Effect of High Pressure on the Crystalline Structure of Various Starch Granules

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ABSTRACT

High-pressure treatment was applied to suspensions of starch and water (1:1, w/v) at 50–500 MPa for 20 or 60 min, and the effect of the high pressure on the crystalline structure of several kinds of starch granules (corn, rice, and potato) was investigated by microscopic observation, X-ray diffraction, and measurement of specific gravity. Corn starch granules clearly showed the swelling, disappearance of polarization cross, and destruction of the A-type X-ray diffraction pattern by high-pressure treatment at 500 MPa for 20 min. A faint B-type X-ray diffraction pattern appeared simultaneously with this change. In contrast, potato starch granules retained the native granular features and increased the B-type X-ray diffraction pattern, even after application of pressure up to 500 MPa for 60 min. Pressure application with hexane in place of water never changed X-ray diffraction patterns of starches, so the changes of the crystalline structures of pressure-treated starches may absolutely require the presence of water. Rice starch also showed changes similar to those of corn starch. For corn and potato starch granules, either untreated or pressure-treated, the higher the relative crystallinity, the smaller the specific gravity.

High pressure denatures protein and forms gel (Hayashi 1989), and denaturation can be explained in terms of the hydration change of protein (Gekko 1990).

The effect of high-pressure treatment at 600 MPa for starches was studied on the granular structure and the susceptibility to amylase. The resultant change depended on moisture content in the starch granules (Mercier et al 1968). Applying high pressure resulted in an upward shift of the gelatinization temperatures of potato (Thevelein et al 1981, Muhr and Blanshard 1982, Muhr et al 1982), wheat, and smooth pea (Muhr and Blanshard 1982, Muhr et al 1982) starches. Another study showed that, after high-pressure application (400–500 MPa at 45–50°C), the susceptibilities of wheat and corn starches to amylase were much increased, but the susceptibility of treated potato starch increased only a little because of its resistance to high-pressure treatment (Hayashi and Hayashida 1989). Recently, Kudla and Tomasik (1992a,b) observed that air-dried potato starch was more compressible than oven-dried potato starch, which could be due to the lower level of moisture penetration into oven-dried starch granules. The dextrinization of potato starch, caused by the high-pressure treatment, was accelerated by increased moisture content. However, there have been few studies on high-pressure-treated starches, except for one on depolymerization of starch by high pressure combined with heat and acid (Kim and Hamdy 1987). In this study, we investigated the effect of high pressure on the crystalline structures of various starch granules. We found that high pressure changed the A-type crystalline structure of starch to B-type and that water played an important role.

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MATERIALS AND METHODS

Materials

Potato, normal corn, and waxy corn starches were purchased from Kyoto Wako Pure Chemical Industry Co., Kyoto, Japan. Normal rice and waxy rice starches were supplied by Shimada Chemical Industry Co., Niigata, Japan.

Defatted normal corn and rice starches were each prepared by Soxhlet extraction with 85% methanol for 30 hr. The amount of lipids extracted from corn and rice starches was 0.42 and 0.65% (w/w), respectively.

Methods of Pressurization

Starch and water suspensions (1:1, w/v) were put into a thin plastic bag and sealed with no air. Pressure of 50–500 MPa was applied at room temperature with a water pump (MFP-7000, Mitsubishi Heavy Industry Co., Tokyo, Japan). The temperature changed from 17 to 23°C during pressurization. After 20 or 60 min of compression, the pressure was released and the starches were analyzed.

Preparation of Ground Potato Starch

Dry potato starch granules easily suffer destruction of crystalline structure during grinding (French 1984). Predried potato starch granules, in a desiccator containing silica gel, were ground in a mortar for specific periods of time to produce different degrees of crystallinity.

Light Microscopy

Light microscopy was performed using an Olympus light microscope (Olympus Optical Co., Tokyo, Japan) operating in normal and polarization modes, with crossed-nicol prisms.

X-Ray Diffraction and Relative Crystallinity

X-ray diffraction patterns were taken with a Rigakudenki X-ray diffractometer RAD-1A, using the following conditions: target, Cu-Kα radiation (Ni filter); voltage, 35 kV; current, 20 mA; scanning speed, 2° 2θ/min; time constant, 1 sec. Because X-ray intensity of starch is affected by moisture content, samples were conditioned at 75% rh for three days before taking the X-ray diffraction patterns (Hizukuri et al 1964, Nara et al 1978, Buleon et al 1982).

A relative crystallinity of pressure-treated starch was determined by the ratio of the peak height observed in the X-ray diffraction pattern to that of an untreated starch. Peak height was measured from a base line drawn through the minimum points of X-ray scattering intensities: 6.8 and 29° 2θ for cereal starches; 4, 6.8, and 29° 2θ for potato starch. Another relative crystallinity index (Lelievre 1974) was used for ground potato starch granules. In the X-ray diffraction pattern, three points of minimum scattering intensities, 4, 6.8, and 29° 2θ, were joined by lines. The upper region was normalized by dividing the X-ray scattering intensity at every 0.2° 2θ by the scattering intensity integrated over the total angular range from 4 to 29° 2θ. The normalized scattering intensities of the crystalline $(Ic)_{2\theta}$, amorphous $(Ia)_{2\theta}$, and unknown $(Iu)_{2\theta}$ specimens were each given at corresponding angles. The crystallinity index was defined by the slope χ of:

$$(Iu - Ia)_{2\theta} = \chi\,(Ic - Ia)_{2\theta} + b$$

where b is the intercept given by the regression line of the plot $(Ic - Ia)_{2\theta}$ versus $(Iu - Ia)_{2\theta}$. It is always zero, indicating that the normalization has functioned correctly.

Acid Hydrolysis

Two grams of starch were hydrolyzed in 300 ml of 15% sulfuric acid aqueous solution at 35°C and stirred twice a day. The amount of solubilized carbohydrate was measured every day; when it came to equilibrium, starch residue was removed, washed, and air-dried.

The crystalline nature of acid-treated starch granules was analyzed by X-ray diffraction, and the amount of carbohydrate solubilized in the solution was measured by the phenol-sulfuric acid method (Hodge and Hofreiter 1962).

Specific Gravity

Specific gravity was measured by the floatation method (Wu and Sarko 1978a, Nara 1979, Rabek 1980), using a mixture of carbon tetrachloride and hexane, at room temperature (23–25°C). Samples were conditioned at 0, 75, and 90.6% rh for one week before measurement because specific gravity of starch granules is affected by moisture content (Nara 1979, Buleon et al 1982, Haine et al 1985). As an index of precision, average deviation of density was ±0.002.

Moisture Content

Moisture content was measured in starch granules dried at 135°C for 5 hr.

RESULTS AND DISCUSSION

Figures 1 and 2 show micrographs of normal corn and potato starch granules, respectively. Untreated corn and potato starch granules show native features with polarization crosses. After high-pressure treatment, many swollen corn starch granules lost their polarization crosses. In contrast, potato starch granules did not lose their polarization crosses, even after high-pressure treatment.

The crystalline structures of these pressure-treated starches were revealed by X-ray diffraction (Fig. 3). The X-ray peak intensity for the pressure-treated normal corn starch clearly showed a decrease. In contrast, the pressure-treated potato starch retained its native X-ray diffraction pattern and showed an increase in X-ray peak intensity.

Thus, it was supposed that the high pressure induced different effects on corn and potato starch granules, as previously reported by Hayashi and Hayashida (1989). The changes of the crystalline structures were further investigated by soaking untreated and pressure-treated starches in 15% sulfuric acid aqueous solution

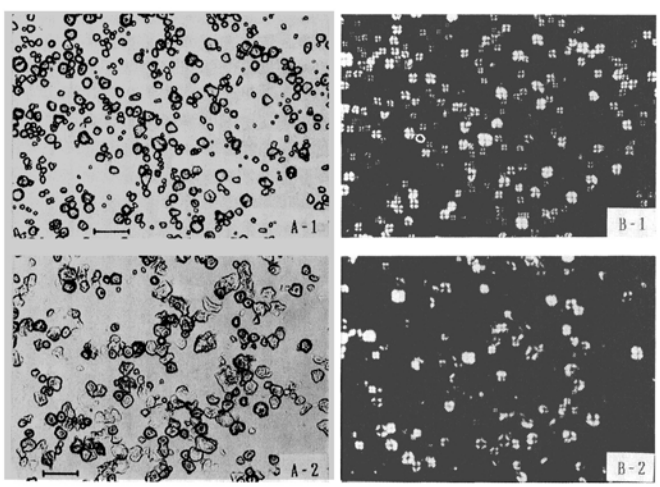

Fig. 1. Micrographs of normal corn starch granules under normal light (**A**) and under polarized light (**B**): untreated (**1**) and pressure-treated at 500 MPa for 20 min (**2**). Bar = 50 μm.

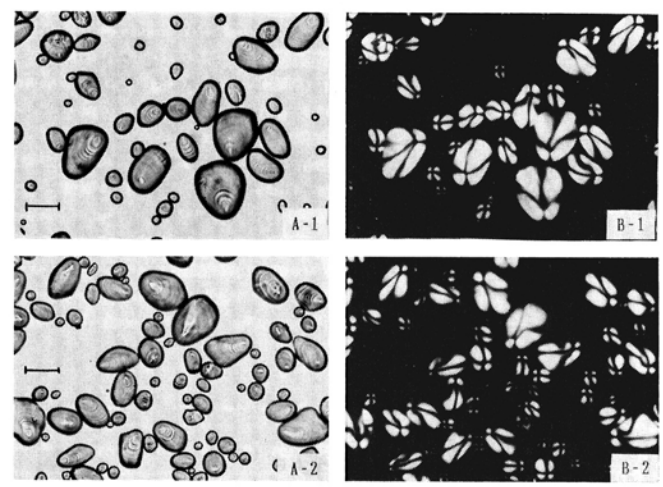

Fig. 2. Micrographs of potato starch granules under normal light (**A**) and under polarized light (**B**): untreated (**1**) and pressure-treated at 500 MPa for 20 min (**2**). Bar = 50 μm.

to preferentially remove the amorphous region from starch granule and increase the proportion of crystalline region (Kainuma and French 1971). Figure 4 shows the X-ray diffraction patterns of untreated and pressure-treated normal corn and potato starches, after the acid hydrolysis. Every sample clearly shows an increase in X-ray peak intensities after the hydrolysis. By comparing these results, we saw that the X-ray peaks of native corn starch tended to be dull, while those of native potato starch tended to be sharp. Hence, we concluded that the crystalline structure of normal corn starch was destroyed by the pressure but that of potato starch was not. This difference corresponds with the previous observations using microscopy (Figs. 1 and 2). In addition, the percentage of solubilized carbohydrates from the acid-treated starches (Table I) increased in normal corn starch but decreased in potato starch. This is another indication that the high-pressure treatment destroys the crystalline structure of normal corn starch but improves the crystalline structure of potato starch.

The effect of increasing pressure on the crystalline structures of various other starch granules was also studied. Figure 5 shows the changes in the X-ray diffraction patterns of corn, rice, and potato starches after pressure applications of 50–500 MPa for 20 min and 500 MPa for 60 min. In microscopic observations (data not shown) for corn and rice starches (normal, defatted normal, and waxy), the swollen granules without polarization crosses (such as those shown in Fig. 1) were rarely found in samples treated at 150 or 200 MPa for 20 min, but many swollen granules appeared in samples treated at 500 MPa for 20 min and more. On the other hand, potato starch did not show the swollen granules at any stage of pressure application; their polarization crosses remained clear. The X-ray diffraction patterns of normal, defatted normal, and waxy corn and rice starches also showed few changes after the treatment up to 200 MPa for 20 min. However, after treatment at 500 MPa for 20 and 60 min, they showed destruction of the A-type crystalline structure. The A-type X-ray diffraction pattern tended to change into a faint B-type pattern. We ascertained this by the appearance of peak 1 and the unity of peaks 4a and b (Fig. 3). The B-type X-ray diffraction pattern of potato starch hardly changed at any stage of the high-pressure treatment.

The changes in the relative crystallinities can be evaluated by comparing the heights of pressure-treated starch granules with those of untreated starch granules (Fig. 6, peaks 6a and 1). For this evaluation, we used samples conditioned at 75% rh because X-ray diffraction patterns become sharper with increased moisture content (Hizukuri et al 1964, Nara et al 1978, Buleon et al 1982). For corn and rice starches, the relative crystallinities estimated by the height ratios of peak 6a for the A-type X-ray diffraction patterns changed slightly at pressure up to 200 MPa, but it greatly decreased at 500 MPa for 20 min. Further prolonged treatment produced little effect on the relative crystallinities, although Kudla and Tomasik (1992a,b) observed some time-dependent changes during high-pressure treatment. Peak 1, which is particular to B-type X-ray diffraction and has never been seen in untreated corn and rice starches, appeared in treatment at 500 MPa for 20 or 60 min. These changes of crystalline structures by high-pressure treatment slightly accelerated in defatted corn and rice starches; this agrees with the opinion of Fukui and Nikuni (1969) that lipid protects crystalline structure of starch. However, the relative crystallinity of potato starch, estimated by the height ratio of the B-type pattern (peak 1), was different from those of corn and rice starches; it increased, even at 50 MPa, and remained

TABLE I
Solubility After Acid Hydrolysis of Pressure-Treated Starches

Starch	Solubilized Carbohydrate, %	
	Untreated	Treated[a]
Corn	20.7	40.3
Potato	27.2	25.0

[a] Pressure-treated at 500 MPa for 20 min.

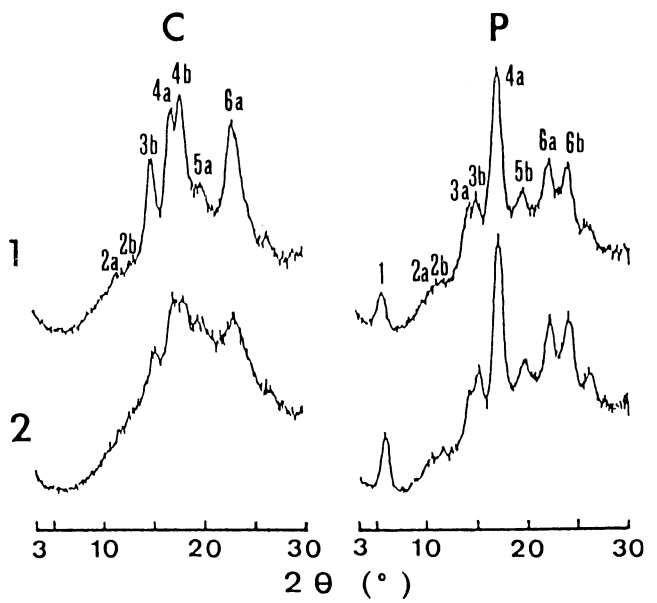

Fig. 3. X-ray diffraction patterns of normal corn (**C**) and potato (**P**) starches: untreated (**1**) and pressure-treated at 500 MPa for 20 min (**2**).

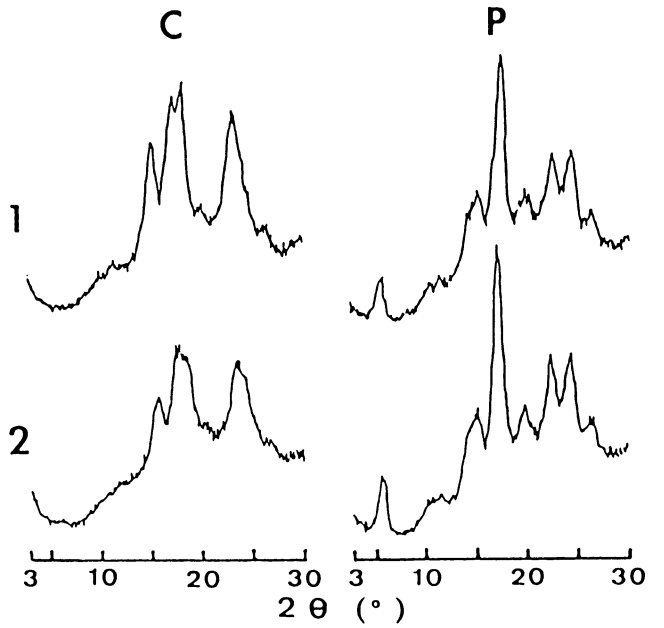

Fig. 4. X-ray diffraction patterns, after acid hydrolysis, of normal corn (**C**) and potato (**P**) starches: untreated (**1**) and pressure-treated at 500 MPa for 20 min (**2**).

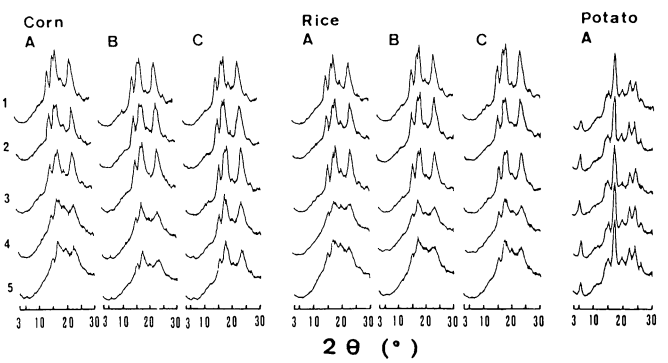

Fig. 5. Changes in the X-ray diffraction patterns of various starches with increased pressure: untreated starch (**1**); pressure-treated for 20 min at 50, 200, and 500 MPa (**2–4**, respectively), and pressure-treated for 60 min at 500 MPa (**5**). **A**, normal starch; **B**, defatted normal starch; **C**, waxy starch.

unchanged, even after treatment at 500 MPa for 60 min.

B-type crystalline structures have more space available for water than A-type does (Wu and Sarko 1978a,b). As shown in Table II, treated normal corn and potato starches had more moisture than untreated starches did, both before and after moisture conditioning. These results are similar to those of Kudla and Tomasik (1992a,b). Therefore, the increase in B-type X-ray intensity would be related to the increase of the moisture content. Mercier et al (1968) and Kudla and Tomasik (1992a,b) also showed the influence of water on the change in starch after pressurization. To elucidate the role of water in these phenomena, we used an organic solvent in place of water. Normal, defatted normal, and waxy corn starches and potato starch were each suspended in hexane and treated at 500 MPa for 60 min. These samples were predried in a desiccator containing silica gel for three weeks; during that time, their moisture contents decreased from 11.6–15.3% to 5.6–7.6%. Even after the pressure treatment in hexane at 500 MPa for 60 min, both the A-type and B-type X-ray diffraction patterns of the starches remained the same as those that were untreated (Fig. 7). These results clearly showed that, without water, the crystalline structure of the starch granule could not be changed by high-pressure treatment. The reason why water is absolutely necessary for the change is not fully clarified, but the fact that B-type crystalline structures contain more water than A-type does (Wu and Sarko 1978a,b) must be considered as one of the reasons. Also, the compressibility ($-dV/dP$) of B-type crystalline structures may be less than that of the A-type. Further investigation is needed on this point.

The relationship between crystalline structure and density was further determined by measuring specific gravity using the density gradient solution made from carbon tetrachloride and hexane. Normal corn and potato starches were conditioned at 75% rh before measuring specific gravity. As shown in Table II, in untreated starch granules, the specific gravity of corn starch granules was larger than that of potato. After high-pressure treatment, the specific gravity of corn starch granule became larger and that of potato became smaller. This might imply that the higher crystallinity of the starch, the smaller the specific gravity. Potato starch, with high crystallinity, includes more moisture.

Crystalline structure of potato starch granule can be easily destroyed by dry grinding (French 1984). Specific gravity indicated a large value (1.473) for ground potato starch granules conditioned at 75% rh; they had little crystalline structure left, as judged by the X-ray diffraction pattern. This seemed very interesting, so the relationship among moisture, specific gravity, and crystallinity was further investigated. Haine et al (1985) also reported on it.

The X-ray diffractions of potato starch granules ground by different degrees are shown in Figure 8. The crystallinity indexes of these samples are given in Table III. When the crystallinity index of nonground potato starch granules and those of highly ground potato starch (showing low X-ray peak intensity) are designated as 1 and 0, respectively, we found that the crystallinity index of the intermediately ground sample was 0.430.

TABLE II
Moisture Content and Specific Gravity of Pressure-Treated Starch Granules

	Moisture Content, %		Specific Gravity	
Starch	Untreated	Treated[a]	Untreated	Treated
Corn	11.6[b]	12.4[b]		
	14.5[c]	14.6[c]	1.464[c]	1.475[c]
Potato	15.3[b]	18.4[b]		
	17.1[c]	19.8[c]	1.453[c]	1.449[c]

[a] Pressure-treated at 500 MPa for 20 min.
[b] Before conditioning.
[c] After conditioning at 75% rh.

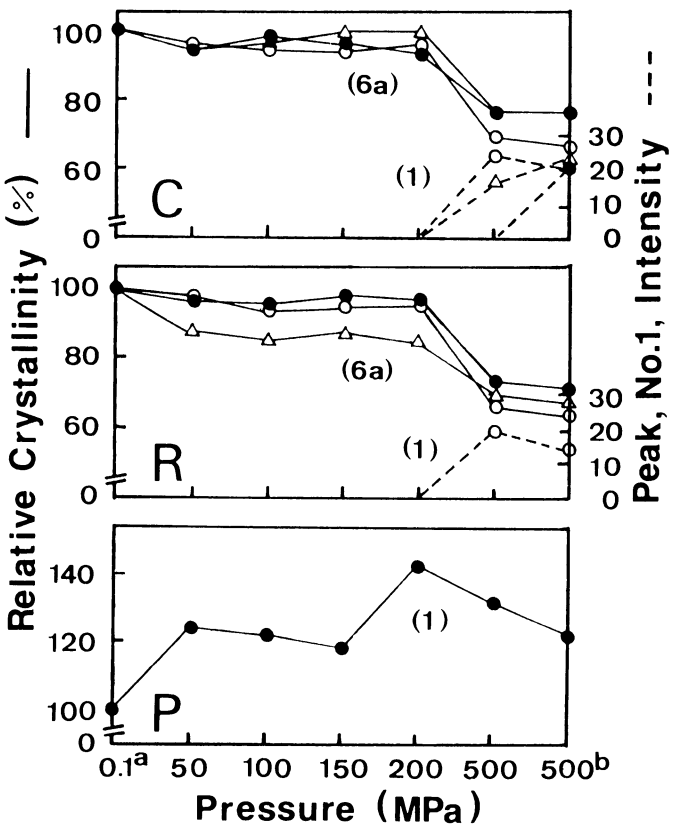

Fig. 6. Changes in the relative crystallinity of corn (**C**), rice (**R**), and potato (**P**) starch granules by pressure applications of 50–500 MPa for 20 min and 500 MPa for 60 min (where 0.1[a] = untreated, and 500[b] = pressure-treated for 60 min at 500 MPa). ● = normal starch, ○ = defatted normal starch, △ = waxy starch.

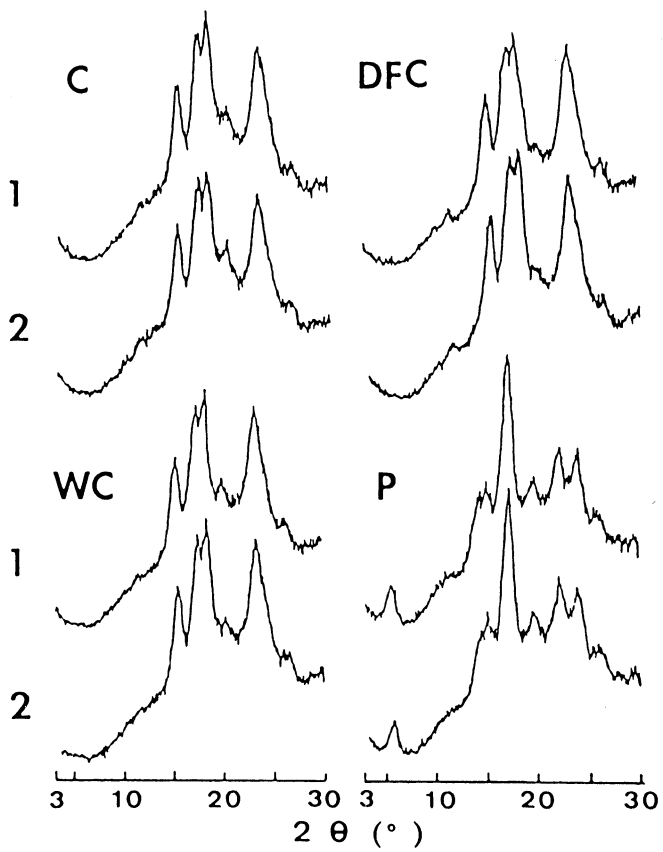

Fig. 7. X-ray diffraction patterns of various starches either untreated (**1**) or pressure-treated in hexane at 500 MPa for 60 min (**2**). **C**, Normal corn starch; **DFC**, defatted normal corn starch; **WC**, waxy corn starch; and **P**, potato starch.

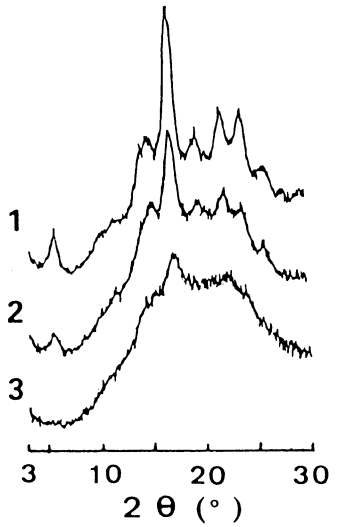

Fig. 8. X-ray diffraction patterns of nonground (**1**), intermediately ground (**2**), and highly ground (**3**) potato starch granules.

TABLE III
Crystallinity of Ground Potato Starch Granule

Sample	Crystallinity Index[a]
Nonground	1.000
Intermediately ground	0.430
Highly ground	0.000

[a] X-ray crystalline indexes of nonground and highly ground starch granules were designated as 1 and 0, respectively.

TABLE IV
Moisture Content of Ground Potato Starch Granules After Conditioning at Various Relative Humidities

	Moisture Content, %		
Sample	0% rh	75% rh	90.6% rh
Nonground	3.0	17.1	22.6
Intermediately ground	1.1	15.7	21.1
Highly ground	1.1	15.8	20.8

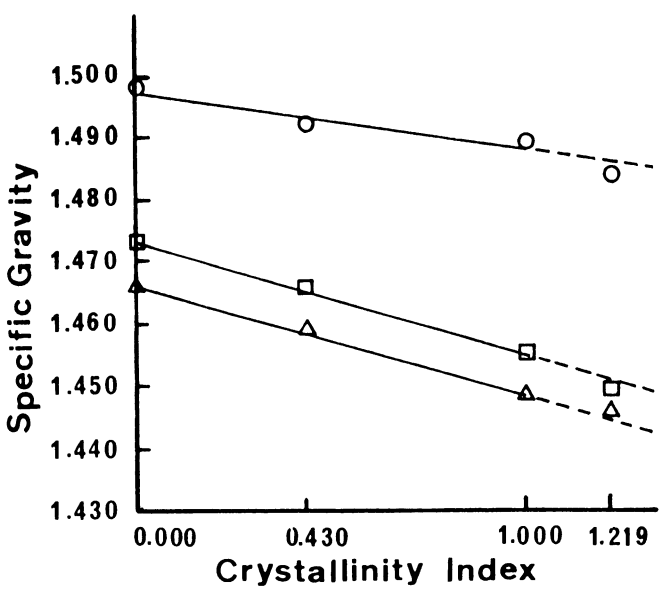

Fig. 9. Relationship among moisture, crystallinity, and specific gravity of potato starch conditioned at 0% rh (○), 75% rh (□), and 90.6% rh (△).

As moisture content affects specific gravity (Nara 1979, Buleon et al 1982, Haine et al 1985), ground potato starches were conditioned at 0, 75, and 90.6% rh before measurement of specific gravity. Moisture contents of these starches are given in Table IV. The moisture content of nonground potato starch (high crystallinity) was slightly higher than those of intermediately and highly ground samples. Figure 9 shows the relationship among moisture, crystallinity, and specific gravity of ground potato starch granules. The higher the moisture contents and crystallinity indexes of the starches, the smaller the specific gravities. This result was similar to the results of Haine et al (1985): the specific volume (or the specific gravity) of potato starch granules increased (or decreased) with more hydration and with higher crystallinity. As linear dependencies were observed between crystallinity indexes and specific gravities at various relative humidities, they were used for potato starch granules pressure-treated at 500 MPa for 20 min. The crystallinity index of the pressure-treated potato starch granules was 1.219; the measured specific gravities of those conditioned at 0, 75, and 90.6% rh were 1.483, 1.449, and 1.446, respectively. The experimental results obviously fell in with the linearity of the crystallinity index and the specific gravity at each relative humidity. We concluded that destroying the crystalline structure of the starch granule increases its density and improving the crystalline structure decreases its density.

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