Effect of β-Amylase Stability and Starch Gelatinization during Heating on Varietal Differences in Maltose Content in Sweetpotatoes[†]

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To elucidate the factors affecting varietal differences in maltose contents in sweetpotatoes, changes in maltose content, β-amylase activity, and gelatinization of starch during heating were studied using lines of high, moderate, and low maltose content. Although β-amylase activity decreased with an increase in temperature in all lines, it had a greater heat stability in the high maltose line than in the other lines. However, there was not a large difference between the moderate and low lines. Starch gelatinization of high maltose lines occurred at a lower temperature than did that of other lines. These results indicate that the heat stability of β-amylase and early gelatinization of starch granules during heating have an important role in the high maltose lines.

Keywords: *Sweetpotato; β-amylase; heat stability; starch gelatinization; maltose*

INTRODUCTION

It is well-known that a heated sweetpotato possesses a large amount of maltose produced from the inner starch by β-amylase hydrolysis (Sistrunk et al., 1954; Deobald et al., 1969; Walter et al., 1975; Kiribuchi and Kubota, 1976; Walter and Purcell, 1976). The maltose content remarkably affects the eating quality of baked sweetpotato and industrial products such as puree or flakes. In addition, the principal reason for consumer purchase of sweetpotatoes in developed contries is sweetness. For selection of a sweetpotato variety, therefore, it is indispensable to obtain information on the varietal differences in maltose content and the mechanism of its formation.

Some researchers have reported varietal differences in maltose content in heated roots or processed products and discussed the reason for the difference. On the relationships of maltose contents and raw root β-amylase activities, Walter et al. (1975) reported that β-amylase levels did not seem to directly affect the amount of maltose produced during baking. Baba et al. (1987) also mentioned that there was no significant correlation between the β-amylase activities and the amount of increase in reducing sugar during cooking of sweetpotatoes.

On the other hand, Picha (1986) reported that the order of rank in maltose concentration among cultivars paralleled that of alcohol-insoluble solids that were negatively correlated with moisture content. However, among cultivars that had similar levels of moisture contents, there was a significant varietal difference in maltose contents over several years' productions (Table 1; Takahata et al., 1992). Table 2 summarizes the results of ANOVA for maltose and dry matter content. The factor "line" is far less influential on the dry matter content than on the maltose content. Actually, the fact that the maltose content increased with increasing dry matter content is due to the increase in the starch content. Thus, maltose production increased because the substrate content increased. However, this cannot explain the whole mechanism of varietal differences in maltose contents. McArdle and Bouwkamp (1986) studied the relationships between heating temperature and carbohydrate changes in the mash from two sweetpotato cultivars; however, their results did not conclusively suggest a probable cause of the cultivar difference.

Table 1. Varietal Differences in Maltose Content in Steamed Sweetpotato Root over Several Years[a]

		1992		1990		1989	
type of line	name of line	DM	maltose	DM	maltose	DM	maltose
high maltose lines	Kyushu91	36.8	145.1	37.0	146.9	42.5	188.2
	Kyushu104	37.9	153.7	39.7	146.1	43.7	174.1
moderate maltose lines	Shiroyutaka	36.3	119.7	37.5	110.4	40.1	142.0
	Koganesengan	38.2	133.3	38.0	117.8	41.7	131.9
low maltose lines	Naeshirazu	34.5	91.7	39.2	61.8	40.1	85.1
	Shirosatuma	37.0	97.6	38.6	92.7	43.9	112.7

[a] Data are expressed as a mean of at least three replicates. DM, dry matter content (%); maltose, maltose content (mg/g, fresh weight basis of heated root).

Table 2. ANOVA of Maltose Content and Dry Matter Content

trait	factor	df	sum of squares	variance	*F* value[a]
maltose content					
	line (A)	5	41820.5	8364.1	66.6**
	year (B)	2	6173.1	3086.6	24.6**
	A × B	10	2363.4	236.3	1.88
	error	37	4647.6	125.6	
dry matter content					
	line (A)	5	48.7	9.74	6.52**
	year (B)	2	249.3	124.6	83.4**
	A × B	10	38.6	3.86	2.58*
	error	37	55.3	1.49	

[a] **, *, significant at 1 or 5%, respectively.

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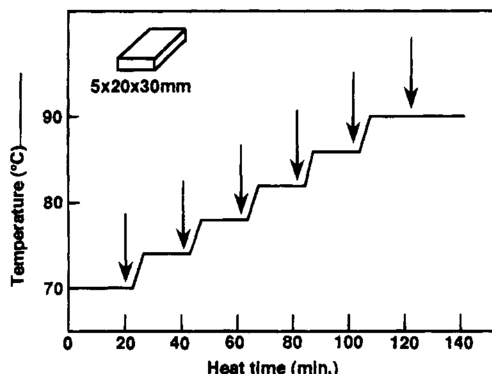

Figure 1. Schematic illustration of heating procedure. Each arrow indicates sampling time.

As mentioned above, the causes of varietal differences in maltose content are still obscure. In the present study, to shed more light on the factors affecting the varietal differences in maltose content of heated sweetpotato roots, we investigated the inactivation of β-amylase and gelatinization of starch granules during heating using six sweetpotato lines that had similar moisture contents.

MATERIALS AND METHODS

Sweetpotato Materials. The six lines shown in Table 1 were cultivated in an experimental field under the same conditions at Nishigoshi, Kumamoto, Japan. The lines were harvested on October 7, 1992, and then stored at 17–20 °C until use.

Sample Preparation and Heating Procedure. Figure 1 illustrates the sampling procedure. Seven pieces of cut tissue that had a size of 5 × 20 × 30 mm were prepared from the central portion of a storage root. All of the pieces were tightly wrapped with aluminum foil; one piece was used as a raw sample, and the other six pieces were placed in a time-programmed convection oven for heated samples. The latter were put in the oven at 70 °C for 20 min. One piece was taken out from the oven and was defined as the 70 °C sample. The oven temperature was then raised at 1 °C/min to 74 °C and held at 74 °C for 20 min. One piece was taken out and was defined as the 74 °C sample. These procedures were repeated to 90 °C in 4 °C steps. Hence, including the raw piece, various materials that were heated at seven different temperature levels were obtained. Each heated piece taken from the oven was left at room temperature for about 20 min to allow its heat to diffuse and was used for the following analysis. Three roots were used per line, and all data were averaged.

Extraction and Assay of β-Amylase. All extraction procedures were carried out at 0–4 °C in ice, unless otherwise described. Samples were ground in 50 mM HEPES–NaOH buffer (pH 7.4) containing 12.5% glycerol, 4 mM $MgCl_2$, 2 mM EDTA, and 50 mM β-mercaptoethanol with a mortar and pestle and centrifuged at 15000*g* for 10 min. The supernatant was dialyzed three times with 50 mM sodium acetate buffer (pH 4.8) containing 12.5% glycerol for 2–3 h and diluted properly with the same buffer to prepare it for assay of β-amylase and native polyacrylamide gel electrophoresis (native PAGE). A 50 μL portion of enzyme preparation was added to 200 μL of 50 mM sodium acetate buffer (pH 4.8, 12.5% glycerol) containing 1% soluble starch and incubated in a water bath (40 °C) for 10 min. The reaction was stopped by the addition of 750 μL of DNSA reagent (Luchsinger and Cornesky, 1956). The mixture was heated for 5 min in boiling water and cooled; 4 mL of distilled water was added, and absorbance at 540 nm was measured.

Native PAGE and β-Amylase Active Stain. Native PAGE was performed on slab gels prepared with 7% (resolving gel) and 4% (stacking gel) acrylamide according to a modification of the method of Davis (1964). Electrophoresis was carried out at 4 °C at a constant current of 12 mA. Active stain of β-amylase was carried out according to a modification of the method of Lin et al. (1988). After electrophoresis, the gel was rinsed with distilled water and imbibed in 50 mM sodium acetate buffer (pH 4.8, 12.5% glycerol) containing 1% soluble starch. The gel was gently shaken for 1 h at room temperature. The gel was then rinsed with distilled water to remove the excess starch solution and left in a capped container for 1.5 h at 40 °C. The gel was stained with an iodine solution that consisted of 0.1% iodine and 1% potassium iodide. The mobility of β-amylase was confirmed with authentic β-amylase purchased from Sigma (type I-B).

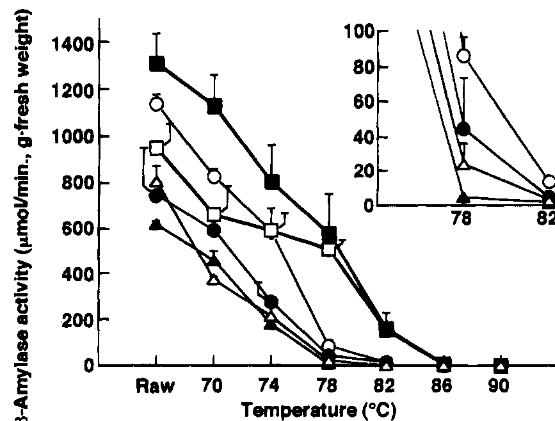

Figure 2. Change in β-amylase activity during heating: (□) Kyushu91; (■) Kyushu104; (○) Shiroyutaka; (●) Koganesengan; (▲) Naeshirazu; (△) Shirosatuma. Each value is the mean of three replications. Results are reported as micromoles of reducing sugar (as maltose) produced per minute per gram of fresh weight. Vertical bars indicate standard deviation. (Inset) Magnification at 78 °C

Microscopic Examination of Starch Granules. A small amount of each sample tissue was suspended with glycerol–water solution and was mounted on a slide glass. Examination of the degree of starch gelatinization was carried out on the basis of the evaluation of loss of starch granule birefringence using polarization microscopy (Schoch and Maywald, 1956). Gelatinization degree was defined as the percentage of starch granules that lost their birefringence.

Extraction and Determination of Maltose. Extraction of maltose was performed according to the method of Picha (1985). Determination of maltose was performed according to the procedure given in the previous paper (Takahata et al., 1992).

RESULTS

Inactivation of β-Amylase. Figure 2 shows the inactivation profile of β-amylase of each line during heating. β-Amylase activities of raw roots of the six lines ranged from 600 to 1300. During heat treatment, β-amylase activity decreased with the increase in temperature in all lines. The most striking feature found was the difference between high maltose lines and the others above 74 °C. One of the high maltose lines, Kyushu91, retained its β-amylase activities of 510 (54%, compared to the activity of the raw root) at 78 °C and 150 (16%) at 82 °C. Another high maltose line, Kyushu104, showed almost the same tendency with activities of 578 (44%) at 78 °C and 160 (12%) at 82 °C. However, moderate maltose lines, Shiroyutaka and Koganesengan, retained activities of only 86 and 44 (8% and 6%) at 78 °C, respectively, and they had almost no activity at 82 °C. The lowest maltose line, Naeshirazu, completely lost its β-amylase activity even at 78 °C. Another low maltose line, Shirosatuma, also had less activity (24, 3%) at 78 °C than moderate lines and lost it at 82 °C. All of the lines lost their β-amylase activities at 86 and 90 °C.

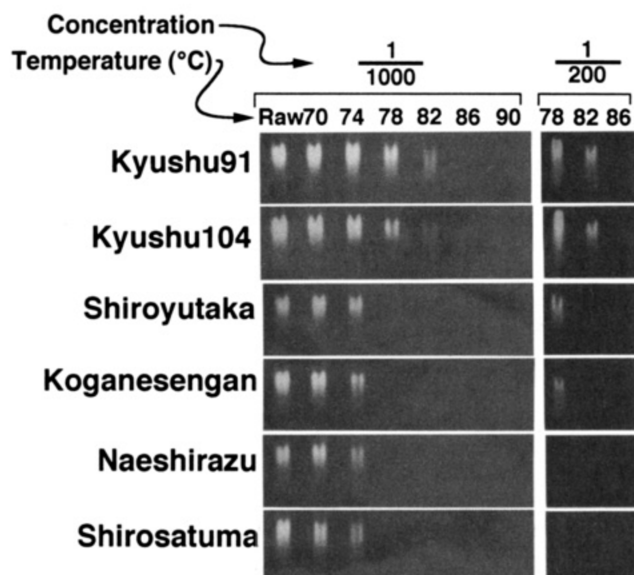

Figure 3. Electrophoretogram of native PAGE active stain of β-amylase during heating. Concentration of enzyme preparation: (left) 1/1000; (right) 1/200.

Table 3. Change in Starch Gelatinization Degree of Six Sweetpotato Cultivars during Heating[a]

type of line	name of line	gelatinization degree[b] raw	70 °C	74 °C	78 °C
high maltose lines	Kyushu91	<1	29 ± 5	58 ± 10	>80
	Kyushu104	<1	31 ± 14	62 ± 4	>80
moderate maltose lines	Shiroyutaka	<1	<1	<1	38 ± 3
	Koganesengan	<1	<1	24 ± 6	61 ± 4
low maltose lines	Naeshirazu	<1	<1	<1	32 ± 7
	Shirosatuma	<1	<1	7 ± 5	38 ± 8

[a] Data are expressed as a mean of three replicates (±SD). [b] Percentage of starch granules that lost their birefringence. For details, see text.

The electrophoretogram of native PAGE active stain of β-amylase is shown in Figure 3. No band was detected except for the β-amylase active band. The active band pattern of native PAGE closely corresponded to the result in Figure 2. Specifically, the right side of Figure 3 (1/200 concentration) clearly shows the distinct varietal differences in β-amylase inactivation. At 78 °C, the high and moderate lines had significant activities, whereas the low lines had no or negligible activity. At 82 °C, only the high lines had significant activity. Thus, it was confirmed that the reducing power assayed in Figure 2 was due exclusively to β-amylase.

Gelatinization of Starch Granules. Starch granule birefringence disappeared by gelatinization with an increase in temperature in all lines; however, the temperature at which birefringence began to disappear was different among the lines. Table 3 shows the varietal differences in starch gelatinization degree during heating. Accurate figures could not be obtained above 78 °C for all lines or at 78 °C in the high lines, because parts of the starch granules were aggregated or completely destroyed. In any event, certain parts of the starch granules were gelatinized above 74 °C in all lines. Figures 4 and 5 depict examples of the disappearance in two representative lines. Some starch granules of Kyushu91 (high maltose line) began to gelatinize even at 70 °C, and more than half of them gelatinized at 74 °C. Another high maltose line, Kyushu104, had similar starch gelatinization properties. However, starch granules of the low and moderate maltose lines gelatinized above 70 °C. Shiroyutaka, one of the moderate maltose lines, and Naeshirazu, one of the low maltose lines, began to gelatinize at 78 °C. On the other hand, Koganesengan (moderate) and Shirosatuma (low) began to gelatinize at 74 °C; whereas the gelatinization degree of Shirosatuma was smaller than that of Koganesengan.

Change in Maltose Content. Figure 6 shows the change in maltose content during heating. In the high maltose lines, maltose content linearly increased up to 86 °C and finally reached the same level as that of steamed whole roots. In the case of the moderate maltose lines, the largest increase was seen in the stage from 74 to 82 °C and remained constant at greater than 82 °C. Compared to the moderate lines, the maltose increases in the low maltose lines were gradual up to 82 °C and remained constant above 82 °C. The final maltose contents (90 °C sample) of low and moderate maltose lines were lower than those of steamed whole roots. However, the order of the rank of maltose contents of the 90 °C samples was the same as that of steamed whole roots.

DISCUSSION

Shen and Sterling (1981) reported the difference in amylolytic activity between moist and dry type sweetpotato roots. Using crude extracts of air-dried (25 °C) flour, they compared the amylolytic activities of the two varieties at different temperatures. The assay method they adopted involved direct incubation of the reaction mixtures in glass tubes at various temperatures. They found a more active enzyme in the moist type at all temperatures, but the temperatures of the activity peak were almost the same in both varieties. They also reported a significant decrease in the enzyme activity of the dry type compared to that of the moist type above the peak temperature. However, this decrease did not reflect the inactivity of β-amylase exactly, because their data contained amylolytic activity that was generated from the initial temperature up to the set temperature. In addition, they did not investigate starch gelatinization with an increase in temperature. In contrast, our results were obtained from intact tissues that were heated with a gradual increase in temperature. Moreover, using the technique of native PAGE active stain, we confirmed that the amylolytic activities of all cases were exclusively derived from β-amylase. Thus, we clearly demonstrated that the varietal differences in heat stability of β-amylase were distinct between high maltose lines and the others.

Takeda et al. (1986) reported that the gelatinization behaviors of suspensions of the sweetpotato starches heated at constant temperature were different among the cultivars. They indicated that cv. Norin2 starch showed a slightly lower gelatinizing tendency than the others at 65–73 °C by means of the two methods of glucoamylase digestion and iodine titration. In our study, it was not possible to use the methods of glucoamylase digestion and iodine titration to obtain an accurate degree of gelatinization because the gelatinized starch granules within each intact tissue were already partially hydrolyzed by β-amylase. In addition, these methods are less convenient than the method of polarization microscopy. Thus, we used polarization microscopy to evaluate the gelatinization of starch granules in intact tissues. We successfully showed the difference

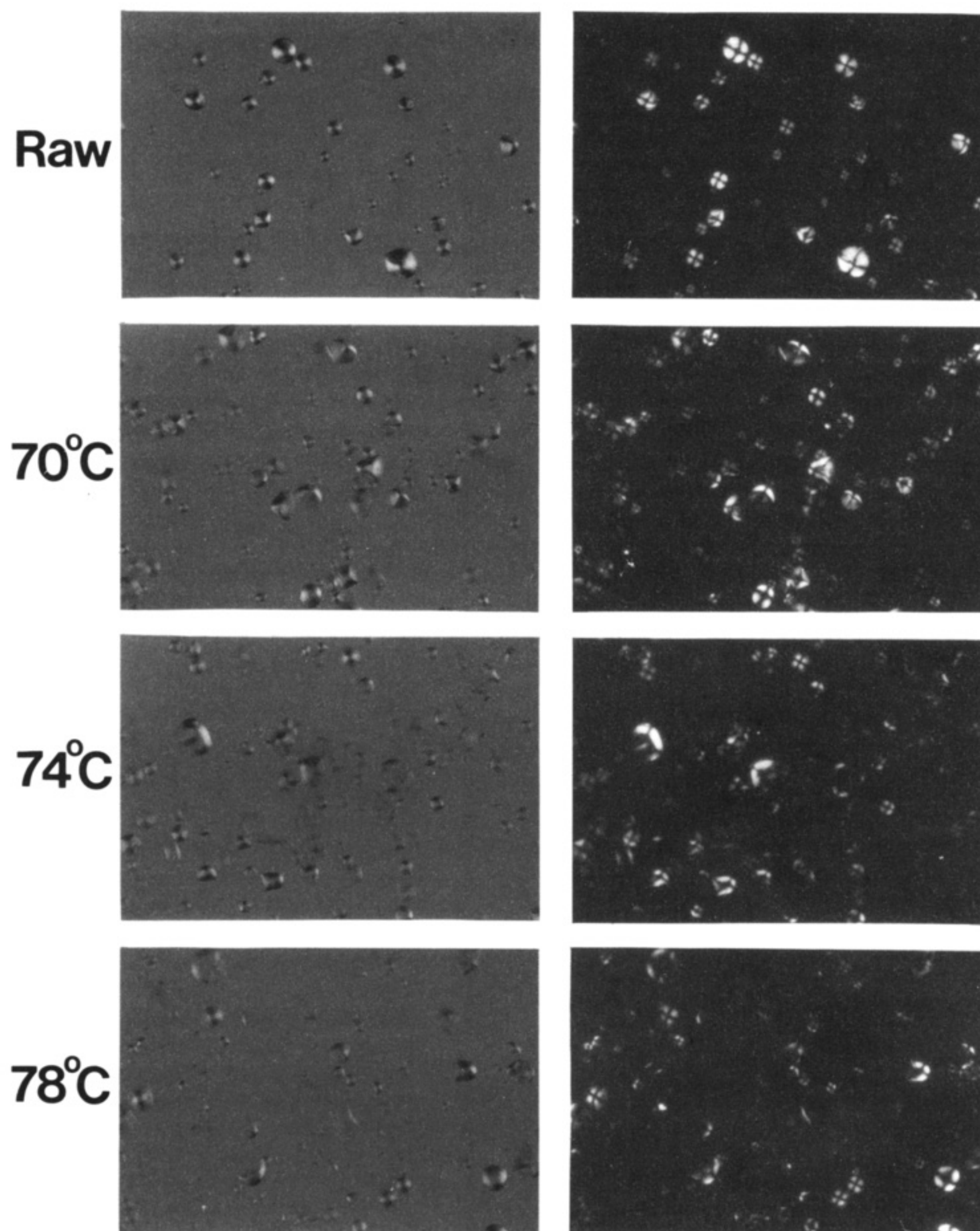

Figure 4. Micrographs of starch granules of Kyushu91 during heating: (left) normal light; (right) polarized light.

in the starch gelatinization property during heating between high maltose lines and the others.

When the dry matter contents differed greatly, as seen in Picha's (1986) study, the amount of substrate, starch, which paralleled the dry matter contents, seemed to have the most important influence on maltose contents. However, among the lines that showed similar dry matter contents in this study, the stability of β-amylase and the gelatinization of starch granules during heating have an important role, especially for the high maltose lines. The residual β-amylase activities of the high maltose lines were much higher than those of the other lines at 78 and 82 °C. On the other hand, the starch granules of the high maltose lines began gelatinization even at 70 °C and earlier than those of the other lines. Both of these traits of the high maltose lines are thought to be the cause of the great increase in maltose up to 86 °C and are considered to be the reason for the high maltose content in steamed whole roots.

Comparing the low and moderate maltose lines, there were no large differences of changes in the maltose content, inactivity of β-amylase, and gelatinization of starch granules. However, that the cultivar Naeshirazu completely lost its β-amylase activity even at 78 °C when starch began to gelatinize is considered to be the reason for the lowest maltose content of steamed whole roots for three different years. Another low maltose line, Shirosatuma, had a similar β-amylase inactivation profile compared to the cultivar Naeshirazu. However, the maltose content in Shirosatuma during heating was higher than that of Naeshirazu. This difference may be caused by the starch gelatinization that occurred in Shirosatuma, but not in Naeshirazu, at 74 °C. The largest increase in the maltose content of Siroyutaka, one of the moderate maltose lines, from 74 to 82 °C was due to the higher residual activity compared to the low maltose lines. On the other hand, the inactivation profile of β-amylase of Koganesengan was similar to that of the low maltose lines, whereas the maltose content above 74 °C was relatively higher than that of Shiroyutaka, Naeshirazu, and Shirosatuma. The largest starch gelatinization degree of Koganesengan during heating among these four cultivars is considered to be the cause.

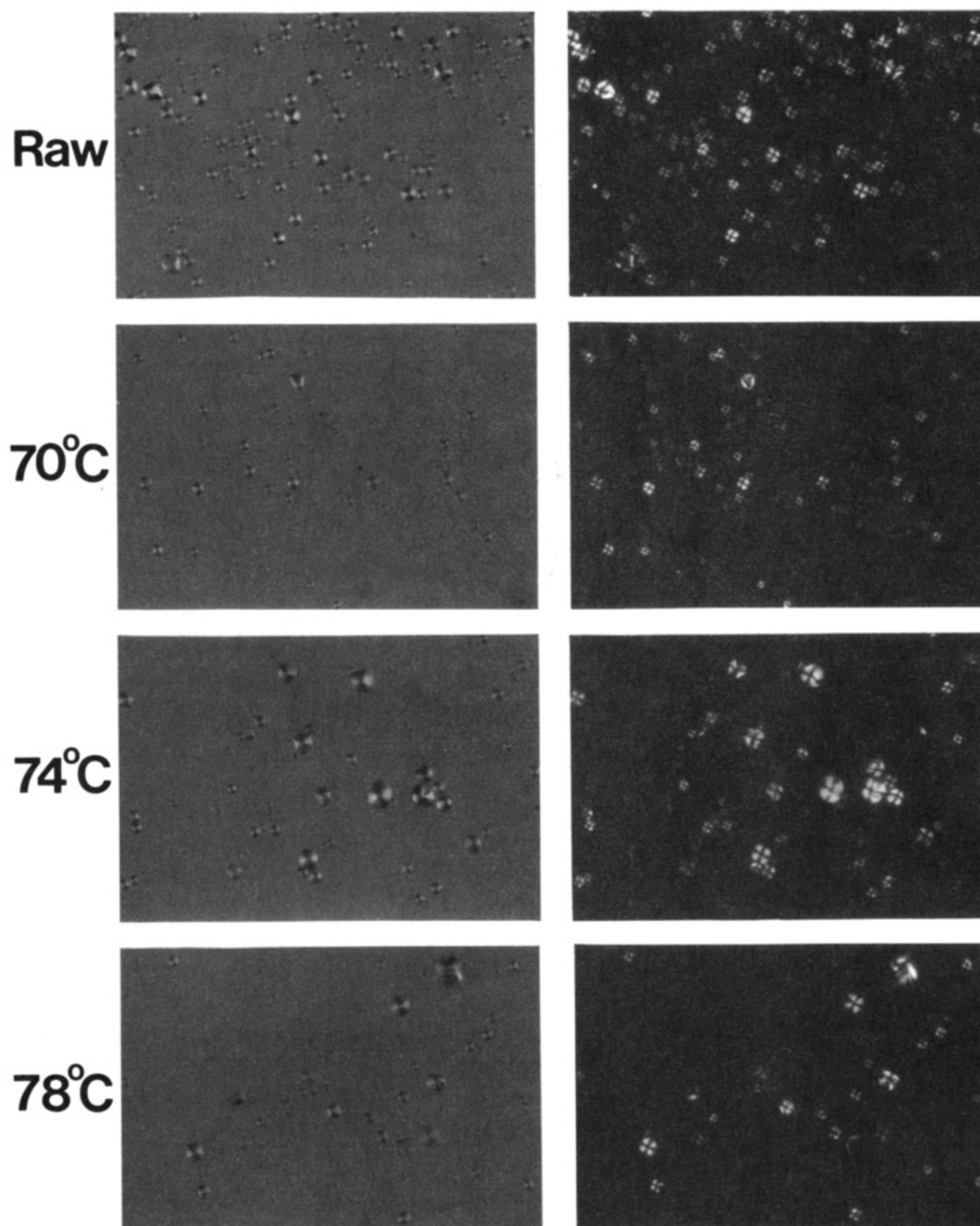

Figure 5. Micrographs of starch granules of Shiroyutaka during heating: (left) normal light; (right) polarized light.

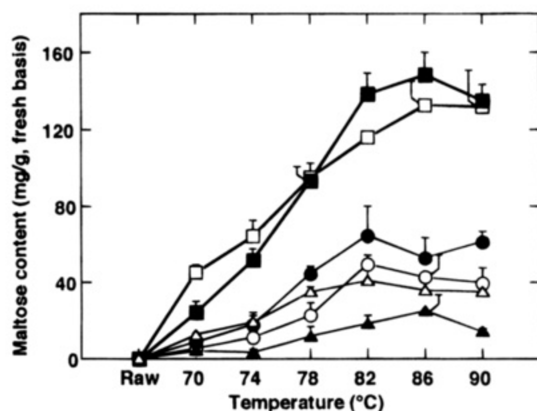

Figure 6. Change in maltose content during heating. Each value is the mean of three replications. Vertical bars indicate standard deviation. Symbols are the same as in Figure 2.

Although the final maltose contents (90 °C sample) of the low and moderate maltose lines were lower than those of steamed whole roots, the differences both in residual activity of β-amylase and in starch gelatinization degree may affect the maltose content of steamed whole roots.

On the other hand, our results showed that the β-amylase activities of raw roots of the six lines were not significantly correlated with the maltose contents of the heated roots. This is the same as in previous studies (Walter et al., 1975; Baba et al., 1987) and indicates the small contribution of raw root β-amylase activity to the varietal differences in maltose content in steamed roots.

Finally, it should be noted that a slight but significant production of maltose was seen in low and moderate maltose lines even at 70 and 74 °C when the starch gelatinization had not yet occurred. The reason for this phenomenon was thought to be endoamylolysis of α-amylase that was not assayed in this study. The endoamylolysis of starch increases the number of sites for attack by β-amylase. Deobald et al. (1969) discussed the relationship between α-amylase activity and maltose content during flake production. They indicated that the maltose content decreased with decreasing α-amyl-

ase activity. Also in the present study, α-amylase presumably played a significant role in maltose production, especially in low and moderate maltose lines during heating at 70−78 °C. The endoamylolysis by α-amylase presumably occurred in high maltose lines, but its effect was thought to be masked by the early starch gelatinization. The optimum temperature of sweetpotato α-amylase was reported as 70−75 °C (Ikemiya and Deobald, 1966); however, its role in varietal differences in maltose content is still unknown in this study.

In conclusion, the properties of β-amylase inactivity and starch gelatinization during heating were distinctly different between the high maltose lines and the others. These traits are considered to greatly affect the varietal differences in maltose content in sweetpotatoes.

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