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Sucrose Metabolism in Stored Green Peas

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Changes in the sugar content and sucrose metabolizing enzymatic activities of stored green peas were studied. Green pea seeds were removed from their pods, and the pods without seeds or whole peas were stored at 1°C or 20°C. Their sucrose content significantly decreased, especially in seeds without pods stored at 20°C, but the stachyose and verbascose contents increased. Glucose and sucrose were the major components in the pods. The pod sucrose content significantly decreased, especially in pods with seeds stored at 20°C. Sucrose synthase (sucrose synthesis, sucrose cleavage) and invertase activities in the seeds decreased during storage, but sucrose phosphate synthase activity increased after 1 or 2 days of storage and then decreased. According to these results, we suggest that sucrose might be utilized not only in the synthesis of starch, but also in the synthesis of the raffinose family of oligosaccharides such as stachyose and verbascose.

Keywords: oligosaccharide, storage, pea, sucrose phosphate synthase, sucrose synthase, invertase

In Japan, immature peas and soybeans are used as vegetables. The quality of these green peas and green soybeans immediately changes after harvest. A decrease in sweetness, increase in hardness and decrease in sugar, amino acids and ascorbate content occur. Ito *et al.* (1972) demonstrated that sucrose is utilized in starch biosynthesis during storage. Saravitz *et al.* (1987) reported that raffinose and stachyose levels increased during the development of soybean seeds. Matsushita (1968) showed a decrease in sucrose content and increase in starch content during pea seed development. Changes in the sugar and starch content of stored green peas (Ito *et al.*, 1972; Shono *et al.*, 1990, 1996) and stored green soybeans (Iwata & Shirahata, 1979; Shono, 1987; Masuda *et al.*, 1988; Terai *et al.*, 1995) were also reported, but little is known of the changes in the activities of sucrose metabolizing enzymes and oligosaccharide content during storage.

In this paper, we examined the changes in sugar content and the activities of sucrose metabolizing enzymes in order to elucidate the sucrose metabolism of stored green peas.

Materials and Methods

Plant material Fresh 'Usui' green peas (*Pisum sativum* L.) were obtained from a local grower in Haga-cho, Hyogo. One hundred grams each of seeds removed from pods, pods without seeds, or whole peas were stored in a polyethylene film bag (20×16 cm size, 0.03 mm thickness, having 4.6-mm diameter holes) at 1°C or 20°C. The extract (70% ethanol) for sugar analysis was prepared by immersing 10 g of seeds without their seedcoat or pods in 40 ml of hot 90% ethanol and heating the mixture in a boiling water bath for 15 min. The mixture was homogenized for 2 min in a Waring blender, and the homogenate was then filtered through Toyo #2 filter paper. The filtrate was passed through a Sep Pak C_{18} cartridge and a 0.22 μm membrane filter. A 20 μl aliquot of the sample was injected onto an HPLC system cosisting of an LC-5A pump (Shimadzu, Kyoto), a guard column, an Asahipak NH_2-50 4E column (250×4.6 mm) and refractive index detector. The column was isocratically eluted with acetonitrile : water (66 : 34, v/v) at a flow rate of 1.0 ml/min.

Enzyme extraction For the enzyme analyses, 5 g of pods or seeds without their seedcoat were frozen in liquid N_2 and stored at −85°C. Frozen peas were ground in a chilled mortar using a 1 : 4 tissue-to-buffer ratio. The buffer contained 50 mM MOPS-KOH (pH 7.5, product from Nacalai Tesque Co., Ltd., Kyoto), 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, and 0.05% Triton X-100. The homogenate was centrifuged at 10,000×g for 10 min and 2.5 ml of the supernatant was desalted on a Sephadex G-25 column equilibrated with the appropriate buffer (Nielsen *et al.*, 1991).

Enzyme assays For the determination of enzyme activities involved in sucrose cleavage, the desalting column was equilibrated with 25 mM phosphate buffer (pH 7.0). The eluate was used for each enzyme reaction. One unit of enzyme is defined as the amount which catalyzes the cleavage of 1 μmol sucrose/min/1 mg protein.

Neutral invertase: Reaction mixtures (1 ml) contained 100 μM phosphate buffer (pH 7.6), 100 mM sucrose, and 200 μl of desalted extract. Reaction mixtures were incubated at 37°C for 40 min. The reaction was stopped by placing reaction tubes in boiling water for 2 min. The glucose and fructose formed was determined using the Somogyi-Nelson method (Somogyi, 1952).

Sucrose synthase (SS; Pressey, 1969): Reaction mixtures (1 ml) contained 50 mM Tris phosphate buffer (pH 7.3), 250 mM sucrose, 10 mM UDPG, 10 mM NaF, and 50 μl of desalted extract. The reaction was incubated for 30 min at 37°C, and stopped by adding 1.0 ml of 0.5 M K_2HPO_4 and placing the tubes in boiling water for 2 min. The fructose formed was

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Abbreviations used in this paper: RFO, raffinose family oligosaccharides; SPS, sucrose phosphate synthase; SS, sucrose synthase; MOPS, 3-(*N*-morpholino)-propanesulfonic acid; SE, standard error of the mean.

determined by the Somogyi-Nelson method (Somogyi, 1952).

For the determination of enzyme activities involved in sucrose synthesis (Nielsen *et al.*, 1991), the desalting column was equilibrated with 50 mM MOPS-KOH buffer (pH 7.5) containing 2.5 mM DTT, 5 mM $MgCl_2$ and 1 mM EDTA. The eluate was used in each enzyme reaction. One unit of enzyme is defined as the amount which catalyzes the synthesis of 1 μmol sucrose/min/1 mg protein.

SS (Nielsen *et al.*, 1991): Reaction mixtures (70 μl) contained 50 mM MOPS-KOH buffer (pH 7.5), 15 mM $MgCl_2$, 1 mM EDTA, 15 mM KCl, 2.5 mM DTT, 4 mM UDPG, 60 mM fructose and 45 μl of desalted extract. The reaction proceeded at 25°C for 10 min and was stopped by adding 70 μl of 30% KOH. Reaction tubes were placed in boiling water for 10 min and then cooled to room temperature. One milliliter of anthron reagent was added and the color was allowed to develop for 20 min at 40°C. The reaction tubes were then cooled to room temperature and the absorbance determined at 620 nm.

Sucrose phosphate synthase (SPS): Reaction mixtures (70 μl) contained 50 mM MOPS-KOH buffer (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, 10 mM UDPG, 5 mM F6P, 15 mM G6P and 45 μl of desalted extract. Reactions and the determination of sucrose formed were performed similar to SS.

Protein analysis The protein content of the crude enzyme was determined according to the procedure of Bradford (1976).

Results

Sugar levels Sucrose was the major sugar component in the seeds of green peas. During storage, the levels of fructose and glucose were low (data not shown). Figure 1 shows the changes in sugar content of seeds during storage. The sucrose content significantly decreased, especially in seeds stored without pods during storage at 20°C, but the stachyose and verbascose contents increased. On the other hand, in the seeds stored at 1°C, the sucrose level slightly increased but later decreased. The stachyose and verbascose contents were low during storage at 1°C. The raffinose content in seeds with pods stored at 20°C showed almost no change, but in seeds without pods, raffinose gradually decreased during storage. The raffinose content of seeds with or without pods increased following a decrease for the first 2 days of storage at 1°C.

When stored without seeds at 20°C, pods withered and browned. Different from seeds, pods contained glucose and

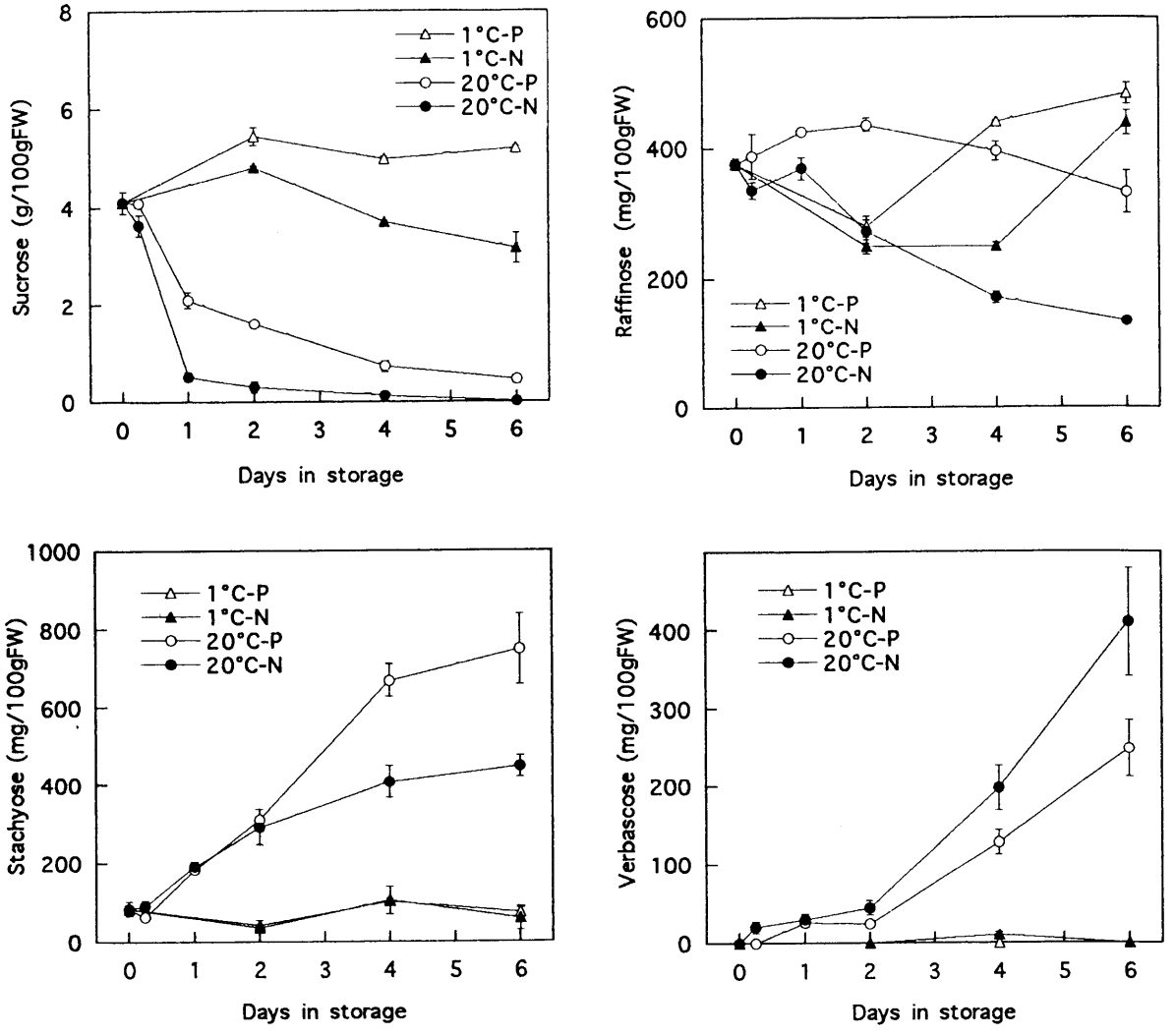

Fig. 1. Changes in sugar content of green pea seeds during storage. 1°C-P: stored with pods at 1°C, 1°C-N: stored without pods at 1°C, 20°C-P: stored with pods at 20°C, 20°C-N: stored without pods at 20°C. The vertical bars represent the average values with SE (standard error of the mean) (n=3).

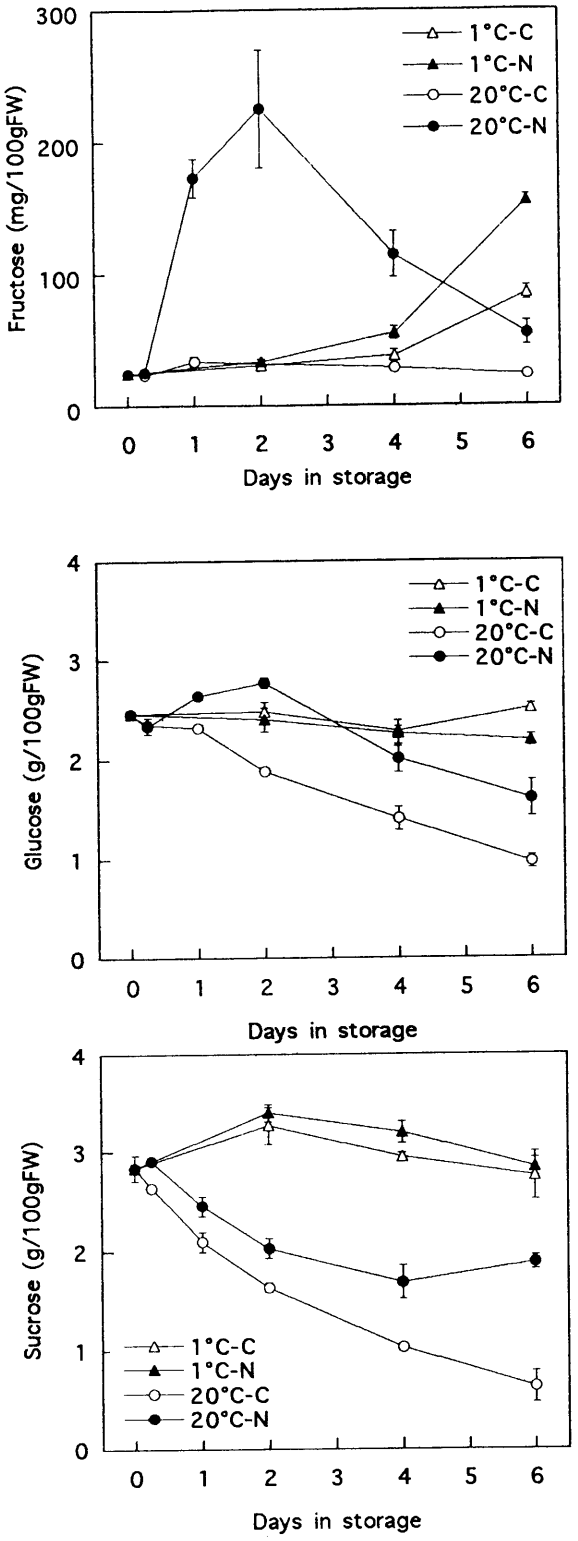

Fig. 2. Changes in sugar content of green pea pods during storage. 1°C-C: stored with seeds at 1°C, 1°C-N: stored without seeds at 1°C, 20°C-C: stored with seeds at 20°C, 20°C-N: stored without seeds at 20°C. The vertical bars represent the average values with SE (n=3).

sucrose as their major components. The sucrose content markedly decreased at 20°C (but not at 1°C), especially in pods stored with seeds (Fig. 2). In pods stored with seeds at 20°C, the glucose content significantly decreased, but in pods without seeds, the glucose level slightly increased for the first 2 days of storage and then slowly decreased. The fructose content of pods without seeds significantly increased during storage at 20°C, peaked after 2 days of storage and then sharply decreased. However, no change was observed in the fructose content in pods stored with seeds at 20°C. The sucrose content of pods slightly increased after 2 days of storage at 1°C and then decreased. The glucose showed almost no change during storage at 1°C.

Enzyme activities Figure 3 shows changes in the enzyme activities of seeds during storage. The SS (synthesis and cleavage of sucrose) and invertase activities decreased during storage at 20°C, but SPS activity increased after 1 or 2 days of storage and then continuously decreased. Invertase activity was low during storage, one-tenth lower than that of SS. On the other hand, four enzyme activities showed almost no change for the first 4 days of storage at 1°C.

Discussion

The sucrose content of seeds and pods immediately decreased during storage at 20°C, and as shown in Figs. 1 and 2, the decrease in seeds without pods or pods with seeds was significant. The difference in the sucrose content of seeds stored with or without pods is presumed to be dependent on the sugar translocation from pods. Flinn & Pate (1970) demonstrated that pods supply sugars to seeds with the advance of ripening. Lanfermeijer *et al.* (1991) also reported sucrose transport in developing pea cotyledons. We reported in a previous paper (Shono *et al.*, 1996) that the starch content in seeds with pods increased during storage at 20°C concurrently with a decrease in sugar content. Furthermore, the increase in starch content of seeds with pods was higher than that without pods. We also observed that the metabolic products consisting of stachyose and verbascose, which are formed from raffinose, significantly increased in seeds with or without pods during storage at 20°C. Castillo *et al.* (1990) reported that raffinose and stachyose levels increased during the later stages of green soybean seed development. Matsushita (1968) demonstrated a decrease in sucrose content and an increase in raffinose family oligosaccharides (RFO) with ripening of green peas. A possible explanation for these findings is that the sugar metabolism of stored seeds at 20°C was almost similar to that of seed ripening.

In contrast to the decrease in sucrose content in stored seeds, no increase in invertase and SS activities was observed. SPS activity increased for the first 2 days of storage at 20°C. This transitory increase in enzyme activity seems to show that sucrose synthesis might be carried out using reducing sugar translocated from pods at the beginning of storage at 20°C in spite of a decrease in the sucrose content of stored seeds. It is known that sucrose is involved in RFO metabolism and that RFO biosynthesis is initiated by the transfer of a galactosyl unit from galactinol to sucrose to produce raffinose (Lehle & Tanner, 1973). Castillo *et al.* (1990) noted that in developing soybean seeds, raffinose synthase was detected after 5 days of flowering and activity increased until 15 days after flowering, after which it remained constant. On the other hand, Ito *et al.* (1972) demonstrated that sucrose was utilized in starch synthesis. Akazawa & Okamoto (1980) postulated that both SS and starch synthase played a role in sucrose-starch

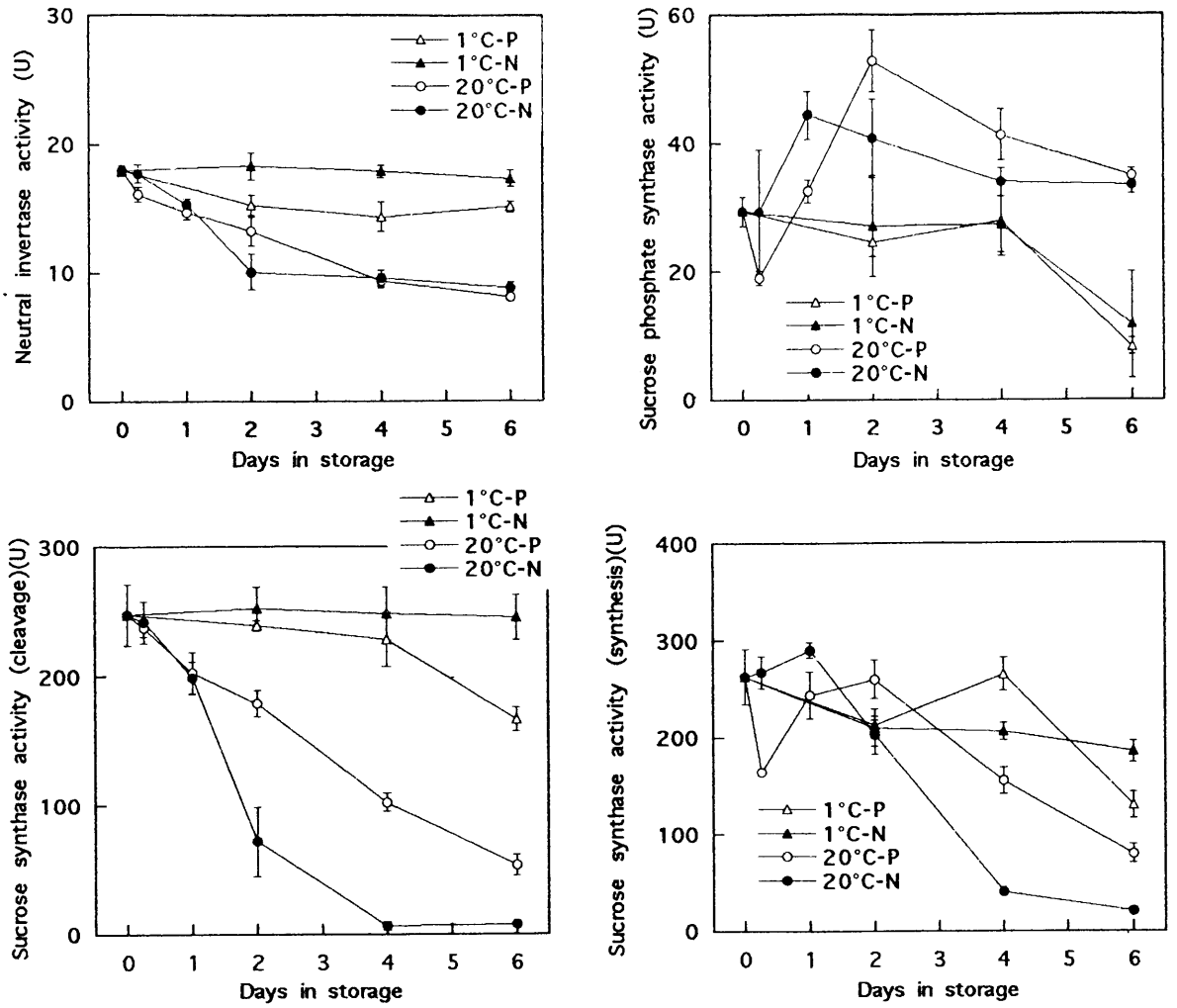

Fig. 3. Changes in enzyme activities of green pea seeds during storage. 1°C-P: stored with pods at 1°C, 1°C-N: stored without pods at 1°C, 20°C-P: stored with pods at 20°C, 20°C-N: stored without pods at 20°C. The vertical bars represent the average values with SE (n=3).

conversion in plant tissues. We suggest from these results that sucrose might be utilized in both the starch synthesis and RFO synthesis during storage at 20°C. Further studies on the enzyme activities in relation to RFO synthesis might be necessary to elucidate sucrose metabolism.

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