04ICES-136

Biomass, Flavonol Levels and Sensory Characteristics of *Allium* cultivars Grown Hydroponically at Ambient and Elevated CO_2

Leslie Thompson
Animal and Food Sciences, Texas Tech University, Lubbock TX

Ellen Peffley, Cary Green
Plant and Soil Sciences, Texas Tech University, Lubbock TX

Paul Paré
Department of Chemistry and Biochemistry, Texas Tech University, Lubbock TX

David Tissue
Department of Biology, Texas Tech University, Lubbock TX

ABSTRACT

Nine onion cultivars of four *Allium* species (*Allium cepa, A. fistulosum, A. schoenoprasum,* and *A. tuberosum*) were evaluated for use in Advanced Life Support (ALS) applications by hydroponic propagation in environmental growth chambers to evaluate the effect of elevated CO_2 (1200 ppm) versus ambient CO_2 (400 ppm) on biomass, phytochemical and folate levels, and sensory characteristics of scallions harvested at 49, 63 and 77 days after planting (dap). Regardless of cultivar or dap, plants grown at elevated CO_2 had greater biomass and % edible biomass than plants grown at ambient CO_2. Of the cultivars evaluated from the 63-dap-harvest, all, with one exception, exhibited increased total flavonols when grown at elevated CO_2. Consumer panelists were able to discern differences in sensory characteristics between ambient- versus elevated-CO_2-grown 'Choesty' but not the other three cultivars evaluated. Based on biomass and phytochemical levels 'Cal 296' and 'Purplette' were the best candidate cultivars for further testing for ALS applications using a single harvest strategy.

INTRODUCTION

As the National Aeronautics and Space Administration (NASA) works toward the development of food systems to accommodate the needs of astronauts on long-duration missions beyond low Earth orbit, the Advanced Food System will require a two phase approach one phase focusing on the Transit Food System, the other phase focusing on a Lunar/Planetary Surface Food System (Perchonok et al., 2001) with the goal of both providing palatable, nutritious, and safe foods while minimizing volume, mass and waste (Perchonok et al., 2002). Salad crops are being considered as an integral component of both systems, and one crop under consideration is onion (Barta et al., 1999).

Research efforts at the Texas Tech University Center for Space Science are underway to provide NASA's Advanced Life Support (ALS) program with horticultural strategies and cultivar screening for onions and related *Allium* spp. (Peffley et al., 2003) for use in Controlled Ecological Life Support System (CELSS), which consist of closed growth systems for crop production (Kliss et al, 1993). These closed growth systems create conditions different from those encountered in the field (Kliss et al., 1996). Factors that vary include solid support matrix, nutrient delivery and availability, relative humidity, photoperiod, accumulation of volatiles, and CO_2 partial pressure (Kliss et al., 1996). Such differing conditions have been associated with variability in composition compared to field grown crops (Alamazan et al., 1997; McKeehen at al., 1996; Steinberg et al., 2000; Wheeler et al., 1993, 1994 & 1996). Some of the constituents that have been altered in wheat, soybeans, lettuce, potatoes or sweet potatoes by hydroponic propagation include ash, protein, carbohydrate, Ca, Mg, K, Na, S, and crude fiber (Alamazan et al., 1997; McKeehen at al., 1996;

Steinberg et al., 2000; Wheeler et al., 1993, 1994 & 1996).

Most of the information available on the nutritional composition of edible plant tissues has been derived from field-grown crops and as such may not be a reliable indication of the nutritional value of crops grown in closed growth systems. Additionally few studies have examined vitamin content of crops grown in these systems. We chose to evaluate folate levels, as green onions could potentially be a good source of folate.

Previous research by our group has demonstrated that green onions are particularly rich in phytochemicals specifically, flavonols (Thompson et al., 2004). Health benefits associated with phytochemical consumption has prompted the examination of these secondary plant metabolites, which are non-nutritive plant polyphenols with potent antioxidant properties (Hertog et al., 1992; Leighton et al., 1992). When consumed in sufficient quantities these phytochemicals could contribute positive health benefits on long-term missions.

This study will focus on four species of *Allium* with distinct growth and developmental characteristics: *Allium cepa* L., *A. fistulosum* L., *A. schoenoprasum* L., and *A. tuberosum* L. Bulbing (*A. cepa*) and non-bulbing Japanese bunching (*A. fistulosum*) onions have markedly different harvest indices (proportion of edible biomass to total yield). For bulb-forming species with the onset of bulb formation, leaf production ceases, and the subsequent allocation of photosynthate to the bulb lowers the renewal as well as maintenance of the crop canopy. Thus, bulb onions have a high harvest index because the bulb serves as a sink while non-bulbing *Alliums* have a lower harvest index, which will vary according to how much green leaf is harvested in addition to the pseudostems. In contrast, non-bulbing species like Japanese bunching onions, chives (*A. schoenoprasum*) and Chinese chives (*A. tuberosum*) can develop a very high leaf area index, because they have mainly vegetative parts. Chive species and bunching onion are perennial in growth habit and have a tendency to form multiple stems (Peffley 1992). In salad onions and chives nearly 100% of the shoot may be harvested. An attribute of these latter species is that the leaves can be clipped for shoot consumption leaving the bulb/rhizome to continue to grow reducing the need for crop replanting, thus conserving crew time devoted to the maintenance of the crop system. Thus two possible strategies could be used for harvesting, single harvest or multiple harvest strategies. The single harvest strategy is the focus of this study.

It is important to identify factors that contribute to altered composition and to create a database of crop composition in order to adequately plan for the nutritional and health requirements of crews. In addition to identifying cultivars with high harvest indices and those that are nutrient and phytochemical rich, crops must also be palatable and desirable. Research by (Jasoni et al., 2004) indicated that emissions of volatile organic compounds (VOC) from green onions were significantly higher when plants were grown under elevated CO_2 compared to those grown at ambient CO_2 suggesting that volatile flavor and aroma compounds may also be altered when grown under elevated CO_2, possibly modifying the palatability and acceptability.

The objectives of this research were to evaluate biomass production, phytochemical and folate levels of nine onion cultivars grown at elevated or ambient CO_2. A second objective was to determine if growth at ambient versus elevated CO_2 influences sensory characteristics of *Allium* cultivars.

METHODS

PLANT MATERIALS AND GROWTH CHAMBER CONDITIONS - Plants were grown in two environmental growth chambers (EGCs) under either ambient (400 ppm) or elevated (1200 ppm) CO_2 in hydroponic units. Cultivars studied were *A. cepa* 'Cal 296', 'Deep Purple', 'Purplette'; *A. fistulosum* 'Kinka' and 'Choesty'; *A. schoenoprasum* 'Fine Leaf', 'Purly', 'Staro'; and *A. tuberosum* 'Garlic' chives. Parameters in the EGCs were 16 h/8 h light/dark, 24/20°C, 75/99% relative humidity, light - 660 $\mu mol \cdot m^{-2} s^{-1}$, and modified Hoagland's nutrient solution. Twenty-three Oasis pucs were sown with 9 seeds of each cultivar for a total of 207 pucs per EGC. At 49, 63 and 77 dap, 4 to 7 pucs per cultivar per EGC were harvested.

PLANT BIOMASS AND PERCENTAGE EDIBLE BIOMASS - Upon harvest Oasis was removed as completely as possible from the roots of each plant and the entire plant (roots, bulb and shoots) was weighed and averaged across treatment to determine biomass per plant. Total biomass from each cultivar was composited and the roots from each plant were removed at the basal plate along with any wilted, discolored, dehydrated materials. The remaining edible biomass was weighed. The percentage edible biomass was calculated by dividing edible biomass by total biomass, then multiplying by 100%.

TOTAL FLAVONOLS - Flavonols were extracted from plant tissues using 80% ethanol (Lombard et al., 2002). Filtrate was collected in 2-ml Eppendorf tubes and stored in a –20°C freezer. Immediately prior to analysis, extracts were warmed to room temperature, and a 0.5-ml sample was diluted with 4.5 ml of 80% ethanol. Absorbance of the diluted solution was determined on a Bausch & Lomb Spectronic 20 spectrophotometer Model 95 (Richmond, BC, Canada) at 362 nm in 1-cm cuvettes (Fisher Scientific; Houston, TX, USA; Bausch & Lomb; Richmond, BC, Canada) (Lombard at al., 2002). Duplicate readings were preformed on each sample and averaged. Isoquercitrin (Extrasynthese; Genay, France) was used as a standard (Lombard, 2000) and nine different standards with concentrations ranging from 0.00625-0.125 mg/ml were used to create a standard curve (absorbance vs. concentration) using linear regression. TF content was calculated on a wet matter basis using the equation for the best fitting line.

FOLATE ANALYSIS – Total folate was determined in duplicate on 50-g composite samples by microbiological assay using *Lactobacillus rhamnosus* and trienzyme digestion by a commercial laboratory according to AOAC method 960.46.

SENSORY ANALYSIS - Whole green onions of the cultivars *A. cepa*, ‘Purplette’ and ‘Cal 296’, and *A. fistulosum*, ‘Choesty’ and ‘Kinka’ were harvested at 63 dap. Insufficient plant material was available for the other cultivars (‘Deep Purple’, ‘Garlic chives’ ‘Fine Leaf’, ‘Purly’, ‘Staro’) to be included in the sensory screening. After the plants were rinsed in tap water, sanitized in a 50-ppm sodium hypochlorite solution and drained, they were chopped into about 0.5-cm long portions and mixed with iceberg lettuce in a 1:1 ratio providing a 5-g “salad” sample. Samples were placed in plastic portion control cups with lids and labeled with 3-digit random numbers. Four consumer panels with 16 to 18 members per panel performed triangle tests, in which elevated CO_2- and ambient-grown onions from each cultivar were presented to panelists. In each triangle test two like samples and one odd sample were presented, and panelists were asked to select the odd sample. In between samples panelists cleansed their palettes with distilled water and unsalted soda crackers. Chi-square analysis at the $P<0.05$ level were used to determine if panelist could discern differences between onions grown at elevated or ambient CO_2.

BIOMASS PRODUCTION

Plants grown at elevated CO_2 had greater total biomass than plants of the same cultivar grown at ambient CO_2 (Figure 1). Plants with the greatest increase in biomass were *A. cepa* cultivars and of those, ‘Cal 296’ exhibited the greatest increase, 276 % (12 g/plant, 400 ppm vs. 33.2 g/plant, 1200 ppm CO_2). Minimal effects on biomass as a result of elevated CO_2 were observed in *A. tuberosum* ‘Garlic’.

Regardless of cultivar or harvest age, onions grown under elevated CO_2 exhibited a higher percentage of edible biomass than those grown at ambient CO_2 (Table 1). The older plants also consistently had a higher edible biomass with the exception of ‘Garlic’. For this single harvest situation the *A. cepa* cultivars had the highest percentage edible biomass, over 91%, when grown at elevated CO_2 and harvested at 77 dap. ‘Garlic’ produced the lowest percentage of total edible biomass averaging 29.8%.

With a single harvest strategy *A. cepa* cultivars clearly produced plants with the largest amount of biomass and the greatest percentage of edible biomass. The Japanese bunching onions ‘Choesty’ and ‘Kinka’ produced plants that were smaller than *A. cepa*, but both had relatively high percentage edible biomass of 70 to 86%. The *A. schoenoprasum* cultivars produced small plants with highly variable percentage of edible biomass. Under a multiple harvest system however, the Japanese bunching onions or the common chives might produce greater total edible biomass over time and save time and labor associated with replanting.

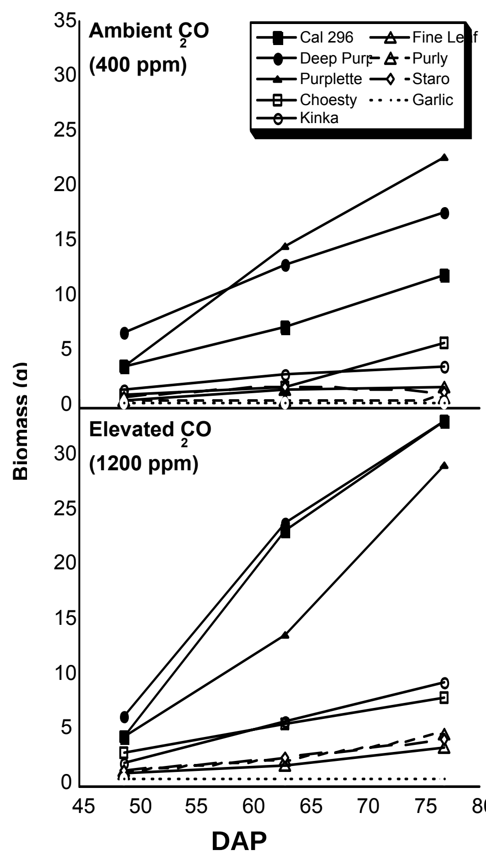

Figure 1. Average total biomass (g) of onion plants 49, 63 and 77 days after planting (dap). Plants were grown hydroponically in EGCs under ambient (400 ppm) or elevated (1200 ppm) CO_2.

PHYTOCHEMICAL AND FOLATE LEVELS

For three of the cultivars, growth under elevated CO_2 increased the TF content in the edible biomass (Figure 2). Two of the *A. cepa* cultivars, ‘Purplette’ and ‘Cal 296’, contained the highest concentrations of TF regardless of CO_2 concentrations, and growth under elevated CO_2 increased phytochemical levels 41% in both cultivars. Results were not consistent for the Japanese bunching onions with ‘Choesty’ exhibiting a significant increase in TF as a result of elevated CO_2 (48.9%), but the same was not noted for ‘Kinka’ with the elevated and ambient treatments having similar TF levels. TF levels of the common chives (*A. schoenoprasum* L. ‘Fine Leaf’, ‘Purly’, ‘Staro’) appeared to be unaffected by elevated CO_2.

Table 1. Percentage edible biomass of nine *Allium* cultivars 63 and 77 days after planting (dap) grown hydroponically in EGCs at elevated (1200 ppm) or ambient CO_2 (400 ppm).

	Edible biomass (%)			
	63 dap		77 dap	
Cultivar	400 ppm	1200 ppm	400 ppm	1200 ppm
Purplette	80.69	81.87	88.35	91.27
Cal 296	79.77	82.68	88.89	91.67
Deep Purple	71.10	76.49	85.04	91.22
Kinka	70.18	74.77	82.68	85.23
Choesty	70.77	81.27	74.74	86.30
Purly	48.98	71.75	65.00	81.16
Staro	60.28	67.51	74.36	80.55
Fine Leaf	63.46	64.58	75.00	86.30
Garlic	26.67	38.10	21.00	33.37

In addition to phytochemical analysis, folate content was estimated on plant material including leaf, pseudostem and bulbs. Folate levels did not appear to be affected by CO_2 level with perhaps the exception of ‘Choesty’ (Figure 3). Definitive conclusions are limited however by the fact that each value represented in Figure 3 is only an average a 50-g sample analyzed in duplicate.

The species average of folate reported in this study for ambient CO_2-grown bunching onions (‘Kinka’ and ‘Choesty’) was slightly lower than folate levels previously reported by Fenwick and Hanley (1990) for bulbs and leaves of *A. fistulosum* cultivars (16.1 mcg vs. 21.7 mcg/100 g, respectively). These differences could be due to growth conditions or plant age. From a nutritional standpoint, using ‘Purplette’ as an example, a 100-g serving of the green onions would provide 5.8% of the reference daily intake (RDI) of folate. The cultivar producing the highest levels of folate, ‘Staro’, would provide 7.8% of the RDI.

SENSORY TESTING

Panelists were unable to discern differences between onions grown at elevated and ambient CO_2 for three of the cultivars – ‘Purplette’, ‘Cal 296’, and one *A. fistulosum* cultivar, ‘Kinka’ (Table 2). Panelists were however, able to discern differences in sensory attributes of ‘Choesty’ onions grown at elevated or ambient CO_2 (Table 2).

The triangle tests conducted in this study were the first step in a series of tests to evaluate the sensory characteristics of the cultivars under investigation, and to determine how growth conditions in EGCs might influence the palatability and acceptability of onions. Further research needs to be conducted determine if the sensory characteristics of other cultivars are affected by CO_2 concentration, the exact character of the differences if they are present and the to identify the chemical nature of the volatile compounds that might be altered by CO_2 concentrations.

The *A. cepa* and *A. fistulosum* cultivars under examination have discernibly different sensory characteristics (data not shown). Generally U.S. consumers tend to favor onions that are less pungent, aromatic, and bitter but still have characteristic mild onion flavor (Alysworth, 1991). One concern in utilizing onions for NASA missions is striking a balance between flavor intensity and pungency such that onions are flavorful but not overly pungent. Additionally, onions will contribute volatile compounds to the atmosphere in the closed environment and, if overly aromatic, may create objectionable lingering odors. Conversely, physiological changes that occur in astronauts, such as fluid shifts, dehydration, and loss of taste/flavor acuity (Perchonok and Bourland, 2002) may necessitate the use of cultivars with more intense aromas and flavors. These studies are important initial steps in identifying cultivars most desirable for use, by basic selections on biomass production, nutritional value and desirable sensory characteristics.

Table 2. Chi-square analysis of sensory panel triangle tests of four onions cultivars harvested 63 dap grown in EGCs under ambient (400 ppm) or elevated CO_2 (1200 ppm).

Cultivar	Number of respondents	Number of correct judgments	α at $P \leq 0.05$
‘Choesty’	16	10	9
‘Kinka’	17	9	10
‘Purplette’	16	4	9
‘Cal 296’	16	2	9

CONCLUSIONS

Growth under elevated CO_2 increased plant biomass and percentage edible biomass for every cultivar with perhaps the exception of ‘Garlic chives’. For a single harvest strategy, the *A. cepa* cultivars in particular ‘Cal 296’ and ‘Deep Purple’ produced the greatest total biomass per plant and highest percentage edible biomass. The percentage edible biomass data collected in this study evaluated not only total biomass minus roots, but also excluded any leaf or bulb material that would be deemed undesirable and not likely to be consumed. Such estimates are necessary to adequately evaluate the waste material that might be generated and the material available for consumption.

The bulb onions examined in this study were rich sources of TF with two cultivars, ‘Cal 296’ and ‘Purplette’ containing more that 4000 mg/kg TF when grown under elevated CO_2. Varying CO_2 concentration during propagation influenced TF concentration of some cultivars, but the affect was not necessarily consistent

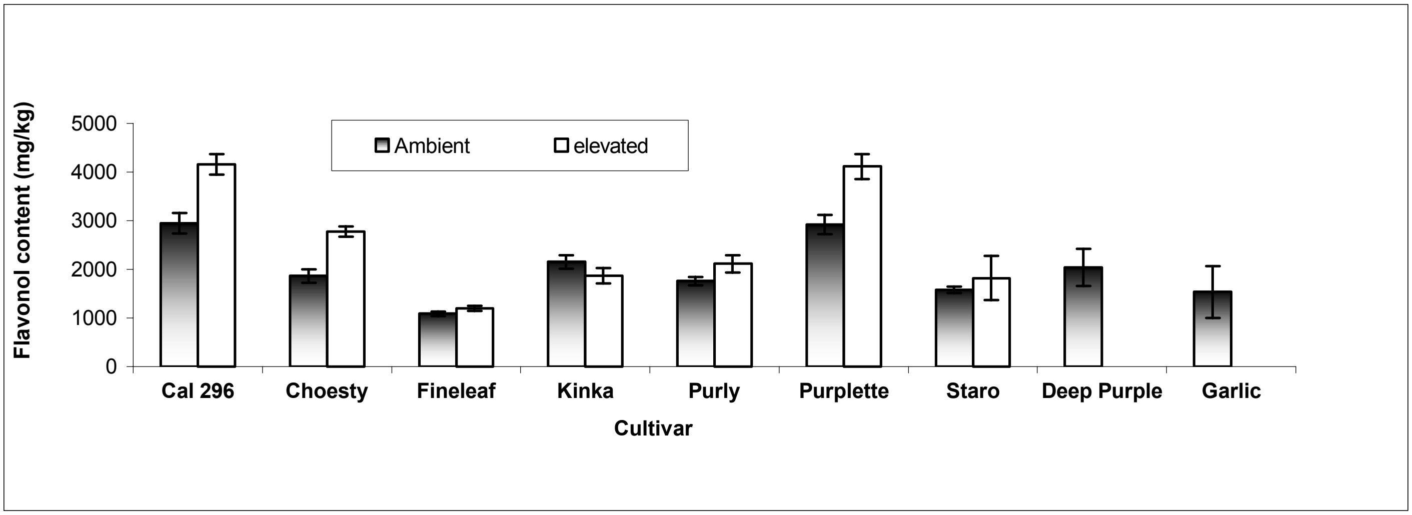

Figure 2. Flavonol content of nine cultivars of *A. cepa* L. (bulb onions; ‘Purplette’, ‘Deep Purple’, ‘Cal 296’), *A. fistulosum* (Japanese bunching onions; ‘Choesty’, ‘Kinka’), *A. tuberosum* (Chinese chives; ‘Garlic chives’), *A. schoenoprasum* L. (chives; ‘Fine Leaf’, ‘Purly’, ‘Staro’) grown in hydroponically in EGCs at elevated (1200 ppm) or ambient CO_2 (400 ppm) harvested at 63 days after planting.

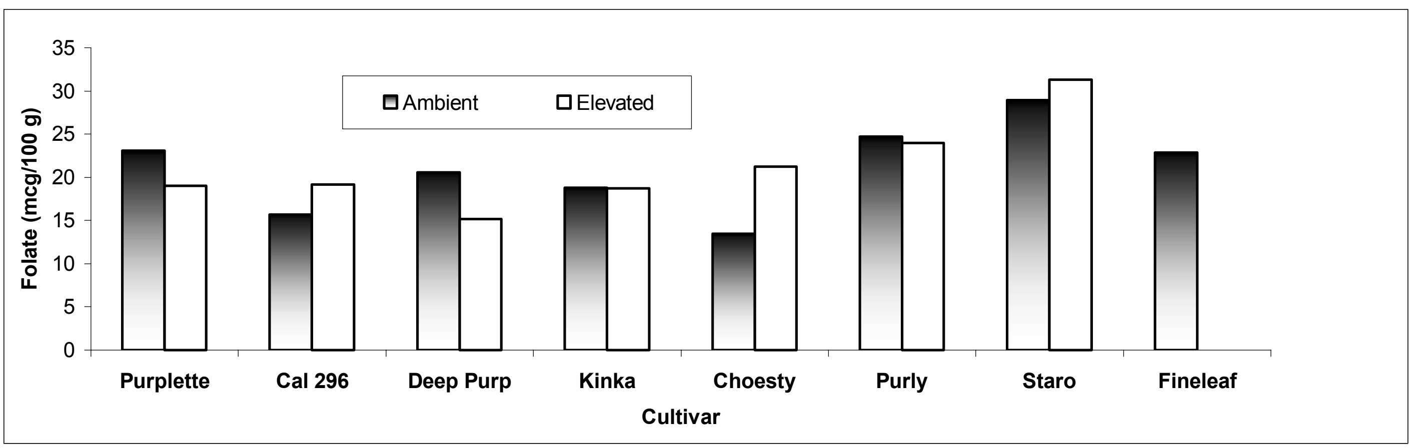

Figure 3. Folate content of nine cultivars of *A. cepa* L. (bulb onions; ‘Purplette’, ‘Deep Purple’, ‘Cal 296’), *A. fistulosum* (Japanese bunching onions; ‘Choesty’, ‘Kinka’), *A. tuberosum* (Chinese chives; ‘Garlic chives’), *A. schoenoprasum* L. (chives; ‘Fine Leaf’, ‘Purly’, ‘Staro’) grown in hydroponically in EGCs at elevated (1200 ppm) or ambient CO_2 (400 ppm) harvested at 63 days after planting.

within species. Data also indicated that sensory characteristics of some cultivars may be affected by growth at elevated CO_2. Further work needs to be conducted to determine the nature of these potential modifications and the chemical basis of changes.

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CONTACT

Leslie Thompson, Associate Professor, Animal and Food Sciences, Texas Tech University, Box 42141, Lubbock, TX 79409. The author can also be reached at leslie.thompson@ttu.edu or 806-742-4862.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

ALS: Advanced Life Support

CELSS: Controlled Ecological Life Support System

Dap: Days after planting

EGC: Environmental Growth Chamber

NASA: National Aeronautics and Space Administration

RDI: Reference Daily Intake

TF: Total Flavonols

VOC: Volatile Organic compounds