tomato juice was treated with Pronase. The increase in viscosity after Pronase digestion was greater in Chico III AIS than in Homestead-24 suspensions.

Enzymatic digestion with cellulase and pectinase caused a decrease in the viscosity of a 1% suspension of AIS from both cultivars. Pectinase decreased the viscosity to a greater extent than did cellulase. This may be attributed to the cellulase contamination inherent in commercial pectinase preparations. As was the case with Pronase, the change in viscosity was greater in the suspensions of Chico III AIS.

The AIS levels tended to be indicative of the shape of tomato. The levels of protein and pectin in Chico III and Homestead-24 AIS were not sufficiently different to account for the previously noted differences in characteristics (Stephens et al., 1970); however, protein levels in the AIS were generally lower in cultivars with high AIS. The compositions of the various pectic fractions of Chico III and Homestead-24 AIS were not different. Viscosity of 1% suspensions was considerably higher for Chico III than Homestead-24. This shows that AIS content alone does not explain the attributed differences. Enzymatic digestion of AIS suspensions of the Chico III and Homestead-24 tomatoes indicated a combination of factors may affect the character of processed tomato products.

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Lipids of *Penicillium roqueforti*. Influence of Culture Temperature and Age on Unsaturated Fatty Acids

James L. Shimp and John E. Kinsella*

The lipids of *Pencillium roqueforti* were influenced by culture age and temperature. Maximum lipid accumulation in the mycelium occurred at a culture age of 120 h at 25 °C. During the log growth phase the relative proportions of both protein and polar lipid decreased, while carbohydrate and triglycerides increased. Palmitic, stearic, and oleic acids increased to a peak level at 120 h of incubation and then declined, whereas linoleic acid increased throughout the incubation period. A decrease in the growth temperature resulted in a decrease in the amount of all components in the mycelium. The relative proportions of protein, polar lipid, and free fatty acid increased while triglycerides decreased as growth temperatures were lowered. As temperature was decreased from 25 to 11.5 °C, linoleic acid content increased from 20.6 to 26.0%, and linolenic acid from 0 to 9.1% of total fatty acids. These increases occurred principally in the polar lipids. The increase in linoleic acid during the log growth phase occurred almost entirely within the polar lipids, whereas during the stationery phase, the increase occurred mostly within the triglycerides.

Penicillium roqueforti is the principal microorganism involved in the biochemistry of blue-type cheeses where lipid catabolism and oxidation are essential events in the ripening process (Kinsella and Hwang, 1976, 1977). However, there is little information available regarding the composition and metabolism of the lipids of mycelium of *P. roqueforti* and the factors which affect these.

There are data available on the lipids of other fungi (Weete, 1974), though most analyses are not comprehensive. The effects of changes in growth temperature on fungal lipid content and fatty acid composition have been investigated (Kates and Baxter, 1962; Meyer and Bloch, 1963; Brown and Rose, 1969; Kates and Paradis, 1973). The effects of culture aging on composition of fungi have been reported in relation to protein and carbohydrate (Gottlieb and VanEtten, 1964), lipid content (Weete et al.,

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1973), lipid composition (Bhatia et al., 1973), and fatty acid composition (VanEtten and Gottlieb, 1965; Dawson and Craig, 1966; Safe, 1974).

The present paper reports the effects of culture age and growth temperature on protein and lipid content, and on the detailed lipid composition of the mycelium of *Penicillium roqueforti*. An understanding of the factors affecting lipid metabolism is necessary to gain some understanding of the metabolic activities occurring in the mold. This is of particular interest in the case of *P. roqueforti*, where knowledge of lipid composition and factors affecting lipid metabolism may help elucidate the interrelationships between lipids originating in the cheese and endogenous mold lipids in relation to fatty acid oxidation and flavor generation in mold ripened cheese (Kinsella and Hwang, 1977). Furthermore we were interested in determining if the increased level of linoleic acid found in blue cheese following ripening (Cruces and Kinsella, 1977) was possibly contributed by the mold lipids.

EXPERIMENTAL SECTION

Culture Conditions. *Penicillium roqueforti* spores were obtained from Dairyland Food Laboratories, Inc. (Waukesha, Wis.). The fungus was grown and collected as previously described (Shimp and Kinsella, 1976). Liquid-submerged cultures of vegetative mycelia, cultured in corn steep liquor/sucrose media (each 1.5% by weight), were constantly agitated on magnetic stir plates.

The effects of varying temperatures on mycelial composition were evaluated by growing the mold at temperatures of 11.5, 20, 25, and 35 °C. All cultures were maintained at a uniform stirring rate for 120 h.

Two series of tests concerning the effects of aging on mycelial composition were run, one at 11.5 °C and the other at 25 °C. Cultures were maintained at a uniform stir rate for 24, 42, 60, 120, 240, and 480 h in each of these tests.

Solvents. Chloroform, petroleum ether, and diethyl ether (Mallinckrodt, AR grade) were used as supplied. Methanol (AR grade) was purchased from Fisher Scientific Co. and redistilled prior to use.

Analysis of Components. After two extractions of the harvested mycelium by the method of Folch et al. (1957), the chloroform layers containing the extracted lipids were pooled, the solvent was evaporated, and the lipids were weighed. Lipid samples were dissolved in chloroform containing antioxidants and stored at −35 °C.

The nitrogen content of dry mycelial samples was determined by the microkjeldahl method (AOAC, 1970). A modification of the Biuret test described by Norris and Ribbons (1969) was used to determine the amount of protein in the mycelium. The carbohydrate content of the mycelium was computed by difference.

Analytical Procedures. Phospholipids were determined colorimetrically by the method of Raheja et al. (1973), using phosphatidylcholine (Nutritional Biochemicals Corp., Cleveland, Ohio) for preparation of a standard curve. Other lipids were quantified by gas–liquid chromatography after separation by thin-layer chromatography. Silica gel containing specific classes of lipids was scraped from the chromatographic plates and the lipids were then eluted with diethyl ether. Tripentadecanoin, dipentadecanoin, and heptadecanoic acid were added as internal standards for quantification of triglycerides, diglycerides, and free fatty acids, respectively (Litchfield, 1972). Transmethylation of the fatty acids was performed using boron trifluoride (Morrison and Smith, 1964). A Hewlett-Packard Model 5830A gas chromatograph equipped with a flame ionization detector was used for separation of fatty acid methyl esters. Stainless steel

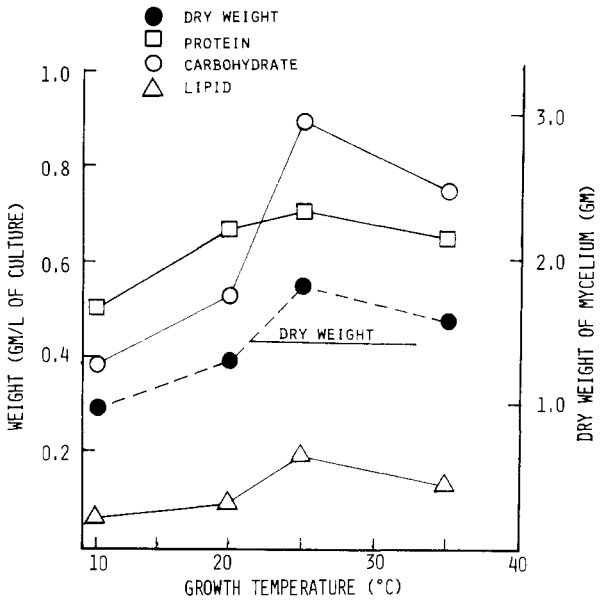

Figure 1. Proximal composition of *Penicillium roqueforti* mycelium at various growth temperatures when incubated for 120 h.

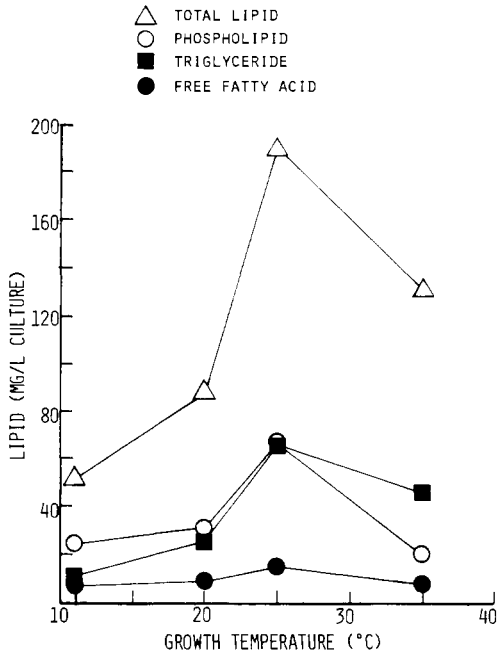

Figure 2. Differences in lipid classes of *Penicillium roqueforti* mycelium when incubated for 120 h at various growth temperatures.

columns (1.9 m × 2.16 mm) packed with 10% EGSS-X on Gas Chrom P (100/120 mesh) were used in the dual column system. Isothermal operating conditions were: nitrogen pressure, 40 psi (31 mL/min); air pressure, 22 psi; hydrogen pressure, 20 psi; oven temperature, 180 °C; injection port temperature, 250 °C; detector temperature, 300 °C. The quantity of fatty acids in the total lipid fraction was determined using heptadecanoic acid as an internal standard.

RESULTS

A. Effects of Growth Temperature on Composition. When cultures were incubated for 120 h at different temperatures, maximum dry weight (1.84 g of mycelium/L) was obtained at 25 °C (Figure 1). Protein, carbohydrate, and lipid levels were also highest at 25 °C,

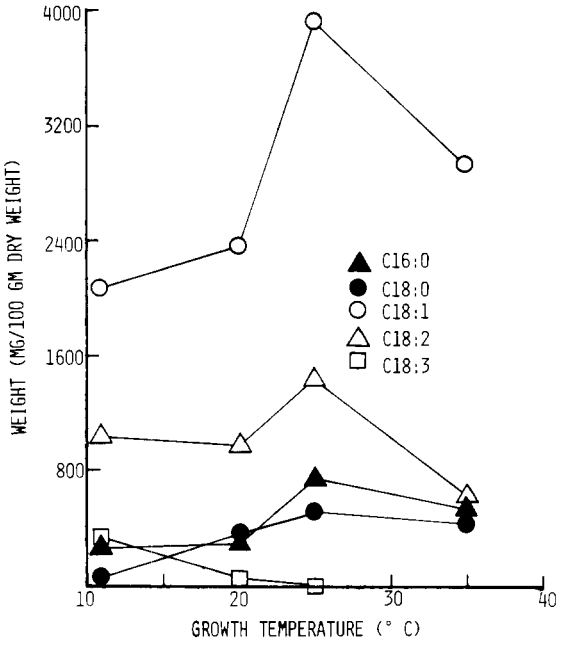

Figure 3. Fatty acid composition of lipids from *Penicillium roqueforti* mycelium when incubated for 120 h at various growth temperatures.

although on a percentage basis the level of protein was higher at lower temperatures.

Culturing at low temperatures apparently favored formation of polar lipids, while increasing the growth temperature to 25 °C resulted in increased levels of triglycerides (Figure 2). Thus, at 11.5 and 25 °C, the percentage of phospholipids was 40.4 and 35.3%, respectively. A further temperature increase of 10 degrees to 35 °C diminished the percentage of phospholipid in the mycelium to 15.2%. Triglyceride changes were oppositely affected, i.e., a decrease in temperature from 35 to 20 °C caused a decrease in triglycerides from 36 to 27% of total lipids. Growth at 11.5 °C resulted in a decrease in triglycerides to 15.8% of total lipids. The quantity of fatty acids (saponifiable lipid) was greatest at 25 °C, and it was also at this temperature that the proportion of fatty acids in the total lipid fraction (68%) was highest.

Formation of unsaturated fatty acids in the mycelium of *P. roqueforti* was favored by culturing at low temperatures (Figure 3). At 11.5 °C, 9% of the total fatty acid was present as linolenic acid, while at 25 °C linolenic acid occurred in trace amounts. The level of stearic acid was particularly depressed at 11.5 °C. The increased proportion of polyunsaturated fatty acids in response to lower growth temperature was associated almost entirely with concurrent increases in the polar lipid fraction since neither triglycerides nor free fatty acids reflected the higher levels of linoleic and linolenic acids found in the total lipid fraction under these conditions (Table I).

B. Effects of Age on Composition. *P. roqueforti* mycelia were grown for 24, 42, 60, 120, 240, and 480 h at growth temperatures of 11.5 and 25 °C to examine the effects of duration of incubation on composition. The previous studies demonstrated that 25 °C was optimum for mold growth and lipid accumulation, and therefore this temperature was chosen for studies concerned with culture aging. Studies involving culture aging were also made at 11.5 °C because this is the storage temperature usually recommended for growth of the mold in Bleu and Roquefort cheeses (Kosikowski, 1966).

At 25 °C dry weight of the mold increased exponentially up to 60 h of incubation (Table II). Lipid content increased gradually up to 120 h of incubation and then remained constant. Protein also remained at a constant level after the log phase of growth; however, carbohydrate continued to increase, accounting for dry weight increase as the culture aged. At 11.5 °C a decreased rate of growth was evident (Table II). The dry weight increase was nearly linear throughout the incubation period. There was no evidence of an exponential growth phase.

At 25 °C polar lipids were the major lipid class present in the early stages of growth of the mycelium. An exponential rise in phospholipid content similar to that which occurs for proteinaceous material was evident during the log phase (Figure 4). After an initial rise in the phospholipids, a stable level was reached at 120 h of incubation. The weight of phospholipid per unit dry weight of mycelium remained nearly constant until late in the stationary growth phase when carbohydrates accumulated.

The percentage of fatty acids in the total lipid fraction of *P. roqueforti* mycelium increased progressively during incubation at 11.5 and 25 °C. At 25 °C, fatty acid levels ranged from 56% of the total lipid weight at 24 h, to 73%

Table I. Fatty Acid Composition of the Lipids of *Penicillium roqueforti* Mycelium at Various Growth Temperatures When Incubated for 120 h

	Weight percent of total fatty acid			
	Growth temperature, °C			
Lipid component	11.5	20	25	35
Total lipid				
Palmitic (C16:0)	7.8	7.9	10.7	11.8
Stearic (C18:0)	1.9	8.8	7.6	9.4
Oleic (C18:1)	52.1	54.5	56.0	61.4
Linoleic (C18:2)	26.0	23.4	20.6	13.4
Linolenic (C18:3)	9.1	1.5		
Triglyceride				
Palmitic (C16:0)	13.2	9.9	10.8	11.5
Stearic (C18:0)	11.9	14.8	11.0	11.2
Oleic (C18:1)	59.5	62.4	64.3	63.3
Linoleic (C18:2)	12.8	8.5	9.6	9.4
Linolenic (C18:3)				
Free fatty acid				
Palmitic (C16:0)	10.3	7.7	5.8	10.7
Stearic (C18:0)	4.4	9.0	8.2	13.2
Oleic (C18:1)	62.8	65.5	70.6	68.7
Linoleic (C18:2)	17.2	14.0	10.7	5.5
Linolenic (C18:3)				

Table II. Proximal Composition of *Penicillium roqueforti* Mycelium at Progressive Stages of Incubation When Grown at 11.5 and 25 °C

	g/100 g of dry mycelium					
	Incubation time, h					
Components	24	42	60	120	240	480
25 °C						
Protein[a]	69.1	57.7	42.9	37.8	28.1	26.0
Protein[b]	47.0	37.3	36.0	34.9	26.2	24.9
Carbohydrate	22.9	32.8	45.1	48.3	59.5	62.3
Lipid	4.0	5.5	7.5	10.4	8.8	7.2
Ash	4.0	4.0	4.5	3.5	3.6	4.5
Dry weight (g/L of culture)	0.30	0.94	1.45	1.84	2.34	3.30
11.5 °C						
Protein[a]	73.1	72.2	70.8	51.2	44.3	32.3
Carbohydrate	16.1	17.4	20.3	38.9	46.0	58.2
Lipid	6.9	6.9	5.4	6.4	6.2	5.4
Ash	3.9	3.5	3.5	3.5	3.5	4.1
Dry weight (g/L of culture)	0.10	0.09	0.20	0.87	1.71	2.75

[a] Protein determined by Kjeldahl nitrogen × 6.25. [b] Protein determined by Biuret, Norris, and Ribbons, 1969.

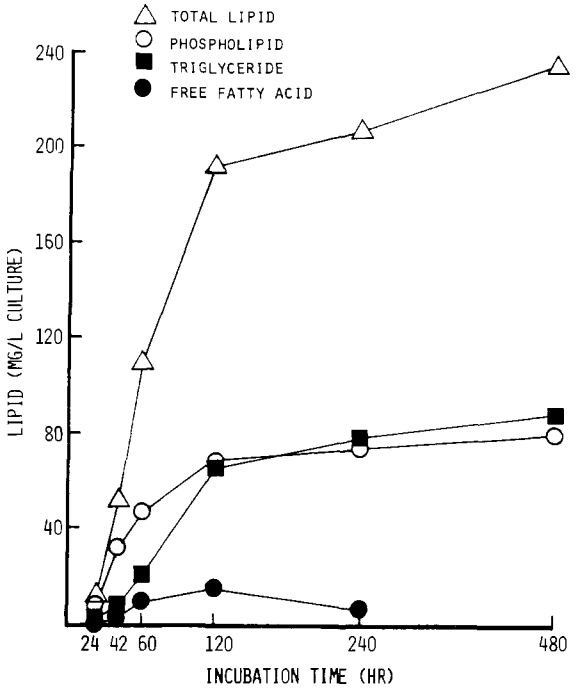

Figure 4. Changes in the composition of lipids from *Penicillium roqueforti* mycelium at progressive stages of incubation when grown at 25 °C.

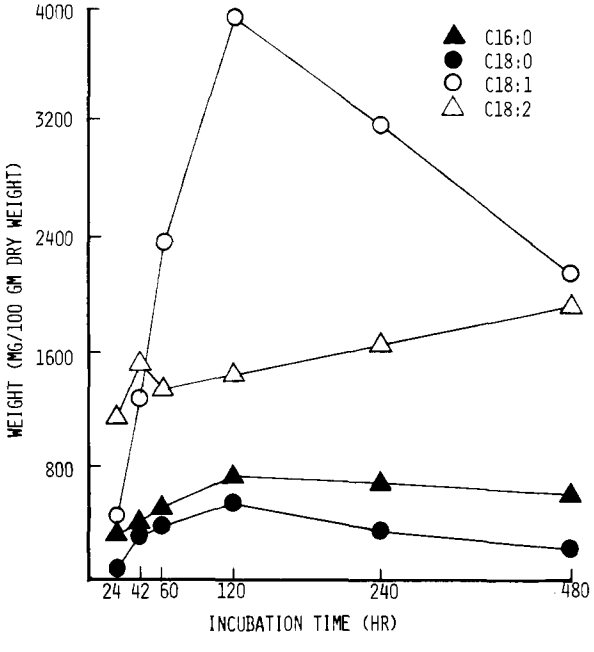

Figure 5. Fatty acid composition of *Penicillium roqueforti* mycelium at progressive stages of incubation when grown at 25 °C.

after 480 h. This indicated increased synthesis of glycerides over the course of incubation and in fact, triglycerides increased from 6.9% of the total lipid weight at 24 h, to 33.8% after 120 h of incubation (Figure 4). The amount of triglycerides present in the mycelium began to increase only midway through the log phase, but most of the lipid accumulation in the mycelium over the duration of the incubation period could be attributed to increased synthesis of triglycerides (Figure 4).

Total fatty acids (mg/100 g of dry weight) rose until 120 h of incubation and then declined as the mycelium accumulated carbohydrate during the stationary phase of growth (Figure 5). The major fatty acid present was oleic

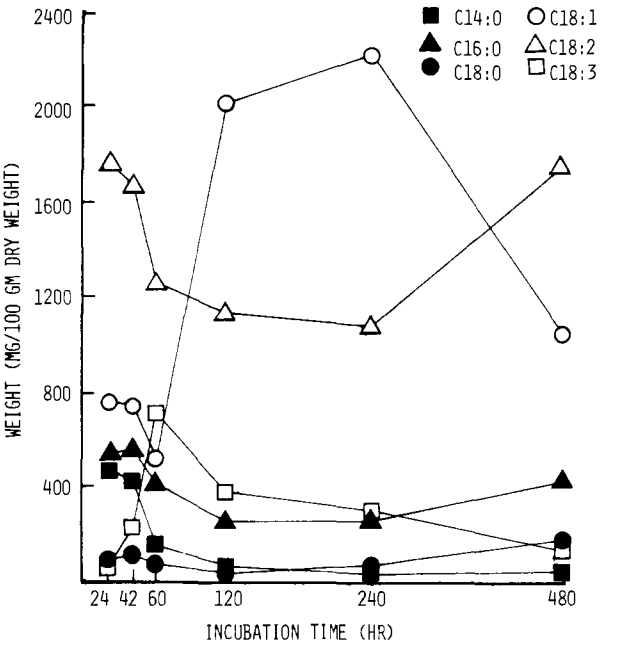

Figure 6. Fatty acid composition of *Penicillium roqueforti* mycelium at progressive stages of incubation when grown at 11.5 °C.

acid, which reached a peak of 56% of total fatty acids at 120 h of incubation and then declined. Palmitic and stearic acids followed the same general pattern. In contrast, linoleic acid levels gradually increased throughout the incubation period. At 11.5 °C a lower mycelial growth rate resulted in lower levels of fatty acids than at 25 °C (Figure 6). A decrease in oleic and linoleic acids accompanied by a concurrent increase in linolenic acid was observed in mold grown at 11.5 °C. The level of linolenic acid increased from zero at 24 h, to a peak of 22.5% of the total fatty acid weight at 60 h, and thereafter declined fairly rapidly. The mycelium apparently adapts to low temperatures by increasing the production of unsaturated fatty acids.

An increase in unsaturation of fatty acids independent of any temperature effect seemed to occur after the mycelium had been incubated (had aged) for some time. At 25 °C an increase in linoleic acid began at approximately 60 h of incubation, whereas at 11.5 °C the increase was not apparent until nearly 240 h of mold growth.

Most of the linoleic acid in the mycelium of *P. roqueforti* was present in the polar lipid class (Shimp and Kinsella, 1976). Since polar lipid levels remained nearly constant and eventually declined after termination of the log growth phase (Figure 4), it was not expected that the levels of linoleic acid in the total lipid fraction should increase at progressive incubation stages at 25 °C, however, the data in Figure 5 indicate an increase. Phospholipid levels changed from 3230 mg/100 g of dry mycelium (47 mg/L of culture) at 60 h to 2420 mg/100 g (80 mg/L) at 480 h, while linoleic acid increased from 1340 to 1930 mg/100 g of dry mycelium (19.4–63.8 mg/L) over the same time period (Table III). Thus, considerable desaturation occurred during this period of incubation.

Analysis of the triglycerides of the mycelium revealed a pattern of increase and subsequent decline of palmitic, stearic, and oleic acids, and a progressive increase in linoleic acid in a manner paralleling the changes of these fatty acids in the total lipid. However, the level of linoleic acid (mg/100 g of dry mycelium) in the triglyceride fraction increased at a greater rate than in the total lipid fraction (Table III). Linoleic acid (mg/100 g of dry mycelium)

Table III. Content of Lipid Classes of *Penicillium roqueforti* Mycelium at Progressive Stages of Incubation When Grown at 25 °C

Lipid class	Incubation time, h					
	24	42	60	120	240	480
	mg/100 g of dry mycelium					
Phospholipids	2730	3450	3230	3670	3170	2420
Triglycerides	380	780	1390	3520	3230	2560
	mg of linoleic acid/100 g of dry mycelium					
Total lipids	1130	1520	1340	1440	1670	1930
Triglycerides	110	150	180	360	670	620
Polar lipids[a]	1020	1370	1160	1080	1000	1310
	mg of linoleic acid/L of culture					
Total lipids	3.4	14.2	19.4	26.5	39.2	63.8
Triglycerides	0.3	1.4	2.7	6.7	15.8	20.4
Polar lipids[a]	3.1	12.8	16.7	19.8	23.4	43.4

[a] By difference.

increased by 44% (44.4 mg/L of culture) in the total lipid fraction from 60 to 480 h of incubation, while the corresponding increase within the triglyceride class was 237% (17.7 mg/L) over the same period (Table III). Thus, linoleic acid associated with the triglycerides increased from 2.7 to 20.4 mg/L of culture from 60 to 480 h, while linoleic acid associated with polar lipids increased from 16.7 to 43.4 mg/L over the same period (Table III). This indicated that a significant proportion of the unsaturated fatty acids formed during the stationary growth phase were concentrated in the triglyceride portion of the mycelial lipid.

The marked increase in linoleic acid content of the triglycerides is revealed by a typical chromatogram (Figure 7).

DISCUSSION

A. Temperature. The present study indicated that a growth temperature of 25 °C resulted in higher yields of mycelia and lipid material than were obtained at 11.5 or 20 °C, while a higher culture temperature, i.e., 35 °C, caused a decline in yield. Weete (1974) and Safe (1974) similarly reported that lipid levels increased in fungi as growth temperature was increased from suboptimum to optimum for fungal growth, but beyond this optimum temperature lipid accumulation was depressed.

Though the greatest amounts of phospholipid and triglyceride were present at a growth temperature of 25 °C, the present study demonstrated that the proportion of phospholipid and free fatty acid decreased while the proportion of triglycerides in mycelium of *P. roqueforti* increased as temperature was increased. Higher proportions of protein and phospholipid obtained at suboptimal growth temperatures are attributable to decreased growth rates at the lower temperatures. Increasing the growth temperature for *P. roqueforti* resulted in an increased rate of growth which hastened cellular reproduction and which subsequently led to carbohydrate and triglyceride accumulation. Safe (1974) noted an increase in the proportion of polar lipid in *Mucor rouxii* with decreased temperatures. Parmegiani and Pisano (1974) compensated for the different growth rates of fungi cultured at different temperatures by harvesting when equal mycelial yields were obtained. They observed a higher absolute level of phospholipids at the lower temperature for *Cephalosporium chrysogenum* but not for *Paecilomyces persicinus* nor *Emericellopsis salmosynnemata*.

B. Age of Culture. *P. roqueforti* mold develops and ages in a manner similar to the lag, log, stationary, and death phases described for most microorganisms (Weete, 1974). In the present study, high levels of nitrogenous compounds were observed early in the growth of mycelia

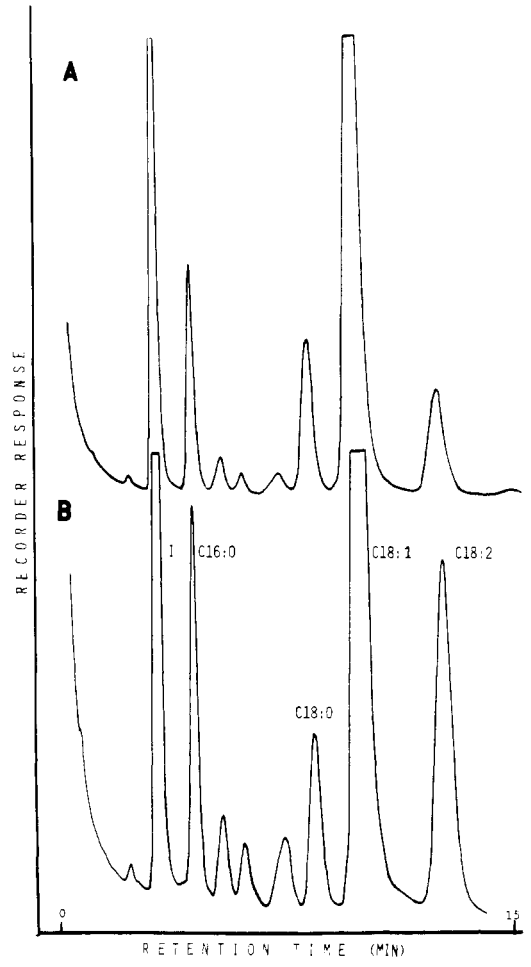

Figure 7. Chromatograms showing fatty acids associated with triglycerides isolated from mycelia after 120 (A) and 240 (B) h culturing at 25 °C. Legend: I = internal standard pentadecanoate; C16:0, C18:0, C18:1, C18:2 denote palmitic, stearic, oleic, and linoleic acids, respectively.

of *P. roqueforti*. Nonprotein nitrogen was particularly high during the lag growth phase which may reflect the presence of nucleic acids and free amino acids which are present at high levels in most organisms prior to extensive protein synthesis. The dry weight increase after termination of the log phase of growth was due almost exclusively to an increase in carbohydrate content of the mycelium. This is in agreement with findings of Blumenthal and Roseman (1957), Fluri (1959), and Gottlieb and VanEtten (1964). Carbohydrate is the major storage component in most fungi, except for a few species such as *Pythium irregulare*

(Bhatia et al., 1973) which accumulate appreciable quantities of lipid during the stationary growth phase.

At progressive stages of incubation at both 11.5 and 25 °C, an increase in the fatty acid content of the total lipid fraction of *P. roqueforti* was observed. This was attributed primarily to an increase in the triglyceride fraction which occurred over the course of incubation. The level of phospholipid (mg/100 g of dry mycelium) in the mycelium remained fairly constant until accumulation of large amounts of carbohydrate in the mycelia of *P. roqueforti* resulted in some decline in the relative proportion of phospholipid during the stationary growth phase. Bhatia et al. (1973) reported an increase in triglycerides and a relatively stable level of polar lipid for *Pythium irregulare*, while Weete (1974) reported that the triglycerides increased in nearly all fungi as the mycelia aged.

C. Factors Influencing Unsaturated Fatty Acids. In the present study we observed an increased level of linolenic acid when *P. roqueforti* was cultured at low temperatures, and an increased level of linoleic acid when cultures were aged. These unsaturated fatty acids are probably produced by desaturase enzymes as shown in other fungi, e.g., *Candida utilis* (Yuan and Bloch, 1961), *Penicillium chrysogenum* (Bennett and Quackenbush, 1969; Richards and Quackenbush, 1974), and *Neurospora crassa* (Baker and Lynen, 1971). The increased level of unsaturated fatty acids may have been caused by increased enzyme levels or increased enzyme activity. The pathways involved in biosynthesis of unsaturated fatty acids in filamentous fungi appear similar to those found in most organisms, though elements common to both plant and animal pathways are present (Weete, 1974; Richards and Quackenbush, 1974).

a. Effects of Temperature. We observed an increase in linolenic and to a lesser extent linoleic acid in *P. roqueforti* in response to decreased growth temperatures. Numerous other reports indicate a higher proportion of unsaturated fatty acids in fungi as growth temperatures are decreased (Kates and Paradis, 1973; Sumner, 1969; McMurrough and Rose, 1971; Chang and Matson, 1972). The effects resulting from a change in growth temperature may be attributed to changes in growth rate and/or the content of dissolved oxygen in the culture media. It is unclear from most reports, however, whether the reported high levels of unsaturated fatty acids were attributable directly to a decrease in culture temperature, or increased oxygen solubility which results from decreased temperatures.

Oxygen is a requirement for fatty acid desaturase activity in fungi and most microorganisms (Weete, 1974; Baker and Lynen, 1971; Gurr, 1974). At a constant temperature, increased oxygen tension resulted in an increase in the content of unsaturated fatty acids for *Candida utilis* (Brown and Rose, 1969). Harris and James (1969) have concluded that the increase in unsaturation accompanying growth at low temperatures is due primarily to increased oxygen solubility with decreasing temperature.

Oxygen solubility changes alone, however, may not be the only factor stimulating fatty acid dehydrogenation at low temperatures. When a constant oxygen tension was maintained in chemostatically grown cultures, decreasing temperatures were accompanied by an increase in the absolute levels of linoleic and linolenic acids in *Candida utilis* (Brown and Rose, 1969). However, even when oxygen tensions are maintained at what appeared to be a constant level in a chemostat, intracellular oxygen availability may still increase as growth temperatures are decreased. Thus, the effects noted by Brown and Rose may be attributable, at least in part, to increased oxygen availability at low temperatures.

Fulco (1972) also observed temperature regulation of fatty acid desaturation in *Bacillus megaterium*. He found that a temperature change of the culture from 21.5 to 19.6 °C resulted in a more than twofold increase in the half-life of the desaturase. Thus lower temperatures may result in increased levels of acyl desaturase.

In the present study, an increase in the absolute amount of linolenic acid was obtained in mold grown at 11.5 °C. Linolenic acid accumulation began at 24 h of incubation, peaked at 60 h of growth (23% of total fatty acid), and then gradually decreased throughout the incubation period. It therefore appears that the decreased temperature and/or increased oxygen solubility resulting from the decreased temperature, was responsible for the observed increase in linolenic acid in this case. *P. roqueforti* apparently adapts to low growth temperatures by increasing the synthesis of unsaturated fatty acids in order to maintain membrane lipids in a fluid state.

b. Effects of Culture Age. Preferential synthesis of unsaturated fatty acids early in the growth of fungi (lag and log phases) gives way to increased formation of saturated fatty acids after the rate of cellular reproduction subsides and the accumulation of storage lipids occurs (stationary phase). The present study revealed a pattern of increased saturation of fatty acids from 0 to 60 h of growth in the mycelium of *P. roqueforti* grown at 25 °C. However, from 60 to 480 h of incubation linoleic acid (mg/100 g of dry weight) increased at the expense of oleic acid. A general trend toward increased saturation of fatty acids with aging of fungi has been reported (Bhatia et al., 1973; VanEtten and Gottlieb, 1965; Leegwater et al., 1962). The increase in linoleic acid with aging which has been reported for *P. roqueforti* in the present study has not been widely reported in fungi, which may be partially because most fungal aging studies are of considerably shorter duration than the present study. In *Choanephora cucurbitarum*, linoleic acid increased gradually throughout the incubation period studied (White et al., 1962).

The pattern of linoleic acid accumulation observed during the stationary phase for *P. roqueforti* may offer some insight into what is occurring in the mold cells. When the dry weight increase accompanying aging of the culture is taken into account, it is apparent that the level of linoleic acid (mg/100 g of dry mycelium) in the phospholipids remained nearly constant throughout the incubation period, while the considerable increase in linoleic acid which occurred in the total lipid fraction during the stationary growth phase was reflected predominantly within the triglycerides. This is different from the pattern of linoleic acid accumulation observed during the log growth phase, where the increase was confined to the polar lipid class. Likewise at suboptimal growth temperatures, linoleic and linolenic acid accumulation also occurred primarily within the polar lipid class.

Increased levels of linoleic acid in the mycelium are of interest in relation to previous studies conducted in our laboratory which showed an increase in the level of linoleic acid in the triglyceride fraction of blue cheese during aging (Cruces and Kinsella, 1977). It appears that such increases are attributable to increased desaturase activity which results in elevated linoleic acid levels in the triglycerides of the mold in response to aging and culturing at low temperatures.

The variability in linoleic and linolenic acid in the mycelium may be significant in relation to nutritive value of cheese and also have an impact on cheese flavor. The

compound 1-octen-3-ol has been observed in mold ripened cheese (Adda and Dumont, 1974; Groux and Moinas, 1974) and this compound can be produced by *Penicillium* species (Kaminski et al., 1974). At high concentrations this compound possesses a musty, mushroomy flavor. Ney et al. (1975) found that the inclusion of octenol in a simulated blue cheese flavor improved the overall flavor quality. This compound can be generated from linolenic acid via autoxidation (Forss, 1972). Conceivably the levels of linolenic and possibly linoleic acid in mold mycelium may influence the amount of octenol produced and thereby influence the flavor quality of cheese. Because aged mycelium contains much greater levels of polyunsaturated acids, aged mold-ripened cheeses may have a greater tendency to generate octenol.

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Lipids in the Exterior Structures of the Hen Egg

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Studies are reported on the composition of lipids of shell with cuticle (SC) and shell membrane (SM) from hen egg. Total lipids were approximately 0.045% of SC and 1.35% of SM. The ratios of neutral lipid to polar lipid in SC and SM were 5:1 and 6:1, respectively. The neutral lipid fractions of SC and SM were found to contain mono-, di-, and triglyceride, cholesterol, cholesteryl ester, and free fatty acid as well as fairly large amounts of bis(2-ethyl hexyl) phthalate. The major neutral lipid (excluding the phthalates) was cholesterol, and the levels of triglyceride were very low in the neutral lipids of SC and SM. The polar lipid fractions of SC and SM were found to contain very low levels of phosphatidylethanolamine and phosphatidylcholine. The predominant phospholipid were sphingomyelin. Significant amounts of ceramide mono- and dihexoside were also detected in the polar lipid fractions of SC and SM. At least 17 different fatty acids were present in SC and SM lipids. The level of linoleic acid was higher in SM than in SC neutral lipid. The fatty acid distributions of polar lipids of SC and SM were similar.

The exterior structures of the hen egg (inner membrane, outer membrane, shell, and cuticle) have significant chemical, biological, and mechanical roles in preservation (Baker and Balch, 1962). Little has been reported about the nature of the lipids in these structures (Hasiak et al., 1970a,b; Maesso et al., 1974). One report (Hasiak et al., 1970b) on the fatty acid composition of lipid in these structures included up to 67.7% of nonadecenoic acid and up to 24.0% of heneicosanoic acid. Recently, Pascal and Ackman (1976) have pointed out that these odd numbered

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