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Scallion (*Allium fistulosum* L.) Chemistry Affected by Variety and Environmental Conditions (Light and CO_2)

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ABSTRACT

To facilitate the selection of palatable and functional foods, eight green onions grown under either cool-white fluorescent lamps (CWF) or high pressure sodium lamps (HPS) were compared for their pungency, tissue nitrate and sulfate status. The influence of light intensity and atmospheric CO_2 levels on pungency of the selected cultivars was also investigated. Results demonstrate that there was a difference in pungency not only among cultivars, but also between tissue types and developmental stages. Pseudobulbs were more pungent than leaves except for cultivar Pacific Pearl that had similar levels in both pseuodbulbs and leaves. EG Hardy White was the most pungent, while Deep Purple was the least pungent. The pungency was inversely correlated with nitrate level in the tissue. Light sources (CWF versus HPS) did not influence plant growth or pungency, but had a clear impact on tissue nitrate levels with nitrate in CWF-grown onions higher than HPS-grown plants. Light and CO_2 levels had positive effect on pungency, i.e. increasing light and CO_2 levels increased the net accumulation of flavor precursors and/or alliinase activity (pungency factors). This study raised several interesting questions. For instance, should green onion be consumed immediately after harvest due to the high nitrate level, or should different horticultural practices (nutrient and light regime) be implemented to reduce nitrate levels? What effect would these strategies have on the vegetable's other quality traits? Does alternative lighting have repercussion for food quality, particularly in nitrate levels?

INTRODUCTION

Plants are an integral component of our sustainable life support system on Earth, and will play an important role in space exploration to initially provide dietary supplements, and eventually a more complete diet with concomitant air and water revitalization (Wheeler *et al*. 2001). Vegetables with minimum processing requirements are considered for both short term (e.g. ISS, CEV) and long term planetary based habitats. Scallion or bunching onion *(Allium fistulosum* L.) has been considered as one of the NASA's Advanced Life Support candidate crops because of its characteristic flavor, short cropping cycle, suitability in dense planting compared with bulb onion, and ease of hydroponic culturing (Thompson *et al*. 2004). Onion is also well known for its health benefit. Consumption of onion and related *Alliums* is associated with a reduction in blood lipids, cholesterol and platelet activity (Block 1992), contributing to decrease risk of cardiovascular disease (Sainani *et al* 1976, Kendler 1987, Augusti 1990). The biological activity and human health benefit are thought to be attributable to a suite of organosulfur compounds (Munday and Munday 1999 and 2004). However, are all onions equally effective? In an effort to select onion cultivar for inclusion in a space menu, in addition to yield, quantum efficiency (g yield mol^{-1} light) and growth characteristics, onion flavor or palatable feature and its health benefits including potential protection against ionizing radiations need be evaluated.

Studies on *Allium* production (mainly bulb onion, *Allium cepa L.*) indicate that onion flavor and pungency are governed by both genetic and environmental factors such as water supplies, temperature and growth medium fertility especially nitrogen and sulfur (Randle 1992, 1999). However bunching onions are less studied; little is known whether or how artificial lighting (quality and intensity), and atmospheric CO_2 levels that are likely encountered in space environment could affect these quality traits. Moreover, differences in food composition exist between field grown and closed environments (Thompson *et al.* 2004 and references cited within). Preliminary studies by (Thompson *et al.* 2004) suggested that elevated CO_2 increased total flavonols content in bulb onions, but not in chives or bunching onions with an exception of cultivar "Choesty". Elevated CO_2 has also been shown to increase onion hydrocarbon emissions (Jasoni *et al*. 2004).

Onion develops its flavor and pungency when the enzyme alliinase interacts with sulfur-substituted amino acids, collectively known as S-alk(en)yl-L-cysteine sulfoxide (ACSO) after cutting or crushing the onion tissue (Lancaster and Boland 1990). The products of this reaction and subsequent spontaneous rearrangements generate pyruvic acid, ammonia, and thiosulfinates. In the early 1960s, a significant correlation between enzymatically developed pyruvic acid and sensory evaluation of onion pungency was reported (Schwimmer and Guadagni 1962). Since then, enzymatically developed pyruvic acid has been used as a quantitative pungency indicator, and is often used to assess the culinary quality of onions. Pungency may also be an indirect measure of medicinal quality (Briggs and Goldman 2002). In order to facilitate the selection of a palatable and functional food, a comparative study on eight green onions in terms of their pungency, tissue nitrate and sulfate status under either cool white fluorescent lamp (CWF) or high pressure sodium lamp (HPS) were conducted in relation to commercial bulb onions. The effect of lighting intensity and atmospheric CO_2 levels on these attributes was also investigated.

MATERIALS AND METHODS

PLANT MATERIALS

Cultivar evaluation – Eight green onions (*Allium fistulosum* L. cv. Kinka, Kruncho, Choetsu, Guardsman, Choho, Pacific Pearl, Evergreen Hardy White, Deep Purple) were hydroponically grown in ½ strength Hoagland's solution using a recirculating nutrient film technique within an environmental chamber. The environmental conditions were controlled to 23 ^{0}C, 1200 µmol mol^{-1} CO_2, and 300 µmol m^{-2} s^{-1} light using either cool white fluorescent lamp (CWF) or high pressure sodium lamps (HPS) with a photoperiod of 16/8 light/dark (i.e. daily integral light 17.3 mol m^{-2} d^{-1}). Detailed horticultural conditions were described in Edney *et al*. (2003). On 34 day after planting (DAP), three representative onion plants were harvested. The third leaf and 3 cm pseudobulb above the root were dissected from each plant and immediately frozen in LN_2 and stored at – 80 ^{0}C for pungency assays. In addition, the third leaf and 3 cm pseudobulb segments from 4-6 plants were pooled for each cultivar, frozen in LN_2 and freeze-dried followed by grinding to pass a 40 mesh screen. Freeze-dried ground samples were analyzed for inorganic nutrient composition. Two experiment replicates were sampled.

Spatial distribution of pungency – Green onion *cv*. Kruncho grown under the conditions described in cultivar evaluation was sampled. Pseudobulbs were dissected into five 1 cm-long segments starting from the interface between the root and stem, and leaf numbers 2, 3 and 4 (progressively younger) were collected from two experimental replicates, three plants per experiment.

Effect of light intensity and atmospheric CO_2 level – For this experiment, only cultivar Kinka was investigated. Green onion, Kinka, was grown under a combination of three lighting intensities (150, 300, 450 µmol m^{-2} s^{-1} of CWF and three CO_2 levels (400, 1200, 4000 µmol mol^{-1}) with a photoperiod of 16/8 light/dark at 25 ^{0}C (Richards *et al*. 2004). These conditions corresponded to daily light integral 8.6, 17.3, 25.9 mol m^{-2} respectively. At DAP 34, samples were taken exactly the same way as in cultivar evaluations for pungency, while the entire plant except the root was frozen in LN_2 freeze-dried and ground to fine powder for determination of inorganic nutrients.

Bulb Onions (*Allium cepa* L.) – Red onion and sweet white onion were purchased from a local grocery store. Three firm red or white onions were selected, and the dried shell along with the immediately adjacent layer was peeled off; the top and base were also removed .. The onion was cut into four quarters, and each quarter was further cut into three approximately equal wedges. One wedge was used for pungency assays, one for inorganic nutrient measurements and the third section for water content determinations.

PUNGENCY ASSAY

Pungency development – Enzymatically developed pyruvic acid was used as a measure of pungency. Briefly, 1-3 gram of frozen green onion was ground in LN_2 with a pestle and mortar; subsequently, 5 ml of 0.1 M sodium pyrophosphate buffer (pH 9.0) was added. The thawed slurry was quantitatively transferred to a 15 ml conical centrifuge tube. The slurry was then incubated for 30 min in a 37 ^{0}C water bath to allow complete enzymatic conversion of flavor precursors to flavor compounds and pyruvic acid. The reaction was terminated by incubating in a 100 ^{0}C water bath for 10 min. The mixture was cooled to room temperature, and filtered through two layers of pre-washed cheesecloth fitted onto a Buckner funnel followed by two rinses. The filtrate and rinses were combined and diluted to a total volume of 50 ml with DI water.

For bulb onion, a freshly cut wedge (15-25 g) was directly blended in 90 ml of the sodium pyrophosphate buffer for 30 s. Homogenized onion was then incubated the same way as above. The filtrate was diluted for the determination of pyruvate concentration.

Quantification of the enzymatically developed pyruvic acid (Schwimmer and Weston 1961) – 1.0 ml of the above filtrate that has been further clarified by centrifugation was mixed with 1.0 ml of 0.0125% 2, 4-dinitrophenylhydrazine in 2*N* HCl and 1.0 ml de-ionized water. After incubation in water bath at 37 ^{0}C for 10 minutes, 5 ml of 0.6 *N* sodium hydroxide was added.

Following thorough mixing and temperature equilibration to room temperature, absorbance at 420 nm was read. Five different concentrations of pure sodium pyruvate were treated the same way as samples and a correlation between pyruvate amount and absorbance was established to determine the enzymatically developed pyruvate in the test samples.

ANION CONCENTRATION IN TISSUES

Freeze-dried and ground tissue (25-30mg) was quantitatively extracted with de-ionized water. The water extract was filtered through a 0.45 µm syringe filter and diluted 5 times with ion chromatographic analysis eluent (3.5 mM Na_2CO_3 + 1 mM $NaHCO_3$). The filtered and diluted extract was analyzed for anion concentrations by an ion chromatography/suppressed conductivity detector (Dionex DX-500 IC/HPLC system, Dionex, Sunnyvale, CA) using a procedure described in Dionex application note 135 (Dionex).

DATA ANALYSIS

Plant cultivars were arranged in growth chambers in a randomized design under defined light sources and carbon dioxide concentrations. A minimum of four replicate measurements were made for each treatment. Data were analyzed by the GLM procedure in the SAS statistical program and means separated using t-test Least Significant Difference (LSD) at the 5% level.

RESULTS AND DISCUSSION

PUNGENCY DIFFERS IN CULTIVARS AND TISSUE TYPES – Enzymatically developed pyruvate in eight green onions grown under HPS and CWF as shown in Figure 1 for pseudobulbs and Figure 2 for the third leaf , were used as pungency indicators. In general, there was no difference in pungency of either tissue types between CWF and HPS, but pungency differed among cultivars and tissue types. The level of pyruvate in pseudobulbs was significantly higher than that in leaves with an exception of Pacific Pearl that had a roughly equal intensity of pungency in the pseudobulb and leaf. EG Hardy White pseudobulbs were the most pungent, while Deep Purple pseudobulbs (under CWF only) were the least pungent among the eight cultivars. The pseudobulbs of other six cultivars had similar level of enzymatically developed pyruvate. On the other hand, the leaves of Kinka, Kruncho, Choetsu, Pacific Pear and EG hardy were more pungent than Choho, Guardsman and Deep Purple, and Deep Purple leaves under CWF were once again the least pungent. When statistical analysis was performed on the combined pungency of pseudobulbs and leaves of an individual cultivar, and compared among cultivars, EG Hardy White also turned to be the most pungent overall.

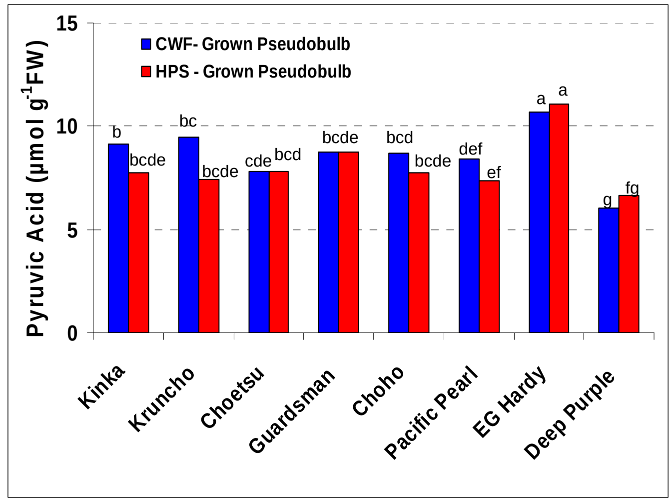

Figure 1: Enzymatically developed pyruvate in green onion pseudobulbs grown under cool white fluorescent and high pressure sodium lamps. The letters above the bars designate the outcome of statistical analysis.

The formation of pyruvate upon maceration or chewing of onion is also accompanied by the release of thiosulfinates, the primary flavor compounds for *Allium* plants (Block 1992, Lawson 1998). Thiosulfinates not only render onion the characteristic flavor, but also various biological activities including anticarcinogenecity (Randle 1997, and references therein, Sainani *et al* 1976, Kendler 1987, Augusti 1990). Although thiosulfinates were not directly measured, it is hypothesized that a more pungent onion may be more flavorful and may provide a greater health benefit. Considering a plant's composition/abundance of beneficial phytochemicals when selecting salad crops as part of a supplemental diet for astronauts may be especially important. Flight crews are at an elevated health risk due to increased exposure to ionizing radiation and microgravity (Todd, 2003; Schimmerling *et al*., 2003; Schimmerling, 2003). Indeed phytochemicals from fruits and vegetables over recent years have gained stature for their general health promoting effects and specifically as radioprotectors to alleviate radiation damage (Weiss and Landauer 2003). In addition, anecdotal evidences imply that astronauts with extended stay in space experience some shifts in the threshold levels for detection of tastes and flavors and desire for more flavorful food (Lane and Schoeller 2000). From the perspectives of palatability and potential health benefit, apparently EG Hardy White outperformed other cultivars. Ironically Kinka was previously selected and recommended for further testing under a space-relevant environment (Edney *et al.* 2003) at Kennedy Space Center based on other criteria (more uniform and compact growth characteristics). Our findings here and the fact that there was no statistical difference in biomass production or quantum efficiency between EG Hardy White and Kinka prompt the need for investigation of both productivity and food quality side by side during cultivar evaluations.

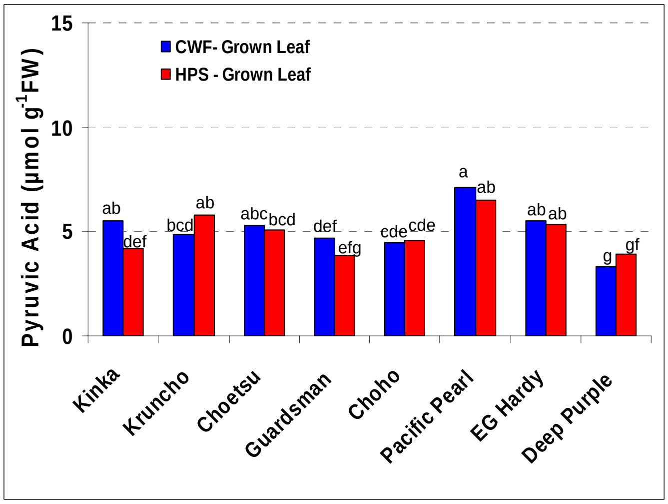

Figure 2: Enzymatically developed pyruvate in green onion leaves grown under cool white fluorescent and high pressure sodium lamps. The letters above the bars designate the outcome of statistical analysis.

SPATIAL DISTRIBUTION OF PUGNENCY – Figure 3 shows that the first cm of the pseudobulb was the least pungent and that there was no significant difference in the remaining stem. The younger leaves (i.e. leaf 4) were more pungent than the older leaves (leaf 2 and 3); which agrees with findings by Schwimmer and Weston (1961). There was no difference between leaves 2 and 3 that were both fully expanded and had not yet reached senescence.

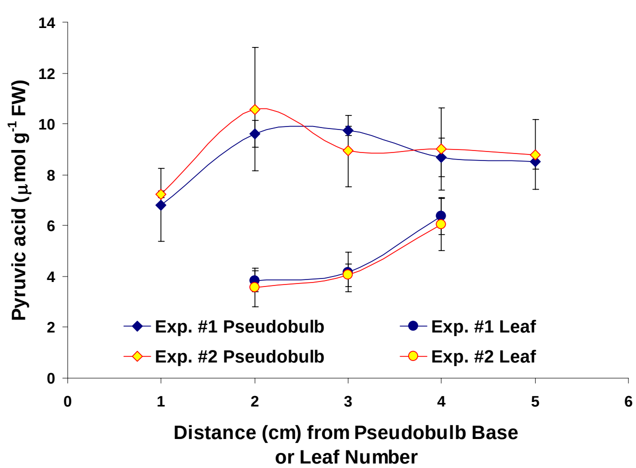

Figure 3. Pungency gradient along the pseudobulb and development stage of leaves in cv Kruncho. Bars represent standard deviation of three plant replicates.

PUNGENCY IN BULB ONIONS – How does the level of green onion pungency compare to that of bulb onion? Although it is known that the pungency of bulb onion varies greatly from 2 to 20 µmol g^{-1} (Schwimmer and Weston 1961, Kopsell and Randle 1997), in order to validate our procedure and provide a direct comparison, two types of bulb onion with distinct sensory value were analyzed. Table 1 indicates that the store-purchased red onion was strongly pungent with a value of 12.2 µmol g^{-1}, while store-purchased sweet white onion was relatively mild with a value of 5.4 µmol g^{-1}. Most of green onions tested in this study fell between these two extremes. EG Hardy White pseudobulbs were equivalent to the red onion bulb in terms of pungency. This implies that some green onions could measure up to bulb onion in terms of pungency and potential health benefit without the disadvantages such as low planting density, twice the growing cycle (12 weeks *vs.* 6 weeks or short for green onions) and longer light/dark photoperiod (Lancaster 1996) required in producing bulb onions. More interestingly, the more pungent onion coincided with lower nitrate and sulfate, but higher phosphate concentration in the tissue (Table 1). At first glance, this finding appears to contradict with previous observation that increasing nitrogen and sulfur fertility results in more flavorful onion (Randle 2000; Randle *et al*. 1995). It is important to note that the bulb onions examined in this study have been commercially stored and shipped post harvest, and that more pungent onion cultivars may have greater genetic potential to continuously utilize reserved nitrate and sulfate for the production of nitrogen and sulfur containing compounds, resulting in lower nitrate and sulfate concentration in tissues.

Table 1. Pyruvate and Anion Concentration of Commercial Bulb Onion. Values are the means of 9 replicates and standard deviations.

	Red Onion	Sweet White
Pyruvic Acid*	12.2 ± 1.8	5.4 ± 0.9
Nitrate**	0.6 ± 0.7	2.4 ± 0.6
Sulfate**	2.2 ± 0.7	2.6 ± 0.6
Phosphate**	8.1 ± 1.4	4.6 ± 1.1

* µmol g^{-1} fresh weight; ** mg g^{-1} dry weight

TISSUE NITRATE AND SULFATE STATUS IN GREEN ONIONS – In all instances, nitrate level (Table 2) in the leaves were higher than that in pseudobulbs, which is consistent with findings in other vegetables (Maynard *et al* 1976), and inversely correlated with the pungency. The inverse correlation between pyruvate concentration and nitrate was also found among cultivars. Pseudobulbs of Deep Purple with the lowest pungency had the highest nitrate concentration, while the most pungent green onion (EG Hardy White pseudobulbs) had the lowest nitrate level excluding Kinka. The nitrate in Kinka may be underestimated because of some procedural difficulties. In addition, CWF lamps consistently increased the nitrate level in green onions, but the enhancement varied from 20-35% in pseudobulbs and 4-58% in the leaves of different cultivars. The tissue nitrate level represents the difference between absorption and assimilation and is affected by genetic programming, environmental factors and nutrient supply. It is well known that the light intensity has a profound impact on nitrate levels, primarily due to the alteration of nitrate reductase (NR) activity (Maynard *et al* 1976). On the other hand, the evidence and interpretation of the effect of light quality, on nitrate level is tenuous. A study by Harper and Paulsen (1968) indicates that nitrate concentrations of wheat leaves (*Triticum aestivum* L.) were lower under blue light (380-470 nm), and NR activity was markedly higher than in red light (680-740 nm). However, in Harper and Paulsen's report, neither light level nor growth data were shown. Overlay of the light spectra of two lighting sources used in our experiment shows that HPS has more red, while CWF has more blue, which would result in lower nitrate level in CWF-grown plants based on Harper and Paulsen's observation. It is important to note that the light intensity of both HPS and CWF in our experiment was adjusted to the same level (300 µmol m^{-2} s^{-1}), and there was not significant difference in biomass accumulation under these lights (Edney *et al* 2003). The finding here is significant and requires further investigation to understand the biochemical and physiological implications of this enhanced accumulation under CWF. Lastly the nitrate level in these green onions was much higher than in bulb onions. Although differences in uptake potential between bulb onions and green onions could not be ruled out, the discrepancy might be partly due to the fact that the green onions investigated here were grown under excess nitrate supply (nitric acid was used to maintain the pH of growth medium), and biological activities were arrested at the time of harvest, preventing any post harvest assimilation.

Table 2. Nitrate Concentration (mg g^{-1} DW) in Green Onion Tissue (n=4). Superscript letters designate the outcome of statistical analysis

	HPS-Bulb	CWF-Bulb	HPS-Leaf	CWF-Leaf
Kinka	22.1^{g}	43.6*	48.8^{ef}	67.0
Kruncho	28.8^{fg}	38^{cde}	69.2^{c}	90.7^{ab}
Choetsu	30.8^{f}	38.1^{cde}	66.4^{cd}	94.9^{ab}
Guardsman	33.8^{def}	41.2^{c}	59.9^{cde}	30.3^{cde}
Choho	31.1^{ef}	38.9^{cd}	69.1^{c}	100.9^{a}
Pacific Pearl	38.0^{cde}	51.4^{b}	30.6^{g}	48.4^{ef}
EG Hardy	29.5^{f}	33.4^{def}	83.5^{b}	87.0^{ab}
Deep Purple	57.7^{b}	78.1^{a}	36.1^{fg}	53.4^{de}

*Insufficient degree of freedom for meaningful statistic analysis.

These results raise the question whether green onion should be consumed immediately after harvest as well as whether storage can have an adverse effect on onion flavor and health promoting properties. The ingestion of vegetables with high nitrate levels presents a potential risk to human health due to the formation of nitrite, which causes methaemoglobinemia (Wright and Davison 1964). The presence of nitrite in blood might also result in the formation of nitrosamine, a carcinogenic compound (Craddock 1983). Previous studies on bulb onions (Smittle 1998, Lancaster and Shaw 1991, Debaene *et al.* 1999, Galmarini *et al.* 2001) demonstrated that pungency increases over post harvest storage, however the response varies among cultivars (Kopsell and Randle 1997, Galmarini *et al.* 2001). Based on previous findings on what controls the nitrate accumulation, it is conceivable that nitrate level in edible biomass may be lowered by implementing one of the following practices a few days prior to harvest: a) increasing light level, b) increasing photoperiods, and/or c) withdrawing the nitrogen supply from the growth medium. How these practices affects the onion's quality traits remains to be determined.

Table 3 shows the sulfate concentration in tissues of eight green onions grown under either HPS or CWF. Sulfate concentrations exhibited the same trend as nitrate, and were higher in leaves than in pseudobulbs. Unlike nitrate, there were no significant cultivar differences or lighting source effects. The genetic potential of a cultivar to absorb and utilize sulfate for the synthesis of the flavor precursors determines onion flavor to a larger extent (Randle 1997). The inverse correlation between pungency and nitrate in different cultivars, and the lack of difference in tissue sulfate at the same time leads us to conclude that higher pungency of EG Hardy White is due to the higher efficiency of sulfur utilization and/or alliinase activity

Table 3. Sulfate Concentration (mg g^{-1} DW) in Green Onion Tissue (n=4); Superscript letters designate outcome of statistic analysis

	HPS-Bulb	CWF-Bulb	HPS-Leaf	CWF-Leaf
Kinka	6.3^{bcd}	6.5*	7.0^{e}	6.8*
Kruncho	7.3^{a}	7.1^{ab}	8.4^{abcd}	8.7^{abc}
Choetsu	6.6^{abc}	7.1^{ab}	7.5^{bcde}	8.7^{ab}
Guardsman	7.0^{ab}	5.7^{defg}	9.2^{a}	7.5^{cde}
Choho	6.4^{bcd}	6.3^{bcdef}	7.4^{de}	7.2^{de}
Pacific Pearl	5.0^{g}	5.9^{cdef}	6.9^{e}	8.9^{a}
EG Hardy	5.9^{cdef}	6.3^{bcde}	8.9^{a}	9.2^{a}
Deep Purple	5.4^{efg}	5.4^{efg}	6.9^{e}	8.1^{abcde}

* Insufficient degree of freedom for meaningful statistic analysis.

EFFECT OF LIGHT INTENSITY AND ATOMSPHERIC CO_2 LEVEL ON ONION BIOMASS AND PUNGENCY – Richards *et al* (2004) previously reported the results of 400 ppm (ppm was used in text instead of µmol mol^{-1} for its simplicity) and 1200 ppm CO_2 treatments. Figure 4 included the results of 4000 ppm CO_2, and shows that biomass production increased with increasing light intensity regardless of CO_2 levels. At any of three given light levels, biomass accumulation increased as CO_2 level increased from 400 ppm to 1200 ppm, but not when it increased from 1200 to 4000 ppm, which suggests that CO_2 saturation and yield potential was reached at 1200 ppm under these light intensities. Pungency of the onion pseudobulbs grown under 400 ppm CO_2 increased with the increase of light level from 8.6 to 17.3 mol m^{-2} d^{-1} (blue graphic bars in Figure 5). Further increases of light levels from 17.3 to 25.9 mol m^{-2} d^{-1} failed to enhance pungency. These results suggest that pungency increased at a greater rate than biomass accumulation from light levels of 8.6 to 17.3 mol, while at a similar rate from 17.3 to 25.9 mol. At 1200 ppm (red graphic bars) and 4000 ppm (yellow graphic bars) CO_2 treatments, pungency per gram basis in the pseudobulbs remained unchanged regardless of light intensity. Based on the near linear increase in biomass with the increase of light intensity, the pungency must have increased at the same rate as the biomass at these two CO_2 levels.

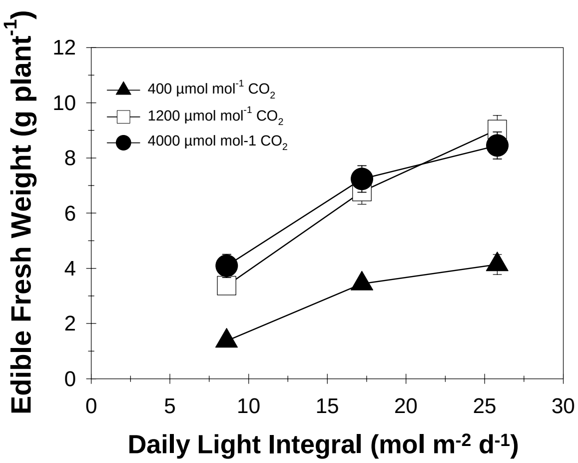

Figure 4. Edible biomass accumulation (g $plant^{-1}$) of onion grown with a daily integral light set at 8.6, 17.3, and 25.9 mol m^{-2} d^{-1} and either 400, 1200 or 4000 µmol mol^{-1} CO_2 levels.

When a comparison is made on pungency of onion pseudobulbs between different CO_2 levels at a given light level, elevated CO_2 had no negative or positive impact on pungency at either 8.6 or 17.3 mol m^{-2} d^{-1} light. This suggests the pungency increased in parallel with the biomass. At a higher light level (i.e. 25.9 mol m^{-2} d^{-1}), increasing CO_2 from 400 ppm to 1200 ppm resulted in decreased pungency, while further increase of CO_2 level from 1200 ppm to 4000 ppm resulted in enhanced pungency in pseudobulbs. Considering that biomass doubled when CO_2 levels increased from 400 to 1200 ppm (Figure 4), the decrease of pungency in these pseudobulbs is likely a dilution effect. On the other hand, even though increasing CO_2 from 1200 to 4000 ppm failed to promote continued enhancement of biomass (Figure 4), this CO_2 increase significantly enhanced pungency, restoring pungency to a level of that under 400 ppm CO_2.

In leaf tissue (Figure 6), the effect of light intensity and CO_2 levels on pungency was markedly different. Under 8.6 mol m^{-2} d^{-1} light, there was a sharp decline in pungency as CO_2 increased from 400 ppm to 1200 ppm, but leveled off between 1200 ppm and 4000 ppm CO_2 coinciding with the CO_2 saturation of growth. The decline in pungency is not a net decrease of pungency precursors or alliinase activity, rather a greater dilution effect due to the fact that leaves account for more than 80% of the total edible biomass, and consequently account for a major portion of the biomass increase resulted from tripled CO_2 level. No CO_2 impact on pungency at other two light intensities signifies a parallel increase in flavor precursor and biomass.

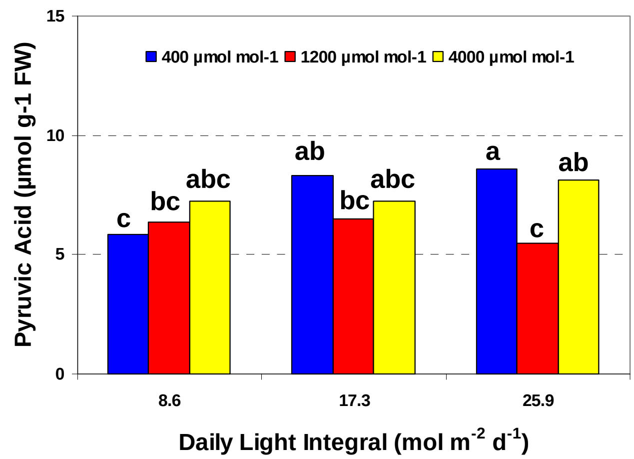

Figure 5: Enzymatically developed pyruvate in green onion *cv*. Kinka pseudobulbs developed under daily integral light 8.6, 17.3, 25.9 mol m^{-2} d^{-1} CWF and 400, 1200 and 4000 µmol mol^{-1} CO_2. The letters above the bars designate statistic treatment outcomes.

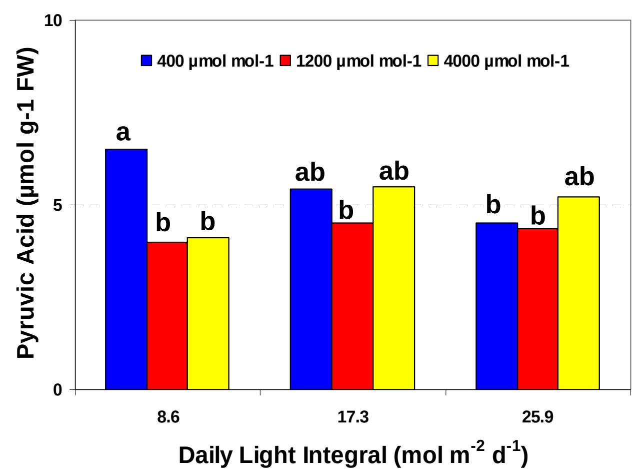

Figure 6: Enzymatically developed pyruvate in green onion *cv*. Kinka leaf developed under 150, 300 and 450 µmol m^{-2} s^{-1} cool-white fluorescent lamps and 400, 1200 and 4000 µmol mol^{-1} CO_2. The letters above the bars designate statistic treatment outcomes.

These initial experimental data indicate that the combination of low light (due to power constraints) and high CO_2 conditions anticipated in enclosed space environment have no adverse effect on onion biomass and quality in terms of pungency equivalent to that produced under the conditions of high light and ambient CO_2.

CONCLUSIONS

Results indicate the onions tested in this study were relatively strong, especially the pseudobulbs of the majority of cultivars were one level more pungent (6-8 µmol g^{-1} FW) than that of the leaf (4-6 µmol g^{-1} FW). EG Hardy White was the most pungent (8-10 µmol g^{-1} FW in pseudobulbs), and outperformed other cultivars in terms of pungency and probable health benefit.

The nitrate level in green onions was markedly higher than bulb onion (10-50 folds depending on cultivars). For instance, nitrate-N in Kinka leaves accounts for 1.1% dry mass. Because of the health hazard of nitrite from consuming vegetables with high nitrate content, further study is deemed to address questions whether green onion should be consumed immediately after harvest or horticultural manipulation of light level, photoperiod or nitrogen withdrawal prior to harvest should be exercised to reduce nitrate level in edible biomass; as well as what effect would these strategies have on green onion flavor and health enhancing traits.

Nitrate level in edible biomass was also dramatically influenced by light quality. Although light sources did not have any effect on the biomass accumulation or onion pungency, CWF-grown onions had 20-35% higher nitrate in pseudobulbs and 4-58% higher in leaves compared to HPS-grown onions.

Light and CO_2 levels had positive effect on pungency, i.e. increasing light and CO_2 levels increased the net accumulation of flavor precursors and/or alliinase activity (pungency factors). However, the rate of accumulation of pungency factors seemed to lag behind biomass before the CO_2 saturation point (1200 ppm), resulting in an apparent decrease in pungency. Accumulation of pungency factors continued beyond 1200 ppm after biomass reached its yield potential. The environment of low light and high CO_2 anticipated in enclosed space habitats could still provide salad crops such as onion with equivalent quantity and quality (in terms of pungency) to those grown under ample high light and ambient CO_2.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

- ACSO: S-alk(en)yl-L-cysteine sulfoxide
- CEV: Crewed Exploration Vehicle
- CWF: cool-white fluorescent
- DAP: days after planting
- HPS: high pressure sodium
- ISS: International Space Station
- LN_2: liquid nitrogen
- PPF: photosynthetic photo flux
- ppm: parts per million (this unit was used in the text interchanging with $\mu mol\ mol^{-1}$)