

Methylation of the Polysaccharide. Hakomori methylation [8] was performed three times. The completeness of methylation was checked by thin-layer chromatography on alumina and by the IR spectrum of the methylated product. The completely methylated glucan did not absorb in the region of hydroxy groups.

Hydrolysis of the Methylated Glucan. The methylated product was hydrolyzed with 90% formic acid at 100°C for 1 h and then with 0.25% sulfuric acid [7]. The hydrolysis products were separated in parallel by paper chromatography in the solvent 1-butanol-ethanol-water (5:1:4) and by thin-layer chromatography on silica gel in the solvent benzene-acetone (2:1.5). By comparison with markers and by their R_f values, the 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-O-methyl derivatives of glucose were identified in a ratio of 5:14:4. The methylated polysaccharide was subjected to methanolysis with 4% HCl in absolute methanol, and the methanolysis products were separated by GLC. Three pairs of peaks were obtained which were identified by comparison with markers as α - and β -methyl 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-O-methyl-D-glucosides.

SUMMARY

The blue-green alga *Microcystis aeruginosa* contains a branched glucan constructed of D-glucopyranose residues with α -1,4 bonds in the main chain and α -1,6 bonds at the points of branching.

LITERATURE CITED

1. A. A. Elenkin, Blue-Green Algae of the USSR [in Russian], Moscow (1938).
2. L. Chao and C. C. Bown, J. Bacteriol., 105, 331 (1971).
3. L. Houg, J. K. H. Jones, and W. H. Wadmen, J. Chem. Soc., 3393 (1952).
4. M. A. Preshkov, in: The Biology of Blue-Green Algae [in Russian], Moscow (1964), p. 25.
5. R. M. Giesy, Am. J. Bot., 51, 388 (1964).
6. K. Nakanishi, Infrared Absorption Spectroscopy. Practical, Holden-Day, San Francisco (1962).
7. N. K. Kochetkova (editor), Methods of Carbohydrate Chemistry [in Russian], Moscow (1967), p. 466.
8. S. Hakomori, J. Biochem. (Japan), 55, 205 (1964).

THE USE OF THE SEPARATING CAPACITY OF UNIFORM-PORED SILICA GEL IN THE REFINING OF COTTONSEED OILS

A. Ganiev, U. I. Tros'ko, A. N. Mironova,
and E. P. Kolobova

UDC 665.3036.7:62.001.6

At the present time, the refining of cottonseed oil is performed with aqueous solutions of sodium hydroxide. In this method of purification, ~10% of the triglycerides, the gossypol, the fatty acids, the phosphatides, the steroids, the tocopherols, etc., are lost. In order to eliminate this defect, it is desirable to use the selective adsorption of the substances composing the oil or ultrafiltration of a solution of the oil through pores or channels with definite dimensions of the separating layers.

The clarification of cottonseed oil with zeolites, ion-exchange resins, and bentonites has been described [1]. Analysis of the experimental results has shown that the types of sorbents studied have a number of defects. However, silica gel is favorably distinguished from the other sorbents by its chemical inertness, its high specific surface, its great

All-Union Scientific-Research Institute of Fats, Leningrad. Translated from Khimiya Prirodnikh Soedinenii, No. 4, pp. 422-426, July-August, 1976. Original article submitted October 22, 1975.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for \$7.50.

TABLE 1. Adsorption Capacity and Separating Capacity of Uniform-Pored Silica Gels with Respect to the Individual Components of Cottonseed Oil, g/100 g of Silica Gel

Sam- ple No.	Component	KSM-6	KSS-4	KSK-2	KSK-1	KSK-1
		$\frac{460}{10,9}$	$\frac{400}{32,0}$	$\frac{338}{70,0}$	$\frac{220}{100,0}$	$\frac{160}{127,5}$
Static capacity						
1	Gossypol	0,09	0,07	1,91	3,71	2,47
2	Free fatty acids	0,44	6,98	2,32	1,55	1,42
	Gossypol	0,02	0,04	0,38	2,17	0,29
3	Free fatty acids	0,59	5,65	1,98	1,14	2,00
	Gossypol	0,03	0,05	0,90	2,18	0,59
	Phosphatides	0,02	0,11	0,01	0,99	4,01
Dynamic capacity						
4	Gossypol	0,05	0,02	1,68	2,98	1,07
5	Free fatty acids	0,37	6,90	2,03	1,39	1,02
	Gossypol	0,03	0,06	0,19	2,71	0,24
6	Free fatty acids	0,61	7,18	2,26	2,30	1,50
	Gossypol	0,02	0,11	0,87	2,90	0,58
	Phosphatides	0,05	0,05	0,10	0,92	3,63
7	Free fatty acids	0,38	7,28	2,26	1,50	1,22
	Gossypol	0,04	0,12	0,24	2,65	0,25
	Phosphatides	0,03	0,11	0,47	1,15	2,90

*Samples 1-6 were model mixtures of the components with triglycerides and hexane, and sample 7 was an industrial gasoline miscella.

†The numerator gives the average surface of the silica gel, m²/g, and the denominator the effective pore radius, Å.

mechanical strength and thermal stability, and the ease with which its porous structure can be regulated.

We have attempted to determine the possibility of the fractional purification of cottonseed oil by using the adsorption activity and separating capacity of silica gels with different porosities.

The activity of sorbents with respect to the main groups of constituents accompanying the triglycerides was determined under static and dynamic conditions using two- to five-component mixtures of these substances in the form of their hexane or gasoline solutions, and also industrial samples of gasoline miscellas of cottonseed oils.

The ratio of the substances in the solutions of these mixtures was made close to their ratio in industrial miscellas. The weight fraction of solvent was varied between 60 and 90%. The lipid part of the mixture contained 0.2-1.0% of gossypol, 1.5-2.0% of phosphatides, and 2-5% of fatty acids.

The amount of sorbed substances was determined:

under static conditions, from the change in the amount of the given substance in the solution before the addition of the sorbent and after the establishment of the adsorption equilibrium, and

under dynamic conditions, from the amount of component retained by the sorbent from the filtrate up to its "breakthrough" beyond the layer of sorbent.

The amount of adsorbed fatty acids, in the mixture with the other components was determined from the change in the true acid number of the oil, the amount of gossypol by the aniline-pyridine method and from the change in the intensity of absorption at a wavelength of 366 nm, the amount of phosphatides by the dry combustion method, the amount of solvent gravimetrically, and the amount of triglycerides from the difference between the total amount of lipids and the total amount of components found [2]. To determine the amounts of tocopherols in the initial oils and those purified by the adsorption and the usual alkaline methods, we used thin-layer chromatography on silica gel [3].

TABLE 2. Content of Biologically Active Substances in Cottonseed Oil after Alkaline and Adsorption Refining

Sample	Tocopherol, mg %			Sterol, %	Phosphorus calculated as stearo-oleocithin, %	Yield, %
	"	γ	total amount			
Initial	43,0	95,5	138,5	0,32	1,31	—
After alkaline refining	17,0	18,0	35,0	0,19	0,020	91,0
After purification on silica gels	19,2	23,8	43,0	0,13	0,027	93,5

The adsorption activity and separating capacity of 19 types of domestic uniform-pored silica gels produced on the industrial scale and differing by the mean pore radius and method of treatment were studied.

The experimental results for the five most specific of them are given in Table 1. The uniform-pored silica gels with an average pore radius of 10-12 Å are ineffective in the purification of cottonseed oils, since they possess only a small absorption capacity for free fatty acids (0.005 g/g), scarcely adsorb gossypol and phosphatides, and have a weak clarifying capacity.

Silica gel with a pore radius of 30-35 Å possesses a considerable separating capacity which is shown in its relatively high absorption capacity for free fatty acids (0.07 g/g) while the sorption of gossypol and phosphatides is slight (0.001-0.002 g/g). Silica gel with an effective pore radius of 50-70 Å has a smaller adsorption capacity with respect to fatty acids (0.02 g/g), apparently because of a decrease in the specific surface of the sorbent, and a somewhat greater capacity for the adsorption of gossypol and phosphatides (0.002 and 0.005 g/g, respectively). At a pore radius of 100 Å silica gels show, in addition to a high capacity with respect to free fatty acids and phosphatides, an extremely high capacity for the adsorption of gossypol (0.026 g/g) and colored substances. As is well known, the latter consist mainly of products of the alteration of gossypol and of its interaction with phosphatides, pheophytins, etc. Silica gel with a pore radius of the order of 127 Å is characterized by an increased capacity for phosphatides (0.029 g/g) — the most polar of the substances accompanying the triglycerides, the molecular volume of which is fairly great.

The results obtained confirm the hypothesis put forward previously for other systems that fine-pored silica gels possess a considerably greater capacity for separating the components of the mixture than coarse-pored silica gels [4]. In this case, also, the separating capacity and absorption activity of definite types of fine-pored silica gels for the free fatty acids of cottonseed oil is extremely high, which is apparently due not only to the great rate of diffusion of the comparatively small molecules of the free fatty acids but also to the impossibility of the penetration of the comparatively large molecules of gossypol and phosphatides into the pore system of the fine-pored silica gel. Coarse-pored silica gels also show a definite selectivity with respect to the larger molecules of gossypol and the phosphorus-containing substances apparently because of the combined influence of the porous structure of the silica gel and the polar properties of the substances undergoing sorption.

The experimental results show that with the use of a definite sequence of layers of silica gel and the passage of a solution of the initial cottonseed oil in gasoline (hexane) through them, it is possible to achieve the separation from the oil of the whole complex of impurities that are separated from cottonseed oil by alkaline refining at the present time. At the same time, the degree of their separation exceeds that achieved at the present time in the last stages of the purification of the oil using special reagents and equipment, with the removal of not more than 2-3% of the triglycerides present in the initial oil.

The samples of industrial solutions of cottonseed oil in gasoline (miscellas) were purified by alkaline treatment and by subsequent passage through a number of columns with various types of uniform-pored silica gels until indices had been reached that corresponded to the requirements of GOST [All-Union State Standard] 1128-55 and to edible cottonseed oil of the highest quality. The weight ratio of silica gel and oil was 1:1, and the concentration of the miscella 30% by volume.

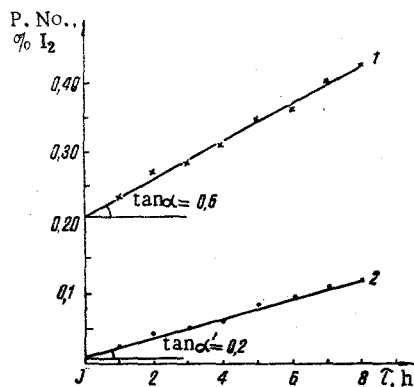


Fig. 1. Intensity of the rise in the peroxide numbers (P. Nos.) of the oils as a function of the time of heating at 80°C: 1) after alkaline refining; 2) after adsorption refining.

We determined the amount of biologically active substances in the oils obtained and checked the resistance of the oils to oxidation. The analytical indices obtained give an idea of the edible value of the oil and its stability on storage.

The use of uniform-pored silica gels for purifying cottonseed oils does not involve the predominant elimination of the α - and γ -tocopherols, sterols, and phosphatides (Table 2) in comparison with the method of alkaline refining, in consequence of which the finished oil retains its high edible properties and resistance to oxidation. The higher stability to oxidation of the oil from adsorption purification is confirmed by the increase in the peroxide numbers of the oils as a function of the time of heating at a definite temperature (Fig. 1). The oil purified with the aid of silica gels has a considerably smaller tendency to undergo oxidation ($\tan \alpha = 0.2$ as compared with 0.6 for the oil from alkaline refining). The very low content of primary oxidation products in the oil after adsorption purification (P. No. 0.01% as compared with P. No. 0.22% for the oil from alkaline refining) is perhaps one of the prerequisites for its greater stability on heating.

The ultraviolet absorption spectra of both oils confirm the insignificant tendency of the uniform-pored silica gels to cause the isomerization of the unsaturated fatty acids of cottonseed oil during its purification.

The ash contents of the oils after adsorption purification and alkaline refining were 0.0008% and 0.0018%, respectively.

SUMMARY

1. Uniform-pored silica gels produced in the Soviet Union possess a fairly pronounced separating capacity with respect to the main groups of substances accompanying cottonseed oil and eliminated during its purification.

2. The adsorption activity of the uniform-pored silica gels is sufficient for the refining of cottonseed oil at a ratio of the total amount of silica gel to the weight of oil of 1:1.

3. The purification of cottonseed oil with uniform-pored silica gels does not lead to appreciable chemical changes in the oil that could have an unfavorable influence on its edible value or its resistance to oxidation.

LITERATURE CITED

1. I. V. Gavrilenko and I. E. Bezuglov, Tr. Vses. Nauchno.-Issled. Inst. Zhirov, No. 14, 32 (1952).
2. Handbook on Methods of Investigation, Technical and Chemical Control, and the Accounting of Production in the Oils and Fats Industry [in Russian], Vol. 1, Book 2, Vses. Nauchno.-Issled. Inst. Zhirov (VNIIZh), Leningrad (1967), p. 833.

3. Handbook on Methods of Investigating, Technical and Chemical Control, and the Accounting of Production in the Oils and Fats Industry [in Russian], Vol. 6, VNIIZh, Leningrad (1974), p. 24.
4. I. E. Neimark and R. Yu. Shenfain, Silica Gel, Its Production, Properties, and Applications [in Russian], Kiev (1973), p. 156.

OPTIMIZATION OF THE ELIMINATION OF LIPIDS FROM THE MICROALGA

Chlorella vulgaris

T. V. Ryazanova, É. D. Levin, and I. I. Astapkovich*

UDC 663.1:547.96.05

The use as food of the biomass of *Chlorella* without chemical treatment is impossible because of the presence of toxic substances in the lipids. According to the literature [1], the lipid fraction contains a number of specific substances including fatty acids of the hexadecatrienoic type and substances possessing biological activity — chondrillosterol and ergosterol. It has been shown [2, 3] that the use of native *Chlorella* in the fodder and food ration leads to pathological changes in the organs of animals and to signs of allergy in man. These changes are the result of the toxic action of the ethanol-soluble substances of the lipid fraction. The possibility has been shown previously of eliminating the lipids by oxidation with peracids [4, 5]. Hydrogen peroxide is a specific oxidizing agent of lipids, since in the absence of catalysts it has practically no effect on the other components of the cell.

The aim of the present investigation was to find the optimum conditions for eliminating the lipids by peroxide oxidation. Since definite requirements are also set for the color of the food product, the color of the protein-carbohydrate product was chosen as a second optimization parameter.

EXPERIMENTAL

For oxidation we used the pasty biomass of the microalga *Chlorella vulgaris* (moisture content 80%) obtained in the L. V. Kirenskii Institute of Physics of the Siberian Branch of the Academy of Sciences of the SSSR. The experiment was based on a Box-Hunter second-order rotatable plan, which corresponds to the requirements of central composition planning. This satisfies well-known criteria for the optimum nature of the plans. The optimization parameters selected were the lipid content in the protein-carbohydrate product [Y_1] and its color [Y_2]. In view of the fact that in the cell the magnesium is present mainly in the chlorophyll, the color of the complex was expressed in terms of its magnesium content.

In a series of preliminary experiments, we worked out the three main factors affecting the process, their basic levels, and the intervals of variation:

Factor	Symbol	Basic level, X_{cch}	Interval of variation, λ	Upper level	Lower level
Time of oxidation,	X_1	5.5	2.5	8.0	3.0
Oxidation temperature, °C	X_2	45	15	60	30
Concentration of H_2O_2 , %	X_3	17.0	8.0	25.0	9.0

Oxidation was performed in a glass reactor fitted with a stirrer, a jacket for heating, and two nozzles for the feed of hydrogen peroxide and the removal of the reaction gases, all

*N. D. Barabash took part in the work.

Siberian Technological Institute, Krasnoyarsk. Translated from *Khimiya Prirodnikh Soedinenii*, No. 4, pp. 426-430, July-August, 1976. Original article submitted October 23, 1975.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for \$7.50.