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Changes in Some Physio-Biochemical Characteristics in Lettuce During Storage at Low Temperature

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Abstract: To investigate the effects of low temperature on the physiological and biochemical processes, we held two crisphead lettuce (*Lactuca sativa* L.) cultivars 'Shizuka' and 'Cisco' at 5°C for 10 days. Minimum weight loss and lower respiration rate along with a higher hue angle value were recorded from both cultivars. However, cultivar 'Cisco' showed higher relative weight loss, respiration rate and accumulation of ammonia compared to that of 'Shizuka'. Except in the leaf portion of 'Shizuka', glutamine synthetase (GS, 6.3.1.2) activity did not change significantly. The accumulation of ammonia was supposed to be below the critical level to repress GS activity. However, the highest amount of ammonia content was found at the end of the storage period in all cases. Amination activity of glutamate dehydrogenase (GDH, 1.4.1.2) decreased by day 4 and slightly increased thereafter while the deamination activity did not show any significant change. Overall, GDH-amination activity was remarkably higher than GDH-deamination activity and the leaf portion showed higher GDH-amination activity while the midrib portion showed higher GDH-deamination activity. Between the two cultivars, 'Cisco' could have a shorter shelf life than 'Shizuka'. The results suggested that although the visual quality of lettuce was maintained at low temperature, some physio-biochemical changes occurred in this period.

Key words: Ammonia, glutamate dehydrogenase, glutamine synthetase, lettuce, storage

INTRODUCTION

After harvesting, horticultural products are subjected to continual changes of a complex series of physiological transitions that influence product quality and further processing operations. Such changes cannot be stopped but can be controlled within certain limits by using various postharvest procedures. Among many others postharvest handling procedures, temperature management is one of the most important tools to extend shelf-life and maintain quality of fresh vegetables. Cantwell *et al.* (1998) reported that temperature is the single most important factor determining the postharvest quality of leafy green vegetables. It is noteworthy that the warmer the temperature, the faster the deterioration and the shorter the storage life; conversely, the cooler the temperature, the slower the deterioration and the longer the storage life. For instance, qualities of lambs lettuce like respiratory substrates are retained longer at low temperature than at 20°C (Enninghorst and Lippert, 2003). Other leafy vegetables, such as Chinese cabbage stored at 20°C showed higher respiration rate, weight loss and trimming loss, as well as lower quality than cabbages

stored at 0 and 2°C (Porter *et al.*, 2003). Based on the consumers acceptance, Nam and Kwon (1999) showed that the shelf life of leaf lettuce was only 4 days when it was stored in a room set at 20°C and 50% RH and the visual acceptance was prolonged up to 10 days when stored at 4°C in a refrigerator.

In leafy vegetables like lettuce, harvesting causes a complete detachment from the mother plant that resulted a sharply increase in respiration within minutes. Consequently, the rates of other biochemical reactions also increase that causes changes in color (including browning), flavor, texture and nutritional quality. Controlling such biochemical changes to a minimum level during storage can provide a longer shelf life of perishable products. However, the depletion of carbon sources and accumulation of ammonia have been reported in some horticultural products after few days of storage (Escribano and Merodio, 2001; Enriquez *et al.*, 2000; Pramanik *et al.*, 2004; Baclayon *et al.*, 2004). Since the accumulation of ammonia in plant tissues at excessive concentration are toxic to plant (Lancien *et al.*, 2000), its assimilation or disposal at least up to an innocuous level is very important for plant growth. In biochemical

literature, it was reported that many reactions occurred in plant tissue which involve ammonia as one of the reactants and could be considered as a possible point for the entry of ammonia into organic form (Mifflin and Lea, 1980). These reactions are mediated by some key enzymes, such as glutamine synthetase (GS, 6.3.1.2), glutamate synthase (GOGAT, EC. 1.4.7.1) and glutamate dehydrogenase (GDH, 1.4.1.2). In the presence of glutamic acid, the enzyme GS binds ammonia as glutamine. GS works in conjunction with GOGAT which removes the newly-assimilated nitrogen atom from the amide group of the glutamine and catalyses the formation of glutamic acid in the presence of the carbon substrate α -ketoglutarate. As a result, in addition to ammonia α -ketoglutarate, ATP and reductant are used as input in this reaction and the output consists of the amide glutamine or alternatively glutamic acid or other amino acids such as glutamate. Besides this key reaction cycle in ammonia assimilation, another enzyme GDH might come into operation at high levels of ammonia. GDH mediates the combination of α -ketoglutarate with ammonia to yield glutamic acid and also perform the oxidative deamination of glutamate. Thus the enzyme GDH may fulfill an important anapleurotic function between carbon and nitrogen metabolism.

In our previous study (Chandra *et al.*, 2006), we found significant changes in some physiological and biochemical traits that occurred in lettuce during storage at ambient temperature. A temperature of 5°C is a refrigerated condition under which many salad products including lettuce are commercially handled during transport and market display. However, there are few reports on the postharvest changes of lettuce that occurred during this condition in relation to ammonia accumulation and its assimilation. Hence, the objective of this study was to further understand some physiological and enzymatic changes that occur in lettuce during storage at 5°C. The results would offer a fresh insight on the postharvest management and handling of this perishable commodity.

MATERIALS AND METHODS

Plant materials: Crisphead lettuce (*Lactuca sativa* L.) cultivars, 'Shizuka' and 'Cisco', were harvested in December, 2005 from Kagawa prefecture, Japan. The cultivars were grown under commercial conditions and the heads were harvested when they reached at commercial maturity. After harvest, the heads were immediately brought to the Postharvest Laboratory, weighed and stored at 5°C in perforated polyethylene bags. At every 48 h interval, lettuce heads were taken out from the storage and fully expanded leaves were chosen from the

middle of the heads, eliminating the outermost and immature inner leaves. The midrib tissues were separated from leaves and both the leaf and midrib portions were cut into small pieces (ca. 2×2 cm) and immediately stored at -30°C until needed for analysis.

Weight loss measurement: The individual lettuce heads were weighed on the day of harvest and considered as initial fresh weight. The percentage of relative fresh weight loss was calculated by the formula:

$$\text{Relative fresh weight (\%)} = \frac{\text{Fresh weight at measurement day}}{\text{Initial fresh weight}} \times 100$$

Color assessment: Color changes in lettuce heads were performed using a chromameter (Nippon Denshoku Kogyo Co. Ltd.) equipped with an 8 mm measuring head and a C illuminant. The meter was calibrated using the manufacturers standard white plate. Color changes were quantified in 'L' 'a' and 'b' color spaces. Parameter 'L' refers to the lightness index scale of the head and ranges from 0 (black) to 100 (white). Parameter 'a' indicates the degree of greenness (negative value) or red-purple (positive value) color, while positive and negative values of 'b' indicate yellow and blue color, respectively (McGuire, 1992). The results were expressed as 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 $b > 0$) and calculated from the 'a' and 'b' values (Lancaster *et al.*, 1997). On each head, three readings were taken from different positions and averaged the value.

Respiration rate measurement: Using a Gas Chromatograph (GC) respiration rate was measured at every 48 h interval by the production of carbon dioxide from an intact lettuce head sealed for 1 h in a 10 L desiccator jar held at 5°C. The lettuce head was weighed carefully before placing into the desiccator jar. Ten milliliter gas sample was taken after 1 h and injected to a Thermal Conductive Detector (TCD) that is equipped with a 1 m activated charcoal column at 70°C (GC-8AIT, Shimadzu Co. Ltd.). The results were expressed as mL CO₂ kg⁻¹ h⁻¹.

Enzyme extraction and assay: Five grams of fresh lettuce sample from leaf and midrib portion was homogenized with 1% polyvinylpolypyrrolidone (PVPP) proportional to the sample weight, 1 g sea sand and 5 mL buffer A solution using a cooled mortar and pestle. Extraction was done as described by Hurst and Clark (1993), in which buffer A contained 50 mM tris-HCl (pH 7.6), 10 mM MgSO₄·7H₂O, 1 mM EDTA, 1 mM dithiothreitol (DTT),

12 mM 2-mercaptoethanol, 5 mM L-glutamate and 100 mL glycerol L⁻¹. The homogenate was filtered 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 x g at 2°C for 10 min. The resulting supernatant was used for enzyme assay.

Both of GS and GDH activities were measured using a double beam spectrophotometer (Shimadzu model UV-150-02) at 540 and 350 nm, respectively. In a 1 mL assay mixture, GS activity was measured with 80 mM Na-L glutamate, 100 mM tricine-KOH buffer (pH 7.0), 6 mM hydroxylammonium chloride (HONH₂Cl), 20 mM MgSO₄·7H₂O, 1 mM diethylenetriamine pentaacetic acid (DTPA), 8 mM ATP and 8 mM mercaptoethanol. After incubating the assay mixture at 35°C for 8 min, 1 mL ferric chloride reagent that contains 0.37 M FeCl₃, 0.67 N HCl and 0.2 M trichloroacetic acid (TCAA) was added to the mixture to stop the reaction. Soluble protein contents were determined following the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

The aminating and deaminating activities of GDH were determined according to NADH oxidation or NAD⁺ reduction at a temperature of 30°C. For GDH amination, 1.0 mL assay mixture contained 10 mM α-ketoglutaric acid, 100 mM tris-HCl (pH 8.0), 200 mM NH₄Cl, 1 mM CaCl₂ and 0.2 mM NAD(P)H and 200 μL crude extract solution. The same volume was also used for GDH deamination which consisted of 100 mM L-glutamate, 100 mM tris-HCl (pH 9.3), 1 mM NAD(P)⁺ and 0.5 mM CaCl₂ and 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) min⁻¹ at 30°C.

Ammonia assay: Two grams of fresh sample was used to assess ammonia from each portion of lettuce tissue. Ammonia was extracted with 10% trichloroacetic acid at 1:10 ratio(w/v) in an ice bath (0- 4°C) and centrifuged at 11,000 x g at 2°C for 10 min. Ammonia content was assayed following the method 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 GDH (10 mg 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.

Statistical analysis: A randomized complete block design was used with three replications. From the *F*-value of ANOVA, the level of significance between the means was calculated.

RESULTS AND DISCUSSION

The quality of fresh produce in storage depends to a great extent, on the storage temperature. In this study, some physiological and biochemical changes were observed when lettuce heads were stored at 5°C for up to 10 days. As lettuce contained high percentage of water, loss of water is one of the important features that cause elastic and wilted leaf. The highest relative weight loss was observed on the second day of storage for both cultivars (Fig. 1). However, at the end of the storage period, only 2.3 and 5.7% relative fresh weight loss was recorded for the cultivar ‘Shizuka’ and ‘Cisco’, respectively. A variation in weight loss between the two cultivars could be a varietal effect as reported by Siomos *et al.* (2002), where they also found remarkable weight loss after 9 days of storage at 1°C. Despite the minimum weight loss, due to the higher hue angle as well as *L** value and lower respiration rate (Fig. 2, 3), the visual quality of the commodity was maintained. Higher hue angle value that represents a lower degree of degreening was also observed in broccoli when the samples were stored at low temperature (Pramanik *et al.*, 2004). However, the gradual but small decrease in *L** value in the cultivars ‘Cisco’ indicates that the leaf become darker when the storage progressed. Respiration rate, on the other hand, declined rapidly in both cultivars on day 2 of storage and almost unchanged thereafter up to the end of the storage period. The high initial respiration rate in both cultivars might be due to the physical stress imposed by harvest. The lower rate of respiration indicates slower metabolic

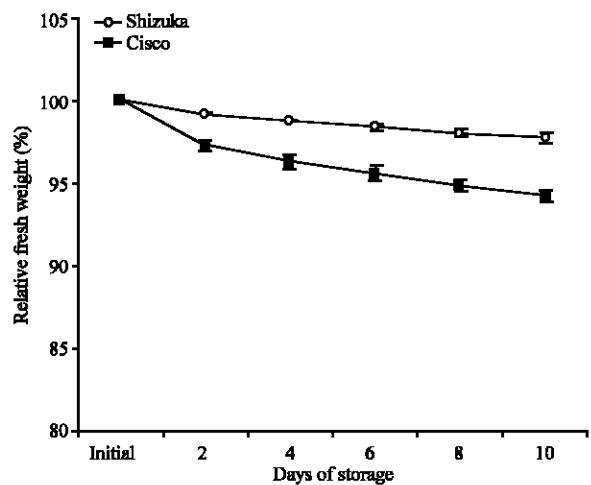


Fig. 1: Changes in relative fresh weight of two lettuce cultivars stored at 5°C for 10 days. Each point represents the mean of 3 replications. Vertical bars indicate SE, which, when absent is concealed by the graph symbols

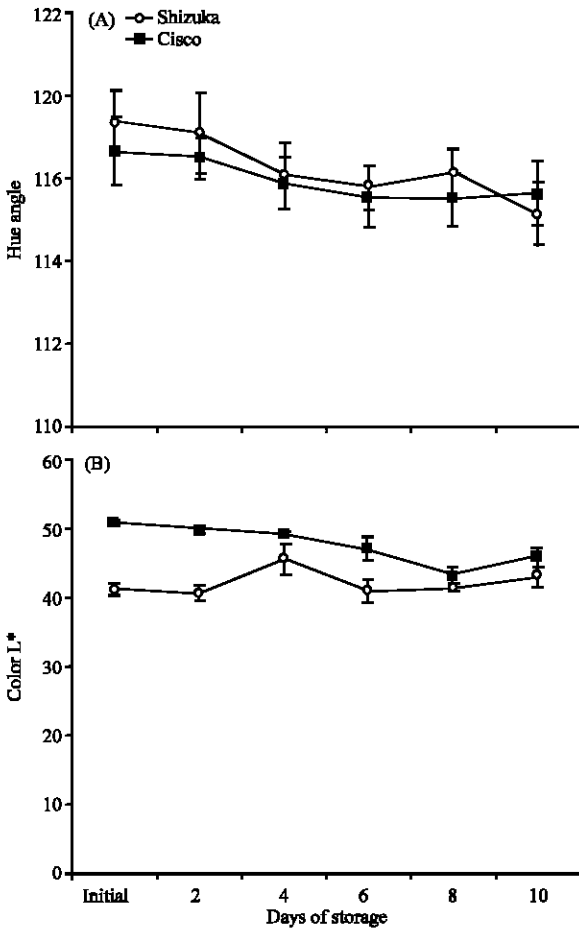


Fig. 2: Changes in hue angle (A) and color L* (B) of lettuce head stored at 5°C for 10 days. Each point represents the mean of 3 replications. Vertical bars indicate SE

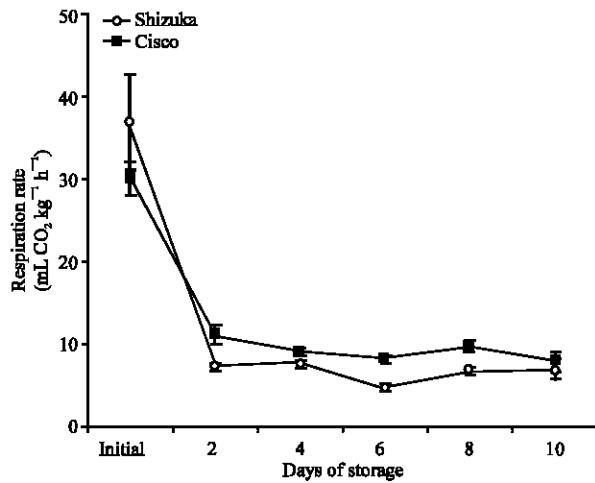


Fig. 3: Changes in respiration rate of intact lettuce head of the cultivar 'Shizuka' and 'Cisco' during storage at 5°C for 10 days. Vertical bars indicate SE, which, when absent is concealed by the graph symbols

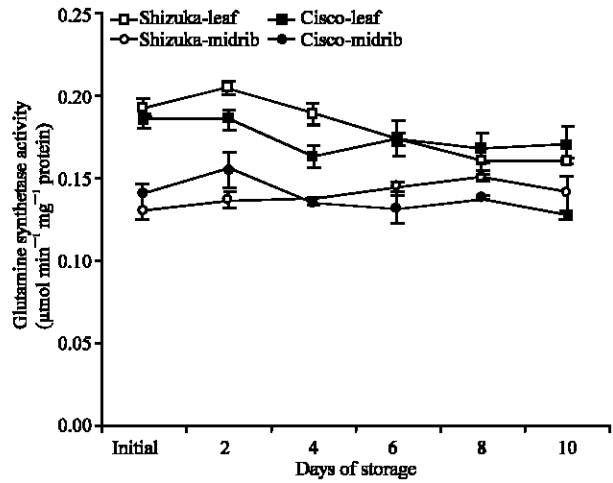


Fig. 4: Changes in the activities of glutamine synthetase in the leaf and midrib portions of two lettuce cultivars stored at 5°C for 10 days. Each point represents the mean of 3 replications. Vertical bars indicate SE, which, when absent is concealed by the graph symbols

rates which are the basis for product deterioration. It is evident that the shelf life of perishable plant products is inversely related to their respiration rate during storage (Hardenburg *et al.*, 1990). Similar to our result, decreases in respiration rate was also reported as the storage temperature decreases (Geysen *et al.*, 2005).

Of the two ammonia assimilating enzymes, glutamine synthetase activity in the leaf and midrib portion of two cultivars slightly increased on the second day of storage and after that the activity did not change significantly until the last day of storage, except in the leaf portion of 'Shizuka' in which activity declined significantly (Fig. 4). In case of 'Cisco', GS activity decreased on day 4 and almost maintained thereafter. However, the leaf portion showed considerably higher activity than the midrib portion as reported in our previous study (Chandra *et al.*, 2006). GDH activity, on the other hand, did not change significantly except in the midrib portion of 'Cisco' and 'Shizuka' in amination and deamination direction, respectively (Fig. 5). Amination activity was higher than deamination activity which was similar to the result of Enriquez *et al.* (2000) in asparagus. Overall, GDH amination activity decreased by day 4 and increased thereafter and again slightly decreased at the end of storage, while the deamination activity almost constant up to day 6 and slightly increased at the end of storage. It has been reported that lower temperature adversely affects GDH activity in the roots of wheat (Srivastava and Fowden, 1972) and soybean (Duke *et al.*, 1979). They also concluded that a decrease in GDH activity in soybean

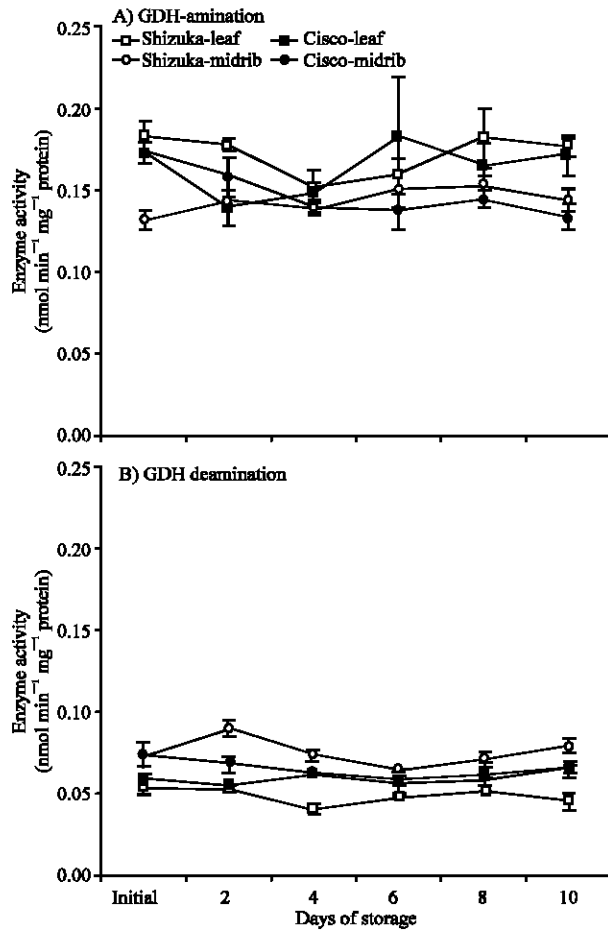


Fig. 5: Changes in the activities of GDH-amination (A) and GDH-deamination (B) in the leaf and midrib portions of two lettuce cultivars held at 5°C for 10 days. Each point represents the mean of 3 replications. Vertical bars indicate SE, which, when absent is concealed by the graph symbols

roots grown at lower temperature is possibly caused by a relative decrease in enzyme protein content as well as by changes in the ratio between NADH/NADPH forms. Moreover, in some studies, it was also demonstrated that at cold temperature, GDH activity may lost, gained or remained at the same level and the activity closely associated with the fluctuation in soluble protein concentration (Krasnuk *et al.*, 1976). The fluctuating activity of GDH observed in this study might be an effect of low temperature during storage. Because, the aminating activity of GDH may not be significant at lower temperature as the energy of activation (E_a) and $K_{NH_4^+}$ are higher at 12 than 25°C or lower temperature as reported in Triticum roots (Alekhina *et al.*, 1984). In leaves, ammonia caused the GS activity to decrease slightly (Cammaerts and Jacobs, 1985) and the explanation in the

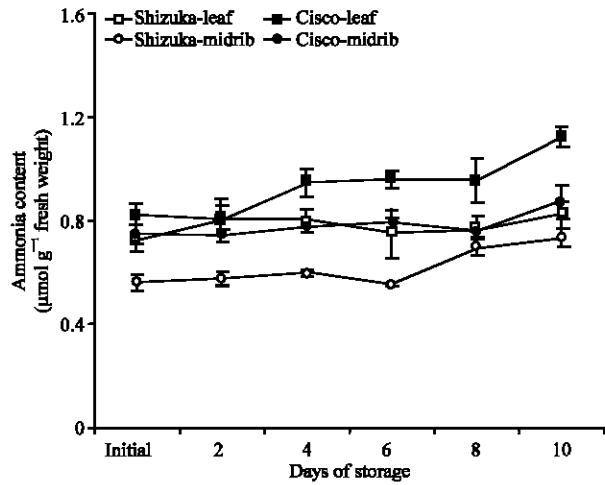


Fig. 6: Ammonia content in the leaf and midrib portions of two lettuce cultivars stored at 5°C for 10 days. Vertical bars indicate SE, which, when absent is concealed by the graph symbols

changes of GS/ GDH activity suggest that at low ammonia levels, ammonia is assimilated via the glutamine (GS) pathway, while at high ammonia levels there is an increase in assimilation via the glutamate (GDH) pathway (Rhodes *et al.*, 1976). However, the trends in ammonia accumulation (Fig. 6) and enzyme activities observed in this study are not agreed with these of those findings. The accumulation of ammonia was only evident in the leaf and midrib portions of ‘Cisco’ and ‘Shizuka’ on day 10, respectively, wherein the GS activity in these portions was maintained. It implies that the accumulated ammonia is not sufficient to repress GS or may be there is a critical level for ammonia to become repressive for GS. However, in all cases, the highest ammonia contents were observed in 10-day stored samples. Similar to our previous report (Chandra *et al.*, 2006), both the portions of ‘Cisco’ contained higher amount of ammonia than ‘Shizuka’ which may enhance the rapid deterioration of this cultivar.

In conclusion, at low temperature, minimum weight loss and lower respiration rate along with a higher hue angle value could be obtain from both cultivars of lettuce. This study also demonstrated that though the visual quality of lettuce stored at low temperature are maintained, some physiological and biochemical activities occurred in this period. Moreover, the symptom of product deterioration like senescence or yellowing appears after some major enzymatic changes. Based on the measured parameter, cultivar ‘Cisco’ showed higher weight loss, respiration rate and accumulation of ammonia which may caused a shorter shelf life of this cultivar. Cool temperature treatment of lettuce head for several days before exposure to room temperature would be a further research interest to extend lettuce shelf life.

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