terpenes from pinane (Fraction A) differ from those of dihydro-*allo*-ocimene and dihydromyrcene (Table I). In addition, the refractive index and density of the completely hydrogenated Fraction A are somewhat greater than those of 2,6-dimethyloctane, the hydrogenation product of both *allo*-ocimene and myrcene (Table II). This increase could be caused by the presence of hydrocarbons having a relatively more compact structure, probably trimethylheptanes. Accordingly, the dihydroacyclic terpenes resulting from the isomerization of pinane are apparently mixtures of dimethyloctadienes and trimethylheptadienes. Since the mixture does not react with maleic anhydride, appreciable conjugation is not present.

Fractions B and D consist of dihydromonocyclic terpenes. Fraction B could not be a dihydropyronene, since the physical constants of its hydrogenation product are dissimilar to those of tetrahydro-α- and β-pyronenes (Table II). By comparing the physical constants of the hydrogenation product of Fraction D with those of the tetrahydropyronenes and menthanes, it is apparent that Fraction D could consist of mixtures of dihydromonocyclic terpenes, probably mostly menthenes.

Fraction C is saturated, and its physical constants (Table I) indicate that it is unchanged pinane.

Summary

Vapor-phase thermal isomerization of pinane at temperatures of 495–515°, and a contact time of about five seconds has been carried out, in order to determine whether dihydroacyclic terpenes are formed.

About 30–40% of dihydroacyclic terpenes are formed by the isomerization. In addition, about 20–40% of dihydromonocyclic terpenes, probably including some menthenes are formed. The remainder of the identified material (20–30%) in the reaction mixture is unchanged pinane.

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[CONTRIBUTION FROM STARCH AND DEXTROSE DIVISION, NORTHERN REGIONAL RESEARCH LABORATORY,[1] PEORIA, ILLINOIS]

Extraction of Fatty Substance from Starch

BY ROY L. WHISTLER AND G. E. HILBERT

Considerable importance has been attached to the "fatty" constituents in starch. Fatty material interferes with the fractionation of starch[1a] into amylose and amylopectin and is responsible, at least in part, for the initial flocculent precipitate (starch, hemicellulose, amylocellulose, γ-amylose, etc.) which forms when cereal starch solutions are treated with amylases.[2] Many other properties of cereal starch pastes, such as their opacity, viscosity, gelling power, retrogradation rate, and iodine absorption capacity are affected by fats.

Before the modifying influence of native fatty material can be fully determined for corn starch, it is necessary to understand the type of combination which exists between the fatty substance and the starch. Considerably different effects would be expected if the fat were bound by primary valence bonds than if it were bound by secondary valence forces.

That the fatty material of cereal starches is not removed by a typical fat solvent, such as ether or carbon tetrachloride, has been known for some time. This, in part, caused Taylor and coworkers[3] to believe that the fatty acids are present as esters of starch, the esters being associated with the *a*-amylose fraction. Schoch[4] has shown that the fatty substance of cereal starches can be largely removed by extraction with neutral hydrophilic solvents such as methanol and aqueous dioxane. Such extraction reduces the fat content of corn starch to about 0.1 to 0.2%. These results were interpreted by Schoch as indicating that most of the fat is combined mechanically with the starch. The fact that palmitic acid is strongly adsorbed by starch has been shown by Lehrman.[5] The adsorption follows a typical Freundlich isotherm and the fatty acid is not removed by extraction with carbon tetrachloride. It is interesting to note that, according to Lehrman's results, the adsorption of palmitic acid by potato, corn, and rice starches is roughly proportional to their surface areas rather than to their original content of fatty acid. Although the evidence is strong that most of the fatty acid in starch is held by forces other than primary valence bonds, the nature of the combination of the residual non-extracted fat with starch is less well known.

Information on this point was obtained from experiments dealing with the extraction of intact and of disintegrated corn starch granules with methanol. The rate of removal of fatty material from raw, intact corn starch granules by Soxhlet extraction with methanol was determined and

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(1a) Schoch, THIS JOURNAL, **64**, 2957 (1942).

(2) Wilson, Schoch and Hudson, *ibid.*, **65**, 1380 (1943).

(3) Taylor and Nelson, THIS JOURNAL, **42**, 1726 (1920); Taylor and Iddles, *Ind. Eng. Chem.*, **18**, 713 (1926); Taylor and Werntz, THIS JOURNAL, **49**, 1584 (1927); Taylor and Walton, *ibid.*, **51**, 3431 (1929); Taylor and Sherman, *ibid.*, **55**, 2584 (1933).

(4) Schoch, *ibid.*, **64**, 2954 (1942); see also Kerr, *Cereal Chem.*, **20**, 299 (1943). Evans and Briggs, *ibid.*, **18**, 443 (1941).

(5) Lehrman, THIS JOURNAL, **64**, 2144 (1942); **61**, 212 (1939).

the results are shown in Fig. 1. The major portion of the fat was removed within ten hours. The rate of removal thereafter was extremely slow, one hundred and thirty six hours of extraction being required to reduce the fat content of the starch to 0.04% as determined by acid hydrolysis. Lehrman[5] similarly found that the fat content of corn starch was reduced to 0.03% by Soxhlet extraction with methanol for two weeks.

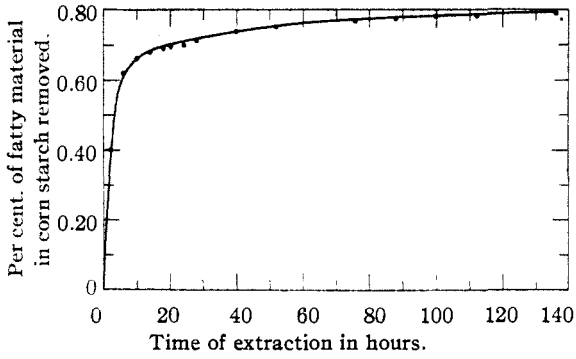

Fig. 1.—Removal of fatty material from raw corn starch by methanol extraction.

Since the starch granule is a compact mass in which the molecules are to a large degree oriented and in close proximity to each other, it would be expected that fatty acids deeply imbedded in the granule would be very difficult to remove because of their relatively large molecular volume. Fat, however, should be easily removed from disintegrated granules. This was found to be the case. All the fat was easily and completely removed by a short extraction of disintegrated granules. But, under more strenuous extraction conditions, fat was not removed completely from granules that had been gelatinized in hot water and then dehydrated with alcohol. In addition to extensive disorganization of the granules, disintegration apparently is necessary for ready removal of fat. The results as a whole show that practically all, if not all, the fat in starch granules is held mechanically, presumably by secondary valence forces.

On removal of fat from starch by methanol extraction, the phosphorus content of the starch is appreciably reduced while the nitrogen content is affected to a lesser extent.

Although cereal starches, such as corn starch and wheat starch, usually contain up to 1.0% of fatty material, glutinous corn starch contains only about 0.06%, and, in this respect, is similar to the root starches.

Experimental

Analyses for fatty material were run by hydrolyzing the starch with acid[6] and removing the fat from the hydrolyzate by repeated extraction with petroleum ether. Analytical data on the determination of fat in starch from which most of the fatty material had been removed are accurate to ±0.02%. Results on the determination of "fat by hydrolysis," in general, are somewhat high in the case of starch due to the extraction of some non-fatty material. Extracted fatty material, that is, the "fat by hydrolysis," in some cases, was titrated with sodium hydroxide—"fat by titration"—it being assumed that the acid present had the molecular weight of oleic acid.

A raw corn starch prepared by mild processing conditions was utilized to obtain the data shown in Fig. 1. In the other experiments described in this section, the corn starch before use was subjected to a preliminary extraction with 85% methanol. After this treatment, it contained 0.23% fat by hydrolysis, 0.05% nitrogen, and 0.017% phosphorus (anhydrous basis).

To obtain the data shown in Fig. 1 a quantity of starch (moisture content about 12%; 61.8 g. dry weight) was extracted with methanol of less than 1% water content in a Soxhlet extractor for a definite period of time after which the methanol extract was removed, evaporated, and the total residue weighed. This value was used for calculation of the per cent. fatty material extracted. Fresh methanol was then added to the extractor and the extraction continued. After repeated extraction over a period of one hundred and thirty-six hours, the starch still contained 0.04% fat by hydrolysis and 0.02% fatty acid by titration.

The following three experiments illustrate the effect of pretreatment of starch on the relative ease and extent of fat removal by methanol extraction:

1. The starch was pasted by heating a 5% suspension in water on a steam-bath for thirty minutes. The paste was poured with stirring into five times its volume of ethanol and the precipitate was filtered and triturated twice with fresh portions of ethanol. The starch was dried in a vacuum desiccator over calcium chloride. After Soxhlet extraction for thirty hours with 85% methanol, the starch contained 0.10% fat by hydrolysis, 0.01% phosphorus, and 0.04% nitrogen.

2. A paste, prepared as described under paragraph 1, while still hot (80–90°) was subjected to the action of a Waring Blendor for thirty minutes to disintegrate the swollen granules before precipitating in ethanol. After Soxhlet extraction for ten hours, this starch contained 0.02% fat by hydrolysis, and 0.02% fat by titration. After thirty hours of extraction, the fat value was unchanged; the phosphorus and nitrogen contents were 0.01 and 0.04%, respectively.

3. A starch paste was autoclaved for two hours at 120° and then passed through a Sharples supercentrifuge. Under these conditions, the starch granules were dispersed. The starch was precipitated by pouring the dispersion into ethanol. After Soxhlet extraction for thirty hours, the starch was found to contain 0.02% fat by hydrolysis and 0.01% fat by titration. The phosphorus content of the product was reduced to 0.00% and the nitrogen content to 0.03%.

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Summary

The fraction of fatty material difficult to extract from intact corn starch granules with methanol is easily removed after disintegration of the granules. All the fatty material in corn starch, apparently, is bound by associative forces rather than by primary valence bonds.

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(6) *Assoc. Official Agr. Chem., Official and Tentative Methods of Analysis*, 5th ed., p. 359 (1940).