assume the presence of valeric and butyric acids in amounts as low as 0.74% and Suzuki, Hastings and Hart have drawn conclusions based on results which indicate the presence of several acids in proportions of less than 5%.

Finally, one of the sources of error in the method arises from the fact that substances do not behave the same when distilled from a mixture as when distilled from pure solutions. The method is therefore not based on sound theoretical principles.

Summary.

1. Our experiments show that unavoidable variations in experimental results, while small, may be sufficient to vitiate the results.

2. When more than two acids are present in a mixture practically identical series may be calculated from mixtures of different acids in varying proportions.

3. Results which indicate the presence of one acid may just as well be calculated in terms of three or more acids.

4. Small amounts of acids may be distributed just as well between the acids next higher and lower in the series.

5. The theory of the method is not sound.

6. The method, therefore, does not deserve either quantitatively or qualitatively for determining the composition of unknown mixtures of fatty acids.

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THE DETERMINATION OF GELATINIZATION TEMPERATURES OF STARCHES BY MEANS OF AN ELECTRICALLY HEATED CHAMBER ON THE MICROSCOPE STAGE.

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It has long been known that starches prepared from different species of plants show differences not only in microscopic appearance but also in gelatinization temperature. There is some evidence also that starches prepared from different varieties or strains of the same species vary in this respect. The differences in many cases are so slight, that in order to serve as a means of identification the determinations must be made by a method capable of giving duplicates that check within a fraction of a degree.

The method commonly used for determining the gelatinization temperature of starch consists in heating small samples with water in test tubes immersed in a water bath. The temperature is gradually raised and a sample removed for microscopic examination with every rise in

temperature of one degree. Numerous modifications of this method have been used, but the principle is essentially the same in each case.

Reichert, in his monumental work,[1] used a long quadrangle water bath heated at one end. At the other end was inserted an accurate thermometer, also a test tube containing a small amount of the starch to be examined, about 10 cc. of water, and a second thermometer. Samples were withdrawn from the test tube by means of a clean pipet at each degree rise in temperature and transferred to a microscope slide. The point of disappearance of anisotropy of practically all the grains was taken as the gelatinization temperature.

La Wall and Graves[2] used a triple water bath composed of three beakers and applied the heat gradually with constant stirring of the starch. Samples were examined at each degree rise in temperature. The point of disappearance of double refraction in the majority of the grains was taken as the gelatinization temperature.

Such methods are necessarily slow and tedious and require several series in order to get results closer than one degree. A much more convenient method was recently described by Francis and Smith.[3] This consists in gelatinizing the starch directly on a slide under the microscope, the heat being supplied by the circulation of hot water through a small device containing the slide.

The method used by the writers is based upon the same principle, *viz.*, gelatinization directly under the microscope, but the chamber containing the slide is electrically heated. The advantages of the electric current over a hot water system in point of convenience need no discussion. The necessary apparatus is already on the market and requires but a simple change in the wiring in order to convert the incubator into an oven. It consists of the "No. 8, Electric Incubator for the Microscope Stage," specially adjusted for temperatures up to 80°. A piece of asbestos inserted between the stage and the chamber will prevent considerable loss of heat.

After trying several different types of slides it was found that a thin hanging drop slide gave the best results. This was trimmed down so that it would easily fit inside the incubator. In order to keep the temperature the same on both sides of the slide, the latter was supported upon two small rods that rested upon the bottom of the chamber.

The procedure which we found most satisfactory was as follows: The temperature of the chamber is brought to 60°. A small amount of starch is placed on the cover glass with the point of a knife, moistened with a small amount of water, and the cover glass placed over the depression in the hanging drop slide. The mica cover is placed on the chamber,

[1] "Differentiation and Specificity of Starches," I, 298, *Pub. Carnegie Inst.*, 1913.

[2] *Trans. Wagner Free Inst. Sci. Phila.*, 7, pt. 2.

[3] *J. Ind. Eng. Chem.*, 8, 509 (1916).

care being taken that the hole for the objective comes directly over the starch. The microscope is now adjusted and focused on the starch. The temperature is increased slowly until the disappearance of anisotropy in all the grains that are large enough to show the characteristic shape and markings. This is not taken as the correct temperature but merely as a preliminary orientation. The operation is now repeated, starting 5 degrees below the previously noted gelatinization temperature, and increasing the temperature at the rate of 1 degree a minute. The gelatinization temperature will be found to be slightly lower than the preliminary observation. Duplicates will check within a few tenths of a degree. Reichert took as the gelatinization temperature, that point at which anisotropy disappeared in the majority of the grains. Francis and Smith took the point at which it disappeared in all the grains. We take the temperature at which anisotropy disappears in all the grains that are large enough to show the characteristic shape and markings. According to Whymper,[1] the larger grains of any given starch gelatinize at a lower temperature than the smaller ones.

It is essential that the proper amount of water be used in preparing the slides. Too small an amount of water will tend to evaporate to dryness. If too much water is used, the starch granules will have a tendency to collect in a clump. Our experience is in accordance with the statement of Francis and Smith that prolonging the operation tends to give high results.

The use of a short thermometer is recommended. This obviates the necessity of introducing a correction for cooling of the mercury column.

A variation in temperature in different parts of the chamber was to be expected, and since the thermometer is nearer the source of heat than is the starch sample, a correction must be made for this difference in temperature. This correction was determined by observing the melting points of three organic substances, whose melting points cover the range of gelatinization temperatures. The substances were melted under precisely the same conditions that prevailed during the gelatinization determinations, except that the water was omitted. In determining the actual melting point, the same thermometer was used in the melting-point apparatus.

Substance.	Apparent m. p.	Average apparent m. p.	Actual m. p.	Correction.
Palmitic acid.....	65.3–65.5–65.2–65.3–65.2	65.3	61.5	3.8
Azobenzene.....	72.0–71.8–71.6–71.7–71.7	71.8	68.1	3.7
Phthalide.....	76.4–76.7–76.3–76.2–76.4	76.4	72.6	3.8

The average apparent gelatinization temperature must, therefore, be reduced 3.8° to give the actual temperature.

[1] *J. Soc. Chem. Ind.*, **28**, 806 (1909).

The above method was tried out with samples of starch prepared from a number of different varieties of corn. Being uncertain of the effect treatment with dilute sodium hydroxide might have upon the gelatinization temperature, we avoided the use of this reagent. The grains were allowed to stand a few hours in alcohol until soft, the germs were picked out by means of a sharp knife, and the remainder of the kernel ground in a mortar and washed through a 100-mesh brass sieve. The starch was allowed to settle, the supernatant liquid being poured off. It was again shaken up with water and allowed to settle, the supernatant liquid again being poured off, the starch then shaken up with 75% alcohol and allowed to settle out. The starch was filtered on a hardened filter in a Hirsch funnel, washed with 75% alcohol, 95% alcohol, and finally with ether. The product was free from color, and completely stained by iodine. As viewed through the microscope it appeared to be very pure.

Below are given the gelatinization temperatures of starches from thirteen different varieties of corn.

Variety.	Apparent gelatinization temperature.					Average.	Corrected.
Stovall's Evergreen (sweet corn)	68.0	67.9	68.0	67.7	68.1	67.9	64.1
Golden Bantam (sweet corn)	68.5	68.4	68.1	68.3	68.1	68.3	64.5
Legal Tender	70.5	70.6	70.4	70.4	70.3	70.4	66.6
Boone Co. White	70.5	70.8	70.8	70.9	70.6	70.7	66.9
Flint	71.6	71.4	71.2	71.0	71.5	71.3	67.5
Bloody Butcher	71.8	71.5	71.6	71.4	71.5	71.6	67.8
Silver Mine	71.5	71.6	71.4	71.8	71.5	71.6	67.8
Iowa Ideal	72.2	72.5	72.2	72.3	72.0	72.2	68.4
Reid's Yellow dent	73.2	73.1	72.9	73.0	72.9	73.0	69.2
Minn. No. 13	73.0	73.2	72.8	73.1	73.0	73.0	69.2
Golden Eagle	74.4	74.1	73.9	74.0	73.9	74.0	70.2
Silver King	74.0	73.9	74.4	74.1	73.9	74.1	70.3
Wolf's Yellow dent	74.7	75.0	75.1	74.9	74.7	74.9	71.1

Previous investigators have reported widely varying results for corn starch. Lintner[1] gives 75°, Lippmann[2] 62.5°, Reichert[3] reports a range of 62.25 to 67.5° for several varieties, La Wall and Graves[3] report 70–71°, and Francis and Smith 70.9°. These wide variations may be attributed partly, at least, to the fact that different varieties of corn were probably used, and partly no doubt to differences in method. We had no difficulty in obtaining very concordant results.

[1] *Wochenschr. Brau.*, **6**, 285 (1888).
[2] *J. prakt. Chem.*, **83**, 51 (1861).
[3] *Loc. cit.*