BANANA GEL.

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(Received for publication, January 12, 1922.)

INTRODUCTION.

The change of a soluble sucrase preparation from bananas into an insoluble preparation was described in a previous paper.[1] This change was found to occur on dialysis against running tap water. The insoluble form showed many of the characteristics of a gel. In this paper, a more complete study of the factors influencing the formation of this gel will be presented together with some of its properties but without considering its possible connection with enzyme activity.

EXPERIMENTAL.

Methods.

The banana extract was prepared by passing the pulp of ripe bananas through a fine food-chopper, mashing it in a mortar with the requisite amount of water or sodium chloride solution, and filtering through paper in large funnels. Toluene was added before the filtration. The extract had a brown tinge. It had a pH value in the neighborhood of 5.0. Bananas in the same stage of ripeness were used as far as possible. Individual minor variations in the gelling properties of extracts were observed at times, just as the constituents of bananas may differ to small extents.

Collodion bags were used for dialysis. These were prepared in the usual way but were not standardized. However, the conditions for their preparation were kept uniform so that possible errors from this source need not be considered in the present connection. The volume of liquid in any one bag was always less than one-fourth the capacity of the bag, to allow for the increase in volume due to the dialysis.

[1] Falk, K. G., and McGuire, G., *J. Gen. Physiol.*, 1920–21, iii, 595.

Hydrogen ion concentrations were determined by use of indicators and suitable standard solutions.

The various salts which were used were either purified, or the impurities determined, since in the work to be described, minute amounts of certain substances were found to produce striking phenomena.

Results.

I. Dialysis of Banana Extract against Tap Water and against Distilled Water.

The results obtained in a large number of experiments in the dialysis of banana extracts may be summarized briefly in the following paragraphs. Essentially the same results were obtained whether the extracts were prepared with water or with molar sodium chloride solution. As a rule, in the preparations 1 part by weight of liquid was used with 4 parts of banana pulp.

1. Dialysis of extract against tap water.

(*a*) Gel formation began in 24 to 48 hours. With small volumes more rapid changes including formation of gel occurred than with large volumes.

(*b*) Volume increased about 100 per cent. Most of the increase occurred before gel formation began. Changing bags during the dialysis had no effect.

(*c*) After two or three days dialysis the liquid still had a pH of 5.0 to 5.5.

(*d*) Gel formation began on the inner walls of the bags.

(*e*) Continued dialysis caused the dark colored gel to flake or separate out, leaving the liquid clear and colorless. As the flakes were formed, the pH of the supernatant liquid was 5.0 to 5.5 at first, but after 4 to 6 days became that of the tap water (pH 7.0).

2. Dialysis of extract against distilled water.

(*a*) No gel formation at any time.

(*b*) Continued increase in volume. In one experiment an increase from 25 cc. to 178 cc. in 4 days was observed.

(*c*) After dialysis pH of liquid unchanged (about 5.0).

(*d*) Liquid became cloudy and light colored (perhaps because of dilution).

3. Dialysis of gel from 1 (e) against distilled water.

(*a*) Gel disappeared on continued dialysis (up to 6 days) against renewed distilled water.

(*b*) Increase in volume as in 2 (*b*) in every case.

(*c*) Liquid became cloudy and lighter colored as in 2 (*d*) if the gel disappeared.

4. Dialysis of liquid from 2 (d) against tap water.

(*a*) Cloudiness disappeared.

(*b*) No further increase in volume.

(*c*) Some flakes of gel separated.

Toluene was present in all these experiments. However, long continued shaking of the extracts with toluene produced no gel. Bubbling air through the extracts also did not result in gel formation.

Most of the experiments were carried out by dialyzing against running tap water or distilled water. Some experiments dialyzing against tap water or distilled water in large beakers gave the same results.

The difference in the behavior of the banana extract relative to the gel formation upon dialysis against tap water and distilled water, was evidently due to differences in the compositions of the two. The pH of the tap water was found repeatedly to be 7.0 to 7.2: that of the distilled water about 5.0. An average analysis of the inorganic constituents, as parts per million of the tap water was as follows:[2]

$CaCO_3$	24	NaCl	5
$MgCO_3$	5	KNO_3	1
$MgSO_4$	10	SiO_2	9

The distilled water was practically salt-free.

II. Dialysis of Banana Extract against Solutions of Definite Hydrogen Ion Concentrations and Salt Contents.

Banana extract was dialyzed for 48 hours at 10–15°C. in collodion bags against the following solutions contained in large beakers with the indicated results. Sodium hydroxide or hydrochloric acid were

[2] The analysis was obtained through the courtesy of Mr. Merritt H. Smith, Chief Engineer of the Department of Water Supply, Gas and Electricity, New York City.

used when necessary to produce the required pH values. That sodium chloride did not play a positive part in the gel formations was evident from the fact that the banana extracts in many experiments were prepared with sodium chloride solution.

When gel formation occurred, the volume increases were 100 per cent or less, when no gel formation occurred the volume increases ranged from 100 to 400 per cent in 48 hours. In a number of experiments, the pH of the outside liquid changed from 7.0 to 6.0 or even less. Whenever this occurred, no gel was formed inside the bags.

These results showed that for the formation of a gel from banana extract by dialysis against tap water, the presence of a calcium salt

Solutions.	pH of outside liquid.	Results.
$CaCO_3$ 0.00020 to 0.00024 M[3]	7.0	Gel.
$CaCO_3$ 0.00020 to 0.00024 M; phosphate buffer[4], 35 cc. : 2 liters	7.2	"
$CaCO_3$ Saturated, excess solid	7.0	"
$CaCO_3$ 0.00024 M	5.0	No gel.
Phosphate buffer,[4] 35 cc. : 2 liters	7.2	" "
$MgSO_4$ 0.00024 M	7.0	" "
$MgSO_4$ 0.00024 M	5.0	" "
$Ca(OH)_2$ Saturated, excess solid CaO	More alkaline than 10.0	Gel (white).

and an alkalinity corresponding to pH 7.0 or more were the important factors.

In order to determine whether the collodion bag as such played a part in the gel formation or whether possibly interfering substances were removed by dialysis, the following experiments, in which a number of different salts were added directly to banana extract at different hydrogen ion concentrations, were carried out.

III. Actions of Salts on Banana Extract at Definite Hydrogen Ion Concentrations.

No satisfactory quantitative methods for the comparison of gels, the readiness of their formation, and their chemical compositions,

[3] 20 to 24 parts per million.

[4] Clark, W. M., The determination of hydrogen ions, Baltimore, 1920, 76.

are at hand. In the following experiments, in comparing different gels, their consistency is taken to be the greater or smaller resistance to deformation, the ability to retain the shape of the vessel in which they were prepared, even when separated from it, etc. In any one series a satisfactory comparison can be made, but in different series carried out at different times, anything more than a rough qualitative study is difficult.

Since calcium salts were found to be involved in the gel formation in the dialysis experiments, a number of series of experiments were carried out in which solutions of calcium salts were added directly to banana extracts under different conditions without dialyzing.

The acidity of the mixture was the first determining factor. A banana-water extract (4 parts of banana pulp with 1 part of water and filtered in the usual way) gave no indication of gel formation at the pH of the juice (about 5.0) with 0.29 mg. of calcium, added as calcium oxide, or 0.22 mg. of calcium, added as calcium chloride, per cc., in 48 hours at 5°–10°C. The same extract at pH 7.5 with 0.04 mg. of calcium as calcium oxide added per cc. formed a firm gel in 5 minutes which retained the shape of the container. These results were confirmed repeatedly. It appeared to be impossible to obtain gel formation with calcium salts with solutions more acid than about pH 6.0. With solutions at pH 7.0 or more alkaline, gels were obtained readily. The greater the concentration of calcium the more rapid the formation of a firm gel, and *vice versa*. For example, with a calcium concentration of 0.03 mg. per cc. and the juice mentioned above, gel formation had only begun after 20 minutes, while a firm gel was present in 7 hours. With 0.02 mg. of calcium per cc. at 5° in 18 hours, definite gel formation had occurred, while with 0.01 mg., the gel formation was doubtful. Low temperatures favored the formation of the gel. While extracts obtained at different times differed slightly, the general conclusion that with calcium salts no gel formation at pH 6.0 and more acid, and gel formation at pH 7.5 and more alkaline, was found to hold in every case.

It is of interest to note that if the banana extract is boiled for a few minutes, some solid shreds forming, a gel could not be obtained either by dialysis against tap water or at pH 7.5 by the addition of calcium salts.

These results are of importance in connection with the preparation of banana extract and subsequent treatment of the latter. As described previously, the banana extract was prepared with sodium chloride solution for a number of experiments. It was found necessary to purify the sodium chloride, since a number of samples of the latter were found to contain small quantities of calcium salts. If the calcium was not removed, on bringing the extract to pH 7.0 or more alkaline condition for various purposes, gel formation would take place very rapidly. Similarly, if sodium hydroxide which contained calcium as impurity, was used for neutralization, gel formation might occur. The results described above were therefore obtained by neutralization with ammonium hydroxide. An added complication was found in the fact that the banana itself contains very small amounts of calcium salts[5] which may appear in the extracts. Long standing (48 hours or more) in solutions of water extract brought to pH 7.0 with ammonium hydroxide resulted in the formation of some gel presumably from this cause.

Sodium chloride extracts showed smaller gelling actions with calcium salts than did water extracts. Thus, with two banana extracts, one prepared with water, the other with sodium chloride solution, treated with 0.02 mg. of calcium in the form of calcium chloride per cc. under the same conditions, formed a firm gel in the first case in 10 minutes, and very little gel in the second.

A comparative study of the gelling actions of certain salts was made. Two series were run, one at pH 5.0, and the other at pH 7.5. To 30 cc. portions of banana-water extract were added 0.5 cc., 1.0 cc., and 1.5 cc. of 0.044 M solutions of calcium chloride, strontium chloride, barium chloride, magnesium chloride, and lithium chloride. None of the solutions at pH 5.0 gelled even after 48 hours. Of those at pH 7.5, the calcium chloride showed the most marked gelling action, the strontium chloride showed somewhat less, and the barium chloride still less. The magnesium chloride and lithium chloride gave no indication of gel formation until after 7 hours, after 48 hours there were soft gels formed but even less than that formed in the control, water extract plus ammonium hydroxide to pH 7.5. The relations

[5] Colby, G. E., *California Agric. Exp. Sta. Report*, 1892–94, 275.

in the gel formations with time and concentration of salt with the first three salts were similar to those already given.

IV. The Action of Pancreatine on Banana Extract.

As a result of some experiments carried out for a different purpose, the formation of a gel by the action of pancreatine on banana extract was observed. For example, one 40 cc. portion of banana sodium chloride extract was treated at pH 5.0 with 0.2 gm. of a commercial pancreatine preparation, and another with 0.1 gm. In 24 hours at 38°, the former formed considerable gel, the latter formed less gel. At 5°, the same mixtures produced no gels. If either the pancreatine or the extract was boiled before mixing the solutions, no gel was obtained. The gel which was obtained by the action of the pancreatine appeared to be less firm than that obtained by the action of calcium salts.

A number of the gels prepared by dialysis were filtered on paper, washed with alcohol and ether and dried and analyzed. The analyses, however, mean nothing. The gels as obtained carried down mechanically or otherwise much of the dissolved material present. Attempts to wash the gels with water were not successful, swelling occurring in almost every case. Until methods of purifying the gels, or at least of removing extraneous material are developed, nothing definite can be stated relative to their chemical composition. It may be mentioned, however, that the nitrogen content of the dried gel was in the neighborhood of 4.5 per cent, pointing to the presence of about 25 per cent protein material.

DISCUSSION.

The question of the relation of this work to other work of similar nature can be disposed of briefly. The source of the material and some of the reactions may indicate that the gels are of the nature of pectins.[6] However, against this view are the facts that boiling destroys the gel-forming property and that only very small quantities of calcium and strontium salts are required to yield the gels under suitable conditions.

[6] *Cf.* Haynes, D., *Biochem. J.*, 1914, viii, 553.

The formation of the gels by the direct addition of certain salts under suitable conditions as well as by dialysis against solutions of such salts is significant. It raises the question whether it would not be possible in many cases to bring about reactions such as precipitation, solution, etc., by direct chemical actions involving the addition of reagents under definite conditions, in place of dialysis against solutions of more or less accidental salt contents. The fact that such solutions as the tap water which was used in the present investigation may contain dissolved substances in minute concentrations only serves to obscure the possible actions. These small concentrations of dissolved substances which may react, but which are constantly renewed as the dialyzing liquid changes, are capable of producing effects which larger initial concentrations would produce in shorter times. Thus, the banana gel-forming substance inside the bags was able to react with the calcium salts from the outside liquid and over the extended period of time which was used here and is also used in most dialysis studies, the reaction occurred, first along the inner walls of the bags and finally throughout the liquid. The different hydrogen ion concentrations inside and outside the bags also served to confuse the exact conditions at which the reactions could take place.

These relations make it advisable in every case in which dialysis is used to study the composition of the dialyzing liquid, as small amounts of dissolved substances and also apparently unimportant differences in hydrogen ion concentrations may result in the occurrence of definite chemical changes. Also, the material of which the dialyzing bags are made may exert profound effects upon the changes.[7] The plea may be made, therefore, to replace dialysis wherever possible by direct additions of chemical reagents at the same time controlling the hydrogen ion concentrations of the mixtures, and in this way substituting more or less accidental and to some extent unknown methods of treatment by known and definite additions of chemical reagents.

The results obtained therefore point to chemical reactions and combinations involving specific elements under definite and limited conditions. Although it was not found possible to obtain these compounds in pure condition as chemical individuals, the results can best be interpreted on the basis of the formation of such chemical compounds.

[7] Brown, W., *Biochem. J.*, 1915, ix, 591; 1917, xi, 40. Eggerth, A. H., *J. Biol. Chem.*, 1921, xlviii, 203.

SUMMARY.

The conditions for the formation of gels from banana extracts were studied.

Gels were obtained with extracts more alkaline than pH 7.0 with very small quantities of calcium, strontium, and barium salts, the gel formation with these salts decreasing in the indicated order.

In solutions more acid than pH 6.0, no gels were obtained with these salts.

Magnesium, lithium, and sodium salts did not cause gel formation either in acid or alkaline solutions.

Pancreatine gave a gel on incubation with banana extract at pH 5.0.

The gel-forming property of banana extracts was destroyed on boiling.

We wish to thank the Fruit Dispatch Company for supplying the greater part of the bananas used in this investigation.