THE COMPONENT FATTY ACIDS OF A YEAST FAT

by

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INTRODUCTION

Few reports have been published with regard to the proportions of the fatty acids present in yeast lipids. In 1933 NEWMAN and ANDERSON[1], in the course of an examination of the lipids of baker's yeast, observed that their saturated fatty acids were made up of 75% of palmitic acid and 25% of stearic acid; but that the unsaturated acids present were apparently composed of about 25% of hexadecenoic ("palmitoleic") acid and 75% of oleic acid (or other unsaturated acids containing 18 carbon atoms), since the unsaturated acids yielded, after hydrogenation, a mixture of palmitic and stearic acids in these proportions. In 1936 TÄUFEL, THALER, and SCHREYEGG[2] examined fatty acids from a specimen of brewer's yeast; they separated the unsaturated from the saturated acids by means of their lead salts, and from the analytical characteristics of the two groups of acids they deduced that the component acids of the yeast lipids included lower (steam-volatile) acids 7.3, palmitic 13.4, stearic 8.3, oleic 66.9 and linoleic 4.1% (wt). TÄUFEL *et al.* did not, apparently, determine the proportion of hexadecenoic acid present, which is presumably included in the oleic acid figure recorded.

Some time ago Dr. A. KLEINZELLER, of the Czechoslovak State Institute of Health, Prague, sent to us a specimen of yeast fat in order that its component fatty acids might be determined. This fat was from a yeast strain "No. 72" which had been grown on molasses and raw sugar and then hydrolysed with dilute hydrochloric acid; the hydrolysed product, after washing and drying in a vacuum, was extracted with light petroleum (b.p. 40–60° C) when it gave a pasty, semi-solid mass of yeast lipids amounting to 25–33% of the weight of the dried hydrolysed yeast.

The specimen which we received was a yellowish, soft semi-solid at room temperature, had an iodine value of 62.6, and contained 33.0% free fatty acid (as oleic) and 2.4% of unsaponifiable matter (which was not further examined). The greater part of the specimen was saponified and converted into its mixed fatty acids, the components of which were then determined by the methods described below.

EXPERIMENTAL

The mixed fatty acids (63.8 g) of the yeast fat were first resolved by crystallisation from ether at —45° and —40° C into three groups differing widely in unsaturation, following the general outlines of the technique described by HILDITCH and RILEY[3] for partial separation of fatty acids by crystallisation from appropriate solvents at low temperatures. Each group of fatty acids was converted into the corresponding mixture of methyl esters, which was fractionally distilled at 0.2 mm pressure through an electrically-heated and packed fractionating column. In the two more unsaturated groups of

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mixed acids (*B* and *C*, below) the proportions of linoleic and linolenic acids present were determined by observing the values of $E^{1\%}_{1\,cm}$ produced respectively at 234 mμ after isomerisation with alkali at 180° C for 60 minutes, and at 268 mμ after isomerisation with alkali at 170° C for 15 minutes; this procedure has been described in detail by HILDITCH, MORTON, and RILEY[4]. The component acids in each group of mixed acids were calculated from the equivalents and iodine values of the ester-fractions obtained in the respective distillations by methods which have been discussed by HILDITCH[5] (due allowance being made for the octadecadienoic and octadecatrienoic acids indicated by the spectrophotometric analyses).

Preliminary separation of the yeast fat mixed fatty acids by low-temperature crystallisation. The total fatty acids (63.8 g) were first crystallised from ether (638 ml) for five hours at −45° C, when 26.7 g were left in solution (group *C*, below); the solids deposited at −45° C were further crystallised from ether (371 ml) for five hours at −40° C, when 15.3 g (group *B*) were left in solution and 21.8 g (group *A*) deposited. The results of this preliminary separation are summarised in Table I.

TABLE I

SEPARATION OF YEAST FATTY ACIDS FROM ETHER SOLUTIONS AT −45° C AND −40° C

Group		g	%	Iodine Value	$E^{1\%}_{1\,cm}$ * 234 mμ	$E^{1\%}_{1\,cm}$ * 268 mμ
A	Insoluble in ether at −40° C . .	21.8	34.2	3.5	Not determined	
B	Soluble in ether at −40° C . . .	15.3	24.0	89.8	30.5	—
C	Soluble in ether at −45° C . . .	26.7	41.8	109.2	126.6	10.1

* After isomerisation with alkali under the standardised conditions described (*loc. cit.*).

Fractional Distillation of Methyl Esters of acids A, B, C. The equivalents and iodine values of the ester-fractions obtained from each group are shown in Table II.

TABLE II

ESTER FRACTIONS FROM METHYL ESTERS OF EACH GROUP OF ACIDS

Fraction	g	Equivalent	Iodine Value
	Methyl esters of fatty acids in group A		
A1	2.44	269.3	0.4
A2	2.50	270.0	0.6
A3	2.40	271.4	0.9
A4	2.74	271.5	1.6
A5	2.90	274.2	3.3
A6	2.33	275.1	4.6
A7	2.68	287.1	8.0
A8	3.73	336.1	7.4
	21.72		
(A8, esters free from unsaponifiable		322.1	5.9)
	Methyl esters of fatty acids in group B		
B1	2.52	286.3	73.9
B2	2.46	291.6	86.3
B3	2.57	295.4	86.6
B4	2.61	295.8	86.8
B5	2.20	295.8	86.9
B6	2.07	321.0	92.1
	14.43		
(B6, esters free from unsaponifiable		299.5	80.3)

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TABLE II (continued)

Fraction	g	Equivalent	Iodine Value
Methyl esters of fatty acids in group C			
C1	2.65	290.1	83.2
C2	3.34	292.1	94.9
C3	2.95	294.3	96.6
C4	2.63	293.9	97.4
C5	2.70	293.6	97.7
C6	2.95	294.9	98.0
C7	2.99	294.9	97.6
C8	2.32	518.1	154.5
	22.53		
(C8, esters free from unsaponifiable		304.6	89.0

The fatty acids in the residual fraction *A8* were isolated and crystallised from light petroleum, when about half remained in solution at room temperature; the deposited solids were further crystallised several times from ethyl acetate, when various fractions which melted at 78–79°, 78.5–79.5° and 81° were obtained. These fractions showed depressions in melting point of 4–5° on admixture with either arachidic acid (m.p. 75.5°) or behenic acid (m.p. 81°). It was therefore concluded that arachidic, behenic and probably lignoceric acids were all present in small proportions in group A of the yeast fatty acids.

The acids from ester-fraction *B4* were oxidised with alkaline permanganate solution, following the procedure of LAPWORTH and MOTTRAM[6], and a dihydroxystearic acid m.p. 130.5° (unchanged when mixed with authentic 9.10-dihydroxystearic acid, m.p. 131.5°) was produced, indicating the presence in this fraction of oleic acid.

The acids (2.18 g) from ester fraction *C5* were dissolved in light petroleum and treated with bromine, when a very small quantity (0.01 g) of crystalline bromo-adducts separated on standing overnight at 0°. Recrystallisation of these furnished tetrabromostearic acid, m.p. 115–116°, and a minute amount of ether-insoluble crystals which melted at 181–181.5° (hexabromostearic acid). These tests showed the presence of linoleic and linolenic acids in very small proportions in this fraction.

From the above data the percentage compositions by weight of the fatty acids in groups *A*, *B* and *C* were calculated to be as shown in Table III.

TABLE III

COMPOSITION (% WT.) OF FATTY ACIDS IN GROUPS A, B AND C (TABLES I AND II)

Acid	*A* % (wt)	*B* % (wt)	*C* % (wt)
Myristic	0.4	—	—
Palmitic	67.0	3.6	2.8
Stearic	13.6	1.2	1.9
Saturated C_{20},C_{22},C_{24} (as arachidic)	14.5	—	—
Hexadecenoic	1.4	2.7	0.3
Oleic	2.3	86.7	75.7
Octadecadienoic	—	3.2	11.4
Octadecatrienoic	—	—	1.7
Unsaturated C_{20-22}	—	1.6	1.7
Unsaponifiable matter	0.8	1.0	4.5

Thus it follows that the component fatty acids of the original yeast fat are as given in Table IV.

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TABLE IV

COMPONENT ACIDS OF THE YEAST FAT

Acid	*A* (34.2 %)	*B* (24.0 %)	*C* (41.8 %)	Total	Excluding unsaponifiable	
					% (wt)	% (mol)
Myristic	0.1	—	—	0.1	0.1	0.2
Palmitic	22.9	0.9	1.2	25.0	25.6	27.6
Stearic	4.6	0.3	0.8	5.7	5.9	5.7
Saturated C_{20},C_{22},C_{24} (as arachidic)	5.0	—	—	5.0	5.1	4.5
Hexadecenoic	0.5	0.6	0.2	1.3	1.3	1.4
Oleic	0.8	20.8	31.6	53.2	54.5	53.4
Octadecadienoic	—	0.8	4.7	5.5	5.7	5.6
Octadecatrienoic	—	—	0.7	0.7	0.7	0.6
Unsaturated C_{20-22}	—	0.4	0.7	1.1	1.1	1.0
Unsaponifiable	0.3	0.2	1.9	2.4	—	—

DISCUSSION

The final results in Table IV have little resemblance to those in the partial analyses recorded by NEWMAN and ANDERSON[1] and by TÄUFEL *et al.*[2], except that they accord with the former in showing that palmitic acid amounts to about 70 % of the saturated acids in the yeast fat; but they also indicate an extremely, and somewhat surprisingly, low content of hexadecenoic acid. This acid has been noted in quantity in the fats of several lower forms of vegetable life, for instance, in diphtheria bacilli[7], a *Penicillium* species[8], and *Lycopodium* spores[9], and as already mentioned NEWMAN and ANDERSON (*loc. cit.*) found evidence of considerable proportions of hexadecenoic acid in the yeast fat which they examined. A most curious feature of the composition of the present specimen of yeast fat is the close resemblance of its component acids to those of many animal depot fats: thus, especially, the contents of palmitic, hexadecenoic, octadecadienoic and unsaturated C_{20-22} acids are those typical of most land animal depot fats and, although the stearic acid content is lower than in some (but by no means all) of such fats, the combined content of stearic and oleic acid is slightly over 60 %, again within the normal range of animal depot fats. Indeed, the only marked differences between the component acids of this yeast fat and those of animal depot fats are the comparatively large proportion of arachidic and higher saturated acids, and the much larger amount of unsaponifiable or non-fatty constituents, some of which is evidently very unsaturated (this is in harmony with the observation of TÄUFEL *et al.* (*loc. cit.*) that squalene accompanies yeast lipids).

The resemblance between the component acids of this specimen of yeast lipids and those of animal depot fats is probably largely fortuitous. Taken in conjunction with the implications afforded by the earlier publications cited, the chief value of the present work may rather lie in the general indication that yeast fats of different origin, or from yeasts grown under varying conditions, may be very different in composition. Dr. KLEINZELLER has informed us that the average unsaturation of yeast fat depends, *inter alia*, on the speed of fat formation, and that the iodine value of the fat decreases during rapid fat formation. He has also informed us that the fats from different species or strains of yeast grown under the same conditions may vary in iodine value from about 40 to about 90.

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It would therefore appear that further detailed studies, similar to those now described, of yeast fat component acids are urgently needed, and it is hoped that the analytical techniques employed in the present work may be found useful to those engaged in investigating this field.

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SUMMARY

1. The component acids of a yeast fat, studied in detail by esterfractionation after preliminary separation by crystallisation from ether at low temperatures, were-found to include myristic 0.1, palmitic 25.6, stearic 5.9, arachidic and higher saturated 5.1; hexadecenoic 1.3, oleic 54.5, octadecadienoic 5.7, octadecatrienoic 0.7, and unsaturated C_{20-22} 1.1 % (wt).

2. This composition has a close resemblance (probably fortuitous) to those of many land animal depot fats, but differs (especially in its low content of hexadecenoic acid) from those indicated by the partial analyses of yeast fats by other investigators.

3. It is evident that further detailed studies of the component acids of fats from different strains of yeasts, and from a given strain grown under different conditions, are required in order to obtain adequately comprehensive knowledge of the range of variability of composition of yeast fats, and of the factors which influence these differences.

RÉSUMÉ

1. Les acides constituant la graisse de levure, étudiés en détail par fractionnement de leurs esters, après une séparation préliminaire par cristallisation à partir de l'éther à basses températures, sont représentés par les acides suivants: myristique 0.1; palmitique 25.6; stéarique 5.9; arachidique et acides saturés de poids moléculaire plus grand: 5.1; hexadécénoïque 1.3; oléique 54.5; octadécadiénoïque 5.7; octadécatriénoïque 0.7; et acides non saturés en C_{20-22} 1.1 % (en poids).

2. Cette composition montre une ressemblance étroite (qui n'est probablement qu'une coïncidence) avec celle des graisses de réserve de beaucoup d'animaux terrestres. Mais elle diffère (particulièrement en ce qui concerne la faible proportion d'acide hexadécénoïque) des données fournies par les analyses partielles de graisse de levure par des autres auteurs.

3. Il est évident qu'il conviendrait d'avoir les résultats d'études détaillées des acides constituant les graisses de différentes souches de levures et d'une souche donnée cultivée dans des conditions différentes, pour connaître d'une façon convenable les possibilités de variations de la composition de la matière grasse des levures ainsi que les facteurs qui jouent sur cette composition.

ZUSAMMENFASSUNG

1. Die Säurekomponenten eines Hefefettes, die detailliert durch Esterfraktionierung nach einer vorläufigen Scheidung durch Kristallisation aus Aether bei niedrigen Temperaturen untersucht wurden, enthielten, wie gefunden wurde, 0.1 (Gew.) % Myristin-, 25.6 % Palmitin-, 5.9 % Stearin-, 5.1 % Arachidin- und höhere gesättigte Säuren; 1.3 % Hexadecen-, 54.5 % Öl-, 5.7 % Octadecadiën-, 0.7 % Octadecatriën und 1.1 % ungesättigte C_{20}–C_{22} Säuren.

2. Diese Zusammenstellung ähnelt (wahrscheinlich zufällig) sehr der von vielen Landtierdepotfetten, unterscheidet sich aber (besonders durch ihren niedrigen Gehalt an Hexadecensäure) von denjenigen, die durch partielle Analysen des Hefefettes von anderen Untersuchern angegeben werden.

3. Es ist deutlich, dass weitere detaillierte Untersuchungen der Säurekomponenten von Fetten aus verschiedenen Hefestämmen, und von demselben Stamm nach Wachstum unter verschiedenen Bedingungen, nötig sind, um eine einigermassen umfassende Kenntnis des Variabilitätsbereiches der Zusammenstellung von Hefefetten, und der Faktoren, die diese Unterschiede beeinflussen, zu erlangen.

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