Effect of Culture Temperature on Fatty Acid Composition of *Chlorella sorokiniana*[1]

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ABSTRACT

Chlorella sorokiniana was grown for extraction of fatty acids at seven temperatures ranging from 14 C to 38 C. The predominant fatty acids in *C. sorokiniana* grown at 38 C were saturated (46% of total); at 22 C, triunsaturated (40% of total); and at 14 C diunsaturated (47% of total). Increasing temperature resulted in an increase in the degree of unsaturation from 14 C to 22 C, but further increases in temperature always resulted in a decrease in unsaturation. At any point in the temperature range used, an increased temperature always resulted in fatty acids with a lower average chain length. Total fatty acid production was greatest at the extremes of temperature and lowest at 26 C. The chain length and degree of saturation of fatty acids increased at temperatures lower than 22 C. Therefore, the fatty acids of *C. sorokiniana* do not have an increasingly lower melting point when the culture temperature is reduced at temperatures 22 C or below.

INTRODUCTION

Environmental temperature is an important factor in the life of plants and animals. Several authors (1-3) have reported that animals respond to a lower environmental temperature by the accumulation of an increased percentage of unsaturated fatty acids in their body lipid. This is true in animals in controlled-diet experiments (4,5) as well as in those in which lipid content of the diet was not reported. The phenomenon of lower environmental temperature resulting in the presence of a greater percentage of unsaturated fatty acids is also apparent in microorganisms such as *Anacystis nidulans* (6), *Candida* sp. (7) and *E. coli* (8), although sometimes an opposite temperature effect is noted (9). Marr and Ingraham (8) studied the effect of temperature on fatty acid content at eight temperatures ranging from 10 C to 43 C, but most authors have chosen to compare data from only two temperatures. The value of such data is thus limited by the lack of more points for comparison. It is quite possible that the results could depend upon the level of the two temperatures chosen for study. Lewis (3) suggested that an increase in unsaturated fatty acids, in response to lower temperatures, could act to preserve protoplasmic viscosity in colder habitats. To determine whether a lipid mixture more resistant to solidification at low temperatures is being synthesized, we need to examine both the molecular weight of the fatty acids as well as the degree of unsaturation and not just the percentage of unsaturated fatty acids. To meet these requirements, *Chlorella sorokiniana* was chosen as a plant which would grow over a wide range of temperature. This paper reports the content of fatty acids in *C. sorokiniana* at seven temperatures ranging from 14 C to 38 C.

MATERIALS AND METHODS

Cells of *C. sorokiniana* (Shihira and Krauss) were grown in the dark in large test tubes containing 400 ml of growth medium which was bubbled with 1% CO_2 in air. The medium was composed of 1 g/liter KNO_3, 0.5 g/liter KH_2PO_4, 0.5 g/liter K_2HPO_4, 0.25 g/liter $MgSO_4 \cdot 7H_2O$, and the micronutrients described for *Chlorella* culture by Thomas and Krauss (10). Glucose at 0.5% provided the carbon source for heterotrophic growth. Temperature was controlled by a Forma controlled temperature bath and did not differ more than 0.5 C from the set temperature. *C. sorokiniana* was cultured at increasingly lower temperatures until growth ceased. The lowest temperature at which consistent and measurable growth occured was 14 C. Cells were grown at 18 C for extraction by taking an inoculum from the 14 C culture, and then making three or more transfers at 18 C. An inoculum from 18 C, was then used for inoculation at 22 C, etc. Cells were grown for extraction at seven temperatures at four-degree intervals from 14 C to 38 C. A culture was always harvested when its optical density reached 0.6 on a Coleman Junior Spectrophotometer (or a dry wt of 0.8-0.9 g/liter), since preliminary work showed some variation in fatty acid content with age of culture. At harvest, an

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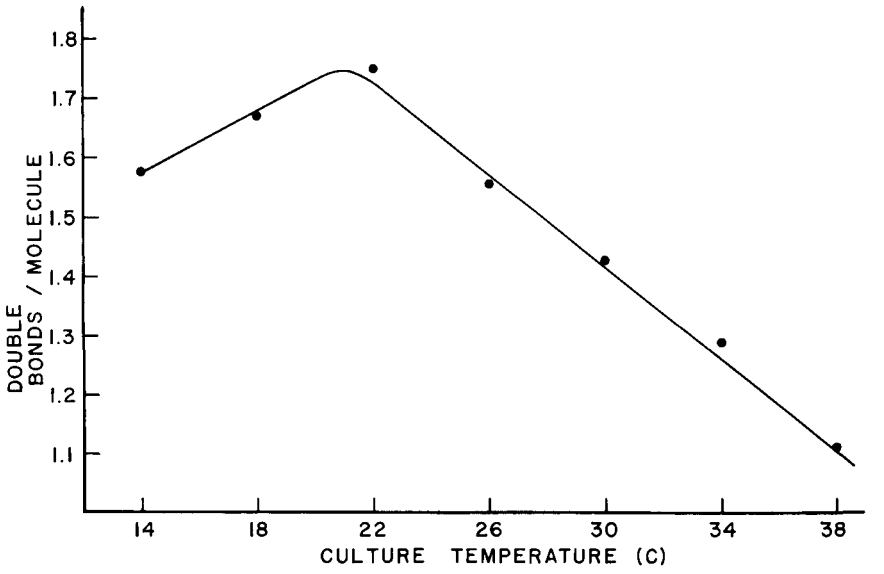

FIG. 1. Effect of culture temperature on the degree of unsaturation (double bonds per molecule) of *C. sorokiniana* fatty acids.

aliquot of cells was removed for dry weight determination. The remainder was centrifuged, resuspended in methanol, transferred with a pipette to an extraction thimble and extracted overnight with chloroform-methanol (2:1) in a Soxhlet apparatus.

Total lipid was determined by evaporating the solvent, resuspending the lipid in chloroform, and filtering the lipid into a weighted beaker to remove nonlipid particulate material. After evaporation of the chloroform, the total lipid weight was obtained. The lipid was saponified with a 100% excess of KOH in 80% ethanol, diluted threefold with water, acidified with HCl, and extracted overnight with diethyl ether in a liquid-liquid extraction apparatus. After evaporation of ether to dryness, the fatty acids were methylated by heating for 3 min with BCl_3-methanol reagent (Applied Science Labs). Fatty acid methyl esters were partitioned into hexane, and the hexane was evaporated to dryness under N_2. The sample was redissolved in hexane and transferred to a vial, leaving behind the last traces of BCl_3.

The fatty acid esters were analyzed by a Glowall model A-110 gas chromatograph. The operating conditions were: (a) column 1.8 m x 3.4 mm i.d., 15% HiEff 1BP on Gas Chrom P, (Applied Science Labs) 20 psi, and 165 C; (b) argon ionization detector, 900 v, 200 C; and (c) flash heater, 205 C. The fatty acids were identified by thin layer chromatography on Silica Gel G, developed with 20% ether in hexane, and followed by gas chromatography using known fatty acids as standards. Quantitative data were obtained by measuring peak areas using a disc integrator.

RESULTS AND DISCUSSION

The major fatty acids of *C. sorokiniana* were identified as 16:0, 16:1, 16:2, 16:3, 18:0,

TABLE I

Effect of Culture Temperature on the Relative Proportions of Various Fatty Acids in *C. sorokiniana*

Culture temperature, C	Individual fatty acids as percentage of total fatty acids							
	16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
14	21.7	3.3	10.8	10.3	0.5	4.7	36.4	11.6
18	26.1	3.1	7.9	12.8	0.3	4.9	29.5	15.2
22	29.3	2.4	4.2	16.9	0.3	3.8	19.7	23.3
26	33.1	2.6	6.0	13.4	1.0	4.0	20.9	18.3
30	37.5	2.7	6.8	11.5	1.2	4.3	20.1	15.8
34	39.2	3.4	7.7	9.6	2.6	4.4	21.0	11.8
38	40.7	4.4	10.2	5.4	5.3	4.7	21.3	7.5

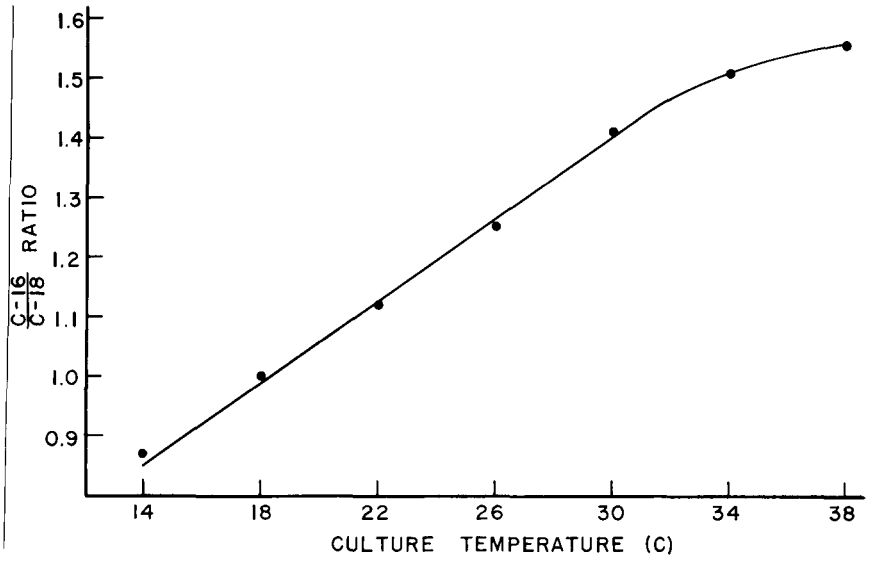

FIG. 2. Effect of culture temperature on the C_{16}/C_{18} ratio of *C. sorokiniana* fatty acids.

18:1, 18:2 and 18:3. Traces of 14:0 and other unidentified acids were detected, but they made up less than 1% of the total fatty acids and were not studied further.

Table I indicates that as the culture temperature increased, the saturated fatty acids made up an increased percentage of the total fatty acids. At the lower temperatures the percentage of triunsaturated acids also increased. These increases were balanced at the lower temperatures by a significant decrease in the proportion of diunsaturated acids. At temperatures above 22 C, the relative proportion of saturates increased, that of triunsaturates decreased and that of diunsaturates remained relatively constant. The proportion of monounsaturates remained essentially constant regardless of temperature.

It can be seen, then, that the predominant acids at 14 C were diunsaturated (47.2% of total), at 22 C were triunsaturated (40.2% of total) and at 38 C were saturated (46.0% of total). Figure 1 shows how the degree of unsaturation of *Chlorella* fatty acids changed with culture temperature. The degree of unsaturation was greatest at about 22 C. From this point there was apparently a linear decrease in unsaturation with either an increase or decrease in temperature. From this graph it can be understood how confusion in the literature could have arisen on the effect of temperature on the degree of unsaturation of fatty acids. If 14 C and 22 C were the temperatures selected for study, it would appear that increased temperature increased the degree of unsaturation. If 22 C and 30 C were selected, it would appear that increased temperature decreased the degree of unsaturation. Unfortunately, only two temperatures have been selected for study in many published works of this kind. The conclusions

TABLE II

The Effect of Culture Temperature on the Absolute Concentrations of Fatty Acids and Total Lipid in *C. sorokiniana*

Culture temperature, C	Per cent dry weight									
	Individual fatty acids								Total fatty acids	Total lipid
	16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:2		
14	1.26	0.19	0.63	0.60	0.03	0.27	2.11	0.67	5.8	10.5
18	1.59	0.19	0.48	0.78	0.02	0.30	1.80	0.93	6.1	10.2
22	0.76	0.06	0.11	0.44	0.01	0.10	0.51	0.61	2.6	9.1
26	0.43	0.03	0.08	0.17	0.01	0.05	0.27	0.24	1.3	10.2
30	0.75	0.05	0.14	0.23	0.02	0.09	0.40	0.32	2.0	10.5
34	1.42	0.12	0.28	0.35	0.09	0.16	0.76	0.42	3.6	10.8
38	2.46	0.27	0.62	0.33	0.32	0.29	1.30	0.46	6.1	11.9

that can be drawn from these studies are thus limited.

It is apparent from Table I that the ratio of 16-carbon acids to 18-carbon acids must also be increasing with increasing temperature. Figure 2 shows that this increase was linear up to 30 C, at which temperature it began to level off.

Although data on relative proportions of various fatty acids are helpful, in order to get a true picture of the changes in fatty acid composition with changing temperature, one must also calculate the data on a dry weight basis to obtain absolute concentrations of fatty acids. Table II shows that when one goes from 38 C to lower temperatures, all fatty acids decrease in absolute concentrations. Changes in relative proportions of fatty acids are seen in Table I, because various fatty acid concentrations were decreasing at different rates. At 26 C all fatty acid concentrations reached their lowest point, and further decreases in temperature generally resulted in an increase in the content of all fatty acids.

The total fatty acids made up from 1.3% to 6.1% of the dry weight of the algal cell and the higher values were obtained at the extremes of temperature. The growth rate of *C. sorokiniana* is highest (about six doublings per day under conditions given) at 38 C and declines with lower temperatures. The total fatty acids made up only 13% of the total lipid at 26 C, but were 50% to 60% of the total lipid at the extremes of temperature. Since the total lipid was relatively constant at about 10% of the dry weight, some other component of the lipid fraction must be changing too. Since chlorophyll makes up a large portion of *Chlorella* lipid, changes in chlorophyll concentration are suspected.

It is clear that culture temperature is an important environmental factor affecting fatty acid content of *Chlorella*. The maximum degree of unsaturation of fatty acids was attained at 22 C with less unsaturation at lower or higher temperatures, although the proportion of total unsaturated fatty acids was always increased at lower temperatures (Table I). Chain length of fatty acids always decreased with increasing temperature within the limits used in this study. From the data presented here, it appears that fatty acid composition does change in a way which would tend to reduce protoplasmic viscosity as temperatures are reduced to 22 C. If temperatures are reduced below 22 C, the fatty acid composition changes in a direction which would tend to increase protoplasmic viscosity. However, these changes in viscosity could also be counterbalanced by changes in chlorophyll content or content of other lipids.

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