STUDIES ON THE FATTY ACID COMPOSITION OF YEAST

by

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INTRODUCTION

The control of cellular activity can be observed by studying the changes in the cellular components when cell activity has been altered by changing the cultural conditions. If a culture is in a physiologically steady state resulting from prolonged growth in an unchanging environment, it is experiencing balanced growth. By this is meant that, on the average, each cellular constituent doubles in mass between successive divisions. When a culture in balanced growth is subjected to a new and chemically different environment, the intracellular composition may undergo various changes. The degree of these changes may be a reflection of how drastically the growth conditions have been altered.

In this investigation a study was made of the effect of environmental changes on the chemical composition of the yeast cell. The work described herein concerns the nature and amounts of the various fatty acids produced by Saccharomyces cerevisiae strain MCC under various conditions. Extensive studies have been made by others on the ribonucleic acid (RNA), desoxyribonucleic acid (DNA), and protein fractions and how these are affected by changing the growth rate. Work of this nature concerning

the lipid fraction of the cells has not previously been reported.

Generally this study can be grouped into four separate phases: (1) effect of changes in the growth medium and temperature of incubation; (2) effect of prolonged incubation on cellular composition; (3) effect of temperature on reconstituted cells; (4) effect of biotin deficiency.

HISTORICAL

The fat composition of yeast is of both academic and economic interest. Some yeast species (Torulopsis lipofera, Endomyces vernalis, Rhodotorula gracilis, and Oospora lactis) may contain a fat content in excess of 50 per cent of the dry weight. These so-called fat yeasts have been exploited as a foodstuff, particularly during the war years. Brewer's and baker's yeasts contain considerably less fat, however, as will be shown subsequently. The fatty acid fraction of yeast encompasses several lipid groups: triglycerides, glycerophosphatides, cerebrins, sterol esters, protein bound and free fatty acids. In this study no attempt will be made to distinguish between these lipid states because, presumably, the fatty acid ester linkages are broken during saponification. The chemical structures and names for the common biological fatty acids are given in Table 1.

Several investigators have shown species and even strain differences in the fatty acid content of yeast. Newman and Anderson (21, p. 219-228) studying S. cerevisiae, found the saturated acids were made up of 75 per cent palmitic and 25 per cent stearic; the unsaturated acids yielded 25 per cent palmitoleic and 75 per cent C-18 unsaturated fatty acid. The yeast was grown in vigorously aerated molasses containing ammonium phosphate.

Table 1

Chemical structures and names of the common biological fatty acids

Common Name	Shorthand designation	Number of carbon atoms	Number of double bonds	Position of double bonds
Caprylic	8:0	8	0	-
Capric	10:0	10	0	-
Undecanoic	11:0	11	0	-
Lauric	12:0	12	0	-
Myristic	14:0	14	0	-
Pentadecanoic	15:0	15	0	-
Palmitic	16:0	16	0	-
Palmitoleic	16:1	16	1	9
Magaric	17:0	17	0	-
Stearic	18:0	18	0	-
Oleic	18:1	18	1	9
Linoleic	18:2	18	2	9,12
Linolenic	18:3	18	3	9,12,15
Chaulmoogric	18:1	18	1	2
Lactobacillic	19:0	19	0	-
Arachidic	20:0	20	0	-
Arachidonic	20:4	20	4	8,11,14,17

Taufel et al. (27, p. 394-404) in a later study reported on an unidentified Saccharomyces species which contained a fatty acid composition of: lower acids volatile in steam 7.3 per cent, palmitic 13.4 per cent, stearic 8.3 per cent, oleic 66.9 per cent, and linoleic 4.1 per cent.

A more detailed analysis, using the ester fractionation technique, was made of the fatty acids in the following yeasts: Torulopsis utilis by Reichert (22, p. 484), Rhodotorula of unknown strain by Hilditch and Shrivastava (8, p. 80-85), and Rhodotorula gracilis by Holmberg (10, p. 14-20). Their data suggest that ordinary yeasts with lipid contents of less than seven per cent may contain fatty acids low in palmitic and oleic and relatively high in palmitoleic and polyethenoic C-18 acids, while yeasts of high lipid content (Rhodotorula) produce a fatty acid mixture primarily of palmitic and oleic acids with small amounts of palmitoleic and linoleic acids. Bass and Hospodka (2, p. 243-245) have shown that Rhodotorula gracilis produces a more saturated fat when grown at higher than at lower temperatures. The total fatty acids produced by S. cerevisiae at 30°C both anaerobically and aerobically was determined by Klein (12, p. 620-627). Under anaerobic conditions he found 12 to 15 mg fatty acid per gram of cells dry weight, while aerobically 52 to 200 mg per gram of cells were produced.

Hofmann and Panos (9, p. 687-693) have shown that a number of long chain cyclopropane fatty acids have the ability to substitute for biotin in the nutrition of various Lactobacillus species. Two cyclopropane acids (C-17 and C-19) in Escherichia coli were reported by Law (15, p. 129). Croom and McNeill (4, p. 170) found the cyclopropane acid, lactobacillic acid, in L. arabinosus but not in a biotin mutant of E. coli. Lactobacillic and dihydrosterculic acids can satisfy the lipid requirement of L. acidophilus, an organism not requiring external biotin (9, p. 687-693). No reports of cyclopropane fatty acids in yeast have appeared.

Kleinzeller et al. (13, p. 470-474) studied the effect of D-xylose and D-glucose on the fats of R. gracilis. They found 70.8 per cent of the dry weight was fat when grown on glucose and 61.2 per cent fat when cultured on xylose. A mixture of both sugars gave a fat content of 65.7 per cent. Glucose is utilized preferentially in the mixture; however, if the cells were previously adapted to xylose, both sugars were assimilated at practically the same rate. The only difference in the fat content noted was in linoleic and linolenic acids.

Many types of control mechanisms operate at varying degrees of efficiency within the cell. These can generally be grouped as genetic and environmental controls. The

expression of genetic regulation of cellular activity is mediated by small molecules which may act as inducers or repressors in the expression of an observed phenotype.

Only in recent years have environmental controls been subjected to extensive study. Schaecter et al. (23, p. 592-606) studied the effect of changes in temperature and medium on the RNA, DNA, average number of nuclei, and protein content of S. typhimurium. Immediate "shifts" in the cellular composition were observed but after 3 or 4 generations the cell returns to balanced growth and the ratios of DNA, RNA, and protein return to the preshifting state. It was concluded that within the temperature range of 25 to 37°C the size and chemical composition of the cells are related to the growth rate only in so far as it depends on the medium. Thus at a given temperature the composition of the cell can be described as exponential functions of the growth rate. The sole effect of temperature by their criterion is alterations in the generation time of the organisms.

Many cultures have an increase in nutrient requirements at supra- and sub-optimal temperatures. This observation is usually explained by the inactivation or inhibition of the requisite enzymes at these incubation temperatures. Sherman (25, p. 37-52) has shown that incubation of Saccharomyces cerevisiae at 40°C for one day

produces a population of which over 99 per cent are of a respiratory deficient type. This higher incubation temperature is characterized by an initial death phase similar to thymine-less death. Under certain conditions oleic acid can completely eliminate the death phase. It was shown that the elevated incubation temperature does not affect the chemical composition of the growth medium in the experiments. The inhibition of growth is readily reversible.

Oxygen tension affects the nutritional requirements of many microorganisms. Andreasen and Stier (1, p. 271-281) have found that both ergosterol and oleic acid were necessary for growth of S. cerevisiae in a defined medium under anaerobic conditions. The requirement for unsaturated fatty acids suggests that these organisms are incapable of desaturating any saturated acids that may be formed. Subsequently, Bloomfield and Bloch (3, p. 337-345) demonstrated an absolute requirement for molecular oxygen and TPNH for the conversion of palmitate to palmitoleate in yeast. These workers also showed that resting yeast cells, under strictly anaerobic conditions, convert up to 10 per cent of trace amounts of C^{14}-acetate to long chain fatty acids.

MATERIALS AND METHODS

Organism and Media Used:

A wild-type diploid clone of *Saccharomyces cerevisiae* strain MCC was used throughout this study. Stock cultures were maintained at 30°C on slants containing 2 per cent tryptone, 2 per cent glucose, 1 per cent yeast extract, and 1.5 per cent agar. Three media were employed in this work. Yeast Complete Medium (YCM) contained 2 per cent tryptone (Difco), 2 per cent glucose, and 1 per cent yeast extract. Yeast Medium A (YMA) contained 1 per cent glucose, 0.1 per cent ammonium chloride, 0.1 per cent yeast extract, and 0.1 molar monobasic potassium phosphate. The last medium, a modified Wickerham's (31, p. 293-301), was completely synthetic, with the following composition:

Wickerham's Complete Medium

All Amounts Per Liter

H_3BO_3	0.01 mg	KH_2PO_4	0.875 g
$CuSO_4 \cdot 5H_2O$	0.01 mg	K_2HPO_4	0.125 g
KI	0.01 mg	$MgSO_4 \cdot 7H_2O$	0.50 g
$FeCl_3 \cdot 6H_2O$	0.05 mg	NaCl	0.10 g
$ZnSO_4 \cdot 7H_2O$	0.07 mg	$CaCl_2 \cdot 2H_2O$	0.10 g
Biotin	0.002 mg	Calcium pantothenate	0.4 mg

Thiamin·HCl	0.4 mg	Adenine sulfate	10.0 mg
Pyridoxine·HCL	0.4 mg	L-Histidine	5.0 mg
Uracil	10.0 mg	L-Methionine	5.0 mg
Inositol	2.0 mg	L-Tryptophan	5.0 mg

$(NH_4)_2SO_4$ 1.0 g

Glucose 20.0 g

A starter culture was prepared by inoculating from a slant 100 ml of the appropriate medium and incubating for 18 hours on a rotary shaker at 30°C. One milliliter of the resulting cell suspension was inoculated into each 100 ml of medium in a 250 ml Erlenmeyer flask.

Treatment of Cells:

Cells in standing culture were prepared by inoculating 20 to 25 two-hundred and fifty milliliter flasks containing 100 ml of medium per flask. These were incubated, without shaking, at 20°, 30°, and 40°C for 72 hours.

Reconstituted cells are those grown anaerobically in a complete medium and then incubated aerobically with glucose in phosphate buffer. Under these conditions large increases of fatty acids and unsaponifiable lipids may be obtained.

Anaerobic cells were prepared by inoculating a two liter Erlenmeyer flask containing 1800 ml of YMA fortified with 2 per cent yeast extract, and fitted with a

Bunsen valve. After flushing the flask with nitrogen gas for ten minutes, the cultures were incubated at 30°C for 80 hours. The nitrogen gas was bubbled through alkaline pyrogallol and filtered through cotton during the flushing procedure.

For experiments in which the anaerobically grown cells were to be aerated, the cultures after incubation were centrifuged in the cold and refrigerated for 24 hours. The cells were washed twice in ice cold distilled water and resuspended in 100 ml of 2 per cent glucose and 0.1 molar KH_2PO_4. The resulting suspension was equally divided into 3 flasks which were incubated (one each at 30°, 40°, and 50°C) with shaking for 30 hours.

Biotin is a metabolite known to be involved in fatty acid synthesis, and in some systems it has been shown to participate in desaturation of fatty acids. Since biotin is essential for growth of Saccharomyces cerevisiae in a synthetic medium; the biotin deficient medium used represents a 1000 fold decrease in the concentration of the vitamin normally supplied in the synthetic broth.

Analysis of Fatty Acids:

For the isolation of yeast fatty acids, the cells from 20 to 25 flasks were harvested by centrifugation, washed twice with distilled water, and resuspended in

water to 40 to 75 mg dry weight per milliliter. A known aliquot of suspension was transferred to glass-stoppered test tubes; three ml of 40 per cent KOH was added together with a few Boileezers (Fisher Scientific) to facilitate even boiling and the tubes then were placed in a steam bath for 6 hours. Upon cooling the non-saponifiable material was extracted with three ten ml portions of petroleum ether. These extracts, which contained the nonsaponifiable fraction, were discarded. To the residue was added sufficient 4 N sulfuric acid to bring the suspension to a pink color using methyl red indicator. The fatty acids were extracted with 3 ten ml volumes of petroleum ether. The extracts were pooled and evaporated to dryness under a blanket of nitrogen gas.

Polyunsaturated fatty acids were determined enzymatically by the method of MacGee (19, p. 298-302). In this procedure the potassium salts of polyenoic fatty acids are oxidized by atmospheric oxygen in the presence of the enzyme lipoxidase, and the absorption of the conjugated diene hydroperoxide is measured at 234 mμ in a Beckman model DU spectrophotometer.

The total fatty acids were quantitatively determined as their methyl esters. Isolated fatty acids were converted to methyl esters by a slight modification of the method of Metcalfe and Schmitz (20, p. 363-364). The

procedure as modified is as follows. To the solvent-free fatty acids is added 3 ml of boron trifluoride-methanol reagent. The reaction mixture is boiled for 3 minutes in 80° water bath and allowed to cool. Ten ml of distilled water is added and the fatty acid esters are extracted and combined. The fatty acid esters were estimated colorimetrically by hydroxamate formation, using the procedure of Snyder and Stephens (26, p. 244-245).

The presence of cyclopropane groups in long-chain fatty acids was determined by infrared spectroscopy (18, p. 830-831). The methyl esters were dissolved in spectro-grade carbon tetrachloride and analyzed using a Beckman IR 7, grating spectrophotometer.

Identification of the individual fatty acids was accomplished by gas-liquid partition chromatography. Fatty acid separations were carried out in part on a Barber-Colman model 20 gas chromatograph equipped with an argon ionization detector. The radiation source was strontium-90. A stainless steel capillary column, 100 feet in length, and coated with Apiezon L, was used. Several chromatograms were run on a Beckman GC-2 with a thermal conductivity detector. A copper packed column, one-fourth inch in diamter and six feet in length, was used. The inert support was 60 to 80 mesh chromosorb (Wilkins Instrument and Research, Inc., Walnut Creek,

Calif.) coated with 10 per cent Apiezon L (Wilkins Instrument and Research, Inc.).

Retention times of known methyl esters of fatty acids of 99+ per cent purity (Applied Science Laboratories, State College, Pa.) were used as standards to identify the unknown peaks. The standards were run each day to check reproducibility of operating conditions. No attempts were made to recover the fatty acid esters after chromatographic separation. The relative amounts of each fatty acid ester was determined by the peak area method.

METHODOLOGICAL PARAMETERS

Work on microbial lipids has been concerned mainly with the problems of lipid chemistry. In view of the notable variations in yeast fats reported in the literature it became necessary to investigate the parameters of the various methods of analyses used throughout this study.

Varying the amount of 40 per cent KOH and distilled water added had little effect upon the quantity of fatty acids obtained from 100 to 200 mg of cells as shown in Table 2. The precision of the method was noteworthy, indicative of quantitative extractions.

The methylation procedure was subjected to variations in the temperature of the water bath as well as duration of heating. The results (Table 3) show the recovery of reagent palmitic acid to be 82 to 84 per cent under the conditions specified. Metcalfe and Schmitz (20, p. 363-364) in their original communications report esterficiation efficiencies of between 83 and 95 per cent for most naturally occuring fatty acids. Palmitic acid was esterified with 83 per cent efficiency. Our data appear to be in good agreement with these authors. No corrections for incomplete methylation were made in this study. Fatty acids containing conjugated unsaturation have not been examined for changes after methylation with

Table 2

Effect of variations in amount of KOH and distilled water added upon fatty acids recovered

Tube number	Cells in aliquot (mg)	40% KOH (ml)	H_2O ml	Fatty acids in aliquot (μmoles)
1	100	5	4	10.38
2	100	5	4	10.86
3	100	3	0	11.33
4	100	3	0	11.56
5	100	3	2	9.44
6	100	3	2	12.27
7	200	3	2	23.13
8	200	3	2	24.54
9	100	4	4	11.56
10	100	4	4	10.15

Table 3

Variations in esterification procedure

Tube number	Palmitic acid (mg)	Time in water bath	Temp. of water bath (C)	Fatty acid recovered (mg)	% recovery
1	3	2 min.	boiling	2.45	82
2	3	2	boiling	2.50	83
3	3	3	80°	2.45	82
4	3	3	80°	2.50	83
5	3	9	60°	2.55	84
6	3	9	60°	2.45	82

boron trifluoride-methanol. The advantages of this method are simplicity of equipment, rapidity, and stability of reagent.

The test for ester linkages by hydroxamate formation suggests complete evaporation of solvent. As mentioned above, this was done in a water bath under a blanket of nitrogen gas. As the methyl esters of the shorter-chain fatty acids are quite volatile, evaporation of a solvent at 50° or 60°C, with agitation by the nitrogen, could vaporize a sizeable amount of the sample. Reproducibility in the method was accomplished only when the samples were not completely evaporated. It was found that a volume of n-hexane up to 0.5 ml does not interfere with hydroxamate formation as shown in Table 4. These data were obtained using methyl laurate. Subsequent ester fractions were evaporated to approximately 0.5 ml before analysis.

Gas chromatographic detectors of the argon-ionization type are theoretically linear in response. However, recent evidence (5, p. 399) has shown a slight variation among fatty acids of differing chain lengths and degrees of unsaturation. Thermal conductivity detectors are subject to greater differences in relative response than are ionization types (11, p. 92-94). These variations

Table 4

Effect of solvent on hydroxamate formation

Tube #	Standard Soln.* (ml)	mg. ester	n-Hexane (ml)	Total (ml)	Optical Density at 530 m
1	0.0	0	0.1	0.1	0.00
2	0.0	0	0.5	0.5	0.00
3	0.01	0.095	0.09	0.1	0.14
4	0.01	0.095	0.49	0.5	0.135
5	0.03	0.286	0.07	0.1	0.415
6	0.03	0.296	0.47	0.5	0.422
7	0.05	0.477	0.45	0.5	0.710
8	0.05	0.477	0.45	0.5	0.710
9	0.09	0.859	0.01	0.1	1.3
10	0.09	0.859	0.41	0.5	1.3

*Standard solution contains 9.54 mg per ml of methyl laurate.

are small and have little significance in a comparative study of this type. Thus no corrections were made for non-linearity of detector response. A representative gas chromatogram of a sample of yeast fatty acid esters is shown in Figure 1.

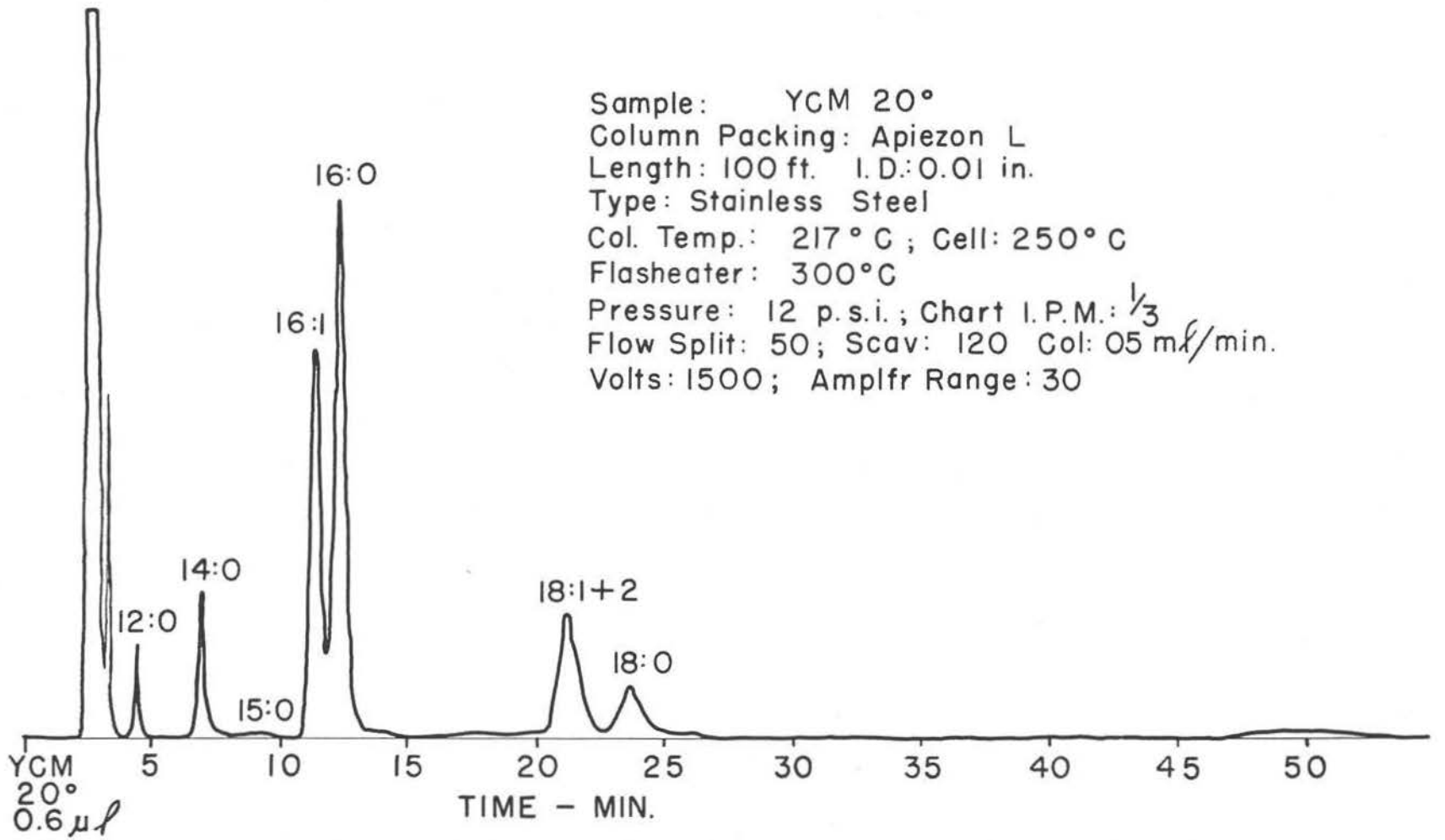

Figure 1. Fatty acid methyl esters of Saccharomyces cerevisiae as shown by gas chromatography.

EXPERIMENTAL RESULTS

Effect of Medium and Temperature

The fatty acid content of S. cerevisiae varies markedly with the culture medium as well as the temperature of incubation (Table 5). It is interesting to note that at 30°C, the near-optimal temperature for growth, the fatty acid content is lowest.

Cells incubated at 40°C in the complete media produce approximately twice as much fat as those grown at the lower temperatures. Growth at 40°C in the synthetic media, however, yields less fat than those grown at 20° or 30°C.

Infrared analysis of pooled yeast fatty acid esters gave a C-H stretching band at 2925 wave numbers (cm^{-1}) and a strong band at 1742 cm^{-1} indicative of aliphatic esters. Lactobacillic acid shows bands at 3058 cm^{-1}, 2988 cm^{-1} and 1008 cm^{-1} (18, p. 830-831).

Sixteen and eighteen carbon fatty acids are the most prevalent acids as revealed by gas chromatography (Table 6). Nine different acids were identified forming a relatively complete fatty acid spectrum. The ratio of palmitic to palmitoleic acid is reversed between 30°C and 40°C in the most complete medium (YCM). No acids with retention times longer than stearic were identified, however, a small peak which could be either a higher boiling

Table 5

Effect of medium and temperature on the fatty acid content of *Saccharomyces cerevisiae**

Medium	Temp. of incubation (C)	Fatty acid content	
		μmoles fatty acid per gram of yeast (dry weight)	mg fatty acid** per gram of yeast (dry weight)
YCM	20°	71.39	19.31
YCM	30°	57.86	15.65
YCM	40°	176.82	47.83
YMA	20°	116.64	31.55
YMA	30°	94.71	25.62
YMA	40°	153.01	41.39
Synthetic	20°	87.73	23.73
Synthetic	30°	68.58	18.55
Synthetic	40°	66.25	17.92

*All cells were grown for three days in standing culture

**Computed using palmitic acid as the representative fatty acid.

Table 6

Effect of medium and temperature on the fatty acid composition* of Saccharomyces cerevisiae**

Fatty Acids	Medium								
	YCM			YMA			Synthetic		
	20° (%)	30° (%)	40° (%)	20° (%)	30° (%)	40° (%)	20° (%)	30° (%)	40° (%)
10:0	+	+	+	+	+		1.0	+	+
11:0	+	-	-	-	+		+	+	-
12:0	2.6	3.6	5.9	1.4	1.0	0.6	2.5	2.0	+
14:0	6.1	5.6	2.1	0.9	0.4	1.0	5.0	2.0	+
15:0	+	-	-	-			-	-	-
16:0	39.1	47.5	29.2	16.8	17.8	17.6	4.5	13.5	+
16:1	26.0	21.6	41.0	47.1	39.1	46.6	64.4	57.9	+
18:0	7.5	7.5	5.1	3.9	7.3	4.2	3.6	5.5	+
18:1 + 18:2	18.0	14.0	16.2	27.7	34.1	29.7	19.0	18.2	+

*Quantitative determination by peak area of resultant gas chromatography.
**Grown in standing culture for three days.

organic compound or a fatty acid dimer, is shown in Figure 1.

The polyunsaturated fatty acid content in cells grown under the cultural conditions covered in Table 5 was always less than four per cent.

Effect of Prolonged Incubation

Incubation of cells for several days apparently does not have a significant effect on the fatty acid content of yeast. At various intervals up to 20 days after inoculation samples were analyzed for fatty acid content (Table 7).

It is interesting to note that at 20 days the fatty acid content was highest of the samples analyzed. At this time the yeast cells contained the lowest amount of polyunsaturated fatty acids. Gas chromatographic analysis showed a relatively constant amount of sixteen and eighteen carbon acids over the interval of time tested (Table 8). Slight deterioration of the 10 per cent Apiezon L column prevented adequate separation of the saturated from the unsaturated acids. The quantity of esters was so low in some instances that very small peaks were recorded. As these peaks do not lend themselves to accurate quantification plus and minus signs were used to denote the presence of a particular ester.

Table 7

Changes in fatty acid content with age of culture in Saccharomyces cerevisiae*

Age of culture (days)	Fatty acid content		
	Per cent polyunsaturated F.A.**	μmoles fatty acid per gram of yeast (dry weight)	mg fatty acid per gram of yeast*** (dry weight)
3	3.992	94.67	25.61
10	1.843	107.39	29.05
15	2.585	102.40	27.70
20	0.3369	133.30	36.06

*Cells were grown in standing culture of YMA medium at 30°C.
**Determined by lipoxidase oxidation
***Computed using palmitic acid as the representative fatty acid.

Table 8

Changes in the fatty acid composition with age of culture in Saccharomyces cerevisiae*

Fatty acids	Age of culture			
	3 days (%)**	10 days (%)	15 days (%)	20 days (%)
10:0	+	+	+	+
11:0	+	+	-	+
12:0	+	+	+	+
14:0	+	+	+	+
16:0 16:1	66.6	69.1	60.6	61.9
18:0 18:1 18:2	33.4	30.9	39.4	38.1

*Cells were grown in standing culture of YMA medium at 30°C.

**Percentage determined by peak areas of resultant gas chromatograms.

Effect of Temperature on Reconstituted Cells

Temperature has a marked influence on fat production in reconstituted cells. This effect was subjected to great extremes as evidenced by Table 9. At 30° and 50°C the fat content is of the same order of magnitude, however, a doubling of component fatty acids occurs at 40°C.

It can be seen (Table 10) that the same fatty acids were produced at all temperatures. The 50°C sample did not contain enough C=18 acids to give a measurable peak on the chromatogram.

Effect of Biotin Deficiency

The involvement of biotin in fatty acid production is clearly demonstrated in Table 11. A one-thousand fold decrease in the biotin concentration causes a concomitant decrease in fat content of 63.8 per cent. The fatty acids obtained from normal and biotin deficient cells is shown in Table 12. The results show no significant change in the quantities of sixteen and eighteen carbon acids produced.

Table 9

Effect of temperature on fatty acid content of reconstituted cells*

Temperature during aeration (C)	Fatty acid content		Polyunsaturated fatty acids*** %
	μ moles fatty acid per gram yeast (dry weight)	mg fatty acid** per gram yeast (dry weight)	
30°	18.89	5.11	0.938
40°	38.33	10.37	2.255
50°	14.89	4.03	0.090

*Cells were grown anaerobically for 80 hours at 30°C, harvested, and washed twice in cold distilled water. The cells were then suspended in 2% glucose and 0.1 M phosphate buffer. The suspensions were aerated for 30 hours at the designated temperatures.

**Computed using palmitic acid as the representative fatty acid in yeast.

***Determined by lipoxidase oxidation.

Table 10

Effect of temperature on fatty acid composition of reconstituted cells*

Temperature during aeration (C)	Fatty acids** (%)						
	10:0	11:0	12:0	14:0	15:0	16:0+16:1	18:0+18:2
30°	+	+	+	+	-	55.9	44.1
40°	+	+	10.2	9.4	-	57.9	22.4
50°	+	+	12.8	14.3	-	72.9	+

*As shown in Table 9.
**Percentage determined by peak areas of resultant gas chromatograms.

Table 11

Effect of biotin deficiency on fat content of Saccharomyces cerevisiae*

Biotin Concentration (Mμg/ml)	Incubation period (days)	Fatty acid content		Polyunsaturated fatty acids*** (%)
		μmoles fatty acid per gram yeast (dry weight)	mg fatty acid** per gram yeast (dry weight)	
2.0	3	50.05	13.54	3.623
0.002	4	18.11	4.90	3.443

*Cells were grown in standing culture at 30°C in synthetic medium.
**Expressed using palmitic acid as the representative fatty acid.
***Determined by lipoxidase oxidation.

Table 12

Effect of biotin deficiency on the fatty acid composition of *Saccharomyces cerevisiae**

Biotin concentration (Mμg/ml)	Fatty Acids** (%)					
	10:0	11:0	12:0	14:0	16:0+16:1	18:0+18:1+18:2
2.0	+	+	+	+	73.4	26.5
0.002	+	+	+	+	81.7	18.2

*Cells were grown in standing culture at 30°C in synthetic medium.
**Percentage determined by peak areas of resultant gas chromatogram.

DISCUSSION

In the experiments described herein an attempt was made to resolve the effect of certain environmental factors on fatty acid production in the yeast cell. It was anticipated that these results would shed some insight on the relationship of environmental control to intracellular composition. The effect is pronounced in some instances and almost negligible in others.

The relatively low fat content of cells grown at 30°C (Table 5) may suggest that at this temperature cellular processes occur at a near-maximal rate. Subsequently, storage compounds will be most readily utilized after the organism has passed the phase of logarithmic growth. Presumably all the available glucose has been assimilated before 24 hours of incubation.

Growth yield at 40°C in the synthetic medium was greatly reduced, and the cells contained less fat than those grown in the complete media. A consideration of the cultural conditions employed may explain this observation. Sherman (24, p. 29-35) has shown that S. cerevisiae will not grow normally at 40°C in a synthetic medium analogous to the one used in this study. The starter culture used in this investigation was grown in

the appropriate medium for one day at 30°C with shaking prior to inoculation into the standing culture flasks. The yield of cells after incubation at 40°C was low enough to suggest that few cell divisions may have occurred and that the cellular composition would closely resemble that of cells grown at 30°C.

As mentioned before, the fatty acid content in the two complete media was highest in cells grown at 40°C. Franke and Heinen (6, p. 359-378) have isolated a fatty acid dehydrogenase from mold mycelia which rapidly loses activity above 35°C. In this light the possibility that stored fatty acids cannot undergo degradation at 40°C is a plausible explanation for the results obtained.

The fat content of mold mycelia is directly proportional, within limits, to the concentration of substrate according to Lockwood et al. (16, p. 411-425). If this relationship applies to yeast a higher fat content would be expected in YCM and the synthetic medium than in YMA. Such was found to be the case.

The degree of complexity and the quantity of complex and unknown substances present in a complete medium has a marked effect on the cellular composition. This is exemplified in the YCM and YMA media. Since yeast extract is present in both but at different concentrations (1 per cent and 0.1 per cent respectively), the possibility of

cellular absorption of exogenous fatty acids was considered. One batch of yeast extract (Difco) was analyzed and found to contain 1.5 mg fatty acid per gram. If this was absorbed intracellularly or adsorbed onto the cell wall, the richer medium (YCM) would be expected to give the higher fat content in growing cells, as was clearly demonstrated.

A second possibility for the differences in fat content observed would be the presence of a fatty acid permease which would concentrate exogenous fatty acids within the cell. Here, also, the most complex medium would produce cells with the highest fat content. If a fatty acid permease can be demonstrated, the temperature effect observed could be a reflection upon the activity of this enzyme. If yeast extract served as a fatty acid donor to the yeast cell, the organism would have to possess a phenomenal scavenging ability.

The accuracy of the infrared analysis is commendable; the Beckman IR7 can resolve within one wave number the actual absorption frequency. The band at 2925 cm^{-1} was probably due to C-H bonds in the fatty acid esters plus a slight contribution from contaminating hexane molecules. The second absorption band (1725 cm^{-1}) is characteristic of ester linkages. The cyclopropane ring absorbs radiation of 1008 cm^{-1}. Absorption observed at 3056 to 3058

cm^{-1} and 2988 to 2990 cm^{-1} is attributed to stretching frequencies of C-H groups contained in the cyclopropane ring (18, p. 830-831). No demonstrable peaks were observed in these ranges. From these analyses it was concluded that cyclopropane fatty acids, if present, do not constitute a significant quantity of the fatty acids isolated.

The effect of age of the cells on fat content found in this study agrees quite closely with results obtained from other microbial systems. Lockwood, et al. (16, p. 411-425), studying the changes in fat content with age of the culture in Penecillium javanicum, showed increasing amounts from four to eight days followed by a decrease at twelve days and then progressive increases to a maximum concentration at 20 days. This level was maintained for several days thereafter. This relative stability may suggest that utilization of stored fat has been completed, or at least to the point of equilibrium, within a short time.

Under anaerobic conditions the lipids produced by Saccharomyces cerevisiae strain LK2G12 are considerably reduced as shown by Klein (12, p. 620-627). Further work revealed that after aeration of resting cellular suspensions of anaerobically grown cells the fatty acid content had increased approximately ten fold. Table 9

shows results obtained in this laboratory in a similar system. At 30°C aeration of reconstituted cells markedly reduced the fat content which was less than one-twentieth that observed by Klein. This discrepancy may have been caused by: (a) the difference in yeast strain used, (b) the culture medium employed in this laboratory contained 2 per cent yeast extract, whereas Klein's medium contained 0.1 per cent yeast extract, (c) the difference in aeration time - eight hours to thirty hours, and (d) the difference in incubation temperature - room temperature (approximately 23°C) to the 30°C used in this study.

A possible explanation for the great differences in fat content observed in reconstituted cells could be that at 40°C a fatty acid dehydrogenase loses activity causing an increase in fat content. However, at 50°C the biosynthetic mechanism could lose a significant amount of activity, resulting in less fat synthesis. At 50°C, supposedly, the dehydrogenase would still be inactivated.

The demonstration of a biotin requirement for adequate fatty acid synthesis has been demonstrated in other biological systems. The fatty acid synthesizing system isolated by Wakil, et al. (28, p. 225) has been found to contain biotin in the amount of one mole per three to five times 10^5 gm of protein which represents

the highest concentration of bound biotin yet recorded in the literature. Avidin combines stoichiometrically with biotin and will reversibly inhibit the synthetic system isolated. Biotin has been implicated in carboxylation reactions by whole cell experiments. However, Wakil, Gibson and Harper (Unpublished, 1959) found that in biotin deficient chicks or rats apparently the apoenzyme for the biotin-containing enzyme is not synthesized unless biotin is available (7, p. 33). Kurtz and Miramon (14, p. 514-516) showed in flax seedlings that in the absence of biotin synthesis of saturated acids and oleic proceeded normally; however linoleic and linolenic acid synthesis was inhibited indicating a different mechanism at some stage in the two biosynthetic pathways.

The effect of environmental factors on the fatty acid biosynthetic system merits mention in this discussion. Lipid synthesis is for the most part dependent upon the intracellular supply of reduced triphosphopyridine nucleotide (TPNH). The pathway for fatty acid synthesis summarized by Wakil (28, p. 2597-2598) involves the utilization of two moles of TPNH per mole of acetate incorporated. Several systems may serve as TPNH donors within the cell but the hexose monophosphate shunt (HMP) is probably of the most importance. The oxidation of glucose to pentose yields two moles of TPNH. This pathway

operates to an extent of about 10 per cent in Saccharomyces cerevisiae according to the radiorespirometric method of Wang et al. (30, p. 207-216). The isocitric dehydrogenase reaction may also convert TPN to TPNH.

Pyridine nucleotide transhydrogenase can catalyze the following reaction:

$$TPN^+ + DPNH \longleftrightarrow TPNH + DPN^+$$

The ratios of reduced to oxidized coenzyme are such as to suggest that this reaction is of minor significance under physiological conditions. The ratio of TPNH to TPN is usually greater than 5.0 whereas the DPNH to DPN ratio is usually less than 0.7 (17, p. 98-103).

Coupled reactions of two consecutive dehydrogenases each involving one coenzyme can function essentially the same as the transhydrogenase reaction. An example of this would be:

(i) α-ketoglutarate + DPNH + H^+ + NH_4^+ $\longleftrightarrow$ Glutamate + DPN^+ + H_2O

(ii) Glutamate + TPN^+ + H_2O $\longleftrightarrow$ α-Ketoglutarate + TPNH + H^+

Sum: $TPN^+ + DPNH \longleftrightarrow TPNH + DPN^+$

The effect of a supra-optimal temperature on the enzymes involved in TPNH supply is a problem the solution of which could contribute greatly to clarify the results observed in this study. The significance of a shift in

the ratio of oxidized to reduced coenzyme is not clear, and it remains to be determined if the ratio is indicative of the cellular respiratory pattern.

It seems highly probable that the synthesis or breakdown of storage materials depends on the endogenous concentrations of relatively few small molecules such as phosphate, coenzymes, glucose, etc. Alterations in the concentration of these can shift chemical equilibria in the direction of synthesis or breakdown. This was demonstrated in the experiment implicating biotin in production of storage lipides.

The chemical composition of the cell can vary within wide limits, and until environmental effects on the biosynthetic and degradative pathways are actually demonstated, generalizations will continue to be regarded as speculation.

SUMMARY

Environmental control of the intracellular composition was the subject of this investigation. Saccharomyces cerevisiae strain MCC grown under various cultural conditions exhibits marked variations in the nature and quantity of fatty acids produced.

Recent advances in analytical techniques have made possible the determination of the fatty acid composition of micro quantities of biological lipids. The fatty acids were isolated after saponification and determined spectrophotometrically and gas chromatographically.

When grown under optimal conditions yeast contain less fat than when grown at either sub-optimal or supra-optimal conditions. At 40°C more fatty acids are formed than at 20°C or 30°C. Generally, the richer the medium the more fat will be produced. Aging of the yeast culture has no significant effect on the cellular composition. Biotin plays an important role in fat synthesis. A one-thousand fold decrease in extracellular biotin causes a three-fold decrease in intracellular fatty acid production. Aeration in the presence of glucose of anaerobically grown cells results in a fat content far lower than that found in cells grown in standing culture. Temperature determines the lipid composition of reconstituted cells.

About twice as much fat is produced at 40°C as at 30°C or 50°C in aerated cells.

Sixteen and eighteen carbon acids are the most abundant found under all cultural conditions employed in this study. However, nine different fatty acids were identified. Cyclopropane fatty acids were not found in any of these experiments.

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