The degree of gelatinization of potato starch was measured by the apparent optical density of starch-water suspensions at 530 nm during isothermal gelatinization at 52.5 ~ 72.0°C. From the temperature dependence of the gelatinization rate, the activation energy of gelatinization was determined to be $22 \pm 6$ kcal/mol, being in good agreement with the value obtained by the enzymic digestion method in the previous paper. The gelatinization temperature as measured from the half-transition was found to be 61.1°C which is slightly higher than the value of 59.1°C determined by the glucoamylase method. From the transition curve, the van't Hoff enthalpy was evaluated to be $117 \pm 3$ kcal/mol.

The heating rate is an important factor in the measurement of the gelatinization temperature of starch, since the process proceeds relatively slowly but in a cooperative manner. In the previous paper the isothermal gelatinization of potato starch was investigated at various temperatures ranging from 50 to 65°C, and the degree of gelatinization at each temperature was measured by the enzymic digestion method. It was found that the starch gelatinization reaches a limit at each temperature after 20 ~ 30 min of incubation. From the results of both the enzyme method and X-ray diffraction patterns, the starch gelatinization was even found to take place at a temperature as low as 52.5°C, which is lower than generally reported for the gelatinization temperature of potato starch. This discrepancy is assumed to arise from the fact that most of the previous studies have been made by employing heating rates which are faster than the rate of gelatinization. On the other hand, Shibukawa and Fukuba have studied starch gelatinization by various methods and found that the glucoamylase method gave a higher value for the degree of gelatinization than that estimated from turbidity measurements, the latter being very close to the value found by viscosity measurements.

From this fact, together with the results obtained by the enzymic digestion method in the previous paper, the isothermal gelatinization studied by other methods may provide further information about the gelatinization characteristics. This paper deals with the gelatinization of potato starch undertaken isothermally as measured from turbidity readings.

**MATERIALS AND METHODS**

**Preparation of gelatinized starch.** Commercial potato starch (Wako Chemical Co., Ltd.), with a water content of 13.6% was used. Starch–water suspensions were isothermally incubated at sixteen temperatures from 52.5 to 72.0°C for between 2 and 60 min. After the timed incubation, the suspensions were dehydrated with ethanol, washed with ethanol and ether, and dried in a desiccator. The concentration of the starch–water suspensions during incubation was either 4.3 or 8.6% by dry starch weight. For reference, the 8.6% starch suspension was boiled for 10 min, dehydrated and washed, followed by drying. This preparation was used as a fully gelatinized starch. The other 8.6% starch suspension was incubated at 30°C for 30 min, dehydrated and washed, followed by drying. This preparation was used as an ungelatinized starch.

**Turbidity measurement of the starch–water suspension.**
The turbidities of the starch suspension and supernatant were measured at 530 nm according to the method of Shibukawa and Fukuba. Thirty mg of potato starch, which had passed an 80-mesh sieve, was dispersed with 10 ml of water in a glass homogenizer. A smooth-surfaced Teflon pestle was used and care was taken so that the starch granules were protected from grinding. The apparent optical density of the homogenized starch–water suspension was measured immediately at 530 nm (turbidity of the suspension). The suspension was then allowed to stand for 100 min at room temperature and the apparent optical density of the supernatant of the suspension was measured (turbidity of the supernatant). Each turbidity was calculated per 30 mg of dry starch. Water contents of samples were measured by drying at 105°C for about 10 hr.

Observation by light microscopy. Wet starch samples incubated at defined temperatures were observed microscopically at a magnification of 50.

RESULTS AND DISCUSSION

Calculation of the degree of gelatinization from turbidity

Figure 1 shows the changes in turbidity of the starch suspension and supernatant during the incubation at 59.7°C for 2 to 60 min. The dotted line represents the turbidity level of the suspension of fully gelatinized starch, which is almost the same as that of the supernatant. The turbidity of a starch–water suspension just after mixing increases in the first 5 min and then decreases as the process proceeds. The turbidity of the supernatant seems to increase slightly with the incubation time, but it is not certain whether this increase is significant or not. The turbidity method for the measurement of degree of gelatinization is based on the principle that the transparency of a starch–water suspension increases with the gelatinization process and the turbidity of a starch–water suspension is regarded to be in proportion to the degree of gelatinization. According to Shibukawa and Fukuba, correction should be made for the turbidity of the supernatant in order to estimate the degree of gelatinization from turbidity measurements on the starch–water suspension. From Fig. 1, it is obvious that this correction term is very small and that the change in turbidity of the suspension actually gives the gelatinization process. The rapid increase of turbidity in the first 5 min is assumed to reflect the increase in light scattering resulting from the increase in granular volume due to swelling. On this assumption, the time course of the change in the first 5 min was neglected and a hypothetical curve of the turbidity change during this earlier stage was drawn by extrapolation on the basis of first-order kinetics. The turbidity at zero-time thus extrapolated was $T_0 = 1.07$ in the experiment given in Fig. 1. With this method it was defined that the starch suspension incubated for 10 min at 100°C is in a 100% gelatinized state and the ratio of the turbidity change of a given sample during a given period of incubation to that of a 100% gelatinized starch suspension was taken to be the degree of gelatinization, $G_x$, according to the equation:

$$G_x = \frac{T_0' - T_x}{T_0' - T_g} \times 100 \text{ (%)}$$

where $T_x$ is the turbidity of the suspension after incubation for $x$ min, and $T_g$ the turbidity of the suspension of fully gelatinized starch which was obtained by maintaining the suspension at 100°C for 10 min.
Study of Starch Gelatinization by the Turbidity Method

It has been a general view that starches separated from such vegetable roots as potato and sweet potato do not exhibit any remarkable expansion in their granular size when they are suspended in water. This conclusion seems to have been deduced mainly from the results obtained by photopastegraphy.\(^3\)\(^{~5}\) However, the present result obtained by turbidity measurement also indicates that the potato starch swells to a certain extent so that the turbidity of the suspension increases at an early stage before it starts to decrease by the disintegration of granules due to gelatinization.

In Fig. 2 the gelatinization process of potato starch at 64.2°C as measured by the turbidity method (●) is shown. The process at the same temperature, which was obtained by the enzyme digestion method\(^8\) (○), is also included in the figure for comparison. In the glucoamylase method, alkali gelatinized starch was used as the 100% gelatinized form following Hisukuri \(et\) al., and in the turbidity method, heat gelatinized starch was used as the 100% gelatinized form, because the degree of gelatinization was too low when alkali gelatinized starch was used instead.\(^6\) However the degrees of gelatinization measured by the glucoamylase method are about 25\% higher than those measured by the turbidity method. This is consistent with the result reported by Shibukawa and Fukuba.\(^6\) As they described, the turbidity method reflects the state of swelling of the starch granule and agrees well with the result of viscometric measurement, showing starch gelatinization at the granule level. On the other hand, the glucoamylase method reflects starch gelatinization at the molecular level. It seems that the starch molecule changes at a lower temperature than that of disintegration of the starch granule. Additionally, since the gelatinized starches are not completely hydrolyzed to yield 100\% glucose by glucoamylase (about 90\%),\(^9\) the fully gelatinized state defined with the glucoamylase method is actually indistinguishable from the state in which a small fraction still remains ungelatinized. These factors seem to result in the fact that the glucoamylase method gives a higher value for the degree of gelatinization than that obtained with the turbidity method.

Figure 3 shows the time courses of potato starch isothermally gelatinized at 54.8~67.7°C, measured by the turbidity method. The starch incubated at 66.5°C undergoes 100\% gelatinization in 10 min, while the starch incubated at 54.8°C is not gelatinized at all within the scope allowed for experimental error. These time courses also indicate that the degree of gelatinization of the potato starch
 incubated at a constant temperature reaches a limit in 30~60 min whose extent is larger as the incubation temperature is increased. This characteristic is essentially the same as that observed by the enzymic method in the previous paper. From this fact it may be reasonably concluded that the extent attained in 30~60 min at each incubation temperature corresponds to the degree of gelatinization of the starch–water suspension, which reaches a limit characterized by a constant value for the ratio of the gelatinized to ungelatinized fractions at a given temperature. Further information about the thermodynamic properties of the gelatinization process will obviously be obtained, if an appropriate analysis is made on this equilibrium state. The equilibrium thermodynamics of gelatinization will be discussed later.

From the time courses given in Fig. 3, the apparent rate constant of gelatinization was estimated on the basis of the first-order kinetics employed in the previous paper, and the apparent activation energy was evaluated by using the Arrhenius equation. In Fig. 4, the Arrhenius plot for the rate constant of gelatinization is shown. From the slope of the plot, the activation energy of potato starch gelatinization in water was calculated to be 22 ± 6 kcal/mol (92 ± 25 kJ/mol). This value is in fairly good agreement with the value of 22 ± 5 kcal/mol which was obtained for potato starch by the glucoamylase method.}

In Fig. 5 the degree of gelatinization at the equilibrium state is given as a function of the incubation temperature (∆). The same quantity measured by the glucoamylase method (○) is also included in the figure for comparison. The two lines are sigmoidal and resemble the transition curve of many biopolymers. This fact clearly indicates that gelatinization of starches should not be characterized simply by an initiation or conclusion temperature. In this connection the author proposes that the starch gelatinization process should be discussed in terms of equilibrium thermodynamics and that the half-transition temperature where 50% of starches are in the gelatinized state should be used as one of the index terms to express a gelatinization temperature which is defined from the statistical point of view. According to this aspect the gelatinization temperature of potato starch was estimated to be 59.1°C by the glucoamylase method and 61.1°C by the turbidity method.

Since the lines shown in Fig. 5 can reasonably be regarded as transition curves, the van’t Hoff enthalpy of the starch gelatinization was evaluated by using the van’t Hoff equation in the same manner as employed in the previous
Study of Starch Gelatinization by the Turbidity Method

**FIG. 4.** Arrhenius Plot from the Turbidity Method.

slope = $(-11.1 \pm 3.3) \times 10^3$; $E_A = 22 \pm 6 \text{ kcal/mol (92} \pm 25 \text{ kJ/mol)}$; starch concentration, 8.6%.

**FIG. 5.** Relationship between the Incubation Temperature and the Degree of Gelatinization.

○, glucoamylase method; △, turbidity method; starch concentration, 8.6%.

work.\textsuperscript{1} The value obtained was $+117 \pm 3$ kcal/mol ($+490 \pm 13$ kJ/mol), this being reasonably in agreement with that obtained by the enzymic method. The corresponding entropy change of starch gelatinization at the half-transition temperature ($61.1^\circ \text{C)$ was determined to be $+350 \pm 8 \text{ cal/mol} \cdot \text{K}$ ($+1460 \pm 33 \text{ J/mol} \cdot \text{K}$).

Figure 6 shows light photomicrographs of potato starch incubated at various temperatures for 60 min. The starch granules swell and disintegrate as the temperature of incubation rises. As described in the previous paper,\textsuperscript{1} the starch granules treated at $52.5^\circ \text{C}$ are slightly expanded when compared with native starch granules. This is supported by the result of X-ray diffraction in which the patterns of the starch treated at $52.5^\circ \text{C}$ change slightly when compared with the native starch. The degree of gelatinization of the starch treated at $52.5^\circ \text{C
Study of Starch Gelatinization by the Turbidity Method

Fig. 6. Light Photomicrographs of Potato Starch Incubated at Various Temperatures for 60 min. 
A, native; B, 52.5°C; C, 54.8°C; D, 57.4°C; E, 58.6°C; F, 59.7°C; G, 60.8°C; H, 64.2°C; I, 67.7°C; J, 100°C (10 min).

for 60 min, measured by the glucoamylase method, is about 3% (Fig. 5). Starch granules treated at 54.8°C are definitely expanded, and their degree of gelatinization measured by the glucoamylase method is about 5%. However the result obtained by the turbidity method was that the starches incubated at both 52.5 and 54.8°C undergo no gelatinization as shown in Fig. 5. These facts seem to indicate that the turbidity method is not adequate for measuring the early stages of gelatinization. From the photomicrographic observations shown in Fig. 6, there does not seem to any distinct difference between the gelatinized state of potato starches incubated at 67.7°C for 60 min and at 100°C for 10 min. It seems that the turbidity method is adequate for measuring the final stage of gelatinization.

Acknowledgments. Special acknowledgments are due to Professor K. Takahashi and Professor T. Watanabe, both of the University of Osaka Prefecture, for their helpful discussions and encouragement.

REFERENCES