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Retrogradation of Dilute Starch Dispersion

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희석 전분 현탁액의 노화

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Abstract

Retrogradation characteristics of 1% dilute rice starch dispersion were analyzed. The retrogradation was increased with prolonged storage, however, the trend in increase was more conspicuous during the initial phase of retrogradation period. The Avrami exponent, rate constant, and time constant of 1% dilute Chuchong starch dispersion were 0.96, 0.21 days^{-1}, and 4.77 days, respectively. As the Avrami exponent approaches unity (n=1), it is confirmed that the dilute rice starch dispersion retrogrades following the formation of a rod-like growth of crystals from instantaneous nuclei. When the retrogradation process of dilute rice starch dispersion was traced, the three-dimensional structure of crystals which had different contour from native stach was formed.

Introduction

Retrogradation refers to the spontaneous aggregation and partial crystallization of starch molecules during their normal aseptic aging. The retrograded starch dispersion loses transparancy and decreases in viscosity. Starch-degrading enzymes cannot attack the retrograded starch dispersion well. It is believed that amylose fraction is primarily involved in retrogradation due to its many hydrogen bonding sites(1).

Kim *et al.*(2,3) and Colwell *et al.*(4) have reported retrogradation characteristics of cassava and wheat starch gels, respectively. They have shown that the mechanism of starch retrogradation is instantaneous nucleation followed by rod-like growth of crystals and the retrogradation rate is inversely related to the storage temperature. Del Rosario and Pontiveros(5) have studied retrogradation tendencies of various starches and concluded that values for Avrami exponent and rate constant differ greatly according to the starch type.

The aim of this research is to present the kinetic study of retrogradation in dilute starch dispersion.

Materials and Methods

Starch preparation

1kg of rice grain (Chuchong, Japonica type) was steeped with 6*l* of 0.01M acetate buffer (pH 6.5) at 5℃ for 24 hrs. After steeping, rice grain was grinded in a blender. The resulted slurry was screened through 120 and 325 mesh standard sieves to obtain purified starch suspension. After centrifugation at 6000xg for 30 mins, the supernatant was decanted and the brown, upper layer of sludge scraped off. The starch was resuspended in 0.2% NaOH solution and set still overnight. After the supernatant and the upper layer of sediment were removed, the starch was suspended in distilled water and adjusted to pH 6.5. After repeated sedimentation and decantation with distiled water, white starch layer was air-dried and passed through 60 mesh sieve.

Proximate compositions

Proximate compositions were determined according to the methods described in AOAC(6).

Preparation of gelatinized starch dispersion

The starch solution was prepared by autoclaving as described below(7). The starch was slurried in a small volume of distilled water, and the slurry was poured with stirring into an appropriate volume of hot (80℃) distilled water to the final concentration of 1%. After cooling to the room temperature, the pH of the dispersion was adjusted to 6.5. Then, the dispersion was autoclaved for 90 mins at 121℃. After cooling to temperature below the boiling point, the undispersed material was removed by hot filtration through a glass filter (1G2 type).

Determination of the degree of starch retrogradation

The dispersion was cooled quickly to 5℃. At appropriate intervals, 40 ml of the retrograding dispersion was removed and centrifuged at 3900xg for 30 mins. An aliquot of the dispersion was taken for starch determination following the method of Whistler and Medcalf(8) with slight modification. Into a 100ml weighing bottle were placed approximately 10g of Celite535 and a glass rod which is just shorter than the inside height of the bottle. The bottle was then dried in a dry oven at 105℃ for 3 hrs. The container was cooled in a desiccator and weighed. A 7 ml of the supernatant was pipetted over the Celite uniformly. The Celite was mixed with the glass rod which was kept in the bottle. The container was placed in a dry oven at 105℃ for 7 hrs. The sample was cooled in a desiccator and weighed. Three replicate supernatant samples were measured on each occasion. Percent retrogradation of starch solution was determined as follows.

$$\%\ \text{retrogradation} = \frac{\text{solid content of retrograded fraction}}{\text{solid content of starch dispersion at initial time}} \times 100$$

Scanning electron microscopy

The process of starch retrogradation was traced during the storage period at 5℃. Aliquots (25 ml) of starch dispersion were removed at appropriate intervals and transferred to 50 ml centrifuge tubes. The starch dispersion was centrifuged at 5000 x g for 15 mins at 4℃. The starch precipitate was washed with distilled water and centrifuged. The supernatant was discarded and a portion of the residue was freeze-dried and coated with carbon(9). The mounted specimen was examined in scanning electron microscope at an accerlerating potential of 15kv. The representative area was photographed on Kodak VP 6041 film.

Retrogradation kinetics

The kinetics of starch retrogradation was analyzed following Avrami equation(10,11).

$$Q = \exp -kt^n \quad \cdots\cdots(1)$$

where, Q: the fraction of crystallizable starch remaining uncrystallized at time t
n: Avrami exponent
k: rate constant

Then, the fraction of uncrystallized material can be expressed as follows.

$$Q = \frac{Rm-Rt}{Rm-Ro} \quad \cdots\cdots(2)$$

where,

Rm: the maximum % retrogradation

Rt: the % retrogradation at time t

Ro: the % retrogradation at initial time

Substitution in the Avrami equation yields equation(3)

$$\exp - kt^n = \frac{Rm - Rt}{Rm - Ro} \quad \cdots\cdots(3)$$

After rearrangement, equation (3) is transformed to equation(4)

$$\log\left(-\ln\frac{Rm - Rt}{Rm - Ro}\right) = n\log t + \log k \quad \cdots\cdots(4)$$

Results and Discussion

Chemical compositions of Chuchong starch are described in Table 1. Contents of moisture, ash, protein, and fat were 12.4, 0.23, 0.26, and 0.12%, respectively.

The change of percent retrogradation according to storage period in dilute Chuchong starch dispersion at 5℃ is depicted in Fig.1. With the increase in storage period, the retrogradation was also enhanced. The percent retrogradation after 3, 6, 9 days were 14.9, 21.8, 26.4%, respectively. As shown in Fig. 1, the retrogradation increased most profoundly during the initial phase of retrogradation process. The maximum percent retrogradation was obtained after 9 days. Del Rosario and Pontiveros(5) have previously reported that the percent retrogradation of rice starch dispersion at 10℃ is in its maximum value after 11 days of storage.

The retrogradation kinetics of 1% dilute Chuchong starch dispersion was analyzed following Avrami equation (Fig. 2). The slope of straight line which represents Avrami exponent was 0.96.

Table 1. Chemical compositions of Chuchong starch

Moisture (%)	12.4
Ash (%)	0.23
Protein (%)	0.26
Fat (%)	0.12

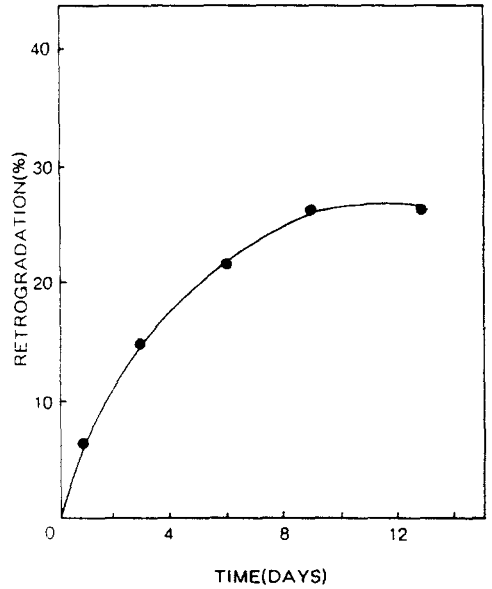

Fig. 1. Retrogradation of 1% dilute Chuchong starch dispersion at 5℃

Since the Avrami exponent (n) was found to have the value n=1 within experimental error, the best value of the rate constant was determined from the graph of $\log_e(Rm - Rt)$ against (Fig. 3)(11).

From the slopes of Fig. 2 and Fig. 3, the Avrami exponent, rate constant, and time constant in dilute Chuchong starch dispersion were determined (Table 2). As shown in Table 2, the Avrami exponent, rate constant, and time constant were 0.96, 0.21days^{-1}, and 4.77 days, respectively. Here, the time constant is defined as the time for any given fraction of starch to be converted to the retrograded form. As clear from Table 2, the Avrami exponent (n) was near unity (i.e., n=1) in dilute Chuchong starch dispersion. This demonstrates that the mechanism of retrogradation in dilute starch dispersion is a rod-like growth of crystals from instantaneous nuclei(5,11,12). From this finding, it is verified that the mechanism of retrogradation in dilute starch dispersion is the same as those in rice starch gel and cooked rice(13,14). Del Rosario and Pontiveros(5) have reported that the retrogradation of 1% rice starch is slower than those of cowpea and mungbean starch.

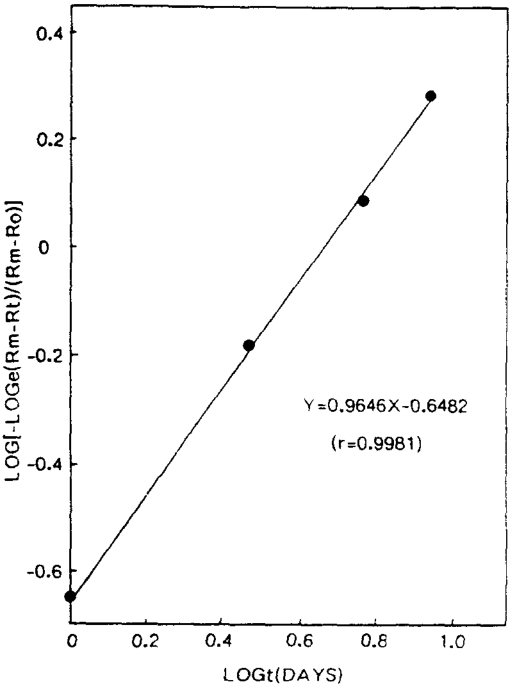

Fig. 2 . Plot of log[-log$_e$ (Rm-Rt)/(Rm-Ro)]against log t in 1% dilute Chuchong starch dispersion at 5℃

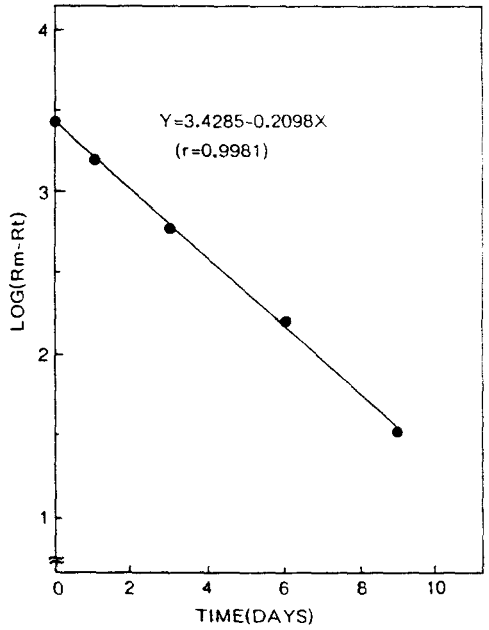

Fig. 3. Plot of log$_e$ (Rm-Rt) against t in 1% dilute Chuchong starch dispersion at 5℃

Table 2. Avrami exponent, rate constant, and time constant of 1% dilute Chuchong starch dispersion stored at 5℃

Avrami exponent(n)	0.96
Rate constant(k)	0.21 days^{-1}
Time constant(1/k)	4.77 days

Fig. 4 shows the process of starch retrogradation in dilute Chuchong starch dispersion observed by scanning electron microscope. The native rice starch granule presented an overal angular outline. After gelatinization, the three dimensional structure of rice starch was almost lost with only vestiges of granule distributed in the field. This seems to be attributed to the fact that the granule structure is destroyed during the successive gelatinization following swelling and volume expansion caused by the imbibition of water in gelatinization process(9,15,16). The micrograph prepared from retrograded starch dispersion after 2 days showed that crystal structure increased slightly, compared with that from gelatinized starch dispersion.

After 6 days, the crystallinity was increased conspicuously and the three-dimensional contour became more evident than 2 days after retrogradation. At this stage, the crystal structure of retrograded starch became compact and dense due to the increase of hydrogen bonds through prolonged retrogradation(1,17). It is reported that the retrograded starch is insoluble unlike natives tarch(18). Hellman et al. studied the change of physical state during gelatinization and retrogradation by X-ray diffraction technique and concluded that the moisture level affected the type of crystallinity produced(17). According to their results, in dilute starch dispersion which has low starch

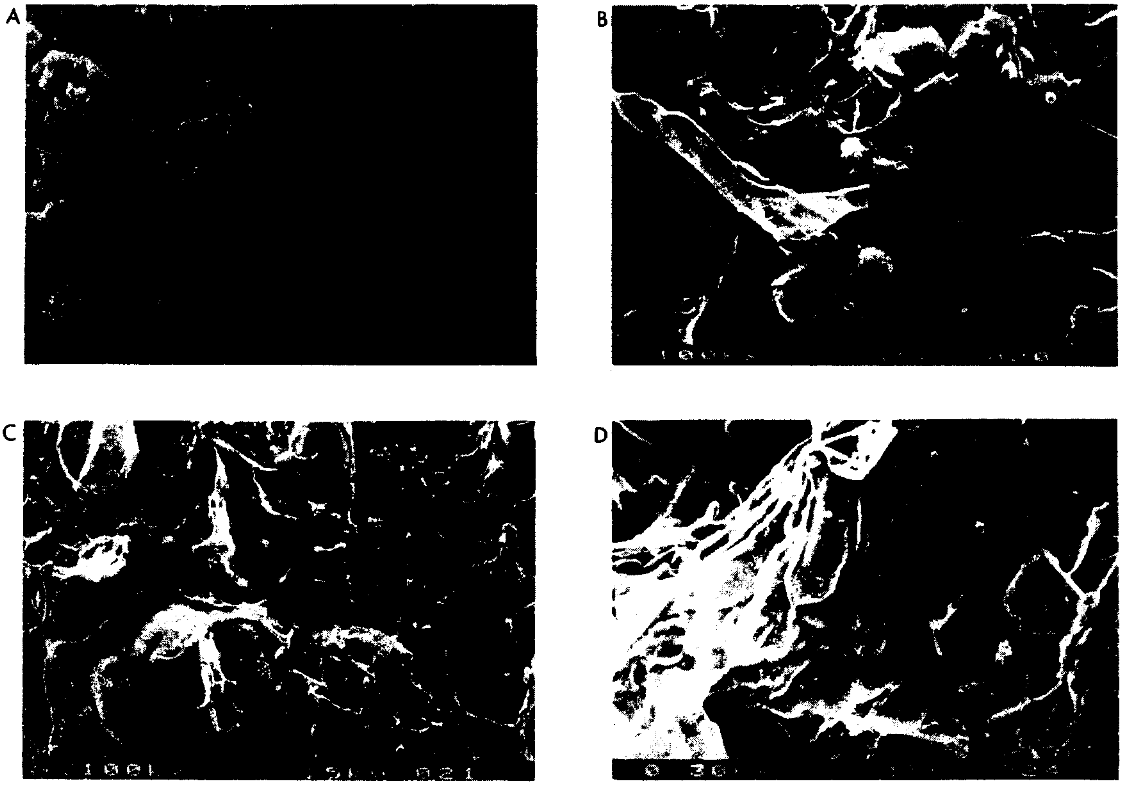

Fig. 4. Scanning electron micrographs of the retrogradation process of Chuchong starch

A: native starch granule, B: gelatinized starch dispersion, C: retrograded starch dispersion (2 days), D: retrograded starch dispersion (6 days). Arrows (→) indicate the crystal structures increased during the retrogradation process

content, the mixed A and B diffraction pattern which is different from A diffraction pattern of native starch is obtained after retrogradation. As expected from above reasoning, the three dimensional structure of retrograded rice starch was significantly different from that of native rice starch in this experiment.

요 약

1% 희석 쌀전분 용액의 노화 특성을 호화후 5℃에서 저장하면서 고찰하였다.

저장기간이 증가함에 따라 노화도도 증가하였는데 그 증가하는 정도는 노화 초기에 가장 현저한 반면 노화가 진행됨에 따라 감소하였다. 추정 전분의 노화 특성을 Avrami식으로 해석하여 노화기작을 살펴본 결과 Avrami지수, 속도상수, 시간상수는 각각 0.96, 0.21 days^{-1}, 4.77 days 였다, 이때 Avrami지수가 단위치 (n=1)에 접근하므로 희석 쌀전분 용액은 즉각적인 핵형성에 이은 간상구조의 생성에 의하여 노화가 진행됨을 알 수 있었다. 주사 전자 현미경에 의하여 노화과정을 관찰한결과 노화기간이 경과함에 따라 생전분과는 다른 형태의 3차원 결정구조가 형성됨을 알 수 있었다.

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