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Changes in Acid Invertase Activity and Sugar Distribution During Postharvest Senescence in Broccoli

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Abstract: Changes in acid invertase activity and sugar distribution were studied during postharvest senescence of broccoli (*Brassica oleracea* L. cvs. 'Hartland' and 'Sairin') stored at 20°C. Broccoli head began to degreen after 3 days of storage and the degree of yellowing gradually increased at the end of the storage period. Respiration rate decreased markedly during the first 24 h of postharvest storage and increased slightly thereafter. In both cultivars and portions, the invertase activity increased gradually at the end of the storage time. The acid invertase in cell wall-bound fraction (CWBF) showed a higher activity than that in soluble fraction (SF). Again, the branchlets showed a significantly higher invertase activity than that of florets. Of the two cultivars, 'Sairin' showed a higher invertase activity in both portions. Sucrose content gradually decreased in both portions of the two cultivars with time. It was negatively correlated with the acid invertase activity in both portions accounting well for the relation between the substrate and the activity. Fructose content was higher than glucose and sucrose in the florets as well as branchlets in both cultivars. Comparing the two cultivars, 'Sairin' showed higher enzyme activities, sugar content and respiration rate than 'Hartland'.

Key words: Acid invertase activity, broccoli, cultivar, senescence, sugar content

INTRODUCTION

Broccoli (*Brassica oleracea* L) head, which is composed of a mass of green floral buds (florets) and thick fleshy stem (branchlets) is harvested at its immature stage for human consumption. The head is highly perishable, with a storage life of 3 to 4 weeks when stored in air at 0°C^[1] and 2 to 3 days when kept in air at 20°C^[2]. Different physiological and biochemical changes occur in broccoli after harvest particularly during storage at ambient temperature that lead to senescence and quality deterioration. The loss of chlorophyll is the first visual sign of senescence^[2]. In broccoli, sepal yellowing commences between 24 and 48 h after harvest and is essentially complete by 96 h^[3] during storage at 20°C. The events of senescence of the commodity are often signaled by changes in respiratory behavior. Broccoli senescence occurred as respiration increased during storage after harvest at temperatures near ambient^[1]. During the first 24 h after harvest sugars (sucrose, glucose, fructose), organic acids and proteins are lost and later increase in the pools of free amino acids and ammonium^[4,5]. In asparagus, the tips of spears lose large amount of sucrose^[6], accumulate asparagines^[7] and undergo major

changes in gene expression^[8,9]. Similar physiological changes have been observed in harvested broccoli^[10], except that application of exogenous sucrose was found to increase the longevity of broccoli^[11]. Sucrose is the major sugar transported through the phloem from source organs to sink organs. It is broken down in plants either by sucrose synthase (EC 2.4.1.13) or invertase (EC 3.2.1.26). Depending on the physiological activities, invertase is a hydrolase, cleaving sucrose into glucose and fructose. Invertase is considered to play a key role in carbohydrate metabolism and in the regulation of sucrose transport in higher plants^[12]. Invertase is responsible for the reduction in sucrose level that accompanies the rapid deterioration of harvested broccoli^[9]. In plants three forms of invertase have been reported, the acid invertase, which has optimum activity at about pH 5, is located in either the vacuolar or cytosolic compartments (soluble fraction) and the insoluble extra-cellular forms which are referred to cell wall-bound fraction. The hydrolysis of sucrose by cell wall-bound invertase and subsequent import of hexoses into target cells appears to be crucial for appropriate metabolism growth and differentiation in plants^[13].

To further understand how respiration and changes in acid invertase activities influence the postharvest life

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of broccoli, we describe and compare the patterns of respiration, color change, invertase activities (SF and CWBF) and sugar distribution during postharvest senescence of two broccoli cultivars stored at 20°C.

MATERIALS AND METHODS

Plant Materials: Two broccoli (*Brassica oleracea*) cultivars 'Sairin' and 'Hartland' were harvested from a commercially grown crop and transported to the laboratory. Harvested broccoli heads were stored at 20°C for 5 days. After 24 h intervals, the broccoli heads of each variety were taken out from storage and florets were shaved off a razor blade and placed in the individual bag and immediately stored at -30°C until use.

Color assessment: Color change in broccoli heads were determined with a chromameter (Minolta CR-200), equipped with an 8-mm measuring head. The meter was calibrated using the manufacturer's standard with white plate. Color changes were quantified in the L, a, b color space. L refers to the lightness of the head and ranges from black=0 to white=100. A negative value of 'a' indicates green, while a positive number indicates red-purple color. Positive b indicates yellow and negative blue color^[14]. Hue angle ($h^\circ = \tan^{-1}(b/a)$) when $a > 0$ and $b > 0$ or $h^\circ = 180^\circ + \tan^{-1}(b/a)$ when $a < 0$ and $b > 0$) was calculated from the a and b values^[15]. On each head, three readings were taken from different portions.

Respiration rate measurement: Each broccoli head was weighed and carefully placed in a 6-liter glass jar held at 20°C. Carbon dioxide production was measured on intact head at 24 h intervals. Production of CO₂ was measured by taking 10 ml gas sample from the glass jar sealed for 1 h and injected to a TCD gas chromatograph equipped with a 1 m activated charcoal column at 60°C (GC-8 AIT, Shimadzu Co. Ltd.). The results were expressed as mL CO₂ kg⁻¹h⁻¹.

Enzyme extraction: Approximately 5 g of sample from each portion was mixed with 1% of polyvinylpyrrolidone (PVPP) and 1g sea sand. The sample was then homogenized using a cooled mortar and pestle with 5 ml of 0.2 M citrate-phosphate buffer (C-P buffer) at pH 5.0. The resulting homogenate was then filtered through four layers of cotton cloth and the filtrate was centrifuged at 11 000 x g for 10 min. The total supernatant was dialyzed with 0.2M C-P buffer (pH 5.0), diluted 40 times for 12 h and the inner solution was designated as soluble fraction. The residual tissues were re-extracted in 5 ml of 0.2 M NaCl C-P buffer for

about 24 h with occasional stirring. The supernatant was dialyzed as described above and dialyzed solution was designated as cell wall-bound fraction. All extraction procedures were carried out at 0-4°C followed immediately by the enzyme assays.

Enzyme assays: The standard assay medium for acid invertase consisted of 0.2 M C-P buffer (pH 5.0), 0.1 ml of 0.5M sucrose, 0.1ml of water and 0.1 ml of crude enzyme solution. The blank experiment contained distilled water instead of sucrose. The assay mixture was incubated at 45°C for 15 min. After the reaction mixture was neutralized with 0.1 N NaOH or 0.1 N HCl, a coloring Somogy's copper reagent was added and the mixture was heated for 10 min in boiling water. The amount of reducing sugars was estimated by the method of Somogy^[16]. Soluble protein content was determined by the method of Lowry^[17]. Bovine serum albumin was used as the standard. The enzyme activity was expressed as the amount of glucose produced per min per mg of protein.

Determination of contents of sucrose, glucose and fructose by HPLC: About 4 g of broccoli sample (for each portion) was mixed with 1 g sea sand and homogenized in a cooled mortar and pestle. Ten ml of distilled water was added to the homogenate and was centrifuged at 11 000 x g for 10 min. The mixture was filtered through a cellulose nitrate membrane filter (0.5 µm pore size). Soluble sugars were analyzed by high performance liquid chromatography (HPLC) using stainless steel column (10.7 mm ID x 30 cm) packed with silica gel (gel pack C 610). The filtered water was pumped through the column at a flow rate of 1.0 ml min⁻¹. The pressure was adjusted to 14-15 kg cm⁻² and the temperature to 60°C. ARI monitor (Hitachi L-3300) was used. Sucrose, glucose and fructose were identified by their retention times and were quantified according to standards.

Statistics: A randomized complete block design was adopted with three replications. The level of significance was calculated from the F value of ANOVA. The relationship between sugar content and invertase activity was described by linear correlation analysis.

RESULTS

Weight loss: Percent weight loss of broccoli heads during storage is presented in Fig.1. In both cultivars, weight loss was very high after 1 day of storage. This level rapidly declined at about 50% of the initial rate during the next 24 h. After that, no significant change was observed throughout the experimental period.

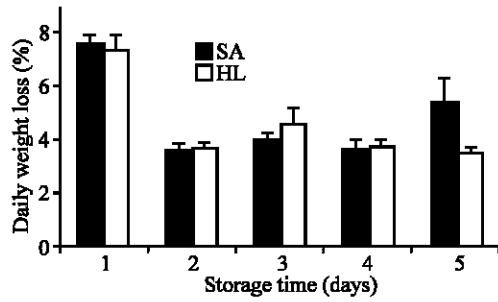


Fig. 1: Weight loss of two broccoli cultivars during storage at 20°C. Vertical bars indicate S.E. when larger than symbols. SA= 'Sairin' HL= 'Hartland'

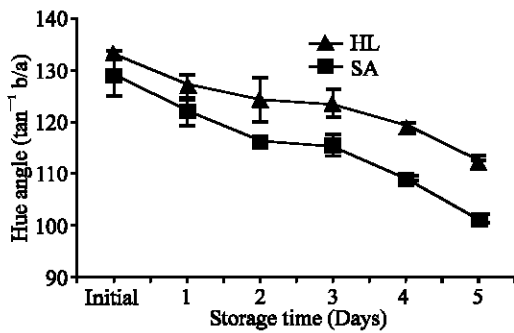


Fig. 2: Color changes of heads of two broccoli cultivars during storage at 20°C. Each point represents the mean of three replications. Vertical bars indicate S.E. when larger than symbols. Symbols as shown in Fig. 1

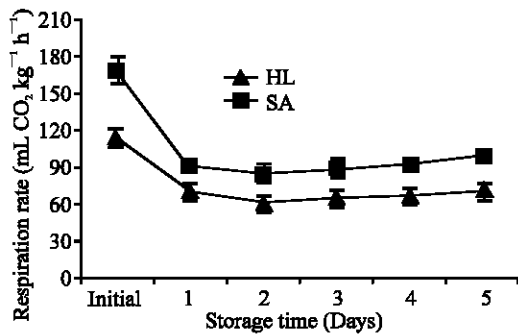


Fig. 3: Changes in respiration rate of two broccoli cultivars during storage at 20°C. Vertical bars indicate S.E. when larger than symbols. Symbols as shown in Fig. 1

Color assessment: Changes in color of broccoli heads during storage is shown in Fig 2. Hue angle (used as a measure of yellowing) gradually declined at end of the storage period. 'Sairin' showed a higher rate of hue angle decline than 'Hartland'.

Respiration rate: In both cultivars, respiration rate was very high initially and these levels rapidly decreased

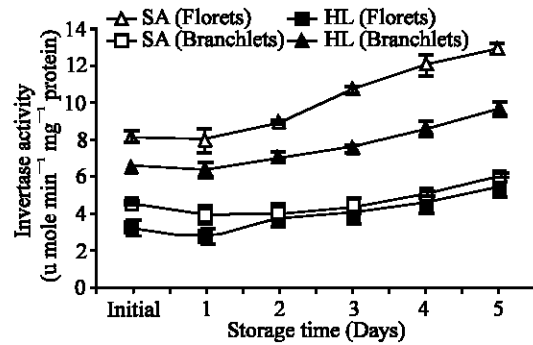


Fig. 4: Changes in acid invertase activity in the soluble fraction in the florets and branchlets of broccoli. Each point represents the mean of 3 replications. Vertical bars indicate S.E. when larger than symbols. Symbols as shown in Fig. 1

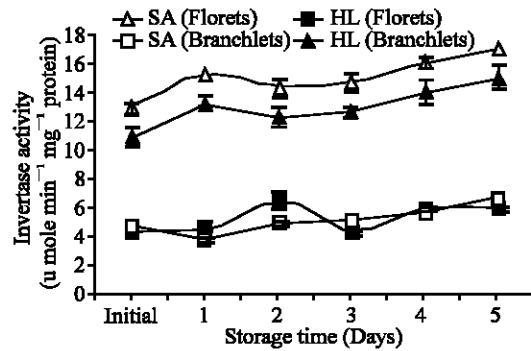


Fig. 5: Changes in acid invertase activity in the cell wall-bound fraction in the florets and branchlets of broccoli. Each point represents the mean of 3 replications. Vertical bars indicate S.E. when larger than symbols. Symbols as shown in Fig. 1

during the first 24 h of postharvest storage and slightly increase at the end of the 5 days of storage (Fig. 3). 'Sairin' heads produced higher rate of CO₂ than 'Hartland'.

Acid invertase activity in soluble fraction: Fig. 4 shows the acid invertase activity in the soluble fraction. In both cultivars, the acid invertase showed a higher activity in the branchlets than in the florets. The activity increased gradually in both the florets and branchlets throughout storage period of the two cultivars. In both portions, 'Sairin' showed a significantly higher activity than 'Hartland'.

Acid invertase activity in cell wall-bound fraction: In both cultivars, invertase activity in cell wall-bound fraction also gradually increased in the branchlets and with few fluctuations in the florets until the end of the experimental period (Fig. 5). But, a significantly higher activity was found in the branchlets of 'Sairin' than 'Hartland'.

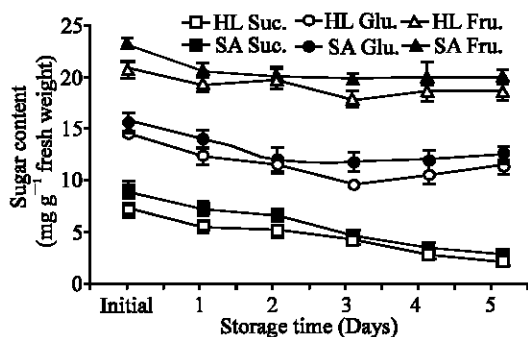


Fig. 6: Changes in soluble sugar contents in the florets of broccoli. Each point represents the mean of 3 replications. Vertical bars indicate S.E. when larger than symbols. Symbols as shown in Fig. 1

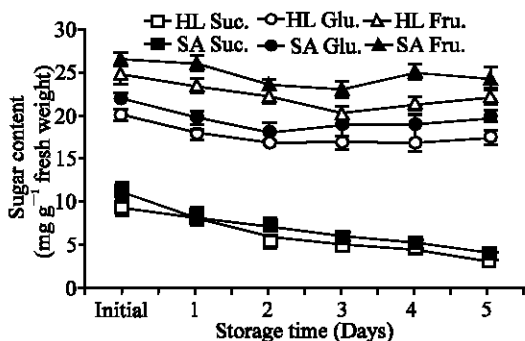


Fig. 7: Changes in soluble sugar contents in the branchlets of broccoli. Each point represents the mean of 3 replications. Vertical bars indicate S.E. when larger than symbols. Symbols as shown in Fig. 1

Changes in soluble sugar content: Figs. 6 and 7 show the changes in soluble sugar content in the florets and branchlets portions of the two broccoli cultivars during storage, respectively. Sucrose content decreased gradually in the florets and branchlets of two cultivars throughout the storage period while there is no remarkable change was observed in glucose and fructose. A higher amount of soluble sugars (sucrose, glucose and fructose) were observed in the branchlets than florets in both cultivars. Among the three sugars, the level of fructose always remained higher than that of glucose and sucrose in the florets as well as in the branchlets of the two cultivars.

Correlation coefficients (*r*) between acid invertase activity and sugar content: Table 1 shows the correlation coefficient (*r*) between the activities of invertase and sugar content in the florets and branchlets of two cultivars. There was a highly significant negative correlation observed between the acid invertase activity

Table 1: Correlation coefficients (*r*) between acid invertase activity and sugar concentration in soluble and cell wall bound-fractions of broccoli florets and branchlets in two cultivars

Character	Cultivar	Portion	Correlation coefficients (<i>r</i>) value	
			SF	CWBF
Sucrose	Hartland	Florets	-0.887**	-0.604*
		Branchlets	-0.821**	-0.651**
	Sairin	Florets	-0.645**	-0.794**
		Branchlets	-0.795**	-0.740**
Glucose	Hartland	Florets	-0.450	-0.260
		Branchlets	-0.440	-0.787**
	Sairin	Florets	-0.240	-0.510*
		Branchlets	-0.320	-0.420
Fructose	Hartland	Florets	-0.410	-0.079
		Branchlets	-0.442	-0.560*
	Sairin	Florets	-0.160	-0.360
		Branchlets	-0.446	-0.290

SF = soluble fraction, CWBF = cell wall-bound fraction, *, ** denote significant at $p < 0.05$ and $p < 0.01$ respectively, $n = 18$

of the soluble and cell wall-bound fraction and sucrose content in the florets and branchlets of both cultivars. No significant correlation was found between the invertase activity and soluble sugars (glucose and fructose) in both portions of each cultivar except florets of 'Sairin' and branchlets of 'Hartland' in cell wall-bound fraction.

DISCUSSION

Many physiological and compositional changes occur during postharvest senescence in broccoli. The major changes that we observed include color, weight loss, respiration, soluble sugar content and invertase activity. Florets of broccoli head commenced yellowing between 3 to 4 days after storage (Fig. 2) as indicated by decreasing hue angle. This is the most striking symptom of the postharvest senescence of broccoli and can be regulated by ethylene^[3]. Weight loss was highest after 1 day of storage which is may be due to moisture loss and loss in reducing substances of the commodity. A decline in respiration rate was observed after 48 h of storage and slowly increased thereafter (Fig. 3). A similar trend has previously been observed in different cultivars of broccoli during storage at 20°C and 16°C^[5,18]. The declining respiration rate could be due to respiratory substrate or respiratory control factors translocated from the other tissues from which the head is separated at harvest, e.g., leaves and roots^[5]. The florets of 'Sairin' became yellow earlier than 'Hartland'. This is probably due to high level of CO₂ production in 'Sairin'. Makhlouf *et al.*^[19] reported that broccoli florets became yellow early as respiration increased.

In the present study, acid invertase activity in the soluble fraction was higher in the branchlets than florets and significantly increased until end of the storage period in both cultivars (Fig. 4). Recent study^[9] demonstrated

that soluble acid invertase activity steadily increased in broccoli florets and branchlets throughout storage for up to 6 day. In the cell wall-bound fraction of both portions, protein content was about 2.0-2.5 fold higher than soluble fraction (data not shown). On the other hand, the amount of glucose produced per min was about 1.10-1.25 fold higher in soluble fraction than cell wall-bound fraction (data not shown). This indicate that the invertase activity was higher in cell wall-bound fraction than soluble fraction since the enzyme activity was expressed as the amount of glucose produced per min per mg of protein. Similar result was also observed in asparagus spears^[20]. Comparatively, higher invertase activity (SF and CWBF) was found in 'Sairin' than 'Hartland'. The higher invertase activity observed in 'Sairin' might be due to the content of anthocyanin, because anthocyanin containing commodity contain higher amount of soluble sugar. Li *et al.*,^[21] found a positive correlation between anthocyanin and soluble sugar (reducing and non- reducing) contain in the skin of apple. Soluble sugars decline substantially during the storage period and concentration was higher in branchlets than florets in both cultivars. King and Morris^[5] reported that soluble sugar concentration (sum of glucose, fructose and sucrose) was lower in floral section than base section (branchlets) in broccoli. Sucrose content change rapidly but the ratio of glucose and fructose did not alter markedly in both florets and branchlets during storage period (Figs. 6 and 7). Sucrose content is low as an enhanced flow of sucrose to the actively respiring cell and this may cause the observed reduction in stored sucrose level seen in the broccoli.

There was a highly significant negative correlation between acid invertase activity (SF and CWBF) and sucrose content in the florets and branchlets in both cultivars (Table 1), suggesting that the decrease of sucrose content was associated with increase in the invertase activity. Such correlation has previously been observed in broccoli florets and top portion of asparagus spear^[22]. They reported that sucrose is the major sugar which is cleaved by invertase to produce glucose and fructose and invertase also shows a high affinity for sucrose^[23].

Based on the above discussion, it appeared that 'Sairin' and 'Hartland' follow similar patterns of postharvest changes in respiration, color, invertase activity and sugar content but 'Sairin' showed significantly higher respiratory metabolism, higher invertase activity (both SF and CWBF), sugar content and became yellow earlier than 'Hartland'. These variability indicate that respiration and changes in invertase activity play an important role in early postharvest senescence in broccoli. Further work is required to clarify the

relationship between respiration and invertase activity during postharvest senescence in broccoli.

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