residue weighed. This lipid extract was fractionated quantitatively by column chromatography on silicic acid according to the procedure of Barron & Hanahan (1958).

Distribution of fatty acids in triglycerides. Triglycerides were purified where necessary by thin-layer chromatography on 0.5 mm silica-gel plates in petroleum ether (b.p. 40 to 60 °C) + diethyl ether + formic acid (85:15:1, by vol.) according to Nichols & James (1964). The technique of lipolysis as described by Coleman & Fulton (1961) was used to compare the fatty acids at the 2-position of the glyceride molecules with the total fatty acids. Monoglycerides were recovered by preparative thin-layer chromatography on 0.5 mm silica-gel plates, developed with *n*-hexane + diethyl ether + chloroform + acetic acid (40:55:5:0.2, by vol.; Desnuelle, Sari & Entressangles, 1966). Formation of 1-monoglycerides was checked by thin-layer chromatography on 5 % boric acid-impregnated silica-gel G plates developed with chloroform + acetone + methanol + acetic acid (71:25:4:0.13, by vol.; Thomas, Scharoun & Ralston, 1965).

The fatty acid composition of the isolated monoglycerides and triglycerides was determined by gas chromatography (using a Wilkens 'Aerograph', Model 600-C, Wilkens Instrument & Research Inc., P.O. Box 313, Walnut Creek, California, U.S.A.) of the methyl esters on a column (5 ft $\times$ $\frac{1}{8}$ in) packed with diethylene glycol succinate at 185 °C. Eluted compounds were identified by comparison of retention times with standard compounds. Relative proportions of fatty acids were calculated from the peak areas estimated from peak height $\times$ peak width at $\frac{1}{2}$ peak height. Methyl esters of fatty acids were formed directly from the lipid by deacylating with 0.2 N-sodium methoxide for 1 h at room temperature under N_2 (Marinetti, 1962). Triglyceride compositions were calculated from lipolysis data according to the 1,3-random, 2-random distribution hypothesis of Vander Wal (1960) and Coleman & Fulton (1961) (see also Coleman, 1963, and Vander Wal, 1964). This hypothesis assumes that the fatty acids are distributed randomly between the 1- and 3-positions so that, knowing the fatty acids of the whole triglycerides and the fatty acids on the 2-position of the glyceride molecule, the triglyceride composition can be calculated.

Fractionation of triglycerides on the basis of unsaturation. This was accomplished by thin-layer chromatography on 12.5 % AgNO_3 - silica-gel plates developed with chloroform +

methanol (99.5:0.5, v/v; Barrett, Dallas & Padley, 1962). Plates were sprayed with a 0.1 % alcoholic solution of Rhodamine 6G and spots visualized under u.v. radiation. Quantitative separation and estimation of each triglyceride class after elution of the bands with 5 % methanol in ether (2 × 2 ml) was then made with a hydroxamic acid procedure (Stern & Shapiro, 1953).

Chemicals. Individual *n*-alkanes, 99.9 % purity based on gas-liquid chromatography, were a generous gift from B.P. Chemicals International Ltd, Sunbury-on-Thames. Other chemicals including chromatography solvents were of highest grade commercially available and were not purified further. Pancreatic lipase was obtained from Koch-Light Laboratories, Colnbrook, Buckinghamshire.

RESULTS

Yeasts grown on glucose. The lipid contents of seven yeasts grown on glucose in shake-culture varied from 41 % to 2.7 % of the total dry weight (Table 1). Fractionation of each lipid by column chromatography on silicic acid showed that the triglyceride + free fatty acids fraction was always the largest component (Table 1). The amount of free fatty acids in this fraction was always very low, as judged from subsequent thin-layer chromatography of this fraction (probably less than 5 % of the amount of triglyceride) except for *Rhodotorula glutinis* which contained a higher proportion of fatty acids (probably about 20 to 30 % of the triglyceride amount). The appearance of free fatty acids in these lipid fractions is probably an artefact caused by action of the lipases of the organism itself during extraction (Barron & Hanahan, 1958; Wills, 1965).

Subsequent analysis of the distribution of fatty acids in triglycerides from five of these yeasts indicated that little, if any, saturated fatty acid was incorporated into the 2-position of the triglyceride (Table 2). The fatty-acid compositions of the triglycerides from *Candida lipolytica* changed between the exponential and stationary phases of growth (Table 2).

From the data of Table 2 the triglyceride compositions were calculated (Table 3): with *Candida* 107 and *Rhodotorula graminis* the largest fractions were 1,3-disaturated-2-unsaturated glycerides because of the relatively high proportion of saturated acids in the total triglyceride.

Yeasts grown on n-alkanes. The appearance of fatty acids in the lipids of micro-organisms of the same chain-length as an *n*-alkane growth substrate (Davis, 1964; Ratledge, 1968) created the possibility of producing acids which are not usually found in microbial lipids. Such acids are those of a short chain-length (C_{12} and C_{14}) or having an odd number of carbon atoms. Accumulation of both these types of acid has already been reported (Ratledge, 1970).

Of the yeasts grown on glucose, two were subsequently used for growth on individual *n*-alkanes from dodecane to hexadecane.

1. *Candida* 107. This yeast grew very slowly in shake-cultures with individual *n*-alkanes from C_{12} to C_{16} but, even in a vortex-aerated fermenter, the growth rate and final yield of biomass were less than those attainable with glucose as carbon source (Table 4). Lipid extracted with chloroform + methanol (2:1, v/v) always contained some residual hydrocarbon which added to the apparent lipid content of the organism and was removed by subsequent column chromatography.

Fractionation of the lipids from the organism grown on the various alkanes showed that, although the triglycerides still constituted the major fraction, their relative proportion was less than when glucose was the growth substrate. Loss in triglycerides was compensated for by corresponding increases in the phospholipids (Table 4). The fatty acids of the whole

Table 1. *Lipid composition of yeasts grown on glucose in shake culture*

Yeast	Growth phase at harvesting	Yield of organism (g dry wt/l)	Total lipid (% dry wt)	Relative percentage composition of lipid			
				Sterol esters	Triglycerides* + free fatty acids	Sterol + di- and mono- glycerides	Phospholipids
<i>Candida</i> 107	Stationary	3.40	41.05	0.5	83.3	16.2	
<i>C. utilis</i>	Stationary	2.53	2.72	3.2	60.2	36.6	
<i>C. lipolytica</i>	Log	0.65	5.39	2.9	54.2		
<i>C. lipolytica</i>	Stationary	3.74	3.52	0.8	74.8	14.6	9.6
<i>C. tropicalis</i>	Stationary	2.09	9.41	5.5	61.2	32.9	
<i>Hansenula anomala</i>	Stationary	1.13	12.51	14.7	76.5	2.6	6.6
<i>Rhodotorula glutinis</i>	Stationary	2.68	3.18	6.9	56.9	15.6	20.6
<i>R. graminis</i>	Stationary	4.20	9.07	1.7	87.0	4.6	6.7

* Except for *R. glutinis*, this fraction contained very little free fatty acid (see text).

Table 2. Distribution of fatty acids in whole triglycerides and at the 2-position after lipolysis from yeasts grown on glucose

		Mole % of fatty acid												Unsaturated acids (% total)
		12:0	13:0	14:0	15:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	
<i>Candida</i> 107	Triglyceride	-	-	1.5	-	36.7	3.8	-	-	8.7	43.1	6.2	-	53.1
	2-Position	-	-	Trace	-	3.0	Trace	-	-	Trace	86.0	11.0	-	97.0
	Percentage of acid in 2-position	-	-	-	-	2.9	-	-	-	-	66.6	59.1	-	-
<i>C. utilis</i>	Triglyceride	0.3	-	Trace	-	15.7	0.3	-	-	5.6	57.4	20.7	Trace	78.4
<i>C. lipolytica</i> (exponential phase)	Triglyceride	-	-	0.4	0.5	17.3	18.1	Trace	1.3	4.0	41.8	16.6	-	77.8
<i>C. lipolytica</i> (stationary phase)	Triglyceride	Trace	Trace	0.7	Trace	11.7	8.5	2.2	2.3	7.7	27.9	26.1	12.9	77.7
	2-Position	Trace	-	Trace	-	1.6	1.3	Trace	Trace	Trace	61.2	30.7	5.2	98.4
<i>C. tropicalis</i>	Percentage of acid in 2-Position	-	-	-	-	4.6	3.7	-	-	-	73.1	39.2	13.4	-
	Triglyceride	Trace	Trace	0.7	Trace	10.3	7.5	1.0	0.9	6.6	35.5	23.4	14.1	81.4
	2-Position	Trace	Trace	3.2	-	Trace	-	-	-	Trace	60.8	36.0	-	96.8
<i>Hansenula anomala</i>	Percentage of acid in 2-position	-	-	100	-	-	-	-	-	-	57.1	51.3	-	-
	Triglyceride	-	-	Trace	-	19.8	2.0	0.9	-	2.4	48.6	26.3	-	76.9
	2-Position	-	-	-	-	2.0	-	-	-	-	66.4	31.6	-	98.0
<i>Rhodotorula glutinis</i>	Percentage of acid in 2-position	-	-	-	-	3.3	-	-	-	-	45.7	39.4	-	-
	Triglyceride	-	-	0.8	-	11.9	2.3	0.5	0.4	7.3	50	20.6	6.2	79.5
	2-Position	Trace	Trace	1.0	-	28.3	1.4	-	-	23.0	43.2	3.1	-	47.7
<i>R. graminis</i>	Percentage of acid in 2-position	-	-	1.4	-	3.6	-	-	-	2.3	79.8	12.9	-	92.5
		-	-	38	-	4.2	-	-	-	3.3	62	100	-	-

Table 3. *Calculated triglyceride compositions in yeasts grown on glucose to stationary phase*

Yeast	Triglyceride fraction*					
	SSS	SSU	SUS	USU	SUU	UUU
<i>Candida</i> 107	1.4	1.4	50.0	0.3	39.0	7.9
<i>C. lipolytica</i>	0.3	1.0	11.9	0.8	44.2	41.6
<i>C. tropicalis</i>	0.3	1.2	7.0	1.6	38.0	51.9
<i>Hansenula anomala</i>	0.3	0.8	11.2	0.8	44.0	42.9
<i>Rhodotorula graminis</i>	4.0	2.0	52.7	0.5	35.0	5.8

* Triglycerides are classed as trisaturated (SSS); 1,2-disaturated-3-monounsaturated glycerides (SSU); 1,3-disaturated-2-monounsaturated glycerides (SUS); 1,3-diunsaturated-2-monounsaturated glycerides (USU); 1,2-diunsaturated-3-monounsaturated glycerides (SUU); and triunsaturated (UUU). The unsaturated acids, palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids are not distinguished.

triglycerides and in the 2-position were determined except when tridecane had been used as growth substrate (Table 5). As may have been expected, the fatty acid composition varied according to the particular *n*-alkane used as growth substrate. Surprisingly, however, the triglycerides from the yeast grown on *n*-tetradecane and *n*-pentadecane contained appreciable amounts of saturated acids in the 2-position so that, upon calculating the triglyceride composition (Table 6), a much higher content of trisaturated triglyceride than usual was indicated.

2. *Candida tropicalis*. This yeast grew faster than *Candida* 107 on all *n*-alkanes used, attaining a high final yield but always with a lower lipid content (Table 7). The proportion of triglyceride in the lipid was generally low. This was compensated for by an increase in sterol content but with *n*-pentadecane as substrate over 50 % of the total lipid was phospholipid (Table 7). The fatty acids in the triglyceride molecules, like those of *Candida* 107, varied according to the chain-length of the *n*-alkane growth substrate (Table 8). There was one notable difference between the two yeasts in that, with *n*-dodecane as substrate, *C. tropicalis* produced 32 % lauric acid within the triglyceride whereas with *Candida* 107 the amount was less than 1 %. From the fatty acid composition at the 2-position in the triglycerides (Table 8) the triglyceride compositions could be calculated (Table 9). Sufficient material to enable accurate analyses to be completed was available only with the triglycerides extracted from *C. tropicalis* grown on *n*-tetradecane, *n*-pentadecane and *n*-hexadecane.

Verification of 1,3-random-2-random distribution hypothesis. There was good agreement between the experimental values obtained for *Candida* 107 growth on either glucose or *n*-tetradecane and those calculated according to the hypothesis advanced independently by Vander Wal (1960) and Coleman & Fulton (1961) (Table 10). As this hypothesis has already been verified with many different types of animal and plant triglycerides (Coleman, 1963; Vander Wal, 1964) and is also applicable to the yeast *Candida* 107 grown on two different types of substrate, we have not repeated this procedure of verification with the other yeasts studied here.

DISCUSSION

Five yeasts, from three genera, when grown on glucose, produced triglycerides containing almost entirely unsaturated fatty acids in the 2-position. This is similar to triglycerides from plant sources (Vander Wal, 1964). *Rhodotorula graminis* and *Candida* 107 produced an unusually high proportion of saturated fatty acids in the lipid (52 % and 46 % respectively) which, therefore, led to a high content of 1,3-disaturated-2-monounsaturated triglyceride in

Table 4. Yield and lipid content of *Candida* 107 grown on individual *n*-alkanes and glucose in a vortex fermenter for 5 days

Substrate	Yield of biomass (g dry wt/l)	Total lipid (% dry wt)	Total lipid — intracellular hydrocarbon (% dry wt)	Relative percentage composition of lipid after <i>n</i> -alkane removal			
				Sterol esters	Triglycerides + free fatty acids	Sterol + partial glycerides	Phospholipids
<i>n</i> -Dodecane	0.67	15	15	1	56	16	27
<i>n</i> -Tridecane	0.84	30	29	4	51	22	23
<i>n</i> -Tetradecane	0.82	37	26	8	61	17	14
<i>n</i> -Pentadecane	1.3	25	22	6	58	18	18
<i>n</i> -Hexadecane	1.2	19	10	7	53	20	20
Glucose	4.2	42	42	2	72	21	5

Table 5. *Distribution of fatty acids in whole triglycerides and at the 2-position of the triglyceride (see Table 4) after lipolysis from Candida 107 grown on n-alkanes and glucose in a vortex fermenter for 5 days*

		Mole % of fatty acid														Unsaturated acids (% total)	Odd-chain acids (% total)
		12:0	13:0	13:1(?)	14:0	14:1	15:0	15:1	16:0	16:1	17:0	17:1	18:0	18:1	18:2		
Dodecane	Triglyceride 2-Position	0.3	—	2.2	1.1	—	—	—	14.1	2.9	—	—	6.1	17.8	55.5	78.4	2.2
	Percentage of acid in 2-position	1.7	—	20.0	2.9	—	—	—	10.0	—	—	—	—	25.2	40.2	85.4	—
Tridecane	Triglyceride	100	—	100	88	—	—	—	24	—	—	—	—	47	24	—	—
	Triglyceride 2-Position	Trace	11.4	2.7	1.0	—	1.1	0.2	19.3	Trace	1.6	Trace	7.6	12.9	42.2	58.0	17.0
Tetradecane	Triglyceride 2-Position	Trace	Trace	—	45.4	6.9	—	—	12.4	Trace	—	—	Trace	11.0	24.3	42.2	0
	Percentage of acid in 2-position	Trace	—	—	29.3	7.8	—	—	17.0	3.9	—	—	—	32.2	9.8	53.7	0
Pentadecane	Triglyceride 2-Position	—	—	—	24	38	—	—	46	—	—	—	—	99	13	—	—
	Percentage of acid in 2-position	0.3	0.3	1.3	0.4	—	38.2	—	7.2	Trace	5.2	1.8	2.1	15.9	27.3	46.3	46.8
Hexadecane	Triglyceride 2-Position	0.5	—	—	—	—	27.6	—	—	—	—	5.8	—	41.4	24.7	71.9	33.4
	Percentage of acid in 2-position	56	—	—	—	—	24	—	—	—	—	100	—	88	30	—	—
	Triglyceride 2-Position	0.8	Trace	—	1.6	Trace	—	—	19.4	3.1	—	—	4.5	17.1	53.5	73.7	0
	Percentage of acid in 2-position	—	—	—	—	—	—	—	21.5	4.1	—	—	—	36.6	37.8	78.5	0
Glucose	Triglyceride 2-Position	—	—	—	—	—	—	—	37	44	—	—	—	72	24	—	—
	Percentage of acid in 2-position	Trace	Trace	—	2.7	Trace	—	—	44.3	4.6	—	—	8.4	31.1	8.9	44.6	0
	Percentage of acid in 2-position	Trace	—	—	Trace	—	—	—	3.5	Trace	—	—	—	66.5	30.0	96.5	0
		—	—	—	—	—	—	—	3	—	—	—	—	71	100	—	—

Table 6. *Calculated triglyceride compositions of Candida 107 grown on n-alkanes*

Derived from data given in Table 5.

	Triglyceride fraction*					
	SSS	SSU	SUS	USU	SUU	UUU
Dodecane	1.0	6.0	5.3	8.3	31.8	48.0
Tetradecane	18.9	22.9	22.1	6.2	28.5	1.4
Pentadecane	13.1	12.3	34.5	2.8	30.5	6.8
Hexadecane	1.7	8.8	6.0	11.5	31.7	40.3
Glucose	2.6	1.6	61.2	0.1	30.8	6.3

* Designations as given in Table 3.

Table 7. *Yield and lipid content of Candida tropicalis grown on individual n-alkanes in a vortex fermenter for 5 days*

Substrate	Yield of biomass (g dry wt/l)	Total lipid (% dry wt)	Total lipid intracellular hydrocarbon (% dry wt)	Relative percentage composition of lipid after <i>n</i> -alkane removal			
				Sterol esters	Triglycerides + free fatty acids	Sterol + partial glycerides	Phospholipids
Dodecane	6.4	0.8	0.8	9	26	44	21
Tridecane	6.9	14.7	2.4	13	35	28	24
Tetradecane	9.2	5.9	1.9	9	36	30	25
Pentadecane	10.1	16.4	2.3	2	20	26	52
Hexadecane	10.4	17.9	6.3	3	51	20	26

the triglyceride fraction (53 % and 50 % respectively). Although this particular triglyceride fraction has only previously been found in considerable proportions in seed oils such as cocoa butter and illipé butter (Coleman, 1963; Jurriens, 1968), in which over 60 % of the total triglyceride acids are saturated acids (Coleman, 1961; Coleman, 1963), there is no reason to assume that other yeasts containing a similarly high content of saturated acids would not likewise have a high proportion of this particular triglyceride fraction. Unfortunately, few yeasts contain more than about 20 to 30 % saturated acids (see Shaw, 1966; Johnson & Brown, 1972; Johnson, Nelson & Brown, 1972) and of the yeasts that are 'oleaginous' (a term usually applied to vegetable seeds or fruit implying an oil content of at least 15 to 20 %; Fedeli & Jacini, 1971) *Candida 107* seems unique in this respect. *Rhodotorula gracilis*, considered to be one of the highest fat-yielding yeasts (Woodbine, 1959), contains only at best about 30 % saturated acids (Kessell, 1968). Some other species of *Rhodotorula*, including *R. graminis* studied here, do, however, contain a much higher proportion of saturated acids (Holmberg, 1948; Bass & Hospodka, 1952; Hartman, Hawke, Shorland & diMenna, 1959) but do not have as high a lipid content as *R. gracilis* (Woodbine, 1959; Hunter & Rose, 1971).

When grown on *n*-alkanes, *Candida 107* and *C. tropicalis* produced less lipid than when grown on glucose but this may only be a reflexion of method of cultivation as lipid contents higher than those obtained when grown on glucose have already been reported for *Candida 107* grown in a stirred fermenter (Ratledge, 1968). The increase in phospholipids in alkane-grown yeasts compared with glucose-grown yeasts probably reflects differences in membrane

Table 8. *Distribution of fatty acids in whole triglycerides and at the 2-position of the triglyceride (see Table 7) after lipolysis from Candida tropicalis grown on n-alkanes in a vortex fermenter*

		Mole % of fatty acid															Unsaturated acids (% total)	Odd-chain acids (% total)
		11:0	12:0	13:0	13:1	14:0	14:1	15:0	15:1	16:0	16:1	17:0	17:1	18:0	18:1	18:2		
Dodecane	Triglyceride	1.3	31.8	—	—	1.0	—	Trace	—	9.0	12.7	—	1.0	Trace	20.2	23.0	56.9	2.3
	Triglyceride	—	10.4	—	1.9	3.1	—	28.5	Trace	3.1	1.9	5.6	35.1	Trace	10.4	—	49.3	71.1
Tetradecane	Triglyceride	Trace	16.0	—	—	19.0	—	—	—	14.9	16.8	—	—	Trace	14.3	19.0	50.0	0
	2-position	—	—	—	—	3.7	—	—	—	4.7	26.2	—	—	—	46.7	18.7	91.6	0
Percentage of acid in 2-position		—	—	—	—	6	—	—	—	10	52	—	—	—	100	32	—	—
Pentadecane	Triglyceride	—	Trace	—	—	—	—	32.1	1.9	2.5	Trace	5.8	46.7	—	10.8	—	59.4	86.7
	2-Position	—	—	—	—	—	—	12.7	5.6	—	12.7	—	56.3	—	12.7	—	81.7	74.4
Percentage of acid in 2-position		—	—	—	—	—	—	13	100	—	100	—	43	—	42	—	—	—
Pexadecane	Triglyceride	—	4.3	—	—	0.9	—	—	—	39.0	26.0	—	—	—	18.2	11.6	55.8	0
	2-Position	—	—	—	—	—	—	—	—	10.5	36.1	—	—	—	42.7	10.7	89.5	0
Percentage of acid in 2-position		—	—	—	—	—	—	—	—	9	46	—	—	—	78	31	—	—

Table 9. *Calculated triglyceride compositions of Candida tropicalis grown on n-alkanes*

Derived from data given in Table 8.

Substrate	Triglyceride fraction*					
	SSS	SSU	SUS	USU	SUU	UUU
Tetradecane	3.6	3.0	46.5	0.6	38.2	8.1
Pentadecane	4.5	9.0	21.0	4.5	41.0	20.0
Hexadecane	3.7	4.6	33.4	1.6	43.0	13.7

* Designations as in Fig. 3.

Table 10. *Comparison of calculated triglyceride composition (from lipolysis data) and experimental triglyceride composition after separation of triglycerides according to number of double bonds present by AgNO₃-thin-layer chromatography*

<i>Candida</i> 107 grown on glucose in vortex fermenter				<i>Candida</i> 107 grown on <i>n</i> -tetradecane in vortex fermenter				
Calculated (%)		Experimental (%)		Calculated (%)		Experimental (%)		
SSS	2.6	SSS	5	SSS	18.9	SSS	23	
SOS	42.0	SOS } SSO } 45	45	SOS	18.0	SOS } SSO } 19	19	
SSO	1.6			SSO	2.9			
SOO	21.2	SOO } SLS } 32	32	OSO	0.1	OSO } SOO } 20	20	
SLS	19.2			SOO	2.8			
OSO	0.1			OSO	4.1			
OOO	2.6	OOO } SLO } 12	12	SSL	18.4			OOO } SOL } 22
SLO	9.6			OOO	0.4			
*Others	3.7	*Others	6	SOL	17.5	SLO } SLL } 14.1	16	
				SLO	2.6			
				SLL	4.0			
				LSL	6.1			
				LLL	1.0			

* Denotes triglycerides having four or more double bonds.

Designations of triglycerides as given in Table 3; additionally triglycerides including 'O' and 'L' indicate the presence of oleic and linoleic acids, respectively, in the positions indicated.

structures. Alkane-grown yeasts have deep invaginations in the cytoplasmic membrane (Ludvik, Munk & Dostalek, 1968) and therefore a higher amount of phospholipids will be needed to maintain its integrity. The wide variation in fatty acid composition in the alkane-grown yeasts can be seen as an attempt to produce triglycerides with acceptable physiological properties, such as melting point, crystal structure and mutual solubilities, to be stored within the fat vacuoles of the yeast. With these substrates, both yeasts contained a significant proportion of triglycerides in which the 2-position was occupied by a saturated acid. This was most evident for *Candida* 107 grown on *n*-tetradecane and *n*-pentadecane, where, although the proportion of unsaturated acids was the same as that when glucose had been used as carbon source, 46% and 28% respectively of the total triglycerides contained a saturated acid in the 2-position. In this respect, the various triglyceride fractions were similar in proportion to those observed in animal fats such as beef tallow (Vander Wal, 1960). Such a commodity has a much lower commercial value than that of many vegetable oils, particularly cocoa and illipé butters (Ratledge, 1970).

As the quality and amount of the triglycerides from the two yeasts grown on *n*-alkanes were less than when the yeasts had been grown on glucose, the main advantage to be gained by growing yeasts on *n*-alkanes is, as far as lipid formation is concerned, the biosynthesis of specific fatty acids rather than the production of plant-like triglycerides.

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