THE EFFECT OF FREEZING ON THE PHYSICAL AND MISCROSCOPIC CHARACTER OF GELS OF CORN AND WHEAT STARCHES[1]

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INTRODUCTION

The number of changes both in the gross and in the microscopic appearance of starch gels brought about by freezing is so great that it suggests many lines of study in connection with the structure of starch granules. It is believed that the facts established in this investigation may be applicable to the problem of low-temperature preservation of food and to that of water removal from food materials containing gelatinized starch. The differences found between corn and wheat starches may be of value in determining the kind of starch for practical use.

METHODS

SOURCE AND PREPARATION OF STARCHES

Starches of both corn (*Zea mays*) and wheat (*Triticum vulgare*) were prepared by methods designed to leave them as nearly in their native state as possible, while at the same time freeing them from other constituents of the kernel. Three varieties of corn, Reid Yellow Dent, Krug Yellow, and Champion White Pearl, were used as sources of starch. So little difference between them was found that photomicrographs for the Reid corn only are shown.

The corn was soaked overnight in cold water and ground several times to medium-fine particles. Its starch was washed out and separated from the other material of the kernel, first on a 60-mesh screen, then on fine bolting cloth. The starch suspension was centrifuged and washed in turn with 2-percent sodium chloride, water, alcohol, and ether. Layers of gluten at the top of the starch layer in the centrifuge bottles were scraped off. This starch was air-dried for use.

Wheat starch was prepared by the method previously reported by Woodruff and Webber (*9*)[2] from 1934 Fulhio wheat flour milled especially by a commercial soft-wheat mill for another experiment station project. Slight physical differences between starches separated from bleached and unbleached flours were found, but microscopic differences were negligible and photomicrographs only of unbleached flour starches are shown.

FREEZING OF STARCH GELS

Starch gels were made by heating suspensions containing 5 percent of starch by weight in a boiling water bath. The starch was first moistened with a little cold water in a conical flask. The boiling water was added and the flask swirled until a thermometer in the

[1] Received for publication Aug. 5, 1935; issued March, 1936. A brief paper covering part of these findings was read before the food and agricultural division of the American Chemical Society in New York City in April 1935.

[2] Reference is made by number (italic) to Literature Cited, p. 237.

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paste reached the temperatures 70°, 75°, 80°, 85°, 90°, and 95° C. The pastes were poured immediately into molds and allowed to cool and set to a gel overnight. One set of molds was placed in the freezing unit of an electric refrigerator where the lowest temperature reached by them was −2° to −3°. After 5 or more hours they were removed from the freezer, allowed to thaw at room temperature, and examined microscopically. Another set of molds was packed in solid carbon dioxide and left for 1 hour, after which they were removed and allowed to thaw at room temperature. Samples were also frozen in liquid air but these were not different from the ones frozen in solid carbon dioxide.

MICROSCOPIC EXAMINATION

Specimens were removed from the gels with a knife tip, special care being taken to avoid cutting the shreds in the frozen ones. The specimens were then mounted in water for microscopic examination. Photomicrographs were made with ordinary light at a magnification of 900 times. Polarized light was also used for the examination of each specimen between crossed nicols.

RESULTS

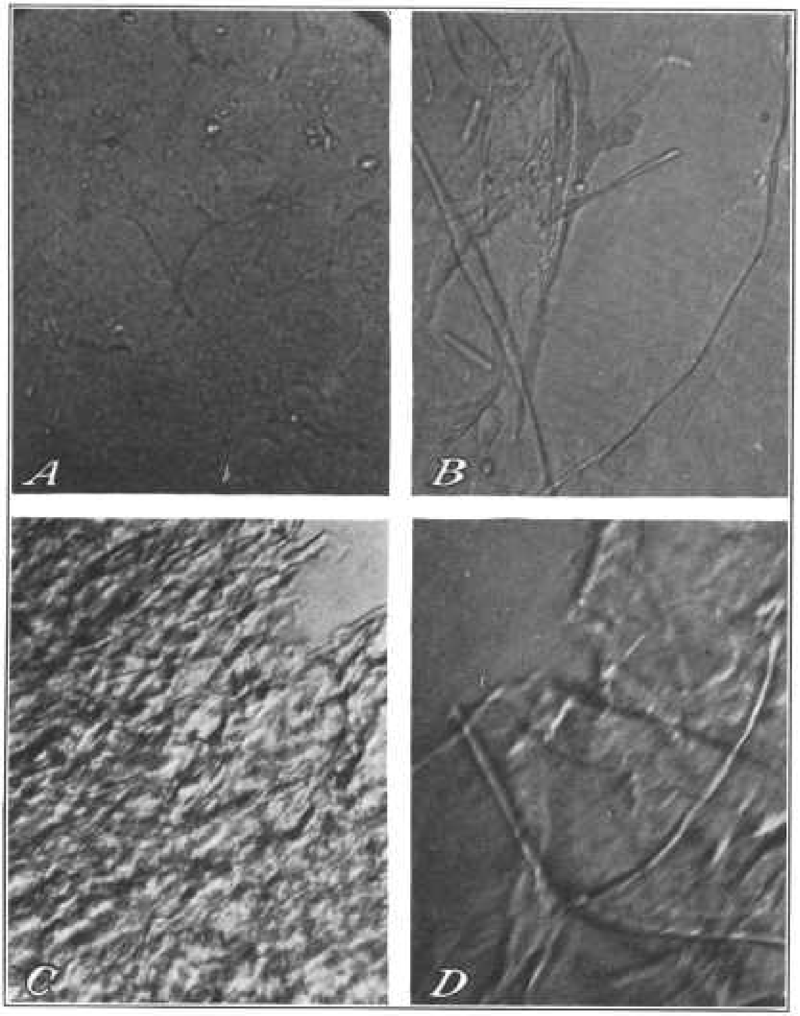
PHYSICAL CHANGES IN GELS CAUSED BY FREEZING

The following discussion is for the most part confined to the behavior of gels made by heating starch suspensions to 95° C. The cornstarch made a well-formed gel even at 75° or 80°, but wheat starch did not until it was heated to a temperature of 95°, for at the lower temperatures some starch granules remained unswollen.

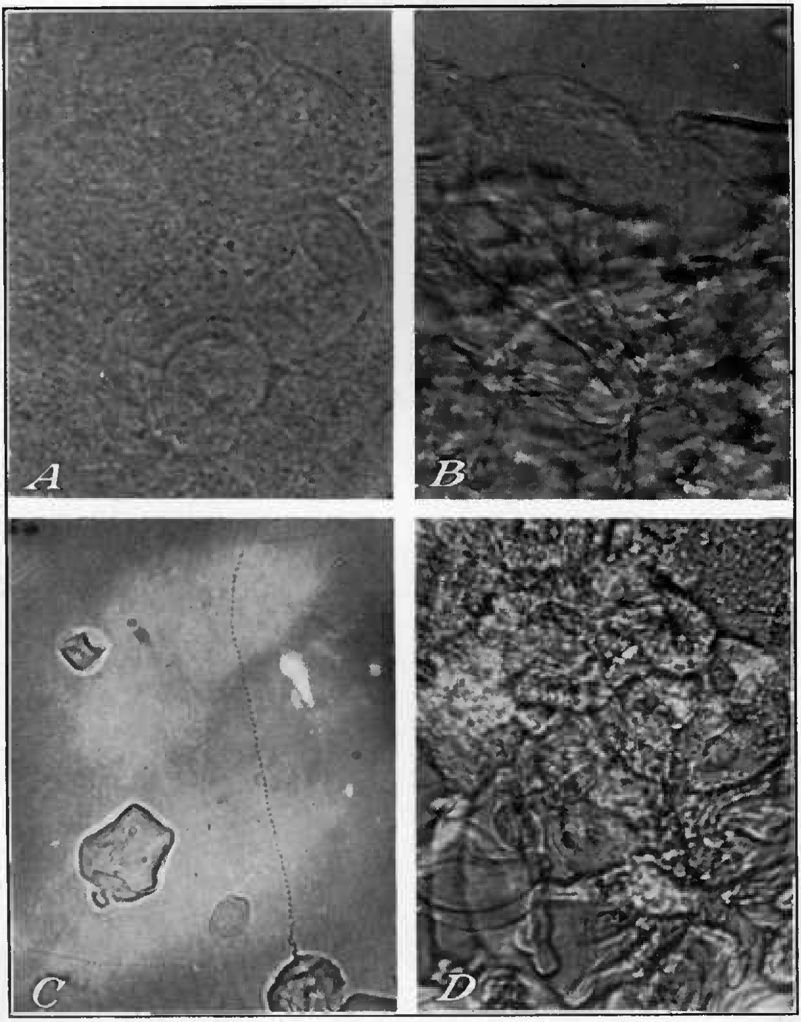
The cornstarch gel after being frozen slowly where it reached a temperature no lower than −2° to −3° C. became coarsely fibrous with some radial orientation of the fibers. It was spongelike in that water could be pressed from it without its losing shape and it greedily absorbed water again. It likewise absorbed water solutions of either iodine or of dyes. The original gel, on the other hand, was not penetrated by an iodine solution dropped on its surface; moreover, it was bluish white, pasty, uniform in consistency so far as the unaided eye could detect, and did not give up water when pressed. It dried in air to a horny film which could be remoistened only with difficulty. The sponge of the frozen gel, pressed free from water, dried in air, giving a porous, fibrous, friable substance which absorbed water as easily when dry as it had when moist. The starch thus retrograded by freezing could be warmed with water and dispersed sufficiently to make a gel again similar in appearance to the original one, though less uniform in consistency. The freezing and dispersing were repeated many times without noticeable loss of power to form a gel. Even in gelatinized suspensions of 1-percent concentration instead of the 5 percent used, the fibrous arrangement could be seen.

Frozen in solid carbon dioxide, the gel changed in gross appearance much less; it retained most of its pasty appearance and though it gave way when pressed, no water came from it. It dried to a horny film very like the original gel.

Wheat-starch gels frozen in the refrigerator were fibrous like those of cornstarch, but the sponge formed was not so tough and water could not be squeezed out so readily. They differed decidedly from



Photomicrographs of frozen cornstarch gels. *A*, Gel before freezing; *B*, 1-percent suspension, frozen at −2° to −3° C.; *C*, gel frozen in solid carbon dioxide; *D*, gel frozen at −2° to −3°. All were gelatinized at 95°; all except *B* contained 5 percent starch. × 900.



Photomicrographs of frozen starch gels and of optically active particles in starch. *A*, Wheat-starch gel before freezing; *B*, wheat-starch gel frozen at −2° to −3° C.; *C*, cornstarch gelatinized at 75°; *D*, wheat starch gelatinized at 85° and then frozen in solid carbon dioxide. Note optically active particles in *C* and *D*. × 900.

the frozen cornstarch gels in that they dried to a horny mass which did not readily reabsorb water. This point was noted by Holton,[3] who worked on a similar problem in this laboratory. Gels frozen in solid carbon dioxide were again more like the original ones and lacked the fibrous structure produced at −2° to −3° C.

MICROSCOPIC APPEARANCE OF FROZEN GELS

Freezing effected changes as pronounced in the microscopic appearance of gels as in the gross. Evidence of these changes is presented in plates 1 and 2.

It was not easy to show the structure of a starch gel microscopically. The swollen granules were very transparent after being heated to 95° C., were packed against each other, and their details were indistinct (pl. 1, *A*). The remarkable change in appearance brought about in cornstarch gels by freezing is shown in plate 1, *C* and *D*, which presents photomicrographs of a gel frozen in solid carbon dioxide and of one placed in the freezing unit of a refrigerator where it reached a temperature of only −2° to −3°. The very heavy veins in the latter seemed to correspond to the fibrous structure noticed on gross examination. Freezing more quickly at the lower temperature produced less change in microscopic as well as in gross appearance, though veining of a light nature was apparent. Plate 1, *B*, shows that even in a 1-percent suspension, gelatinized starch was profoundly altered by freezing; long fibers are apparent there.

Wheat starch became even more indistinct in outline than cornstarch when gelatinized at a temperature of 95° C. Plate 2, *A* and *B*, shows the wheat starch as it appeared in the original gel and after being frozen at −2° to −3°. Definite lines of reorientation are visible in the latter though the heavy veins of frozen cornstarch gel are absent.

Starch grains lost their anisotropy as soon as they swelled slightly, Very small, oval-round particles which transmitted polarized light could be seen, however, after birefringence had disappeared. These particles were somewhat visible at all temperatures of gelatinization though they were more plentiful and more distinct at temperatures under 95° C. In many cases where particles occurred singly they showed a Brownian movement. A few such particles can be seen in the cornstarch gel previously heated to 95°, shown in plate 1, *A*, and a suggestion of their presence is to be noted also in the wheat-starch gel gelatinized also at 95°, of plate 2, *A*, though in the latter case the camera was focused on the outer boundaries of starch granules, hence these small particles were not in focus. Plate 2, *C* and *D*, shows these oval-round particles in corn and wheat starches which had been heated to temperatures of only 75° and 85° respectively. When slight pressure was exerted on the cover glass during the examination of starch gelatinized at temperatures of 70° or somewhat above, such streams of these particles as are shown in plate 2, *C*, were often observed. Short chains of them under or on the surface of the swollen starch granules were noted more frequently than the long streamers just mentioned. The wheat starch of plate 2, *D*, shows a surface on which such particles are visible in great numbers.

[3] HOLTON, M. THE EFFECT OF RETROGRADATION ON THE SUBSEQUENT GELATION OF WHEAT STARCH. Unpublished master's thesis. Univ. Ill. 1934.

The veinlike structure appearing in the photomicrographs of frozen gels, particularly of those frozen at −2° to −3° C., also transmitted polarized light. Likewise did dried shreds of frozen starch gels mounted without water (not shown in the plates).

DISCUSSION

Only a few investigators seem to have mentioned the appearance of frozen starch gels and no photomicrographs of them have come to the writers' attention. Samec (*8*) dialyzed gelatinized and frozen potato starch for soluble starch on which he made determinations of phosphorus. Baldwin (*1*) used freezing as a means of separating alpha and beta amyloses of potato starch. Reilly, O'Donovan, and Murphy (*7*) centrifuged the clear liquid from the fibers formed by freezing a 5-percent suspension of gelatinized potato starch and recovered an amylose on which to make molecular weight determinations.

The literature contains several references to what might correspond to the oval-round particles pointed out in the accompanying plates. Several investigators, Samec (*8*), Hanson and Katz (*3*), and Hess and Rabinowitsch (*4*), in recent years have discussed crystallin or micellar structure in line with the very early conceptions of starch constitution. Hanson and Katz reproduced with drawings the arrangement of blocklike units in potato, arrowroot, and wheat starches, made suitable for observation by swelling with calcium nitrate. Hess and Rabinowitsch photographed by cinematograph the material issuing from a punctured potato-starch granule. The irregular mass consisted of particles having a Brownian movement. Linsbauer (*6*) has followed the gelatinization changes in potato starch with a hot-stage microscope; his drawings, however, do not include such particles as are shown in this paper. There is at least a suggestion in the resemblance in appearance of the particles of the accompanying photomicrographs to the cellulose particles found by Farr and Eckerson (*2*) in cotton fibers, that the particles seen here in starch have something fundamental to do with the structure of the starch granule.

The observation that gels showed a greater change in both gross and microscopic appearance at −2° to −3° C. than they did at much lower temperatures, was in accord with a result of Katz' (*5*) work on bread staling. The staling process which is concerned with changes in starch was found by him to pass through a maximum value at −2° to −3°, when bread was stored at temperatures varying over a wide range.

A study of the photomicrographs made in the course of this investigation lead the writers to believe that the reticulation of the starch gels, frozen at −2° to −3° C. and later thawed, may possibly have resulted from the association of micelles or aggregates which in turn had formed when the dehydration of the swollen starch granules by ice-crystal formation permitted the molecules to be drawn closer together through secondary valence forces. The explanation offered for lesser change at the still lower temperatures of solid carbon dioxide and liquid air is that less injury occurred with rapid formation of smaller ice crystals, and the temperature was, moreover, too low easily to permit of the physical changes of reorientation. Indications of this lie in the fact that if a gel, previously frozen in solid carbon dioxide, was kept for many hours in the freezing unit of the electric refrigerator while its temperature rose it would assume practically the same microscopic characteristics as the gel that had been all the time in the unit at —2° to —3°.

SUMMARY

Corn and wheat starches were gelatinized at temperatures ranging from 70° to 95° C. and gels of 5 percent concentration were then frozen at —2° to —3° and also at the temperature of solid carbon dioxide. After thawing they were examined for physical and microscopic changes. In gross appearance the frozen gels were very different from the original ones. At —2°, where the effect of freezing was greatest, the gel became like a fibrous sponge from which most of the water could be pressed. Other changes in physical properties also were noted.

Photomicrographs are shown of corn and wheat starches which had been first gelatinized, then frozen. Veined areas appeared in gels after slow freezing at —2° to —3° C. Rapid freezing in solid carbon dioxide produced fewer changes both in the gross and in the microscopic appearance of gels than did slow freezing at —2° to —3°. The veinings in the frozen gels were optically anisotropic as were also small oval-round particles seen constantly at different stages of swelling.

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