

Starch Gelatinization at Different Temperatures as Measured by Enzymic Digestion Method[†]

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The gelatinization process of potato starch was isothermally investigated at 52.5~65.3°C. The degree of gelatinization was measured by an enzymic digestion method using glucoamylase. When the starch-water suspension was incubated at a definite temperature the gelatinization reached a limit at each temperature after 30~60 min incubation. So, it can be supposed that starch gelatinization reached an equilibrium state. It was found that gelatinization of potato starch occurred even at 52.5°C, a temperature which is lower than the so-called gelatinization temperature generally reported. Starch gelatinization was found to follow first order kinetics, and from the temperature dependence of the rate constants obtained, the activation energy was calculated to be 22 ± 5 kcal/mol. The relationship between the degree of gelatinization of the starch whose gelatinization reached an equilibrium state at a definite temperature and the incubation temperature gave a transition curve expressed by the fraction of gelatinized potato starch granules as a function of temperature, and the half-transition temperature was found to be 59.1°C. From the transition curve the van't Hoff enthalpy for gelatinization was determined to be $+130 \pm 3$ kcal/mol.

Gelatinization of many sorts of starch has been investigated by various temperature-scanning methods such as amylography,¹⁾ photopastography^{2,3)} and differential thermal analysis or differential scanning calorimetry.^{4~6)} With these methods, the changes in the physicochemical nature of the starch-water suspension were measured during the temperature scan. It has been established that, unlike melting of crystals, gelatinization of starch does not take place at a definite temperature in a short time, but occurs relatively slowly over a broad temperature range. This indicates that for such a study with the temperature-scanning method it is essential to choose as slow a heating rate as possible so that gelatinization is not kinetically limited at any time during the scan. In this respect the heating rates of 1~10°C/min which have been generally used seem to be fairly fast compared with the rate of gelatinization of starch granules in water. One may expect that if a starch-water suspen-

sion is incubated at a definite temperature for long enough, gelatinization may occur at a lower temperature than that previously reported. In this study, potato starch was isothermally incubated in water at various temperatures until an equilibrium state was attained, and the gelatinization process occurring at each temperature was followed by an enzymic digestion method.

MATERIALS AND METHODS

Commercial potato starch purchased from Wako Chemical Co., Ltd., Tokyo, was used. Its water content as measured by oven-drying at 105°C for about 10 hr was 13.6%. The granular size distribution checked by sieving was as follows; 0.4% of the granules have diameters of over 74 μm , 4.1% between 63~74 μm , 8.3% between 53~63 μm , 6.5% between 46~53 μm , 25.0% between 37~46 μm , 28.8% between 25~37 μm , 11.9% between 20~25 μm and 15.1% under 20 μm .

Starch was suspended in a small amount of water at 30°C, and enough water at the proper temperature was added to the suspension to obtain the concentration and final temperature desired. The temperature of the water to

[†] Study of Starch Gelatinization. Part I.

be added was calculated from the intended temperature, and the heat capacities of the starch-water suspension and the vial used. For example to obtain 100 g of a 10% starch-water suspension at 60.0°C, 10 g of potato starch suspended in 20 ml of water was maintained at 30°C, and 70 ml of water at 84.8°C was poured into the suspension. The resulting suspension was incubated at the intended temperature in a thermostat bath. The incubation temperature was maintained at that intended within $\pm 0.5^\circ\text{C}$. At intervals, a portion of the starch suspension was removed from the vial, dehydrated in a ten-fold volume of ethanol with mixing in a blender for 1 min, filtered through a glass filter, washed with ethanol twice and then ether twice, and finally dried in a desiccator. For reference, 10 g of starch was suspended in 90 ml of water at 30°C for 30 min, and then dehydrated, washed and dried as above. This sample was used as the native potato starch. Also, a 10% starch-water suspension was boiled for 10 min, and then dehydrated, washed and dried. This was used as the fully gelatinized potato starch. Concentrations of starch are shown as the weight percentage of dry starch in suspension. The degree of gelatinization was measured by enzymic digestion with glucoamylase according to the method of Hizukuri *et al.*⁸⁾ Glucoamylase of *Aspergillus niger* purchased from Nagase Biochemicals Co., Ltd. (1×10^4 GUN/g) was used without further purification. X-Ray diffraction patterns were obtained at a scanning rate of one degree per minute.

RESULTS AND DISCUSSION

Isothermal starch gelatinization proceeded as shown in Fig. 1. The starch concentration was 8.6% and temperatures were 52.5~65.3°C. Starch was completely gelatinized

as determined by the glucoamylase method at 64.2°C in 30 min, and at 65.3°C starch was completely gelatinized in 2 min. When a starch-water suspension was isothermally incubated for more than 30 min at 52.5~65.3°C, the degree of gelatinization reached a constant value which depended on the temperature used. It is clear that starch reached its gelatinization limit for each temperature when the starch-water suspension was maintained for more than 30 min, that is, within this time, the suspension seemed to reach an equilibrium state.

The changes of X-ray diffraction patterns of potato starch incubated at 59.7°C for different durations are shown in Fig. 2. The results obtained with the ungelatinized and fully gelatinized starches are included in the figure. The numbers on the uppermost diffraction pattern are the designations for the interference rings proposed by Katz and van Italie.⁸⁾ The crystal structure of the starch broke down with time, and the pattern after 30 min incubation was the same as that after 60 min. This observation is consistent with the results given in Fig. 1. From this it was concluded that the degree of gelatinization observed for 30~60 min incubation periods corresponded to an equilibrium state. Then, the gelatinization process was further investigated kinetically as follows. In

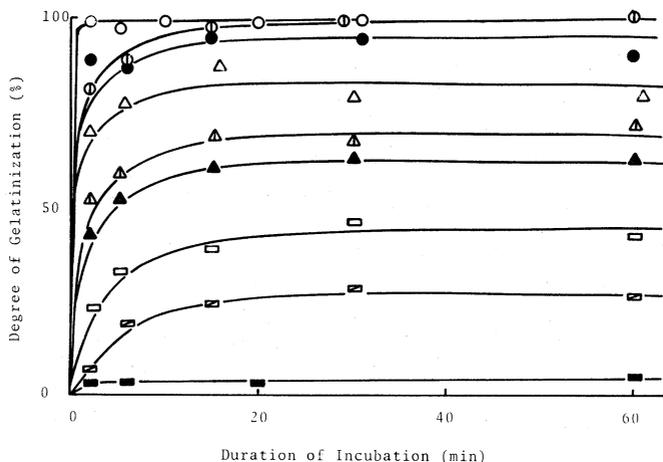


FIG. 1. Time Courses of Gelatinization of Potato Starch Measured by the Glucoamylase Method.

○, 65.3°C; ⊙, 64.2°C; ●, 63.1°C; △, 62.0°C; ▴, 60.8°C; ▲, 59.7°C; □, 58.6°C; ▢, 57.4°C; ■, 52.5°C. Starch concentration, 8.6%.

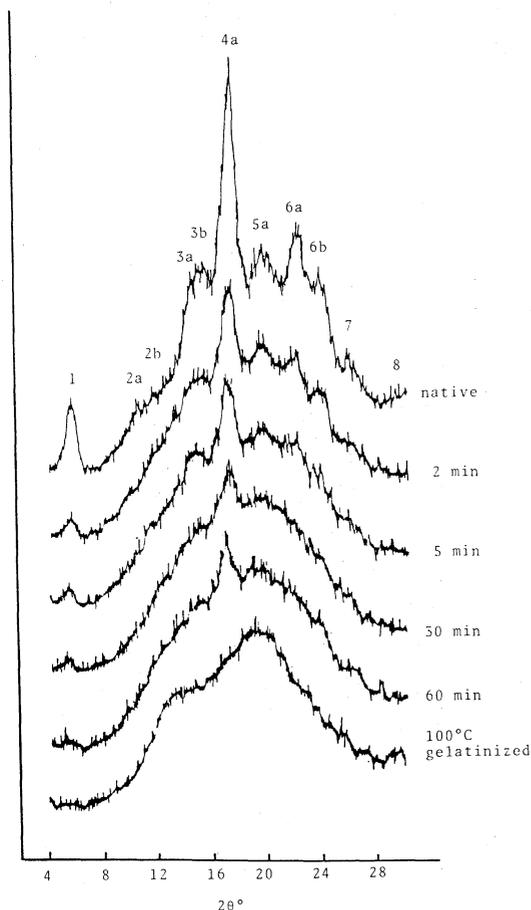


FIG. 2. Changes of X-Ray Diffraction Patterns of Potato Starch Incubated at 59.7°C for Various Periods of Time.

Starch concentration, 8.6%.

the case of starch gelatinization with an excess of water, the velocity of the reaction should be proportional to the ungelatinized fraction, that is, it was expected to be a first-order reaction, and a plot of the logarithm of ungelatinized starch fraction vs. time (not shown) was, indeed, linear. From the slope of this line, the rate constant of gelatinization can be calculated. The rate constant increases as the incubation temperature rises. An Arrhenius plot of potato starch gelatinization is shown in Fig. 3. From the slope of this plot which was obtained by the least-squares method, the activation energy of starch gelatinization was calculated to be 22 ± 5 kcal/mol (92 ± 21 kJ/mol).

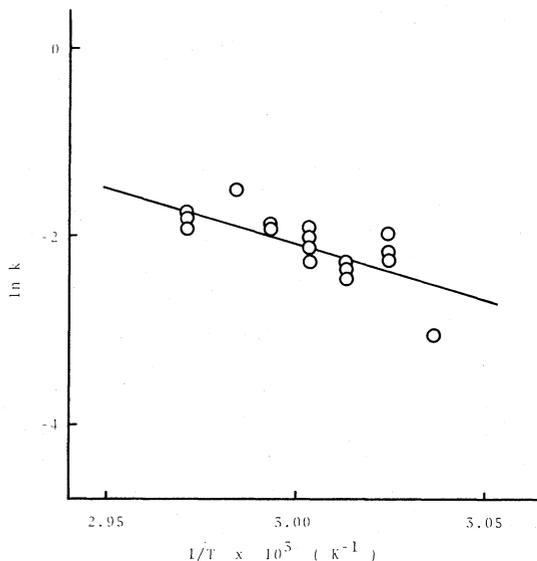


FIG. 3. Arrhenius Plot of Potato Starch Gelatinization Determined by the Glucoamylase Method.

Starch concentration, 8.6%; slope = $(-11.1 \pm 2.6) \times 10^3$; $E_A = 22 \pm 5$ kcal/mol.

In Fig. 4, the degrees of gelatinization observed at various temperatures are plotted against the incubation temperature. The sigmoidal curvature is a characteristic one for the transition of polymers. The curve given in Fig. 4 can be reasonably regarded as a transition curve in that it is expressed by the fraction of gelatinized starch granules as a function of temperature. From this curve, the half-transition temperature was found to be 59.1°C. It is also clear that gelatinization of potato starch starts even at a temperature as low as 52.5°C. Figure 5 shows the X-ray diffraction patterns of starch gelatinized for 60 min at different temperatures. The X-ray diffraction patterns of the starch incubated at 52.5°C were almost the same as that of the native starch, except that interference rings 1 and 7 were weakened. This indicates that the potato starch maintained at 52.5°C for 60 min is very slightly gelatinized. Quantitative evaluation cannot be done with these diffraction patterns, but qualitatively they agree well with the results in Fig. 4. Hitherto gelatinization temperatures of potato starch were re-

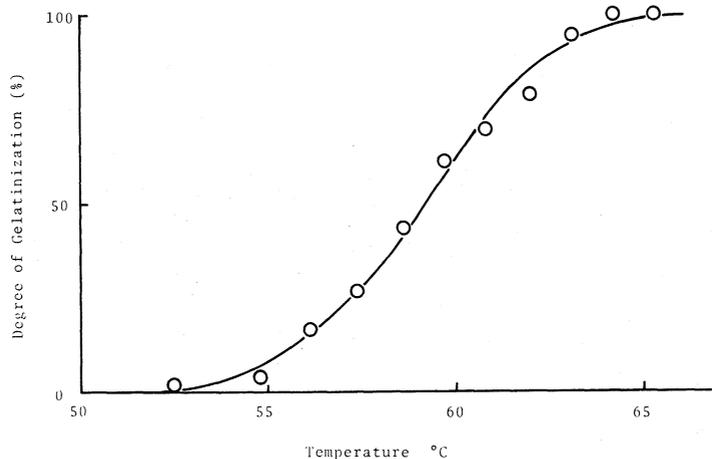
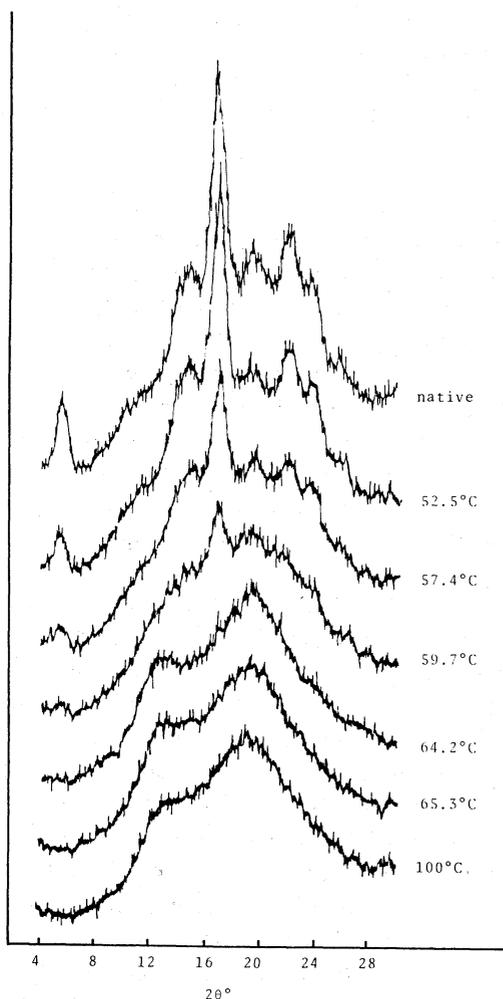


FIG. 4. The Relationship between the Incubation Temperature and the Degree of Gelatinization. Starch concentration, 8.6%.



ported to be 56.0~66.0°C by hot stage microscopy,⁹⁾ over 62°C by photopastography,³⁾ and 66~80°C by amyloviscography.¹⁾ It is most likely that the degree of gelatinization differs according to which changes in the starch were measured. For example, the gelatinization temperature determined by viscometry such as amylography is higher than that obtained with other temperature-scanning methods. But even the gelatinization temperature measured by microscopy, which can detect changes in starch granules at early stages, is higher than the results shown in Fig. 4. It is clear that by maintaining a starch-water suspension for a long period of time at a definite temperature starch gelatinization starts at a lower temperature than those previously reported.

Since, as described earlier, the curve given in Fig. 4 is regarded as a transition curve, an attempt was made to determine the effective enthalpy change for potato starch gelatinization by using the van't Hoff relation.

$$\left(\frac{\partial \ln f/(1-f)}{\partial T}\right)_p = \frac{\Delta H_{vH}}{RT^2}$$

FIG. 5. Changes of X-Ray Diffraction Patterns of Potato Starch Incubated at Different Temperatures for 60 min.

Starch concentration, 8.6%.

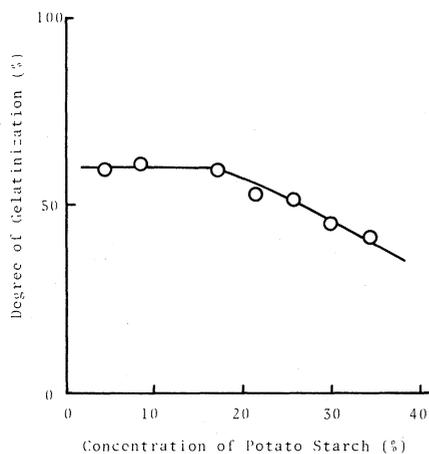


FIG. 6. Effects of Starch Concentration on the Degree of Gelatinization.

Temperature, 59.7°C; incubation time, 60 min.

where f is the gelatinized fraction, T the absolute temperature, R the gas constant and ΔH_{vH} the van't Hoff (effective) enthalpy. From this relation, the van't Hoff enthalpy was determined to be $+130 \pm 3$ kcal/mol ($+544 \pm 13$ kJ/mol). As the Gibbs energy change is zero at the half-transition temperature, the entropy change of starch gelatinization was determined to be $+391 \pm 9$ cal/mol·K ($+1640 \pm 38$ J/mol·K) at 59.1°C.

Figure 6 shows the effects of starch concentration on the degree of gelatinization found for the starch-water suspension incubated at 59.7°C for 60 min. The degree of gelatinization reaches the same value when the concentration of potato starch is 17% or under, but at concentrations higher than 17%, it decreases as the concentration increases. X-Ray diffraction patterns of potato starch of different concentrations which had been incubated at 59.7°C for 60 min are shown in Fig. 7. At concentrations of 4.3, 8.6 and 17%, the diffraction patterns are almost the same. A possible reason for this is that at higher concentrations, the amount of water available for the starch in the system is limited so that the starch granules are not fully gelatinized.

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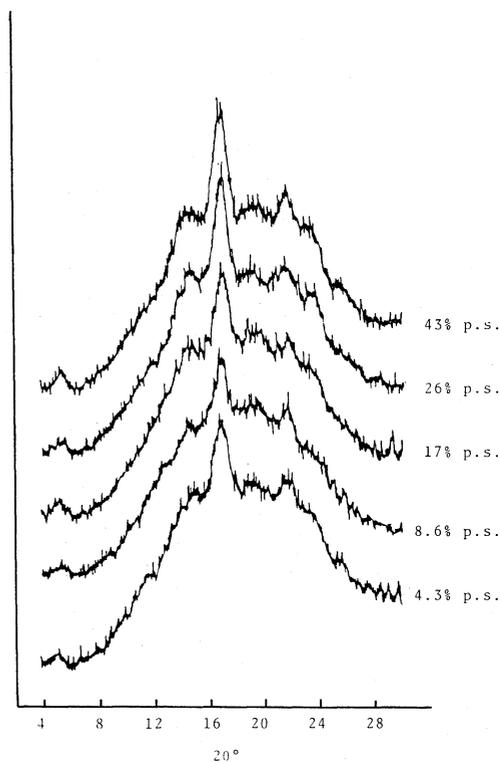


FIG. 7. Effects of Starch Concentration on X-Ray Diffraction Patterns.

Incubation temperature, 59.7°C.

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