Changes in invertase, sucrose-6-phosphate synthase and UDP-glucose pyrophosphorylase activities, and their relations to reducing sugar content in Japanese processing potato varieties stored at low temperature

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Invertase, sucrose-6-phosphate synthase (SPS) and UDP-glucose pyrophosphorylase (UGPase) activities of three Japanese processing varieties, differing in susceptibility to low temperature sweetening (susceptible: Norin-1 ; middle: Toyoshiro; resistant: Hokkai-82), were measured at intervals during storage at 7 °C in order to investigate the role of the three enzyme activities in reducing sugar accumulation during storage. The susceptible variety dramatically increased the reducing sugar content, and invertase, SPS and UGPase activities during storage, whereas the mid-susceptible and resistant varieties progressively increased them. In the three enzymes, both invertase and SPS activities correlated significantly ($p < 0.01$) with the reducing sugar content, implying that invertase and SPS may play an important role in the regulation of the reducing sugar accumulation of processing potatoes stored at low temperature. This information may be useful for potato breeders to develop new resistant processing varieties.

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Sugars accumulate in many plants in the cold, and may act as cryoprotectants¹⁾. Low temperature sweetening (LTS) has been intensively studied especially in potato tubers^{2),3)}. The ancestors of the modern potato grew in the Andes, where low temperatures may render cryoprotectants useful for survival. However, sugar accumulation is an undesirable trait in the crop plant, because tubers that have been stored at low temperature to delay sprouting are unsuitable for the production of crisps and chips; the reducing sugars that they have accumulated react with amino acids during frying, leading to an unacceptably dark color²⁾. The mechanism regulating sugar accumulation in the cold is therefore of ecophysiological and commercial interest⁴⁾.

With respect to the mechanism for LTS, SOWOKINOS⁵⁾ suggested that three enzymes, i.e.,

invertase, sucrose-6-phosphate synthase (SPS) and UDP-glucose pyrophosphorylase (UGPase) are key to the regulation of sugar accumulation. However, this issue remains poorly understood⁴⁾.

Japanese potato farmers, processors and breeders wish to know the role of invertase, SPS and UGPase in sugar accumulation in cold-stored Japanese processing potatoes in order to produce light-colored products. Very limited information is available on the invertase activity of the Japanese fresh-consumption variety of potato (Danshaku)⁶⁾. However, there are no studies on changes in invertase, SPS and UGPase activities of the processing potato varieties stored at low temperature. Therefore, this study was carried out to investigate changes in the above three enzyme activities in the processing potato varieties differing in susceptibility to LTS, and whether the three

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enzymes are responsible for the reducing sugar accumulation.

Materials and methods

1. Source and tuber storage

Three processing varieties (Norin-1 : susceptible ; Toyoshiro : mid-susceptible ; and Hokkai-82 : resistant) were used in this study. These three varieties were grown in the NARCH experiment test fields and harvested in September 1999. After harvesting (the first sampling day), the potatoes were cured for two weeks at 12°C and 90~95% RH. Thereafter, the potatoes were stored at 7°C and 90~95% RH. Approximately 150 g of each variety was used for the experiment.

2. Sample preparation

Eight potatoes were selected at random from each cultivar, washed, peeled and diced into approximately 5 mm cubes.

3. Sugar

Sugar was extracted from tubers using the method described by WILSON *et al.*⁷⁾. Briefly, 50 grams of potato cubes was homogenized in a Waring blender in 50 ml of distilled water for 150 sec. The homogenate was centrifuged at $1,710 \times g$ for 5 min at 4°C. The supernatant was filtered through Toyo No.5 filter paper and then analyzed using high-performance liquid chromatography (HPLC) (Model BIP-1, Nihon Bunko Co.) as described previously⁸⁾.

4. Enzyme extraction

Enzyme extraction and assay were done according to the procedure of SOWOKINOS *et al.*⁹⁾. Potato tubers (25 g) were homogenized in a Waring blender at high speed for 90 sec with 25 ml buffer (pH 6.3). The suspension was centrifuged at $18,000 \times g$ for 15 min at 4°C. The precipitate was washed with 20 ml of the above buffer, recentrifuged, and the two supernatants were combined. The extract was dialyzed overnight against 50 mM Tris-maleate (pH 6.3, 40 volumes) with one change. The dialysate was centrifuged to remove inactivated protein.

5. Invertase (EC 3.2.1.26)

Invertase activity was assayed as previously described by SOWOKINOS *et al.*⁹⁾. The reaction mixture (1 ml) contained 0.25 M sucrose, 40 mM trisphosphate (pH 6.6), 10 mM NaF, 5 mM 2-mercaptoethanol, and diluted enzyme extract containing 0.1 mg protein or less. The incubation

was carried out at 37°C for 30 min, and the amount of reducing sugar liberated was determined by the Somogyi method¹²⁾. One unit of activity for invertase is defined as that amount of enzyme which liberates 1 μ mol of glucose/h.

6. Sucrose-6-P synthase (EC 2.4.1.14)

Sucrose-6-P synthase extraction and assay were done following the procedure of SOWOKINOS *et al.*⁹⁾. The buffer used to extract sucrose-6-P synthase consisted of a solution of 10% NaHSO₃ and 50 mM Tris-phosphate buffer (pH 7.5, 1 : 4 v/v) containing 10 mM GSH and 1 mM EDTA. Extracts were dialyzed against the Tris-phosphate buffer containing 10 mM NaHSO₃. Reaction mixtures (0.25 ml) contained 100 mM Tris-phosphate buffer (pH 7.3), 20 mM fructose-6-P, 10 mM UDPglucose, 50 mM NaF, and diluted enzyme containing between 0.02 and 0.2 units of enzyme. Boiled enzyme blanks and sucrose standards (0.5 μ mol) were run with each assay. After incubation at 37°C for 1 hr, 0.25 ml of 5 N NaOH was added to each reaction, which was then placed into a boiling water bath for 10 min to destroy unreacted fructose-6-P. The concentration of sucrose-6-P was determined by the thiobarbituric acid method¹⁰⁾. One unit of activity is defined as that amount of enzyme which catalyzes the formation of 1 μ mol of sucrose-6-P/h.

7. UDPglucose pyrophosphorylase (EC 2.7.7.9)

UDPglucose pyrophosphorylase activity was also assayed using the method of SOWOKINOS *et al.*⁹⁾. The buffer used to extract and dialyze UDPglucose pyrophosphorylase consisted of 50 mM Hepes (pH 7.5) containing 1 mM EDTA, 2 mM GSH, and 20% sucrose. The incubation was carried out at 37°C for 30 min, and the rate of α -D-glucose-1-P pyrophospholysis from UDPglucose was measured spectrophotometrically (340 nm) in the presence of excess phosphoglucomutase, glucose-6-P dehydrogenase, and NADP. One unit of activity is defined as that amount of enzyme which catalyzes the formation of 1 μ mol of α -D-glucose-1-P/h.

All analyses were performed six times, and results were averaged to obtain mean values.

Results and discussion

Fig. 1~4 show changes in the sugar content of potatoes stored at 7°C. As shown in Fig. 1, the glucose content of the Norin-1 potato increased rapidly, whereas that of the Toyoshiro potato increased progressively. However, there was little

Fig. 1 Changes in glucose content of processing potatoes during storage at 7 °C

Fig. 2 Changes in fructose content of processing potatoes during storage at 7 °C

Fig. 3 Changes in reducing sugar content of processing potatoes during storage at 7 °C

Fig. 4 Changes in sucrose content of processing potatoes during storage at 7 °C

accumulation for the Hokkai-82 potato. Similarly to the behavior of the glucose content in Fig. 1, the fructose content sharply raised in the Norin-1 potato, followed by the Toyoshiro and Hokkai-82 varieties (Fig. 2). With respect to the reducing sugar (glucose+fructose) content, the same behavior was observed as the glucose and fructose contents (Fig. 3). In addition, similar increases in reducing sugar content during storage at low temperatures have been reported for Japanese fresh consumption^{11)~13)} and processing varieties^{14), 15)}.

In Fig. 4, sucrose contents of each potato at harvesting time (0 day in Fig. 4) were considerably high. RICHARDSON *et al.*¹⁶⁾ reported that storage

temperature of 3 °C rapidly increased sucrose content of potatoes within one week after the start of storage. Then, high contents of sucrose might be caused by low soil temperatures around potato tubers in Obihiro. In addition, DEITING *et al.*¹⁷⁾ showed that sucrose content remained almost constant during storage at 11 and 7 °C, whereas that increased rapidly at 5 and 3 °C. Thus, in the present study sucrose contents of each potato decreased gradually during curing at 12 °C for two weeks and subsequent storage at 7 °C, but each potato maintained comparatively high sucrose content during storage (Fig. 4).

Fig. 5 shows changes in invertase activity at 7 °C.

As shown in this figure, the invertase activity of the Norin-1 variety increased sharply, whereas those of the Toyoshiro and Hokkai-82 varieties increased progressively and slightly, respectively. UPPAL and VERMA⁽⁸⁾ also reported the increase in invertase activity of Indian potato varieties stored at low temperature.

Fig. 6 shows changes in SPS activity at 7 °C. The susceptible variety (Norin-1 potato) rapidly increased the activity, whereas the other varieties, mid-susceptible (Toyoshiro) and resistant (Hokkai-82) potatoes, increased gradually. The rise in SPS activity of potatoes stored at low temperatures has been reported by PRESSEY⁽¹⁹⁾ and DEITING *et al.*⁽¹⁷⁾.

Fig. 7 shows changes in UGPase activity at 7 °C. As shown in this figure, the UGPase activity of the Norin-1 potato increased gradually, while that of the Toyoshiro variety decreased at the beginning of storage, and later increased. A similar rapid decrease in UGPase activity during storage at low temperature has been reported in a low sugar variety⁽²⁰⁾.

Concerning starch degradation in potato tubers, the starch is thought to be degraded via a mainly phosphorolytic route, based on the relative activities of phosphorylase and amylolytic enzymes in the tuber⁽³⁾, and on comparison of the rates of respiration, the rate of sugar accumulation, and the energetic requirements of the different routes for the conversion of starch to sucrose⁽²¹⁾. The hexose phosphates are then exported to the cytosol via a phosphate translocator that accepts hexose-phosphates⁽²²⁾, and are converted to sucrose via UGPase, followed by SPS⁽²³⁾. UGPase produces the cytosolic intermediate UDP-glucose, which is a substrate for SPS reaction (sucrose synthesis)⁽²⁴⁾. Thereafter, the sucrose can subsequently be hydrolysed to glucose and fructose by acid invertase⁽²⁵⁾.

In order to investigate whether the three enzymes in the present study (invertase, SPS and UGPase) contribute to the accumulation of reducing sugar content, we attempted to examine correlations between reducing sugar content and the three enzyme activities. Table 1 gives the results, in which the invertase and SPS activity correlated significantly ($p < 0.01$) with the reducing sugar content, implying that invertase and SPS play an important role in the regulation of reducing sugar accumulation of processing potatoes stored at low

Fig. 5 Changes in invertase activity of processing potatoes during storage at 7 °C

Fig. 6 Changes in SPS activity of processing potatoes during storage at 7 °C

Fig. 7 Changes in UGPase activity of processing potatoes during storage at 7 °C

Table 1 Correlation between reducing sugar content and three enzymes in processing potato tubers during storage

Enzyme	Correlation coefficient
Invertase	0.720*
SPS	0.740*
UGPaes	0.515

*significant at P=0.01

temperature. This information may be useful for potato breeders to develop new resistant processing varieties.

Conclusion

The results of the present study demonstrated that invertase and SPS may play an important role in the regulation of reducing sugar accumulation in Japanese processing potatoes stored at low temperature.

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- 低温貯蔵加工用ジャガイモのインベルターゼ、スクロース-6-リン酸シンターゼおよびUDP-グルコースピロホスホリラーゼ活性の推移とそれらの酵素の還元糖含量との関係**
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- 3品種の国産加工用ジャガイモを5および12℃で90日間貯蔵し、インベルターゼ、スクロース-6-リン酸シンターゼ (SPS) およびUDP-グルコースピロホスホリラーゼ (UGPase) 活性を測定した。この研究の目的は、それらの3酵素に及ぼす貯蔵温度の影響の検討である。5℃貯蔵のジャガイモは12℃に比べ多くの還元糖を蓄積した。さらに、低温 (5℃) はインベルターゼおよびSPSは活性を増加させた。これらのことより、加工用ジャガイモの低温における還元糖増加に、この2つの酵素は重要な役割をになっているものと推察された。
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