[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE CITY COLLEGE OF NEW YORK]

The Fatty Acids Associated with Banana Starch

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It has already been shown that the starches of some of the common cereals (corn, rice and wheat)[1] and one of the tubers (cassava)[2] have fatty acids combined with carbohydrate in the α-amylose component. However, the amount in the latter starch is much smaller than in the former and also contains an additional fatty acid. Furthermore, another tuber (potato) starch does not contain any combined fatty acid.[3] Thus it is apparent that the combined fatty acids may vary in kind and amount with the type of starch. In order to gain added information as to the amount and nature of the fatty acids in different starches, a typical fruit starch was investigated. For this purpose the banana was selected, because in the green stage a large percentage of the dry pulp is starch. Specifically, bananas from the port of Limon were used.[4]

At first the dried pulp from the green fruit was used, but it was found that some naturally occurring substance made it very difficult to pulverize the residue, after drying, liberated by acid hydrolysis. As this is necessary in order to completely extract the fatty acids, the pure starch was made instead.[5] A total of about 3500 g. of starch was obtained starting with approximately 350 bananas.

In order to be sure that any fatty acids obtained were not originally present extraneously, all the starch was extracted with alcohol before carrying out the experimental work. Furthermore, to settle any question as to the presence of any glycerides as the source of the fatty material, the aqueous filtrate from the hydrolysis was analyzed for glycerol. As in previous investigations, the liberated fatty acids were examined also for other substances and phytosterol that might occur in the starch.

Experimental Part

Mixed Fatty Acids.—Extraction of a sample of the starch with petroleum ether for several hours showed a negligible amount of extractable material. Two separate portions of the starch weighing about 600 g. each were hydrolyzed with hydrochloric acid[1b] and the liberated fatty acids recovered by extraction with petroleum ether. This "fat by hydrolysis," a light yellow semi-solid, amounted to 0.2% in each case and had an iodine number of 59.4.[1a]

Examination of Filtrate for Glycerol.—Four liters of filtrate, reddish-brown in color, was evaporated on a steam-bath until a moist solid mass, black in color, was obtained. Alcohol was added, the mixture stirred well and filtered. The dark brown filtrate was again evaporated on the steam-bath to moist dryness. This process was repeated several times until a small amount of brown material, soluble in alcohol, resulted. A portion of this brown material gave a negative test for acrolein when heated with potassium bisulfate, indicating the absence of glycerol originally. Other tests[6] likewise showed the absence of glycerol.

Isolation and Identification of Saturated Fatty Acids.—In the separation of the saturated from the unsaturated fatty acids by the magnesium soap-alcohol method,[7] the insoluble magnesium soap was decomposed by heating with concentrated hydrochloric acid. The liberated fatty material, after several crystallizations from alcohol, was a white solid, melting point 62°. The phenylhydrazide was made[8] and had a melting point of 110°.[9]

The molecular weight of the acid was determined by titrating a weighed amount dissolved in alcohol with standardized sodium hydroxide using phenolphthalein as the indicator. Below are also given the results of micro carbon and hydrogen determinations.[10]

Anal. Calcd. for palmitic acid, $C_{16}H_{32}O_2$: C, 74.91; H, 12.59; mol. wt., 256.3. Found: C, 75.01; H, 12.16; mol. wt. (monobasic), 265.

The above data indicate the presence of palmitic acid in the mixed fatty acids.

Identification of Unsaturated Fatty Acids. A. By Oxidation.—The unsaturated fatty acids, separated from the saturated by the magnesium soap-alcohol method, was a light yellow oil having an iodine number of 147.1.

One and one-half grams was oxidized by potassium permanganate in alkaline solution.[11] The white solid oxidized acids were filtered, washed and allowed to dry. Then they were extracted with chloroform to effect a separation.[1b] On evaporation of the chloroform a fatty material was obtained which was re-oxidized and the resultant solid oxidized acids extracted with chloroform.

(1) (a) Taylor and Lehrman, THIS JOURNAL, **48**, 1739 (1926); (b) Lehrman, *ibid.*, **51**, 2185 (1929); (c) **52**, 808 (1930).

(2) Lehrman, *ibid.*, **54**, 2527 (1932).

(3) Lehrman and Kabat, *ibid.*, **55**, 850 (1933).

(4) The authors wish to thank the United Fruit Co., Inc., New York City, for their kindness in supplying the necessary green bananas.

(5) The authors wish to thank the Corn Products Refining Co., Edgewater, N. J., for the use of their technical laboratory and the aid of their technical research staff in making the starch.

(6) (a) Denigès, *Bull. soc. pharm. Bord.*, **49**, 161 (1911); (b) Kolthoff, *Pharm. Weekblad.*, **61**, 1497 (1924).

(7) Thomas and Yu, THIS JOURNAL, **45**, 123 (1923).

(8) Brauns, *ibid.*, **42**, 1480 (1920).

(9) J. van Alphen, *Rec. trav. chim.*, **44**, 1064 (1925).

(10) The authors are indebted to Mr. William Saschek of the College of Physicians and Surgeons, Biochemical Department, for most of the analyses (micro) recorded in this paper.

(11) Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," The Macmillan Company., New York, N. Y., 5th ed., Vol. I, p. 564.

The slight quantity of residues, insoluble in the chloroform extractions above, were combined and extracted with hot water. On cooling a small amount of white solid separated, which was crystallized from alcohol and water and had a melting point of 170–172°.

Anal. Calcd. for tetrahydroxystearic acid, $C_{18}H_{36}O_6$: C, 62.05; H, 10.34. Found: C, 61.95; H, 10.15.

Due to the small amount of material a molecular weight determination by titration could not be made.

The data indicate the presence of α-linoleic (linolic) acid,[12] in the unsaturated fatty acids.

The residue, obtained by the evaporation of the chloroform extracts, was extracted with petroleum ether to remove unoxidized fatty material. It was then extracted with ether in order to make a separation. The residue, insoluble in ether, was crystallized from alcohol and water, yielding a white solid, melting point 154–155°.

Anal. Calcd. for tetrahydroxystearic acid, $C_{18}H_{36}O_6$: C, 62.05; H, 10.34; mol. wt., 348. Found: C, 62.00; H, 9.95; mol. wt. (monobasic), 354.

The above data are added evidence of the presence of α-linoleic (linolic) acid[12] in the unsaturated fatty acids.

The ether extract was allowed to evaporate and the residue crystallized several times from alcohol. It was a white solid, melting point 115–117°.

Anal. Calcd. for dihydroxystearic acid, $C_{18}H_{36}O_4$: C, 68.35; H, 11.39; mol. wt., 316. Found: C, 68.00; H, 11.22; mol. wt. (monobasic), 319.

The data indicate the presence of oleic acid in the unsaturated fatty acids.

The water filtrates from the two oxidations were combined and examined for higher hydroxy acids,[11] with negative results. This indicates the possible absence[2] of acids more unsaturated than linoleic (linolic) in the unsaturated fatty acids.

B. Bromination.—One gram of the unsaturated fatty acids was brominated[13] yielding a small amount of white precipitate in the cold petroleum ether. This precipitate, together with a small amount of similar material recovered from the evaporated petroleum ether filtrate (see below), was extracted with hot petroleum ether. After evaporation of the petroleum ether, the residue was crystallized from ether yielding a white solid, melting point 176.5–177°.

Anal. (Carius) Calcd. for hexabromostearic acid, $C_{18}H_{30}O_2Br_6$: Br, 63.34. Found: Br, 63.55.

The data indicate the presence of linolenic acid in the unsaturated fatty acids. Additional qualitative tests for linolenic acid were obtained in both the mixed and unsaturated fatty acids using arsenotungstic acid.[14] It is interesting to note the detection of this acid by its bromide and the failure to find its oxidized product, hexahydroxystearic acid. This result is similar to that found previously by the first author with linolenic acid from cassava starch.[2] This further confirms his conclusion that bromination is a more delicate method for detecting small amounts of linolenic acid than oxidation.

(12) Green and Hilditch, *Biochem. J.*, **29**, 1552–63 (1935).

(13) Maksimov, *Bull. Far Eastern Branch Acad. Sci. U. S. S. R.*, 99–102 (1934).

(14) Martin, THIS JOURNAL, **58**, 364 (1936).

The petroleum ether filtrate was allowed to evaporate and the residue treated with ether. A small amount of white solid did not dissolve. As the melting point indicated the presence of the hexabromo compound, it was combined with the material insoluble in the cold petroleum ether (see above). The ether was allowed to evaporate and the residue recrystallized first from ether and finally from petroleum ether. It was a white solid, melting point 113°.

Anal. (Carius) Calcd. for tetrabromostearic acid, $C_{18}H_{32}O_2Br_4$: Br, 53.33. Found: Br, 53.00.

These data are added evidence for the presence of α-linoleic (linolic) acid in the unsaturated fatty acids.

Examination for Other Substances.—Tests on the resultant solution from a sodium fusion with a portion of the mixed fatty acids showed the absence of nitrogen, sulfur and halogens. Results obtained from the fusion of another portion of the mixed fatty acids with a mixture of equal parts of sodium carbonate and sodium nitrate indicated the absence of phosphorus.

The mixed fatty acids were tested for phytosterol by the Liebermann–Burchard reaction[11] (p. 270) and the precipitation with 1% alcoholic digitonin,[11] (p. 264) both being positive. The precipitate of the latter, after washing and drying, was silky white platelets having a melting point of 208–212°. However, there was too small an amount to obtain any added data. An attempt was made to isolate enough of the pure phytosterol,[15] for further identification, from a portion of the mixed fatty acids, but the amount again was too small to work with. In order to ascertain whether or not the phytosterol could have its source in some extraneous material, the alcohol used for the extraction of the starch was evaporated to dryness. The residue was then subjected to the same tests with negative results. As a further check 500 g. of alcohol extracted starch was extracted with petroleum ether. After evaporation of the petroleum ether a very small residue was obtained in which the absence of phytosterol was shown by the same tests.

Summary

The amount of fatty acids liberated by the hydrolysis of banana starch free from extraneous fatty material has been determined to be 0.2%.

The fatty acids have been found to consist of a mixture of palmitic, oleic, linoleic (linolic) and linolenic acids together with a very small amount of phytosterol.

The detection of small amounts of linolenic acid in the presence of oleic and linoleic (linolic) by bromination has again been shown to be a more sensitive method than oxidation.

This is the first time that phytosterol has been found combined in a starch.

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(15) Hertwig, Jamieson, Baughman and Bailey, *J. Assoc. Off. Agr. Chem.*, **8**, 439–42 (1925).