低温貯蔵加工用ジャガイモのインベルターゼ活性と還元糖含量との関係

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Relationship between invertase activity and reducing sugar content of cold-stored Japanese processing potatoes

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Invertase activities and sugar contents of four Japanese processing varieties (Norin-1, Toyoshiro, Hokkai-76 and Hokkai-82) were measured at intervals during storage at 5 and 12℃ in order to invetigate the role of invertase activity in sugar accumulation during cold storage. The experimental data revealed that the invertase activity of potatoes stored at 5℃ correlated significantly ($r=0.81$, $p<0.01$) with the reducing sugar content, whereas potatoes stored at 12℃ showed no significant relationship ($r=0.01$, $p>0.05$) between the invertase activity and reducing sugar content. The invertase activity of cold-stored processing potatoes accounts for 66% of the variability in reducing sugar content, implying that invertase is one of the key enzymes for reducing sugar accumulation in cold-stored processing potatoes.

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Potato tubers are an important carbohydrate resource for nutrition and are used as the basis for a variety of processed products. In addition, starch tubers contain high-quality proteins, substantial amounts of vitamins, minerals and trace elements. Potato plants are best grown in moderate climates where the continuous production of tubers throughout the year is impossible. Therefore, the harvested tubers need to be stored for a length of time. To prevent sprouting during this storage period tubers are kept at a low temperature. Cold storage of potato tubers leads to an accumulation of hexoses (reducing sugar) in a process called cold sweetening[1),2)]. Hexose accumulation results in a sweeter taste but also leads to the dark brown coloring of tuber slices after frying, which is a major economic problem for the potato processing industry[3)].

One of the gluconeogenic enzymes, invertase, is a hydrolase that cleaves sucrose into glucose and fructose[4)]. With respect to invertase, Russel and Roy[5)] showed the participation of invertase in the regulation of the conversion of starch to sugars in potatoes. However, the mechanism of the sweetening remains unknown[6)].

Japanese potato farmers, processors and breeders want to know the role of invertase 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)[7)]. However, no reports exist on the invertase activities of the processing potato varieties. Therefore, this study was initiated to investigate changes in and the relationship between invertase activity and sugar content of four processing potato varieties (Norin-1, Toyoshiro, Hokkai-76 and Hokkai-82) stored at 5 and 12℃.

Materials and methods

1. Source and tuber storage

Four processing varieties (Norin-1, Toyoshiro, Hokkai-76 and Hokkai-82) were used in this study. Hokkai-76 and Hokkai-82 potatoes were new varieties that NARCH has recently bred. These four varieties were grown in the NARCH

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experiment test fields and harvested in September 1998. After harvesting, the potatoes were cured for two weeks at 12℃ and 90~95% RH. Thereafter, the potatoes were stored at two temperatures (12 and 5℃) at 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.*[8]. Briefly, 50 g 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 × g for 5 min at 4℃. 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 previously[9].

4. Enzyme extraction

Enzyme extraction and assay were done according to the procedure of SOWOKINOS *et al.*[10]. Potato tubers (25 g) were homogenized in a Waring blender at high speed for 90 sec with 25mℓ buffer (pH 6.3). The suspension was centrifuged at 18,000 × g for 15 min at 4℃. The precipitate was washed with 20mℓ 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. Protein content was determined using Coomasie Brilliant Blue G 250 (CBB) reagent as described by Bradford[11]. Extracts were immediately measured for enzyme activity.

5. Invertase

Invertase activity was assayed as previously described by SOWOKINOS *et al.*[10]. 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 0.1mg protein or less. The amount of reducing sugar liberated was determined by the Somogyi method[12]. One unit of activity for invertase is defined as that amount of enzyme which liberates 1 μmol of glucose/h.

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

Results and discussion

Fig. 1 shows changes in the sugar content of potatoes stored at 5℃. As shown in Fig. 1 a, the glucose content of the Norin-1 variety increased most rapidly, followed by Toyoshiro, Hokkai-82 and Hokkai-76 varieties. The fructose content of each variety also increased (Fig. 1 b) similarly to the glucose content. In addition, the reducing sugar (glucose+fructose) content of each variety also increased during storage, especially, the Norin-1 and Toyoshiro potatoes (Fig. 1c). In contrast to these sugars, sucrose content (Fig. 1d) fluctuated considerably during storage, and Norin-1 and Toyoshiro varieties had the lowest values at the 60 day storage, when the reducing sugar content reached approximately the maximum levels of the storage period, as shown in Fig. 1c

Smith reported that the upper content limits, for reducing sugar content to produce an acceptable color for processed products, such as for chips and French fries, is 0.25%[13,14] and 0.5%[15] of fresh

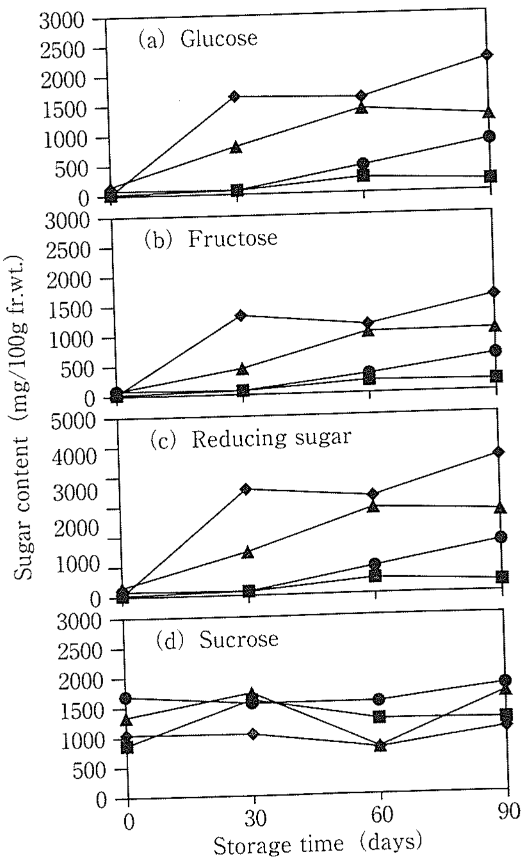

Fig. 1 Changes in sugar content of processing potatoes during storage at 5℃

◆Norin-1 ▲Toyoshiro ■Hokkai-76 ●Hokkai-82 weight, respectively. As shown in Fig. 1c, Hokkai-76 potatoes did not exceed the 0.5% level, while the other varieties exceeded this content, implying that the Hokkai-76 variety is suitable for French fries even when stored at 5℃. In contrast, the other varieties required reconditioning operation[16),17)] to decrease the reducing sugar content.

Sugar change in potatoes stored at 12℃ are shown in Fig. 2. As shown in Figs. 2 a, b and c, glucose, fructose and reducing sugar contents did not change much compared with those at 5℃. Moreover, the sucrose content of each variety gradually decreased during storage (Fig. 2d). This decrease in sucrose content has been reported in tubers stored at 10℃[6)].

As mentioned earlier, the upper limit for chips and French fries are 0.25%[13),14)] and 0.5%[15)], respectively. In Fig. 2c, the reducing sugar content of each variety was lower than 0.25% except for that of Norin-1 potato at 30 days. Therefore, each variety had a reducing sugar content less than the maximum acceptable level for chips and French fries almost throughout the entire storage period.

Fig. 3 a shows changes in invertase activity at 5℃. The Norin-1 and Toyoshiro varieties, which had higher reducing sugar content during storage as previously shown in Fig. 1 c, sharply increased invertase activity compared with the Hokkai-82 and Hokkai-76 potatoes. However, at 12℃ storage, no variety produced much activity (Fig. 3b).

Concerning the reason for the high invertase activity of cold-stored potatoes, Pressey[18)] reported that if a high soluble sugar content protects plant tissue from frost damage, a means for regulating the sugar level must exist; the function of invertase in such a mechanism would be to hydrolyze sucrose to reducing sugars to provide a high concentration of soluble sugars in response to low temperature. In the present experiment at 5℃, the higher activities of invertase may be due to the same reason.

Concerning the relationships between invertase activity and reducing sugar content of potatoes used in this study, a significant relationship ($r=0.81$, $p<$

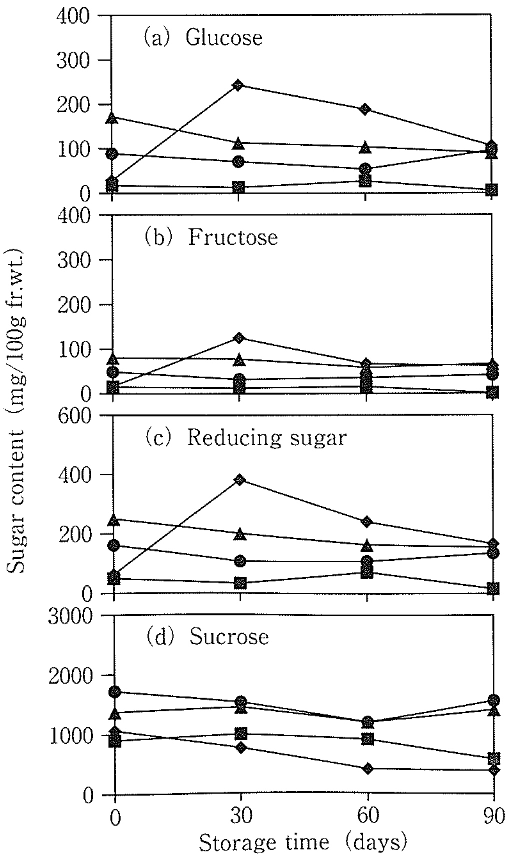

Fig. 2 Changes in sugar content of processing potatoes during storage at 12℃

◆Norin-1 ▲Toyoshiro ■Hokkai-76 ●Hokkai-82

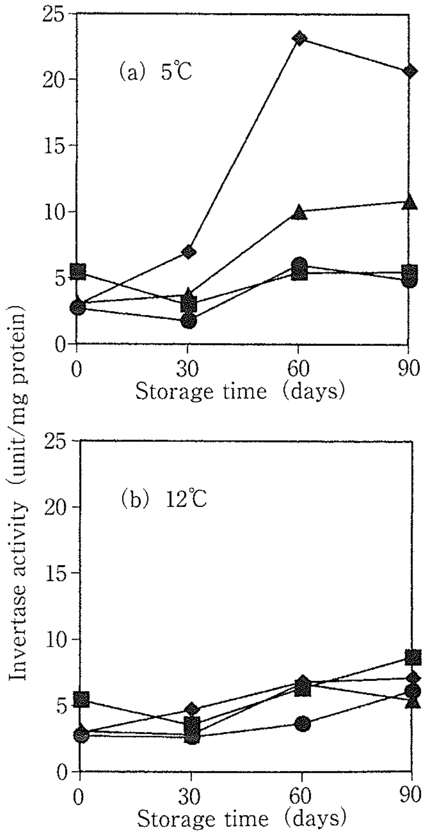

Fig. 3 Changes in invertase activity of processing potatoes during storage at 5(a) and 12℃(a)

◆Norin-1 ▲Toyoshiro ■Hokkai-76 ●Hokkai-82

0.01) between the two parameters was obtained only at 5℃ storage where the invertase activity accounts for 66% of the increased reducing sugar content. Therefore, we may conclude that invertase is one of the key enzymes for the reducing sugar accumulation of cold-stored processing potatoes.

The present study may be useful for potato breeders to develop new cold-resistant processing varieties.

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低温貯蔵加工用ジャガイモの インベルターゼ活性と還元糖含量との関係

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農林1号，トヨシロ，北海76号および北海82号の4品種の加工用ジャガイモを用いて5および12℃で貯蔵実験を行い，インベルターゼ活性と還元糖含量との関係を検

討した。その結果，5℃貯蔵加工用ジャガイモのインベルターゼ活性は還元糖含量との間に1％水準で有意な相関を有し，還元糖増加の66%をインベルターゼ活性の増加で説明できることがわかり，インベルターゼは低温貯蔵加工用ジャガイモの還元糖増加の主要な酵素の一つであることが提示された。

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