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Seasonal changes in texture, sugar and organic acid contents and activities of some ammonia-assimilating enzymes in lettuce

Dulal Chandra[A], Toshiyuki Matsui[A], Haruo Suzuki[A], Yusuke Kosugi[A] and Koichi Fujimura[B]

[A] *Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki-Cho, Kagawa 761-0795, Japan,* [B] *Kagawa Prefectural Agricultural Experiment Station, Busshouzan, Takamatsu, Kagawa 761-8078, Japan. Kagawa University, 2393 Ikenobe, Miki-Cho, Kagawa 761-0795, Japan. E-mail: dchandrajp@yahoo.com*

Abstract

As a cool weather crop, lettuce (*Lactuca sativa* L.) is very sensitive to the changes in temperature during growth. This study investigated the textural, compositional and some biochemical changes in the outer and inner leaf tissues of two crisphead lettuce cultivars ('Bittsu' and 'Cisco') harvested in different seasons. The result demonstrated that in colder months, the crispiness of lettuce leaves reduced significantly and higher amount of sugars, organic acids and ammonia were accumulated. In general, between the two cultivars, 'Bittsu' contained higher amount of sugars and organic acids, while 'Cisco' contained higher amount of ammonia. However, inner leaf tissues contained higher amount of ammonia than outer leaf tissues in both cultivars. The level of fructose was found to be higher than glucose and sucrose in all cases while malic acid was the main component in organic acid fraction. The activities of ammonia-assimilating enzymes such as glutamine synthetase (GS; EC 6.3.1.2) and asparagine synthetase (AS; EC 6.3.5.4) either decreased or nearly remain constant depending on the tissue types during the colder months. Outer leaf portion showed higher GS activity than inner leaf tissues. However, both of aminating and deaminating activity of glutamate dehydrogenase (GDH, EC 1.4.1.2) decreased in the outer leaves whereas deamination activity slightly increased in the inner leaf tissues during warmer harvest months.

Key words: Ammonia, amination, asparagine synthetase, crispness, deamination, glutamine synthetase, *Lactuca sativa,* sugar.

Introduction

Climate limits the agriculture production capacity of any country and nearly every element in planning, producing, harvesting and postharvest operations such as marketing of the products are directly or indirectly linked to the variations of weather. It is commonly accepted that seasonal factors such as temperature influences the composition of plant tissues during growth and development. Total available heat and the extent of low and high temperature are the most important factors in determining growth rate and chemical composition of horticultural crops (Lee and Kader, 2000). For instance, in iceberg lettuce, maximum head weight was found when crops experienced a mean temperature lift of about 2ºC from transplanting to maturity, corresponding to a mean temperature of about 12ºC, while the time to maturity steadily decreased with increased temperature (Wurr *et al.*, 1996). Head density, diameter and solid heading are also affected by fluctuations in temperature during different growth stages of crisphead lettuce (Wurr *et al.*, 1992). High temperature during the summer rainy season is associated with bolting (Glenn, 1984; Wurr *et al.*, 1992) and some physiological disorder like tipburn. Low temperature, on the other hand, slackens the growth of leaf lettuce (Knight and Mitchell, 1983).

Iceberg or crisphead lettuce is more popular mainly due to its crisp texture than others such as butterhead, leaf or romaine. Textural changes are among the main causes of quality loss for lettuce and soft or limp product may be rejected by the consumer. Since temperature has a direct influence on metabolism, it also indirectly affects the cellular structure and other components which determine texture (Sams, 1999). Hence, it is important to know the textural and some compositional properties of the products when it is cut and ready for consumption. Especially, in lettuce, sugar and nitrate contents greatly vary with the seasonal temperature (Behr and Wiebe, 1992).

Recently, Tosun and Ustun (2004) summarized that raw consumed vegetables like lettuce contain high nitrate nitrogen which may be detrimental to human health. The incorporation of inorganic nitrogen, nitrate and ammonium into the carbon skeleton is an important biochemical feature of land plant which might be influenced by the environmental conditions (Inokuchi *et al.*, 2002). Ammonium is a preferred source of nitrogen and a key metabolite situated at the junction between carbon metabolism and nitrogen assimilation, because nitrogen compound can choose an alternative pathway according to the stage of crop growth and environmental conditions (Inokuchi *et al.*, 2002). Therefore, it is desirable to study the enzymes responsible for ammonia assimilation in relation to seasonal environmental variation. The incorporation of ammonium into the pool of N-containing molecules is first catalysed by the enzyme glutamine synthetase (GS; EC 6.3.1.2). Glutamine and glutamate, which are formed through the action of GS from ammonia, serve as nitrogen transport compounds and nitrogen donors in the biosynthesis of many compounds in plant including amino acids and chlorophyll. Nitrogen may also be channeled from glutamine and glutamate to asparagines by another enzyme asparagine synthetase (AS; EC 6.3.5.4) (Suarez *et al.*, 2002). In addition to GS and AS, glutamate dehydrogenase (GDH; EC 1.4.1.2) also plays key role among other enzymes which maintain the balance of carbon and nitrogen

(Miflin and Habash, 2002). GDH is induced by high levels of ammonia (Cammaerts and Jacobs, 1985) and capable of releasing amino nitrogen from amino acids to give keto-acid and ammonia that can be separately recycled to be used in respiration and amide formation, respectively (Miflin and Habash, 2002).

Studies on lettuce have generally been limited to sensory attributes, general appearance, wilting, enzymatic browning, decay and physiological disorders during packaging and storage (Alsadon, 1993; Artes and Martinez, 1996; Toole *et al.*, 2000; Murata *et al.*, 2004). Hence, this study was conducted to measure the changes in texture, sugar, organic acid and ammonia contents as influenced by seasonal temperature, tissue type and cultivar. The activities of enzymes related to ammonia assimilation which have been reported to influence the overall quality and shelf life of lettuce after harvest are also discussed.

Materials and methods

Plant materials: Heads of two crisphead lettuce (*Lactuca sativa* L.) cultivars 'Bittsu' and 'Cisco' grown in field condition were harvested from Kagawa Prefectural Agricultural Experiment Station, Busshouzan, Kagawa, Japan. Harvesting was done monthly intervals from December, 2005 to April, 2006 when the head reached at commercial maturity. Temperature was recorded daily in respect of maximum, minimum and a-day average. Then the values were averaged individually on the calendar month basis (Fig. 1). The harvested heads were packed in a box with crushed ice and immediately transported to the laboratory. Outer (green) and inner (white) leaves were separated from the head, cut into small pieces (ca. 2 × 2 cm) and immediately stored at -30ºC until needed for analysis.

Texture measurement: Texture was measured rheologically based on the measurement of breaking force to puncture the leaf tissue. Breaking force was determined with a creep meter (Yamaden Rheoner RE-33005) equipped with software ver. 2.0 for automatic analysis. With a running load cell of 20 N, the rheometer was mounted with a cylinder like plunger (adapter no. 5) of 5 mm diameter and 18 mm in length. The flat base containing a 12 mm high and 12 mm diameter hole, on which the sample was horizontally placed and tightened the sample with clips, moves upward to the plunger at a speed of 1 mm s^{-1} to measure the puncture or breaking force as an index of crispness of the sample. Lower breaking force indicates higher crispness and conversely higher breaking force is needed to puncture the soft or limp tissues. For each sample, measurements were taken from each of five pieces (approx. 3 × 5 cm) of outer and inner leaf segments separately and the average of 15 measured values was expressed as breaking force of the sample.

Determination of soluble sugars and organic acids: Approximately 4 g of lettuce tissue (for each portion) was mixed with 1 g sea sand and homogenized in a cool mortar and pestle. To make a total volume of 10 mL of the homogenate, required amount of distilled water was added to the homogenate and centrifuged at 11,000 × *g* at 2ºC for 10 min. The supernatant was filtered through a cellulose nitrate membrane filter (0.45µm pore size). Soluble sugars were analyzed using a high performance liquid chromatography (HPLC) containing a stainless steel column (10.7 mm ID × 30 cm) packed with silica gel (gel pack C 610). The mobile phase, filtered air free distilled water, was pumped through the column at a flow rate of 1.0 mL min^{-1}. The pressure was adjusted to 28-30 kg cm^{-2} and the column temperature was maintained at 60ºC. A refractive index (RI) monitor (Hitachi L-3300) was used to record the peak heights. Sucrose, glucose and fructose were identified by their retention times and were quantified according to standard. On the other hand, organic acids were also analyzed by HPLC using a stainless steel column (10.7 mm ID × 30 cm) packed with silica gel (gel pack GL-C610H-S). The mobile phase was 0.1% phosphoric acid adjusted to a flow rate of 0.5 mL min^{-1}. The pressure was adjusted to15-20 kg cm^{-2} and the column temperature was maintained at 60ºC. The ultraviolet (UV) detector (Hitachi L-4200) set at 210 nm was used to record the peak heights. Citric and malic acids were identified by their retention times and were quantified according to standard.

Ammonia assay: To assess ammonia content, 2 g sample from each portion of lettuce tissue was extracted with 10% trichloroacetic acid at 1:10 ratio(w/v) in an ice bath (0- 4ºC) and centrifuged at 11, 000×*g* at 2ºC for 10 min. Ammonia content was assayed as described by Kun and Kearney (1974), where 1 mL assay mixture contained 200 µL 0.5 M tris-HCl buffer (pH 8.0), 100 µL 0.1 M 2-oxoglutarate solution (pH 7.4), 30 µL 8 mM β-NADH solution, 20 µL G*l*DH (10mg mL^{-1}), 150 µL distilled water and 500 µL of neutral extract sample. The decrease in NADH, as determined by the change of extinction at 365 nm was used as a measure of the reaction.

Enzyme extraction: Approximately 5 g lettuce sample from outer and inner leaf portion was homogenized in ice cold condition (ca. 0-4ºC) with 1% polyvinylpolypyrrolidone (PVPP) proportional to the sample weight, 1 g sea sand and 5 mL buffer solution using a mortar and pestle. Extraction was performed as described by Hurst and Clark (1993), in which buffer A contained 50 mM tris-HCl (pH 7.6), 10 mM $MgSO_4.7H_2O$, 1 mM EDTA, 1 mM dithiothreitol (DTT), 12 mM 2-mercaptoethanol, 5 mM L-glutamate and 100 mL glycerol per liter and buffer B contained 50 mM KH_2PO_4 (pH 8.0), 50 mM KCl, 1 mM EDTA, 1mM EGTA, 1 mM DTT, 12 mM 2-mercaptoethanol, and 100 mL glycerol per liter. Buffer A was used for the extraction of GS and GDH while buffer B was used for AS. The homogenate was squeezed through four layers of cotton cloth. The residual tissues were re-extracted with an additional 5 mL of the same buffer and the filtrate was centrifuged at 11,000×*g* at 2ºC for 10 min. The resulting supernatant was used for enzyme assay.

Enzyme assay: The enzymatic activities were assayed in a total volume of 1 mL assay mixture. The activity of GS was determined with 80 mM Na-L glutamate, 100 mM tricine-KOH buffer (pH 7.0), 6 mM hydroxylammonium chloride ($HONH_3Cl$), 20 mM $MgSO_4.7H_2O$, 1 mM diethylenetriamine pentaacetic acid (DTPA), 8 mM ATP and 8 mM mercaptoethanol. For AS, activity was assayed with a mixture of 20 mM Na-L-aspartate monohydrate, 100 mM tris-HCl (pH 7.0), 12mM $MgCl_2.6\ H_2O$, 10 mM ATP-2Na and 800 mM hydroxylammonium chloride ($HONH_3Cl$). After incubating the assay mixture at 35ºC for 8 min and at 30ºC for 10 min for GS and AS, respectively, the reaction was stopped by the addition of 1 mL ferric chloride reagent that contains 0.37 M $FeCl_3$, 0.67 N HCl and 0.2 M trichloroacetic acid (TCAA). Both of GS and AS activity was measured using

a double beam spectrophotometer (Shimadzu model UV-150-02) at 540 nm and soluble protein contents was measured following the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Both aminating and deaminating activities of GDH were determined spectrophotometrically (Shimadzu model UV-150-02) at 350 nm according to NADH oxidation or NAD^+ reduction maintaining a temperature of 30ºC. For GDH amination, a total volume of 1.0 mL assay mixture contained 10 mM α-ketoglutaric acid, 100 mM tris-HCl (pH 8.0), 200 mM NH_4Cl, 1mM $CaCl_2$ and 0.2 mM NAD(P)H. The reaction was started by adding 200 µL crude extract. Likewise, the 1.0 mL assay mixture for GDH deamination consisted of 100 mM L-glutamate, 100 mM tris-HCl (pH 9.3), 1 mM $NAD(P)^+$ and 0.5 mM $CaCl_2$. The reaction was started with the addition of 200 µL crude extract. Blank controls were performed omitting individual substrates. One unit of GDH activity is defined as the reduction or oxidation of one micromole of coenzyme (NADPH/ NADP, respectively) per min at 30ºC.

Statistical analysis: A randomized complete block design was used with three replications. Following ANOVA, the level of significance between the means were calculated using the Duncan's multiple range test (DMRT). Linear correlation was used to evaluate the relationship between enzyme activities and ammonia contents.

Results

Textural changes: The textural measurement showed that breaking force changed significantly in the outer and inner leaf of two lettuce cultivars harvested at different months. Higher ($P <$ 0.05) breaking force was recorded during the colder months (Figs. 1 and 2). Both the tissues followed almost the same pattern in changes, except the outer leaves of both cultivars, which showed the maximum breaking force in February.

Changes in sugar and organic acid contents: Significant changes in soluble sugar contents in both portions of lettuce head were observed at different months (Fig. 3). Comparatively higher amount of sugars (sucrose, glucose and fructose) contents were observed in the cultivar 'Bittsu' than that in 'Cisco'. Generally, inner leaf contained higher amounts of glucose and fructose than outer leaf, while an inverse relation was found for sucrose content. Except in January, sugar content in outer leaf tissues declined (P <0.05) with the increase in temperature. In inner leaf tissues, the same change was observed only for sucrose content. Among the three sugars, the level of fructose was found to be higher than glucose and sucrose in all cases. The content of malic acid, on the other hand, was substantially higher than citric acid in both tissue types (Fig. 4). However, outer leaf tissues contained higher amount of citric acid than the inner leaf tissues. The content of malic acid was significantly higher in 'Bittsu' than that in 'Cisco'. Generally, the quantities decreased (P <0.05) during the warmer harvest period except in January where the content of malic acid declined rapidly. However, malic acid content in 'Bittsu' outer leaf was almost constant during warmer harvest months.

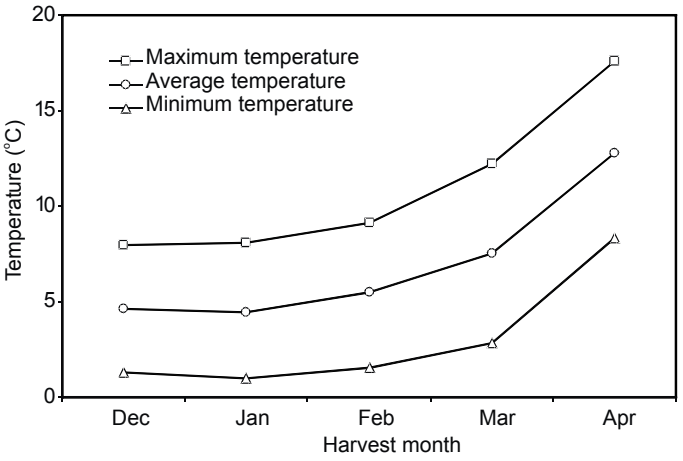

Fig. 1. Temperature of harvest months from December to April.

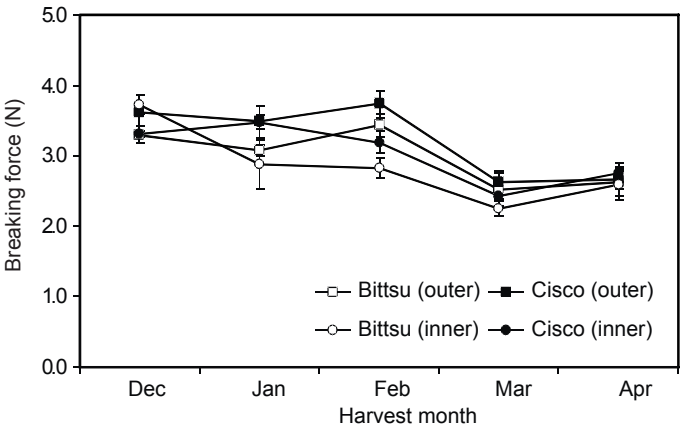

Fig. 2. Changes in breaking force to puncture the leaf tissues (outer and inner) of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE.

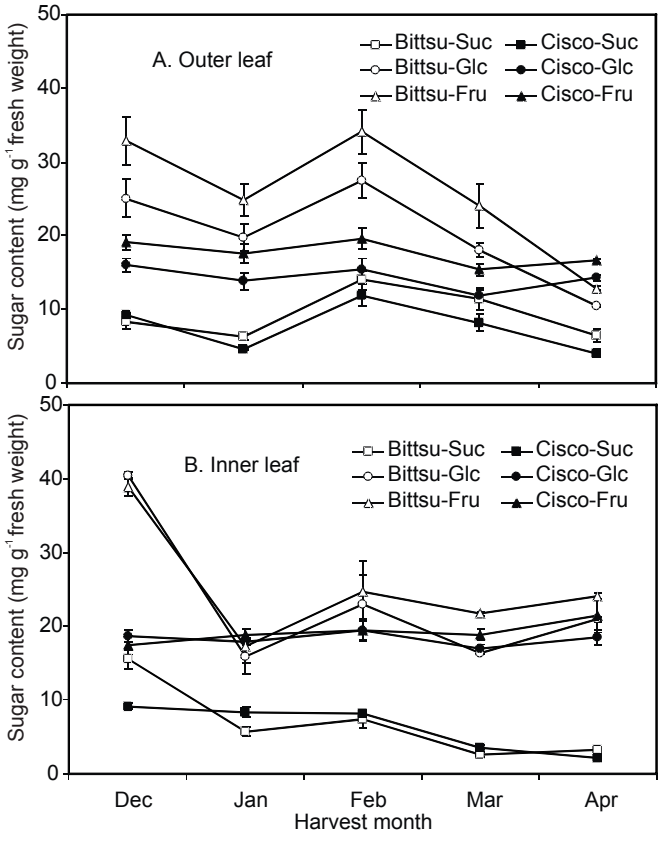

Fig. 3. Seasonal changes in soluble sugar contents in the outer leaf (A) and inner leaf (B) of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE. Suc= sucrose, Glc=glucose, Fru= fructose.

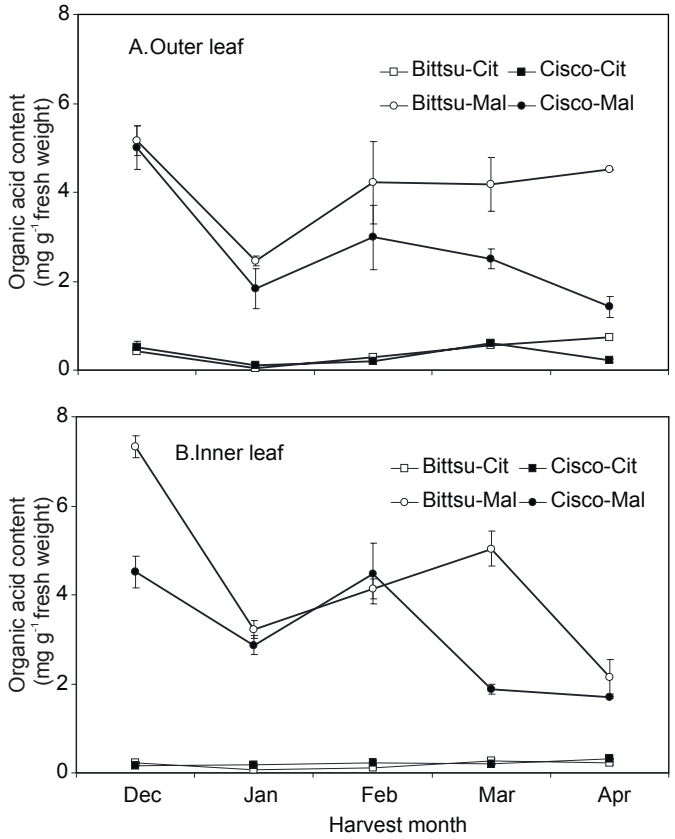

Fig. 4. Seasonal changes in organic acid contents in the outer leaf (A) and inner leaf (B) of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE. Cit = citric acid, Mal = malic acid

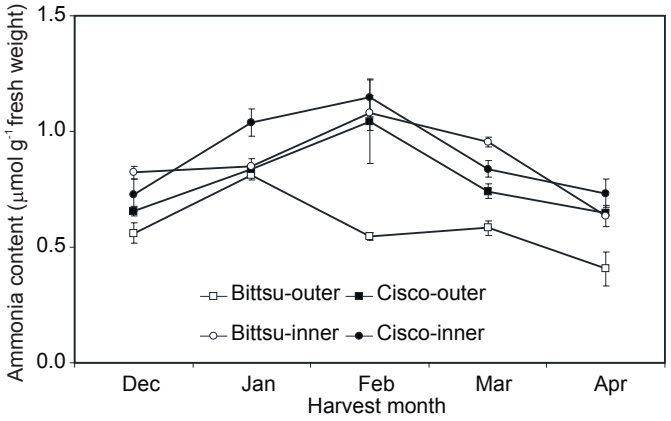

Fig. 5. Ammonia content in the outer leaf and inner leaf of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE.

Ammonia content: Ammonia content of lettuce leaves changed noticeably throughout the harvest months. Higher ($P < 0.05$) ammonia was found in colder months with a maximum level in February, except the outer leaf tissues of 'Bittsu' (Fig. 5). Between the two cultivars, 'Cisco' contained higher amount of ammonia than 'Bittsu'. On the other hand, inner leaf tissues of both cultivars contained higher ammonia than that of outer tissues.

Glutamine synthetase activity: The GS activity in the outer leaf tissues decreased in January and remained unchanged in the following month and slightly increased at the last two harvest months (Fig. 6). In both the cultivars, outer leaf tissues showed considerably higher activity than inner tissues. The highest GS activity of outer tissues was measured in December and April for

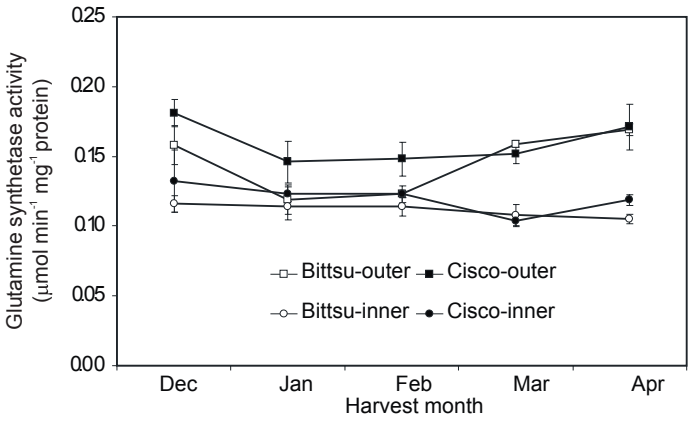

Fig. 6. Changes in the activities of glutamine synthetase in the outer leaf and inner leaf of two lettuce cultivars harvested in different seasons. Data are the means of three replications. Vertical bars represent SE.

'Cisco' and 'Bittsu', respectively. No specific trend in changes of GS activity was found in inner tissues.

Asparagine synthetase activity: The highest AS activities of leaf tissues were obtained when lettuce was harvested in December (Fig. 7). After that the activity of the outer leaf tissues decreased ($P < 0.05$) gradually in the following months and again increased with the rise in temperature. However, in the inner leaf tissues AS activities fluctuated with changing temperature during the harvest months. Cultivar 'Bittsu' showed comparatively higher enzyme activity than 'Cisco'.

Glutamate dehydrogenase activity: GDH-amination activity was higher than deamination activity in both tissue types and cultivars. In outer leaf tissues, GDH-aminating activity declined ($P < 0.05$) with the increase in temperature for both cultivars while inner leaf tissues did not show any trend (Fig. 8). Outer leaf tissues showed noticeably higher aminating activity than inner leaf while an opposite trend was found for GDH-deaminating activity. The deamination activity of inner leaf tissues slightly increased in the warmer harvest month. On the other hand, the deamination activity of 'Bittsu' outer leaf decreased significantly ($P < 0.05$) with the increases in temperature throughout the harvest period.

Correlation between enzyme activities and ammonia content: A significant negative correlation was found between GS activity and ammonia content in outer leaf portion of 'Bittsu', while in

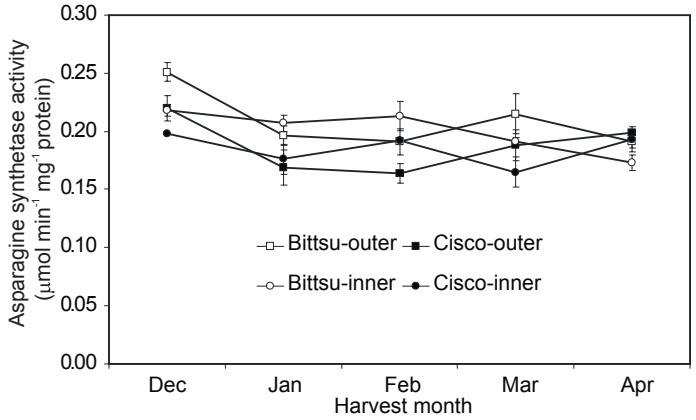

Fig. 7. Changes in the activities of asparagine synthetase in the outer leaf and inner leaf of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE.

other cases, the correlations were poor (Table 1). On the other hand, significantly negative correlation was observed between AS activity and ammonia content in the outer leaf portion of 'Cisco'. However, this relationship was significantly positive in the inner leaf portion of 'Bittsu'. No significant correlation was observed between GDH-amination/ deamination activities and ammonia content, except in the outer leaf portion of 'Bittsu'.

Table 1. Correlation coefficient (r) values computed from linear regression analyses between enzymes' activities and ammonia content in outer and inner leaf tissues of lettuce head harvested in different months

Cultivar	Portion	Enzyme	Correlation coefficient (r)
'Bittsu'	Outer	Glutamine synthetase	-0.592*
		Asparagine synthetase	0.068
		GDH-amination	0.536*
		GDH-deamination	0.637*
	Inner	Glutamine synthetase	0.276
		Asparagine synthetase	0.640*
		GDH-amination	0.041
		GDH-deamination	-0.461
'Cisco'	Outer	Glutamine synthetase	-0.188
		Asparagine synthetase	-0.519*
		GDH-amination	0.204
		GDH-deamination	0.107
	Inner	Glutamine synthetase	-0.086
		Asparagine synthetase	0.033
		GDH-amination	-0.375
		GDH-deamination	-0.307

* Significant at $P \leq 0.05$

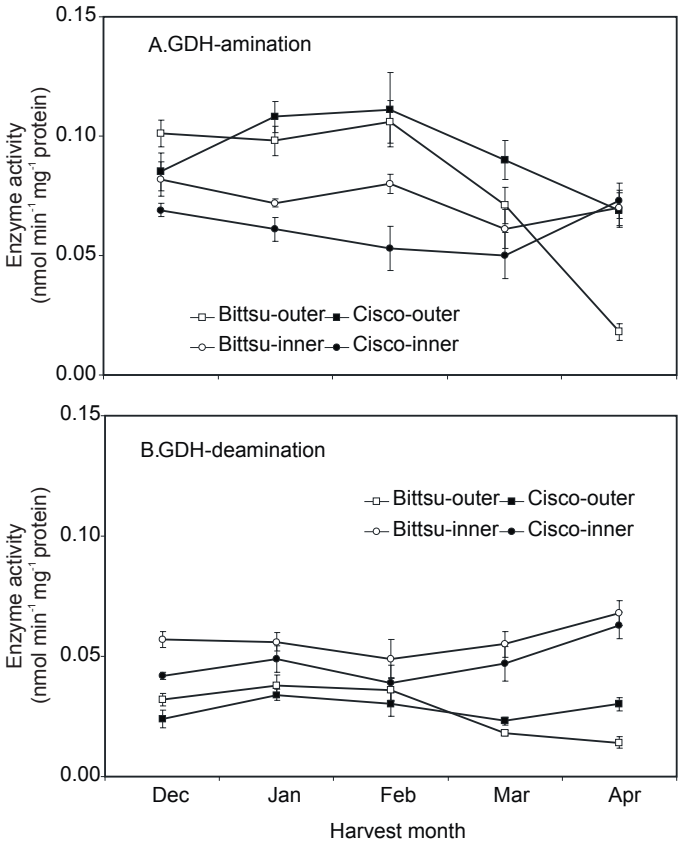

Fig. 8. Changes in the activities of (A) GDH-amination and (B) GDH-deamination in the outer leaf and inner leaf of two lettuce cultivars harvested in different months. Data are the means of three replications. Vertical bars represent SE.

Discussion

Crisphead lettuce has been described as a plant of temperate zone, cool weather crop (Whitaker *et al.*, 1974), so the production and quality of head is most dependent on ambient temperature. This study illustrates some significant qualitative and biochemical changes that occurred in lettuce harvested from winter to spring season. Since lettuce is consumed as raw, mainly for salad, its textural quality is critically important to meet the consumer demand. However, the texture evaluation of lettuce is relatively difficult due to the heterogeneity of the product (Martin-Diana *et al.*, 2006). In this study, the textural properties of outer/ green (photosynthetic) and inner/ white (vascular) leaf tissues showed that winter season's crops are less crispy than spring crops. The maximum breaking force to puncture the leaf tissues was recorded in February and December in outer and inner leaf tissues of both cultivars, respectively (Fig. 2). The higher breaking force which indicates a lower crispness of the tissues might be due to the slow growth during the cold weather as reported in other vegetables like asparagus spears (Bhowmik *et al.*, 2002). Moreover, calcium is associated with maintaining the cell wall structure of vegetables by interacting with pectin to form calcium pectate (Martin-Diana *et al.*, 2006). Due to the action of the enzyme pectin methyl esterase (PME), calcium diffusion into tissues increases at higher temperature (Bartolome and Hoff, 1972) thus reveals a firmer structure of the tissues.

Higher accumulations of soluble sugars were found in the colder months. Specially, sucrose content of both lettuce cultivars decreased gradually in warmer months except with some fluctuations in the outer leaf tissues. This result is an agreement with other results of green vegetables like asparagus spears (Bhowmik *et al.*, 2001) and broccoli (Pramanik *et al.*, 2004). In spinach, leafy vegetable, Guy *et al.* (1992) reported that accumulation of sugars increased 10 to 20 fold at low temperature. It could be argued that sucrose is a storage carbohydrate that can be rapidly mobilized as metabolic needs, and at low temperature photosynthetic energy capture is reduced but to a lesser degree than the metabolic utilization processes. Moreover, active growth is almost always reduced or suspended at low temperature resulting in decreased demand of photosynthate which leads to reserve the excess photosynthate in the form of carbohydrate (Guy *et al.*, 1992). However, all the sugar contents decreased steeply in January without almost any changes in temperature (Figs.1 and 3). The reason might be the foggy weather and less hours of photoperiod prevailing that time. In some previous studies it was reported that lettuce growth is significantly affected by the interaction of solar radiation and temperature (Glenn, 1984; Koontz and Prince, 1986; Wurr and Fellows, 1991). Furthermore, organic acid content followed almost the same trend with sugar and showed similarity with the result of Pramanik *et al.* (2004) in broccoli. In most cases, the immediate precursor of an organic acid is sugar or another organic acid from which organic acid is formed or synthesized (Kays, 1991). However, unlike Pramanik *et al.* (2004) the organic acid fraction consisted of only two acids, where malic acid was the main component and oxalic acid could not be detected (Fig. 4). Blom-Zandstra and Lampe (1985) also reported that the main component of the organic acids in lettuce

is malate and oxalic acid can not be detected either by G.L.C. or other analytical techniques.

Except in December, higher ammonia content was found in the colder harvest months. The reason could be the higher ammonium uptake of the crop at low temperature as reported in barley (Macduff and Jackson, 1991) and in broccoli (Baclayon *et al.*, 2006). The lower ammonia content in December could be explained as a consequence of higher temperature in the previous months. However, significantly higher ammonia contents were observed in the inner tissues compared with the outer tissues. Marsic and Osvald (2002) also reported that inner tissues accumulate considerably higher NH_4^+ than outer leaf tissues. The possible reason might be the inner leaf contains more growing tissues than outer leaf, and growing tissues accumulate higher amount of nutrients like ammonium.

The activity of GS was almost constant in the inner leaf tissues during the harvest period (Fig. 6). However, the activity in outer leaf tissues changed with the changes in temperature. The inverse relationship between ammonia content and GS activity in the outer leaf portion ($r = -0.592$ and -0.188 for 'Bittsu' and 'Cisco', respectively) showed an agreement with Peeters and Van Laere (1992), where they concluded that the accumulation of ammonium coincided with the disappearance of GS activity. The variation of the GS activity between two types of tissues might be due to the possiblity that the inner leaf tissues contain higher GS_1 (cytosolic) and outer tissues contain higher GS_2 (chloroplastic), in which the later one is highly contributing in the total GS activity (Chandra *et al.*, 2006). The activities of AS in the outer leaf of both cultivars followed almost the same trend as GS except in April for the cultivar 'Bittsu', while in inner leaf tissues AS activity fluctuated over the harvest months (Fig. 7). It was suggested that there is a functional GS_1/ AS cycle, where the induction of AS has been observed in parallel with the induction of GS_1 expression (Avila *et al.*, 2001). However, significantly negative correlation was found between ammonia content and AS activity only in the outer leaf portion of 'Cisco' (Table 1). It is evident that AS is capable to use glutamine more efficiently than NH_4^+ in the conversion of aspartate to asparagine (Rognes, 1975).

In both aminating and deaminating directions, GDH activity was measured as this enzyme provides an assimilatory pathway for ammonium under various stress conditions (Srivastava and Singh, 1987). In both directions, the activity of GDH in outer leaf decreased whereas deaminating activity of inner leaf slightly increased in the warmer harvest months. The higher activity of GDH amination in colder months may be a consequence of higher ammonia content during that period. It was reported that, GDH is induced at high levels of ammonia (Cammaerts and Jacobs, 1985). The positive relations between ammonia content and GDH activity in the outer leaf portion suggest that GDH is playing more vital role in ammonia assimilation in this portion (Table 1). However, such relation was stronger in 'Bittsu' than that in 'Cisco'. As deamination activity operates in energy generation (Cammaerts and Jacobs, 1985), very little changes in deamination could be explained in such a way that the tissues contained higher amount of sugar which is supplying energy. Sugar appears to play a more central role in the regulation of GDH than ammonia and other nitrogenous sources (Lea *et al.*, 1990). Moreover, the decrease in GS activity might be compensated by the increased GDH activity during the colder months as the antagonistic relation between GS and GDH have already been reported (Ratajczak *et al.*, 1981).

In conclusion, during colder months the crispness of lettuce leaf reduced and accumulated higher amount of sugars, organic acids and ammonia. In general, between the two cultivars, 'Bittsu' contained higher amount of sugars and organic acids, while 'Cisco' contained higher amount of ammonia. The activities of GS and AS either decreased or remained nearly constant depending on the tissue type during the colder months. However, GDH activity in the outer leaf and GDH deaminating activity in the inner leaf tissues changed, with the changes in temperature throughout the harvest period. Detailed study is needed to confirm the effects of environmental factors such as temperature, photoperiod and light intensity on the textural and compositional quality, and the activities of ammonia assimilating enzymes in lettuce harvested monthly for a longer period. The effect of such growing conditions on the shelf life and storability along with textural and compositional quality would provide an idea for the growers to select the suitable growing season to achieve longer shelf life of this perishable commodity.

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References

Alsadon, A.A. 1993. Sensory quality attributes of butterhead lettuce cultivars grown in arid conditions. *HortScience*, 28: 159-160.

Artes, F. and J.A. Martinez, 1996. Influence of packing treatments on the keeping quality of "Salinas" lettuce. *Lebensmittel Wissenschaft und Technologie*, 29: 664-668.

Avila, C., F.R. Canton, P. Barnestein, M.F. Suarez, P. Marraccini, M. Rey, J. Humara, R. Ordas and F.M. Canovas, 2001. The promoter of a cytosolic glutamine synthetase gene from the conifer *Pinus sylvestris* is active in cotyledons of germinating seeds and light regulated in transgenic *Arabidopsis thaliana*. *Physiol. Plant*., 112: 388-396.

Baclayon, D.P., T. Matsui, H. Suzuki, Y. Kosugi and M. Tomohiro, 2006. Fluctuation in the activities of some ammonia-assimilating enzymes and ammonia content in broccoli harvested at different seasons. *Asian J. Plant Sci*., 5: 54-60.

Bartolome, L.G. and J.E. Hoff, 1972. Firming of potatoes: biochemical effect of preheating. *J. Agril. Food Chem*., 20: 266-270.

Behr, U. and H.J. Wiebe, 1992. Relation between photosynthesis and nitrate content of lettuce cultivars. *Scientia Hortic*., 49: 175-179.

Bhowmik, P.K., T. Matsui, K. Kawada and H. Suzuki, 2001. Seasonal changes of asparagus spears in relation to enzyme activities and carbohydrate content. *Scientia Hortic*., 88: 1-9.

Bhowmik, P.K., T. Matsui, T. Ikeuchi and H. Suzuki, 2002. Changes in storage quality and shelf life of green asparagus over an extended harvest season. *Postharvest Biol. Technol*., 26: 323-328.

Blom-Zandstra, M. and J.E.M. Lampe, 1985. The role of nitrate in the osmoregulation of lettuce (*Lactuca sativa* L.) grown at different light intensities. *J. Exp. Bot*., 36: 1043-1052.

Cammaerts, D. and M. Jacobs, 1985. A study of the role of glutamate dehydrogenase in the nitrogen metabolism of *Arabidopsis thaliana*. *Planta*, 163: 517-526.

Chandra, D., T. Matsui, H. Suzuki and Y. Kosugi, 2006. Postharvest changes in some physiological traits and activities of ammonia-

assimilating enzymes in lettuce during storage. *Asian J. Plant Sci*., 5: 378-384.

Glenn, E.P., 1984. Seasonal effects of radiation and temperature on growth of greenhouse lettuce in a high insolation desert environment. *Scientia Hortic*., 22: 9-21.

Guy, C.L., J.L.A. Huber and S.C. Huber, 1992. Sucrose phosphate synthase and sucrose accumulation at low temperature. *Plant Physiol*., 100: 502-508.

Hurst, P.L. and C.J. Clark, 1993. Postharvest changes in ammonium, amino acids and enzymes of amino acid metabolism in asparagus spear tips. *J. Sci. Food Agric*., 63: 465-471.

Inokuchi, R., K. Kuma, T. Miyata and M. Okada, 2002. Nitrogen-assimilating enzymes in land plants and algae: phylogenic and physiological perspectives. *Physiol. Plant.*, 116: 1-11.

Kays, S.J., 1991. Secondary metabolic processes and products. In: *Postharvest Physiology of Perishable Plant Products*. Van Nostrand Roinhold. New York, pp: 157-161.

Knight, S.L. and C.A. Mitchell, 1983. Stimulation of lettuce productivity by manipulation of diurnal temperature and light. *HortScience*, 18: 462-463.

Koontz, H.V. and R.P. Prince, 1986. Effect of 16 and 24 hours daily radiation (light) on lettuce growth. *HortScience*, 21: 123-124.

Kun, E. and E.B. Kearney, 1974. Ammonia. In: *Methods of Enzymatic Analysis*. (Bergmeyer, H.U., ed.), Vol. 4, 2nd edn.., Verlag Chemie, Weinheim, Academic. Press, New York, pp: 1802-1806.

Lea, P.J., S.A. Robinson and G.R. Stewart, 1990. The enzymology and metabolism of glutamine, glutamate and asparagines. In: *The Biochemistry of Plant*: *A Comprehensive Treatise* (Stumpf, P.K. and E.E. Conn, eds.), Vol.16, Academic Press Inc., New York, pp. 121-152.

Lee, S.K. and A.A. Kader, 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol*., 20: 207-220.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin-phenol reagent. *J. Biol. Chem*., 193: 265-275.

Macduff, J.H. and S.B. Jackson, 1991. Growth and preferences for ammonium or nitrate uptake by barley in relation to root temperature. *J. Exp. Bot*., 42: 521-530.

Marsic, N.K. and J. Osvald, 2002. Effects of different nitrogen levels on lettuce growth and nitrate accumulation in iceberg lettuce (*Lactuca sativa* var. *capitata* L.) grown hydroponically under greenhouse conditions. *Gartenbauwissenschaft,* 67: 128-134.

Martin-Diana, A.B., D. Rico, J. Frias, G.T.M. Henehan, J. Mulcahy, J.M. Barat and C. Barry-Ryan, 2006. Effect of calcium lactate and heat-shock on texture in fresh-cut lettuce during storage. *J. Food Engineer*., 77: 1069-1077.

Miflin, B.J. and D.Z. Habash, 2002. The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. *J. Exp. Bot*., 53: 979-987.

Murata, M., E.Tanaka, E. Minoura and S. Homma, 2004. Quality of cut lettuce treated by heat shock: prevention of enzymatic browning, repression of phenylalanine ammonia-lyase activity, and improvement of sensory evaluation during storage. *Biosci. Biotechnol. Biochem*., 68: 501-507.

Peeters, K.M.U. and A.J. Van Laere, 1992. Ammonium and amino acid metabolism in excised leaves of wheat (*Triticum aestivum*). *Physiol. Plant.*, 84: 243-249

Pramanik, B.K., T. Matsui, H. Suzuki, Y. Kosugi and M. Tomohiro, 2004. Seasonal fluctuation of some sucrose metabolizing enzymes and sugar and organic acid contents in broccoli. *Asian J. Plant Sci*., 3: 549-555.

Ratajczak, L., W. Ratajczak and H. Mazurowa, 1981. The effect of different carbon and nitrogen sources on the activity of glutamine synthetase and glutamate dehydrogenase in *lupin* embryonic axes. *Physiol. Plant*., 51: 277-280.

Rognes, S.E. 1975. Glutamine-dependent asparagine synthetase from *Lupinus luteus*. *Phytochemistry*, 14: 1975-1982.

Sams, C.E. 1999. Preharvest factors affecting postharvest texture. *Postharvest Biol. Technol.*, 15: 249-254.

Srivastava, H.S. and R.P. Singh, 1987. Role and regulation of L-glutamate dehydrogenase activity in higher plants. *Phytochemistry,* 26: 597-610.

Suarez, M.F., C. Avila, F. Gallardo, F.R. Canton, A. Garcia-Gutierrez, M.G. Claros and F.M. Canovas, 2002. Molecular and enzymatic analysis of ammonium assimilation in woody plants. *J. Exp. Bot*., 53: 891-904.

Toole, G.A., M.L. Parker, A.C. Smith and K.W. Waldron, 2000. Mechanical properties of lettuce. *J. Mater. Sci*., 35: 3553-3559.

Tosun, I. and N.S. Ustun, 2004. Nitrate content of lettuce grown in the greenhouse. *Bull. Environ. Contam. Toxicol*., 72: 109-113.

Whitaker, T.W., E.J. Ryder, V.E. Rubatsky and P.A. Vail, 1974. *Lettuce production in the United States*. United States Department of Agriculture: Washington, D.C., Agriculture Handbook No. 221.

Wurr, D.C.E. and J.R. Fellows, 1991. The influence of solar radiation and temperature on the head weight of crisp lettuce. *J. Hortic. Sci*., 66: 183-190.

Wurr, D.C.E., J.R. Fellows and A.J. Hambidge, 1992. Environmental factors influencing head density and diameter of crisp lettuce cv. Saladin. *J. Hortic. Sci*., 67: 395-401.

Wurr, D.C.E., J.R. Fellows and K. Phelps, 1996. Investigating trends in vegetable crop response to increasing temperature associated with climate change. *Scientia Hortic*., 66: 255-263.