is presumed to be due principally to the slow esterification of sterols. The increase in ester value is noticeable within 24 hr. of liberating the fatty acids and the ester value continues to increase slowly for a period of a month or two before leveling off.

Over an extended time period of 4 mo., the acid value of a sample of tallow fatty acids decreased from an initial 205.2 to 203.2 while the saponification value increased from 205.6 to 207.3. The ester value originally was 0.4 and increased to 4.1.

In all work of this nature, involving as it does small differences between acid and saponification values, it is necessary to operate with great care. In this laboratory it has been found possible by rigorous adherence to prescribed experimental conditions to run replicate saponifications that do not differ by more than ± 0.2 saponification value units (5).

The preceding discussion has shown that the acid and saponification values of commercial fatty acids, as commonly determined, can only be approximations. Fortunately the variations in the acid and saponification value are limited in magnitude and do not seriously interfere in the normal course of analytical work. For example, in calculating the anhydrous soap content of a soap containing 72.05% of fatty acids, a negligible difference of 0.05% in the anhydrous soap content results depending upon whether acid value (222.1) or saponification value (224.2) is employed in the calculation.

On the other hand, it does make quite a difference whether the acid value or saponification value is employed in determining the composition of two component fatty acid mixtures. Thus a table of acid values constructed for a mixture of coconut oil (saponification value 266.1) and tallow (saponification value 205.0) gives a result for coconut oil content of 28% versus 31% depending upon which value is used. It is more realistic to employ the acid value (222.1) even though a corrected acid value, if it were practical to correct it, would fall somewhere between 222.1 and 224.2. It would not, except in a minority of cases, constitute an advantage to utilize instrumental methods of analysis in determining the exact nature of the compounds which determine the magnitude of the ester value. However, unless such a study is made, it is improper to apply such corrections to the acid and saponification value, as have been proposed from time to time (1,6). Fortunately for the analytical chemist the advent of vapor phase chromatography provides a far more accurate technique for the determination of the composition of mixed fatty acids than the use of the acid and saponification value.

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Fatty Acid Composition of *Choanephora Cucurbitarum*[1]

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Crude lipid extracts were prepared from *Choanephora cucurbitarum* which had been grown on a glucose-glutamic acid medium for periods up to 12 days. Methanolysis of the extracts and gas chromatography of the esters revealed that palmitate accumulated continuously at a rate 7-fold that of palmitoleate. Oleate accumulation followed a different pattern at a rate nearly 6-fold that of stearate. The esters included linoleate and four others which were not identified.

MANY FUNGI, particularly when grown under conditions of high carbohydrate and low nitrogen supply, synthesize large amounts of lipid (1,2, 3,4,5). *Choanephora cucurbitarum*, a carotene-producing phycomycete (6) which attacks squash blossoms and fruits, can produce as much as 50% of the mycelial dry weight as lipid (2). This report is concerned with the fatty acid composition of this lipid and some effects of culture conditions and age of fungus on the proportions of the acids.

Materials and Methods

Stock cultures of *Choanephora cucurbitarum* NRRL-A-6097 (+) and NRRL-A-6098 (−) were maintained on glucose-yeast agar slants. Transplants were made once a month.

An inoculum was prepared by subculturing from the stock cultures into flasks containing aliquots of a solution composed of 10 g. glucose and 3 g. yeast extract in a liter of water. After 3 days at 28C the cultures were combined in a sterilized Waring Blendor and macerated for 10–20 sec. Unless otherwise stated a 1-ml. sample of the homogenate from the combined + and − cultures was used to inoculate each 100 ml. of the basal medium.

The basal medium contained glucose, 20 g.; L-glutamic acid, 2 g.; KH_2PO_4, 1 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; thiamine, 100 mg.; Fe^{+++}, 0.2 mg.; ZN^{++}, 0.2 mg.; Mn^{++}, 0.1 mg.; Ca^{++}, 10.0 mg.; and water to 1 liter. The pH was adjusted to pH 6.0 before autoclaving.

Cultures were incubated in air at 28C in continuous darkness on a reciprocating-platform shaker operating with 4-in. strokes 86 times a min. Trays were rotated daily.

Two or more cultures were harvested at the desired intervals (7). After extraction of the cultures with 50–100 ml. of hexane-acetone (10:7) in a Virtis homogenizer, they were re-extracted at least twice with 50–100 ml. of acetone, and the homogenate filtered.

The combined hexane-acetone extracts were washed with 50 ml. each of water, methanol-water (9:1), and water in that order. Following passage of the hexane solution through an anhydrous sodium sulfate column the solvent was removed in vacuo and the crude lipid weighed on an analytical balance.

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Gas liquid partition chromatography on a 5-ft. stainless steel column of ethylene glycol succinate polyester on 60-80 mesh firebrick operating at 210C and a helium flow rate of 72 ml./min. was used to determine the fatty acid composition of the lipid. Sensitivity of the recorder was 2 mv. Maintaining the injector part approximately 100C hotter than the column helped to give good resolution of the samples.

The mixed fatty acid methyl esters for injection onto the column were prepared as follows: to 50 mg. of crude lipid in a test tube was added 10 ml. of a mixture which contained ethyl ether-methanol-0.5*N* KOH in methanol (5.0:3.5:0.2), all in the anhydrous state (8). Fifteen hr. later the reaction mixture was neutralized with 0.1*N* H_2SO_4, washed with 0.1*N* NaOH and then water. After drying and removal of the solvent, benzene was used to dissolve the sample before injection of 5 μl.

For quantitative estimation of the various components the areas beneath peaks were cut from the chart and weighed.

Highly purified methyl esters (Hormel) were used as reference standards in the gas chromatographic analyses.

Results and Discussion

Eleven fatty acids were found in the crude lipid of *C. cucurbitarum*. A comparison of the retention times of their methyl esters with those of authentic samples using palmitate as a reference standard showed that laurate, myristate, palmitate, palmitoleate, stearate, oleate, and linoleate were present (Table I). Four other acids were not identified.

A plot of the logarithm of the retention time versus the fatty acid chain length gave a straight line (9,10). Peaks X_1, X_2, and X_3 fell at the points which would be expected for the methyl esters of a C_{14}-monounsaturated, a C_{15}-saturated, and a C_{17}-monounsaturated acid, respectively. The position of Peak X_4 was not correct for either linolenic or arachidic. None of the four were isolated for positive identification.

TABLE I

Retention Times of Methyl Esters of Some Known Fatty Acids and Those from *Choanephora cucurbitarum*

Methyl esters	Retention time relative to that of methyl-palmitate	
	Known esters	Esters from *C. cucurbitarum* oil
Laurate	0.34	0.34
Myristate	0.58	0.59
X_1	.....	0.67
X_2	.....	0.78
Palmitate	1.00	1.00
Palmitoleate	1.09	1.12
X_3	.....	1.50
Stearate	1.74	1.76
Oleate	1.88	1.93
Linoleate	2.24	2.31
X_4	.....	2.62
Linolenate	2.80	.....

Growth of the fungus was much faster in the shaking than in the static type culture (Table II). Addition of molasses also stimulated fungal development. Although the addition of β-ionone to the culture medium is known to increase carotene production (1,2), no consequence of this addition on fatty acid composition was found. Palmitate and oleate were the major fatty ester components in all experiments. The fatty acid compositions of the +, −, and ± cultures were very similar. As the cultures aged, total lipid increased and all fatty acids increased in the glycerides, with the exception of X_3 which was not detectable on the chromatogram after the third day (Table III). However, the pattern of increase differed markedly with the result that, percentagewise, some acids decreased. Thus, the percentage of palmitate and linoleate increased while that of palmitoleate and oleate

TABLE II

Effects of Some Nutritional and Environmental Culture Conditions on the Fatty Acid Ester Composition of *Choanephora Cucurbitarum* Oil [a]

Medium	Glucose-asparagine [b]			Molasses-glutamic [c]		Glucose-glutamic [d]	
Culture Condition	+ Strain	− Strain	± Strains	SHC [e]	SHCB [f]	SHCT [g]	STC [h]
Mycelium weight (mg./culture)	466.3	469.8	477.5	647.3	632.0	282.9	53.7
Lipid (%)	23.0	30.0	33.7	37.8	39.3	22.0	9.1
Fatty ester composition (wt. %)							
Laurate	0.6	0.7	0.8	.....	.....	0.1	0.1
Myristate	2.4	1.9	2.8	1.7	2.0	1.7	1.4
X_1	1.2	1.3	1.1	0.7	1.2	0.2	0.4
X_2	.....	.....	.....	.....	.....	0.2	0.4
Palmitate	21.7	22.2	22.2	12.2	12.4	33.9	29.7
Palmitoleate	11.2	10.2	9.3	9.6	10.3	3.4	6.8
X_3	.....	.....	.....	0.6	0.7	.....	0.7
Stearate	4.8	6.1	4.2	1.2	3.1	7.1	8.9
Oleate	37.3	33.9	39.6	58.8	58.6	35.4	22.7
Linoleate	15.5	17.3	12.7	10.5	9.3	11.3	9.6
X_4	5.2	6.6	7.2	4.8	2.5	9.3	19.3

[a] The data are for 2-day-old cultures except for those under STC which are for 3-day-old cultures.
[b] Basal medium except that asparagine replaced L-glutamic acid as nitrogen source and 20 ml. of β-ionone and 5 g. Tween 80 were added per liter.
[c] Basal medium except that 10 rather than 20 g. of glucose, 30 ml. of molasses and 5 g. of Tween 80 were added per liter.
[d] Basal medium.
[e] Shaken culture. (See Materials and Methods section.)
[f] Shaken culture; 20 mg. of β-ionone were added per culture flask.
[g] Shaken culture; 0.22 g. Tween 80 was added per liter of basal medium.
[h] Static culture.

TABLE III

Changes in Fatty Acid Composition of *Choanephora Cucurbitarum* Oil During Incubation [a]

Incubation time (hr.)	20	28	36	44	56	68	80	96	120	144
Mycelium wt. (mg./culture)	9.74	51.6	214.8	282.9	365.9	425.0	447.2	480.0	489.2	521.8
Lipid (mg./culture)	1.82	8.3	31.8	64.0	82.5	98.5	99.0	112.5	120.0	128.0
%	18.7	16.1	14.85	21.9	22.3	23.2	22.1	23.5	24.6	24.2
Fatty ester composition (wt. %)										
Laurate	0.2	0.14	0.29	0.08	0.13	0.17	0.07	0.07	0.13	0.06
Myristate	2.65	1.80	1.74	1.68	1.98	1.64	1.97	1.48	1.27	1.42
X_1	1.61	0.9	0.2	0.15	0.13	0.06	0.13	0.2	0.06	0.19
X_2	0.7	0.42	0.29	0.23	0.33	0.39	0.59	0.61	0.51	0.68
Palmitate	10.96	13.08	29.7	33.9	34.4	31.8	32.2	31.8	32.2	30.31
Palmitoleate	17.17	10.17	4.15	3.42	3.7	3.74	4.75	3.96	4.08	4.37
X_3	1.81	0.83	1.34	.....	.....	.....	.....	.....	.....	.....
Stearate	2.51	2.63	6.15	7.05	5.94	4.31	4.67	3.76	3.52	3.2
Oleate	42.01	49.76	36.7	35.4	28.4	25.75	22.8	21.15	21.3	20.46
Linoleate	9.28	8.65	11.3	11.27	14.8	18.1	19.7	20.5	20.4	20.95
X_4	11.1	11.63	9.25	6.76	10.0	14.0	13.5	16.4	16.7	18.36

[a] Basal medium including the addition of 0.22 gram of Tween 80 per liter.

decreased. The small amount of oleate added as Tween 80 was neglibible in the final lipid accumulation.

Patterns of fatty acid increase were shown further by calculation of total amounts present (wt. % x mg. of lipid per culture). In the procedure used, the methyl esters measured by gas chromatography were derived from the esterified fatty acids of the crude lipid. The amount of nonesterified substance was apparently small, since typical crude lipid produced by the organism contained 2.4% of free fatty acid and 0.3%of carotenes. The results showed a common pattern of increase for the C_{16} acids (Fig. 1), in which palmitic increased at a rate about 7-fold that of palmitoleic, and a different pattern for the C_{18} acids (Fig. 2) in which oleic increased at a rate nearly 6-fold that of stearic. Stearate reached a maximum after 56 hr., then appeared to diminish measurably, while oleate held steady or increased slightly. Linoleate, slow at start, continued to increase.

The significance of these observations is uncertain, although they do suggest an interrelationship between the acids of a common chain-length. Interconversion by desaturation has been reported in experiments with *Fusarium lini* (11) and yeast (12). However, such interconversion was not observed in experiments with *A. nidulans* (5) or *Phycomyces Blakesleeanus* (13). Observations with seeds from higher plants (14) lend support to the suggestion. Isotopic studies with *C. cucurbitarum* may prove the point.

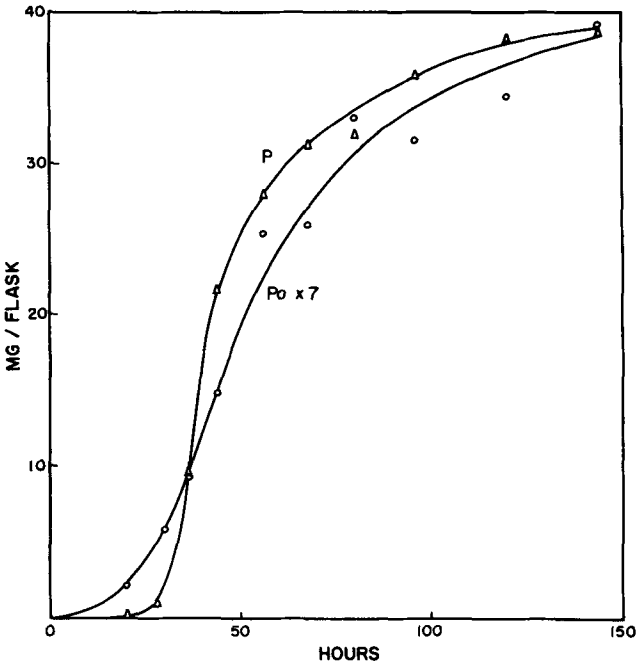

FIG. 1. Accumulation of C_{16}-acids in the crude lipid of *C. cucurbitarum*. P, palmitate; Po x 7, palmitoleate (each value multiplied by seven).

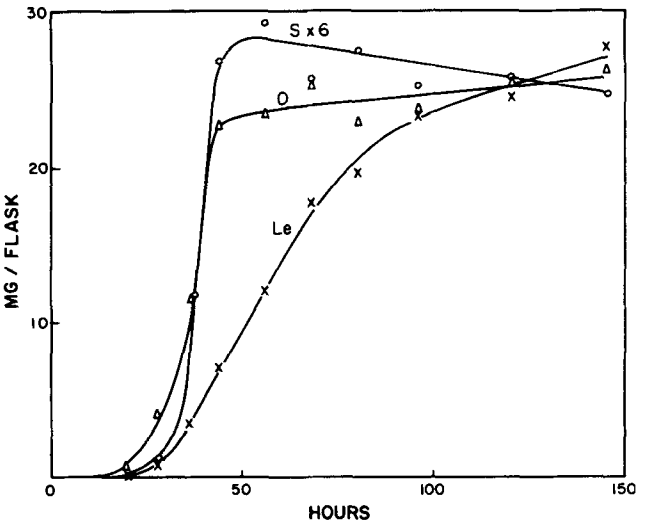

FIG. 2. Accumulation of C_{18}-acids in the crude lipid of *C. cucurbitarum*. Le, linoleate; O, oleate; S x 6, stearate (each value multiplied by six).

Summary

1. Gas chromatography of the methyl esters prepared from the crude lipid of *Choanephora cucurbitarum* revealed the presence of laurate, myristate, palmitate, palmitoleate, stearate, oleate, linoleate, and four unidentified esters.

2. The fatty ester compositions of +, −, and ± cultures were qualitatively and quantitatively very similar.

3. Accumulation of total lipid in the culture was continuous throughout a 12-day period of incubation. Palmitate accumulated at a rate about 7-fold that of palmitoleate, and by a different pattern than oleate which accumulated at a rate nearly 6-fold that of stearate.

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