

TABLE II Cliffordian Different Property Minimum of Bellin Andre

potential one in the indirect determination and characterization of the higher alcohols oxidized to the corresponding acids.

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## Possibilities in Photosynthetic Methods for Production of Oils and Proteins<sup>1</sup>

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HERE are innumerable ways in which green plants are useful to and necessary for the existence of man. Every bit of fuel we burn to cook our meals, to warm our homes, to drive our cars, to power our industries, all these originated from plants which grew in the past. A very great number of important industrial products are derived from plants. Every bite of food we eat can be traced directly back to plants, whether we eat the plants or plant products as such, or whether we eat an animal which, in turn, has fed on plants. The availability of an adequate food supply is of more urgent importance to the welfare of the individual man than is the production of fuel and of industrial raw materials.

Since the day, ages ago, when someone first conceived the idea of planting seeds and growing a crop. man has become increasingly dependent on farm crops for his daily food. As there were more men, there had to be more farms to feed them. The end of this sort of expansion is already in sight. There is a limit to the amount of land on earth which can be used to grow food. Some improvement, but not nearly enough, has been made in the yield of food per acre. This was mostly due to the development of new strains of plants and better methods of farming. If the world food supply is to keep pace with an increasing population, new and more efficient methods of food production must be sought.

Only the green plant can live on a strictly inorganic diet, that is, carbon dioxide, water, and a few mineral salts. Out of these it makes the organic matter without which all other living things would starve. Energy is required to convert the inorganic compounds into organic matter. A wealth of energy in the form of sunlight pours onto the surface of the earth every day. The only part of that energy which we know is converted to chemical energy and stored for future use is the light used by green plants in the process of converting carbon dioxide and water into organic matter. This process of photosynthesis upon which man depends for his very existence is grossly inefficient.

Every minute each square centimeter of the earth's surface exposed normally to the sun's rays receives 1.9 calories. A simple calculation shows that the world receives a million calories from the sun for each calorie that is available to mankind as food. There is obviously no shortage of energy for making food. Of course, much of this solar energy does not fall on green plants, and of that which does, only about half is available to the plant for use in photosynthesis. The plant can use only the wavelengths in the visible spectrum, not those in the infrared. Considering only the sunlight falling on a cultivated acre in a year, the very best crops can convert only one-half of 1% of the available solar energy into chemical energy stored as organic matter.

Most crops do not utilize nearly as much as  $0.5\%$ of the sunlight which falls on the farm because farm-

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ing is essentially a discontinuous batch process. The growing season of the usual crop is but a few months out of the year. During a large part of the growing season itself, much of the sunlight strikes bare ground because the plants have not attained sufficient size to intercept all the light. It is only during the short interval after the plants have reached full size and before they are harvested that most crops can reach even 0.5% efficiency in utilization of sunlight.

**L** ET us consider the possibility of using plants to convert a larger percentage of the solar energy into forms suitable for man's use. In the first place we would want to absorb all of the sunlight by having plants cover all of the area all of the time. Then, if possible, we would like to increase the efficiency of utilization of light by the plant. Laboratory studies of photosynthesis demonstrate that the plant growing under controlled experimental conditions can utilize a much higher percentage of the available light than it does under natural conditions. The third possibility is to control the chemical composition of the material produced by the photosynthetic activity of the plants.

The bulk of man's food is composed of carbohydrates, proteins, and fats. There is still enough carbohydrate, but the world supply of protein and fat is not large enough for the proper nourishment of the world population. It has been said that one-half the people in the world now are hungry all of the time! If we cannot grow enough more food of the old type to feed all mankind, perhaps we can grow more efficiently plants containing higher proportions of the needed proteins and fats.

When Dr. Spoehr and I started looking for an answer to the question: can the chemical composition of a plant be changed by altering the environmental conditions under which it grows ? we were first faced with the choice of a good experimental plant. The higher plants would probably not be very flexible in their organic composition because elaborate structural elements form so great a part 6f their total mass. Moreover the major constituents of higher plants are carbohydrates, of which we already have plenty. The higher plants grow slowly, and complex apparatus would be needed for the control of their environment. There are more plants growing in the waters of the earth than there are on the land. Some of the water plants, particularly the unicellular green algae, grow rapidly, have a minimum of structural elements, and require only simple apparatus for the control of their environment. For our purpose we chose *Chlorella pyrenoidosa,* which is a unicellular, green, fresh-water alga (not a seaweed, as has been erroneously stated in the press). This species of Chlorella has long been used in studies of photosynthesis, and a great deal was already known about cultural conditions required to obtain plant material of uniform properties. We set out to determine what would happen to the composition of this same alga when the conditions for its growth were varied over a wide range.

The environmental factors which we varied were: the concentrations and relative proportions of the different salts in the nutrient medium, the composition of the gas stream which was bubbled continuously through the cultures, the temperature of the culture, and the light intensity incident on the culture.

The Chlorella cells from each pure culture were dried and analyzed for percentage of carbon, hydrogen, nitrogen, and ash. A very simple way was found for calculating the proportion of carbohydrate, protein, and lipide in the Chlorella samples from their elementary analysis (1). Different lots of Chlorella had a protein content ranging from 78% down to 7% of the dry weight. Carbohydrate varied from 6% to 37%. The most striking variation was in the lipid content, from as little as  $4.5\%$  up to  $86\%$  of the dry weight of the Chlorella.

The cultures were grown in an inorganic medium and the carbon dioxide in the gas stream bubbling through them was their only source of carbon. Cultures receiving only air grew very slowly. As the carbon dioxide content of the air was increased, the rate of growth of Chlorella was increased. This was true up to about 5% carbon dioxide, where the growth rate was maximum. With an increase to 10% carbon dioxide, the rate of growth began to fall off again. Other conditions being equal, the concentration of carbon dioxide supplied was reflected in the amount of plant material produced, without causing very much difference in its composition. Temperature and intensity of illumination also appeared to affect mainly the quantity of Chlorella produced rather than its composition.

**T** HE concentration of fixed nitrogen in the medium proved to be the key factor in influencing the yield of cells and in determining whether or not a Chlorella culture could attain a high lipid content. The fixed nitrogen content of the medium for each culture was computed from the nitrogen content of the salts added. The fixed nitrogen remaining in the medium after the cells were harvested was called residual fixed nitrogen.

When the residual fixed nitrogen in the medium is plotted against the lipid content of the Chlorella cells, certain relations become clear. No culture reached a lipid content higher than  $35\%$  when the residual fixed nitrogen was greater than 0.001M. Below 0.001M residual fixed nitrogen, the lipid content appears to be controlled by environmental factors other than fixed nitrogen concentration. A number of cultures grown under low light intensity and for relatively short time reached residual fixed nitrogen concentrations between 0.001M and zero without attaining a lipid content as high as 35%. But, with high enough light intensity and long enough time, all of the cultures in which the residual fixed nitrogen was less than 0.001M reached a high lipid content. Up to fixed nitrogen concentrations of about 0.0025M initially present in the medium, with high light intensity and with long enough time for growth, so that in each case the residual fixed nitrogen fell below 0.001M, the yield of cells was roughly proportional to the initial nitrogen concentration and all cultures reached a high lipid content. If, however, the concentration of fixed nitrogen put into the medium was so large that the Chlorella by its growth could not exhaust it below 0.001M, large yields of cells were obtained, but the lipid content was always below 35%.

After having once established a set of experimental conditions to produce a given yield of Chlorella having a stated lipid content, it was easy to reproduce such a culture within very close limits both with respect to yield and lipid content. In four larger scale experiments conditions were selected to produce Chlorella having 20, 35, 60, and  $75\%$  lipid. The lipid content of the four lots actually obtained was 23, 33, 63, and 75%.

The extracted lipid from each of the four lots was subjected to analysis with particular reference to its component fatty acids (2). The true fat content of the four lots was found to be 7, 17, 55, and 69% of the dry Chlorella. The fatty acids were nearly all of the  $C_{16}$  and  $C_{18}$  series, with roughly 15% saturated and 85% unsaturated acids. The principal saturated acid was palmitic, stearic acid constituting only 0.4% to  $4\%$  of the total fatty acids.  $C_{1s}$  unsaturated acids constituted 54-67% of the total and  $C_{16}$  unsaturated acids 18-29%. The degree of unsaturation in the different fractions of liquid acids ranged from 1.6 to 2.25 double bonds per molecule. Thus the presence of triply unsaturated acids is shown. An unusual feature of Chlorella fat was the presence of triply unsaturated  $C_{16}$  acids, 17% in the liquid acid fraction from one lot of cells. As the lipid content of the Chlorella increased from 23% to 75%, the iodine number of the total fatty acids decreased from 163 to 125. There was no clear relation between the different proportions of  $C_{16}$  and  $C_{18}$  solid and liquid acid fractions and the total fatty acid content of the different samples of Chlorella. In general, Chlorella fat does not seem to differ markedly from other highly unsaturated plant fats in its composition. Its high iodine number suggests that it might be classed as a drying oil although this property was not tested. It seems safe to predict on the basis of analysis that Chlorella fat should be suitable for use as food.

The protein of Chlorella compares favorably with other good plant proteins on the basis of amino acid analysis. Merck and Company found all of the essential amino acids to be present in Chlorclla protein, in amounts totalling 42% of the protein. Vitamin assays of Chlorella place it among the richest plant sources with respect to several vitamins.

**H** AVING seen that it is possible to control within wide limits the composition of Chlorella by choice of conditions for its growth, to produce at will a product high in protein or one very rich in fat, the next question is, can it be done on a large scale and with high efficiency of light utilization ?

These cultures were grown by the batch method, wherein the utilization of light is very inefficient. At the beginning there are too few Chlorella cells to absorb all the light, and at the end there are so many cells that some are shaded by others. The situation is similar to another batch process, the growing of a farm crop, where much of the time the plants do not cover all of the illuminated area. If a Chlorella culture is maintained continuously in the middle stage where growth is most rapid and where there are just enough cells to absorb all the light, a marked improvement in yield per day is realized. Such continuous cultures of Chlorella have been grown on a laboratory scale by Myers (3) and by Cook (4).

Supposing that you could cover an acre with such a continuous culture, how much Chlorella could you grow in a year ? Extrapolation from a laboratory culture a few liters in size to one covering an acre is a somewhat hazardous sort of calculation, but the results so obtained are very challenging. Figuring conservtively, it seems reasonable to expect an annual production of 40 tons of dry, high-protein Chlorella per acre. Considering that 40 tons of such Chlorella contains 20 tons of protein plus three tons of fat, the present agricultural production of protein and fat per acre per year is dwarfed in comparison. If it were desired to increase the fat production, six tons per acre per year appears possible. Since the production of a gram of fat requires more than twice the energy input needed to produce a gram of protein the total tonnage of high-fat Chlorella would be much less than that of high-protein Chlorella.

The culture of Chlorella, or a similar organism, on a vast scale in a factory-farm appears entirely feasible from the biological point of view. The major difficulties in setting up such a project are of an engineering nature. It is not easy to design large scale equipment and processes to fit the biological requirements of Chlorella, and it is even more difficult to build from such designs at a reasonable cost. Probably on an emergency basis, where cost is a minor consideration, a Chlorella factory-farm could be put into operation. In order for ChIorella to enter a free economic market in competition with present supplies of fats or highprotein feeds, the alga would have to be produced at a cost of a few cents per pound.

Before the suitability of Chlorella as either a food or an industrial raw material can be assessed, considerable quantities will be needed for testing. And, once such quantities are available, there is the chance of discovering some by-product worth so much more per pound than either protein or fat that it could pay a significant part of the production cost of Chlorella.

Without complete engineering data it is not possible to draw a blueprint for the construction of a factoryfarm for the production of tons of Chlorella. It seems reasonable to assume that such an enterprise would have to be very big in order to pay, big enough to produce tens of tons of Chlorella per day. It is much easier to state the problems that need to be solved than it is to tell how to solve them. Let us try to define the sort of system which will fit the biological requirements for growing Chlorella at the maximum rate and with the maximum efficiency of utilization of light.

**I** T does not seem worthwhile to consider any light source other than the sun. Artificial illumination for great areas of Chlorella culture would no doubt be prohibitive in cost. Except for the small amount of light utilized in photosynthesis, all of the sun's energy reaching a Chlorella culture will appear as, or be converted into heat, which must be dissipated in order to avoid raising the temperature of the culture above that suitable for good growth. The removal of heat from the culture might be accomplished by evaporation if growth took place in a pond, but in a closed system cooling would have to be provided.

A closed system appears necessary in order to supply the culture with 5% carbon dioxide to promote rapid growth. If the growth depends on the 0.03% carbon dioxide in ordinary air, the yield of Chlorella is only one-tenth to one-fifteenth that with  $5\%$  carbon dioxide in air. The closed system is desirable to prevent excessive loss of water by evaporation in a large scale plan for culturing Chlorella. We have considered only pure cultures of Chlorella. Any nonautotrophie contaminant will live at the expense of the Chlorella and will contribute materials of its own manufacture to the harvest. Some contaminants might be harmless and others very objectionable. Only in a closed system that can be sterilized at the start and maintained aseptic in operation will it be possible to maintain a pure culture of Chlorella.

The architecture of the closed system is extremely important. It should be arranged to cover as much as possible of the area of the site so that nearly all of the sunlight will fall on the Chlorella culture. The geometric shape of the culture containers and their orientation, whether vertical, inclined normal to the sun's rays or horizontal will depend on the material chosen for their construction and on engineering considerations, such as the ease of maintaining continuous flow at the proper rate. The dimensions of the culture containers have to be considered in relation to the density of the culture. If, at a given culture density, the container is too thin, light will be transmitted and wasted. If the container is too thick, light will not penetrate all the way through the culture and some of the cells will be in darkness.

Below the optimum intensity of illumination the rate of growth of Chlorella will be approximately proportional to the light intensity. Above the saturating light intensity the growth rate is independent of intensity over a limited range. If the light intensity is above that limit, as full sunlight is, the Chlorella cells suffer damage and the growth is retarded. Alternate exposure of the Chlorella cell to intense light and weak light or darkness not only eliminates the harmful effect of the intense light but gives a growth rate even higher than that at the optimum continuous intensity.

So, added to the requirement that the depth of the culture container be in proper relation to the culture density, is the desirability of turbulence within the culture so that no cells remain too long exposed to intense light or too long in semidarkness. Turbulence is also desirable in order to prevent settling of the cells and to insure that each cell has equal access to the mineral nutrients, including carbon dioxide, in the culture medium.

The best culture density for large scale production of Chlorella would be determined first by the relation between culture density and growth rate and second by the weight of water to be handled per unit weight of Chlorella. Even the densest Chlorella cultures which have been grown were mostly water, and the growth rate is maximum at only a small fraction of the greatest density attainable. Data now available indicate that growth of Chlorella is most rapid when there are less than 0.5 gram of cells, dry weight, per liter of culture. At that level our factory-farm will have to handle over 2,000 tons of water for every ton of Chlorella produced. It might be that a higher culture density with a slower growth rate and lower water : Chlorella ratio would be more economical.

While the culture medium contains only 0.5% to 1% by weight of nutrient salts, the quantity of these is not negligible when dealing with thousands of tons of solution. Since the Chlorella cells remove from solution only a small part of the available salts, with the exception of fixed nitrogen, re-use of the medium after appropriate replenishment of the salts is indicated both to conserve on the cost of salts and the cost of water. A fair-sized chemical plant will be needed for harvesting of the Chlorella, preparation of new medium, replenishment of used medium, and sterilization of all medium.

Besides water the two principal raw materials needed for the production of Chlorella are carbon dioxide and fixed nitrogen. For high-protein Chlorella, which has 50% carbon, 1.8 tons of carbon dioxide per ton of cells are required. For high-lipid Chlorella, with 65% carbon, the requirement is 2.4 tons of carbon dioxide per ton of Chlorella. A large amount of fixed nitrogen must be supplied in order to make protein. If complete conversion of the fixed nitrogen taken from the medium to protein in the Chlorella is assumed, the production of one ton of protein will require 1.1 tons of potassium nitrate, or 0.75 ton of ammonium sulfate, or 390 pounds of ammonia. It is evident therefore that the success of large scale Chlorella culture is dependent on an ample and cheap supply of carbon dioxide and the availability of low-cost fixed nitrogen in quantity.

**T** HERE is another problem to be solved in connec-tion with re-use of the eulture medium. The yield of Chlorella in a given lot of medium is limited in part by a self-inhibitory substance produced by the cells. Pratt (5) ealled this substance ehlorellin. Even when all the controllable factors are kept favorable to growth, there is a limit to the number of Chlorella cells which will be produeed in a unit volume of culture. Presumably the accumulation of chlorellin prevents cell division after this limit is reached, but the individual Chlorella eells remain alive and undergo a slow increase in size for a long time after reproduction stops (6). Pratt separated the cells and medium of old cultures which had reached the limiting cell count due to accumulation of ehlorellin. The medium still contained adequate amounts of all the mineral nutrients but, when inoculated with fresh Chlorella cells, very little growth took place. The cells from the old culture however resumed normal growth when placed in newly prepared medium. It appears essential, then, to include a treatment for removal of chl0 rellin before the medium is re-used in a faetory-farm for Chlorella production.

As separated from the culture, say by centrifugation, the Chlorella cells are about 75% water and 25% dry weight. By virtue of their chemical composition the fresh Chlorella cells are a wonderful food for a host of other microorganisms, including bacteria and fungi. For this reason it would be necessary to dry the Chlorella cells to prevent spoilage unless the product of the Chlorella factory-farm were to be used immediately at the site of production.

There are, of course, many more engineering details to be worked out than the ones which have been mentioned. It is not difficult though to visualize in a general way the sort of installation that would be needed to produce tons of Chlorella per day. There would be a large area nearly completely covered with a system of closed transparent conduits through which Chlorella culture would flow continuously under automatic regulation. The rate of flow, the depth of culture, and the cell density of the culture would be balanced to give maximum utilization of the light at each point in the system. A small part of the area would be occupied by the processing plant in which the culture medium is handled and the crop of Chlorella harvested and dried.

The ideal geographical location for a Chlorella factory-farm would be in a region where there is a high percentage of clear days, on land which is not valuable for other purposes, and in a place where adequate supplies of water, carbon dioxide, and fixed nitrogen are available. It should be borne in mind that even should high capital investment and labor costs make the process uneconomical in this country, it might still be a success in parts of the world where it is most needed, in those areas of cheap labor where the spectre of famine is ever present.

The Stanford Research Institute under a grant from the Research Corporation has made an engineering study of the possibilities in large scale culture of Chlorella (4). They considered plant designs best

fitted to meet the biological requirements of Chlorella which have been outlined here, and they also made estimates of the cost of producing Chlorella on a tonnage basis. These studies need to be continued on a larger scale. Several agencies in Europe and in this country are interested in continuing work along these lines. The commercial production of oils and proteins from Chlorella may not take place in the immediate future, but the possibility of its eventual accomplishment cannot be disregarded.

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## **ABSTRACTS ............. vo. Whyte, Editor**

## **• Oils and Fats R. A. Reiners, Abstractor**

The occurrence of highly unsaturated  $C_{20}$  and  $C_{22}$  fatty acids in **plant phosphatides.** E. Klenk and Hildegard Debuch (Univ. Cologne, Ger.). *Z. physiol. Chem.* 286, 33-7(1950). Phosphatides from soybeans and rape seeds were precipitated from concentrated ether solution by acetone, refluxed 1 hr. with 4% HC1 in methanol and the methyl esters of the unsaturated fatty esters extracted with petroleum ether. From the higher-boiling fractions (distilled in vacuo)  $C_{20}$  and  $C_{22}$  unsaturated acids were isolated. *(Chem. Abs.* 45, 4311)

**Reactions of tertiary butyl hypochlorite with vegetable oils and their derivatives. IV. Conversion of alkyl oleates** and methyl **linoleate to derivatives of ketostearic acids. H. M. Teeter, L. E.**  Gast, Dolores Raleigh and L. C. Woods (Northern Reg. Res. Lab., Peoria, Ill.). *J. Am. Chem. Soc.* 73, 2302(1951). Alkyl oleates are reacted with t-butyl hypochlorite in the presence of an alcohol to form alkyl alkoxychlorostearates. These compounds may be decomposed at 285 ° and 100 mm. pressure to form a mixture of alkyl 9-ketostearate and alkyl 10-kctostearate in 70% yield.

**Texas** plant marks **new trend in oilseed processing.** Anon. *Am. Miller # Processor* 79(5), 26(1951). A new plant in Sherman, Texas, is described in which the oil seeds are preexpelled prior to solvent extraction.

**Spontaneous heating and ignition in stored palm kernels. VI.**  Summary and conclusions. J. H. Burgoyne (Imperial College, London). *J. Sci. Food Agr.* 2, 157(1951). Spontaneous heating of jute and palm kernels due to microbiological activity does occur. It can be minimized by excluding water from the stacks.

Sell **that oil in citrus seed.** E. E. Wright. *Food Eng.* 23(5), 102(1951). Recovery of oil from citrus seeds will not be economically advantageous unless the oil can be sold for about 25c a pound.

**The saponification of a-monomyristin, a-monostearin and**  ~-mono-olein. H. H. G. Jellinek and A. Gordon (Lyons Labs., London). *J. Appl. Chem.* 1, 185(1951), The saponification of a-monomyristin, a-monostearin and a-mono-olein in 75% aqueous alkaline acetone was found to be a second-order reaction. Energies of activation of the three monoglycerides were 8,900, 11,100 and 10,800 g-cal., respectively.

**The component acids and glycerides of Pentacletbra** (leguminosae) and Lophira (ochnaceae) seed fats. T. P. Hilditch, M. L. Meara and C. B. Patel (Univ., Liverpool). *J. Sci. Food*  Agr. 2, 142(1951). The seed fats of these West African species of plants contain 10-20% of saturated acids (mainly behenic and lignoceric) of higher molecular weight than stearic acid. The unsaturated acids are confined to oleic and linoleic in varying proportions. The composition of the mixed glycerides in the fats conforms closely to even distribution.

**The seed fat of** Parinarium laurinum. **Part II. Component glycerides of the seed fat. J. P.** Riley (Univ. Liverpool). *J. Chem. 8oe.* 1951, 291. The *component* acids of the fat of *P. laurinum* are parinaric 55.8%, elaeostearic 27.0%, conju gated dienoic 1.3%, linoleic 2.2%, oleic 7.6%, and saturated 6.1%. The principal glycerides are triparinarin 22%, elaeostearo-diparinarin 27%, oleo-elaeostearo-parinarin 16%, saturated-elaeostearo-parinarin 15% and dielaeostearo-parinarin The component acids, except parinaric, appear to be combined in the glycerides according to the rule of even distribution. In the case of parinaric acid, however, far more triparinarin is present than would be accounted for by either even or random distribution principles.

**Analysis of fat acid oxidation product by countercurrent distribution methods. Model compounds. K. T.** Zilch and **H. J.**  Dutton (Northern Reg. Res. Lab., Peoria, Ill.). Anal. Chem. 23, 775(1951). The distribution in the Craig countercurrent apparatus between hexane and 80% ethanol was studied for a series of compounds similar to those which might be produced on oxidation of fatty acids. Their weight distribution curves were predictable from their partition coefficient and the binomial theorem.

**Chromatographic separation of choline-containing phospholipids from phospholipid mixtures.** T. H. Bevan, G. L Gregory, T. Matkin and A. G. Poole (Univ., Bristol, England). *J. Chem. Soc.* 1951, 841. Choline containing phospholipids (lecithin and sphingomyelin) may be separated from other phospholipids using a column of powdered cellulose.

**Formation of cyclic compounds in polymerization of methyl esters of fatty acids from linseed oil. H. I. Waterman, C. J.**  Kips and J. van Steenis (Tech. Univ., Delft, Holland). *Research* 4, 96(1951). Polymerization of the methyl esters of linseed oil fatty acids at  $300-310^\circ$  in the presence of  $SO_2$ yields a monomer and polymer fraction which contain rings. Ring analysis was performed by direct catalytic high *pressure*  hydrogenation at 300°. The monomer fraction contained 0.3 ring per molecule, the polymer fraction about 2.

**Utilization of** olive-oil foots. J. M. M. Moreno. *Ion* 10, 579-  $85(1950)$ . Extraction of olive-oil press cake with  $CS<sub>2</sub>$  produces a low-acidity (8-10% free fatty acid) and high-acidity oil, and cake which can be used for fuel, destructively distilled to pyroligneous acid, or hydrolyzed to furfurah Low-acidity oils can be blended with olive oil, or hydrogenated (after S removal), and trans-esterified to give margarine and shortenings. High-acidity oil is produced in the greatest amount and is the least valuable. Oils containing more than 50% free fatty acid are used in soap manufacture without glycerol recovery; those containing less are first hydrolyzed in the Twitchell process. Hydrogenation of high-acidity oils is possible after removal