Effects of Various Carbon and Nitrogen Sources on Fungal Lipid Production

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ABSTRACT

The efficiency of lipid production by *Tolyposporium ehrenbergii* and *Spacelotheca reiliana* (family Ustilaginaceae) cultivated on a fat-free medium was evaluated. The fungi were artificially cultured on media containing various mono- or disaccharides and urea or peptone as basic sources for carbon and nitrogen, respectively. Some natural and industrial byproducts (glycerol, glucose syrup, potato hydrolysate and molasses hydrolysate) were also used as principal carbon sources for fungal growth. Media containing fructose/peptone or glucose/peptone were the most efficient for fungal fat production during one week of incubation. After a two-week incubation period, highest phospholipid concentrations were found in *S. reiliana* and *T. ehrenbergii* mycelia obtained from media containing molasses hydrolysate/peptone and glycerol/urea, respectively. Fatty acid analysis of the fungal growth indicated the presence of a wide range of fatty acids, i.e., odd- and even-carbon numbered acids, both saturated and unsaturated. The results demonstrated that the variability in the fatty acid composition largely depends on the type of sugar and nitrogen sources and the age of the culture.

INTRODUCTION

The demand for oils and fats for consumption or industry is increasing annually. The price of these materials has increased tremendously in the past few years. The concept of producing food from waste has become of great interest as a result of increasingly frequent food shortages and increases in prices. The high yields from classical crops are dependent on different inputs which are expensive and scarce, for instance, fertilizer and water. The energy crisis and the dramatic increase in the cost of oil is causing the green revolution to wither. During the last few decades, a new biotechnological industry has emerged and developed based on the large-scale commercial culture of microorganisms. This new knowledge and expertise in biotechnology is being applied to microbial biosynthesis as a means of adding to our food supply.

Some microorganisms have the ability to produce fat and can be considered as a possible alternative source for classical oil crops. The choice of growth substrates as well as growth conditions is of utmost importance in order to direct the microorganism to synthesize certain compounds. However, acceptance of microbial food must be preceded by extensive clinical and toxicity feeding tests.

The main objective of the present work was to direct the fungi to produce fats by culturing them on a fat-free medium containing various mono- or disaccharides and peptone. Some natural and industrial byproducts as cheap and abundant substrates, e.g., sugar cane molasses (a waste of the sugar industry), potato, glucose syrup (a byproduct from the starch industry), glycerol (from soap industry) and urea as basic carbon and nitrogen sources were also used. The influence of the aforementioned compounds on composition of fungal fatty acids was also studied.

MATERIALS AND METHODS

Fungi

Two fungi, namely *Tolyposporium ehrenbergii* and *Sphacelotheca reiliana* belonging to the Ustilaginaceae family were used in the present study in order to direct their metabolic pathways towards lipid production. Fungi were isolated by a single spore technique (1) from mature sacs obtained from infected corn plants. Inoculum was prepared by first growing the organism on a potato-dextrose agar (PDA) at 30 C ± 1 C to obtain sufficient fungal growth.

The fungi were artifically cultured on Shaw medium (2). The medium consisted of 5.0 g KH_2PO_4, 1.0 g $MgSO_4$, 10.0 g peptone, 40.0 g glucose, 20.0 g maltose and 1 mL of trace metal solution per liter. The trace metal solution contained 5.0 g $Fe_2(SO_4)_3$, 1.0 g $ZnSO_4$, 0.5 g $CuSO_4$, 0.1 g $MnSO_4$, 0.1 g boric acid and 0.1 g $Ca(OH)_2$ per liter. The cultures were grown in 1000-mL Erlenmeyer flasks containing 330 mL medium using a rotary shaker (25 oscillations/min) and incubated at 30 C ± 1 C for one- and two-week periods. In some experiments, urea (ca. 3.50 g/L) was added instead of peptone and other sugars (80 g/L as monosaccharides) to maintain the ratio of carbon sources (sugars) and nitrogen sources (urea and peptone) in the substrates of 20:1.

Substrates

Extra pure mono- and disaccharides (glucose, fructose, maltose and sucrose) used in the present work were "Merck" grade. Several natural and industrial byproducts were used as basic carbon sources for fungal growth. These included sugar cane molasses, potato, glucose syrup and glycerol. Egyptian can molasses obtained from El-Hawamdia Sugar and Distillate Company at Cairo was clarified and hydrolyzed by HCl (3). Potato obtained from local market was used as a substrate for fungal growth after being hydrolyzed. The hydrolysis process was carried out by using HCl (2N, 400 mL) to mashed potato (1 kg) and the mixture was heated under reflux using boiling water bath for 7 hr. Glucose syrup obtained from Starch and Glucose Company at Cairo was hydrolyzed by 2% of conc HCl for 3 days at room temperature. The pH of any hydrolyzed substance was adjusted to 7.0 before adding it to the culture medium. Glycerol obtained from El-Nasr Chemical Company at Cairo was added to culture medium without any pretreatments. Urea (Merck grade) and peptone (Oxoid Limited Company, England) were used in some experiments as a source of nitrogen for fungal growth.

Work-Up of Fermentation Products

The fungal growth was separated from the medium by filtration, then washed several times with distilled water and dried at 60 C under vacuum. Fungal lipids were extracted using a chloroform-methanol mixture (2:1, v/v) (4).

The fungal lipids were saponified by 20% (w/v) alcoholic KOH at room temperature overnight. The unsaponifiables were extracted three times with petroleum ether (bp 60–80 C) and discarded. The aqueous layer was acidified with HCl (1:1, v/v) and the liberated fatty acids were extracted three times with petroleum ether. The combined extract was dried over anhydrous sodium sulfate and the dried fatty acids were methylated with diazomethane (5).

Analyses

The reducing sugars expressed as invert sugar in the hydrolyzed molasses, potato and glucose syrup were determined (3). The nitrogen content of peptone and urea was determined by the usual Kjeldahl method. Spectrophotometric methods were used for the quantitative determination of total lipids and phospholipids (6,7).

Standard methyl esters of 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2, 18:3 and 20:0 fatty acids (Nu-Chek Prep) with a purity not less than 99% by gas liquid chromatography (GLC) were used as authentic materials to characterize the unknown fungal fatty acids.

A GCV Pye Unicam gas chromatograph equipped with dual flame ionization detectors and coiled glass column (1.5 m × 4 mm) packed with 10% PEGA supported on alkali-acid washed and silanized Diatomite C (100–120 mesh) was used. The gas chromatographic conditions for separating the methyl esters of fatty acids were similar to those described by Farag et al. (8).

Peak identification was performed by comparing the relative retention times of each peak with those of standard materials. The relative retention time of methyl oleate is given a value of 1.00. The linear relationship between log retention times of the standard monoenoic and dienoic acids and the number of carbon atoms of these compounds was used to characterize 11:1, 12:2, 13:1, 14:1, 14:2, 15:1, 15:2, 16:2, 17:1 and 20:1 fatty acids in fungal lipids.

The area of each peak was measured by triangulation and the corrected peak area was obtained by dividing the peak area by the detector response factor suitable for each fatty acid. The percentage composition of fungal fatty acids was calculated by using the following equation:

$$\text{fatty acid percentage composition} = \frac{\text{corrected area of peaks}}{\text{corrected area of all peaks}} \times 100$$

RESULTS AND DISCUSSION

There are several factors which encourage the use of fungi for lipid production. For instance, fungi need a limited space for cultivation, a short time to reach maximum lipid yield, and the cost is low. A number of restrictions were imposed in the present investigation including the concentrations of sugar and nitrogen, the culture conditions (shaking rate, incubation period, temperature and volume of medium per flask) and the sugar:nitrogen molar ratio (C:N ratio). The variables in the culture ingredients were restricted only to the sources of carbon (glucose, fructose, maltose, sucrose, glycerol, molasses, potato and glucose syrup) and nitrogen (peptone and urea) used for fungal growth. There are several parameters influencing the lipid production from fungi. The most important factors are the type of fungi, culture media and fungal lipids.

Fungi

The microbial production of lipids requires careful selection of fat-accumulating species as not all microorganisms have this ability. The fungi may excrete mycotoxins, such as aflatoxins, or produce spores which may contaminate the environment. In spite of the many variations in culture conditions or age of the cultures, the fatty acid analysis of Basidiomycetes indicated that they produce the essential fatty acids, i.e., linoleic and α-linolenic (9). In addition, some Basidiomycetes are used as food for human beings, e.g., mushroom and some smut species. From these considerations, two fungi (*Tolyposporium ehrenbergii* and *Sphacelotheca reiliana*) were used in the present work. However, several carbon and nitrogen sources were used in order to govern their fat-accumulating capacity.

Culture Media

Fungi were grown on fat-free medium to avoid possible incorporation of prior synthesized fatty acids into fungal lipids. Pure sugars and some cheap natural and industrial byproducts were used as basic sources for carbon and nitrogen for fungal growth. The C:N molar ratio was maintained constant in all our experiments (20:1).

Effect of Sugar and Nitrogen Sources on the Dry Weight, Lipid and Phospholipid Contents of *Tolyposporium ehrenbergii*

The dry weights of fungal growth after one and two-week incubation periods are shown in Table I. When a mixture of glucose and maltose (2:1, w/w) was used as a major carbon source, it accelerated the fungal growth and produced the highest fungal dry weights after a one-week period in comparison with other pure sugars. The application of glycerol, molasses hydrolysate and glucose syrup as cheap natural and industrial byproducts promoted the fungal growth. Molasses hydrolysate and urea in the medium were found to be the best ingredients for fungal growth since they gave the highest fungal growth in the whole set of experiments.

The influence of various sugars in the medium containing peptone or urea on the lipid content of *T. ehrenbergii* is shown in Table I. This fungus gave promising results in media containing fructose/peptone or glucose/peptone after one and two-week periods, respectively, and the lipid yield was quite comparable to that of fat-rich vegetable seeds. The industrial byproducts, though rich in sugar content, did not afford adequate substrate for mycological fat formation. Urea prevented the fungal growth on glycerol or potato hydrolysate incubated for one week. Hence, the fungus responded differently with the change in the nitrogen source. This conclusion is in accordance with the results of other investigators (10,11).

For evaluation of this set of experiments towards directing the fungus for fat production, one must take into account the incubation period, the amount of mycelium produced and the quantity of synthesized lipids. Three terms can be used to evaluate the fungus fat accumulating

TABLE I

Effect of Various Carbon and Nitrogen Sources on the Dry Weight, Total Lipids (TL) and Phospholipids (PL) of *T. ehrenbergii* and *S. reiliana*

Carbon/nitrogen source	Dry weight (mg/100 mL) medium	TL (g%)	PL (g%)	Dry weight (mg/100 mL) medium	TL (g%)	PL (g%)
			One-week incubation period			
	T. ehrenbergii			*S. reiliana*		
Glucose/peptone	19.7	25.03	0.64	33.8	27.26	0.60
Fructose/peptone	31.9	37.89	0.48	90.9	27.70	0.56
Glucose + maltose/peptone	448.3	10.80	7.46	62.0	22.20	1.04
Sucrose/peptone	11.4	23.64	2.44	48.5	16.62	1.52
Maltose/peptone	9.6	22.28	1.05	43.8	23.80	0.58
Glycerol/peptone	508.6	9.88	3.91	0.0	0.0	0.0
Molasses hydrolysate/peptone	810.0	5.71	1.59	0.0	0.0	0.0
Glucose syrup/peptone	577.1	13.25	2.37	0.0	0.0	0.0
Potato hydrolysate/peptone	0.0	0.0	0.0	0.0	0.0	0.0
Glycerol/urea	0.0	0.0	0.0	0.0	0.0	0.0
Molasses hydrolysate/urea	333.5	1.66	2.78	0.0	0.0	0.0
Glucose syrup/urea	454.6	5.63	2.84	0.0	0.0	0.0
Potato hydrolysate/urea	0.0	0.0	0.0	0.0	0.0	0.0
			Two-week incubation period			
Glucose/peptone	157.6	40.56	0.1	241.1	40.43	0.15
Fructose/peptone	168.7	18.52	0.4	460.1	29.78	0.07
Glucose + maltose/peptone	1142.8	12.08	2.12	366.7	32.61	0.11
Sucrose/peptone	126.2	11.45	0.08	365.0	32.28	0.09
Maltose/peptone	121.9	27.27	0.29	165.2	40.65	0.09
Glycerol/peptone	981.7	5.61	1.52	302.0	4.41	5.47
Molasses hydrolysate/peptone	1903.4	3.61	0.93	0.0	0.0	0.0
Glucose syrup/peptone	1518.2	5.51	0.98	0.0	0.0	0.0
Potato hydrolysate/peptone	911.4	6.21	4.99	0.0	0.0	0.0
Glycerol/urea	149.5	4.61	7.55	62.7	8.40	18.65
Molasses hydrolysate/urea	2365.4	3.29	0.65	0.0	0.0	0.0
Glucose syrup/urea	842.0	5.17	1.68	44.2	27.98	20.98
Potato hydrolysate/urea	959.4	5.47	4.84	0.0	0.0	0.0

capacity, i.e., lipid content, lipid percentage and efficiency of fat production. Lipid content refers to the quantity of lipid synthesized in a fixed amount of the media. Lipid percentage indicates the proportion of the lipid to total fungal growth. Efficiency of fat production refers to mg fungal growth per 100 mL of medium X lipid content^{-1} X incubation period (week)$^{-1}$ X 100. By applying this equation for all systems, the medium containing fructose/peptone or glucose/peptone after one week incubation period was the most efficient medium for fat production. *T. ehrenbergii* showed great variation with respect to mycelial phospholipids according to sugar source and incubation period. In general, the fungus with a low amount of lipids was invariably rich in phospholipids.

Effect of Sugar and Nitrogen Sources on the Dry Weight, Lipid and Phospholipid Contents of *Spacelotheca reiliana*

Table I shows the influence of various carbon sources and incubation periods in fungal dry weight. *S. reiliana* fungus showed great variation with respect to the amounts of mycelium by using various pure sugars and nitrogen sources. Generally speaking, the amounts of the synthesized mycelium were enormously increased with increasing the incubation period. In dealing with the natural and industrial byproducts, the fungus was only grown on medium containing glycerol/peptone, glycerol/urea or glucose syrup/urea after a two-week incubation period.

The effect of various sugar and nitrogen sources on the lipid content of *S. reiliana* is shown in Table I. The fungus growth on media containing peptone showed significant variations with respect to mycelial lipids. The fungal lipid percentage was increased by prolonging the incubation period. No fungal growth was achieved using less expensive sugar sources after one- or two-week incubation periods except the medium containing glycerol. The substitution of peptone by urea produced only fungal growth after a two-week period using glucose syrup and glycerol. It is worth mentioning that glucose syrup/urea synthesized mycelium containing much lower lipid content than pure sugars at a comparable incubation period.

The values for fat production efficiency indicated that under the influence of *S. reiliana* glucose or fructose with peptone in the medium were the most suitable ingredients for lipid production. The one-week incubation period was superior to the two-week incubation period for lipid production. Various pure sugars with peptone had a marked influence on phospholipid composition. Highest concentration of phospholipids was synthesized after a one-week period by *S. reiliana* grown on sucrose. Once again, there was a reversible correlation between lipid and phospholipid percentages. Fungal growth contained a higher amount of phospholipids when a lower total percentage was present. By prolonging the incubation period, the phospholipid percentages were enormously decreased in this set of experiments. If interest is focused on obtaining higher phospholipids the medium containing glucose syrup/urea would be the most productive.

TABLE II

Influence of Various Sugars and Peptone on the Fatty Acid Composition of *T. ehrenbergii* Incubated for One- and Two-Week Periods

Fatty acid	Fatty acid composition									
	Glucose	Fructose	Glucose + maltose	Sucrose	Maltose	Glucose	Fructose	Glucose + maltose	Sucrose	Maltose
	One-week period					Two-week period				
10:0	5.0	5.6	0.6	5.1	8.9	2.3	–	0.4	–	–
11:0	0.1	0.1	0.6	–	0.3	0.5	0.2	0.2	–	0.2
11:1	0.3	0.3	–	0.4	0.7	–	0.4	–	–	–
12:0	1.0	1.7	–	1.0	2.3	7.2	0.2	–	–	–
12:1	0.9	0.7	–	0.8	0.8	0.3	0.2	–	0.1	0.3
13:0	2.2	2.9	0.3	2.3	3.8	0.9	0.3	–	2.1	1.4
13:1	–	0.2	–	0.2	0.2	–	0.2	–	0.4	0.3
14:0	0.9	6.1	0.2	6.5	10.1	1.1	4.1	0.5	7.7	8.0
14:1	1.1	–	19.3	0.9	0.5	12.8	0.2	4.0	–	–
15:0	1.8	3.6	–	1.7	0.7	–	1.1	–	0.1	0.6
15:1	–	–	–	–	0.2	–	0.8	–	0.1	0.2
16:0	32.8	15.4	2.7	25.1	5.0	58.2	39.8	12.5	14.4	21.9
16:1	3.2	1.3	0.2	2.0	0.6	0.4	1.0	0.9	0.4	0.6
16:2	–	–	–	–	–	–	–	–	0.7	0.4
17:0	3.8	5.9	–	6.5	17.9	–	5.2	–	8.7	10.8
17:2	–	–	11.6	–	–	–	–	1.5	–	–
18:0	11.0	3.8	0.6	5.0	2.2	0.5	3.2	2.6	2.0	2.7
18:1	14.0	19.1	3.0	9.4	3.1	14.4	35.1	35.8	53.6	43.9
18:2	2.8	4.0	1.3	9.7	2.6	1.2	5.4	22.5	1.1	1.1
20:1	19.1	29.3	59.6	23.4	40.1	0.2	2.6	19.1	8.6	7.6

Influence of Cultural Ingredients on the Fatty Acid Composition of *T. ehrenbergii* Fungus

Table II shows the fatty acid composition of *T. ehrenbergii* incubated for one and two weeks on various sugars and peptone. The fatty acid analysis of fungal growth was qualitatively and quantitatively different using various types of sugars. For example, the glucose/maltose mixture produced fungal growth characterized by the highest amount of eicosenoic acid (59.6%), ca. 3.1, 2.6, 2.0 and 1.5 times as high as those in media containing glucose, sucrose, fructose and maltose, respectively.

Lipids produced from the glucose/maltose mixture were also characterized by the highest total unsaturation and polyunsaturated fatty acids. Therefore, there is a good chance in hand to select the culture ingredients for having lipids of specific properties.

In all fungal lipid, even number acids predominated, but small amounts of odd acids (13:0, 15:0 and 17:0) were also present and 17:0 was the most abundant of these. The extent of changes in the fatty acid composition was entirely dependent upon the sugar type. The fatty acid composition of the fungal growth in media containing various pure sugars were not qualitatively and quantitatively similar in young and old cultures.

Table III shows the fatty acid composition of *T. ehrenbergii* incubated for one and two weeks on some natural and industrial byproducts with peptone or urea as basic sources for carbon and nitrogen, respectively. With molasses and urea or peptone, the fatty acids of the fungal lipids were qualitatively the same, while remarkable differences in the amounts of various acids were observed. The basic feature of *T. ehrenbergii* lipids from glycerol/urea substrate was the presence of 14:1 (32.7%) as the most widely distributed acid. As a result of changing the nitrogen source from urea to peptone, the concentration of total unsaturated acids decreased by a factor of 1.6. Here again, the results of this set of experiments indicated that the natural and industrial byproducts as basic source for carbon remarkably influenced the fatty acid make-up by *T. ehrenbergii*. Also, the fungus responded differently, according to the type of nitrogen source.

Influence of Various Carbon and Nitrogen Sources on the Fatty Acid Composition of *S. reiliana* Fungus

Table IV shows the fatty acid composition of fungal growth incubated for one and two weeks on media containing various pure sugars and peptone as basic carbon and nitrogen sources. The lipids of *S. reiliana* contained a wide range of fatty acids (10:0-20:0). Maltose in the medium produced fungal growth rich in saturated fatty acids, while fructose produced fungal growth characterized by having approximately equal proportions of saturated and unsaturated fatty acids. Hence, the variability of fatty acid composition of the fungus largely depended on the type of sugar in the medium. The application of this study might be implemented along two different lines. At first, the use of certain sugar might produce oil containing low percentage of unsaturated fatty acids in order to increase its keeping quality. For instance, maltose in the growth medium produced oil containing the lowest amounts of unsaturated fatty acids. Secondly, the use of a particular sugar in the culture medium to produce oil having a high percentage of unsaturated acids is quite desirable in paintings and varnishes. Fructose in the medium was a good representative in this respect.

Extending the incubation period (two weeks) led to a decrease or an increase in the amounts of certain fatty acids, appearance of some new fatty acids and disappearance of some mono- and diunsaturated fatty acids. As a result of these changes various lipids differing in their degree of unsaturation were obtained. Several investigators (12,13) have studied the fatty acid composition of most widely consumed edible oils, i.e., corn and cottonseed oils. The

TABLE III

Influence of Some Industrial Byproducts on the Fatty Acid Composition of *T. ehrenbergii* Incubated for One- and Two-Week Periods

Fatty acid	Fatty acid percentage composition												
	Molasses/ urea	Molasses/ peptone	Glucose syrup/ urea	Glucose syrup/ urea	Glucose peptone	Glucose syrup/ peptone	Glucose syrup/ urea	Molasses + peptone	Molasses + urea	Glycerol + peptone	Glycerol + urea	Potato + peptone	Potato + urea
	One-week incubation period							Two-week incubation period					
10:0	2.7	0.7	2.7	0.2	0.9	0.2	0.2	0.1	0.3	–	0.1	0.4	2.6
11:0	1.1	0.4	1.2	–	–	0.1	0.1	0.1	0.2	0.1	0.7	–	–
11:1	–	–	–	–	–	–	–	–	–	–	1.1	–	–
12:0	2.7	1.4	3.8	0.2	–	–	–	0.1	0.1	0.1	–	0.1	0.5
12:1	–	–	–	–	–	–	–	–	–	–	–	0.1	0.1
13:0	6.9	4.0	10.3	0.7	–	–	0.1	0.3	0.3	–	0.7	–	0.1
13:1	–	–	–	–	–	–	–	–	–	–	–	0.2	1.2
14:0	0.2	0.6	0.4	1.3	2.2	1.0	1.3	0.5	2.2	1.6	1.9	1.0	0.8
14:1	18.4	4.1	27.3	0.7	–	–	–	1.8	0.6	0.3	36.1	0.2	1.2
15:0	–	–	–	–	–	0.2	0.2	–	0.1	0.2	–	0.5	0.7
16:0	8.2	22.2	8.0	53.8	27.8	27.8	34.1	6.7	30.5	26.8	6.8	35.4	40.0
16:1	–	–	0.2	0.2	0.6	0.5	0.8	0.6	0.3	0.5	–	0.4	0.3
17:0	1.6	2.1	2.0	0.4	–	0.5	–	0.4	0.7	0.5	–	0.8	0.7
17:1	–	–	–	–	–	–	–	0.3	–	–	–	0.4	0.1
17:2	23.0	27.7	25.5	–	–	–	–	1.6	–	–	32.7	–	–
18:0	1.6	5.3	1.6	14.6	19.7	14.6	11.0	4.6	21.0	17.8	3.8	11.0	11.0
18:1	6.2	16.7	11.0	19.6	26.0	32.4	93.7	80.8	19.1	33.2	10.5	24.1	23.1
18:2	1.2	5.3	2.2	1.9	16.0	21.2	17.5	2.1	24.6	17.9	5.5	23.1	16.4
18:3	–	–	–	–	–	1.5	1.0	–	–	1.0	–	2.3	1.2
20:1	26.2	9.5	3.8	6.4	6.8	–	–	–	–	–	–	–	–

TABLE IV

Influence of Various Sugars on the Fatty Acid Composition of *S. reiliana* Incubated for One and Two Weeks

Fatty acid	Fatty acid percentage composition									
	Glucose	Fructose	Glucose + maltose	Sucrose	Maltose	Glucose	Fructose	Glucose + maltose	Sucrose	Maltose
	One-week incubation period					Two-week incubation period				
10:0	4.8	2.4	0.5	6.2	7.3	0.1	0.6	0.2	0.1	–
11:0	0.2	0.2	2.5	0.5	0.4	–	0.2	0.5	0.2	0.3
11:1	–	3.9	–	0.6	0.6	0.2	–	0.1	–	–
12:0	1.4	3.8	6.7	1.9	2.6	0.3	0.5	0.5	0.1	0.4
12:1	1.1	–	0.1	0.9	0.1	–	–	0.2	0.1	0.1
13:0	3.8	3.5	16.8	2.8	4.4	1.2	0.6	0.1	0.3	0.3
13:1	0.6	0.3	–	0.2	0.2	0.5	0.3	2.1	–	0.1
14:0	5.0	5.8	6.4	8.4	14.8	7.6	8.3	3.3	2.3	8.4
14:1	0.3	0.4	–	0.2	0.3	0.3	0.6	0.2	0.1	0.2
15:0	3.2	2.1	0.6	0.7	2.5	0.7	1.3	0.6	0.4	1.1
15:1	–	0.1	0.6	–	0.2	0.5	1.7	0.9	0.8	1.7
15:2	–	–	0.4	0.1	–	–	–	–	–	–
16:0	23.9	27.9	3.0	21.9	10.4	23.4	33.0	45.3	20.9	30.0
16:1	1.2	0.5	0.1	0.4	0.4	0.6	0.8	3.4	2.1	3.7
16:2	–	–	1.3	0.1	0.4	–	–	–	–	–
17:0	4.9	2.6	22.1	14.7	28.0	15.7	9.7	2.3	2.0	2.7
17:1	0.4	–	–	–	–	–	–	–	–	–
18:0	6.1	4.3	1.2	2.8	2.0	2.5	2.5	7.9	2.9	7.3
18:1	12.8	13.5	6.3	5.2	4.4	37.4	23.6	23.4	22.6	35.0
18:2	2.6	2.2	3.8	1.8	1.9	1.4	2.0	4.3	25.7	5.6
18:3	–	–	–	–	–	–	–	–	–	0.2
20:0	–	–	–	–	–	–	–	1.8	–	1.6
20:1	27.9	26.5	27.6	30.6	19.1	7.6	14.3	3.0	19.4	1.3

TABLE V

Influence of Some Industrial Byproducts on the Fatty Acid Composition of *S. reiliana* Incubated for Two Weeks

Fatty acid	Fatty acid percentage composition		
	Glycerol/urea	Glycerol/peptone	Glucose syrup/urea
10:0	0.2	–	0.1
11:0	0.3	–	0.1
11:1	–	1.1	–
12:0	0.1	0.2	0.2
13:0	0.4	0.3	–
13:1	0.1	0.1	–
14:0	0.7	1.6	1.2
14:1	17.8	11.5	7.0
15:0	0.1	1.6	0.2
15:1	0.2	0.2	–
16:0	4.3	13.5	24.0
16:1	0.3	1.3	0.3
17:0	–	4.4	–
17:1	–	–	1.1
17:2	13.7	17.4	20.8
18:0	1.0	8.6	1.0
18:1	2.9	14.4	20.7
18:2	0.6	23.9	15.9
20:1	57.5	–	–

fatty acid composition of these oils were not far away from the fatty acid composition of fungal lipids. By altering the culture conditions, e.g., temperature, pH and growth ingredients, one could achieve approximately the same fatty acids as in oils obtained from natural sources.

The fatty acid composition of *S. reiliana* fungus cultivated on some natural and industrial byproducts after a two-week incubation period are shown in Table V. When peptone was used instead of urea it dramatically changed the fungal fatty acid pattern, since the substitution of urea by peptone directed the fungal metabolic processes to synthesize 18:2, 18:1, 18:0 and 17:2 at the expense of 20:1. Total unsaturation for the lipids produced from urea or peptone were equal. However, the profiles of fatty acids were quite different from these ingredients. It has been found that organic and inorganic nitrogen sources in the culture media significantly changed the fungal fatty acid pattern (2,10). The displacement of glycerol by glucose produced lipids characterized by the higher concentrations of 14:0, 16:0, 17:2, 18:1 and 18:2. Although the levels of unsaturation for the lipids produced from the medium containing glycerol/urea or glucose/urea was similar, the distribution of unsaturated fatty acids was quite different. In general, the variability in fatty acid pattern of different growth media give a good chance to select the appropriate medium to produce lipids with certain properties.

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[Received December 28, 1981]