

Heating A-, B-, and C-Type Starches in Aqueous Sodium Chloride: Effects of Sodium Chloride Concentration and Moisture Content on Differential Scanning Calorimetry Thermograms

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ABSTRACT

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The effects of moisture and NaCl content on the phase transitions of different granular starches were examined by differential scanning calorimetry. Samples were adjusted to starch-water weight ratios of 1:4, 2:3, and 3:2 in NaCl concentrations of 0, 1, 2, 3, 4, 5M. The gelatinization temperatures (GTs) of seven starches increased and then decreased as the NaCl concentration increased. However, the salt effects on starches of different X-ray diffraction patterns varied because of their different granular structures. The GTs of the B-starches with salt decreased to

a level lower than that of the individual original starch, whereas the GTs of A-starches were always higher than those of their respective native samples. The increment of GT in the A-starch was also greater than that in the B-starch. The influence of NaCl on lotus tuber starch (C-starch) was a combination of the modes of A- and B-starches. The amylose content of starch did not affect the influence of NaCl on gelatinization. NaCl created similar effects on the endotherms in excess water content and on the first endotherm with limited water content.

Electrolytes are known to affect the gelatinization temperature (GT) of starch. Anions or cations can cause changes in the GT that are closely related to the effect on the water structure of the aqueous phase (Zobel 1984). Strongly hydrated ions (e.g., sulfate and lithium ions) tend to increase the order of the water structure and to decrease the concentration of "free" water molecules, resulting in the raising of the GT. Takahashi et al (1980) used the critical concentration index to demonstrate the quantitative influence on the GT and correlated it with the factors (i.e., lyotropic value, the hydration number of ion, and the β -coefficient of salt) that could influence the interaction between water and the solute. Evans and Haisman (1982) illustrated the relationship between initial GT, water activity, and the volume fraction of water in the starch granule for the selected solute. Chinachoti et al (1991a) reported that the change in GT resulting from the added solute was correlated with various physical properties, such as water activity, solute-solvent average molecular weight, and the D₂O mobility (T_2) of ¹⁷O nuclear magnetic resonance.

However, it seems that not only the interaction between the salt and water but also the interaction between the salt and starch could affect the gelatinization of starch. NaCl and NaBr decreased the sharpness of X-ray diffraction of potato starch when Takahashi et al (1981) tested the correlation between the gelatinization and the salt retained in the starch granule. Oosten (1982, 1990) viewed starch as a weak ion-exchanger; also, the cation stabilized the starch structure while the anion promoted the rupture of the hydrogen bond. The interaction of salt and starch was also evidenced by the data of ¹³C and ²³Na nuclear magnetic resonance (Chinachoti et al 1991b).

Although many studies concerning the effects of various ions on the gelatinization of starch have been reported, few studies have investigated whether the different granular structures of starches would alter such influence. Hence our study of the effect of NaCl on starch granules with various X-ray diffraction patterns heated under different water contents was initiated to further elucidate the mechanism of the salt effect on starch.

MATERIALS AND METHODS

Starches

Corn, potato, and canna starches were purchased from the starch factory located in Central Taiwan and were purified by

washing with distilled water (Chang and Lii 1981). Three varieties of rice starches including indica (KSS7, 25.6% amylose content), japonica (TNU67, 14.8% amylose content), and waxy (TCW70, a trace of amylose content) rice were isolated by the modified alkaline method (Yang et al 1984). The starch of lotus tuber (*Nelumbo nucifera* Gaertner var. white flower) was separated by the method of Sung et al (1978). All starches were ground to pass a 100-mesh sieve for the differential scanning calorimetry (DSC) investigations and a 200-mesh sieve for the X-ray diffraction studies.

X-Ray Diffraction

The starch was saturated with water vapor overnight in a closed container. The X-ray diffraction patterns of the samples were determined by using an X-ray diffractometer (Riga-ku/MAX-III A, Rigaku Keisoku Co, Ltd., Tokyo, Japan) at 35 kV and 25 mA and were scanned from 30° to 4° of 2 θ .

DSC

Samples were adjusted to starch-water weight ratios of 1:4, 2:3, and 3:2, and concentrations of NaCl were prepared at 0, 1, 2, 3, 4, and 5M in the aqueous phases of starch suspensions. Samples (7-12 mg) were hermetically sealed in a coated aluminum pan (Du Pont, Wilmington, DE) and were held at room temperature for 1 hr. A Du Pont 1090B thermal analyzer and the 990 DSC cell base were used for the analysis. Samples were scanned from 25 to 140°C at a rate of 10°C/min. Each test was run in triplicate, and the standard deviation of the temperature measurement was below 0.5°C in all samples. The characteristic temperatures—onset, peak, and conclusion were determined by computer (Lund 1983).

RESULTS AND DISCUSSION

X-Ray Diffraction Patterns of Various Starches

The X-ray diffraction patterns of starches used in the studies are shown in Figure 1. Cereal starches (corn and rice) could be classified into type A. Two tuber starches (potato and canna) belonged to type B, but lotus tuber starch was type C (Zobel 1964, French 1984). However, two peaks, lines 2a and 2b were not observed on the diffractograms of A-type starches, except those of waxy rice starch (TCW70) and the rice starch with low amylose content (TNU67). The different diffraction patterns of starch samples implied that various granule structures existed among the starches (Sarko and Wu 1978, Imberty and Perez 1988).

Effects of NaCl Content on A- and B-Type Starches

Generally, as the concentration of NaCl increased, the GT increased to a certain level and then decreased as the concentration

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increased (Fig. 2). Oosten (1982) attributed such results to the fact that starch is a weak acid ion-exchanger. The OH-group of starch could dissociate protons into the aqueous phase. Consequently, the starch granule carries a negative charge and the water phase a positive charge. This results in building the so-called Donnan potential between the starch granule and the water phase. The negative potential of the starch granule attracts the cation and repels the anion. Oosten (1990) also suggested that the anion is the gelatinizing agent and could promote the gelatinization of starch by rupturing the hydrogen bond between starch molecules. Thus, the high Donnan potential in the starch suspension retards the gelatinization of starch. When a swelling-inhibitor (e.g., sodium chloride) was added to a starch suspension, some protons of alcohol groups in the starch granule were exchanged by the sodium ions. These alcoholates were better dissociated, thus causing a rise in the Donnan potential. However, the exchange capacity of starch for the sodium ion was limited, so the increase of the GT was also restricted. When the concentration of NaCl was raised beyond a certain level, the influence of the anion became dominant. This would contribute to decreasing the GT of starch (Oosten 1979, 1982, 1983, 1990).

The influence of the NaCl varied on the starches with different

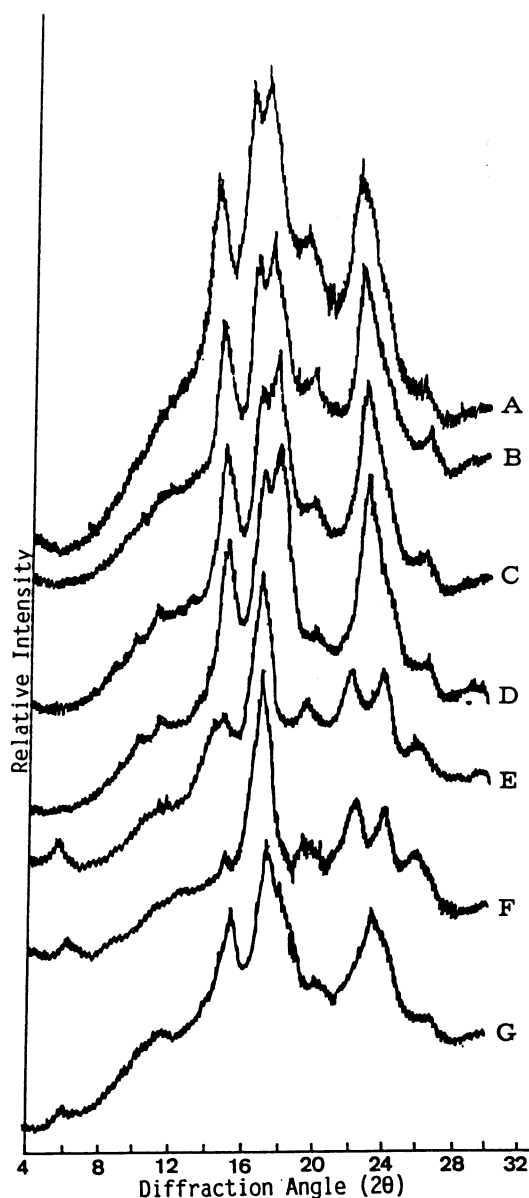


Fig. 1. X-ray diffraction patterns of different starches: corn (A), KSS7 rice (B), TNU67 rice (C), TCW70 waxy rice (D), potato (E), canna (F), and lotus tuber (G).

patterns of X-ray diffraction. The A-starches, which included corn and three varieties of rice, showed the highest peak temperatures of the endotherms at 2–3M concentrations of NaCl (Fig. 2). The peak temperatures were raised about 10°C. At concentrations of 3–5M, the peak temperatures of endotherms decreased with the increase of the salt concentration but were still higher than those of their respective starches without added salt. The peak temperatures of the tuber B-starches (potato and canna) were highest at concentrations of 1–2M and only increased less than 2°C (Fig. 2). In the 2–5M NaCl solutions, the temperature decreased with increasing salt concentration to 10°C less than the temperature of the original starch.

The results showing the influence of NaCl on A-starch agreed with the effects of salt on wheat starch (Sandstedt et al 1960) and on corn starch (Oosten 1979), both by Brabender amylography. The effects on B-starches were similar to those of studies on potato starch by Gerlsma (1970) with polarized-light microscopy, Takahashi et al (1980) with differential thermal analysis, and Evans and Haisman (1982) with DSC. The crystalline region of B-starches is made up of a hexagonal arrangement in which the left-handed parallel-stranded double helices are packed in a parallel fashion, and the center portion is packed with structural water (Imberty and Perez 1988). The proposed structure of A-starches is more compact, with a double helix occupying the central open space instead of the water proposed for B-starch (Sarko and Wu 1978). The more compact granular structure of the A-starch could be much more capable of resisting the gelatinizing effect of the chloride ion. This contributed to NaCl decreasing GT less in A-starch than in B-starch. In addition, it might imply that A-starch could create a higher Donnan potential than B-starch, judging by the effect of NaCl on the pH value of the starch suspension (Oosten 1990). The high Donnan potential would restrict the gelatinization process.

The effects of NaCl on the three varieties of rice starches were similar (Fig. 2), which suggests that the various amylose contents did not affect the influence of the salt.

Effects of NaCl on the Starches at Various Water Contents

The profiles of the DSC thermal curves of starches at low water content (starch-water ratio = 3:2) were not influenced by NaCl (Fig. 3). Potato starch showed two endothermic transitions,

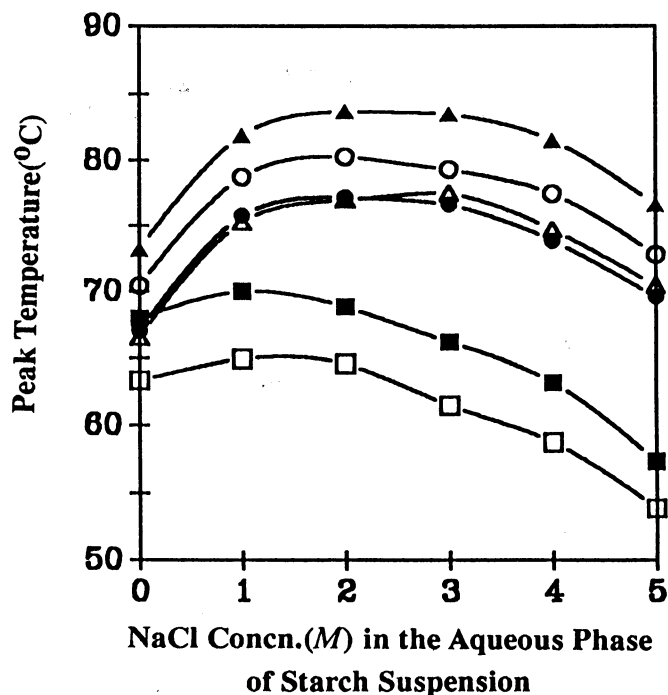


Fig. 2. Effect of salt on peak temperature of phase transition with a 2:3 starch-water ratio. ○ = corn, ● = TCW70 waxy rice, △ = TNU67 rice, ▲ = KSS7 rice, □ = potato, ■ = canna.

and the other starches had three. The DSC thermogram of potato starch was similar to the result reported by Donovan (1979) in the presence of limited amounts of water (water to starch = <1.5:1, w/w). The sharp third endotherm observed on the waxy rice starch might be attributed to the melting of annealed or reorganized material, and the third endotherms of corn and KSS7 starches might be due to the melting of the lipid-amylose complex (Biliaderis et al 1986). However, both results require further study for elucidation.

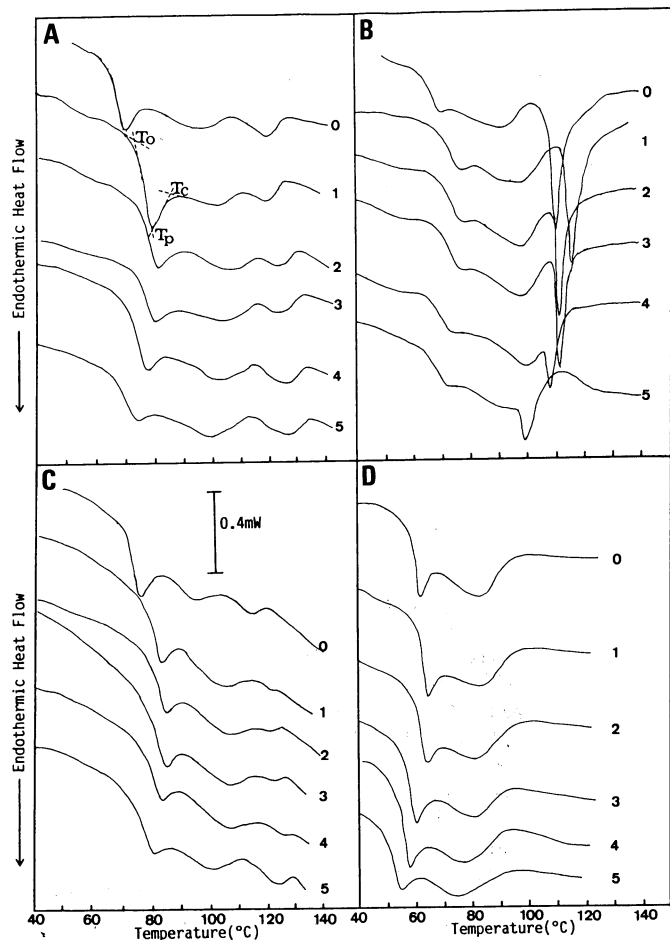


Fig. 3. Differential scanning calorimetry thermograms of starches at a 3:2 starch-water ratio. A, corn; B, TCW70 waxy rice; C, KSS7 rice; D, potato. Numbers indicate the molarity of the NaCl in the aqueous phase of the starch suspension. T_o = temperature of onset, T_p = peak temperature, T_c = conclusion temperature.

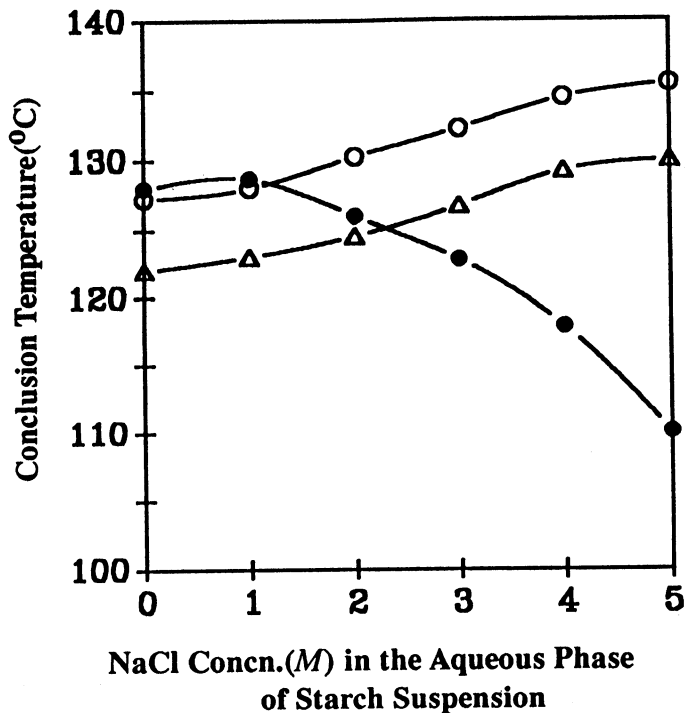


Fig. 5. Effect of salt on the conclusion temperature of the third phase transition with a 3:2 starch-water ratio. \circ = corn, \bullet = TCW70 waxy rice, Δ = KSS7 rice.

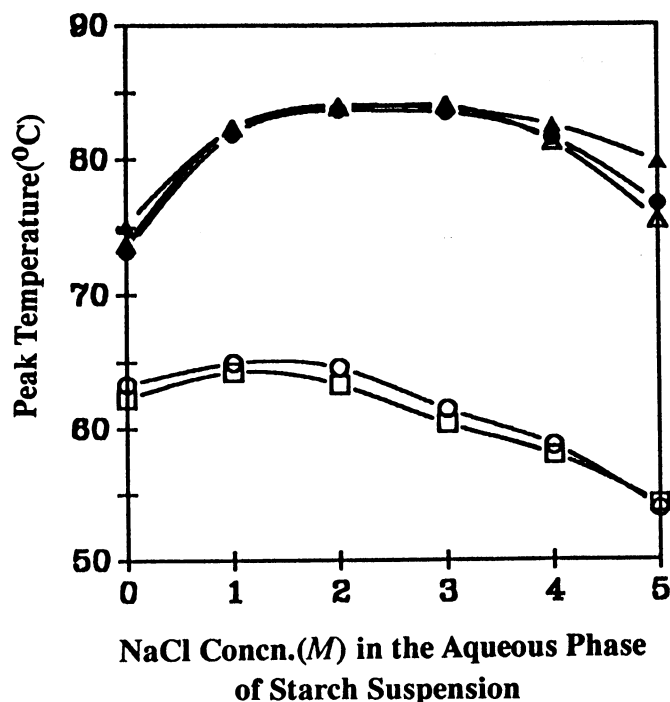


Fig. 4. Effect of salt on peak temperature of the first endotherm of KSS7 rice and potato starches with various water contents. \bullet = KSS7 rice-water, 1:4, Δ = KSS7 rice-water, 2:3, \blacktriangle = KSS7 rice-water, 3:2, \square = potato-water, 2:3, \circ = potato-water, 3:2.

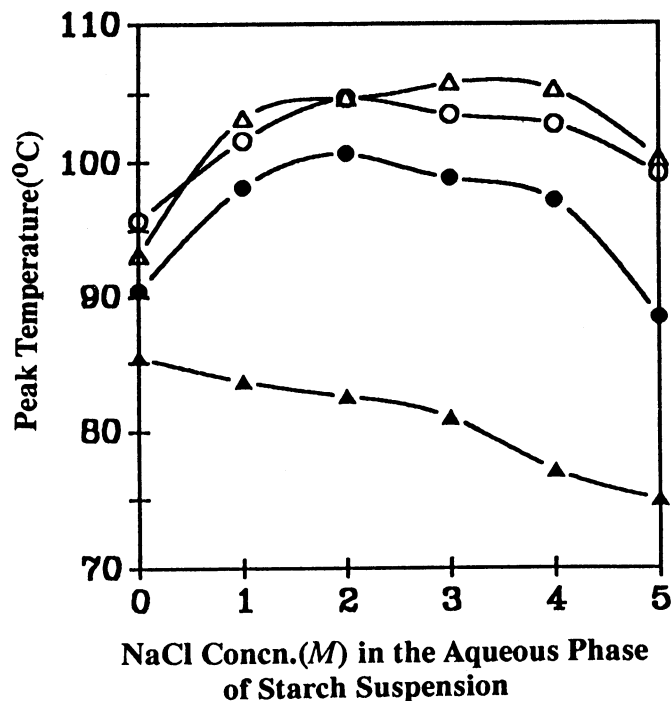


Fig. 6. Effect of salt on peak temperature of the second endotherm with a 3:2 starch-water ratio. \circ = corn, \bullet = TCW70 waxy rice, Δ = KSS7 rice, \blacktriangle = potato.

The effects of sodium chloride on the peak temperatures of the first endotherms with limited water content (starch-water ratio = 3:2) (Fig. 4) were similar to those of the first endotherms with excess water (starch-water ratios = 1:4 and 2:3). The first endothermic peak could reflect an entropy-driven swelling process (Donovan 1979, Blanshard 1987), which implies that changes in water content would not affect the influence of sodium chloride on the swelling of granules during starch gelatinization.

However, the mode of salt influence on the third endotherm was highly different. The third peak of waxy rice TCW70 was smaller and appeared at lower temperature with high salt concentration ($>2M$). NaCl might decrease the stability of the reorganized materials (Figs. 3 and 5). Although the peak of the lipid-amylose complex overlapped with the second peak in the KSS7 rice sample, the conclusion temperature of the endotherm in corn and KSS7 rice were increased by NaCl (Fig. 5). Such results agreed with the data of Biliaderis and Seneviratne (1990), who found that the effectiveness of anions and cations in stabilizing the ordered-chain domains of the complex follows the classical Hofmeister series. The influence of salt on the second endotherm was intermediate between the effects on the first and third endotherms in the A-starch. Nevertheless, the peak temperature of the second endotherm in potato starch was decreased when NaCl was added, even at low concentration (Fig. 6). It might be due to the influence of Donnan potential in the first endotherms of all starches and in second endotherms of

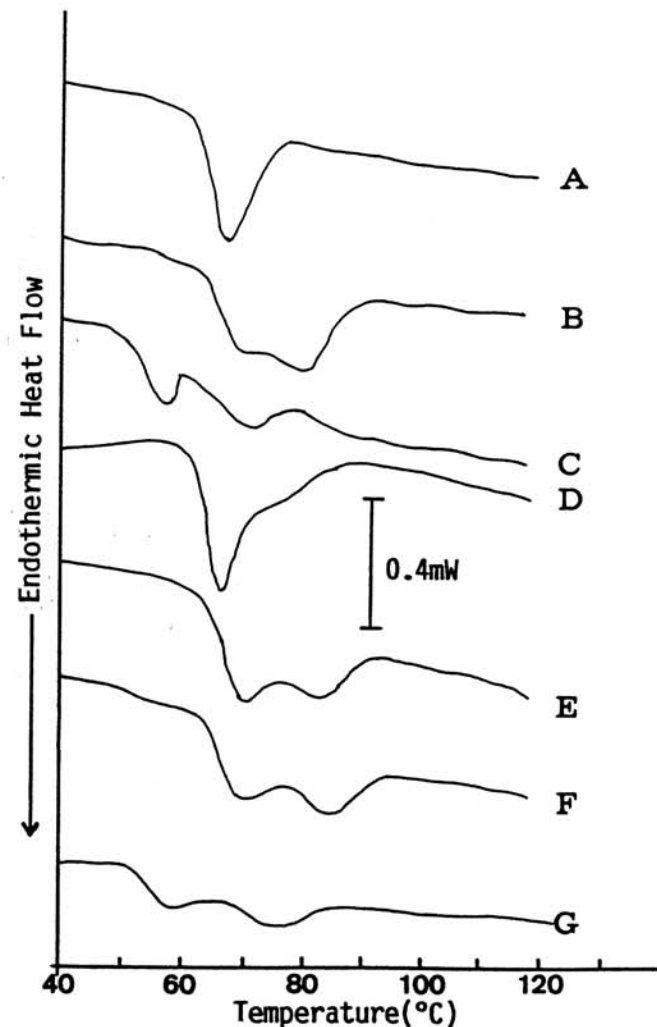


Fig. 7. Effect of salt on the differential scanning calorimetry thermogram of lotus tuber starch at a 1:4 starch-water ratio without NaCl (A), with 2M NaCl (B), and with 5M NaCl (C); and at a 2:3 starch-water ratio without NaCl (D), and with 1M NaCl (E), 2M NaCl (F), and 5M NaCl (G).

A-starches at limited water contents. Since the chloride ion already entered into the crystalline region and underwent "true melting," such Donnan potential influence did not show in the second endotherm of potato starch.

Effect of NaCl Concentration on the C-Type (Lotus Tuber) Starch

NaCl had some peculiar influences on the lotus tuber starch, with the type C X-ray diffraction pattern, as shown in Figure 7. One simple endothermic peak appeared on the sample with a starch-water ratio of 1:4. A second peak developed as the concentration of the NaCl increased. At lower water content (starch-water = 2:3, w/w), a small shoulder showed on the end of the peak. The shoulder grew into the second separate peak as more salt was added. The effect of salt on the transition temperature of the first endotherm was similar to that of B-starch, and the second one was like that of A-starch (Table I). It could be concluded that the effect of salt on lotus tuber starch was a combination of those on A- and B-starches.

Since the X-ray diffraction pattern of C-type starch can be obtained from mixtures of A- and B-type starch granules or from each individual granule consisting of A- or B-type crystalline structures (Hizukuri and Nikuni 1957), the following experiment was designed to determine the crystal type of lotus tuber starch. The pan packed with the mixture of starch and 5M NaCl solution at a ratio of 2:3 (w/v) was heated to the conclusion temperatures of the first (67°C) and second (87°C) endotherms by DSC. The heated starch sample was examined by microscope. The granules were highly swollen, but the tips of the granules were still intact at the conclusion temperature of first endotherm (Fig. 8C). Most granules did not lose birefringence completely under polarized light. Residual birefringence was observed in the unswollen part

TABLE I
Effect of NaCl on the Differential Scanning Calorimetry Characteristics of Lotus Starches at 60% Water Contents

NaCl Concentration in the Aqueous Phase (M)	First Endotherm ^a			Second Endotherm ^a		
	T_o	T_p	T_c	T_o	T_p	T_c
0	61.8	66.6	86.5	... ^b
1	64.6	69.9	77.0	77.9	85.1	92.0
2	64.4	69.5	77.0	78.0	85.4	95.0
3	61.7	66.3	73.5	75.3	83.8	93.5
4	57.4	63.5	71.0	72.0	81.6	94.0
5	51.9	58.2	66.5	67.4	74.8	86.0

^a T_o = onset temperature, T_p = peak temperature, T_c = completion temperature.

^b Not detectable.

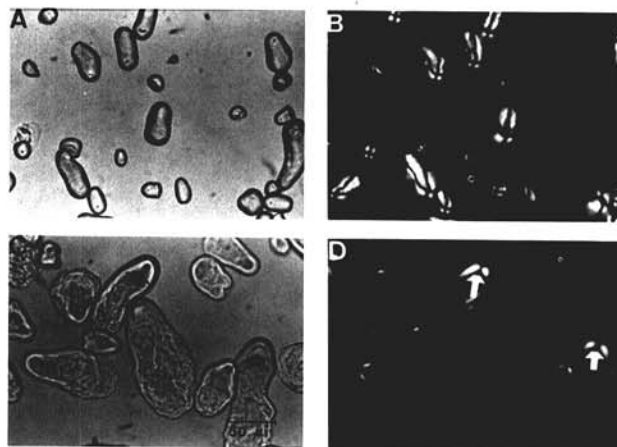


Fig. 8. Effect of NaCl on the birefringence of lotus tuber starch. Light micrograph (A) and polarized-light micrograph (B) of native starch; light micrograph (C) and polarized-light micrograph (D) of the sample (2:3 starch-water with 5M NaCl added) after heating to the conclusion temperature of the first endotherm by differential scanning calorimetry.

of the granule (Fig. 8D). No birefringence was detected in the starch sample at the conclusion temperature of the second endotherm. The same treatment (heating to the conclusion temperature of first and second endotherms) to the mixture of starch and 5M NaCl solution at a ratio of 1:4 showed the same results (data not shown). Such results might lead to the conclusion that lotus tuber starch consisted of crystalline regions A and B within each individual granule. The Donnan potential could be produced by NaCl not only between the granule and the water phase but between these two crystalline regions.

CONCLUSION

When starches were heated in sodium chloride solution, B-starches showed a small increase and then a significant decrease in GT (lower than that of the original sample) as the concentration of NaCl was raised to 5M. A-starches were more resistant to chloride ions because of their compact structures. Furthermore, A-starches could produce a higher Donnan potential, which contributed to the higher maximum temperature and reduced the effect of chloride ions on gelatinization.

The contours of the DSC thermograms of starches with NaCl at low water content (starch-water ratio = 3:2) were not changed significantly. The similar effect of salt on the first endotherm in limited water content and the endotherm for gelatinization in excess water content was because of the same melting mechanism of these phase transitions. The peak temperature of the second endotherm in potato starch was not increased further by the addition of NaCl, since the chloride ion had already entered the crystalline region and undergone "true melting."

The results of studies of lotus tuber starch (C-type) further proved the difference in action of salt on A- and B-starches. The heterogeneity of crystallinity within individual granules resulted in two endotherms in excess water in the presence of salt. The effect of the salt on the first endotherm was similar to the effect on B-starch, and the second one was like that on A-starch. Such phenomena suggested the Donnan potential could be produced by NaCl not only between the granule and the water phase but also between the A and B crystalline regions inside the granule.

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