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# Composition of Salad Greens: A Comparison of Locally-Grown and Supermarket Produce

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**Table 2.** Analysis of variance of composition of lettuce and spinach due to commercial source of the sample, and time-of-year. There were two degrees of freedom for variance due to source in lettuce, and one in spinach, and one degree of freedom for effect of season. There were 11 degrees of freedom in the error term for lettuce and 6 for spinach.

| Species | Lettuce | | | | Spinach | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Source | | Season | | Source | | Season | |
| Component | F ratio | Prob-ability | F ratio | Prob-ability | F ratio | Prob-ability | F ratio | Prob-ability |
| Dry matter | 5.8 | 0.006 | 0.0 | ns | 9.7 | 0.005 | 1.0 | ns |
| Nitrogen | 2.4 | ns | 1.7 | ns | 4.7 | ns | 0.3 | ns |
| Potassium | 9.6 | 0.004 | 0.8 | ns | 0.0 | ns | 0.5 | ns |
| Phosphorus | 30.0 | 0.000 | 7.2 | 0.02 | 0.7 | ns | 2.6 | ns |
| Calcium | 1.6 | ns | 0.1 | ns | 12.6 | 0.012 | 0.2 | ns |
| Magnesium | 4.0 | 0.05 | 0.4 | ns | 39.7 | 0.001 | 0.0 | ns |
| Sodium | 1.3 | ns | 2.7 | ns | 0.3 | ns | 0.0 | ns |
| Nitrate | 6.1 | 0.006 | 0.3 | ns | 1.5 | ns | 0.0 | ns |
| Chloride | 3.9 | 0.03 | 0.3 | ns | 0.6 | ns | 2.7 | ns |
| Malate | 7.5 | 0.003 | 0.4 | ns | 2.4 | ns | 1.6 | ns |
| Oxalate | 2.2 | ns | 0.6 | ns | 1.9 | ns | 0.6 | ns |
| Sum soluble Sugars | 1.2 | ns | 0.4 | ns | 0.0 | ns | 11.9 | 0.003 |
| Sucrose | 7.2 | 0.003 | 11.7 | 0.002 | 0.9 | ns | 10.4 | 0.005 |
| Glucose | 0.2 | ns | 0.6 | ns | 0.0 | ns | 15.5 | 0.001 |
| Fructose | 0.3 | ns | 0.0 | ns | 0.5 | ns | 7.1 | 0.017 |
| Starch | 3.1 | ns | 14.3 | 0.001 | 4.2 | 0.05 | 1.2 | ns |
| Sum Amino acids | 20.0 | 0.000 | 15.2 | 0.001 | 66.8 | 0.000 | 3.9 | ns |
| Aspartate | 26.4 | 0.000 | 0.3 | ns | 24.1 | 0.000 | 3.6 | ns |
| Glutamate | 6.5 | 0.006 | 11.6 | 0.003 | 0.4 | ns | 6.0 | 0.03 |
| Asparagine | 25.1 | 0.000 | 4.6 | 0.04 | 35.7 | 0.000 | 0.2 | ns |
| Glutamine | 4.3 | 0.03 | 13.0 | 0.002 | 21.9 | 0.000 | 5.0 | 0.04 |

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## ABSTRACT

Locally-grown produce is fresher and may have a different composition than produce that has been in storage or transit for days or weeks. The greater content of nutrients has been used to promote the sale of locally grown food. Production of salad greens in hydroponics in Connecticut has the potential to significantly increase the economic returns for Connecticut greenhouse operations. We determined the nutritional value of locally-grown salad greens and compared these values to those of the same crops that were produced in distant regions and sold in supermarkets. Samples were obtained from all sources in each season of the year. Locally grown produce had higher concentrations of sucrose and starch, and lower concentrations of free amino acids than produce shipped from distant regions. Sugars were increased in summer in lettuce, and in winter in spinach. The local produce likely retained more of the sugars found in plants immediately after harvest. The high amino acids in non-local produce may be an early sign of tissue breakdown due to prolonged storage. However, we found no instances in which mineral- or organic acid composition differed between local compared to distance production. These concentrations were not affected by seasonal changes in environment. Most differences in composition could be attributed to production conditions and/or the size of the plants.

## INTRODUCTION

About 4000 acres in Connecticut are devoted to vegetable production (USDA-NASS, 2011), but most of these farms are small: most of them are less than 50 acres in size. Small farms need to grow high-value crops in order to succeed. They tend to sell directly to stores, restaurants and the general public. Many farms sell directly to the public through roadside stands, sales rooms, and farmers' markets (CT Dept. of Agriculture, 2011). Farmers' markets make Connecticut-grown, farm-fresh produce available to city dwellers that do not have direct access to farms. In 2010, there were more than 100 farmers' markets (CT Dept. of Agriculture, 2010). Farmers' markets encourage growers to supply vegetables for a longer duration of the year. Consumers are used to the wide variety of fruits and vegetables that are available year-round in large supermarkets, and they expect the same at farmers' markets and roadside stands.

Protected cultivation in greenhouses extends the production season of vegetables in Connecticut beyond the few summer and fall months allowed by open field production. Even in unheated high tunnels, salad greens can be produced from late March to late December (Gent, 2002), and tomato and peppers can be produced from June through November (Gent, 1991). Greenhouses can provide locally grown, fresh vegetables to Connecticut consumers year-round. It is possible to manipulate the environment and fertilization within a heated greenhouse to increase the production of these crops, and the quality of specific compounds in plant tissue that are important for human nutrition. Plants can be kept free of pesticides since most pathogens and diseases can be addressed using integrated pest management techniques in the contained environment. Hydroponics allows fine control of the concentration and composition of fertilizer available to the plants, and thus provides another level of control of plant tissue composition.

Marketing studies and food sales indicate that consumers are concerned about the nutritional value and safety of the food they eat. They will pay more for fresher vegetables and food produced under safe conditions that maximize nutritional value. More and more consumers prefer locally grown produce that is fresh, to produce grown and shipped from distant regions. Consumers want to know the conditions under which their vegetables are grown, rather than purchasing produce from anonymous farms, under unknown conditions. Local growers who sell their own produce directly to the public can personally vouch for the safety and freshness of their produce.

One occurrence of a human health problem associated with non-locally grown food was publicized in 2009 (Schreck, 2009). Approximately 22,000 cartons of romaine lettuce were recalled because the product may have been contaminated with salmonella, an organism that can cause serious and sometimes fatal infections. The cartons of bulk or wrapped romaine were harvested June 25-July 2 in Salinas, CA, and sold to retail, wholesale and food service outlets across the United States, Canada and Puerto Rico. However, the recall was not announced until July 22. This illustrates the potential danger of consumption of food grown in distant regions under unknown conditions. It also illustrates the time-delay this produce had in shipment and storage (up to four weeks). This delay may diminish the nutritional value of the crop.

Locally grown vegetables are fresh and may have a different nutritional composition than vegetables that have been in storage or transit for weeks before reaching the consumer. The presumption of higher nutrient levels appeal to consumers and enhances the desirability of locally grown food. In this study, we determined the amounts of various nutrients in representative samples of locally grown lettuce and spinach, and compared these nutritional values to those of greens that were transported from distant regions of the U.S. or other countries, and sold through a nationally-branded supermarket chain. Fresh greens were obtained from local growers, including an organic farmer, and a farmer who produces salad greens in hydroponics. The samples from these growers were compared to samples obtained from a large supermarket chain. The supermarket samples were presumed to have come from large-scale operations in the south and southwest USA. The plant tissues were preserved by freeze-drying and then analyzed for total nitrogen (protein), other essential elements, soluble anions such as nitrate, amino and organic acids, and sugars.

## METHODS

Salad greens were obtained from two growers in Connecticut and from a supermarket chain. ‘Starlight Gardens’ is a certified organic farm in Durham, CT, which grows a variety of salad greens in unheated high tunnels, and under low tunnels covered with plastic or spun bonded row cover. Samples of lettuce and spinach from this farm were selected at a relatively early growth stage, baby salad green size. ‘Two Guys from Woodbridge’ is a greenhouse hydroponic operation in Hamden, CT. It is operated according to organic principles. The nutrients added to the hydroponic solution are approved for organic use. Plants are grown in a heated greenhouse under supplemental light. Samples of lettuce from this farm were bouquets of mixed cultivars of lettuce. The bouquet had a single root mass, and was on the order of 200 g fresh weight. These Connecticut-grown samples were obtained directly from the growers and returned to the laboratory. Lettuce and spinach samples were also obtained from ‘Stop and Shop’, a nation-wide supermarket chain. Lettuce was sold as an ‘Organic Spring Mix’. The lettuce was separated from other greens before drying and analysis. The spinach was sold in bags as ‘Baby Spinach’. These samples were presumed to come from California, Arizona, or Mexico. Samples were chosen with the latest available ‘best used by’ date. Samples were collected on five dates through the year: Dec 9, 2009; Mar 25, 2010; June 29, 2010; Sep 28, 2010; and Dec 16, 2010. These dates corresponded to winter, spring, summer, fall, and a second winter harvest. Samples were collected from all sources on the same date. Samples were brought to the laboratory and immediately washed in tap water, dried on paper towels and weighed. The samples were then frozen and freeze-dried. After drying, they were re-weighed, and ground to a fine powder, which was stored at -20 $^{o}$C until chemical analysis was done.

Sub-samples of the dried tissue were extracted and analyzed for nitrate, soluble sugars, and organic acids using liquid chromatography (LC) on a model 1100 instrument (Agilent Corp, Palo Alto, CA). For nitrate analysis, a 50 mg subsample of freeze-dried plant tissue was transferred to a 7 mL homogenizer cooled in ice and ground with 5 mL ice-cold water. This sample was diluted 2:1, centrifuged and stored at -20 $^{o}$C in a micro-centrifuge tube until analysis. Just prior to analysis, the sample was thawed, diluted 12:1 and filtered into a Target vial. A 20 uL subsample was injected onto a 4.1 x 100 mm ion exchange column with pre-column (model PRP-X110S, Hamilton, Reno, NV) and eluted at 1.5 $mL\cdot min^{-1}$ with a buffer of 1.7 m$M$ $NaHCO_3$, 1.8 m$M$ $Na_2CO_3$, and 0.1 m$M$ KSCN. Nitrate and other anions were detected by UV absorbance at 212 nm. This LC method and dilution were calibrated with external standards of nitrate.

Part of the sample described above was diluted 2:1 into 4% v/v trichloroacetic acid for amino acid analysis. A subsample was injected onto a 4.6 x 150 mm reverse phase silica column with pre-column (model Adsorbosphere OPA HR, Alltech Assoc., Deerfield, IL). The column was maintained at 40 $^{o}$C and eluted at 1.5 $mL\cdot min^{-1}$ with a gradient obtained from mixing methanol with a 50 m$M$ acetic acid buffer at pH 4.9 containing 2% v/v tetrahydrofuran. The methanol fraction was increased from 10 to 60%

**Table 1.** Composition of lettuce and spinach averaged over five sample dates.
Dry matter is stated as a fraction of fresh weight. Concentrations are in moles per kilogram dry tissue.
Values in a row followed by different letters differ significantly at P<0.05 among sources, either within the sources of lettuce and/or within the sources of spinach.

| Species | Lettuce | | | Spinach | | Least |
|---|---|---|---|---|---|---|
| Source | Starlight | Stop&Shop | TwoGuys | Starlight | Stop&Shop | significant |
| Component | | | | | | difference |
| Dry matter | 0.063 a | 0.056 ab | 0.046 b | 0.092 a | 0.072 b | 0.011 |
| Nitrogen | 2.8 | 3.4 | 3.4 | 3.9 | 4.2 | 0.6 |
| Potassium | 1.6 b | 1.4 b | 2.2 a | 1.9 | 2.0 | 0.4 |
| Phosphorus | 0.16 b | 0.15 b | 0.21 a | 0.18 | 0.16 | 0.03 |
| Calcium | 0.17 | 0.24 | 0.22 | 0.09 b | 0.26 a | 0.10 |
| Magnesium | 0.11 | 0.16 | 0.12 | 0.28 b | 0.56 a | 0.06 |
| Sodium | 0.4 | 0.3 | 0.1 | 0.7 | 0.5 | 0.3 |
| Nitrate | 0.22 b | 0.31 b | 0.54 a | 0.23 | 0.30 | 0.02 |
| Chloride | 0.26 b | 0.31 b | 0.47 a | 0.18 | 0.24 | 0.09 |
| Malate | 0.26 a | 0.10 b | 0.11 b | 0.08 | 0.04 | 0.07 |
| Oxalate | 0 | 0 | 0 | 0.09 | 0.13 | 0.05 |
| Sum soluble Sugars | 0.27 | 0.24 | 0.21 | 0.13 | 0.10 | 0.10 |
| Sucrose | 0.07 a | 0.03 b | 0.03 b | 0.06 a | 0.03 b | 0.03 |
| Glucose | 0.07 | 0.08 | 0.06 | 0.03 | 0.03 | 0.03 |
| Fructose | 0.12 | 0.13 | 0.11 | 0.03 | 0.04 | 0.05 |
| Starch | 0.10 a | 0.11 a | 0.07 b | 0.07 a | 0.03 b | 0.03 |
| Sum Amino acids | 0.052 c | 0.168 a | 0.097 b | 0.078 b | 0.199 a | 0.040 |
| Aspartate | 0.006 b | 0.019 a | 0.008 b | 0.012 b | 0.020 a | 0.005 |
| Glutamate | 0.010 b | 0.019 a | 0.016 b | 0.023 | 0.021 | 0.006 |
| Asparagine | 0.008 b | 0.036 a | 0.018 b | 0.003 b | 0.047 a | 0.013 |
| Glutamine | 0.010 b | 0.032 a | 0.033 a | 0.018 b | 0.039 a | 0.015 |

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from 2 to 22 min, and then decreased from 60 to 10% from 26 to 29 min. A 3 uL subsample was mixed with 9 uL of o-phthaldialdehyde reagent for 1 min before injection on the column, and the OPA-derivatives were detected by fluorescence, with excitation at 350 nm and emission at 450 nm. This method was calibrated with external standards of mixtures of amino acids.

For sugar analysis, 100 mg subsamples of dried tissue were extracted twice with 5 mL of a solution of 240 mL methanol: 100 mL chloroform: 20 mL water: and 10 mL formic acid. Six mL of water was added to the extract and mixed to separate liquid phases. The chloroform layer containing pigments was discarded. The water phase was dried at 50 $^{o}$C under a stream of air. Just prior to analysis, the dried residue was resuspended in 10 mL water and filtered into a Target vial. A 50 uL volume was injected on an 7.8 x 300 mm LC column with a calcium form of exchange resin (model HPX-87C, Bio-Rad, Hercules, CA.) with both anion and cation pre-columns to trap all except neutral compounds. Deionized water at 0.6 $mL\cdot min^{-1}$ flowed through the pre-columns at room temperature, and the column at 70 $^{o}$C. Sugars were detected by refractive index and calibrated with external standards of sucrose, glucose and fructose.

Total nitrogen and elemental composition were determined in 250 mg subsamples digested by boiling for 10 min in a reagent of 4 mL $H_2SO_4$ then adding 10 mL $H_2O_2$. The sample was cooled and diluted to 100 mL. Most elemental concentrations were determined by inductively coupled plasma atomic emission spectroscopy (Atom Scan 16, Thermo-Jarrell Ash, Franklin, MA). Reduced nitrogen was determined by reacting 0.4 mL of the digest with 1.0 mL Nessler reagent diluted to 25 mL. Optical absorbance was measured at 460 nm using a spectrometer (Evolution 60, ThermoFisher Scientific, Pittsburgh, PA). The method was calibrated with mixtures of starch and bovine serum albumin.

A statistical analysis was done separately for the lettuce and spinach samples, as these two species had different composition. There were three different sources of lettuce; two growers in Connecticut and supermarket samples originating from Arizona, California, or Mexico. There were two sources of spinach; a grower in Connecticut and samples obtained from the supermarket. Analysis of variance used the source of the greens as the main effect. A variable corresponding to day length was used as a covariate to account for environment effects, such as seasonal variation in light and temperature. This variable had a minimum on December 21 and a maximum of June 21.

## RESULTS AND DISCUSSION

There were many nutrients that differed among the sources of salad greens. However, only amino acids and sugars varied according to the season. Table 1 summarizes the effect of the source or farm on composition when averaged over samples from all five harvest dates. Table 2 presents an analysis of variance of these data due to source, and using a covariate with a single degree of freedom to account for changes in environment due to season.

### *Dry matter*

The dry matter content, the dry weight as a fraction of fresh weight, differed between the sources or farms, for both spinach and lettuce. In part, these differences were likely related to the method of growing. The lettuce grown locally in hydroponics had a lower dry matter than the other local sample grown in the ground in high tunnels, or the supermarket sample grown in the field. Samples of spinach grown locally in high tunnels had higher dry matter than the supermarket samples.

### *Protein and minerals*

The source or farm had no significant effect on total nitrogen or protein content in either lettuce or spinach. The protein tended to be lowest in lettuce or spinach grown locally in high tunnels. In part, this may be because the plants were harvested at a smaller size than those from other sources, and nitrogen content of vegetables tends to increase with plant size (Greenwood and Hunt, 1986). Lettuce from a local grower using hydroponics had more potassium and phosphorus than the other sources of lettuce. There were no differences in these elements in spinach from local or distant farms. This suggests that the plants in hydroponics had more of the nutrients available to them than the plants grown in soil.

Calcium and magnesium did not differ among lettuce samples. However the spinach grown locally in high tunnels had less of these elements than spinach from the supermarket. Strangely, both sources of spinach had less calcium than magnesium, which is contrary to the usual pattern in plant tissue (Roorda van Eysinga and Smilde, 1981). There was less sodium in lettuce than in spinach, when comparing similar sources or farms. Moreover, the lettuce grown in hydroponics had the lowest amount of sodium.

### *Nitrate and soluble ions*

The lettuce samples differed in the concentration of nitrate and chloride anions. The levels were highest in the plants produced in hydroponics. There were no differences among the sources of spinach. Accumulation of nitrate in hydroponic production of lettuce has long been recognized as a problem in northern Europe (Santamaria, 2006). The European Economic Community has set upper limits of 3500 and 4500 parts per million of nitrate by fresh weight, for lettuce harvested in summer and winter, respectively (Santamaria, 2006). The concentrations seen here averaged 1500 parts per million, so they are far less than the high values observed in Europe. In part this is because the hydroponic grower used supplemental light, which lowers the concentration of nitrate in tissue (Gaudreau et al., 1995). Other studies found that high concentrations of nitrate in lettuce occur primarily under low light conditions in winter (Van der Boon et al., 1990). There was a large variation in nitrate that could not be attributed either to source or environment. This is because within one source or farm, nitrate varied from one harvest date to another, in a manner that was not correlated with time of year. This suggests that tissue nitrate is very sensitive to the timing of fertilizer application or some other factor that is not easily related to seasonal effects.

### *Organic acids*

Whereas malic acid was the predominant organic acid in lettuce tissue, there was more oxalic than malic acid in spinach. The malic acid differed among the sources of lettuce, because the plants from the local organic grower had more than other sources. It is not clear whether this is due to the smaller size of the plants, or due to the difference in environment in high tunnels compared to the greenhouse or open field.

### *Amino acids*

There were effects of source or grower as well as season on the free amino acids in lettuce. For both lettuce and spinach, the supermarket samples had the highest concentration of amino acids. The highest concentrations in these samples occurred in winter. In an earlier study, amino acids varied with season in a variety of salad greens grown in high tunnels, and concentrations were highest in early spring and late fall, under cool temperatures (Gent, 2005). However, in the present study, season had no effect on amino acid composition of spinach samples from Connecticut or those obtained from the supermarket. Free amino acids in samples obtained from the supermarket were not depleted due to storage and transport. In fact, supermarket samples had 4- to 10-fold higher concentrations of asparagine, than in samples grown locally in high tunnels. This pattern may be an early indication of tissue degradation due to the time spent in storage and transport.

### *Sugars and starch*

Sucrose and starch differed among the sources of lettuce and spinach, but there was no difference in other sugars. The concentration of sucrose was highest for both lettuce and spinach grown locally in high tunnels. I have noted that small lettuce plants tend to have a higher concentration of sugars than larger plants (Gent, 2011). This may explain the difference between sources noted in the present survey. There were also significant differences in starch content. Lettuce from a local hydroponic grower has the lowest amount of starch, and the supermarket sample had the lowest amount in spinach. These results could be a genotype effect, as many cultivars of lettuce have no starch. For spinach, the low amount of starch could be indicative that the tissue had been in storage or transit for so long that the sugars were depleted. All of the sugars in spinach samples from the supermarket were lower than in any other samples. Our assay reported more starch in lettuce than in spinach. It is possible that anthocyanins in lettuce interfered with the starch assay, as many cultivars of lettuce do not contain starch, but starch was detected in all samples assayed in this study.

### *Seasonal effects*

Sugars and amino acids were the only components that showed a seasonal variation (Table 2). For both lettuce and spinach, the concentration of sucrose was higher in winter than in summer. The same was true for glucose, fructose, and the sum of sugars in spinach. There were no significant seasonal variations of starch or sugars other than sucrose in lettuce. The highest concentrations of amino acids occurred in winter in supermarket samples. Whereas the samples grown locally in high tunnels showed a similar pattern, with the highest amino acids in winter, there was no clear trend for the samples grown in hydroponics. Consequently this generated an interaction of effects of source an season.

In general, the season in which the samples were harvested had little effect on their composition. This trend was noted in an earlier study of the composition of various salad greens grown in high tunnels in Connecticut (Gent, 2002). This is in contrast to the results obtained from experiments in heated greenhouses, which often show a seasonal variation in both nitrate and sugars (Drews et al., 1995). Nitrate in plant tissues tends to be higher in winter under low light conditions and lower in summer, and the concentration of sugars, at least in lettuce, shows an opposite trend with season. However, the one heated greenhouse that was involved in the present study used supplemental light in winter, which likely alleviated the effects of low light, such as high nitrate and low sugars. All of the other samples were either grown in unheated high tunnels or in the field. For these particular samples, there must be offsetting effects of temperature and light, such that most nutrients are relatively little affected by seasonal trends. It is interesting that lettuce and spinach differed in the response to season of their sugar contents. Spinach is generally considered a cool-season crop, and it tends to grow poorly in hot weather. It is likely that this is related to a sugar metabolism and the fact that sugars tend to be depleted under warm temperatures. This was definitely the trend we found in the spinach sampled here. In contrast, sugar content of lettuce was relatively unaffected by warm temperatures, except for sucrose, which was increased due to the higher available light. Lettuce grows much faster in warm, compared to cool weather. It appears that lettuce is better able to tolerate high temperature because sugars are not depleted.

### *Other effects*

Although some of the variation in composition of tissue samples could be correlated to a simple function of time of year, there were other variations among samples from each source that could not be readily explained by this factor. For instance, one sample of ‘Stop and Shop’ lettuce obtained in winter had the highest value of protein from this source, while another sample collected the next winter had the lowest. The sample with high nitrogen also had high nitrate and low concentrations of sugars, while the sample with low nitrogen had the highest concentration of sugars from this source. The latter pattern is indicative of a crop with insufficient nitrogen fertilizer. A similar pattern was found among lettuce samples grown locally in high tunnels. In this case the lowest concentrations of nitrogen and nitrate, and the highest concentrations of sugars were found in the plants sampled in summer. These variations in composition are likely due to the timing of fertilizer application, more than to seasonal changes in environment. We did not see such variation in the samples from the local hydroponics production. In general, the supply of nutrients does not fluctuate in hydroponics, unless there are unrecognized problems with the hydroponics system. The concentrations of nitrogen and nitrate in spinach were much more stable over the various sample dates than those in lettuce.

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