Consequences of Cool Storage of Broccoli on Physiological and Biochemical Changes and Subsequent Senescence at 20 °C

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ABSTRACT. In most broccoli (*Brassica oleracea* L. var. *italica*) cultivars studied, the loss of chlorophyll was marginal after 5 weeks cool storage (1 °C) + 2 days at 20 °C, but there was significant loss of chlorophyll from some poor-storing cultivars, particularly after 10 weeks cool storage (+2 days at 20 °C). Soluble sugars were depleted rapidly during cool storage (especially sucrose) and were essentially exhausted after 10 weeks at 1 °C. Losses of total proteins were only 20% after 10 weeks cool storage. There is preferential catabolism of carbohydrates (sucrose, glucose, and fructose) at low temperatures, whereas, at 20 °C, protein and carbohydrate levels decline concomitantly. The patterns of sugar and protein depletion suggest that all soluble sugar is potentially accessible for metabolism, but protein catabolism is targeted to specific tissues or organs. After 5 weeks at 1 °C and placing at 20 °C, ethylene production and respiration increased to steady-state levels. Peak production of wound ethylene usually occurs 4 to 6 hours after harvest at 20 °C. After 5 or 10 weeks cool storage, this peak of production was not detected at 20 °C. After 10 weeks at 1 °C, recovery of ethylene production was delayed and the respiration rate only partially recovered to the steady level. However, chlorophyll loss is the major determinant of marketable life without cool storage, and, after 5 weeks at 1 °C, postharvest decay is the major determinant of marketable life after cool storage, particularly after 10 weeks at 1 °C.

Harvest and storage at 20 °C of the rapidly growing floral apices of broccoli impose stresses and unsustainable demands for a limited supply of nutrient reserves (King and Morris, 1994b), and the inability to maintain homeostasis leads to senescence (Huber, 1987). The most visible sign of senescence is loss of chlorophyll from the sepals. At harvest, broccoli florets respire almost 1% of their dry mass per hour. In contrast, the stem tissue respiration is almost an order of magnitude lower and relatively stable (King and Morris, 1994a, 1994b). Rapid senescence can be reduced by low-temperature storage or reduced oxygen supply to moderate respiration and energy consumption. Rapid cooling to low temperature for subsequent shipping and handling is essential for maintaining postharvest quality (Brennan and Shewfelt, 1989), and beneficial effects of low temperature and high relative humidity in reducing the chlorophyll degradation rate have been reported (Deschene et al., 1991; Isenberg, 1979; Klieber and Wills, 1991).

There has been recent scientific interest in investigating the process of senescence in chlorophyll-containing vegetables, such as broccoli and asparagus, and the concomitant respiratory and metabolic changes (Brady et al., 1992; Clarke et al., 1994; Davies and King, 1993; Deschene et al., 1991; King and Morris, 1994a, 1994b; Makhoul et al., 1989; Pogson et al., 1995a). The effects of exogenous phytohormones and endogenous ethylene biosynthesis on the senescence of heterogeneous tissues have been investigated.

Cytokinin and ethylene appear to be the principal hormonal antagonists, delaying and advancing senescence rate, respectively (Aharoni et al., 1985; Brady et al., 1992; Clarke et al., 1994; Dedolph et al., 1963; Makhoul et al., 1989; Rushing, 1990; Tian et al., 1994; Wang, 1977). Measurements of ACC oxidase activity and associated mRNA levels indicate that the increase in endogenous ethylene production is initiated in the reproductive structures of the immature floret and removal of these reproductive structures delays sepal yellowing (Tian et al., 1994; Pogson et al., 1995a). The physiological and biochemical changes that occur at 20 °C also have been investigated (Deschene et al., 1991; King and Morris, 1994a, 1994b). There is a rapid shift in the respiratory quotient toward more oxidative metabolism and an associated shift in substrates from carbohydrate to protein and lipids. Protein and lipid depletion begins well in advance of complete depletion of carbohydrates. Hypotheses explaining the shift in metabolite usage include the following: 1) the initial rapid decline in carbohydrates is perceived as a stress and results in the metabolite shift (King and Morris, 1994b), 2) accessible sugars are depleted and the remainder are inaccessible (Saglio and Pradet, 1980), and 3) the changes in hormone balance, such as the rapid induction of ethylene biosynthetic enzymes within 2 h of harvest in the reproductive tissue (Pogson et al., 1995a, 1995b), and loss of root derived cytokinins.

The aforementioned studies have advanced our understanding of the senescence process in harvested floral apices at ambient temperatures. However, marketing of broccoli routinely involves cool storage, and, to our knowledge, the physiological and biochemical changes that occur during cool storage have not been detailed. Neither has the effect of cool storage on postharvest senescence processes, when the tissue warms to room temperature. In this study, we investigate the effect of cool storage on senescence using a wide range of cultivars. We also try to gain further understanding into the process of postharvest senescence in chlorophyll-rich vegetative tissue, such as broccoli.

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Materials and Methods

PLANT MATERIAL AND STORAGE. Field-grown broccoli was harvested, rapidly cooled to $\approx 1^{\circ}\text{C}$ by top icing in the cartons, and transported overnight by refrigerated (1°C) road transport. 'Green Belt' was used in the experiment, with a range of code-numbered breeding lines (Arthur Yates Seeds Pty., Ltd., Sydney). On arrival at the laboratory, all broccoli heads were surface-sterilized in 2.7 mmol sodium hypochlorite for 1 min, rinsed in 27 μM sodium hypochlorite, and shaken dry (Rushing, 1990). A minimum of three and a maximum of seven heads were used per treatment for each cultivar examined. The three storage treatments were 0, 5, and 10 weeks at 1°C . The cool-stored broccoli was placed in loose, low-density polyethylene bags with the top folded over and was stored at 1°C in a high-humidity cool room at 95% to 100% humidity. After removal to 20°C , broccoli branchlets were cut from heads, weighed, surface-sterilized, and placed in 1.5-L jars with an air flow of humidified, ethylene-free air at $1.2 \pm 0.2 \text{ L}\cdot\text{h}^{-1}$ (King and Morris, 1994b).

ASSESSMENT. Floret color was measured nondestructively by reflectance following King and Morris (1994b) as hue angles (Shewfelt et al., 1984). A calibration curve between hue angle and chlorophyll extracted from florets was produced using a subsample of heads using *N*-dimethylformamide (Petermann and Morris 1985). The calibration equation accounted for 86% of the variability, and the equation $\text{Chl } (\mu\text{g}\cdot\text{g}^{-1}) = \text{hue angle} \times 0.0687 - 6.02$ was viable between hue angles of 90° and 125° . Carbon dioxide production, O_2 consumption, and ethylene production were measured by infrared gas analyzer and paramagnetic O_2 analyzer respectively as described by King and Morris (1994a). Each day, assessments were made of the overall postharvest infection (fungal and bacterial) of the florets of intact heads of broccoli using a 1 to 5 severity scale, with 1 being no infection, 5 being severe infection, and 2.5 being the limit of commercial acceptability. Variability due to examiner and examination time was minimized by using photographs of samples representing each unit on the 1 to 5 severity scale. Assessments also were made of overall acceptability, color, head rot, stem rot, and turgor on a 1 to 5 severity scale using reference photos.

SUGAR AND PROTEIN ANALYSIS. Sugar and protein contents were determined for a limited range of cultivars using powdered, freeze-dried florets plus pedicel subsamples. For each treatment, florets from three heads were lyophilized. For each head, three subsamples were extracted and the assay was repeated twice for each extract. Soluble sugars were measured by high-performance liquid chromatography by the method of Wade and Morris (1982) with quantification of sucrose, glucose, and fructose. Protein was extracted twice for 10 min in 0.1 M NaOH, 1% SDS at 100°C , precipitated in 20% trichloroacetic acid, redissolved in 0.1 M NaCl, 0.1% SDS, and measured using a protein analytical kit (Biorad, Sydney) based on a modified Bradford method (Bradford, 1976).

GEL ELECTROPHORESIS AND WESTERN BLOTS. Four milligrams of freeze-dried tissue samples were ground in 100 μL 50 mM sodium phosphate, pH 6.8, 5% SDS, 6 M urea, 5% glycerol, 10% mercaptoethanol, and the homogenates were centrifuged for 15 min. The supernatant was assayed for protein as previously described. Equivalent amounts of total protein per lane were fractionated by 14% polyacrylamide gel electrophoresis (SDS-PAGE) as described by Pogson and Brady (1993).

STATISTICAL ANALYSIS. Three to seven heads or branchlets were analyzed per cultivar per treatment. Analyses of variance were performed using Minitab for Windows, release 9.2 (Minitab, State College, Pa.). Significant differences between treatments were

assessed using the Waller-Duncan-Baysian k-ratio least significant difference rule (Steel and Torrie, 1980). In some figures, an average of six cultivars is presented; these are Arthur Yates Pty., Ltd., breeding lines 43, 44, 54, 59, 60, and 68. For postharvest infection, the average scores of 13 cultivars are given; this group includes the previously mentioned six lines plus 32, 35, 36, 61, 64, 65, and 72.

Results

The two major pathogens in our study were *Botrytis cinerea* and *Alternaria brassicae*; the bacterial pathogen *Erwinia carotovora* also was present. Infection was not visible at harvest, and remained low for nonstored material after 4 d at 20°C (data not shown). On removal from 5 weeks cool storage, infection also was low; however, over the next 4 d at 20°C , the level of infection increased slowly (Fig. 1). After removal from 10 weeks cool storage, many cultivars had significant levels of infection, and, at 20°C , the level of infection increased rapidly. The visual assessments of the broccoli were analyzed by multiple regression using all the data for 5 and 10 weeks. The most important contributor to the decline in overall acceptability was head rot, which accounted for 48% of the variability, followed by color, which accounted for 40% of the variability. Stem rot and turgor both contributed <5% each to explaining the decline of overall acceptability.

The chlorophyll decline of sepal tissue was measured for six cultivars after storage for 0, 5, or 10 weeks at 1°C and then during holding at 20°C (Fig. 2). For nonstored samples, the decline in hue angle was minor during the first 48 h and then the samples yellowed rapidly. Negligible chlorophyll was lost during 5 weeks at 1°C , and, over the subsequent days at 20°C , there was no difference in the rate of chlorophyll loss compared to nonstored broccoli. During 10 weeks at 1°C , there was a loss of $\approx 25\%$ chlorophyll with substantial variability among cultivars. Cultivars stored for 10 weeks lost significantly more chlorophyll in the first 48 h at 20°C than either freshly harvested or cultivars stored for 5 weeks. By 3 d at 20°C , the difference between the treatments was diminished since all were yellowing. The visual assessment of color was not surprisingly highly correlated to the hue angle measured by reflectance. Since hue angle data were less variable than visual color assessment, the latter data is not presented.

In the absence of cool storage, ethylene production was similar to that previously reported (King and Morris, 1994a, 1994b). A harvest- or wound-induced peak occurred after 4 to 6 h at 20°C (Fig. 3). Over subsequent days, ethylene production was relatively constant (20 to 50 $\text{nmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$). During storage at 1°C , ethylene production was reduced by 10-fold to 2 to 5 $\text{nmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. Subsequent to cool storage, there did not appear to be a "wound ethylene" peak at 4 h. Stored broccoli had low rates of ethylene evolution when removed from storage; however, after a lag period of ≈ 2 d, rates were comparable to those of freshly harvested broccoli.

There was a rapid decline in respiration rate during the first 24 h for nonstored samples (Fig. 4). After 24 h the average respiration rate as CO_2 released was $\approx 6.5 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. There was an increase on the second day, followed by a gradual decline over days 3 and 4. During storage at 1°C , the respiration rate of the broccoli was measured during the 6 to 28 d of cool storage and was ≈ 10 -fold lower (0.5 to 0.7 $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) than at 20°C . After 5 weeks and 10 weeks, samples were moved to 20°C , and their respiration rate recovered to 4 to 5 $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ within 6 h. By 24 h, the respiration rate for broccoli stored for 5 weeks was not significantly different from nonstored broccoli. During the next 3 d, the material stored for 5 weeks continued to respire at a slightly

reduced rate compared to freshly harvested material. Broccoli stored for 10 weeks respired at significantly lower rates on removal to 20 °C than nonstored broccoli.

The respiratory quotient (RQ) for nonstored material declined from 1.05 to 0.95 over the first 24 h and then fluctuated between 0.9 and 1.0 over subsequent days (Fig. 5). On removal from 1 °C, RQ was ≈ 0.95 for broccoli stored 5 and 10 weeks. RQ of samples stored for 5 weeks fluctuated in a similar manner to nonstored samples. RQ of samples stored for 10 weeks dropped to <0.9 by day 2. RQ values after day 3 and 2 increased for samples stored for 5 and 10 weeks, respectively. This increase in RQ was associated with increased postharvest infection in these heads. For nonstored broccoli, there was a decline in soluble sugar levels during the first 2 d after harvest, but the subsequent rate of decline slowed after 2 d (Fig. 6). During cool storage, there was a net loss of glucose, fructose, and sucrose, the magnitude being $>50\%$ after 5 weeks storage and $>90\%$ after 10 weeks storage. At 20 °C, sugar loss continued at similar rates for each storage time; virtually complete depletion of sugars occurred for broccoli stored 10 weeks after 2 d at 20 °C. In all cases, sucrose was depleted before glucose, which was depleted before fructose.

Concomitant with the loss of sugar and chlorophyll was a 70% decline in protein by 4 d at 20 °C for nonstored broccoli (Fig. 7). Of the total protein, 20% was degraded during 5 and 10 weeks at 1 °C. After placing broccoli at 20 °C, protein rapidly degraded over the first 2 to 3 d. Then, the rate slowed. After 4 d at 20 °C, the amount of protein remaining was similar, whether or not the tissue had been cool stored.

Discussion

To determine the effect of cool storage on the process of senescence, two cool-storage periods of 5 and 10 weeks were imposed. We confirm that low temperature markedly delays senescence of broccoli (Forney et al., 1989; Toivonen, 1992). Five weeks of cool storage did not result in chlorophyll loss or postharvest infection on removal or during a subsequent 4 d at 20 °C. The only change during 5 weeks of cool storage was a loss of soluble sugar. Apparently, 10 weeks of cool storage was at or beyond the limit of acceptable quality for most, but not all cultivars, reducing the average marketable life of broccoli lines at 20 °C from 3 to 1.5 d for yellowing and from >4 to 0 d due to postharvest infection. Whether or not there is a change in susceptibility to postharvest infection over time or not requires further investigation. Thus, the limiting factor for prolonged storage (5 to 10 weeks) was postharvest infection. Because three of the most important postharvest pathogens, namely *Botrytis cinerea*, *Alternaria brassicae*, and *Erwinia carotovora* (Geeson, 1983), were observed in this study, these results should be generally applicable in most broccoli growing areas.

The visual quality assessment data clearly demonstrated that the major limitations to cool storage of broccoli was head rots (48% of quality decline) and yellowing (40%). Stem rot was only a minor contributor to quality decline during storage ($<5\%$). This result may be due to the greater effectiveness of the chlorine sterilization against rots on the cut surface of the stem. Similarly, turgor was only a minor contributor to quality decline during storage ($<5\%$). This small contribution was due to using a high-humidity cool room and wrapping the broccoli in plastic during storage.

For periods of cool storage of <5 weeks, the limiting factor was chlorophyll loss (Figs. 1 and 2). Chlorophyll degradation can be accelerated by exogenous ethylene or retarded by either reducing

Fig. 1. Postharvest infection of broccoli held at 20 °C after 5 (■) and 10 (▲) weeks at 1 °C and 95% relative humidity. Data points are means for 13 cultivars.

Fig. 2. Chlorophyll loss of broccoli sepals held at 20 °C after 0 (■), 5 (□), and 10 (▲) weeks at 1 °C and 95% relative humidity. Data points are means for six cultivars.

Fig. 3. Ethylene production of broccoli branchlets held for 0 (■), 5 (□), and 10 (▲) weeks at 1 °C and 95% relative humidity, followed by 0, 1, 2, or 4 d at 20 °C. Data points are means for six cultivars.

Fig. 4. Respiration rates of broccoli held at 20 °C after 0 (■), 5 (□), and 10 (▲) weeks at 1 °C and 95% relative humidity. Data points are means for six cultivars.

Fig. 5. Respiratory quotients of broccoli held at 20 °C after 0 (■), 5 (□), and 10 (▲) weeks at 1 °C and 95% relative humidity. Data points are means for six cultivars.

Fig. 6. Loss of sugars from florets of broccoli breeding line 60 held at 20 °C after 0 (■), 5 (□), and 10 (▲) weeks at 1 °C and 95% relative humidity. Data points are means from three heads. The inset shows the loss of individual carbohydrates from selected time points.

ethylene biosynthesis or ethylene sensitivity (Tian et al., 1994). The initial induction of ACC oxidase is specific to the reproductive structures, suggesting that ethylene-induced senescence of florets may be initiated by reproductive tissues (Pogson et al., 1995a, 1995b; Tian et al., 1994). A possible reason for induction of ethylene production may be maintenance of the reproductive organs through mobilizing metabolites from surrounding tissue, such as sepals. Reproductive development often is associated with leaf senescence in intact plants (Thayer et al., 1987), and the harvest-induced senescence of the sepals may be analogous to this phenomenon. The effect of ethylene produced during cool storage on chlorophyll loss requires further investigation to separate it from other effects of cool storage. The most obvious effect of cool storage on subsequent ethylene production at 20 °C was its ability to inhibit harvest- or wound-induced peak. Ethylene production has been inhibited by chilling injury in cucumber fruit (Wang and Adams, 1982). The recovery of ethylene production after 2 d at 20 °C on removal from cool storage to levels produced by nonstored broccoli indicates no effect of chilling injury on the ethylene biosynthetic pathway. However, in this study ethylene production was only measured at 4 h, so it may be that the peak is delayed or the maximum has shifted.

During cool storage, the respiration rate was an order of magnitude lower than at 20 °C. The main metabolic substrate during this period is apparently soluble sugar, since after 10 weeks cool storage, >90% of the sucrose was depleted but only 20% of the protein was lost (Figs. 6 and 7). Protein degradation during storage is limited and may even be absent during the first 2 weeks if the absence of chloroplast phospholipid degradation can be taken as a guide (Deschene et al., 1991). The relatively slow rate of protein and phospholipid degradation (Deschene et al., 1991) during 2- to 5-weeks cool storage is reflected in the maintenance of chlorophyll. After 10 weeks, chlorophyll degradation was significantly greater than that in nonstored broccoli (Fig. 2).

The second aim of this study was to further elucidate the postharvest senescence processes in broccoli. At harvest, initial respiration rates and RQs are high, dropping after 12 to 24 h to steady-state levels (King and Morris, 1994a, 1994b). The steady-state respiration rate at 20 °C is similar with or without prior cool storage. These changes suggest possible causes of the switch in metabolite usage during senescence. One possibility is that switching was substrate driven. Rapid loss of soluble carbohydrate, particularly sucrose, and the correlated change in RQ support this hypothesis. However, after 5 weeks cool storage, RQ and postharvest respiration rates were only slightly reduced despite a large reduction in soluble sugar levels at 5 weeks compared to nonstored (Fig. 6) and, in nonstored tissue, the RQ shifts well in advance of carbohydrate exhaustion from oxidative substrates, such as carbohydrate and organic acids, to other substrates, such as protein and lipids, all of which decline in a harvested broccoli (Figs. 4–7) (Deschene et al., 1991; King and Morris, 1994b). These results mitigate against this hypothesis in terms of total substrates. Catabolism of protein before carbohydrate exhaustion, has occurred in excised barley leaves (*Hordeum sativum* Jess.), excised maize roots (*Zea mays* L.), and asparagus (*Asparagus officinalis* L.), and it has been proposed that this may reflect inaccessible carbohydrate pools (Platenius, 1942; Saglio and Pradet, 1980; Yemm, 1937). In this study, adding a cool-storage period enabled the complete exhaustion of soluble sugar from harvested broccoli (Fig. 6). This indicates that under certain conditions, such as cool storage, all soluble carbohydrate pools are accessible as respiratory metabolites. In all treatments, sucrose was used before fructose and glucose, despite the fact that sucrose only formed 10% of

the total carbohydrate pool. Previous work has shown that less use of carbohydrates at 20 °C occurred in branch tissue below the florets (King and Morris, 1994b). Overall, however, the shift in metabolic substrates at 20 °C suggests that, under these conditions, the change in metabolite usage may be regulated by factors other than the presence or absence of total carbohydrates, although the exhaustion of all accessible carbohydrates (especially sucrose) may exert a major effect. It is possible that under cool-storage conditions, the lower respiratory demand means that slower conversion of carbohydrates to sucrose and intra- or intercell transport is no longer rate limiting, permitting a more complete access to the total carbohydrate pool.

The switch in metabolic use, together with a relatively constant respiration rate, suggest that a certain respiration rate is required for organ and cellular maintenance. Besides this evidence for regulatory regulation, there is evidence of a strong relationship between total respiratory activity and the onset of the final stages of senescence (Brash et al., 1995). This may suggest that, once the availability of accessible sugars can no longer match the required metabolic demand, other metabolic pools, such as proteins and lipids, are used.

Alternative hypotheses for the shift in metabolite usage include the following: 1) the depletion rate of the sugars may, in itself, be a signal for the shift since asparagine synthetase is induced in broccoli and asparagus within 2 h of harvest, and, in asparagus, asparagine synthetase expression may be a response to sucrose stress (Davies and King, 1993) or 2) the shift may be a direct response to harvest-induced stress or an indirect response via, for example, increased ethylene production.

Broccoli sepals initially contain fully differentiated chloroplasts with grana stacks, but during senescence the chloroplasts lose internal structure (unpublished observations) coincident with the decline in protein levels. The fact that protein levels only decline to a certain level, even in a rapidly senescing system, such as broccoli after 10 weeks (Fig. 7), suggests that there may be two pools of protein—namely “available” and “unavailable” as respiratory metabolites. Two sets of observations appear consistent with this concept: 1) the chlorophyll loss from reproductive tissues, pedicels, and supporting branchlets is far less than that from the sepals. The sepals are typical of green tissue, with the bulk of the protein being RuBisCO and other chloroplast proteins. In contrast, the spectra of proteins in broccoli reproductive tissue and cauliflower (*Brassica oleracea* L.) are quite different. 2) Perhaps, florets are still actively respiring after 4 d when chlorophyll degradation is essentially complete, which implies maintenance of mitochondrial integrity and mitochondrial proteins (Romani, 1987). However, SDS PAGE gel protein profiles do not suggest any relative turnover of any of the more abundant proteins. Although it has been proposed that RuBisCO is more rapidly degraded in senescing green tissues (Thayer et al., 1987; Vierstra, 1989), such an effect was not apparent for broccoli florets. Considering that more than half of the proteins of green tissues are chloroplast localized (Hikosaka et al., 1993), if total protein levels are declining, it may be difficult to distinguish differences in the relative rate of RuBisCO turnover. Further analysis of chloroplast protein breakdown in broccoli will require more detailed analysis with antisera specific for major chloroplast, cytoplasmic, and mitochondrial proteins.

Patterns of protein and chlorophyll loss are presumed to be linked because of their concomitant degradation. These data show that the linkage between protein and chlorophyll loss is not always tight. After 5 weeks cool storage and then during subsequent senescence at 20 °C, protein levels are only 70% to 80% of those

Fig. 7. Loss of protein from florets of broccoli breeding line 60 held at 20 °C after 0 (■), 5 (□), and 10 (▲) weeks at 1 °C and 95% relative humidity. Data points are means from three heads.

in fresh-harvested broccoli. However, chlorophyll contents are essentially identical during senescence at 20 °C for freshly harvested and broccoli stored for 5 weeks (Figs. 2 and 7). A lack of correlation between protein and chlorophyll loss has previously occurred in a mutant of meadow fescue (*Festuca pratensis* L.), with chlorophyll retained while protein levels decline in senescing leaves (Thomas and Stoddart, 1975).

In this study, cool storage prolonged the chlorophyll retention of broccoli from several days at 20 °C to 5 and 10 weeks at 1 °C. The limitation on acceptability of broccoli either nonstored or after 5 weeks at 1 °C is chlorophyll loss. However, after 10 weeks storage at 1 °C, the limiting factor is susceptibility to postharvest pathogens. At 1 °C, the respiration rate is an order of magnitude lower than at 20 °C, and, during cool storage, the tissue loses proportionally more soluble sugars and less protein than at 20 °C. When sugars are completely depleted (after 10 weeks at 1 °C) there is evidence that the rate of protein catabolism is declining. It is not known if the decline in the rate of protein catabolism reflects exhaustion of accessible proteins, for example the chloroplast proteins in sepals. The fact that substantial changes in sugar and protein levels do not result in similar large changes in the respiration rates provides support for the idea that the respiration rate is tightly regulated. However, the tight control of respiration rate does not necessarily directly relate to chlorophyll loss, which becomes more rapid after removal from cool storage when sugars are essentially exhausted. The ability of florets to metabolize virtually all soluble sugars during cool storage suggests that there is a rate-limiting enzyme or transport step at 20 °C, which doesn't occur under cool storage.

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