# SENSORY ANALYSIS OF PAC CHOI AND TOMATO GROWN UNDER ORGANIC AND CONVENTIONAL SYSTEMS

by

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B.S., Universidad San Ignacio de Loyola, 2002
M.S., Kansas State University, 2006

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Human Nutrition
College of Human Ecology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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# Abstract

Vegetables are popular among consumers because of their versatility of preparation, unique sensory characteristics, and exceptional health benefits. Trends such as organic farming and breeding to increase nutrition and functional health components have increased interest in understanding the flavor of vegetables, such as leafy greens. A lexicon of thirty-two flavor attributes was created to help describe the flavor of fresh leafy vegetables. This lexicon includes five "green" attributes; mouth feel characteristics such as pungent, bite, tooth-etch, and heat/burn; fundamental tastes including bitter and umami; seven terms that describe unique flavors related to specific vegetables such as cabbage, celery, lettuce, spinach, parsley, beet, and radish leaves; and a group of other terms including citrus, piney, woody, water-like, musty/earthy, floral, sulfur, metallic, soapy, petroleum-like, and overall sweet. In addition, our study encompassed a series of sensory tests which will aid in better understanding the effects of several production variables on the sensory characteristics of pac choi and tomato. Variables evaluated were production systems (i.e. organic and conventional), fertilizer amount (i.e. high, low, and no fertilizer), environment (i.e. field and high tunnel), maturity level (i.e. 2.5, 4.5, and 6.5-week old plants at the time of harvest), and shelf life (i.e. 1, 4, 9, 18 days of refrigerated storage). Samples were grown at the Kansas State University Horticulture Research Center located in Olathe, Kansas. Highly trained descriptive panelists from the Sensory Analysis Center at Kansas State University evaluated the samples. There do not appear to be major sensory differences between organic and conventional products specific to the crops and seasons studied. Furthermore, when differences were present, they generally were quite small and showed no clear trends or patterns favoring one production system over the other even after refrigerated storage. However, it is suggested that differences in flavor and volatile composition between organic and conventional pac choi may be more evident at early stages of growth.

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# Table of Contents

# List of Figures

# List of Tables

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# Dedication

A mi esposa María José y a mi hijo Lucas, este trabajo es para ustedes.

# CHAPTER 1 - Literature Review

## Vegetable Market in the United States

While the harvested area of vegetables and melons decreased 3.4% in the United States in the 2006 – 07 periods, production increased 4.7% in the same period (United States Department of Agriculture – Economic Research Service [USDA-ERS], 2008). At the same time, per capita utilization increased 2% moving from 179.1 pounds in 2006 to 182.8 pounds in 2007 and was projected to be at 180.5 in 2008 (USDA-ERS, 2008). It is evident from these values that production processes need constant change to improve yields and be able to increase production and fulfill increasing demand even though fields are less available for production. Some of the reasons for these increases in yield are (1) increased knowledge that certain areas are more suitable for production of certain vegetables, (2) increased use of irrigation, (3) increased knowledge in fertilization practices and plant nutrition, (4) new methods to control pests and diseases, and (5) the introduction of superior cultivars (Peirce, 1987).

In 2007, the vegetable crops that showed the largest production were onion, head lettuce, watermelon, and tomato (Figure 1) mainly from states such as California, Florida, and Georgia. Texas also showed an important production of watermelon, Michigan was important for squash, and New York for cabbage (USDA-ERS, 2008).

The United States also imports much of its vegetable supply to be able to fulfill an increasing demand. In 2007, the products that were imported the most were tomato (all varieties), cucumber, cantaloupe, and watermelon (Figure 2). Most of the U.S. imports come from countries such as Mexico and Canada. Other countries that also sell their products to the United States are Guatemala (cantaloupe, green peas, and snap beans), Peru (Asparagus, onions, and green peas), Chile (onions), and The Netherlands (bell peppers, tomatoes, and eggplant) (USDA-ERS, 2008).

In relation to their exports, the United States exports mainly to Mexico, Canada, and Japan. In 2007, the main exports were lettuce, onions, tomatoes, and broccoli (Figure 3). Other destinations of U.S. products are the United Kingdom (onions, lettuce head, and sweet corn), The Netherlands (sweet corn and carrots), Switzerland (asparagus), South Korea (head lettuce and sweet corn), Hong Kong (celery), and Taiwan (broccoli, head lettuce, and onion).

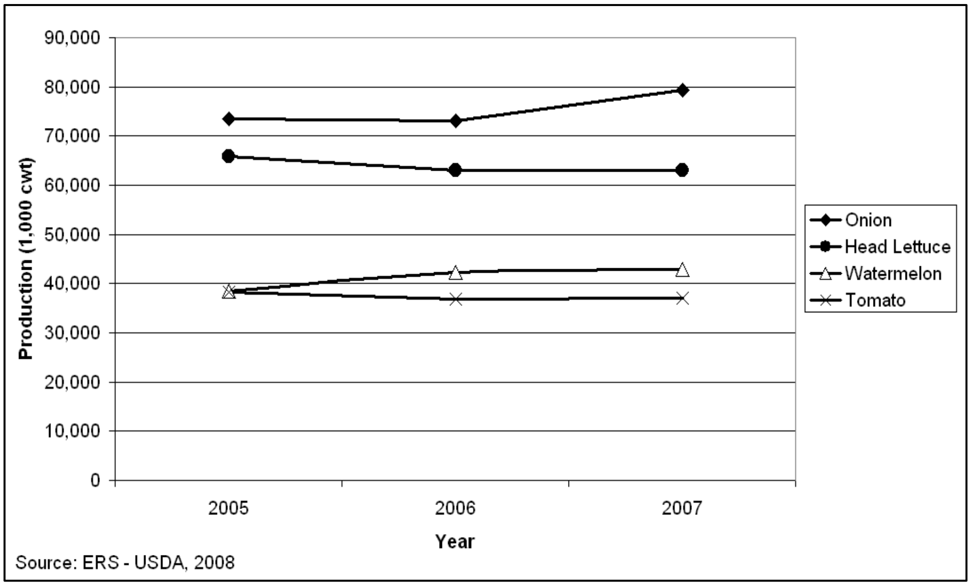


Figure 1.1. Major vegetable crops produced in U.S. in the 2005-07 period

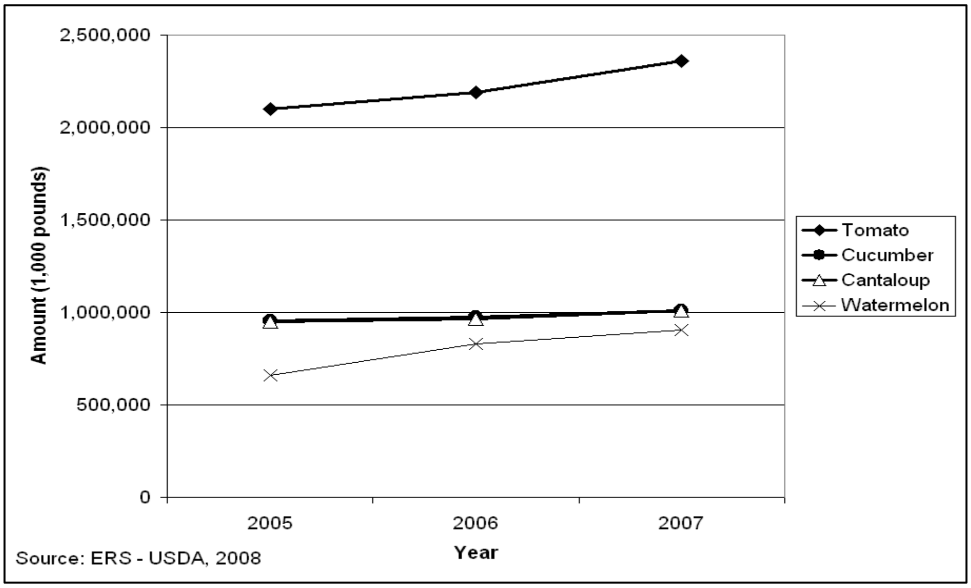
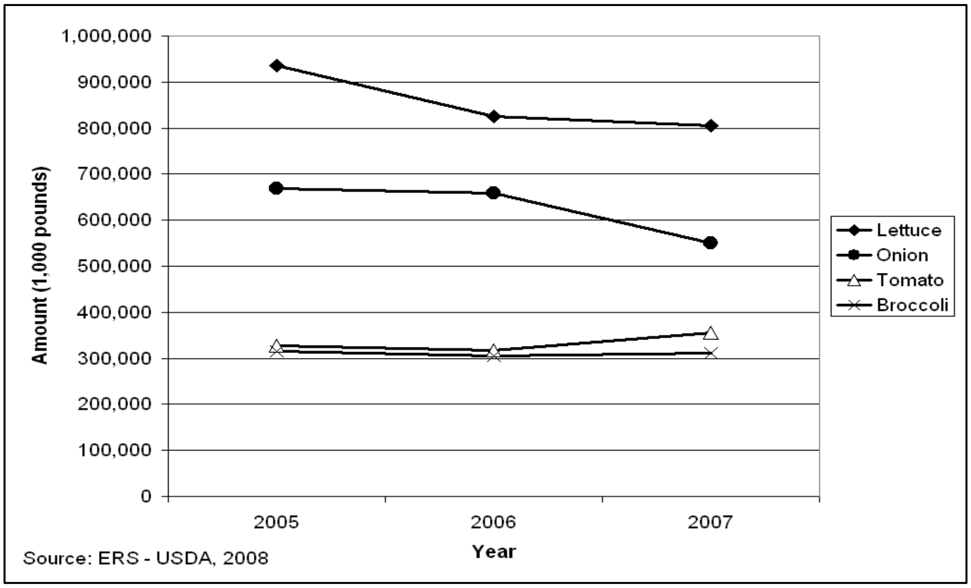


Figure 1.2. Major U.S. imports of vegetable crops in the 2005-07 period



Source: ERS - USDA, 2008

**Figure 1.3. Major U.S. exports of vegetable crops in the 2005-07 period**

## Vegetable Classification & Nutrition

Many vegetables that are widely used in the occident (and specifically the United States) have their origin in Asia or are commonly used in that part of the world (Table 1). Vegetable products from Japan, India, Korea, Philippines, and Southeast Asia are the main focus of Asian cuisine, which is very popular in many countries (Creasy, 2000).

**Table 1.1. Vegetables commonly used in the Asian cuisine**

- Amaranth
- Bamboo
- Basil
- Beans
- Bitter melon
- Bunching onions
- Burdock
- Carrots
- Chinese broccoli
- Chinese cabbage
- Chinese celery
- Coriander
- Baby corn
- Cucumbers
- Daikon
- Eggplants
- Garlic
- Garlic chives
- Chinese leek
- Ginger
- Lemon grass
- Lime leaf
- Luffa
- Mitsuba
- Mibuna
- Mizuna
- Mustard
- Pac choi
- Peas
- Peppers
- Perilla
- Sesame
- Shungiku
- Spinach
- Turnips
- Water chestnuts
- Winter melon
- Winter squash

Source: Creasy, 2000

To organize vegetables is essential because of the large amount of species that exist in the world and specifically in the United States. Different criteria exist to classify vegetables. According to Yamaguchi (1983) there are eight different ways of classifying vegetables. They can be classified by (1) botanical information, (2) optimum growing temperatures, (3) relative resistance to frost or low temperatures, (4) part of the plant used for food, (5) number of seasons the plant may live, (6) storage temperature and storage life, (7) optimum soil conditions, and (8) water requirements.

From these classification schemes, the most exact is the classification by botanical information because it combines plants of similar characteristics according to flower type and structure. This can be of use to horticulturists because plants of similar characteristics will most likely be affected in the same way by the growing environment (Yamaguchi, 1983). Classifying vegetables by botanical information is also universal and can be used in any part of the world regardless of language. For the purposes of this dissertation, where leafy vegetables will be the basis of our work, we will use the classification based on the edible part, which is detailed below (Table 2).

Yamaguchi (1983) also groups the different vegetables according to their source of nutrients. The author classifies vegetables into (1) high in carbohydrates (including potatoes, cassava, dry beans, taro, and yam); (2) high in oils (legume seeds and mature vegetable seeds); (3) high in proteins and amino acids (legumes, sweet corn, and most leafy vegetables); (4) high in vitamin A value (carrot, sweet potato, cucurbits, peppers, green leafy vegetables, green beans, and green peas); (5) high in vitamin C (crucifers, peppers, tomato, melon, seeds at immature stages, bean sprouts, freshly harvested white potatoes, and most leafy vegetables) and (6) high in minerals (including most leafy vegetables such as crucifers and root crops which are particularly rich in minerals).

It is then important to notice that when specifically referring to green leafy vegetables, we are referring to products rich in proteins, amino acids, vitamin A, vitamin C, and minerals. This information is corroborated by some studies. Ejoh *et al*. (2007) investigated the nutritional composition of some non-conventional leafy vegetables consumed in Cameroon. The plants investigated were *Vernonia calvoana* var. bitter, *V. amygdalina, V. colorata*, and *V. calvoana* var. non-bitter. According to the authors, these plants are resistant to drought, have bitter taste, and are a good nutritional complement in the diets of Cameroon's families.

**Table 1.2. Classification of vegetables based on edible part**

| Type | Vegetables |
|---|---|
| Root | a. Enlarged taproot<br>Beet, carrot, radish, salsify, rutabaga, turnip, parsnip, and celeriac.<br>b. Enlarged lateral root<br>Sweet potato, winged bean, cassava, and arracacha. |
| Stem | a. Above ground, not starchy<br>Asparagus, celtuce, and kohlrabi.<br>b. Below ground, starchy<br>White or Irish potato, yam, Jerusalem artichoke, and taro. |
| Leaf | a. Onion group, leaf bases eaten (except chive)<br>Onion, leek, shallot, garlic, and chive.<br>b. Broad-leaved plants<br>*1. Salad use*<br>Lettuce, cabbage, chicory, Chinese cabbage, celery (petiole only), and endive.<br>*2. Cooked (may include tender stem in some)*<br>Spinach, chard, New Zealand spinach, Jew's mallow, dandelion, rhubarb (petiole only), kale, edible amaranth, chicory, Chinese cabbage, mustard, and cardoon (petiole only). |
| Immature flower bud | Cauliflower, broccoli, broccoli raab, and artichoke. |
| Fruit | a. Immature<br>Pea, snap bean, lima bean, broad bean, chayote, summer squash, cucumber, zucca melon, okra, sweet corn, and eggplant.<br>b. Mature<br>*1. Gourd family (cucurbits)*<br>Pumpkin, winter squash, muskmelon, Chinese wax gourd, and watermelon.<br>*2. Potato family*<br>Tomato, pepper, pepino, and husk tomato. |

Source: Yamaguchi, 1983.

The authors found that these plants are good sources of proteins, carotenoids, vitamin C, and minerals such as iron, calcium, phosphorus, potassium, magnesium, and zinc. The authors also mentioned the non-bitter species (*V. colorata*, and *V. calvoana* var. non-bitter) as particularly good sources of dietary fiber and minerals such as iron, potassium, and magnesium. The *Vernonia amygdalina* variety, which may be the plant most used for food and medical purposes in tropical Africa, has also shown an important fatty acid content and antioxidant activity (Erasto *et al*., 2007). Oduro *et al*. (2008) conducted a study on *Moringa oleifera* and seven varieties of sweet potato leaves (*Ipomoea batatas*). These are leafy vegetables with an important nutritional content and are highly consumed in some poor countries, especially in Africa. The authors found that both species are very nutritious and high in proteins, fiber, and minerals. However, the *M. oleifera* species showed higher levels of calcium, iron, and proteins compared to the sweet potato leaves varieties.

It also has been suggested that green leafy vegetables should be included in the diet to overcome iron and vitamin A deficiencies (Singh *et al*., 2001). Those authors investigated the nutrient composition of green leafy vegetables and herbs such as spinach, amaranth, Bengal gram, cauliflower, mint, coriander, and carrots. They found that these vegetables are good sources of ascorbic acid, β-carotene, and iron. From the vegetables evaluated in this study, Bengal gram leaves showed higher contents of all three nutrients compared to the other vegetables evaluated. A study by Gupta and Wagle (1988) highlights the importance of green leafy vegetables as good sources of minerals. They evaluated chickpea, chenopodium, spinach, mustard, and cauliflower and investigated their content of different minerals such as copper, iron, zinc, manganese, sodium, potassium, magnesium, calcium, and phosphorus. Chickpea had the highest amount of iron, copper, manganese, and calcium, while mustard had the highest amount of phosphorus. Spinach, on the other hand, had the lowest content of copper, calcium, and phosphorus and the highest content of zinc and sodium compared to the other vegetables evaluated by these authors.

Vegetable crops also contain certain phytochemicals used by the plant as defense mechanisms against predators, pests, and environmental stress that may provide some negative characteristics to the plant (Table 3). These chemicals are also known as anti-nutritional factors (ANF) (Enneking and Wink, 2000). Gupta and Wagle (1988) studied anti-nutritional factors such as nitrate, saponin, oxalate, and trypsin inhibitors in chickpea, chenopodium, spinach, mustard, and cauliflower. They found that spinach was highest for nitrate, saponin, and oxalate, while cauliflower was higher for the trypsin inhibitor. Ejoh *et al*. (2007) also found oxalic acids, certain polyphenols, and saponins when evaluating leafy vegetables consumed in Cameroon. Some of these anti-nutritional factors will decrease the bioavailability of important nutrients by increasing complexity of compounds and decreasing digestibility (Enneking and Wink, 2000).

The contents of the different nutrients in plants as well as phytochemicals will vary according to genotype, pest incidence, soil condition, fertilization, irrigation, pesticide application, season, location, climatic conditions, time of harvest, and storage conditions (Zhao *et al*., 2006).

**Table 1.3. Categories of anti-nutritional factors (ANF) and their effect on consumers**

| Categories | Compounds |
|---|---|
| Toxic | Lectins<br>Cyanogenic glycosides<br>Non-protein Amino acids<br>Alkaloids |
| Unpalatable | Saposins<br>Tannins<br>Non-protein Amino acids<br>Bitter alkaloids |
| "Anti-nutritive" (Reducing fitness or growth of consumer by nutrient complexation) | Phytates |
| Metabolic inhibition | Non-protein Amino acids<br>Cyanogenic glycosides<br>Isoflavones<br>Alkaloids |
| Reduction of digestion | Protease inhibitors<br>Lectins<br>Oligosaccharides |

Source: Enneking and Wink, 2000

## Leafy Vegetables Consumed in the United States

The following is a review of different leafy vegetables that are commonly consumed in the United States.

### *Arugula (Eruca sativa)*

Also known as Rocket salad, Roquette, Gargeer, White pepper, or Koka, this plant has its origin in southern Europe and western Asia. This is a cool season crop, because growing it on warm temperatures will cause its leaves to be bitter and pungent (Yamaguchi, 1983). This plant belongs to the Cruciferae family and is a close relative of the mustards and has a characteristic spicy flavor (Lovelock, 1972). Because of its strong and spicy flavor, arugula may be rejected by consumers if consumed alone in comparison to other green fresh vegetables (Zhao *et al*., 2007a). This crop is mostly consumed in salad combinations with other green leafy vegetables. This crop is a good source of protein, thiamin, riboflavin, vitamin B6, pantothenic acid, zinc and copper. It is also a very good source of dietary fiber, vitamin A, vitamin C, vitamin K, folate, calcium, iron, magnesium, phosphorus, potassium and manganese (Nutrition Data, 2008).

### *Beet (Beta vulgaris)*

Table beets are believed to descend from sea beets (*Beta maritima*) native to southern Europe and transported to northern Europe by invading armies spreading its consumption throughout Europe, the Middle East, and India (Yamaguchi, 1983). The red-violet color of the root and the petioles are betacyanins, pigments with similar chemical properties to anthocyanins (specifically betanin, isobetanin, betanidin, and isobetanidin) and the yellow pigments betaxanthins (specifically vulgaxanthin I and vulgaxanthin II). The content ratios between the two pigment classes vary among varieties and are beneficial to human health because of their antioxidant capacity as well as valuable for the production of food colorants (Nagy Gasztonyi *et al*., 2001). This crop belongs to the Chenopodiaceae family and has a characteristic earthy flavor which is thought to be caused by trans-1,10-dimethyl-trans-9-decalol, also know as geosmin. This is an organic compound which is believed to be synthesized by the beet root itself (Lu *et al*., 2003). The roots are commonly used in salads and the preparation of pickles and chutney while the leaves are mostly used as cooked vegetables (Tindall, 1983). Beets are a good source of vitamin C, iron, and magnesium, and a very good source of dietary fiber, folate, potassium and manganese. In addition, beet greens are good sources of pantothenic acid, phosphorus, zinc, β-carotene, vitamin E (alpha tocopherol), vitamin K, thiamin, riboflavin, and Vitamin B6. As negative points, it is indicated that much of the caloric content of the beet roots come from sugars and that the leaves have a high content of sodium (Nutrition Data, 2008).

### *Swiss Chard (Beta vulgaris* **var.** *cicla)*

Swiss chard and beet greens are very similar, when cooked, in appearance, flavor and nutritional value. They both belong to the Chenopodiaceae family and both are *Beta vulgaris*. However, Swiss chard belongs to the Cicla group which is a leafier form than the beets with prominent enlarged midribs (Peirce, 1987). A serving of Swiss chard provides 87% of the average adult daily requirements of vitamin A and 25% of the required vitamin C. Also, as related to spinach and beet greens, Swiss chard is high in minerals (Peirce, 1987). See table 1.5 for details on the nutritional content of Swiss chard. It also has been found that Swiss chard is an important dietary source of phenolic antioxidants. The major phenolic acid and flavonoid found in Swiss chard leaves are syringic acid and kaempferol (Pyo *et al*., 2004).

Because Swiss chard is considered a very perishable product, many studies have investigated the effect of different storage conditions on the quality of Swiss chard. It has been shown that refrigeration and high relative humidity are required to minimize weight, water, and chlorophyll losses as well as for maintaining sensory properties (Roura *et al*., 2000). Furthermore, other factors such as organic fertilization have shown positive effects on sensory characteristics such turgidity, color and brightness of the leaves after storage. However, these results were not conclusive (Moreira *et al*., 2003). Colored varieties of Swiss chard are also being investigated for their betalain content and its potential as food coloring (Kugler *et al*., 2004). This study reported nineteen betaxanthins and nine betacyanins found in different varieties of colored Swiss chard. Swiss chard is also a very good source of vitamin E (alpha tocopherol), vitamin K, riboflavin, vitamin B6, calcium, iron, magnesium, phosphorus, potassium, copper, manganese, and sodium (Nutrition Data, 2008).

### *Spinach (Spinacia oleracea)*

Spinach is considered the most important of the greens in the United States. It originated in Iran but was not introduced in the new world until the colonial era (Peirce, 1987). Spinach can be consumed cooked or raw. It is high in minerals and vitamins, particularly β-carotene, calcium, phosphorus, iron, and potassium, and contains moderate amounts of protein (Peirce, 1987). See table 1.5 for the nutritional content of spinach among other vegetables consumed in the United States. Spinach also has some anti-nutritional properties. For example, calcium in spinach is not as readily available for human consumption as in other products such as milk probably due to the presence of oxalic acid (Heaney *et al.*, 1988). Oxalic acid, a normal constituent in leafy vegetables, has also shown to negatively affect the absorption of zinc and magnesium but not the absorption of iron (Storcksdieck genannt Bonsmann *et al.*, 2008). On the positive side, spinach has also shown to have high levels of carotenoids (Mueller, 1997), L-ascorbic acid (Davey *et al.*, 2000), and flavonoids (Cho *et al.*, 2008) which have shown an inverse relationship with chronic diseases such as cardiovascular diseases or certain types of cancer. The main carotenoids present in spinach are lutein, violaxanthin, β-carotene, and neoxanthin. The content of these components in spinach is dependant upon stage of development and storage conditions (Bergquist *et al.*, 2006). Furthermore, the glycolipid fraction from spinach has been shown to have potential in the prevention of cancer which would make spinach a functional food with "anti-cancer activity" (Maeda *et al.*, 2007). The most important world producers of spinach are China, United States, and Japan (FAO, 2005).

### ***Cabbage (Brassica oleracea* var. *capitata)***

Cabbage is part of the cole group which also includes cauliflower, broccoli, brussel sprouts, kohlrabi, and curly kale. It is thought that the cabbages and kales originated in Western Europe while the cauliflower and broccoli came from the Mediterranean region (Yamaguchi, 1983). All Cole crops are high in vitamin C. Even though cabbage has a high nutritional value, it is not particularly high in vitamins and minerals compared to other cole crops such as broccoli or Brussels sprouts (Table 1.4). However, because it is consumed in relatively large amounts, it does serve the necessary adult daily requirements (Peirce, 1987). White and Portuguese cabbages have shown higher content of soluble sugars content compared to broccoli (Rosa *et al.*, 2001). On the other hand, because leaves and stems can accumulate a large amount of nitrates, leafy vegetables such as cabbage are found to be major sources of nitrate intake by humans (Wang *et al.*, 2008) which at high concentrations may pose a risk for human health (Boink and Speijers, 2001).

**Table 1.4. Nutritional comparison among Cole crops and cabbage cultivar types** [a]

| Crop | Water (%) | Vitamin A (IU)[b] | Vitamin C (mg)[c] | Ca (mg) |
|---|---|---|---|---|
| Broccoli | 89.0 | 2,500 | 113 | 103 |
| Brussels Sprouts | 85.0 | 550 | 102 | 36 |
| Green Cabbage | 92.4 | 130 | 47 | 49 |
| Red Cabbage | 90.2 | 40 | 61 | 42 |
| Savoy Cabbage | 92.0 | 200 | 55 | 67 |
| Napa Cabbage | 95.0 | 150 | 25 | 43 |

Source: Peirce, 1987

[a] data per 100g sample

[b] 1 IU = 0.03 µg vitamin A alcohol

[c] Ascorbic acid.

Sensory quality of cabbage can be affected by many production variables such as irrigation or production time (Radovich, *et al*., 2004; Rosa *et al*., 2001). For example, it has been shown that panelists could perceive a difference among cabbage samples irrigated at different times during the development process (Radovich, *et al*., 2004). Cabbage has a very distinctive taste reminiscent of hard boiled eggs due to the presence of many sulfur compounds (Bailey *et al*., 1961).

In the year 2005, China was the largest world producer of cabbage, followed by India and Russia. The same year, the United States was the sixth largest world producer accounting for 2.2 million MT (Food and Agricultural Organization of the United Nations [FAO], 2005).

### *Collards and Kale (Brassica oleracea* var. *acephala)*

Native to the eastern Mediterranean region of Europe, collards and kale are the oldest forms of cabbage. Collards are very popular in the southern diet of the United States whereas Kale's consumption is more widespread (Peirce, 1987). Even though they both belong to the same taxonomic group they are very different in appearance and nutritional value. Collard greens leaves are broad and flat while Kale leaves have abundant curls. Both crops have a very good nutritional value (Table 1.5). However, kale is nutritionally superior to most vegetables in protein, vitamin, and mineral content (Peirce, 1987). Furthermore, kale has been found to be a very good dietary source of phenolic antioxidants and its phytochemicals can be potentially used for pharmaceutical research (Ayaz *et al*., 2008). Collard greens are also an important source of phenolic compounds as found by Huang *et al*. (2007). Those authors conducted research on both kale and collard greens and suggested that as good sources of phenolic compounds, these vegetables can be of use in the prevention of cardiovascular and other chronic diseases. Flavonoids such as isorhamnetin, quercetin, and kaempferol were found in kale. Collard greens also contained these flavonoid with the exception of Isorhamnetin. Ferulic acid was another phenolic compound common in both collard and kale (Huang *et al.*, 2007). In addition to these constituents, both kale and collards are a very good source of vitamin K (Nutrition Data, 2008). As green leafy vegetables, collard and kale are a good source of phylloquinone (Vitamin $K_1$), which is an important nutrient involved in the regulation of blood coagulation (Booth *et al.*, 1995).

**Table 1.5. Nutritional comparison among green leafy vegetables** [a]

| Crop | Water (%) | Protein (g) | Vitamin A (IU)[b] | Vitamin C (mg)[c] | Ca (mg) |
|---|---|---|---|---|---|
| Spinach | 91 | 3.2 | 8,100 | 51 | 93 |
| Kale | 83 | 6.0 | 10,000 | 186 | 249 |
| Collards | 85 | 4.8 | 9,300 | 152 | 250 |
| Mustard | 90 | 3.0 | 7,000 | 97 | 183 |
| Chard | 91 | 2.4 | 6,500 | 32 | 88 |
| Turnip greens | 90 | 3.0 | 7,600 | 139 | 246 |
| Beet greens | 91 | 2.2 | 6,100 | 30 | 119 |

Source: Peirce, 1987

[a] data per 100g sample

[b] 1 IU = 0.03 μg vitamin A alcohol

[c] Ascorbic acid.

### *Mustard Greens (Brassica juncea)*

Native to central Asia and the Himalayas, different varieties of mustard (Table 1.6) are used in many parts of the world as spice, flavoring, oilseed, fodder, and greens or salads (Peirce, 1987). Mustard greens (Brassica juncea) are cultivated in eastern Europe, Malaysia, India, Indonesia, China, and Africa (Sierra Leone and Guinea) and are one of the most pungent of the mustards and are very important for oilseed production (Tindall, 1983). In the United States, mustard greens are mostly concentrated in Texas, California, Florida, Georgia, Mississippi, Tennessee, Arkansas, and Alabama (Peirce, 1987).

**Table 1.6. Varieties and characteristics of Mustard greens**

| Variety | Taxonomic group | Characteristics |
|---|---|---|
| White Mustard | *Sinapsis alba* | Pungent |
| Brown Mustard | *Brassica juncea* | Pungent, important for oilseed crops, and mostly used as greens |
| Black Mustard | *Brassica nigra* | Pungent, important for oilseed crops |
| Ethiopian Mustard | *Brassica carinata* | Important for oilseed crops and resistant to extreme environmental conditions (Rakow and Getinet, 1998) |

Source: Peirce, 1987

Mustard greens, as well as all plants from the *Brassica* family, are also known for having a high concentration of glucosinolates, which are a group of more than a 100 sulfur compounds. These sulfur compounds are normally stable inside the plant cells. However, if tissue damage occurs, sulfur compounds are released and hydrolyzed by plant myrosinase. The breakdown products of glucosinolates are responsible for the “hot and spicy” flavors of mustard, radishes, and other plants from the *Brassica* family (Johnson, 2001). At the same time, breakdown products of glucosinolates, such as nitriles and isothyocianates, have shown positive effects against lung and gastrointestinal cancer (Johnson, 2001). Processing factors such as storage and cooking may alter the concentration of both glucosinolates and myrosinase enzyme and alter the positive effects that these phytochemicals have on human health (Rungapamestry *et al*., 2007). Among the different varieties of Chinese *Brassica* vegetables, collards have the highest content of glucosinolates followed by mustard, kale, and pac choi (He *et al*., 2003). Table 1.7 shows the glucosinolate compounds present in different varieties of Chinese *Brassica* vegetables. Additionally, mustard greens are a very good source of dietary fiber, provitamin A, vitamin C, vitamin E, vitamin K, thiamin, riboflavin, vitamin B6, folate, calcium, iron, magnesium, potassium, copper, and manganese (Nutrition Data, 2008).

**Table 1.7. Individual glucosinolate contents (µmol·100 g$^{-1}$ FW) in Chinese *Brassica* vegetables**

| **Glucosinolate** | **Pac Choi** | **Mustard** | **Kale** | **Collard** |
|---|---|---|---|---|
| 3-methylsulfinylpropyl-GS | --- | --- | 96.69 | --- |
| 2-propenyl-GS | --- | 423.16 | 53.30 | 146.12 |
| 2-hydroxy-3-butenyl-GS | 2.18 | --- | 6.77 | 314.16 |
| 4-methylsulfinylbutyl-GS | --- | --- | 2.17 | 2.86 |
| 3-butenyl-GS | 24.36 | 21.64 | --- | 69.11 |
| 4-pentenyl-GS | 13.26 | --- | --- | --- |
| 2-phenylethyl | 8.32 | 3.23 | --- | --- |
| 4-hydroxy-3-indolylmethyl-GS | 0.46 | 2.86 | 2.43 | 0.89 |
| 3-indolylmethyl-GS | 13.35 | 6.02 | 210.52 | 166.61 |
| 4-methoxy-3-indolylmethyl-GS | 2.17 | 1.67 | 7.97 | 6.68 |
| 1-methoxy-3-indolylmethyl-GS | 20.64 | 19.69 | 49.57 | 24.19 |

*GS: glucosinolates
Source: He *et al*., 2003

### *Pac Choi (Brassica rapa* Chinensis group*)*

Chinese cabbage probably originated in Asia and it was not introduced in the United States until late nineteenth century. It is grown throughout the year in California, Florida, and Hawaii and in the spring and fall seasons in New Jersey. Pac Choi is the non heading form of Chinese cabbage and is characterized by dark green leaves and white petioles (Peirce, 1987). There are two main varieties of Pac Choi. A green-leafed, white stemmed pac choi or “Joi Choi” and a green-stemmed pac choi which includes many cultivars such as “Chinese Pac Choi,” “Mei Qing Choi,” “Shanghai Pak Choi,” and Tatsoi (Tah Tsai)” (Creasy, 2000). From these sub varieties, Chinese Pac Choi has the most intense taste followed by Mei Quing Choi, and Joi Choi has the least taste intensity (Schnitzler and Kallabis-Rippel, 1998). The leaves can be cooked or eaten raw in salads. It also has been shown that the process of cooking causes changes in the taste going from sweet, sour, bitter, and spicy in the raw form to sweet and cabbage-like in the cooked form (Schnitzler and Kallabis-Rippel, 1998). The flavor change is caused by the action of heat on the enzyme myrosinase and the altered availability of glucosinolates and breakdown sulfur products, which are highly available in plants from the *Brassica* family (Rungapamestry *et al*., 2007). The leafy cultivars have twice the nutritional value of white cabbage. However, the heading forms (also known as pe tsai) have lower nutritional value compared to the leafy forms (Tindall, 1983). Table 1.8 shows the nutritional differences between the heading form of Chinese cabbage or pe tsai and the leafy form (pac choi).

**Table 1.8. Nutritional comparison between two varieties of Chinese cabbage**

| Crop | Water (%) | Protein (g) | Vitamin A (IU) | Vitamin C (mg) | Ca (mg) |
|---|---|---|---|---|---|
| Pac choi | 95 | 1.5 | 3,000 | 45 | 105 |
| Pe-tsai | 91 | 1.2 | 1,200 | 27 | 92 |

Source: Yamaguchi, 1983

Pac choi, as part of the Brassica family, is also an important source of polyphenols. A study detected eleven flavonoid derivatives and seventeen hydroxycinnamic acid derivatives in pac choi samples of the communis variety (Harbaum *et al*., 2007). Those authors found that the main flavonoids present in pac choi are kaempferol and isorhamnetin. Polyphenols can be beneficial to human health against coronary disease and certain types of cancer (Harbaum *et al*., 2008). The stability of these compounds during storage has also been of interest for many studies. Kevers *et al*. (2007) found that phenolic content is relatively stable in a selected group of fruits and vegetables during storage and that the sample is more likely to spoil before any significant change in antioxidant capacity occurs. Leafy greens are perishable mostly due to mechanical damage during post harvest handling (Mahmud *et al*., 1999). Some of the most important defects are yellowing (Lu, 2007) caused by degradation of the chlorophyll, and water-loss, which is increased by the high surface area to volume ratio (Burton, 1982). These factors are related to each other and are increased when not refrigerated (Lazan *et al*., 1987). Able *et al*. (2005) estimated that the shelf life of detached pac choi leaves is ~27 days when stored at 2°C, ~8 days when stored at 10°C, and ~3 days when stored at 20°C. End of shelf life was estimated by measuring yellowing, rot, and damage.

The taste of Pac Choi has been previously studied by Scnitzler and Kallabis-Rippel (1998) in which plants of different varieties were evaluated by a trained sensory panel both cooked and fresh. Terms used by these authors were sweet, sour, bitter, spicy, and cabbage-like. Other studies focused on instrumental analysis of Pac Choi leaves to evaluate flavonoid composition (Rochfort *et al*., 2006), phenolic content in organic plants (Young *et al*., 2005), and the effect of packaging on their shelf life (Lu, 2007).

### *Turnip greens (Brassica campestris)*

Turnip greens originated in central and southern Europe, probably in the Mediterranean area, and are now distributed throughout the tropics (Tindall, 1983). There are many varieties of turnips which are cultivated for their roots. These varieties are Nozawana, Scarlet Ball, Shogoin, and Tokio Cross. From these varieties, Nozawana is breed also for their greens which can be cooked, pickled or used fresh in salads (Creasy, 2000). As a member of the *Brassica* family, this plant also has a high concentration of glucosinolates. These are phytochemicals that when broken down by enzyme myrosinase produce sulfur compounds which have been associated with reducing the risk of cancer (Rungapamestry *et al*., 2007). Glucosinolate degradation products specifically related to turnip greens are benzene acetonitrile, benzene propane nitrile, 1H-indole-3-acetonitrile, and benzene ethyl isothiocyanate. It has been shown that the concentration of these compounds vary according to variety and maturity. The concentration increases as the plant matures (Jones *et al*., 2007). Other than the health benefits, isothiocyanates (the breakdown products of glucosinolates) provide turnip greens, and all vegetables from the *Brassica* family with unique flavor characteristics such as sulfurous aroma, pungent flavor and bitter taste (Jones and Sanders, 2002). Those researchers suggest that a mustard green aroma and taste, bitterness, and bitter aftertaste are specific attributes that vary across varieties and maturity of turnip greens. The authors also suggest that liking of turnip greens decreases as the plant gets older, probably linked to the increasing bitterness of the sample, which at the same time, is linked with the increased concentration of phytochemicals (Jones *et al*., 2007). Turnip greens are also a good source of provitamin A, vitamin C, vitamin E, vitamin K, vitamin B6, folate, calcium, magnesium, potassium, copper, and manganese (Nutrition data, 2008). See Table 1.5 for a comparison of nutritional characteristics between turnip greens and other green leafy vegetables.

### *Watercress (Nasturtium officinale)*

Watercress is known to be one of the oldest leaf vegetables consumed by humans. Records exist of watercress being used as a medicinal plant even in the times of Christ. However, it has been cultivated for only 200 years as before it was obtained from the wilds only (Yamaguchi, 1983). The young shoots and the leaves are normally eaten raw in salads and used as garnishing but they are also cooked in South East Asia and added to soups (Tindall, 1983). Watercress belongs to the Cruciferae taxonomic group, and is a very good source of provitamin A, vitamin C, vitamin E, vitamin K, thiamin, riboflavin, vitamin B6, calcium, magnesium, phosphorus, potassium and manganese (Nutrition Data, 2008). Also, watercress is known to be rich in isothiocyanates (the product of glucosinolates hydrolysis) and is believed to be an effective agent in reducing cancer risk in humans by reducing lymphocyte DNA damage (Boyd *et al*., 2006; Gill *et al*., 2007). In the same way, these compounds are known to inhibit Phase I enzymes, which are responsible for activating many carcinogens in animals, and induce phase II enzymes, which are associated with excretion of carcinogens (Rose *et al*., 2000).

Many production variables have been found to have an effect on the concentration of phytochemicals in watercress. For example, It has been found that the concentration of these compounds can be manipulated by varying the photoperiod, temperature, and light quality of the light source therefore increase the health benefits and overall quality of watercress (Engelen-Eigels *et al*., 2006). Stage of harvest also has an effect on the concentration of nutritive components in watercress. Palaniswamy *et al*. (2003) evaluated the concentration of PEITC (Phenethyl isothiocyante, a secondary metabolite with anti-cancer properties product of the hydrolysis of gluconasturtiin), and vitamin C (ascorbic acid) on watercress leaves at different stages of maturity. Those authors found that PEITC increased almost 3 times at 60 days after transplant while ascorbic acid had a peak concentration at 40 days to decrease at 60 days after transplant to an amount of almost half compared to the control samples (at 0 days after transplant). Finally, fertilization has also shown to have an effect on the concentration of chemicals in watercress. Kopsell *et al*. (2007) found that the variation of concentration of both nitrogen (N) and sulfur (S) during fertilization altered the final amount of several phytochemicals present in watercress leaves such as gluconapin, glucobrassicin, 4-methoxyglucobrassicin, and gluconasturiin as well as carotenoid and chlorophyll pigment accumulation.

### *Endive (Cichorium endivia)*

Cultivated in Egypt over 2000 years ago, Endive has spread around the world and today it is cultivated mainly in the Caribbean area, the Philippines, Central and West Africa (Tindall, 1983). Endive belongs to the Compositae family, the same as lettuce, and is very popular in Europe and is increasing in popularity in the United States.

There are two types of endive known as endive and chicory. Both types differ from each other in both appearance and use (Table 1.9), and nutritional content (Table 1.10). Endives also contain moderate levels of certain minerals such as phosphorous, potassium, magnesium, iron, zinc, cooper, and manganese (Peirce, 1987; Nutrition Data, 2008).

Endives have been researched for their flavonoid content; a compound with protective benefits against certain chronic diseases especially due to their antioxidant capabilities (DuPont *et al*., 2000).

**Table 1.9. Appearance and use of the two cultivars of Endive**

| **Type** | **Varieties** | **Characteristics** |
|---|---|---|
| **I.** Endive | Escarole | **A.** Leaves broad, coarse, and crumbled; plant medium large, deep-hearted; inner leaves well blanched. |
| | Curly Endive | **B.** Narrow leaf; leaf margins curled and deeply cut; plant broad in diameter, with creamy inner leaves. |
| **II.** Chicory | | **A.** Leaves used as a salad or green, or for forcing. |
| | Radichetta | **1.** Green type: leaves dark green, narrow and Notched; petiole and leaf harvested for potherb. |
| | Witloof, Belgian endive | **2.** Forcing type: resembles Cos lettuce, but smaller; leaves, narrow on broad stalks; may be used for Salads; roots are enlarged as in Magdebourg chicory; forcing produces tight, blanched heads. |
| | Magdebourg chicory | **B.** Roots enlarged, may be ground for use as coffee adulterant or substitute; leaves resemble dandelion and can be used in salad. |

Source: Peirce, 1987

**Table 1.10. Nutritional comparison between endive cultivars and lettuce[a]**

| Crop | Water (%) | Protein (g) | Vitamin A (IU)[b] | Vitamin C (mg)[c] | Ca (mg) |
|---|---|---|---|---|---|
| Witloof Chicory | 95 | 1.0 | Trace | -- | 18 |
| Endive Curly | 93 | 1.7 | 3,300 | 10 | 81 |
| Endive Escarole | na | na | 14,000 | 100 | na |
| Lettuce[d] | 96 | 0.9 | 330 | 6 | 20 |

Source: Peirce, 1987
[a] data per 100g sample
[b] 1 IU = 0.03 μg vitamin A alcohol
[c] Ascorbic acid.
[d] Crisphead type
na = Not available

Regarding the flavonoid content, it is important to know that endive and lettuce do not have as high a flavonoid concentration compared to products such as onion, broccoli or green beans. However, because endive and lettuce are consumed in such large amounts, their role in providing these nutrients becomes significant (DuPont *et al*., 2000). Specifically, endive is considered a dietary source of the flavonoid kaempferol-3-glucuronide and its absorption through the digestive tract has been studied (DuPont *et al*., 2004). Those researchers found that kaempferol-3-glucuronide from endive soup was absorbed at the latter part of the small intestine.

Lastly, the volatile constituents of endive have also been studied by Götz-Schmidt and Schreier (1986). Those authors identified 119 volatiles and found that $C_6$ aldehydes comprised about 70% of the total amount of volatiles found in endive.

Radicchio (*Chicorium intybus*) is an Italian leafy vegetable related to chicory. It has a rich maroon color and a characteristic peppery flavor and bitter taste. Both radicchio and endive have shown an important concentration of phenolic compounds even at prolonged stages of shelf life (Di Venere *et al*., 2005).

### ***Lettuce (Lactuca sativa)***

Lettuce is native to Europe and Asia. It is not especially rich in vitamins and minerals (ranks 26th among major vegetables) but because it is consumed in large amounts it actually ranks as the 4th most important crop nutritionally (Peirce, 1987). Among the different types of lettuce, Romaine and leaf are the ones with higher amounts of vitamin A and C (Table 1.11) (Peirce, 1987).

**Table 1.11. Nutritional comparison among four varieties of lettuce[a]**

| Crop | Vitamin A (IU)[b] | Vitamin C (mg)[c] | Ca (mg) |
|---|---|---|---|
| Crisphead (Iceberg) | 330 | 6 | 20 |
| Butterhead (Boston) | 970 | 8 | 35 |
| Romaine (Cos) | 1,900 | 18 | 68 |
| Leaf | 1,900 | 18 | 68 |

Source: Peirce, 1987

[a] data per 100g sample

[b] 1 IU = 0.03 μg vitamin A alcohol

[c] Ascorbic acid.

As with other leafy vegetables, lettuce is also an important source of antioxidant compounds which offer health benefits against chronic diseases such as cancer (Nicolle *et al*., 2004). Other than vitamin C, polyphenols (such as flavonols and anthocyanins) increase the antioxidant activity in lettuce (Llorach *et al*., 2008). Caffeic acid derivatives are the main polyphenols in green varieties of lettuce while flavonols were found mostly in red varieties and escarole (Llorach *et al*., 2008). The same study reports that anthocyanins were only found in red varieties of lettuce. Overall, red varieties showed higher concentrations of flavonols, caffeic acid, and vitamin C compared to green varieties of lettuce which translates in a higher antioxidant activity for the red-leaved varieties (Llorach *et al*., 2008; Liu *et al*., 2007). Flavonoid content has also been found to vary among different varieties of lettuce. Specifically, the content of conjugated quercetin has been found to differ among varieties of lettuce grown in the United Kingdom (Crozier *et al*., 1997). The content of carotenoids and vitamin E also have been found to influence positively the antioxidant capacity of lettuce of different varieties increasing their health properties (Nicolle *et al*., 2004). Both the hydrophilic and lipophilic sources of antioxidant activity in lettuce vary depending on the part of the plant consumed (Table 1.12) (Cano and Arnao, 2005).

**Table 1.12. Antioxidant activity in different sections of three types of lettuce**

| Plant Section | Iceberg | | Romaine | | Baby head | |
|---|---|---|---|---|---|---|
| | HAA[a] | LAA | HAA | LAA | HAA | LAA |
| Stem | 7.3ab[b] | 1.4a | 60.3a | 1.7a | 4.9a | 1.5a |
| Inner Leaf | 8.8b | 1.5a | 111.1c | 3.6a | 9.0b | 2.4b |
| Medium Leaf | 5.6a | 3.3b | 73.6b | 9.6b | -- | -- |
| Exterior Leaf | 6.7a | 5.5c | 129.7d | 15.4c | 4.8[a] | 3.1c |

Source: Cano and Arnao, 2005

[a] Values are mg Trolox equivalent / 100gr of fresh weight (HAA = Hydrophilic LAA = Lipophilic).

[b] If different letters, values are significantly different at the 0.05 level

In 2005, China and the United States were the largest producers of lettuce in the world, followed by Spain, Italy, and India (FAO, 2005).

### *Cilantro (Coriandrum sativum* L.*)*

Cilantro or Chinese parsley is the leaf portion of an annual herb that also produces a fruit known as coriander which is a very popular spice around the world as well. Coriander has a very different aroma compared to cilantro. This culinary herb is native to the eastern Mediterranean region and was introduced through cultivation to other regions of the world where is very popular today. Cilantro is used predominantly in places such as India, Thailand, China, Mexico, and South America (Teuscher, 2006). Cilantro leaves have a very distinctive soapy taste and pungent aroma caused by essential oils and volatile aromatics such as alkanals, 2-alkenals, 2-alkenols, aliphatic aldehydes, alcohols, and nonane (Potter and Fagerson, 1990). However, it has been shown that the chemical profile of the plant changes according to its age and that quality and aroma decreases as the plant gets older (Potter, 1996; Kohara *et al*., 2006). Cilantro is mostly used as a fresh product because its characteristic aroma decreases quickly when they are cooked (Creasy, 2000). In the same way, this plant has a very limited shelf life because its characteristic aroma fades rather fast after its essential oils have been exposed to air (Creasy, 2000). The essential oils of coriander are used by the spirits industry as a flavor component of aperitifs and liqueurs and by the perfume industry as a raw material for the production of linalool (Teuscher, 2006). Cilantro is a very good source of dietary fiber, vitamin A and vitamin C (Nutrition Data, 2008).

### *Parsley (Petroselinum crispum)*

Parsley originated in Southwestern Europe and Western Asia. It has been known in this area for about 2,000 years were it was cultivated by Romans and Greeks mostly as a medicinal plant and for religious purposes (Teuscher, 2006). In the United States, it is produced commercially mostly in Texas, California, New Jersey, Florida, and New York. Parsley can be used as both a salad ingredient and for flavoring and garnishing (Peirce, 1987). Parsley is also known for its diuretic action and for aiding digestion by promoting secretion of bile and gastric juices (Teuscher, 2006). Parsley is also an excellent source of vitamin A, vitamin C, protein, and calcium, and has important amounts of potassium, iron, sodium, and phosphorus (Peirce, 1987; Teuscher, 2006).

As with other culinary herbs, parsley is known for having high antioxidant activity and antimicrobial properties that can aid in preventing chronic diseases in humans, and at the same time, can help prevent food spoilage (Wong and Kitts, 2006). These characteristics are inherent to parsley due to the presence of secondary metabolites which at the same time provide parsley with a distinctive pungent aroma when released (Masanetz and Grosch, 1998).

The essential oil of parsley is constituted mainly by the phenyl propane derivatives myristicin and apiol (Teuscher, 2006; Masanetz and Grosch, 1998). The predominating compound, myristicin, has shown to be of potential use as a cancer chemopreventive agent (Zheng *et al*., 1992).

## Shelf Life of Vegetables

Leafy greens are perishable mostly due to mechanical damage during post harvest handling (Mahmud *et al*., 1999). Some of the most important defects are yellowing (Lu, 2007) caused by degradation of the chlorophyll, and water-loss, which is increased by the high surface area to volume ratio (Burton, 1982). These factors are related to each other and are increased when not refrigerated (Lazan *et al*., 1987). Other defects of prolonged shelf life may be the loss of nutrients such as ascorbic acid (Vitamin C), which is accelerated by water loss (Nwufo, 1994) and high temperatures (Table 1.13) (Lazan *et al*., 1987). Other nutrients such as phenolic acids and flavonoids may be affected during storage. However, this variation is highly dependant on the type and variety of the plant (Amarowicz, 2009). For example, DuPont *et al*. (2000) reported that storing endive and lettuce in the dark at 1°C and 98% humidity resulted in 7 – 46% losses of flavonol glycosides after 7 days of storage. Lower temperatures decrease the respiration rate of vegetables, hence increasing the shelf life (Geronimo and Beevers, 1964). Other than refrigerated storage, other techniques to increase shelf life of minimally processed vegetables are washing with chlorinated water and modified atmosphere packaging (Wiley, 1994; Delaquis *et al*., 2000). In addition, innovative techniques such as gamma irradiation of the vegetables and treating the leaves with natural essential oils have been studied as ways of increasing shelf life of vegetables (Prakash *et al*., 2000; Ponce *et al*., 2004).

It also has been suggested that organic farming increases the shelf life of leafy vegetables because it causes the nitrate contents to decrease in the leaf (Rembialkowska, 2007; Bourn and Prescott, 2002). These authors explain that this is likely due to the lower amounts of nitrogen used in organic fertilization which will generate less nitrogen available for the plant to absorb.

**Table 1.13. Effect of storage on vitamin C (ascorbic acid) and total weigh of selected vegetables**

| Vegetable | TAA at harvest | TAA after storage[a] | % Weight loss[b] |
|---|---|---|---|
| Kohlrabi | 60.0 ± 5.68 | 65.4 ± 1.79 | 0.73[c] |
| Collard | 136.0 ± 1.47 | 114.5 ± 6.14 | 2.26[c] |
| Swiss Chard | 30.6 ± 2.32 | 37.5 ± 2.41 | 3.47[c] |
| Kale | 146.5 ± 7.97 | 103.0 ± 1.00 | 2.67 |
| Cabbage | 63.4 ± 3.80 | 59.2 ± 0.60 | 6.06 |
| Squash | 41.0 ± 1.00 | 21.1 ± 1.11 | 8.08 |

Source: McCombs, 1957

[a] TAA = Total Ascorbic acid (mg/100g) after 5 days of storage at 55°C

[b] Vegetables stored in slowly circulating air with relative humidity of 75 – 80% at 55°C for 6 days.

[c] Stored in perfored polyethylene bags at 55°C for 4 days (kohlrabi and Chard) and 6 days (collard).

The shelf life of vegetables has been studied based on two aspects: sensory characteristics (color, flavor, and turgidity) and microbial population count. Some methods may increase shelf life based on one aspect but may not affect the other. For example, a study by Ponce *et al*. (2004) studied the effect of treating leafy vegetables with natural essential oils. Those authors found that essential oils from eucalyptus, tea tree, and clove are successful in controlling the populations of different microbial groups but do not extend shelf life of vegetables from the sensory stand point. Some studies have focused on assessing the shelf life of vegetables based on sensory characteristics. Able *et al*. (2005) estimated that the shelf life of detached pac choi leaves (Chinese cabbage) is ~27 days when stored at 2°C, ~8 days when stored at 10°C, and ~3 days when stored at 20°C. The end of shelf life was estimated by measuring yellowing, rot, and damage. Aranea *et al*. (2008) estimated that after 11 days, lettuce would be rejected by 25% of the consumers and that 50% of the consumers would reject the lettuce after 15 days of storage. Those authors explored the use of survival analysis as an efficient tool to asses the shelf life of different products.

## Factors Affecting the Final Quality of Crops: Organic Production of Fruits and Vegetables

As described by Zhao *et al*. (2006), foods are organic when have been produced by more "environmentally friendly" conditions. Crop rotation, cover crops, and the use of natural products (such as natural fertilizes and pesticides) are used to enhance or maintain long-term soil fertility, to minimize all forms of pollution, to avoid the use of synthetic fertilizers and pesticides, to maintain genetic diversity of the production system, to consider social and economic impact, and to produce high quality products (Winter and Davis, 2006; Bourn and Prescott, 2002). Synthetic substances are prohibited in organic farming, unless the substance is included in the "National List of Allowed and Prohibited Substances." Emphasis is directed to the use of farmyard manures, crop residues, or composts made of animal manure and plant residues to meet plant nutritional needs (Wang *et al*., 2008). Forbidden substances cannot be used in a land intended for organic production at least 3 years before the harvest of an organic crop (Winter and Davis, 2006).

Several studies have been conducted on the effect that organic fertilization can have on chemical, nutritional, and sensory characteristics of crops (Table 1.14). Results at this point seem inconsistent and show no clear trends of the effects that organic fertilization have on the sensory characteristics of crops (Bourn and Prescott, 2002) or nutritional composition (Zhao *et al*., 2006). Some studies suggest that crops produced using organic practices may be potentially richer in phenolic compounds and vitamin C (Zhao *et al*., 2006; Rembiałkowska, 2007), lower in pesticide residues, and lower in nitrate content (Woese *et al*., 1997). However, it has not been shown yet if these differences are biologically significant (Winter and Davis, 2006). It has been suggested that the inconsistencies of results is because the differences between organic and conventional practices are product specific and that this should be looked in a case-by-case basis (Fillion and Arazi, 2002). With no doubt, the study of organic fertilization effects is difficult, and is complicated even further by the large number of factors that also influence the characteristics of crops. These factors include genotype, plant tissue, fruit size, stage of development, ripening, diseases and pests, soil condition, irrigation, and pesticide application (Zhao *et al.*, 2006). This is the reason why well managed and well controlled cultivation tests are viewed by scientists as the most accurate way of studying the effects of organic farming practices because that way, researchers can control many of the factors that may be affecting the crops final characteristics (Woese *et al.*, 1997).

**Table 1.14. Literature related to the effect of organic fertilization on the chemical, nutritional, or sensory characteristics of crops**

| **Product** | **Object Studied** | **Conclusions** | **Reference** |
|---|---|---|---|
| Apples | Flavor and texture | No consistent quality or sensory differences. | DeEll & Prange, 1992 |
| Carrots | Flavor and texture | Conventional system produced carrots were higher in carrot-taste and less bitter than organic. | Haglund *et al.*, 1999 |
| Swiss Chard | Instrumental, sensory, and shelf life | No differences at initial time point. Organic chard outlasted the conventional chard | Moreira *et al.*, 2003 |
| Banana | Flavor | No differences. | Caussiol & Joyce, 2004 |
| Tomato | Vitamin C, carotenoids, and polyphenols. | Organic tomatoes were higher in vitamin C, carotenoids, and ployphenols compared to the conventional tomatoes. | Caris-Veyrat *et al.*, 2004 |
| Lettuce, collards, and pac choi | Phenolics | No differences in lettuce and collards. Phenolics higher in organic pac choi. | Young *et al.*, 2005 |
| Grapes | Polyphenol Oxidase | Higher content in organic grapes | Nuñez-Delicado *et al.*, 2005 |
| Lettuce | Phenolics | No significant differences | Zhao *et al.*, 2007a. |
| Vegetables | Polyamines, phenols, and flavonoids | Usually higher contents of polyamines and total phenols in organic vegetables | Pereira-Lima *et al.*, 2008 |
| Swiss Chard | Instrumental quality parameters | No significant effect of organic practice in quality of crop | Daiss *et al.*, 2008 |

In addition to fertilizer type, the amount of fertilizer can also have an effect on the chemical profile and sensory characteristics of vegetables. This is because by manipulating nutrient availability, the amount of nitrogen the plant will absorb is modified and can affect its flavor, size of the plants and/or fruit, and shelf life (Mattheis and Fellman, 1999; Rembiałkowska, 2007). Light exposure is another important factor affecting quality and sensory characteristics of crops (Mattheis and Fellman, 1999). For example, a study conducted by Antonious *et al*. (1996) suggested that light reflecting from mulches of different colors into growing turnip plants, affected both chemical content and certain sensory characteristics.

## Sensory Analysis of Vegetables

Vegetable growing trends such as organic farming have sparked interest in the better understanding of flavor of vegetables. Flavor lexicons are widely used to describe and compare products within a category (Drake and Civille, 2003). Lexicons have been used in the past to describe sensory properties of a wide range of different products including natural cheeses (Heisserer and Chambers, 1993), green tea (Lee and Chambers, 2007), floral honey (Galán-Soldevilla *et al*., 2005), frozen vegetable soybeans (Krinsky *et al*., 2006), and soymilks (Day N'Kouka *et al*., 2004). In relation to lexicon development to specifically describe products with green characteristics such as vegetable products, a very important work is the one completed by Hongsoongnern and Chambers (2008a). Those authors developed a lexicon to describe green odor and flavor. They produced fourteen terms which included overall green, green unripe, green peapod, green grassy/leafy, green viney, green fruity, musty/earthy, floral, piney, overall sweet, pungent, and astringent. Those authors also evaluated aroma characteristics of chemicals associated with green. Lee and Chambers (2007) developed another lexicon, this time to describe flavor characteristics of brewed green tea including thirty-one descriptive terms such as green, parsley, spinach, citrus, and astringent. Krinsky *et al.* (2006) developed a lexicon to describe flavor of frozen soybeans. The lexicon was composed of fourteen terms and some of the descriptors included were green complex, sulfur, and astringent. Another study used terms such as crispness, pungent, and the basic tastes salty, acid, bitter, and umami to describe commercial samples of potherb mustard samples and relate these characteristics to their chemical content (Zhao *et al*., 2007b). The sensory characteristics of hearts of palm (palmito), a vegetable popular in Central and South America, was studied by Lawless *et al*. (1993). Those authors used appearance attributes (visible fibers, core color, outer color, core/outer, and appearance of layers); texture attributes (springy, crunchy, core firmness, firmness, fibrousness, and flakiness); taste attributes (sour, bitter, salty, sweet, and umami); and flavor attributes (earthy flavor/aroma, vegetal, briny flavor/aroma, and umami/brothy flavor). Another study by Lyon *et al*. (1992) evaluated the flavor, aroma, and mouth feel sensations of persimmons, a popular crop in Japan, Asia, and South America. Those authors used a lexicon of seventeen terms including aroma (green/grassy, nutty, chlorine, earthy, fall vegetable complex, fresh cut corn, sugar cane, and floral); flavor (fall vegetable, raw vegetable, nutty, melon mango, sweet, sour, and bitter); and mouth sensations (astringent and mouth coating).

The presence of “green” flavors in foods may be beneficial to human health as they may represent “sensory cues” to the presence of free fatty acids which are considered essential to human diet (Goff and Klee, 2006). On the other hand, having a better understanding of flavor characteristics of vegetable products may be useful for producers and scientists who want to know how the different production variables and storage conditions affect the flavor of vegetables. For example, organic farming, as a trend, has increased importance on the research of the flavor of fruits and vegetables as people want to know if these techniques provide a product with different flavor characteristics in comparison to conventional farming. Wszelaki *et al*. (2005) conducted research on soybeans and only used a general “taste” term for comparison. Haglund *et al*. (1999) used more descriptive terms such as hardness, crunchiness, juiciness, sweetness, bitterness, carrot-taste, and aftertaste to compare organically and conventionally grown carrots. DeEll and Prange (1992) used terms such as sweetness, tartness, off-flavor, firmness, and juiciness to compare organic and conventional apples. A single study by Basker (1992) also compared a general “taste quality” term to evaluate organically and conventionally grown vegetables such as tomatoes, carrots, orange juice, spinach, bananas, sweet corn, grapes, and mangoes.

Other genetic, environmental, cultural, and developmental factors may as well affect the flavor of vegetables (Mattheis and Fellman, 1999). The flavor of leafy vegetables can also be altered during storage mainly due to chlorophyll degradation and water loss (Agüero *et al*., 2008), which have an effect on the consumer’s purchase decision (Ares *et al*., 2007).

Instrumental testing has been widely used to predict quality of vegetable products. However, interactions between different compounds will make conclusions about impact in flavor difficult (Mattheis and Fellman, 1999). It is here were sensory analysis can make a meaningful contribution to better understanding the flavor of vegetable products and the effect of preharvest factors.

## Chemical and Sensory Analysis: Determining quality of vegetables

A product of good quality is a product that has positive and "expected" sensory characteristics. In plants, these sensory characteristics are affected by chemical composition. Chemical composition in plants is defined by the presence of many volatile and non-volatile compounds generated from primary or secondary metabolites (Goff and Klee, 2006).

The role of flavor chemistry is to identify and sometimes quantify these compounds to relate their occurrence to flavor characteristics. This science is of special importance to fresh produce because of the large number of factors that can affect chemical concentration and therefore, final sensory characteristics. These factors are genotype, stage of development (ripening), diseases, soil conditions, irrigation, pesticides, location, climate, and management practices among others (Zhao *et al*., 2006). It is by understanding the effect of these factors on chemical composition that researchers will be able to know their effect on sensory characteristics and final produce quality.

Additionally, there is a correlation between human health and volatiles (Goff and Klee, 2006). For example, the most abundant volatiles in tomatoes are derived from fatty acids which are classified as "essential" to human diet. These volatiles also provide tomatoes with "typical" flavors such as "tomato," "green," or "grassy" (Zhao *et al*., 2006).

In the late 1980's, approximately 15,000 different compounds were correlated with sensory characteristics (Teranishi *et al*., 1999). This is thirty times more than the number of compounds in the 50's and possibly less than what we may have right now. This demonstrates the importance and the attention this area has received as better methodologies and more sophisticated technologies are available these days.

However, measuring final sensory characteristics starting from chemical composition is not simple. There are many challenges in this science due to different thresholds and the effect of the food matrix (Drake and Civille, 2003).

When flavor chemistry data and analytical sensory data are collected simultaneously, results can be linked using multivariate analysis methods. Many publications have used different statistical techniques to find relationships between chemistry and sensory data. A very comprehensive publication exists that explores the use of multivariate statistics in understanding wine flavor (Noble and Ebeler, 2002). Those authors discuss and compare the use of three techniques that aid in modeling sensory and volatile data. These are principal component analysis of instrumental data (PCA-IV), generalized procrustes analysis (GPA) and partial least squares regression (PLS). Authors conclude that all three methods of modeling provide “fairly similar results”. This explanation is an oversimplification of the process and the results discussed by the authors. Working with these statistic methods involve many complex steps and some differences may still be found between methods depending on the final use of the results.

Several research groups exclusively use PLS regression as their selected method to find relations between flavor research and sensory data. Some of these studied the effect of processing techniques on the volatile content and sensory properties of navel orange juices (Baxter *et al*., 2005), durian (Voon *et al*., 2007), and rye (Heiniö *et al*., 2003). Correlation, cluster analysis, and multidimensional scaling (MDS) techniques have also been used on virgin olive oil (Morales *et al*., 1995). In addition, the authors used regression techniques to “predict” consumer acceptability from volatile data. It is important to understand that multivariate techniques do not determine if compounds related to certain flavors are actually responsible for those flavors. However, they offer substantial evidence for future work (Noble and Ebeler, 2002).

Prior to the use of these relation techniques, some other univariate and multivariate methods can be used on the individual sets of data with different purposes. Analysis of Variance (ANOVA) can be used to asses for significant differences between samples (volatile data would need to be quantified). If the interest lies on finding what specific differences exist between specific sets of samples, pairwise comparison techniques such as Tuckey’s Honestly Significant Differences (HSD) can be used. All these techniques were used by the authors discussing the relationship between sensory perception and volatile content in rye (Heiniö *et al*., 2003). Another method that can be used to asses for significant differences and the nature of these differences between samples is the Least Significant Different (LSD) method.

Principal component analysis (PCA) may also be used on the individual sets of data if the researcher wants to reduce the number of sensory attributes or volatiles into fewer factors or components which group variables of similar characteristics.

It is evident that a wide range of different techniques can be used to relate flavor chemistry data with sensory data. The selection of specific methods will be in function to the objectives of the study and the questions that need to be addressed. In the specific case of determining produce quality, where the chemical composition is greatly variable and reflective upon final sensory characteristics and quality perception, having flavor chemistry data available may be of great help in understanding quality differences.

# CHAPTER 2 - Materials and Methods

## Part 1: Lexicon development for fresh leafy vegetables

### *Fresh Leafy Vegetable Samples*

Samples of leafy vegetables were purchased from stores in Manhattan, Kansas and Kansas City, Kansas/Missouri one to three days prior to testing. Only fresh leafy vegetables (i.e. not cooked) available in the United States and potentially available in local grocery stores or supermarkets were included in this study (Table 2.1). No attempt was made to select specific variations in harvest, shipping, and storage conditions among the vegetables selected. However, the range of products evaluated has inherent differences in those areas. After purchase, the vegetables were immediately stored in a walk-in refrigerator at 4ºC and 50% relative humidity until ready for testing. To maintain moisture, vegetables were sprayed with tap water once daily until ready for testing.

On the day of testing, samples were retrieved from the refrigerator, rinsed using tap water, and excess water was eliminated using a salad spinner (Oxo International, Ltd., New York, NY). Random leaves of similar visual characteristics and with no deterioration were used for evaluation. Samples were then served to the panelists monadically on 6” foam plates identified with a three-digit code to reduce bias associated with knowing the name of the vegetable. In order to test a consistent sample of each leaf, panelists were instructed to hold the sample with both hands, fold the leaf, and bite one time through the middle of the folded leaf. To focus the evaluation on the leaf section of the vegetable, stems and large ribs were cut from the vegetable. For small leaves, panelists were asked to sample 1 – 3 whole leaves depending on leaf size. Because of different leaf sizes, amounts served to panelists were different across vegetable types. For example, panelists received three sprigs of small leaf vegetables such as parsley, cilantro, and watercress; three leaves of medium leaf plants such as spinach or curly endive; and half a leaf cut lengthwise through the middle (not including the stem or rib sections) was served of larger leaves such as collards or romaine lettuce.

**Table 2.1. Samples used to develop a lexicon to describe flavor characteristics of fresh leafy vegetables**

| Common name | Family* | Genera / Species* | Group* |
|---|---|---|---|
| Beet Greens (Organic) | Chenopodiaceae | *Beta vulgaris* | . |
| Beet Greens, Golden (Organic) | Chenopodiaceae | *Beta vulgaris* | . |
| Swiss chard, Green | Chenopodiaceae | *Beta vulgaris* | Cicla |
| Swiss chard, Red (Organic) | Chenopodiaceae | *Beta vulgaris* | Cicla |
| Spinach | Chenopodiaceae | *Spinacia oleracea* | . |
| Endive, Belgian | Compositae | *Cichorium endiva* | . |
| Endive, Curly | Compositae | *Cichorium endiva* | . |
| Radicchio | Compositae | *Cichorium intybus* | . |
| Lettuce, Butterhead / Boston / Bibb | Compositae | *Lactuca sativa* | . |
| Lettuce, Crisphead / Iceberg | Compositae | *Lactuca sativa* | . |
| Lettuce, Green Leaf | Compositae | *Lactuca sativa* | . |
| Lettuce, Red Leaf | Compositae | *Lactuca sativa* | . |
| Lettuce, Romaine / Cos | Compositae | *Lactuca sativa* | . |
| Mustard Greens, Curly | Cruciferae | *Brassica campestris* | Perviridis |
| Turnip Greens | Cruciferae | *Brassica campestris* | Rapifera |
| Pak Choy | Cruciferae | *Brassica rapa* | Chinensis |
| Pak Choy, Baby | Cruciferae | *Brassica rapa* | Chinensis |
| Cabbage, Napa | Cruciferae | *Brassica rapa* | Capitata |
| Cabbage, Green | Cruciferae | *Brassica oleracea* | Capitata |
| Cabbage, Red | Cruciferae | *Brassica oleracea* | Capitata |
| Cabbage, Savoy | Cruciferae | *Brassica oleracea* | Capitata |
| Collard Greens | Cruciferae | *Brassica oleracea* | Acephala |
| Kale | Cruciferae | *Brassica oleracea* | Acephala |
| Kale, Lacinato | Cruciferae | *Brassica oleracea* | Acephala |
| Kale, Red | Cruciferae | *Brassica oleracea* | Acephala |
| Arugula | Cruciferae | *Eruca sativa* | . |
| Watercress | Cruciferae | *Rorippa nasturtiumaquaticum* | . |
| Cilantro | Umbelliferae | *Coriandrum sativum* | . |
| Parsley, Curly | Umbelliferae | *Petroselinum crispum* | . |
| Parsley, Italian | Umbelliferae | *Petroselinum crispum* | . |

*Source: Yamaguchi, 1983

## *Panelists*

Six highly trained panelists from the Sensory Analysis Center at Kansas State University (Manhattan, KS., U.S.A.) participated in this study. The panelists had completed more than 120 hours of descriptive training, average more than 2000 hours of testing experience, and had prior experience testing vegetables and vegetable products.

## *Evaluation Procedures*

A method adapted from the flavor profile method (Caul, 1957; Keane 1992) was used. The method is a panel consensus method whereby the panelists must agree on attributes, definitions, and key reference products. Our adaptation uses a 0-15 point scale divided in 0.5 point increments, 0 meaning “none” and 15 meaning “extremely high”. This methodology has been previously used to describe a wide variety of products such as cheese (Heisserer and Chambers, 1993; Rètiveau *et al*., 2005), black walnut syrup (Matta *et al*., 2005), rose apples (Vara-Ubol *et al*., 2006) and tomatoes (Hongsoongnern and Chambers, 2008b).

During lexicon development, panelists were asked to review previously developed terms used to describe “green” aroma characteristics (Hongsoongnern and Chambers, 2008a) and green tea (Lee and Chambers, 2007). Those beginning lexicons were used to help start the creation of a lexicon to describe flavor characteristics of a wide variety of fresh leafy vegetables. Discussion of the terms helped ensure the use of terms that are discriminative, descriptive, and nonredundant; key characteristics of a good flavor lexicon (Drake and Civille, 2003). When new terms were needed, panelists were also asked to discuss terminology, select an appropriate term, develop a descriptive definition, and to assign one or more reference materials that could be helpful in understanding the attribute and evaluating intensity. Lexicon development sessions were 90-minutes long and up to six samples were evaluated in each session depending on the complexity of flavor, carry over, and if new terms needed to be discussed, defined, and referenced. Products were reviewed multiple times during the lexicon development phase of the study.

Complete profiles were generated for each of the fresh leafy vegetables used during lexicon development. Using a similar evaluation procedure, samples were evaluated once more and a consensus profile was developed. At that point, a new sample was evaluated until the set of 30 samples was complete. No actions were taken to mask appearance differences between samples.

## *Analysis*

Statistical analysis is required to clarify attribute relationships in the lexicon development process (Drake and Civille, 2003). Principal component analysis (PCA) was used on the consensus profiles to assess the relationships among terms. PCA was conducted on the correlation matrix and the data was orthogonally rotated to facilitate interpretation of the results. This analysis was performed by SAS® (2002, version 9.1.3; SAS Institute, Cary, NC).

## Part 2: Effect of organic production and fertilizer variables on the sensory properties of pac choi and tomato

### *Samples*

Trials were conducted at the K-State Horticulture Research and Extension Center, Olathe, Kansas, on experimental plots established in 2002 for comparison of crops grown under organic and conventional production systems in high tunnels (unheated, passively ventilated greenhouses) and open field plots (Zhao *et al*., 2007a). The soil was a Kennebec silt loam. Six 9.8 m x 6.1 m high tunnels with 1.5m sidewalls (Stuppy, North Kansas City, MO) and six adjacent 9.8 m x 6.1 m field plots were used for this study. High tunnels were covered with single layer 6-mil (0.153mm) K-50 polyethylene (Klerk's Plastic Product Manufacturing, Inc., Richburg, SC). At establishment of the experimental plots, the six high tunnels were divided into three groups (blocks) and the two high tunnels in each block were randomly assigned for long-term conventional or organic management treatments. A similar set-up was used in the field plots. Organic plots were managed in compliance with USDA National Organic Program standards, and were inspected and certified in 2003, 2006, 2007 and 2008.

For this study, beginning in 2007, each high tunnel or open field plot was subdivided into three 3.2 m x 6.1 m plots to which one of three fertilizer levels were assigned (high, low, and no fertilizer) following a latin square design to avoid bias due to position effects in the high tunnels. Fertilizer rates were determined based on soil analysis at the beginning of the study in 2007, and recommendations for vegetable crops in Kansas (Marr *et al*., 1998), with compost applied to organic plots and synthetic fertilizer applied to conventional plots. Compost application rates were based on the assumption that 50% of the nitrogen from compost would be available to plants during the growing season, while 100% would be available from conventional fertilizers (Warman and Havard, 1997). Low and high fertility plots were fertilized with equal amounts of compost or synthetic fertilizer at the beginning of the growing season, and high fertility plots received additional fertilization during the growing season as described below.

Pac choi (*Brassica rapa* L. *chinensis* 'Mei Qing Choi') (Johnny's Selected Seed, Albion, ME, U.S.A.) and tomato (*Lycopersicon esculentum* 'Bush Celebrity') (Totally Tomatoes, Randolph, WI, U.S.A.) were grown in one half of each open field or high tunnel plot (6.8 m x 3 m) in 2007 and 2008, with a rotation between pac choi and tomato plots each year. In our experimental system, a spring and a fall crop of pac choi was grown each year, while a single crop of tomato was grown. Between the spring and fall pac choi crops, plots were seeded with a summer cover crop of buckwheat (*Fagopyrum sagittatum*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 134 kg/ha. In the late fall, all plots were seeded with a cover crop of annual rye (*Secale cereale*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 229 kg/ha.

Conventional high and low fertility plots were fertilized with Jack's Professional Peat-lite N-$P_2O_5$-$K_2O$ 20-10-20 (Allentown, PA, U.S.A.), at a rate of 98 kg/ha. Organic plots received MicroLeverage compost N-$P_2O_5$-$K_2O$ 0.6-0.8-0.5 (Hughesville, MO, U.S.A) at a rate of 197 kg/ha. Starting 2 weeks after planting, high fertility plots received additional fertilization at a rate of 7.2 kg/ha. Fertilizer used on organic plots was fish hydrolyzate N-$P_2O_5$-$K_2O$ 2.23-4.35-0.3 (Neptune's Harvest, Gloucester, MA, U.S.A) and conventional plots received calcium nitrate and potassium nitrate at a rate calculated to apply an amount of calcium equivalent to that present in the fish hydrolyzate. The tomato crop received 6 weekly applications, for a total of 43 kg/ha, and the spring and fall pac choi crops each received three such applications.

Pac choi and tomato transplants were started in a greenhouse in Sunshine Mix Special Blend E6340 (SunGro Horticulture, Bellevue, WA) supplemented with MicroLeverge compost. The pac choi trial was planted on April 1 and harvested on May 5. The tomato trial was planted on May 5 and harvested on July 18. All testing occurred in 2008.

## *Sample Preparation*

### *Pac choi*

Plants were harvested one to three days before testing. After harvest, plants were immediately rinsed using cold tap water to remove excess dirt and stored in a refrigerated container for transport to the Kansas State University campus located in Manhattan, Kansas. Once samples arrived, they were moved to a walk-in refrigerator for storage at 4ºC until testing. The plants were sprayed daily with tap water to maintain moisture. The day of testing the plants were retrieved from the refrigerator. Random leaves of similar visual characteristics were removed from each stalk (not including the stem) of each treatment, rinsed using distilled water and excess water was eliminated using a salad spinner (Oxo International, Ltd., New York, NY). Samples were served to the panelists monadically in 6” foam plates identified with a three-digit code to eliminate potential panelist bias.

### *Tomatoes*

Tomatoes were harvested at the pink stage, three to six days before testing (United States Department of Agriculture [USDA], 1975).When harvested, samples were placed in labeled boxes for their transportation to the Kansas State University campus located in Manhattan, Kansas. Special care was taken when handling the tomatoes to avoid damage because it had been suggested that internal bruising may alter the quality and flavor of tomatoes (Moretti *et al*., 2002). Once tomatoes arrived, they were organized in trays sorted by treatment and placed on a flat surface (no tomato on top of another) to avoid damage. On the day of testing three tomatoes at the red stage (USDA, 1975) with similar visual characteristics were selected from each treatment. Samples were washed thoroughly using tap water at room temperature and then cut in half lengthwise. One half of each tomato was cut in ½-inch wedges and served to the panelists in covered, odor-free 3.25 oz. plastic cups. Cups with the samples were labeled with a 3-digit code to avoid potential bias. The tomatoes were never refrigerated.

## *Panelists*

Six highly trained panelists from the Sensory Analysis Center at Kansas State University (Manhattan, Kans., U.S.A.) were selected for this study. The panelists had completed more than 120 hours of descriptive training, averaging more than 2000 hours of testing experience and had prior experience testing vegetables and vegetable products.

## *Evaluation Procedure*

Previously developed lexicons were used for this study. The lexicon for pac choi was developed by Talavera-Bianchi *et al*. (2009) to describe the flavor of different leafy vegetables and was produced using an adaptation of the flavor profile method (Caul, 1957; Keane 1992) which has been used by many studies in the past to describe a variety of products such as cheese (Heisserer and Chambers, 1993; Rètiveau *et al*., 2005), green tea (Lee and Chambers, 2007), tomatoes (Hongsoongnern and Chambers, 2008b) and green flavors (Hongsoongnern and

Chambers, 2008a). The lexicon for tomatoes was previously developed by Hongsoongnern and Chambers ( 2008b) to describe flavor of fresh and processed tomatoes. Lexicons with definitions and references were presented to the panelists in one 90-minute session prior to the start of testing so they could become familiar with the terminology, test procedures and samples.

For testing, panelists were presented with the lexicon and references used during orientation. Data were collected using a computerized collection system (Compusense Five version 4.4.8, 2002, Guelph, ON, Canada). Intensities for each attribute were recorded using a 0-15 point scale divided in 0.5 point increments, 0 meaning none and 15 meaning extremely high. Panelists evaluated the samples individually and followed a completely randomized block design with replication as the blocking factor. Twelve samples of pac choi were evaluated in each of three 180-minute sessions. Twelve samples of tomatoes also were evaluated in another set of three 180-minutes sessions. Reverse osmosis, deionized, carbon-filtered water and unsalted crackers were used to rinse the palate between the samples. A similar procedure has been used in the past to evaluate the sensory characteristics of four samples of calcium-biofortified lettuce (Park *et al.*, 2009).

### ***Analysis***

Treatments were organized in a split plot design with production system (i.e. organic vs. conventional) as the whole plot and fertilizer amount (i.e. control, low and high) as the sub-plot. Analysis of variance (ANOVA) was used to detect significant differences between treatments for individual attributes. Principal component analysis (PCA) was used to evaluate relationships between treatments and to provide a graphic representation of the results. Cluster analysis (Ward method) was used to separate groups of similar sensory characteristics. This analysis was computed in SAS® (2002, version 9.1.3; SAS Institute, Cary, NC). Because of the complexity of the design, plots for field vs. high-tunnel production were not randomized and thus a statistical comparison of those data is not made.

## Part 3: Relation between developmental stage, sensory properties, and volatile content of organically and conventionally grown pac choi

### *Samples*

Trials were conducted at the K-State Horticulture Research and Extension Center, Olathe, Kansas, on experimental plots established in 2002 for comparison of crops grown under organic and conventional production systems in high tunnels (unheated, passively ventilated greenhouses) and open field plots (Zhao *et al*., 2007a). The soil was a Kennebec silt loam. Six 9.8 m x 6.1 m high tunnels with 1.5m sidewalls (Stuppy, North Kansas City, MO) and six adjacent 9.8 m x 6.1 m field plots were used for this study. High tunnels were covered with single layer 6-mil (0.153mm) K-50 polyethylene (Klerk's Plastic Product Manufacturing, Inc., Richburg, SC). At establishment of the experimental plots, the six high tunnels were divided into three groups (blocks) and the two high tunnels in each block were randomly assigned for long-term conventional or organic management treatments. A similar set-up was used in the field plots. Organic plots were managed in compliance with USDA National Organic Program standards, and were inspected and certified in 2003, 2006, 2007 and 2008.

For this study, beginning in 2007, each high tunnel or open field plot was subdivided into three 3.2 m x 6.1 m plots to which one of three fertilizer levels were assigned (high, low, and no fertilizer) following a latin square design to avoid bias due to position effects in the high tunnels. Fertilizer rates were determined based on soil analysis at the beginning of the study in 2007, and recommendations for vegetable crops in Kansas (Marr *et al*., 1998), with compost applied to organic plots and synthetic fertilizer applied to conventional plots. Compost application rates were based on the assumption that 50% of the nitrogen from compost would be available to plants during the growing season, while 100% would be available from conventional fertilizers (Warman and Havard, 1997). Low and high fertility plots were fertilized with equal amounts of compost or synthetic fertilizer at the beginning of the growing season, and high fertility plots received additional fertilization during the growing season as described below.

Pac choi (*Brassica rapa* L. *chinensis* 'Mei Qing Choi') (Johnny's Selected Seed, Albion, ME, U.S.A.) and tomato (*Lycopersicon esculentum* 'Bush Celebrity') (Totally Tomatoes, Randolph, WI, U.S.A.) were grown in one half of each open field or high tunnel plot (6.8 m x 3 m) in 2007 and 2008, with a rotation between pac choi and tomato plots each year. In our experimental system, a spring and a fall crop of pac choi was grown each year, while a single crop of tomato was grown. Between the spring and fall pac choi crops, plots were seeded with a summer cover crop of buckwheat (*Fagopyrum sagittatum*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 134 kg/ha. In the late fall, all plots were seeded with a cover crop of annual rye (*Secale cereale*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 229 kg/ha.

Conventional high and low fertility plots were fertilized with Jack's Professional Peat-lite N-$P_2O_5$-$K_2O$ 20-10-20 (Allentown, PA, U.S.A.), at a rate of 98 kg/ha. Organic plots received MicroLeverage compost N-$P_2O_5$-$K_2O$ 0.6-0.8-0.5 (Hughesville, MO, U.S.A) at a rate of 197 kg/ha. Only pac choi grown in the outside plots with low amounts of fertilizer were considered for this specific study.

Pac choi transplants were started in a greenhouse in Sunshine Mix Special Blend E6340 (SunGro Horticulture, Bellevue, WA) supplemented with MicroLeverge compost. Pac choi was planted on April 1, 2008 and harvested on April 20 (2.5 weeks old for baby pac choi), May 5 (4.5 weeks old for optimum growth), and May 19 (6.5 weeks old for overgrown pac choi).

## *Sample Preparation*

### *Sensory analysis*

Plants were harvested one to three days before testing. After harvest, the plants were immediately rinsed using cold tap water to remove excess dirt and stored in a refrigerated container for transport to the Kansas State University campus located in Manhattan, KS. Once arrived, the samples were moved to a walk-in refrigerator for storage at 4ºC until testing. The plants were sprayed daily with tap water to maintain moisture. On the day of testing, plants were retrieved from the refrigerator. Random leaves of similar visual characteristics were removed from each stalk (not including the stem) and rinsed using distilled water. Excess water was eliminated with a salad spinner (Oxo International, Ltd., New York, NY). Samples were served to the panelists monadically in 6" foam plates identified with a three-digit code to eliminate potential panelist bias. The sample amount was dependant on leaf size. For example, for baby pac choi (2.5 weeks old plant) one whole sprig comprised of several leaves was served to each panelist, 1-2 leaves were served to each panelist when leaves were 4.5 weeks old and 6.5 week old.

### *Volatile analysis*

The same day of sensory testing, approximately 5-10 g from leaves of each treatment was vacuum sealed and frozen at −80°C for 30 d until volatile analysis. The day of the analysis, samples were retrieved from the freezer and thawed at room temperature (22 ± 1°C) for approximately 30 min. For solid-phase microextraction (SPME) sampling, 4 g pac choi leaves were blended with 200-mL of reverse osmosis, deionized, carbon-filtered water using an electric hand blender (Rival, Peoria, IL) for 20 s. The mixture was then filtered through double layered cheese cloth. From the filtered solution, 1-mL was transferred to a 10-mL clear headspace vial and mixed with 0.2 g of sodium chloride (NaCl). Additionally, 5-µl of 0.2 ppm 1,3 dichlorobenzene in methanol (internal standard) was added. Glass vials were closed using an open-center screw cap with a 1.8 mm silicone/PTFE septum (Varian, Palo Alto, CA).

## *Panelists*

Six highly trained panelists from the Sensory Analysis Center at Kansas State University (Manhattan, KS, U.S.A.) were used for this study. The panelists had completed more than 120 hours of descriptive training, average more than 2000 hours of testing experience, and had prior experience testing vegetables and vegetable products.

## *Experimental procedure*

### *Sensory analysis*

The lexicon for pac choi was used based on Talavera-Bianchi *et al*., (2009) to describe flavor of different leafy vegetables. A lexicon consisting of twenty-nine terms with definitions and references was presented to the panelists prior the start of testing so they could become familiar with the terminology, test procedures, and samples. The original lexicon consisted of twenty-six flavor and mouth feel attributes. However, three texture attributes were added because we believed that this would aid in describing changes in the plant during the maturation process. Similar lexicons have been developed and used for other products such as green tea (Lee and Chambers, 2007), tomatoes (Hongsoongnern and Chambers, 2008b), ice cream (Thompson *et al*., 2009), and brewed coffee (Seo *et al*., 2009).

The day of testing, panelists were presented with the lexicon and references used during orientation. Data were collected using a computerized collection system (Compusense Five version 4.4.8, 2002, Guelph, ON, Canada). Intensities for each attribute were recorded using a 0-15 point scale divided in 0.5 point increments, 0 meaning “none” and 15 meaning “extremely high”. Panelists evaluated the samples individually and followed a completely randomized block design with the stage of development as the blocking factor. Six samples of pac choi were evaluated in each of three 90-minute sessions. Reverse osmosis, deionized, carbon-filtered water and unsalted crackers were used to rinse the palate between the samples. A similar procedure has been used in the past to evaluate the sensory characteristics of four samples of calcium-biofortified lettuce (Park *et al*., 2009).

### *Gas chromatography – mass spectrometry*

Volatile compounds were identified and quantified using a Varian Saturn CP-3800 Gas Chromatograph / Mass Spectrometer 2200 (Varian Inc., Walnut Creek, CA). The sample vials were equilibrated at 40°C/500 rpm for 10 min. SPME was performed using a StableFlex Divinylbenzene / Carboxen / Polydimethylsiloxane 50/30 µm fiber (Sigma Aldrich, Saint Louis, MO) for 20 min at 40°C. The agitation during extraction was of 250 rpm. The extracted compounds were thermally desorbed at 250°C for 3 min in the front injection port of the gas chromatograph. After the injection, the fiber was baked at 270°C for 30 min. An RTX®-5 Capillary Column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness; Restek U.S., Bellefonte, PA) was used to separate the volatiles desorbed from the fiber. The initial temperature of the column was set at 40°C for 2 min and then raised to 200°C at a rate of 5°C $min^{-1}$ and held for 1 min (total GC run time was 35 min). Varian MS Workstation software (version 6.8) was used for system control, data collection, and data processing. Compound identification was based on NIST 2005 version 2.0 Mass Spectra library search. The final compounds concentration was based on the concentration of the internal standard. Three replications were analyzed for each treatment. Kovats retention indices were calculated to aid in the identification of the volatile compounds. A blend of hydrocarbon (HC) mix and carbon disulfide (1 drop of HC mix in 1 ml of $CS_2$ directly injected to the GC) was also run under the same methodology to generate the retention times of the n-alkanes ($C_6$-$C_{20}$) for calculating the Kovats indices. Comparing Kovats indices from chemicals previously identified using the same column and stationary phase under similar conditions has shown to be an accurate method of identification (Moustafa, 2008).

### *Analysis*

Analysis of variance (ANOVA) with PROC MIXED (panelist and replication as the random effects) was used to detect overall differences among treatments for individual sensory attributes. PROC GLM was used to detect differences for individual volatile compounds. ANOVA was computed in SAS® (2002, version 9.1.3; SAS Institute, Cary, NC). Partial least squares regression (PLS2) was used to correlate sensory and instrumental data. PLS is a soft modeling method which is widely used to predict a set of dependant variables (sensory attributes) from a large set of independent variables (volatile compounds) (Noble and Ebeler, 2002). This method has been previously used to correlate instrumental and sensory data in cheese (Hough *et al*., 1996), diced tomatoes (Lee *et al*., 1999), and ice cream (Chung *et al*., 2003). Even though this analysis does not determine which volatile components are actually responsible for specific sensory attributes, it does help in studying the relationship between certain volatiles and sensory characteristics (Noble and Ebeler, 2002). This analysis was performed using Unscrambler (2005, version 9.2; Camo Process AS, Oslo, Norway).

## Part 4: Sensory and chemical properties of organically and conventionally grown pac choi change little during 18 days of refrigerated storage

### *Samples*

Trials were conducted at the K-State Horticulture Research and Extension Center, Olathe, Kansas, on experimental plots established in 2002 for comparison of crops grown under organic and conventional production systems in high tunnels (unheated, passively ventilated greenhouses) and open field plots (Zhao *et al*., 2007a). The soil was a Kennebec silt loam. Six 9.8 m x 6.1 m high tunnels with 1.5m sidewalls (Stuppy, North Kansas City, MO) and six adjacent 9.8 m x 6.1 m field plots were used for this study. High tunnels were covered with single layer 6-mil (0.153mm) K-50 polyethylene (Klerk's Plastic Product Manufacturing, Inc., Richburg, SC). At establishment of the experimental plots, the six high tunnels were divided into three groups (blocks) and the two high tunnels in each block were randomly assigned for long-term conventional or organic management treatments. A similar set-up was used in the field plots. Organic plots were managed in compliance with USDA National Organic Program standards, and were inspected and certified in 2003, 2006, 2007 and 2008.

For this study, beginning in 2007, each high tunnel or open field plot was subdivided into three 3.2 m x 6.1 m plots to which one of three fertilizer levels were assigned (high, low, and no fertilizer) following a latin square design to avoid bias due to position effects in the high tunnels. Fertilizer rates were determined based on soil analysis at the beginning of the study in 2007, and recommendations for vegetable crops in Kansas (Marr *et al*., 1998), with compost applied to organic plots and synthetic fertilizer applied to conventional plots. Compost application rates were based on the assumption that 50% of the nitrogen from compost would be available to plants during the growing season, while 100% would be available from conventional fertilizers (Warman and Havard, 1997). Low and high fertility plots were fertilized with equal amounts of compost or synthetic fertilizer at the beginning of the growing season, and high fertility plots received additional fertilization during the growing season as described below.

Pac choi (*Brassica rapa* L. *chinensis* 'Mei Qing Choi') (Johnny's Selected Seed, Albion, ME, U.S.A.) and tomato (*Lycopersicon esculentum* 'Bush Celebrity') (Totally Tomatoes, Randolph, WI, U.S.A.) were grown in one half of each open field or high tunnel plot (6.8 m x 3 m) in 2007 and 2008, with a rotation between pac choi and tomato plots each year. In our experimental system, a spring and a fall crop of pac choi was grown each year, while a single crop of tomato was grown. Between the spring and fall pac choi crops, plots were seeded with a summer cover crop of buckwheat (*Fagopyrum sagittatum*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 134 kg/ha. In the late fall, all plots were seeded with a cover crop of annual rye (*Secale cereale*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 229 kg/ha.

Conventional high and low fertility plots were fertilized with Jack's Professional Peat-lite N-$P_2O_5$-$K_2O$ 20-10-20 (Allentown, PA, U.S.A.), at a rate of 98 kg/ha. Organic plots received MicroLeverage compost N-$P_2O_5$-$K_2O$ 0.6-0.8-0.5 (Hughesville, MO, U.S.A) at a rate of 197 kg/ha. Starting 2 weeks after planting, high fertility plots received additional fertilization at a rate of 7.2 kg/ha. Fertilizer used on organic plots was fish hydrolyzate N-$P_2O_5$-$K_2O$ 2.23-4.35-0.3 (Neptune's Harvest, Gloucester, MA, U.S.A) and conventional plots received calcium nitrate and potassium nitrate at a rate calculated to apply an amount of calcium equivalent to that present in the fish hydrolyzate. The tomato crop received 6 weekly applications, for a total of 43 kg/ha, and the spring and fall pac choi crops each received three such applications. Only pac choi grown in the outside plots with high amounts of fertilizer were considered for this specific study.

Pac choi transplants were started in a greenhouse in Sunshine Mix Special Blend E6340 (SunGro Horticulture, Bellevue, WA) supplemented with MicroLeverge compost. An amendment of fish hydrolysate was fertigated at a rate 18.1lbs/ hectare. Pac choi was planted on September 4, 2008 and harvested on October 6 of the same year, at approximately 4.5 wks of age, a typical time for pac choi.

## *Sample Preparation*

### *Sensory analysis*

After harvest, the plants were immediately rinsed using cold tap water to remove excess soil and stored in a refrigerated container for transport to the Kansas State University campus located in Manhattan, KS. The samples were placed in a walk-in refrigerator for storage at 4ºC until testing at 1, 4, 9, and 18 days of storage. This temperature was selected to reduce deterioration of the pac choi leaves while recreating normal conditions in the refrigerator of a consumer. The interval selected was based on previous research by Able *et al*. (2005). The plants were sprayed once every two days with tap water to maintain moisture. On the day of testing, plants were retrieved from the refrigerator. Random leaves of similar visual characteristics were removed from each stalk (not including the stem) and rinsed using distilled water. Excess water was eliminated with a salad spinner (Oxo International, Ltd., New York, NY). Samples were served to the panelists monadically on 6” foam plates identified with a three-digit code to eliminate potential panelist bias. One leaf was served to each panelist.

### *Volatile analysis*

Volatile analysis for each shelf life point occurred the same day as sensory testing. For solid-phase microextraction (SPME) sampling, 4 g pac choi leaves were blended with 200-mL of reverse osmosis, deionized, carbon-filtered water using an electric hand blender (Rival, Peoria, IL) for 20 s. The mixture was then filtered through a double layered cheese cloth. From the filtered solution, 1-mL was transferred to a 10-mL clear headspace vial and mixed with 0.2 g of Sodium Chloride (NaCl). Additionally, 5-µl of 0.2 ppm 1,3 dichlorobenzene in methanol (internal standard) was added. Glass vials were closed using an open-center screw cap with a 1.8 mm silicone/PTFE septum (Varian, Palo Alto, CA).

## *Panelists*

Six highly trained panelists from the Sensory Analysis Center at Kansas State University (Manhattan, KS, U.S.A.) were used for this study. The panelists had completed more than 120 hours of descriptive training, averaged more than 2000 hours of testing experience, and had prior experience testing vegetables and vegetable products.

## *Experimental procedure*

### *Sensory analysis*

The lexicon used for pac choi was created to describe the flavor of different leafy vegetables and was developed by Talavera-Bianchi *et al*. (2009) using an adaptation of the flavor profile method (Caul, 1957; Keane, 1992), which has been used by many studies in the past to help describe a variety of products such as cheese (Heisserer and Chambers, 1993; Rètiveau *et al*., 2005), green tea (Lee and Chambers, 2007) or tomatoes (Hongsoongnern and Chambers, 2008b). The previously developed lexicon consisting of thirty-two attributes with definitions and references was presented to the panelists prior to the start of testing so they could become familiar with the terminology, test procedures, and samples. Twenty-six flavor and mouth feel attributes were selected to evaluate pac choi. However, three texture attributes (i.e. crispness, moistness and fiber awareness) and three off-flavor attributes (i.e. stale/refrigerator, cardboard and moldy) were added because they were needed to describe changes in the leaves during shelf life.

The day of testing, panelists were presented with the lexicon and references determined while in orientation. Data were collected using a computerized collection system (Compusense Five version 4.4.8, 2002, Guelph, ON, Canada). Intensities for each attribute were recorded using a 0-15 point scale divided in 0.5 point increments, 0 meaning "none" and 15 meaning "extremely high". Panelists evaluated the samples individually and followed a completely randomized block design with the shelf life stage as the blocking factor. Six samples of pac choi were evaluated in each of four 90-minute sessions at 1, 4, 9, and 18 days after storage. Reverse osmosis, deionized, carbon-filtered water and unsalted crackers were used to rinse the palate between the samples. A similar procedure has been used in the past to evaluate the sensory characteristics of four samples of calcium-biofortified lettuce (Park *et al*., 2009).

### *Gas chromatography – mass spectrometry*

Volatile compounds were identified and quantified using a Varian Saturn CP-3800 Gas Chromatograph / Mass Spectrometer 2200 (Varian Inc., Walnut Creek, CA). The sample vials were equilibrated at 40°C/500 rpm for 10 min. SPME was performed using a StableFlex Divinylbenzene / Carboxen / Polydimethylsiloxane 50/30 µm fiber (Sigma Aldrich, Saint Louis, MO) for 20 min at 40°C. The agitation during extraction was of 250 rpm. The extracted compounds were thermally desorbed at 250°C for 3 min in the front injection port of the gas chromatograph. After the injection, the fiber was baked at 270°C for 30 min. An RTX®-5 Capillary Column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness; Restek U.S., Bellefonte, PA) was used to separate the volatiles desorbed from the fiber. The initial temperature of the column was set at 40°C for 2 min and then raised to 200°C, at a rate of 5°C $min^{-1,}$ and held for 1 min (total GC run time was 35 min). Varian MS Workstation software (version 6.8) was used for system control, data collection, and data processing. Compound identification was based on NIST 2005 (National Institute of Standards and Technology, U.S. Department of Commerce, Gaithersburg, MD) version 2.0 Mass Spectra library search. The final compounds concentration was based on the concentration of the internal standard. Three replications were analyzed for each treatment. Kovats retention indices were calculated to aid in the identification of the volatile compounds. A hydrocarbon (HC) mix (1 drop of HC mix in 1 ml of carbon disulfide – $CS_2$ – directly injected to the GC) was also run under the same methodology to generate the retention times of the n-alkanes ($C_6$-$C_{20}$) for calculating the Kovats indices. Comparing Kovats indices from chemicals previously identified using the same column and stationary phase under similar conditions has shown to be an accurate method of identification (Moustafa, 2008).

## *Analysis*

Analysis of variance (ANOVA) with PROC MIXED (panelist and replication as the random effects) was used to detect overall differences among treatments for individual sensory attributes. PROC GLM was used to detect differences for individual volatile compounds. ANOVA was computed in SAS® (2002, version 9.1.3; SAS Institute, Cary, NC). Partial least squares regression (PLS2) was used to correlate sensory and instrumental data. PLS is a soft modeling method which is widely used to predict a set of dependant variables (sensory attributes) from a large set of independent variables (volatile compounds) (Noble and Ebeler, 2002). This method has been previously used to correlate instrumental and sensory data in cheese (Hough *et al*., 1996), diced tomatoes (Lee *et al*., 1999) and ice cream (Chung *et al*., 2003). Even though this analysis does not determine which volatile components are actually responsible for specific sensory attributes, it does help in studying the relationship between certain volatiles and sensory characteristics (Noble and Ebeler, 2002). This analysis was performed by Unscrambler (2005, Version 9.2; Camo Process AS, Oslo, Norway).

# CHAPTER 3 - Lexicon to describe flavor of fresh leafy vegetables

## Abstract

Practices such as organic farming and breeding to increase nutrition and functional health components have increased interest in understanding the flavor of vegetables, such as leafy greens. The main objective of this study was to select, define, and reference a lexicon for describing the flavor of fresh leafy vegetables. A highly trained descriptive sensory panel determined a list of 32 sensory attributes that was able to describe the flavor of the fresh leafy greens studied. This lexicon includes five "green" attributes; mouth feel characteristics such as pungent, bite, tooth-etch, and heat/burn; fundamental tastes including bitter and umami; seven terms that describe unique flavors related to specific vegetables such as cabbage, celery, lettuce, spinach, parsley, beet, and radish leaves; and a group of other terms including citrus, piney, woody, water-like, musty/earthy, floral, sulfur, metallic, soapy, petroleum-like, and overall sweet.

## Practical applications

Understanding the effects that different breeding, growing, harvesting, shipping, and storage technologies have on properties of leafy vegetables has increased the need for appropriate evaluation tools. Using this lexicon can guide researchers to a better understanding of differences in flavor among various fresh leafy vegetables and can help in understanding changes in flavors of those vegetables resulting from various alterations in the breeding and production systems. This project provides researchers with specific sensory terminology to track changes in fresh leafy greens instead of using generic terms such as "taste" or "typical flavor."

## Introduction

Flavor lexicons are widely used to describe and compare products within a category (Drake and Civille, 2003). Lexicons for a number of product categories have been published over the last 20 years. In recent years, lexicons have been developed or supplemented for flavor and texture of many products such as brewed coffee (Seo *et al*., 2009), tomato (Hongsoongnern and Chambers, 2008b), yogurt (Coggins *et al*., 2008), cheese (Talavera-Bianchi and Chambers, 2008; Drake *et al*., 2007; Karagul-Yuceer *et al*., 2007), green tea (Lee and Chambers, 2007), rose apples, an Asian fruit (Vara-Ubol *et al*., 2006), soy products (Drake *et al*., 2007; Chambers *et al*., 2006; Krinsky *et al*., 2006), chemical compounds (Bott and Chambers, 2006), and honey (Galán-Soldevilla *et al*., 2005).

Recently, several lexicons related to green, leafy, or vegetable materials have been developed. For example, a lexicon was developed specifically to describe products with "green" flavor characteristics such as vegetable products (Hongsoongnern and Chambers, 2008b) and a lexicon for a specific vegetable, tomato, also was published (Hongsoongnern and Chambers, 2008a). As part of those lexicons, terms associated with green vegetative materials such as overall green, green-unripe, green-peapod, green-grassy/leafy, green-viney, and green-fruity were determined.

Lee and Chambers (2007) developed a lexicon, to describe a particular brewed green leaf tea, which included thirty-one descriptive terms such as green, parsley, spinach, citrus, and astringent. Krinsky *et al*. (2006) published a lexicon to describe the flavor of frozen soybeans. The lexicon was composed of fourteen terms and some of the descriptors included were green-complex, sulfur, and astringent. Another study used terms such as crispness, pungent, and the basic tastes salty, acid, bitter, and umami to describe commercial samples of mustard greens and relate those characteristics to their chemical content (Zhao *et al*., 2007b).

The presence of "green" flavors in foods may be beneficial to human health as they may represent "sensory cues" to the presence of free fatty acids which are considered essential to human diet (Goff and Klee, 2006). Having a better understanding of flavor characteristics of vegetable products may be useful for producers and scientists who want to know how different production variables and storage conditions affect the flavor of vegetables. For example, Park *et al*. (2009) conducted descriptive sensory studies to determine whether increasing nutritive value of lettuce, i.e. biofortification with calcium, had an impact on flavor or texture. Additionally, organic farming, as a trend, has increased the importance of research on the flavor of fruits and vegetables because people want to know if these techniques provide a product with different flavor characteristics in comparison to conventional farming. Unfortunately, the sensory vocabulary to describe differences in many vegetables often is quite general. Wszelaki *et al*. (2005) conducted research on soybeans and only used a general "taste" term for comparison. Haglund *et al*. (1999) used more descriptive terms such as hardness, crunchiness, juiciness, sweetness, bitterness, carrot-taste, and aftertaste to compare organically and conventionally grown carrots. DeEll and Prange (1992) used terms such as sweetness, tartness, off-flavor, firmness, and juiciness to compare organic and conventional apples. A study by Basker (1992) also compared a general “taste quality” term of organically and conventionally grown vegetables such as tomatoes, carrots, orange juice, spinach, bananas, sweet corn, grapes, and mangoes. Other genetic, environmental, cultural, and developmental factors may, as well, affect the flavor of vegetables (Mattheis and Fellman, 1999).

The flavor of leafy vegetables also can be altered during storage mainly because of chlorophyll degradation and water loss (Agüero *et al*., 2008). Those changes can affect consumer’s purchasing decisions (Ares *et al*., 2007). However, in order to track those effects it is necessary to have a sensory terminology that is specific rather than general and can be used in various laboratories.

This study was designed to provide a tool for better understanding the flavors present in fresh leafy vegetables commonly consumed in the United States and may be used to clarify the effect of production variables on the flavor of fresh leafy vegetables if used by an appropriately trained sensory panel.

The objectives of this study were (1) to develop a lexicon that will describe the flavor characteristics of fresh leafy vegetables and (2) to assess relationships among the terms to determine if specific terms overlap or are duplicative of other terms in the lexicon.

## Materials and Methods

### *Fresh Leafy Vegetable Samples*

Leafy vegetable samples were purchased one to three days before testing at local stores in Manhattan, Kansas and Kansas City, Kansas/Missouri. Only fresh leafy vegetables (i.e. not cooked) available in the United States and potentially available in local grocery stores or supermarkets were included in this study. No attempt was made to select specific variations in harvest, shipping, and storage conditions among the vegetables selected. However, the range of products evaluated have inherent differences in those areas. After purchase, the vegetables were immediately stored in a walk-in refrigerator at 4ºC and 50% relative humidity until ready for testing. To maintain moisture, vegetables were sprayed with tap water once daily until ready for testing.

On the day of testing, samples were retrieved from the refrigerator, rinsed using tap water, and excess water was eliminated using a salad spinner (Oxo International, Ltd., New York, NY). Random leaves of similar visual characteristics and with no deterioration were used for evaluation.  Samples were then served to the panelists monadically on 6" foam plates identified with a three-digit code to reduce bias associated with knowing the name of the vegetable.  In order to test a consistent sample of each leaf, panelists were instructed to hold the sample with both hands, fold the leaf, and bite one time through the middle of the folded leaf. To focus the evaluation on the leaf section of the vegetable, stems and large ribs were cut from the vegetable.  For small leaves, panelists were asked to sample 1 – 3 whole leaves depending on leaf size. Because of different leaf sizes, amounts served to panelists were different across vegetable types. For example, panelists received three sprigs of small leaf vegetables such as parsley, cilantro, and watercress; three leaves of medium leaf plants such as spinach or curly endive; and half a leaf cut lengthwise through the middle (not including the stem or rib sections) was served of larger leaves such as collards or romaine lettuce.

### *Panelists*

Six highly trained panelists from the Sensory Analysis Center at Kansas State University (Manhattan, Kans., U.S.A.) participated in this study. The panelists had completed more than 120 hours of descriptive training, average more than 2000 hours of testing experience, and had prior experience testing vegetables and vegetable products.

### *Evaluation Procedures*

A method adapted from the flavor profile method (Caul, 1957; Keane 1992) was used. The method is a panel consensus method whereby the panelists must agree on attributes, definitions, and key reference products.  Our adaptation uses a 0-15 point scale divided in 0.5 point increments, 0 meaning "none" and 15 meaning "extremely high". This methodology has been previously used to describe a wide variety of products such as cheese (Heisserer and Chambers, 1993; Rètiveau *et al*., 2005), black walnut syrup (Matta *et al*., 2005), rose apples (Vara-Ubol *et al*., 2006) and tomatoes (Hongsoongnern and Chambers, 2008b).

During lexicon development, panelists were asked to review previously developed terms used to describe "green" aroma characteristics (Hongsoongnern and Chambers, 2008a) and green tea (Lee and Chambers, 2007).  Those beginning lexicons were used to help start the creation of a lexicon to describe flavor characteristics of a wide variety of fresh leafy vegetables. Discussion of the terms helped ensure the use of terms that are discriminative, descriptive, and nonredundant; key characteristics of a good flavor lexicon (Drake and Civille, 2003). When new terms were needed, panelists were also asked to discuss terminology, select an appropriate term, develop a descriptive definition, and to assign one or more reference materials that could be helpful in understanding the attribute and evaluating intensity. Lexicon development sessions were 90-minutes long and up to six samples were evaluated in each session depending on the complexity of flavor, carry over, and if new terms needed to be discussed, defined, and referenced. Products were reviewed multiple times during the lexicon development phase of the study.

After lexicon development, complete profiles were generated for each of the same fresh leafy vegetables used during lexicon development. Using a similar evaluation procedure, samples were evaluated once more and a consensus profile was developed. At that point, a new sample was evaluated until the set of 30 samples was complete. No actions were taken to mask appearance differences between samples.

### *Analysis*

Statistical analysis is required to clarify attribute relationships in the lexicon development process (Drake and Civille, 2003). Principal component analysis (PCA) was used on the consensus profiles to assess the relationships between terms. PCA was conducted on the correlation matrix and the data was orthogonally rotated to facilitate interpretation of the results. This analysis was performed by SAS® (2002, version 9.1.3; SAS Institute, Cary, NC).

## Results and Discussion

### *Lexicon Development and Validation*

A lexicon of thirty-two attributes (Table 3.1) was developed to describe flavor characteristics of fresh leafy vegetables consumed in the United States. From this list of attributes, five attributes specifically describe the green flavor of vegetables and were adapted from Hongsoongnern and Chambers (2008a). Those authors related the occurrence of these flavor characteristics to the presence of certain chemicals such as hexanal, *cis*-3-hexen-1-ol, 1-penten-3-ol, 2-isobutylthiazole, citronellal, *trans*-2-hexenal, *trans*-2-hexen-1-ol,

## Table 3.1. Lexicon to describe flavor characteristics of fresh leafy vegetables

| Attribute | Definition | References and Intensities[1] |
|---|---|---|
| Overall Green[2] | Aromatic characteristics of plant-based materials. A measurement of the total green characteristics and the degree to which they fit together. Green attributes include one or more of the following: green-unripe, green-peapod, green-grassy/leafy, green-viney, and green-fruity. These may be accompanied by musty/earthy, pungent, astringent, bitter, sweet, sour, floral, beany, minty, and piney. | Hexanal in Propylene Glycol (5,000ppm) = 5.0 (aroma) [2]<br>2-isobutylthiazole in Propylene Glycol = 7.0 (aroma) [2]<br>Fresh Parsley Water = 7.0 (flavor)<br>25 g chopped fresh curly parsley soaked in 300 ml room temperature de-ionized water for 15 minutes, filtered. |
| Green-Unripe[2] | A green aromatic associated with unripe or not-fully-developed plant-based materials; characterized by increased sour, astringent, and bitter. | 1:1 Diluted Fresh Parsley Water + 0.2% Alum Solution (1:1) = 3.0 (flavor)<br>Fresh parsley water preparation: Same as above |
| Green-Peapod[2] | A green aromatic associated with green peapods and raw green beans; characterized by increased musty/earthy. | Kroger Frozen Baby Lima Beans (thawed) = 6.0 (flavor, aroma) |
| Green Grassy/Leafy[2] | A green aromatic associated with newly cut-grass and leafy plants; characterized by sweet and pungent characters. | Hexanal in Propylene Glycol = 3.5 (aroma) [2]<br>Kroger Fresh Spinach = 4.5 (flavor)<br>Fresh Parsley Water = 7.0 (flavor)<br>Fresh parsley water preparation: Same as above |
| Green-Viney[2] | A green aromatic associated with green vegetables and newly cut vines and stems; characterized by increased bitter and musty/earthy character. | Hexanal in Propylene Glycol = 3.5 (aroma) [2]<br>1/8 inch Sliced Fresh Cucumber = 5.0 (flavor, aroma)<br>2-isobutylthiazole in Propylene Glycol = 7.0 (aroma) [2] |

| | | |
|---|---|---|
| Cabbage | The green, somewhat sharp, slightly sulfur, sometimes sweet, pungent aromatics associated with raw cabbage and Brussels sprouts. | Green Cabbage water = 5.5 (flavor)<br>25 g chopped fresh green cabbage soaked in 300 ml room temperature de-ionized water for 15 minutes, filtered. |
| Celery | The slightly sweet, green, brown, slightly bitter aromatics associated with dried celery leaves. | Fresh Celery water = 5.5 (flavor)<br>25 g chopped fresh celery soaked in 300 ml room temperature de-ionized water for 15 minutes, filtered. |
| Lettuce | Green, slightly musty and sometimes bitter water-like aromatics associated with lettuce like Bibb and Iceberg. | Lettuce water = 4.0 (flavor)<br>25 g chopped fresh Iceberg Lettuce soaked in 300 ml room temperature de-ionized water for 15 minutes, filtered. |
| Spinach | The brown, green, slightly musty, earthy aromatics associated with fresh spinach. | Spinach water = 3.0 (flavor)<br>25 g chopped fresh spinach soaked in 300 ml room temperature de-ionized water for 15 minutes, filtered. |
| Parsley | The clean fresh green, bitter, pungent aromatics associated with fresh parsley. | Fresh Parsley water = 5.5 (flavor)<br>25 g chopped fresh curly parsley soaked in 300 ml room temperature de-ionized water for 15 minutes, filtered. |
| Beet | The dark, musty, dusty, earthy aromatics reminiscent of fresh beets. | Del Monte Sliced Beets Juice diluted w/ water (1:1) = 6.0 (aroma) |
| Radish | The sharp, pungent, somewhat bitter aromatics associated with a fresh radish. | Fresh Radish water = 3.5 (flavor)<br>25 g chopped fresh radish soaked in 300 ml room temperature de-ionized water for 15 minutes, filtered. |
| Citrus[3] | The aromatics associated with commonly known citrus fruits, such as lemons, limes, oranges, could also contain a peely note. | Lemon Lime Juice = 4.0 (flavor)<br>1 Part of equal amounts of lemon and lime juices with 24 of water<br>McCormick Lemon Grass = 4.5 (aroma)<br>Weight 0.1 g of McCormick Lemon Grass. Place in a medium snifter. Add 100 mL of room temperature water. Cover. |
| Piney[2] | Aromatics reminiscent of resinous pine tree; can be medicinal or disinfectant in character. | Diamond raw Pine Nuts = 4.0 (flavor, aroma) |
| Woody | Brown, musty aromatics associated with very fibrous plants and bark. | Asparagus Stem (fresh 1/2" piece) = 6.0 (flavor) |

|  |  |  |
|---|---|---|
| Water-like | Liquid perception during mastication of some fruits and vegetables such as watermelon, peaches, tomatoes, and lettuce. | Asparagus Stem (fresh 1/2” piece) = 3.0<br>Dole Pineapple Tidbits = 7.5<br>Del Monte Mandarin Oranges = 12.0 |
| Musty/Earthy | Aromatics associated with damp, wet soil. | Hexanal in Propylene Glycol = 2.0 (aroma)[2]<br>2-isobutylthiazole in Propylene Glycol = 2.5 (aroma)*<br>Kroger Frozen Baby Lima Beans (thawed) = 3.0 (flavor)<br>Fresh chopped mushrooms = 8.5 (flavor, aroma) |
| Floral[2] | Sweet, light, slightly perfumey impression associated with flowers | Welch’s White Grape Juice = 5.0 (flavor)<br>Dilute with water 1:1 |
| Sulfur | The aromatics associated with hydrogen sulfide resulting from the heating of eggs or egg products. | Warm chopped hard boiled eggs = 3.0 (flavor), 7.0 (aroma).<br>Hard boil 1 egg for 9 minutes. Peel, chop, and place 1 Tbsp (even yolk-egg white ratio) in 1 oz. cups. |
| Metallic | An aromatic and mouth feel associated with tin cans or aluminum foil. | Dole Canned Pineapple Juice, Unsweetened = 6.0 (flavor) |
| Soapy | An aromatic commonly found in unscented hand soap. | Ivory Bar Soap Dilution = 6.5 (aroma)<br>Place 0.5 g of bar soap in 100 ml of room temperature |
| Petroleum-like | A specific chemical aromatic associated with crude oil and its refined products that have heavy oil characteristics. | Vaseline Petroleum Jelly = 3.0 (aroma) |
| Pungent | The sharp aromatics with a physically penetrating sensation in the nose reminiscent of radish and horseradish. | 2-isobutylthiazole in Propylene Glycol = 4.0 (aroma)*<br>Fresh Radish = 4.0<br>Slice radish thin (1/8”)<br>Heinz White Vinegar (1 part vinegar with 8 parts of water) = 8.0<br>Reese Horseradish = 12.0 |
| Bite | The slight burning, prickling and/or numbness of the tongue and/or mouth surface. | Fresh Radish = 4.0<br>Slice radish thin (1/8”)<br>Heinz White Vinegar (1 part vinegar with 8 parts of water) = 8.0<br>Reese Horseradish = 12.0 |

| | | |
|---|---|---|
| Tooth-etch | A chemical feeling factor perceived as drying/dragging when the tongue is rubbed over the back of the tooth surface. | 0.1% alum solution = 4.0<br>Welch's Grape Juice = 6.0<br>Dilute with water 1:1<br>0.2% alum solution = 9.0 |
| Heat/burn | A chemical feeling factor described as a burning sensation perceived on the tongue and mouth surface. | Radish = 2.0<br>Slice radish thin (1/8")<br>0.4 ppm capsaicin solution = 5.0 |
| Sweet, Overall | Aromatics associated with the impression of sweet substances such as fruit or flowers. (Note: This refers to the aromatics of sweetness rather than the sweet taste). | Asparagus (fresh 1/2" piece) = 2.0 (flavor)<br>Edible pea pods = 5.0 (flavor) |
| Sour | The fundamental taste sensation of which citric acid is typical. | 0.015% Citric Acid solution = 1.5<br>0.025% Citric Acid solution = 2.5 |
| Bitter | A basic taste factor of which caffeine is typical. | 0.01% caffeine solution = 2.0<br>0.02% caffeine solution = 3.5<br>0.035% caffeine solution = 5.0<br>0.05% caffeine solution = 6.5<br>0.06% caffeine solution = 8.5 |
| Salty | The fundamental taste factor of which sodium chloride in water is typical. | 0.15% Sodium Chloride Solution = 1.5 |
| Umami | Flat, salty flavor sometime thought of as brothy naturally occurring in products such as monosodium glutamate | 0.35% Accent Salt Solution = 7.5 |
| Astringent | The drying, puckering sensation on the tongue an other mouth surfaces. | 0.03% alum solution = 1.5<br>0.05% alum solution = 2.5<br>0.1% alum solution = 5.0 |

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[1] Intensities are based on a 15-point numerical scale with 0.5 increments, where 0 means "none" and 15 means "extremely strong."

[2] Adapted from Hongsoongnern & Chambers, 2008a

[3] Adapted from Lee & Chambers, 2007

*trans*-2-pentenal, geranyl formate, and heptyl butyrate. King *et al*., (2006) also mentioned (*E*)-2-hexenal and (*Z*)-3-hexen-1-yl acetate, in addition to hexanal, as responsible for providing an orthonasal "green" perception. The five terms related to "green" in our study are overall green, green-unripe, green-peapod, green-grassy/leafy, and green-viney. Of those attributes, overall green and green-grassy/leafy were present in all of the vegetables at moderate to high intensities while green-unripe, green-peapod, and green-viney were present more sporadically at lower intensities. For example, spinach was moderately green overall and green-grassy/leafy but had no intensity for green-unripe, green-peapod, and green-viney. Conversely, collard greens were moderate in overall green and green-grassy/leafy, but also had low intensities of green-peapod, green-viney, and green-unripe attributes (Table 3.2). The different types of lettuces exhibited similar "green" profiles with the exception of Romaine lettuce which received low intensity scores for both green-peapod and green-viney attributes. This lexicon can also be used to evaluate the effect that different production technologies have on the flavor of a selected variety of vegetables. For example, selected attributes from this lexicon were used by Park et al. (2009) to study differences in genetically modified lettuce.

Other leaf samples scored in the lower range for the green attributes. For example, the different cabbage varieties generally were low in overall green, green-peapod, and green-grassy/leafy. Arugula was moderate in overall green and green-peapod, and low in green-grassy/leafy and green-viney. The term "green" or "green-complex" has been previously used to describe flavor of frozen vegetable soybeans (Krinsky *et al*., 2006) and green tea (Lee and Chambers, 2007). The term "grass" has been used in the past to describe flavor of wine (Vilanova and Soto, 2005) and durian (Voon *et al*., 2007). Additionally, the term "green-leaf" was used to describe the sensory properties of virgin olive oil (Morales *et al*., 1995).

Attributes also were broken down into seven vegetable-like flavors to describe more specific flavor properties. Panelists had been instructed only to develop specific vegetable attributes when they believed that the perceived flavor characteristic was unique and could not be explained by using other terms including green. Lee and Chambers (2007) used six vegetable-like terms to describe flavor of green tea because they believed that using a single "green" term would be too general and may miss to describe more specific characteristics. Those authors used terms such as asparagus, Brussels sprouts, celery, green beans, parsley, and spinach. In this study, the terms used were cabbage, celery, lettuce, spinach, parsley, beet, and radish.

## Table 3.2. Flavor profiles for each of the thirty fresh leafy vegetables evaluated[1]

| Product | Plant Family | Overall Green | Green-Unripe | Green-Peapod | Green-Grassy/ Leafy | Green-Viney |
|---|---|---|---|---|---|---|
| Beet Greens (Organic) | Chenopodiaceae | 6.5 | 0.0 | 1.0 | 5.0 | 1.5 |
| Beet Greens, Golden (Organic) | Chenopodiaceae | 5.5 | 0.0 | 0.0 | 4.0 | 2.0 |
| Spinach | Chenopodiaceae | 6.5 | 0.0 | 0.0 | 4.5 | 0.0 |
| Swiss Chard, Green | Chenopodiaceae | 6.5 | 0.0 | 0.0 | 4.5 | 2.0 |
| Swiss Chard, Red (Organic) | Chenopodiaceae | 5.5 | 0.0 | 0.0 | 4.0 | 1.5 |
| Endive, Belgian | Compositae | 3.5 | 1.0 | 0.0 | 2.0 | 2.0 |
| Endive, Curly | Compositae | 9.0 | 1.5 | 0.0 | 6.0 | 4.5 |
| Lettuce, Butterhead / Boston | Compositae | 5.0 | 0.0 | 0.0 | 4.5 | 0.0 |
| Lettuce, Crisphead / Iceberg | Compositae | 4.5 | 0.0 | 0.0 | 3.5 | 0.0 |
| Lettuce, Green Leaf | Compositae | 6.0 | 0.0 | 1.5 | 5.0 | 0.0 |
| Lettuce, Red Leaf | Compositae | 7.0 | 0.0 | 0.0 | 5.0 | 1.0 |
| Lettuce, Romaine / Cos | Compositae | 6.5 | 0.0 | 1.5 | 5.5 | 2.0 |
| Radicchio | Compositae | 2.5 | 1.0 | 0.0 | 0.0 | 2.5 |
| Arugula | Cruciferae | 6.0 | 0.0 | 4.0 | 2.0 | 2.5 |
| Cabbage, Green | Cruciferae | 4.0 | 0.0 | 2.5 | 2.0 | 0.0 |
| Cabbage, Napa | Cruciferae | 4.0 | 0.0 | 2.0 | 2.0 | 0.0 |
| Cabbage, Red | Cruciferae | 3.0 | 0.0 | 2.5 | 2.0 | 0.0 |
| Cabbage, Savoy | Cruciferae | 5.5 | 0.0 | 3.0 | 2.5 | 0.0 |
| Collard Greens | Cruciferae | 9.0 | 1.5 | 2.0 | 5.5 | 3.5 |
| Kale | Cruciferae | 7.0 | 0.0 | 2.0 | 4.0 | 3.5 |
| Kale, Lacinato | Cruciferae | 8.0 | 1.5 | 1.5 | 5.0 | 2.0 |
| Kale, Red | Cruciferae | 4.0 | 0.0 | 2.0 | 2.5 | 0.0 |
| Mustard Greens, Curly | Cruciferae | 8.5 | 0.0 | 0.0 | 5.5 | 5.5 |
| Pac Choi | Cruciferae | 8.0 | 0.0 | 2.0 | 4.5 | 3.5 |
| Pac Choi, Baby | Cruciferae | 6.0 | 0.0 | 1.5 | 2.5 | 2.5 |
| Turnip Greens | Cruciferae | 8.5 | 2.0 | 0.0 | 5.5 | 5.5 |
| Watercress | Cruciferae | 7.0 | 0.0 | 0.0 | 4.0 | 3.0 |
| Cilantro | Umbelliferae | 7.0 | 0.0 | 0.0 | 4.5 | 2.0 |
| Parsley, Curly | Umbelliferae | 9.0 | 1.0 | 0.0 | 7.5 | 2.5 |
| Parsley, Italian | Umbelliferae | 7.5 | 0.0 | 0.0 | 6.0 | 2.5 |

| Product | Plant Family | Cabbage | Celery | Lettuce | Spinach | Parsley | Beet |
|---|---|---|---|---|---|---|---|
| Beet Greens (Organic) | Chenopodiaceae | 0.0 | 0.0 | 2.0 | 2.0 | 0.0 | 2.0 |
| Beet Greens, Golden (Organic) | Chenopodiaceae | 0.0 | 1.5 | 1.5 | 3.0 | 0.0 | 0.0 |
| Spinach | Chenopodiaceae | 0.0 | 0.0 | 1.5 | 5.5 | 1.5 | 0.0 |
| Swiss Chard, Green | Chenopodiaceae | 0.0 | 0.0 | 2.0 | 0.0 | 1.0 | 0.0 |
| Swiss Chard, Red (Organic) | Chenopodiaceae | 0.0 | 0.0 | 1.5 | 2.0 | 0.0 | 3.5 |
| Endive, Belgian | Compositae | 0.0 | 0.0 | 2.5 | 0.0 | 0.0 | 0.0 |
| Endive, Curly | Compositae | 0.0 | 2.0 | 3.5 | 0.0 | 3.0 | 0.0 |
| Lettuce, Butterhead / Boston / Bibb | Compositae | 0.0 | 1.5 | 5.0 | 0.0 | 0.0 | 0.0 |
| Lettuce, Crisphead / Iceberg | Compositae | 0.0 | 0.0 | 5.0 | 0.0 | 0.0 | 0.0 |
| Lettuce, Green Leaf | Compositae | 0.0 | 0.0 | 5.0 | 0.0 | 0.0 | 0.0 |
| Lettuce, Red Leaf | Compositae | 0.0 | 1.5 | 5.0 | 1.5 | 0.0 | 0.0 |
| Lettuce, Romaine / Cos | Compositae | 0.0 | 1.5 | 5.0 | 1.5 | 0.0 | 0.0 |
| Radicchio | Compositae | 2.5 | 0.0 | 1.5 | 0.0 | 0.0 | 0.0 |
| Arugula | Cruciferae | 2.5 | 0.0 | 0.0 | 2.5 | 0.0 | 0.0 |
| Cabbage, Green | Cruciferae | 6.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Cabbage, Napa | Cruciferae | 4.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| Cabbage, Red | Cruciferae | 5.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Cabbage, Savoy | Cruciferae | 5.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Collard Greens | Cruciferae | 4.0 | 0.0 | 1.5 | 2.0 | 0.0 | 0.0 |
| Kale | Cruciferae | 3.0 | 0.0 | 1.5 | 1.5 | 2.0 | 0.0 |
| Kale, Lacinato | Cruciferae | 4.0 | 0.0 | 0.0 | 2.0 | 1.5 | 0.0 |
| Kale, Red | Cruciferae | 1.5 | 0.0 | 0.0 | 0.0 | 0.0 | 3.0 |
| Mustard Greens, Curly | Cruciferae | 2.5 | 1.0 | 2.0 | 1.5 | 1.5 | 0.0 |
| Pac Choi | Cruciferae | 3.0 | 0.0 | 4.0 | 1.0 | 1.5 | 0.0 |
| Pac Choi, Baby | Cruciferae | 3.0 | 0.0 | 0.0 | 1.5 | 0.0 | 0.0 |
| Turnip Greens | Cruciferae | 2.0 | 0.0 | 1.5 | 1.5 | 1.5 | 0.0 |
| Watercress | Cruciferae | 2.5 | 1.0 | 0.0 | 0.0 | 1.5 | 0.0 |
| Cilantro | Umbelliferae | 0.0 | 1.5 | 0.0 | 1.5 | 3.5 | 0.0 |
| Parsley, Curly | Umbelliferae | 0.0 | 1.0 | 0.0 | 0.0 | 9.0 | 0.0 |
| Parsley, Italian | Umbelliferae | 0.0 | 1.5 | 0.0 | 0.0 | 8.5 | 0.0 |

| Product | Plant Family | Radish | Citrus | Piney | Woody | Water-like | Musty/ Earthy |
|---|---|---|---|---|---|---|---|
| Beet Greens (Organic) | Chenopodiaceae | 0.0 | 0.0 | 0.0 | 2.0 | 1.5 | 3.0 |
| Beet Greens, Golden (Organic) | Chenopodiaceae | 0.0 | 0.0 | 0.0 | 1.5 | 1.5 | 2.0 |
| Spinach | Chenopodiaceae | 0.0 | 0.0 | 0.0 | 2.0 | 2.0 | 2.5 |
| Swiss Chard, Green | Chenopodiaceae | 1.0 | 0.0 | 0.0 | 2.0 | 2.0 | 2.5 |
| Swiss Chard, Red (Organic) | Chenopodiaceae | 0.0 | 0.0 | 1.0 | 3.0 | 2.0 | 4.5 |
| Endive, Belgian | Compositae | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 1.0 |
| Endive, Curly | Compositae | 0.0 | 0.0 | 1.5 | 2.5 | 1.0 | 2.5 |
| Lettuce, Butterhead / Boston / Bibb | Compositae | 0.0 | 0.0 | 0.0 | 1.5 | 4.0 | 2.0 |
| Lettuce, Crisphead / Iceberg | Compositae | 0.0 | 0.0 | 0.0 | 1.0 | 4.5 | 1.5 |
| Lettuce, Green Leaf | Compositae | 0.0 | 0.0 | 0.0 | 1.5 | 3.5 | 2.0 |
| Lettuce, Red Leaf | Compositae | 0.0 | 0.0 | 0.0 | 2.5 | 2.5 | 2.5 |
| Lettuce, Romaine / Cos | Compositae | 0.0 | 0.0 | 0.0 | 2.0 | 2.5 | 2.0 |
| Radicchio | Compositae | 0.0 | 0.0 | 0.0 | 1.5 | 2.0 | 2.5 |
| Arugula | Cruciferae | 2.5 | 0.0 | 3.0 | 2.0 | 1.5 | 2.5 |
| Cabbage, Green | Cruciferae | 1.0 | 0.0 | 0.0 | 1.5 | 2.0 | 2.0 |
| Cabbage, Napa | Cruciferae | 2.0 | 0.0 | 0.0 | 1.5 | 2.5 | 1.5 |
| Cabbage, Red | Cruciferae | 2.5 | 0.0 | 0.0 | 2.0 | 2.0 | 2.5 |
| Cabbage, Savoy | Cruciferae | 1.0 | 0.0 | 0.0 | 2.0 | 2.0 | 2.0 |
| Collard Greens | Cruciferae | 1.5 | 0.0 | 2.0 | 3.0 | 1.5 | 3.0 |
| Kale | Cruciferae | 2.0 | 0.0 | 1.0 | 2.5 | 1.0 | 2.5 |
| Kale, Lacinato | Cruciferae | 1.5 | 0.0 | 1.5 | 2.0 | 1.0 | 2.5 |
| Kale, Red | Cruciferae | 0.0 | 0.0 | 0.0 | 3.0 | 1.0 | 5.0 |
| Mustard Greens, Curly | Cruciferae | 4.0 | 0.0 | 0.0 | 2.5 | 2.0 | 2.5 |
| Pac Choi | Cruciferae | 2.0 | 0.0 | 0.0 | 3.0 | 3.0 | 2.5 |
| Pac Choi, Baby | Cruciferae | 0.0 | 0.0 | 0.0 | 2.0 | 1.0 | 2.5 |
| Turnip Greens | Cruciferae | 3.0 | 0.0 | 2.0 | 2.5 | 1.0 | 3.0 |
| Watercress | Cruciferae | 4.5 | 0.0 | 1.5 | 2.0 | 1.5 | 2.0 |
| Cilantro | Umbelliferae | 0.0 | 1.0 | 2.5 | 2.0 | 1.0 | 3.0 |
| Parsley, Curly | Umbelliferae | 0.0 | 0.0 | 1.5 | 3.0 | 1.0 | 2.5 |
| Parsley, Italian | Umbelliferae | 0.0 | 0.0 | 1.5 | 2.5 | 1.0 | 2.0 |

| Product | Plant Family | Floral | Sulfur | Metallic | Soapy | Petroleum-like |
|---|---|---|---|---|---|---|
| Beet Greens (Organic) | Chenopodiaceae | 0.0 | 0.0 | 0.0 | 2.0 | 0.0 |
| Beet Greens, Golden (Organic) | Chenopodiaceae | 0.0 | 0.0 | 0.0 | 3.0 | 0.0 |
| Spinach | Chenopodiaceae | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Swiss Chard, Green | Chenopodiaceae | 0.0 | 1.5 | 0.0 | 2.5 | 0.0 |
| Swiss Chard, Red (Organic) | Chenopodiaceae | 0.0 | 0.0 | 0.0 | 3.0 | 0.0 |
| Endive, Belgian | Compositae | 0.0 | 0.0 | 1.5 | 0.0 | 0.0 |
| Endive, Curly | Compositae | 0.0 | 0.0 | 0.0 | 2.0 | 0.0 |
| Lettuce, Butterhead / Boston / Bibb | Compositae | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Lettuce, Crisphead / Iceberg | Compositae | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Lettuce, Green Leaf | Compositae | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Lettuce, Red Leaf | Compositae | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Lettuce, Romaine / Cos | Compositae | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Radicchio | Compositae | 0.0 | 1.5 | 1.5 | 2.0 | 1.0 |
| Arugula | Cruciferae | 0.0 | 0.0 | 0.0 | 1.5 | 2.0 |
| Cabbage, Green | Cruciferae | 0.0 | 2.0 | 0.0 | 0.0 | 0.0 |
| Cabbage, Napa | Cruciferae | 0.0 | 1.5 | 0.0 | 0.0 | 0.0 |
| Cabbage, Red | Cruciferae | 0.0 | 2.0 | 0.0 | 0.0 | 0.0 |
| Cabbage, Savoy | Cruciferae | 0.0 | 2.0 | 0.0 | 0.0 | 0.0 |
| Collard Greens | Cruciferae | 0.0 | 0.0 | 0.0 | 1.5 | 1.5 |
| Kale | Cruciferae | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 |
| Kale, Lacinato | Cruciferae | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| Kale, Red | Cruciferae | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Mustard Greens, Curly | Cruciferae | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Pac Choi | Cruciferae | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| Pac Choi, Baby | Cruciferae | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 |
| Turnip Greens | Cruciferae | 0.0 | 2.5 | 0.0 | 2.0 | 3.0 |
| Watercress | Cruciferae | 0.0 | 1.5 | 0.0 | 1.5 | 1.0 |
| Cilantro | Umbelliferae | 1.5 | 1.0 | 1.0 | 1.5 | 3.0 |
| Parsley, Curly | Umbelliferae | 0.0 | 0.0 | 0.0 | 2.0 | 1.5 |
| Parsley, Italian | Umbelliferae | 1.5 | 0.0 | 0.0 | 3.0 | 2.0 |

| Product | Plant Family | Pungent | Bite | Toothetch | Heat/burn | Sweet, Overall |
|---|---|---|---|---|---|---|
| Beet Greens (Organic) | Chenopodiaceae | 0.0 | 1.0 | 2.0 | 0.0 | 1.5 |
| Beet Greens, Golden (Organic) | Chenopodiaceae | 0.0 | 0.0 | 1.5 | 0.0 | 1.5 |
| Spinach | Chenopodiaceae | 0.0 | 0.0 | 2.0 | 0.0 | 1.5 |
| Swiss Chard, Green | Chenopodiaceae | 0.0 | 0.0 | 0.0 | 0.0 | 1.5 |
| Swiss Chard, Red (Organic) | Chenopodiaceae | 0.0 | 0.0 | 1.5 | 0.0 | 1.5 |
| Endive, Belgian | Compositae | 0.0 | 0.0 | 0.0 | 0.0 | 1.5 |
| Endive, Curly | Compositae | 0.0 | 2.0 | 0.0 | 0.0 | 1.0 |
| Lettuce, Butterhead / Boston / Bibb | Compositae | 0.0 | 0.0 | 0.0 | 0.0 | 2.0 |
| Lettuce, Crisphead / Iceberg | Compositae | 0.0 | 0.0 | 0.0 | 0.0 | 2.0 |
| Lettuce, Green Leaf | Compositae | 0.0 | 0.0 | 0.0 | 0.0 | 2.0 |
| Lettuce, Red Leaf | Compositae | 0.0 | 0.0 | 1.5 | 0.0 | 1.5 |
| Lettuce, Romaine / Cos | Compositae | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 |
| Radicchio | Compositae | 1.0 | 2.5 | 1.5 | 0.0 | 1.0 |
| Arugula | Cruciferae | 1.5 | 2.0 | 1.5 | 2.0 | 1.5 |
| Cabbage, Green | Cruciferae | 1.0 | 1.5 | 0.0 | 0.0 | 1.5 |
| Cabbage, Napa | Cruciferae | 1.0 | 2.0 | 0.0 | 0.0 | 2.0 |
| Cabbage, Red | Cruciferae | 0.0 | 2.0 | 1.0 | 0.0 | 1.5 |
| Cabbage, Savoy | Cruciferae | 0.0 | 1.0 | 0.0 | 0.0 | 1.5 |
| Collard Greens | Cruciferae | 1.5 | 2.5 | 2.0 | 0.0 | 1.0 |
| Kale | Cruciferae | 0.0 | 1.5 | 1.5 | 0.0 | 1.5 |
| Kale, Lacinato | Cruciferae | 0.0 | 1.5 | 1.5 | 0.0 | 1.5 |
| Kale, Red | Cruciferae | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 |
| Mustard Greens, Curly | Cruciferae | 4.5 | 6.5 | 0.0 | 4.5 | 1.5 |
| Pac Choi | Cruciferae | 1.0 | 1.5 | 0.0 | 0.0 | 2.0 |
| Pac Choi, Baby | Cruciferae | 0.0 | 1.5 | 1.5 | 0.0 | 2.0 |
| Turnip Greens | Cruciferae | 1.5 | 3.0 | 2.0 | 0.0 | 1.0 |
| Watercress | Cruciferae | 3.0 | 5.0 | 1.5 | 3.5 | 1.5 |
| Cilantro | Umbelliferae | 0.0 | 1.5 | 1.5 | 0.0 | 1.5 |
| Parsley, Curly | Umbelliferae | 0.0 | 0.0 | 1.0 | 0.0 | 1.5 |
| Parsley, Italian | Umbelliferae | 0.0 | 1.0 | 0.0 | 0.0 | 1.5 |

| Product | Plant Family | Sour | Bitter | Salty | Umami | Astr.[2] |
|---|---|---|---|---|---|---|
| Beet Greens (Organic) | Chenopodiaceae | 1.0 | 3.5 | 1.0 | 1.5 | 2.5 |
| Beet Greens, Golden (Organic) | Chenopodiaceae | 2.0 | 4.0 | 3.0 | 2.0 | 2.5 |
| Spinach | Chenopodiaceae | 1.5 | 2.5 | 1.0 | 1.0 | 2.0 |
| Swiss Chard, Green | Chenopodiaceae | 1.5 | 3.0 | 1.5 | 2.5 | 2.0 |
| Swiss Chard, Red (Organic) | Chenopodiaceae | 1.5 | 4.0 | 2.0 | 2.5 | 2.0 |
| Endive, Belgian | Compositae | 1.5 | 5.5 | 1.0 | 0.0 | 2.5 |
| Endive, Curly | Compositae | 1.5 | 7.0 | 1.0 | 0.0 | 2.0 |
| Lettuce, Butterhead / Boston / Bibb | Compositae | 1.0 | 2.5 | 0.0 | 1.5 | 1.5 |
| Lettuce, Crisphead / Iceberg | Compositae | 1.0 | 2.5 | 0.0 | 0.0 | 1.0 |
| Lettuce, Green Leaf | Compositae | 1.0 | 3.0 | 1.0 | 1.5 | 1.5 |
| Lettuce, Red Leaf | Compositae | 1.5 | 2.5 | 1.0 | 1.5 | 2.0 |
| Lettuce, Romaine / Cos | Compositae | 1.0 | 3.0 | 1.0 | 1.5 | 2.5 |
| Radicchio | Compositae | 1.5 | 8.0 | 1.0 | 1.5 | 2.5 |
| Arugula | Cruciferae | 1.5 | 3.5 | 1.5 | 2.5 | 2.5 |
| Cabbage, Green | Cruciferae | 1.5 | 3.5 | 0.0 | 1.0 | 2.0 |
| Cabbage, Napa | Cruciferae | 1.0 | 3.0 | 1.0 | 2.0 | 1.5 |
| Cabbage, Red | Cruciferae | 1.5 | 4.0 | 0.0 | 0.0 | 2.0 |
| Cabbage, Savoy | Cruciferae | 1.5 | 3.5 | 0.0 | 0.0 | 2.0 |
| Collard Greens | Cruciferae | 2.0 | 8.5 | 1.0 | 0.0 | 3.5 |
| Kale | Cruciferae | 1.5 | 5.0 | 1.0 | 2.0 | 2.5 |
| Kale, Lacinato | Cruciferae | 2.0 | 6.0 | 1.0 | 2.0 | 3.0 |
| Kale, Red | Cruciferae | 1.5 | 3.5 | 1.0 | 1.5 | 2.0 |
| Mustard Greens, Curly | Cruciferae | 2.0 | 7.0 | 1.0 | 2.0 | 2.0 |
| Pac Choi | Cruciferae | 1.5 | 4.5 | 1.0 | 2.0 | 2.5 |
| Pac Choi, Baby | Cruciferae | 2.0 | 3.5 | 1.5 | 1.5 | 3.0 |
| Turnip Greens | Cruciferae | 1.5 | 9.0 | 0.0 | 0.0 | 3.5 |
| Watercress | Cruciferae | 1.5 | 6.5 | 1.0 | 1.5 | 2.5 |
| Cilantro | Umbelliferae | 2.0 | 4.5 | 1.0 | 2.0 | 2.0 |
| Parsley, Curly | Umbelliferae | 1.0 | 5.5 | 1.0 | 2.0 | 2.0 |
| Parsley, Italian | Umbelliferae | 1.5 | 6.5 | 1.0 | 2.5 | 2.0 |

[1] Intensities are based on a 15-point numerical scale with 0.5 increments, where 0 means “none” and 15 means “extremely strong.”

[2] Astr. stands for Astringent

Interestingly, these terms did not necessarily describe actual vegetables tested in this study (e.g. celery) and also varied in intensity within vegetable type. For example, among all the different types of cabbages evaluated, green cabbage was considered as having the most cabbage-like flavor while napa cabbage was the least. Other vegetables with cabbage-like notes were pac choi, collard greens, and kale. All of the samples that showed a cabbage-like flavor belong to the Cruciferae family. The only exception to this rule was radicchio which belongs to the Compositae family and was slightly cabbage-like. On the other hand, the different types of lettuces were all scored similarly at the moderate level for the lettuce-like flavor. Cilantro, curly endive, and kale scored at a low intensity for the parsley-like flavor (other than both Italian and curly parsleys which received a high intensity score). Watercress and curly mustard greens scored at a moderate level for radish-like flavor. Other vegetables such as turnip greens, red cabbage, and arugula were low in radish-like flavor. Golden beet greens and arugula scored at a low intensity for the spinach flavor. Celery flavor was present at low levels in a few samples such as curly endive and Italian parsley. Finally, red Swiss chard, red kale, and beet greens were the only ones to score for the beet-like flavor. All the samples with "beet-like" notes were pigmented mainly because of the presence of betacyanins (also present in beets), which also provide an antioxidant benefit to human health (Nagy Gasztonyi *et al*., 2001). A very particular characteristic of the beet-like flavor is the "earthy" note which is believed to be caused by geosmin, an organic compound which is believed to be synthesized by the beet root itself (Lu *et al*., 2003). In our study, beet and earthy/musty flavors were correlated (r = 0.79).

Other terms adapted from Hongsoongnern and Chambers (2008a) are piney and floral. Piney was found in eleven samples at low intensities. Among these samples, the ones with the highest intensities were arugula, cilantro, turnip greens, and collard greens. Floral was found in only two samples, cilantro and Italian parsley, at very low intensities. The citrus attribute was adapted from Lee and Chambers (2007) who used this attribute to describe flavor of green tea. The attributes citrus and floral were found only in cilantro and Italian parsley. Those characteristics are important because they differentiated those two small leafy greens from the other fresh leafy vegetables.

Sulfur is an attribute reminiscent of hard boiled eggs which is present in vegetables mainly from the Cruciferae family including cabbage probably because of the presence of many sulfur compounds in vegetables from that family (Bailey *et al*., 1961). The samples with the highest sulfur notes were turnip greens, and cabbages from the savoy, red, and green varieties. Other samples with lower intensity of sulfur were green Swiss chard, radicchio, and napa cabbage.

Other terminology included was to describe mouthfeel attributes such as pungent, bite, toothetch, heat/burn, and astringent, and the basic tastes sweet, sour, bitter, salty, and umami. All these terms have been previously used to describe flavor of products with "green" characteristics (Hongsoongnern and Chambers, 2008a; Lee and Chambers, 2007; Morales *et al*., 1995).

## *Attribute Relationships*

Principal component analysis was conducted to evaluate the relationships among attributes. Nine factors accounting for 86% of the total variability between samples were used. Selection was based on the Kaiser criterion in which only eigenvalues above one are considered. This criterion has been previously used when evaluating terms used to describe green tea (Lee and Chambers, 2007) and cheese (Talavera-Bianchi and Chambers, 2008). In some instances, when attributes group in a single factor they may be redundant if they are present in the same products, at similar levels, and represent similar sensory experiences. However, attribute grouping may not represent redundancy, but merely the *general* tendency of particular attributes to change similarly over a large group of products. It is necessary to review the correlated attributes to determine whether any are redundant and can be eliminated from further testing.

Factor 1 groups bitter, astringent, green-unripe, and green-viney attributes. Bitter taste and astringent were both correlated to green-unripe (r = 0.76 and 0.57) and green-viney (r = 0.78 and 0.61) respectively. Both green-unripe and green-viney must be kept separate because they explain different characteristics and some samples that were green-viney were not green-unripe. For example, mustard greens and watercress samples were green-viney and bitter at a moderate level but were not considered green-unripe. In the same way, even though bitter scored at a lower level, pac choi and kale samples were green-viney and not green-unripe. On the other hand, turnip and collard greens were very bitter, astringent, green-viney, and slightly green-unripe. Lacinato kale was also bitter, astringent, slightly green-unripe and slightly green-viney. The green, bitter, and astringent attributes previously have been grouped in a single factor (with a positive correlation) when evaluating green tea (Lee and Chambers, 2007), but those authors also suggested that the terms were not synonymous and needed to be evaluated separately. Two more attributes present in this factor were water-like and overall sweet (negatively correlated). That negative correlation suggests that when samples were green-unripe, green-viney, bitter, and astringent, they were not considered as water-like and sweet overall or viceversa. For example, all the lettuce samples scored at a low to moderate level for water-like and overall sweet but generally were not considered green-unripe and/or green-viney. Only the red leaf and romaine samples were considered as green-viney at very low levels. They were also slightly bitter and slightly astringent. Lee and Chambers (2007) also reported that overall sweet was negatively correlated with “green”, perhaps because sugars begin to form as products continue to ripen, which might result in less “green” character.

The second factor groups attributes mostly related to mouth feel sensations such as heat/burn, bite, pungent, and radish-like flavor. This means that these attributes are normally present at the same time. However, they did so at different intensities. For example, turnip greens were considered as radish-like, biting, slightly pungent, with no heat/burn. Curly endive was considered only as slightly biting. Green Swiss chard was perceived only as slightly radish-like. Sometimes, factors can group attributes that scored in the moderate to high range for only a few samples or even a single sample, again indicating that the attributes are not redundant, but are grouped or correlated for other reasons. Watercress and mustard green both scored moderately for the attributes present in factor two which may be the reason why these attributes grouped together.

Factor three grouped attributes such as cabbage-like, green-peapod, and sulfur. This may be caused by the cabbage samples which scored at low to moderate levels for these three attributes. Sulfur may be considered as a characteristic flavor of cabbage because of the high content of sulfur compounds present in this vegetable (Bailey *et al.*, 1961). However, cabbage-like flavor also includes green, sweet, and pungent notes. This is why some vegetables were considered as cabbage-like but did not have a sulfur flavor. For example, kale, baby pac choi, and collard greens were considered as having a cabbage-like flavor with little or no sulfur flavor. On the other hand, green Swiss chard and cilantro had a slight sulfur note and no cabbage-like flavor. Also, green-peapod was not always found in samples that were cabbage-like and sulfurous. For example, green leaf and romaine lettuces were slightly green-peapod and had neither cabbage-like nor sulfur notes. Arugula had a moderate intensity of green-peapod but was only slightly cabbage-like and no sulfur. Celery-like and lettuce-like attributes were negatively correlated to this factor. This is probably due to the lettuce and curly endive samples which were low to moderate on celery-like and lettuce-like but hardly scored for green-peapod, cabbage-like, and/or sulfur.

The fourth factor groups citrus, floral, petroleum-like, and piney. This grouping may be caused by the cilantro sample, which had a unique flavor compared to the rest of the samples evaluated and scored at low to moderate intensities for all these attributes. Some other samples scored sporadically for these attributes. For example, Arugula was piney and petroleum-like but neither citrus nor floral. Both kale samples and curly endive were slightly piney. Italian parsley was slightly floral, slightly petroleum-like, and slightly piney but no citrus. Citrus and floral have also been grouped in the same factor in the past (Lee and Chambers, 2007), but clearly represent different sensory experiences.

The fifth factor grouped green-grassy/leafy, overall green, woody, and parsley-like. "Green" and "grassy" attributes have been previously used together to describe aroma of products such as virgin olive oil (Morales *et al*., 1995) and durian (Voon *et al*., 2007). Panelists perceived that many times, the majority of the overall green character of the samples was specifically related to green-grassy/leafy. This is especially true for the parsley samples that scored at a high intensity for both. This is the reason why the parsley-like flavor also is included in this factor. However, in some cases, overall green was explained by notes other than green-grassy/leafy and parsley-like. For example, the cabbage samples scored moderately for overall green while green-peapod and green-grassy/leafy were scored at a lower intensity. The parsley-like attribute was not present in any of the cabbage samples. Arugula also scored at a moderate level for overall green but was perceived as more green-peapod and green-viney than green-grassy/leafy and was not parsley-like. Parsley samples scored the highest for the woody attribute in comparison to the other samples evaluated which also explains the presence of woody in this factor. Woody was also high in samples such as red kale, red Swiss chard, and pac choi. The metallic attribute correlated negatively in this factor. Cilantro, radicchio, and Belgian endive were the only samples to have a slight metallic note present. It is important to note that radicchio and Belgian endive were the samples with the lowest overall green score among all the samples evaluated. This suggests that samples with "green" characteristics are usually not metallic.

Factor six grouped only beet-like and musty/earthy flavors. Even though all samples with beet-like notes were also musty/earthy, not all samples with the musty/earthy character were considered as beet-like. Only the pigmented samples red Swiss chard, beet greens, and red kale were considered as beet-like. As previously explained, organic compounds in the beet root can explain the earthy flavors in this type of plant.

Factor seven groups salty, umami, and soapy attributes which are mostly present in samples such as golden and red beet greens, Italian and curly parsley, and red Swiss chard. Factor eight grouped spinach-like and tooth-etch flavors which were present in spinach, golden and red beet greens, and red Swiss chard. Some variations were also present. For example, radicchio was perceived as slightly tooth-etch but no spinach-like while romaine lettuce was slightly spinach-like with no tooth-etch. Finally, sour taste is correlated alone with factor nine and was present in all of the samples at low intensities.

In summary, the thirty-two attributes used are important to describe flavor of fresh leafy vegetables and should be kept separate. Some attributes such as overall green, green-grassy/leafy, musty/earthy, overall sweet, and bitter are present in all the samples and describe more general flavor characteristics varying in intensity among samples. Other attributes such as citrus, floral, metallic, petroleum-like, and soapy are more sporadically present and can be used to describe samples with unique flavor characteristics such as cilantro or parsley. The attributes used by certain studies will depend on the type of vegetable used and the need to describe general characteristics such as overall green or more specific characteristics such as citrus or floral.

## Conclusions

Thirty-two attributes were selected, defined, and referenced by a highly trained descriptive panel to explain the flavor characteristics of fresh leafy vegetables commonly consumed in the United States. Additionally, this lexicon was validated and principal component analysis was conducted on the consensus data finding no major redundancies between attributes. This lexicon can be used to evaluate flavor of vegetables in a more specific manner avoiding the use of general terms such as “taste” or “typical flavor.”

# CHAPTER 4 - Effect of Organic Production and Fertilizer Variables on the Sensory Properties of Pac Choi (*Brassica rapa* cv. Mei Qing Choi) and Tomato (*Solanum lycopersicum* cv. Bush Celebrity)

## Abstract

The increased popularity of organic production has amplified the need for research that will help in understanding how this production system affects the final quality of vegetables. The effects of organic and conventional production on the sensory characteristics of pac choi (often called bok choy) and tomato were studied. Samples were grown in two environments at the Kansas State University Horticulture Research Center located in Olathe, Kansas. Highly trained descriptive panelists from the Sensory Analysis Center at Kansas State University used previously developed flavor lexicons for tomatoes and leafy greens to evaluate the samples. Crispness, green-grassy/leafy, piney, and pungent attributes were normally higher in conventional pac choi only for the field samples. Pac choi grown in high tunnels showed slight differences only at certain levels of fertilizer. Organic tomatoes grown in the field were generally juicier and less mealy compared to conventionally grown tomatoes. In the high tunnel, tomatoes were generally stronger in tomato aroma. However, all differences generally were very small. It can be concluded that organic and conventional production systems do not create major sensory differences in the vegetables evaluated. The few differences that were detected were so small they may not be of practical importance.

## Practical Applications

The increased popularity of organic production has amplified the need for research that will help in understanding how this production system affects the final quality of food products and more specifically vegetables. This study suggested no major differences between organic and conventional production systems applied in two environments and using three levels of fertilizer.

# Introduction

Organic foods are produced using more environmentally friendly conditions than foods produced conventionally (Zhao *et al*., 2006). Organic production uses crop rotation, cover crops and natural fertilizers and pesticides to maintain long term soil fertility, minimize pollution and produce high quality products (Winter and Davis, 2006; Bourn and Prescott, 2002). However, studies of food quality seem inconsistent and show no clear trends on the effects that organic fertilization has on the final quality (Bourn and Prescott, 2002; Basker, 1992).

Several studies have been conducted to study the effects that organic production has on the chemical, nutritional and sensory characteristics of foods. For example, some studies that are focused on the nutritional content of vegetables suggest that crops produced under organic methods may be richer in phenolic compounds and vitamin C (Zhao *et al*., 2006; Rembialkowska, 2007), lower in pesticide residues, and lower in nitrate content (Woese *et al*., 1997). Nonetheless, it is unknown if these differences are biologically significant or if they translate into quality differences perceivable by consumers (Winter and Davis, 2006). Thus far, research has shown that consumers do not find sensory difference between organically and conventionally grown vegetables (Schutz and Lorenz, 1976; Zhao *et al*., 2007a).

Similarly, the differences between organic and conventional products for individual sensory characteristics detected by trained panelists remain inconsistent and show no clear patterns at this point. For example, Haglund *et al*. (1999) found that conventional carrots had a higher "carrot taste" while organic carrots were more bitter. DeEll and Prange (1992) suggested that organic apples were firmer than conventional apples at the time of harvest but did not differ in juiciness, sweetness or sourness. Similarly, Caussiol and Joyce (2004) showed that there were no flavor differences between organically and conventionally grown bananas. All these inconsistencies might exist because the differences between organic and conventional practices may be product specific and should be observed in individual categories rather than all together (Fillion and Arazi, 2002).

Certainly to study the effects of organic production on the quality of vegetables is difficult and complicated even further by the large number of factors that influence the quality of crops. Those factors include genotype, plant tissue, fruit size, stage of development, ripening, diseases and pests, soil condition, irrigation, light exposure and pesticide application (Zhao *et al*., 2006). Manipulating the amount of fertilizer can have an effect on the quality of vegetables because the amount of nitrogen the plant will absorb is modified, which could affect the plants' flavor, size and shelf life (Rembialkowska, 2007; Mattheis and Fellman, 1999). Because of the large number of variables involved in the production of vegetables, well managed and controlled cultivation tests are viewed by scientists as the most accurate way of studying the effects of organic farming practices (Woese *et al*., 1997).

The objective of this study is to evaluate the effects of production systems (i.e. organic and conventional) on the sensory characteristics of pac choi (also known as bok choy, pak choy or bai tsai) and tomatoes grown in two controlled environments (i.e. field and high tunnel) and using three concentrations of fertilizer (i.e. high, low and no fertilizer).

## Materials and Methods

### *Samples*

Trials were conducted at the K-State Horticulture Research and Extension Center, Olathe, Kansas, on experimental plots established in 2002 for comparison of crops grown under organic and conventional production systems in high tunnels (unheated, passively ventilated greenhouses) and open field plots (Zhao *et al*., 2007a). The soil was a Kennebec silt loam. Six 9.8 m x 6.1 m high tunnels with 1.5m sidewalls (Stuppy, North Kansas City, MO) and six adjacent 9.8 m x 6.1 m field plots were used for this study. High tunnels were covered with single layer 6-mil (0.153mm) K-50 polyethylene (Klerk's Plastic Product Manufacturing, Inc., Richburg, SC). At establishment of the experimental plots, the six high tunnels were divided into three groups (blocks) and the two high tunnels in each block were randomly assigned for long-term conventional or organic management treatments. A similar set-up was used in the field plots. Organic plots were managed in compliance with USDA National Organic Program standards, and were inspected and certified in 2003, 2006, 2007 and 2008.

For this study, beginning in 2007, each high tunnel or open field plot was subdivided into three 3.2 m x 6.1 m plots to which one of three fertilizer levels were assigned (high, low, and no fertilizer) following a latin square design to avoid bias due to position effects in the high tunnels. Fertilizer rates were determined based on soil analysis at the beginning of the study in 2007, and recommendations for vegetable crops in Kansas (Marr *et al*., 1998), with compost applied to organic plots and synthetic fertilizer applied to conventional plots. Compost application rates were based on the assumption that 50% of the nitrogen from compost would be available to plants during the growing season, while 100% would be available from conventional fertilizers (Warman and Havard, 1997). Low and high fertility plots were fertilized with equal amounts of compost or synthetic fertilizer at the beginning of the growing season, and high fertility plots received additional fertilization during the growing season as described below.

Pac choi (*Brassica rapa* L. *chinensis* 'Mei Qing Choi') (Johnny's Selected Seed, Albion, ME, U.S.A.) and tomato (*Lycopersicon esculentum* 'Bush Celebrity') (Totally Tomatoes, Randolph, WI, U.S.A.) were grown in one half of each open field or high tunnel plot (6.8 m x 3 m) in 2007 and 2008, with a rotation between pac choi and tomato plots each year. In our experimental system, a spring and a fall crop of pac choi was grown each year, while a single crop of tomato was grown. Between the spring and fall pac choi crops, plots were seeded with a summer cover crop of buckwheat (*Fagopyrum sagittatum*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 134 kg/ha. In the late fall, all plots were seeded with a cover crop of annual rye (*Secale cereale*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 229 kg/ha.

Conventional high and low fertility plots were fertilized with Jack's Professional Peat-lite N-$P_2O_5$-$K_2O$ 20-10-20 (Allentown, PA, U.S.A.), at a rate of 98 kg/ha. Organic plots received MicroLeverage compost N-$P_2O_5$-$K_2O$ 0.6-0.8-0.5 (Hughesville, MO, U.S.A) at a rate of 197 kg/ha. Starting 2 weeks after planting, high fertility plots received additional fertilization at a rate of 7.2 kg/ha. Fertilizer used on organic plots was fish hydrolyzate N-$P_2O_5$-$K_2O$ 2.23-4.35-0.3 (Neptune's Harvest, Gloucester, MA, U.S.A) and conventional plots received calcium nitrate and potassium nitrate at a rate calculated to apply an amount of calcium equivalent to that present in the fish hydrolyzate. The tomato crop received 6 weekly applications, for a total of 43 kg/ha, and the spring and fall pac choi crops each received three such applications.

Pac choi and tomato transplants were started in a greenhouse in Sunshine Mix Special Blend E6340 (SunGro Horticulture, Bellevue, WA) supplemented with MicroLeverge compost. The pac choi trial was planted on April 1 and harvested on May 5. The tomato trial was planted on May 5 and harvested on July 18. All testing occurred in 2008.

## *Sample Preparation*

### *Pac choi*

Plants were harvested one to three days before testing. After harvest, plants were immediately rinsed using cold tap water to remove excess dirt and stored in a refrigerated container for transport to the Kansas State University campus located in Manhattan, Kansas. Once samples arrived, they were moved to a walk-in refrigerator for storage at 4ºC until testing. The plants were sprayed daily with tap water to maintain moisture. The day of testing the plants were retrieved from the refrigerator. Random leaves of similar visual characteristics were removed from each stalk (not including the stem) of each treatment, rinsed using distilled water and excess water was eliminated using a salad spinner (Oxo International, Ltd., New York, NY). Samples were served to the panelists monadically in 6” foam plates identified with a three-digit code to eliminate potential panelist bias.

### *Tomatoes*

Tomatoes were harvested at the pink stage, three to six days before testing (United States Department of Agriculture [USDA], 1975). When harvested, samples were placed in labeled boxes for their transportation to the Kansas State University campus located in Manhattan, Kansas. Special care was taken when handling the tomatoes to avoid damage because it had been suggested that internal bruising may alter the quality and flavor of tomatoes (Moretti *et al.*, 2002). Once tomatoes arrived, they were organized in trays sorted by treatment and placed on a flat surface (no tomato on top of another) to avoid damage. On the day of testing three tomatoes at the red stage (USDA, 1975) with similar visual characteristics were selected from each treatment. Samples were washed thoroughly using tap water at room temperature and then cut in half lengthwise. One half of each tomato was cut in ½-inch wedges and served to the panelists in covered, odor-free 3.25 oz. plastic cups. Cups with the samples were labeled with a 3-digit code to avoid potential bias. The tomatoes were never refrigerated.

## *Panelists*

Six highly trained panelists from the Sensory Analysis Center at Kansas State University (Manhattan, Kans., U.S.A.) were selected for this study. The panelists had completed more than 120 hours of descriptive training, averaging more than 2000 hours of testing experience and had prior experience testing vegetables and vegetable products.

## *Evaluation Procedure*

Previously developed lexicons were used for this study. The lexicon for pac choi was developed by Talavera-Bianchi *et al.* (2009) to describe the flavor of different leafy vegetables and was produced using an adaptation of the flavor profile method (Caul, 1957; Keane, 1992) which has been used by many studies in the past to describe a variety of products such as cheese (Heisserer and Chambers, 1992; Retiveau *et al*., 2005), green tea (Lee and Chambers, 2007), tomatoes (Hongsoongnern and Chambers, 2008b) and green flavors (Hongsoongnern and Chambers, 2008a). The lexicon for tomatoes was previously developed by Hongsoongnern and Chambers (2008b) to describe flavor of fresh and processed tomatoes. Lexicons with definitions and references were presented to the panelists in one 90-minute session prior to the start of testing so they could become familiar with the terminology, test procedures and samples.

For testing, panelists were presented with the lexicon and references used during orientation. Data were collected using a computerized collection system (Compusense Five version 4.4.8, 2002, Guelph, ON, Canada). Intensities for each attribute were recorded using a 0-15 point scale divided in 0.5 point increments, 0 meaning none and 15 meaning extremely high. Panelists evaluated the samples individually and followed a completely randomized block design with replication as the blocking factor. Twelve samples of pac choi were evaluated in each of three 180-minute sessions. Twelve samples of tomatoes also were evaluated in another set of three 180-minutes sessions. Reverse osmosis, deionized, carbon-filtered water and unsalted crackers were used to rinse the palate between the samples. A similar procedure has been used in the past to evaluate the sensory characteristics of four samples of calcium-biofortified lettuce (Park *et al*., 2009)

### *Analysis*

Treatments were organized in a split plot design with production system (i.e. organic vs. conventional) as the whole plot and fertilizer amount (i.e. control, low and high) as the sub-plot. Analysis of variance (ANOVA) was used to detect significant differences between treatments for individual attributes. Principal component analysis (PCA) was used to evaluate relationships between treatments and to provide a graphic representation of the results. Cluster analysis (Ward method) was used to separate groups of similar sensory characteristics. This analysis was computed in SAS® (2002, version 9.1.3; SAS Institute, Cary, NC). Because of the complexity of the design, plots for field vs. high-tunnel production were not randomized and thus a statistical comparison of those data is not made.

## Results and Discussion

### *Pac choi*

Results indicated few differences between organic and conventional production systems. In addition, the few differences found were generally small (Table 4.1). Additionally, differences between organic and conventional pac choi were present only in field plants. Green-grassy/leafy, piney and pungent attributes were higher (P-value $\leq 0.05$) in conventional pac choi than organically produced pac choi. However the differences were quite small ranging from 0.2-0.4 points. Organic and conventional production systems applied in the fields also were compared at varying concentrations of fertilizer (Table 4.2). For example, the green-grassy/leafy attribute was significantly different when high and no fertilizer were applied. There were no differences when a low concentration of fertilizer was applied. Crispness and moistness attributes were higher for conventional pac choi only when a high concentration of fertilizer was applied. Again, the differences were small, approximately 0.5.

In the high tunnels a few small differences were present between organic and conventional pac choi for individual fertilizer concentrations only (Table 4.3). For example, Soapy and petroleum-like attributes were higher for organic pac choi only when no fertilizer was applied in a high tunnel. When low concentrations of fertilizer were applied the conventional pac choi was more petroleum-like and bitter. In addition, conventional pac choi was more sweet overall when no fertilizer was applied.

**Table 4.1. Sensory differences between organic and conventional pac choi grown in the field and high tunnel**

| Attribute | Environment | Organic | Conventional |
|---|---|---|---|
| **Crispness** | **Field[1]** | **3.0b** | **3.3a** |
| | High Tunnel | 3.5 | 3.5 |
| Moistness | Field | 4.0 | 4.0 |
| | High Tunnel | 4.0 | 4.1 |
| Fiber Awareness | Field | 4.1 | 4.2 |
| | High Tunnel | 4.4 | 4.5 |
| Green Overall | Field | 5.9 | 6.2 |
| | High Tunnel | 6.3 | 6.3 |
| Green-Unripe | Field | 1.1 | 1.1 |
| | High Tunnel | 0.9 | 0.9 |
| Green-Peapod | Field | 0.4 | 0.5 |
| | High Tunnel | 0.5 | 0.5 |
| **Green-Grassy/Leafy** | **Field[1]** | **4.8b** | **5.2a** |
| | High Tunnel | 5.3 | 5.3 |
| Green-Viney | Field | 1.9 | 1.9 |
| | High Tunnel | 2.0 | 2.0 |
| Cabbage | Field | 2.5 | 2.6 |
| | High Tunnel | 2.7 | 2.7 |
| Lettuce | Field | 1.9 | 1.8 |
| | High Tunnel | 1.8 | 1.8 |
| Spinach | Field | 2.0 | 1.9 |
| | High Tunnel | 2.1 | 1.9 |
| Parsley | Field | 1.3 | 1.5 |
| | High Tunnel | 1.4 | 1.3 |
| Radish | Field | 2.0 | 2.0 |
| | High Tunnel | 2.2 | 2.2 |
| **Piney** | **Field[1]** | **0.5b** | **0.8a** |
| | High Tunnel | 1.0 | 0.8 |
| Woody | Field | 1.5 | 1.7 |
| | High Tunnel | 1.9 | 1.8 |
| Water-like | Field | 1.8 | 1.8 |
| | High Tunnel | 1.8 | 1.8 |
| Musty/Earthy | Field | 2.2 | 2.3 |
| | High Tunnel | 2.4 | 2.5 |
| Sulfur | Field | 1.6 | 1.8 |
| | High Tunnel | 2.1 | 2.0 |
| Soapy | Field | 1.0 | 1.2 |
| | High Tunnel | 1.5 | 1.4 |
| Petroleum-like | Field | 0.3 | 0.5 |
| | High Tunnel | 0.8 | 0.7 |
| **Pungent** | **Field[1]** | **1.9b** | **2.1a** |
| | High Tunnel | 2.3 | 2.2 |
| Bite | Field | 2.0 | 2.1 |
| | High Tunnel | 2.3 | 2.4 |

|  |  |  |  |
|---|---|---|---|
| Tooth etch | Field | 1.9 | 2.0 |
|  | High Tunnel | 2.1 | 2.1 |
| Overall Sweet | Field | 1.3 | 1.4 |
|  | High Tunnel | 1.1 | 1.3 |
| Sour | Field | 1.5 | 1.6 |
|  | High Tunnel | 1.7 | 1.7 |
| Bitter | Field | 6.7 | 6.7 |
|  | High Tunnel | 6.9 | 7.1 |
| Salty | Field | 0.4 | 0.5 |
|  | High Tunnel | 0.5 | 0.5 |
| Umami | Field | 2.0 | 2.0 |
|  | High Tunnel | 2.0 | 2.0 |
| Astringent | Field | 1.8 | 1.8 |
|  | High Tunnel | 1.9 | 2.0 |

[1] Significant differences at 95% confidence.

**Table 4.2. Sensory differences between organic and conventional pac choi grown in the field at three fertilizer levels**

| Attribute | Fertilizer Amount | Organic | Conventional |
|---|---|---|---|
| **Crispness** | Control | 2.9 | 3.0 |
| | Low | 3.2 | 3.3 |
| | **High[1]** | **2.8b** | **3.5a** |
| **Moistness** | Control | 4.1 | 3.8 |
| | Low | 4.1 | 4.2 |
| | **High[1]** | **3.8b** | **4.2a** |
| Fiber Awareness | Control | 3.9 | 3.8 |
| | Low | 4.3 | 4.2 |
| | High | 4.2 | 4.6 |
| Green Overall | Control | 5.9 | 6.0 |
| | Low | 5.9 | 6.1 |
| | High | 6.0 | 6.3 |
| Green-Unripe | Control | 1.4 | 1.3 |
| | Low | 1.0 | 0.9 |
| | High | 0.9 | 1.0 |
| Green-Peapod | Control | 0.4 | 0.6 |
| | Low | 0.4 | 0.4 |
| | High | 0.4 | 0.5 |
| **Green-Grassy/Leafy** | **Control[1]** | **4.7b** | **5.2a** |
| | Low | 5.0 | 5.0 |
| | **High[1]** | **4.8b** | **5.4a** |
| Green-Viney | Control | 1.8 | 1.7 |
| | Low | 1.8 | 2.0 |
| | High | 2.1 | 2.0 |
| Cabbage | Control | 2.4 | 2.5 |
| | Low | 2.6 | 2.6 |
| | High | 2.6 | 2.7 |
| Lettuce | Control | 2.0 | 1.9 |
| | Low | 1.9 | 1.8 |
| | High | 1.7 | 1.7 |
| Spinach | Control | 2.0 | 1.9 |
| | Low | 2.0 | 2.0 |
| | High | 1.9 | 2.0 |
| Parsley | Control | 1.2 | 1.4 |
| | Low | 1.4 | 1.5 |
| | High | 1.3 | 1.5 |
| Radish | Control | 1.9 | 1.9 |
| | Low | 1.9 | 1.9 |
| | High | 2.1 | 2.2 |
| Piney | Control | 0.3 | 0.6 |
| | Low | 0.4 | 0.8 |
| | High | 0.8 | 1.1 |

|  |  |  |  |
|---|---|---|---|
| Woody | Control | 1.2 | 1.4 |
|  | Low | 1.5 | 1.7 |
|  | High | 1.7 | 2.1 |
| Water-like | Control | 1.9 | 1.8 |
|  | Low | 1.9 | 1.8 |
|  | High | 1.8 | 1.8 |
| Musty/Earthy | Control | 1.9 | 2.2 |
|  | Low | 2.3 | 2.3 |
|  | High | 2.3 | 2.5 |
| Sulfur | Control | 1.3 | 1.6 |
|  | Low | 1.7 | 1.7 |
|  | High | 1.9 | 2.1 |
| Soapy | Control | 0.8 | 1.1 |
|  | Low | 1.2 | 1.3 |
|  | High | 1.0 | 1.3 |
| Petroleum-like | Control | 0.2 | 0.3 |
|  | Low | 0.3 | 0.5 |
|  | High | 0.4 | 0.7 |
| Pungent | Control | 1.4 | 1.8 |
|  | Low | 2.0 | 2.1 |
|  | High | 2.1 | 2.4 |
| Bite | Control | 1.7 | 1.9 |
|  | Low | 2.1 | 2.2 |
|  | High | 2.1 | 2.3 |
| Toothetch | Control | 1.8 | 1.8 |
|  | Low | 2.0 | 2.1 |
|  | High | 1.9 | 2.1 |
| Overall Sweet | Control | 1.4 | 1.4 |
|  | Low | 1.3 | 1.5 |
|  | High | 1.3 | 1.3 |
| Sour | Control | 1.4 | 1.5 |
|  | Low | 1.5 | 1.6 |
|  | High | 1.6 | 1.6 |
| Bitter | Control | 6.3 | 6.3 |
|  | Low | 7.0 | 6.6 |
|  | High | 6.8 | 7.2 |
| Salty | Control | 0.4 | 0.4 |
|  | Low | 0.4 | 0.6 |
|  | High | 0.4 | 0.5 |
| Umami | Control | 1.9 | 1.9 |
|  | Low | 2.1 | 2.1 |
|  | High | 2.0 | 2.1 |
| Astringent | Control | 1.8 | 1.7 |
|  | Low | 1.8 | 1.8 |
|  | High | 1.8 | 1.9 |

[1] Significant differences at 95% confidence.

**Table 4.3. Sensory differences between organic and conventional pac choi grown in the high tunnel at three fertilizer levels**

| Attribute | Fertilizer Amount | Organic | Conventional |
|---|---|---|---|
| Crispness | Control | 3.5 | 3.4 |
| | Low | 3.6 | 3.8 |
| | High | 3.6 | 3.3 |
| Moistness | Control | 4.1 | 4.1 |
| | Low | 4.0 | 4.3 |
| | High | 3.9 | 3.9 |
| Fiber Awareness | Control | 4.3 | 4.4 |
| | Low | 4.5 | 4.6 |
| | High | 4.4 | 4.4 |
| Green Overall | Control | 6.4 | 6.3 |
| | Low | 6.2 | 6.3 |
| | High | 6.2 | 6.3 |
| Green-Unripe | Control | 0.9 | 0.9 |
| | Low | 0.9 | 0.9 |
| | High | 1.0 | 0.9 |
| Green-Peapod | Control | 0.4 | 0.6 |
| | Low | 0.5 | 0.4 |
| | High | 0.5 | 0.5 |
| Green-Grassy/Leafy | Control | 5.4 | 5.3 |
| | Low | 5.2 | 5.4 |
| | High | 5.1 | 5.2 |
| Green-Viney | Control | 1.8 | 1.8 |
| | Low | 2.0 | 2.1 |
| | High | 2.1 | 1.9 |
| Cabbage | Control | 2.6 | 2.6 |
| | Low | 2.8 | 2.7 |
| | High | 2.8 | 2.7 |
| Lettuce | Control | 1.8 | 1.8 |
| | Low | 1.9 | 1.8 |
| | High | 1.6 | 1.7 |
| Spinach | Control | 2.0 | 1.9 |
| | Low | 2.1 | 2.0 |
| | High | 2.0 | 2.0 |
| Parsley | Control | 1.4 | 1.4 |
| | Low | 1.4 | 1.3 |
| | High | 1.3 | 1.2 |
| Radish | Control | 2.3 | 2.2 |
| | Low | 2.1 | 2.2 |
| | High | 2.3 | 2.1 |
| Piney | Control | 1.2 | 0.9 |
| | Low | 1.0 | 1.1 |
| | High | 0.9 | 0.7 |

| | | | |
|---|---|---|---|
| Woody | Control | 1.8 | 1.8 |
| | Low | 1.9 | 1.9 |
| | High | 1.9 | 1.7 |
| Water-like | Control | 1.8 | 1.9 |
| | Low | 1.8 | 1.9 |
| | High | 1.8 | 1.8 |
| Musty/Earthy | Control | 2.3 | 2.5 |
| | Low | 2.4 | 2.5 |
| | High | 2.5 | 2.4 |
| Sulfur | Control | 2.1 | 2.0 |
| | Low | 2.1 | 2.2 |
| | High | 2.1 | 1.8 |
| **Soapy** | **Control**[1] | **1.6a** | **1.3b** |
| | Low | 1.3 | 1.6 |
| | High | 1.5 | 1.3 |
| **Petroleum-like** | **Control**[1] | **1.2a** | **0.6b** |
| | **Low**[1] | **0.4b** | **0.9a** |
| | High | 0.8 | 0.6 |
| Pungent | Control | 2.3 | 2.3 |
| | Low | 2.2 | 2.3 |
| | High | 2.3 | 2.0 |
| Bite | Control | 2.3 | 2.4 |
| | Low | 2.3 | 2.5 |
| | High | 2.3 | 2.2 |
| Toothetch | Control | 2.2 | 2.1 |
| | Low | 2.1 | 2.2 |
| | High | 2.0 | 2.0 |
| **Overall Sweet** | **Control**[1] | **1.2b** | **1.5a** |
| | Low | 1.2 | 1.2 |
| | High | 1.0 | 1.3 |
| Sour | Control | 1.7 | 1.6 |
| | Low | 1.6 | 1.8 |
| | High | 1.8 | 1.7 |
| **Bitter** | Control | 7.1 | 7.0 |
| | **Low**[1] | **6.6b** | **7.3a** |
| | High | 6.9 | 7.1 |
| Salty | Control | 0.7 | 0.5 |
| | Low | 0.5 | 0.5 |
| | High | 0.4 | 0.4 |
| Umami | Control | 2.1 | 2.1 |
| | Low | 2.0 | 2.1 |
| | High | 2.0 | 1.8 |
| Astringent | Control | 1.9 | 2.0 |
| | Low | 1.9 | 2.0 |
| | High | 2.0 | 1.9 |

[1] Significant differences at 95% confidence.

Different results between plants grown in the field and high tunnels may exist because plants grown in the field are exposed to a number of environmental factors such as temperature changes, weather, wind, light, and disease which may affect their final quality (Zhao *et al*., 2007a; Peirce, 1987; Antonious *et al*., 1996). In our study it appears that when plants are more protected in the high tunnels, they have even fewer small sensory differences than field grown pac choi perhaps because they are growing more similarly. For example, Antonious *et al*. (1996) suggested that that even a small difference in light reflecting from mulches of different colors had an effect on the flavor strength of growing turnip plants because it varied the amount of glucosinolates, compounds frequently found in plants of the Cruciferous family.

It is important to stress that the few sensory differences found between production systems and among fertilizer amounts were very small and may not be detected by untrained consumers. Previous testing has shown that a panel of untrained consumers did not find sensory differences between organic and conventional produce (Zhao *et al*., 2007a). The few differences found may be related to stage of maturity at the time of harvest which can be affected by many of the factors included in this experiment. After genotype, plant maturity at the time of harvest may be the most important factor affecting quality of vegetables (Kader, 2008).

Principle component analysis (Figure 4.1) shows the distribution of the pac choi samples grown under different treatments in the attribute space. Cluster analysis suggests that the samples are separated into two general groups, based mainly on environmental conditions (field and high tunnel). It has been shown in the past that the effect of a protected environment on final plant quality outweighs the effect of fertilizer (Zhao *et al*., 2007a). In our study all the plants grown in the high tunnel grouped together while the samples grown in the field, with the exception of one sample, grouped together in the other cluster. Interestingly, the sample that failed to group with the other field plants and grouped with the high tunnel plants instead was the one that had been treated with a high amount of conventional fertilizer. This may suggest that, because this plant had higher amounts of nutrients available, it was able to develop more than the other field plants. This translated into flavor characteristics more similar to the plants grown in the high tunnels. The plants grown in the high tunnel, including the field plant produced with conventional high fertilizer, had numerically higher intensities of attributes such as crispness, sulfur, green overall or woody. On the other hand, the field plants and especially the organic plants with no fertilizer had seemingly higher intensities of green-unripe, lettuce and sweet overall. These results may

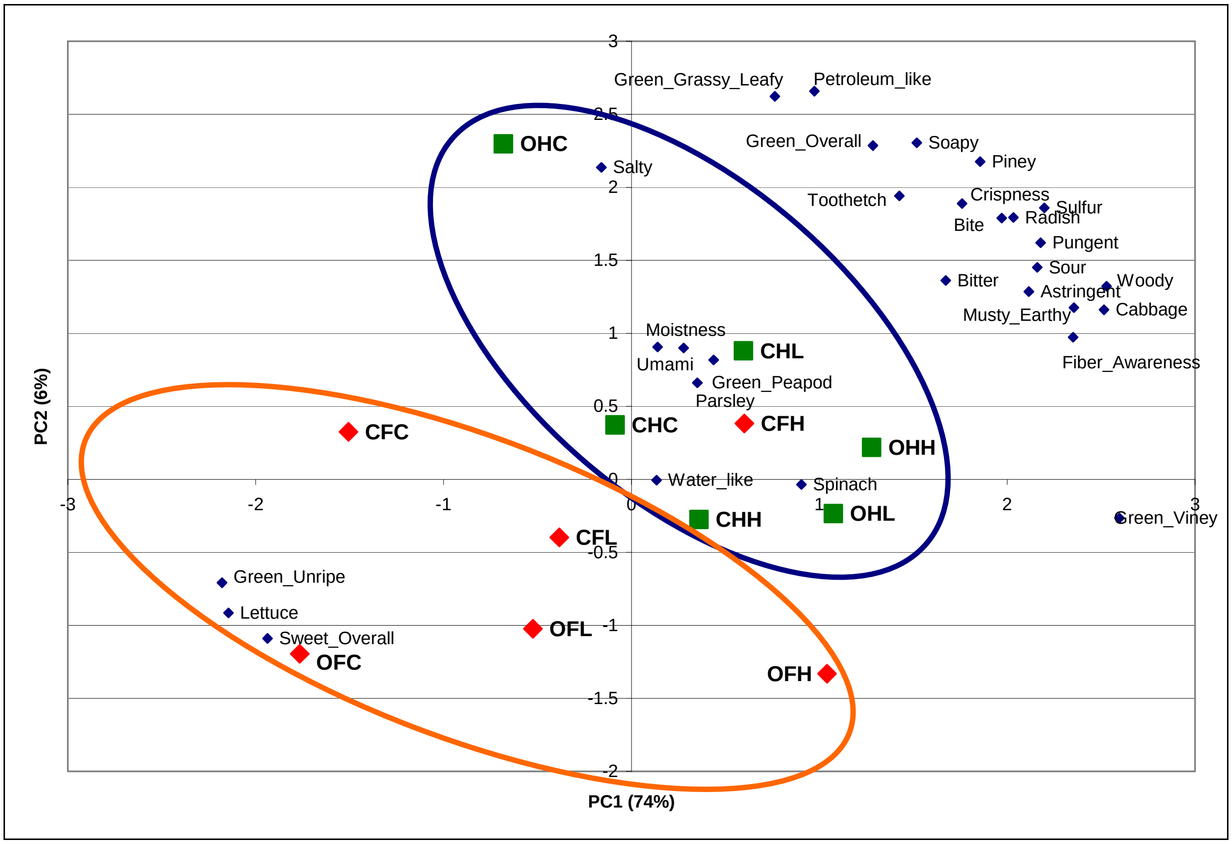

**Figure 4.1. Principal component and cluster analyses of sensory attributes for pac choi grown in two environments (field and high tunnel), two production systems (organic and conventional), and three amounts of fertilizer (high, low, and no fertilizer [control]). CFC=conventional-field-control; CFL=conventional-field-low fertilizer; CFH=conventional-field-high fertilizer; OFC=organic-field-control; OFL=organic-field-low fertilizer; OFH=organic-field-high fertilizer; CHC=conventional-high tunnel-control; CHL=conventional-high tunnel-low fertilizer; CHH=conventional-high tunnel-high fertilizer; OHC=organic-high tunnel-control; OHL=organic-high tunnel-low fertilizer; OHH=organic-high tunnel-high fertilizer.**

suggest that the differences observed in this study are linked to the maturity of the plant at the time of harvest rather than fertilizer amount or environmental conditions. Plants grown in the high tunnel and plants with higher amounts of fertilizer where able to develop more and therefore had different flavor characteristics. The influence of maturity level at the time of harvest is a critical factor as it affects flavor because production of flavor and aroma volatiles changes as ripening progresses (Mattheis and Fellman, 1999).

This study suggests that flavor and texture differences between organic and conventional pac choi are few and quite small if found. Prior research has hypothesized that flavor could be changed by organic production because organic production may increase the chance of insect attack, therefore increasing the concentration of phenolic compounds which effect the flavor of pac choi (Young *et al*., 2005). The content of phenolic compounds has been positively correlated with bitterness and astringency (Mondy *et al*., 1971).

### ***Tomato***

Results indicate that out of the twenty-five sensory attributes evaluated, only three showed significant overall differences (P-value ≤ 0.05) between organic and conventionally grown tomatoes (Table 4.4). When tomatoes were grown in the field, the organic tomatoes generally were more juicy and less mealy compared to conventionally grown tomatoes. When the plants were grown in the high tunnels, organic tomatoes had a higher tomato aroma than conventional tomatoes. However, these differences were small. Differences also were studied for individual amounts of fertilizer (Table 4.5). For plants grown in the field, the difference in juiciness favoring the organic plants was higher for tomatoes to which no fertilizer was applied (control). Similarly, the mealy attribute was higher for conventional tomatoes only when a low concentration of fertilizer was applied. In addition, there was a very slight cardboard flavor in organic tomatoes only when low fertilizer was applied.

For tomatoes grown in a high tunnel, a few minor differences also were noted for individual amounts of fertilizer (Table 4.6). When no fertilizer was applied, conventionally grown tomatoes were higher in seed awareness and green-viney flavor while organic tomatoes were higher in ripeness perception. Organic tomatoes also were more mealy when tomatoes were grown with a high amount of fertilizer. It is important to stress that all of the differences found were rather small and may be related to environment or maturity. Differences between environments may exist because the high tunnels protect the plants against environmental factors such as weather and temperature changes, insect attacks or winds which may affect the quality of the plant by reducing stress (Zhao *et al*., 2007a).

Organic fertilization releases nutrients slower than conventional fertilization, which may cause reduced concentrations of sulfur and phosphorus in the leaves, limiting the yield and growth of organic tomatoes (Heeb *et al*., 2006). However, these limitations have not yet proven to affect the flavor of tomatoes. Our study does not show major differences or tendencies favoring one production system over the other. Organic tomatoes may show superior characteristics because they have a higher tomato aroma and are more juicy than conventional tomatoes. However, these differences are too small to provide clear conclusions. Superior taste of organic tomatoes is possible because

**Table 4.4. Sensory differences between organic and conventional tomato grown in the field and high tunnel**

| Attribute | Environment | Organic | Conventional |
|---|---|---|---|
| **Tomato Aroma** | Field | 7.8 | 7.8 |
| | **High Tunnel[1]** | **8.3a** | **8.1b** |
| Green-Viney Aroma | Field | 5.0 | 5.2 |
| | High Tunnel | 4.7 | 4.9 |
| Overripe Aroma | Field | 0.2 | 0.1 |
| | High Tunnel | 0.2 | 0.3 |
| Color | Field | 7.2 | 7.4 |
| | High Tunnel | 8.1 | 8.2 |
| Uniformity of Color | Field | 9.2 | 8.9 |
| | High Tunnel | 9.7 | 8.9 |
| **Juiciness** | **Field[1]** | **9.9a** | **9.3b** |
| | High Tunnel | 10.0 | 9.8 |
| **Mealy** | **Field[1]** | **2.6b** | **2.9a** |
| | High Tunnel | 3.2 | 2.9 |
| Skin Awareness | Field | 4.2 | 4.4 |
| | High Tunnel | 4.6 | 4.6 |
| Seed Awareness | Field | 2.3 | 2.3 |
| | High Tunnel | 2.6 | 2.9 |
| Fiber Awareness | Field | 4.1 | 4.3 |
| | High Tunnel | 3.8 | 3.9 |
| Tomato | Field | 8.0 | 7.9 |
| | High Tunnel | 8.6 | 8.6 |
| Ripeness | Field | 7.5 | 7.4 |
| | High Tunnel | 8.6 | 8.5 |
| Green-Viney | Field | 5.1 | 4.9 |
| | High Tunnel | 4.4 | 4.6 |
| Umami | Field | 1.9 | 1.8 |
| | High Tunnel | 2.3 | 2.2 |
| Fruity | Field | 2.9 | 2.8 |
| | High Tunnel | 3.3 | 3.2 |
| Cardboard | Field | 0.1 | 0.0 |
| | High Tunnel | 0.0 | 0.0 |
| Fermented | Field | 0.1 | 0.1 |
| | High Tunnel | 0.1 | 0.2 |
| Musty/Earthy | Field | 2.6 | 2.6 |
| | High Tunnel | 2.6 | 2.7 |
| Overall Sweet | Field | 2.7 | 2.6 |
| | High Tunnel | 3.1 | 3.0 |

|  |  |  |  |
|---|---|---|---|
| Sweet | Field | 1.4 | 1.3 |
|  | High Tunnel | 1.5 | 1.6 |
| Sour | Field | 2.9 | 2.8 |
|  | High Tunnel | 2.6 | 2.7 |
| Salty | Field | 1.4 | 1.3 |
|  | High Tunnel | 1.4 | 1.4 |
| Bitter | Field | 2.7 | 2.5 |
|  | High Tunnel | 2.4 | 2.6 |
| Astringent | Field | 1.8 | 1.7 |
|  | High Tunnel | 1.7 | 1.7 |
| Metallic | Field | 0.7 | 0.7 |
|  | High Tunnel | 0.7 | 0.7 |

[1] Significant differences at 95% confidence.

**Table 4.5. Sensory differences between organic and conventional tomatoes grown in the field at three fertilizer levels**

| Attribute | Fertilizer Amount | Organic | Conventional |
|---|---|---|---|
| Tomato Aroma | Control | 7.3 | 7.6 |
| | Low | 8.2 | 8.0 |
| | High | 7.9 | 7.9 |
| Green-Viney Aroma | Control | 5.1 | 5.4 |
| | Low | 5.1 | 5.1 |
| | High | 4.8 | 5.0 |
| Overripe Aroma | Control | 0.0 | 0.1 |
| | Low | 0.2 | 0.0 |
| | High | 0.4 | 0.3 |
| Color | Control | 7.1 | 7.2 |
| | Low | 7.5 | 7.5 |
| | High | 7.1 | 7.4 |
| Uniformity of Color | Control | 8.8 | 8.5 |
| | Low | 9.2 | 9.5 |
| | High | 9.6 | 8.7 |
| **Juiciness** | **Control**[1] | **9.6a** | **8.9b** |
| | Low | 10.0 | 9.4 |
| | High | 10.2 | 9.6 |
| **Mealy** | Control | 2.8 | 3.0 |
| | **Low**[1] | **2.4b** | **3.0a** |
| | High | 2.5 | 2.7 |
| Skin Awareness | Control | 4.2 | 4.2 |
| | Low | 4.1 | 4.8 |
| | High | 4.3 | 4.1 |
| Seed Awareness | Control | 2.2 | 2.1 |
| | Low | 2.3 | 2.6 |
| | High | 2.5 | 2.3 |
| Fiber Awareness | Control | 4.2 | 4.4 |
| | Low | 4.0 | 4.3 |
| | High | 3.9 | 4.0 |
| Tomato | Control | 7.6 | 7.6 |
| | Low | 8.3 | 8.0 |
| | High | 8.3 | 8.1 |
| Ripeness | Control | 7.1 | 7.0 |
| | Low | 7.7 | 7.5 |
| | High | 7.8 | 7.6 |
| Green-Viney | Control | 5.5 | 5.0 |
| | Low | 4.9 | 5.1 |
| | High | 4.8 | 4.5 |
| Umami | Control | 1.7 | 1.7 |
| | Low | 2.1 | 1.8 |
| | High | 2.0 | 1.9 |

| | | | |
|---|---|---|---|
| Fruity | Control | 2.7 | 2.6 |
| | Low | 2.9 | 2.9 |
| | High | 3.1 | 2.9 |
| **Cardboard** | Control | 0.0 | 0.0 |
| | **Low[1]** | **0.2a** | **0.0b** |
| | High | 0.0 | 0.0 |
| Fermented | Control | 0.0 | 0.1 |
| | Low | 0.1 | 0.2 |
| | High | 0.3 | 0.1 |
| Musty/Earthy | Control | 2.6 | 2.4 |
| | Low | 2.7 | 2.7 |
| | High | 2.6 | 2.6 |
| Overall Sweet | Control | 2.5 | 2.4 |
| | Low | 2.9 | 2.6 |
| | High | 2.8 | 2.7 |
| Sweet | Control | 1.3 | 1.2 |
| | Low | 1.4 | 1.3 |
| | High | 1.4 | 1.3 |
| Sour | Control | 3.0 | 2.9 |
| | Low | 2.9 | 2.6 |
| | High | 2.9 | 2.8 |
| Salt | Control | 1.4 | 1.3 |
| | Low | 1.3 | 1.3 |
| | High | 1.4 | 1.4 |
| Bitter | Control | 2.8 | 2.6 |
| | Low | 2.8 | 2.6 |
| | High | 2.5 | 2.3 |
| Astringent | Control | 1.8 | 1.8 |
| | Low | 1.8 | 1.8 |
| | High | 1.8 | 1.7 |
| Metallic | Control | 0.7 | 0.8 |
| | Low | 0.7 | 0.8 |
| | High | 0.8 | 0.7 |
| Chemical | Control | 0.0 | 0.1 |
| | Low | 0.0 | 0.0 |
| | High | 0.1 | 0.0 |

[1] Significant differences at 95% confidence.

**Table 4.6. Sensory differences between organic and conventional tomatoes grown in high tunnel at three fertilizer levels**

| Attribute | Fertilizer Amount | Organic | Conventional |
|---|---|---|---|
| Tomato Aroma | Control | 8.2 | 7.9 |
| | Low | 8.3 | 8.0 |
| | High | 8.5 | 8.2 |
| Green-Viney Aroma | Control | 4.7 | 4.9 |
| | Low | 4.7 | 4.9 |
| | High | 4.6 | 4.9 |
| Overripe Aroma | Control | 0.3 | 0.3 |
| | Low | 0.1 | 0.3 |
| | High | 0.2 | 0.2 |
| Color | Control | 7.9 | 8.3 |
| | Low | 8.1 | 8.2 |
| | High | 8.3 | 8.3 |
| Uniformity of Color | Control | 9.2 | 8.6 |
| | Low | 10.2 | 9.1 |
| | High | 9.6 | 9.0 |
| Juiciness | Control | 9.9 | 9.5 |
| | Low | 10.3 | 9.8 |
| | High | 9.8 | 10.1 |
| **Mealy** | Control | 3.3 | 3.3 |
| | Low | 3.1 | 2.8 |
| | **High**[1] | **3.2a** | **2.6b** |
| Skin Awareness | Control | 4.3 | 5.2 |
| | Low | 5.0 | 4.7 |
| | High | 4.9 | 3.9 |
| **Seed Awareness** | **Control**[1] | **2.6b** | **3.3a** |
| | Low | 2.5 | 2.8 |
| | High | 2.8 | 2.7 |
| Fiber Awareness | Control | 4.0 | 3.9 |
| | Low | 3.8 | 3.9 |
| | High | 4.0 | 3.9 |
| Tomato | Control | 8.6 | 8.2 |
| | Low | 8.7 | 8.6 |
| | High | 8.6 | 9.0 |
| **Ripeness** | **Control**[1] | **8.7a** | **7.8b** |
| | Low | 8.8 | 8.6 |
| | High | 8.3 | 8.9 |
| **Green-Viney** | **Control**[1] | **4.3b** | **5.0a** |
| | Low | 4.4 | 4.3 |
| | High | 4.6 | 4.4 |
| Umami | Control | 2.3 | 2.0 |
| | Low | 2.3 | 2.2 |
| | High | 2.2 | 2.4 |

|  |  |  |  |
|---|---|---|---|
| Fruity | Control | 3.3 | 3.0 |
|  | Low | 3.3 | 3.2 |
|  | High | 3.3 | 3.3 |
| Cardboard | Control | 0.0 | 0.0 |
|  | Low | 0.0 | 0.0 |
|  | High | 0.0 | 0.0 |
| Fermented | Control | 0.1 | 0.2 |
|  | Low | 0.2 | 0.4 |
|  | High | 0.0 | 0.1 |
| Musty/Earthy | Control | 2.5 | 2.7 |
|  | Low | 2.7 | 2.7 |
|  | High | 2.5 | 2.6 |
| Overall Sweet | Control | 3.1 | 2.8 |
|  | Low | 3.1 | 3.2 |
|  | High | 3.0 | 2.9 |
| Sweet | Control | 1.7 | 1.5 |
|  | Low | 1.5 | 1.5 |
|  | High | 1.5 | 1.7 |
| Sour | Control | 2.6 | 2.8 |
|  | Low | 2.7 | 2.5 |
|  | High | 2.6 | 2.8 |
| Salt | Control | 1.4 | 1.4 |
|  | Low | 1.4 | 1.4 |
|  | High | 1.3 | 1.4 |
| Bitter | Control | 2.5 | 2.5 |
|  | Low | 2.4 | 2.5 |
|  | High | 2.4 | 2.6 |
| Astringent | Control | 1.6 | 1.7 |
|  | Low | 1.6 | 1.7 |
|  | High | 1.7 | 1.7 |
| Metallic | Control | 0.7 | 0.7 |
|  | Low | 0.8 | 0.7 |
|  | High | 0.7 | 0.7 |
| Chemical | Control | 0.0 | 0.0 |
|  | Low | 0.0 | 0.0 |
|  | High | 0.0 | 0.0 |

[1] Significant differences at 95% confidence.

plants under organic conditions grow slower, which may generate lower concentrations of water and higher concentrations of sugars, acids, and volatiles, providing more of the characteristic ripe tomato flavor (Heeb *et al.*, 2006; Baldwin *et al.*, 2008). However, there is still no clear evidence to confirm these findings and Zhao *et al.* (2007a) showed that consumers did not find differences among leafy greens grown in organic vs. conventional conditions.

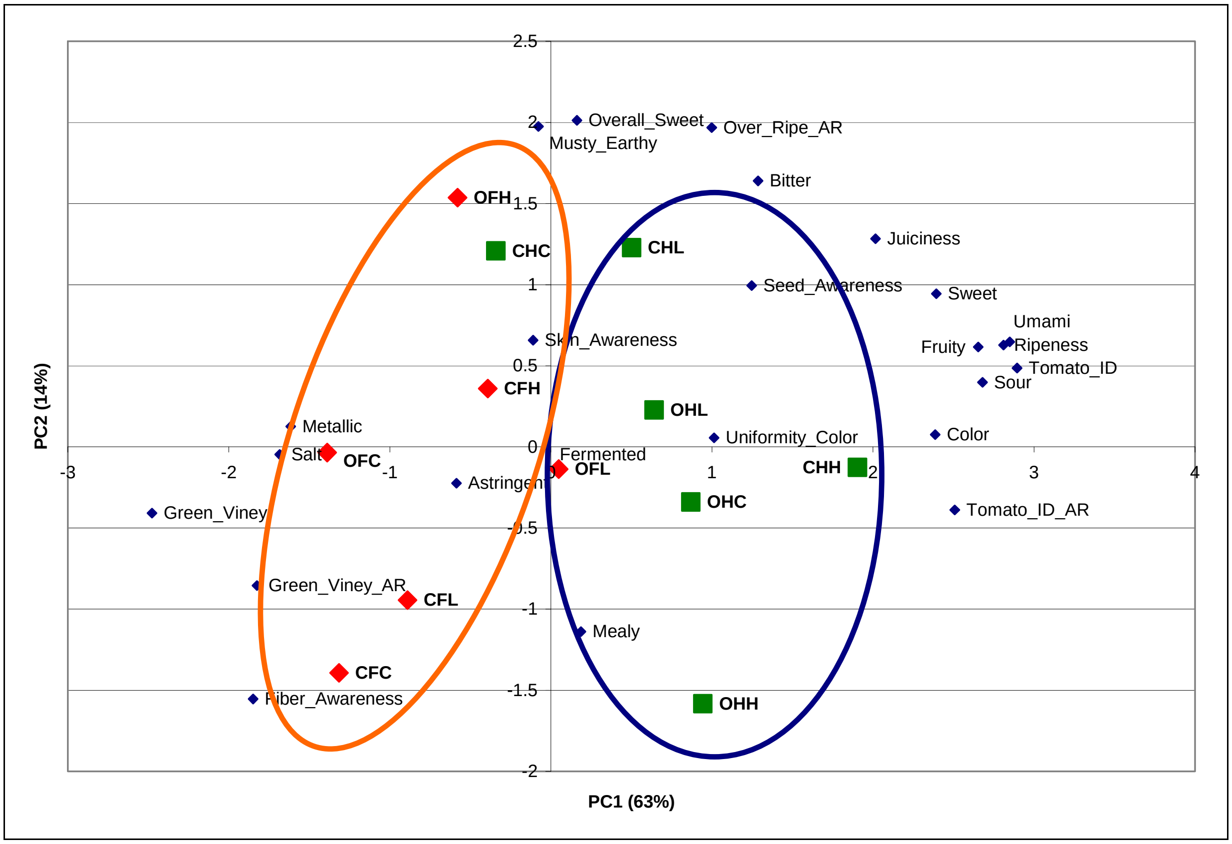

**Figure 4.2 – Principal component and cluster analyses of sensory attributes for tomato grown in two environments (field and high tunnel), two production systems (organic and conventional), and three amounts of fertilizer (high, low, and no fertilizer [control]). CFC=conventional-field-control; CFL=conventional-field-low fertilizer; CFH=conventional-field-high fertilizer; OFC=organic-field-control; OFL=organic-field-low fertilizer; OFH=organic-field-high fertilizer; CHC=conventional-high tunnel-control; CHL=conventional-high tunnel-low fertilizer; CHH=conventional-high tunnel-high fertilizer; OHC=organic-high tunnel-control; OHL=organic-high tunnel-low fertilizer; OHH=organic-high tunnel-high fertilizer.**

Principal component and cluster analyses again were used to map the different treatments and the sensory attributes evaluated in the tomato samples (Figure 4.2). Cluster analysis again suggested the existence of two groups with separation based mainly on the environment. All the tomatoes grown in the field grouped together with the exception of the low organic fertilizer, which was grouped with the high tunnel samples. All the high tunnel samples were grouped together with the exception of the no fertilizer conventional sample, which was grouped with the field samples. This may be related to the fact that the high tunnel sample with no fertilizer was not as ripe as the other samples of the same environment and had flavor characteristics more similar to the field samples. In the same way, the field sample with low fertilizer appears to have a riper flavor and therefore was more similar to the high tunnel samples than the field samples. Differences generally were small. As a general rule, field samples were better explained by attributes such as green-viney flavor and aroma, fiber awareness, metallic, salt and astringent, which may be characteristics of less ripe tomatoes. High tunnel samples are more juicy, sweet, umami, ripe, fruity and had more tomato flavor and aroma. This may be characteristic of tomatoes that are more mature. The flavor of fresh tomatoes is a combination of sugars, organic acids, free amino acids and salts, in addition to volatile chemicals that vary at different stages of maturity (Baldwin *et al*., 2008; Yilmaz, 2001). It also has been suggested that, while ripening, the increase of sugar concentration in tomatoes decreases the perception of green flavors, bitter and sour tastes (Baldwin *et al*., 2008). This may indicate, as observed with the pac choi, that the differences observed are more related to ripeness of the tomatoes. Samples grown in the high tunnel with higher amounts of fertilizer may have flavor more reminiscent of a ripe product (higher intensities of tomato aroma and flavor, ripeness, overall sweet and juiciness) compared to samples grown in the field with lower amounts of fertilizer which will have greener, more unripe flavors.

## Conclusions

There did not appear to be major differences between organic and conventional products at both comparison levels (i.e. overall comparison and at single concentrations of fertilizer). Furthermore, when differences were present, they generally were quite small and showed no clear trends or patterns favoring one production system over the other. Principal component and cluster analyses suggest that some differences existed in plant maturity between field and high tunnel crops. Plants grown in the high tunnel with higher amounts of fertilizer may have sensory characteristics more reminiscent of a mature product compared to samples grown in the field with lower amounts of fertilizer, which may have been less mature at the time of harvest. Results were similar for pac choi and tomato.

# CHAPTER 5 - Relation Between Developmental Stage, Sensory Properties, and Volatile Content of Organically and Conventionally Grown Pac Choi (*Brassica rapa* cv. Mei Qing Choi)

## Abstract

This study was conducted to identify and quantify the sensory characteristics and chemical profile of organically and conventionally grown pac choi (*Brassica rapa* var. Mei Qing Choi), also called bok choy, at three stages of growth (2.5, 4.5, and 6.5 weeks). Sensory and instrumental data were correlated using partial least squares regression (PLS). Pac choi was grown in late spring. Descriptive sensory analysis was conducted by a highly trained panel and compounds were identified and quantified using a gas chromatograph / mass spectrometer. The findings of the study indicated that the differences in sensory characteristics and chemical profiles among stages of growth were more substantial than the differences between organic and conventional production. Green-unripe, musty/earthy, lettuce, and sweet flavors were representative in pac choi at early stages of growth. When older, pac choi has higher intensities of green-grassy/leafy, bitter, cabbage, and sulfur flavors which are associated with the increase of (Z)-3-hexen-1-ol, octyl acetate, 1-nonanol, 2-decanone, 1-penten-3-ol, linalool, camphor, menthol, isobornyl acetate, geranylacetone, and cedrol compounds. Conventional pac choi was higher than organic pac choi in green overall, bitter, and soapy flavors only at 2.5 weeks of age. This may be associated with the presence of (Z)-3-hexenal, 2-hexyn-1-ol, and (E)-2-hexenal compounds.

## Practical Applications

The increased popularity of organic production has amplified the need for research that will help in understanding how this production system affects the final quality of food products. This study suggests that the stage of development has a much larger impact on sensory quality than organic or conventional growing of pac choi. Findings from this study promote consumer choice by showing that comparable sensory quality can be obtained using either production system making the ultimate choice not only based on sensory quality but consumer choice related to environmental beliefs or economics.

## Introduction

Zhao et al. (2006) suggests that organic foods are ones that have been produced by more environmentally friendly conditions. Crop rotation, cover crops, and natural products (such as natural fertilizes and pesticides) are used to enhance or maintain long-term soil fertility, minimize pollution, avoid synthetic fertilizers and pesticides, consider the social and economic impact, and produce higher quality products (Winter and Davis, 2006; Bourn and Prescott, 2002). However, results at this point seem inconsistent and show no clear trends or patterns regarding the effects that organic fertilization have on the crops' final quality (Basker, 1992; Bourn and Prescott, 2002). Those inconsistencies may exist because the differences between organic and conventional practices are product specific (Fillion and Arazi, 2002) or because differences are dependent on confounding factors such as age, picking time, or transportation.

Pac choi (*Brassica rapa* var. Mei Qing Choi) is a variety of Chinese cabbage well known in Asia that is gaining popularity in the United States. The flavor of pac choi has been previously studied by Schnitzler and Kallabis-Rippel (1998). Those authors studied different varieties of cooked and fresh pac choi using a trained sensory panel. Descriptive terms used by these authors were sweet, sour, bitter, spicy, and cabbage-like. Other studies focused on instrumental analysis of pac choi leaves to evaluate flavonoid composition (Rochfort *et al*., 2006), phenolic content in organic plants (Young *et al*., 2005), and the effect of packaging on their shelf life (Lu, 2007). However, stage of development was not included in these studies. Stage of development at the time of harvest should be included when evaluating pac choi because this plant is frequently consumed at different maturity levels (i.e. baby or mature stage) (Rochfort *et al*., 2006).

Plant maturity at the time of harvest is critical for flavor and texture development (Mattheis and Fellman, 1999). It has been suggested that age has an effect on the content of flavor compounds such as catechins and amino acids which tend to create off-flavors in young tea leaves (Kinugasa *et al*., 1997). It also has been suggested that some fruits such as muskmelon must be harvested at their ripening stage for best post-harvest quality (Asghary *et al*., 2005). Tomatoes, where harvest maturity is a critical factor related to sensory properties, have been the object of many studies to assess the relation between fruit ripeness, sensory properties, and chemical composition (Hayase *et al*., 1984; Shewfelt *et al*., 1988; Stern *et al*., 1994; Yilmaz *et al*., 2002).

The relation between sensory properties and chemical composition has received important attention in the past. Studies that relate sensory and instrumental data have been conducted for products such as wheat bread (Quílez *et al*., 2006), virgin olive oil (Morales *et al*., 1995), durian fruit (Voon *et al*., 2007), navel oranges (Baxter *et al*., 2005), and exotic salad crops (Price *et al*., 1990) using different statistical methodologies. A study focused on wine flavor (Noble and Ebeler, 2002) explored and compared three different multivariate methodologies that can be used to relate sensory and instrumental data. These studies used principal component analysis, generalized procrustes analysis, and partial least square regression. Authors concluded that all three methods provide similar results. However, some differences were noted.

Many studies have been able to link aroma volatiles found in foods with sensory characteristics. For example, butyl acetate, 1-hexanal, and camphor are aroma volatiles found in apples linked to fruity, green, and piney odors respectively (Mehinagic *et al*., 2006). Pentanal, heptanal, and octanal are related to nutty, floral, and citrus aromas in rice (Yang *et al*., 2008). Similarly, hexanal, nonanal, and acetaldehyde are related to green, soapy, and fruity aromatics in grapefruit juice (Buettner and Schieberle, 2001). Bott and Chambers (2006) found combinations of chemicals that produced beany odors and Hongsoongnern and Chambers (2008a) related chemicals to “green” characteristics found in various food products.

The objectives of this study are (1) to evaluate the sensory characteristics and the aroma volatile content of organically and conventionally grown pac choi leaves at three stages of development and (2) to correlate sensory and instrumental data by means of partial least squares regression.

## Materials and Methods

### ***Samples***

Trials were conducted at the K-State Horticulture Research and Extension Center, Olathe, Kansas, on experimental plots established in 2002 for comparison of crops grown under organic and conventional production systems in high tunnels (unheated, passively ventilated greenhouses) and open field plots (Zhao *et al*., 2007a). The soil was a Kennebec silt loam. Six 9.8 m x 6.1 m high tunnels with 1.5m sidewalls (Stuppy, North Kansas City, MO) and six adjacent 9.8 m x 6.1 m field plots were used for this study. High tunnels were covered with single layer 6-mil (0.153mm) K-50 polyethylene (Klerk’s Plastic Product Manufacturing, Inc., Richburg, SC). At establishment of the experimental plots, the six high tunnels were divided into three groups (blocks) and the two high tunnels in each block were randomly assigned for long-term conventional or organic management treatments. A similar set-up was used in the field plots. Organic plots were managed in compliance with USDA National Organic Program standards, and were inspected and certified in 2003, 2006, 2007 and 2008.

For this study, beginning in 2007, each high tunnel or open field plot was subdivided into three 3.2 m x 6.1 m plots to which one of three fertilizer levels were assigned (high, low, and no fertilizer) following a latin square design to avoid bias due to position effects in the high tunnels. Fertilizer rates were determined based on soil analysis at the beginning of the study in 2007, and recommendations for vegetable crops in Kansas (Marr *et al*., 1998), with compost applied to organic plots and synthetic fertilizer applied to conventional plots. Compost application rates were based on the assumption that 50% of the nitrogen from compost would be available to plants during the growing season, while 100% would be available from conventional fertilizers (Warman and Havard, 1997). Low and high fertility plots were fertilized with equal amounts of compost or synthetic fertilizer at the beginning of the growing season, and high fertility plots received additional fertilization during the growing season as described below.

Pac choi (*Brassica rapa* L. *chinensis* ‘Mei Qing Choi’) (Johnny’s Selected Seed, Albion, ME, U.S.A.) and tomato (*Lycopersicon esculentum* ‘Bush Celebrity’) (Totally Tomatoes, Randolph, WI, U.S.A.) were grown in one half of each open field or high tunnel plot (6.8 m x 3 m) in 2007 and 2008, with a rotation between pac choi and tomato plots each year. In our experimental system, a spring and a fall crop of pac choi was grown each year, while a single crop of tomato was grown. Between the spring and fall pac choi crops, plots were seeded with a summer cover crop of buckwheat (*Fagopyrum sagittatum*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 134 kg/ha. In the late fall, all plots were seeded with a cover crop of annual rye (*Secale cereale*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 229 kg/ha.

Conventional high and low fertility plots were fertilized with Jack’s Professional Peat-lite N-$P_2O_5$-$K_2O$ 20-10-20 (Allentown, PA, U.S.A.), at a rate of 98 kg/ha. Organic plots received MicroLeverage compost N-$P_2O_5$-$K_2O$ 0.6-0.8-0.5 (Hughesville, MO, U.S.A) at a rate of 197 kg/ha. Only pac choi grown in the outside plots with low amounts of fertilizer were used for this specific study.

Pac choi transplants were started in a greenhouse in Sunshine Mix Special Blend E6340 (SunGro Horticulture, Bellevue, WA) supplemented with MicroLeverge compost. Pac choi was planted on April 1, 2008 and harvested on April 20 (2.5 weeks old for baby pac choi), May 5 (4.5 weeks old for optimum growth), and May 19 (6.5 weeks old for overgrown pac choi).

## *Sample Preparation*

### *Sensory analysis*

Plants were harvested one to three days before testing. After harvest, the plants were immediately rinsed using cold tap water to remove excess dirt and stored in a refrigerated container for transport to the Kansas State University campus located in Manhattan, KS. Once arrived, the samples were moved to a walk-in refrigerator for storage at 4ºC until testing. The plants were sprayed daily with tap water to maintain moisture. On the day of testing, plants were retrieved from the refrigerator. Random leaves of similar visual characteristics were removed from each stalk (not including the stem) and rinsed using distilled water. Excess water was eliminated with a salad spinner (Oxo International, Ltd., New York, NY). Samples were served to the panelists monadically in 6" foam plates identified with a three-digit code to eliminate potential panelist bias. The sample amount was dependant on leaf size. For example, for baby pac choi (2.5 weeks old plant) one whole sprig comprised of several leaves was served to each panelist, 1-2 leaves were served to each panelist when leaves were 4.5 weeks old and 6.5 week old.

### *Volatile analysis*

The same day of sensory testing, approximately 5-10 g from leaves of each treatment were vacuum sealed and frozen at −80°C for 30 d until volatile analysis. The day of the analysis, samples were retrieved from the freezer and thawed at room temperature (22 ± 1°C) for approximately 30 min. For solid-phase microextraction (SPME) sampling, 4 g pac choi leaves were blended with 200-mL of reverse osmosis, deionized, carbon-filtered water using an electric hand blender (Rival, Peoria, IL) for 20 s. The mixture was then filtered through double layered cheese cloth. From the filtered solution, 1-mL was transferred to a 10-mL clear headspace vial and mixed with 0.2 g of Sodium Chloride (NaCl). Additionally, 5-µl of 0.2 ppm 1,3 dichlorobenzene in methanol (internal standard) was added. Glass vials were closed using an open-center screw cap with a 1.8 mm silicone/PTFE septum (Varian, Palo Alto, CA).

## *Panelists*

Six highly trained panelists from the Sensory Analysis Center at Kansas State University (Manhattan, KS, U.S.A.) were used for this study. The panelists had completed more than 120 hours of descriptive training, average more than 2000 hours of testing experience, and had prior experience testing vegetables and vegetable products.

## *Experimental procedure*

### *Sensory analysis*

The lexicon for pac choi was used based on Talavera-Bianchi *et al.* (2009) to describe flavor of different leafy vegetables. A lexicon consisting of twenty-nine terms with definitions and references was presented to the panelists along orientation samples in one 90-minute orientation session prior the start of testing so they could become familiar with the terminology, test procedures, and samples. The original lexicon consisted of twenty-six flavor and mouth feel attributes. However, three texture attributes were added because we believed that this would aid in describing changes in the plant during the maturation process. Similar lexicons have been developed and used for other products such as green tea (Lee and Chambers, 2007), tomatoes (Hongsoongnern and Chambers, 2008b), ice cream (Thompson *et al*., 2009), and brewed coffee (Seo *et al*., 2009).

The day of testing, panelists were presented with the lexicon and references used during orientation. Data were collected using a computerized collection system (Compusense *Five* version 4.6.702, Guelph, ON, Canada). Intensities for each attribute were recorded using a 0-15 point scale divided in 0.5 point increments, 0 meaning “none” and 15 meaning “extremely high”. Panelists evaluated the samples individually and followed a completely randomized block design with the stage of development as the blocking factor. Six samples of pac choi were evaluated in each of three 90-minute sessions. Reverse osmosis, deionized, carbon-filtered water and unsalted crackers were used to rinse the palate between the samples. A similar procedure has been used in the past to evaluate the sensory characteristics of four samples of calcium-biofortified lettuce (Park *et al*., 2009).

### *Gas chromatography – mass spectrometry*

Volatile compounds were identified and quantified using a Varian Saturn CP-3800 Gas Chromatograph / Mass Spectrometer 2200 (Varian Inc., Walnut Creek, CA). The sample vials were equilibrated at 40°C/500 rpm for 10 min. SPME was performed using a StableFlex Divinylbenzene / Carboxen / Polydimethylsiloxane 50/30 µm fiber (Sigma Aldrich, Saint Louis, MO) for 20 min at 40°C. The agitation during extraction was of 250 rpm. The extracted compounds were thermally desorbed at 250°C for 3 min in the front injection port of the gas chromatograph. After the injection, the fiber was baked at 270°C for 30 min. An RTX®-5 Capillary Column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness; Restek U.S., Bellefonte, PA) was used to separate the volatiles desorbed from the fiber. The initial temperature of the column was set at 40°C for 2 min and then raised to 200°C at a rate of 5°C $min^{-1}$ and held for 1 min (total GC run time was 35 min). Varian MS Workstation software (version 6.8) was used for system control, data collection, and data processing. Compound identification was based on NIST 2005 version 2.0 Mass Spectra library search. The final compounds concentration was based on the concentration of the internal standard. Three replications were analyzed for each treatment. Kovats retention indices were calculated to aid in the identification of the volatile compounds. A blend of hydrocarbon (HC) mix and carbon disulfide (1 drop of HC mix in 1 ml of $CS_2$ directly injected to the GC) was also run under the same methodology to generate the retention times of the n-alkanes ($C_6$-$C_{20}$) for calculating the Kovats indices. Comparing Kovats indices from chemicals previously identified using the same column and stationary phase under similar conditions has shown to be an accurate method of identification (Moustafa, 2008).

## *Analysis*

Analysis of variance (ANOVA) with PROC MIXED (panelist and replication as the random effects) was used to detect overall differences among treatments for individual sensory attributes. PROC GLM was used to detect differences for individual volatile compounds. ANOVA was computed in SAS® (2002, version 9.1.3; SAS Institute, Cary, NC). Partial least squares regression (PLS2) was used to correlate sensory and instrumental data. PLS is a soft modeling method which is widely used to predict a set of dependant variables (sensory attributes) from a large set of independent variables (volatile compounds) (Noble and Ebeler, 2002). This method has been previously used to correlate instrumental and sensory data in cheese (Hough *et al*., 1996), diced tomatoes (Lee *et al*., 1999), and ice cream (Chung *et al*., 2003). Even though this analysis does not determine which volatile components are actually responsible for specific sensory attributes, it does help in studying the relationship between certain volatiles and sensory characteristics (Noble and Ebeler, 2002). This analysis was performed using Unscrambler (2005, version 9.2; Camo Process AS, Oslo, Norway).

## Results and Discussion

### *Sensory analysis*

Findings from the study show that stage of development is an important factor affecting sensory characteristics of pac choi. Twenty-one flavor and texture attributes were significantly different among maturity levels ($P\text{-value} \leq 0.05$) (Table 5.1). Most of the attributes' intensities increased as the plants get older although some decreased. For example, attributes such as crispness, fiber awareness, overall green, green-grassy-leafy, woody, sulfur, soapy, toothetch, and bitter had lower intensities in younger plants and higher intensities in older plants. The attributes that remained stable throughout the plant development process were green-viney, radish, water-like, petroleum-like, pungent, bite, and the sour taste. The typical green, bitter, and sulfur flavors of pac choi and other vegetables of the *Brassica* family are believed to be caused by glucosinolate-derived compounds. The main glucosinolates found in pac choi are 3-butenyl- and 1-methoxy-3-indoylmethyl (He *et al*., 2003). It would be expected that as the concentration of these compounds increase when the plant matures, the intensity of typical flavors may increase as well.

**Table 5.1. Analysis of variance showing the significant differences (95% confidence) between stages of development for individual attributes**

| | | **Stage of Development** | | |
|---|---|---|---|---|
| **Attributes[1,2]** | **Fertilization** | **2.5 Weeks** | **4.5 Weeks** | **6.5 Weeks** |
| Crispness | Organic | 2.7b | 3.2ab | 3.5a |
| | Conventional | 2.7b | 3.3a | 3.6a |
| Moistness | Organic | 5.5a | 4.1c | 4.5b |
| | Conventional | 5.7a | 4.2c | 4.6b |
| Fiber Awareness | Organic | 2.9c | 4.3b | 4.7a |
| | Conventional | 3.3c | 4.2b | 4.9a |
| Overall Green | Organic | 5.9b | 5.9b | 7.0a |
| | Conventional | 6.9a | 6.1b | 7.0a |
| Green-unripe | Organic | 1.6a | 1.0b | 0.9b |
| | Conventional | 1.7a | 0.9b | 0.9b |
| Green-peapod | Organic | 1.3a | 0.4b | 1.3a |
| | Conventional | 1.5a | 0.4b | 1.1a |
| Green-grassy/leafy | Organic | 4.6b | 5.0b | 6.0a |
| | Conventional | 5.3ab | 5.0b | 5.8a |
| Green-Viney | Organic | 1.6 | 1.8 | 1.9 |
| | Conventional | 1.6 | 2.0 | 1.8 |
| Cabbage | Organic | 2.4 | 2.6 | 2.7 |
| | Conventional | 2.2b | 2.6ab | 2.8a |
| Lettuce | Organic | 1.8a | 1.9a | 1.4b |
| | Conventional | 1.7 | 1.8 | 1.5 |
| Spinach | Organic | 1.6b | 2.0a | 1.9a |
| | Conventional | 1.7b | 2.0a | 1.8ab |
| Parsley | Organic | 0.9b | 1.4a | 1.1b |
| | Conventional | 1.1b | 1.5a | 1.1b |
| Radish | Organic | 2.0 | 1.9 | 1.8 |
| | Conventional | 1.9 | 1.9 | 2.0 |
| Piney | Organic | 1.1a | 0.4b | 0.9a |
| | Conventional | 1.6a | 0.8b | 0.8b |
| Woody | Organic | 1.3b | 1.5b | 2.0a |
| | Conventional | 1.3b | 1.7a | 1.9a |
| Water-like | Organic | 1.6 | 1.9 | 1.6 |
| | Conventional | 1.6 | 1.8 | 1.7 |
| Musty/Earthy | Organic | 2.3 | 2.6 | 2.3 |
| | Conventional | 2.9a | 2.3b | 2.4b |
| Sulfur | Organic | 1.0c | 1.7b | 2.2a |
| | Conventional | 1.3c | 1.7b | 2.3a |
| Soapy | Organic | 0.5c | 1.2b | 1.6a |
| | Conventional | 1.1b | 1.3ab | 1.5a |
| Petroleum-like | Organic | 0.4 | 0.3 | 1.0 |
| | Conventional | 1.2 | 0.5 | 0.7 |

| | | | | |
|---|---|---|---|---|
| Pungent | Organic | 2.1 | 2.0 | 2.0 |
| | Conventional | 2.2 | 2.1 | 2.1 |
| Bite | Organic | 1.9 | 2.1 | 2.3 |
| | Conventional | 1.9 | 2.2 | 2.2 |
| Toothetch | Organic | 1.1b | 2.0a | 2.3a |
| | Conventional | 1.3b | 2.1a | 2.1a |
| Overall Sweet | Organic | 1.4a | 1.3ab | 1.1b |
| | Conventional | 1.3ab | 1.5a | 1.2b |
| Sour | Organic | 1.6 | 1.5 | 1.6 |
| | Conventional | 1.6 | 1.6 | 1.6 |
| Bitter | Organic | 4.9b | 7.0a | 7.0a |
| | Conventional | 6.3 | 6.6 | 6.7 |
| Salty | Organic | 0.6ab | 0.4b | 0.9a |
| | Conventional | 0.5b | 0.6ab | 0.9a |
| Umami | Organic | 2.1a | 2.1a | 1.7b |
| | Conventional | 2.0 | 2.1 | 1.9 |
| Astringent | Organic | 1.4b | 1.8a | 1.9a |
| | Conventional | 1.8 | 1.8 | 1.9 |

[1] Significant differences are at the 95% confidence level. Different letters indicate statistically significant differences.

[2] Significant differences are at the 95% confidence level. Different letters indicate statistically significant differences.

When glucosinolates are released from the plant cells, they are broken down by enzymatic action into products such as nitriles and isothiocyanates which are also responsible for the "hot and spicy" flavors of mustard, radishes, and other plants from the *Brassica* family (Johnson, 2001). The lower concentration of glucosinolates in pac choi compared to other plants of the *Brassica* family is consistent with its milder flavor (He *et al*., 2003). Contrarily, attributes such as moistness, green-unripe, and overall sweet had higher intensity in younger plants and lower intensities in older plants. It may be that as the plant matures, sugar may be used by the plant and the development of other flavor characteristics such as sulfur or bitterness may mask the sweet taste as well as reducing the perception of unripeness in pac choi. Many of these flavor characteristics have been reported for pac choi in the past. Schnitzler and Kallabis-Rippel (1998) used terms such as sweet, sour, bitter, and spicy to describe flavor of raw pac choi. In our study, we also used the sour and spicy (bite) attributes. However, they were not significantly different among stages of maturity or production system (i.e. organic and conventional).

Few differences were found between organically and conventionally grown pac choi. The few small differences that exist were found only at the 2.5-week stage of development (Table 5.2). In this case, conventionally grown pac choi had significantly higher intensities (P-value $\leq$ 0.05) of overall green, soapy, and bitter attributes. No differences were found at 4.5-week or

6.5-week old pac choi. This suggests that the effect of organic production may be more evident at early stages of development. It has been suggested that organic treatment may increase the opportunity of insect attack in pac choi which may cause the amount of total phenolics to increase affecting its flavor (Young *et al*., 2005). Kobue-Lekalake *et al*. (2007) suggest that phenolic compounds increase the bitterness and astringency of sorghum grains. Another study also reported higher bitterness in organically grown carrots (Haglund *et al*., 1999). In our study, bitterness was lower in the organic pac choi at 2.5 weeks maturity and there were no differences in astringency. It also may be that conventionally grown pac choi was more mature at the time of harvest generating higher intensities for these flavor attributes.

**Table 5.2. Individual attributes that showed significant differences (P-value ≤ 0.05) between organic and conventional pac choi at the baby stage (2.5 weeks)[1]**

| Attributes[2] | Organic | Conventional |
|---|---|---|
| Overall Green | 5.9b | 6.9a |
| Soapy | 0.5b | 1.1a |
| Bitter | 4.9b | 6.3a |

[1]No differences between organic and conventional pac choi were observed at 4.5 or 6.5-week maturity.
[2]Significant differences are at the 95% confidence level. Different letters indicate statistically significant differences.

### *Gas chromatography – mass spectrometry*

Forty-eight volatile compounds were identified and quantified (Table 5.3). The chemicals that were mostly present in pac choi leaves are the aldehydes (Z)-3-hexenal (9), (E)-2-hexenal (11), (E,E)-2,4-hexadienal (15), and benzeneacetaldehyde (21); alcohols such as 2-hexyn-1-ol (10), (Z)-3-hexen-1-ol (12), (E)-3-hepten-1-ol (13), and (E)-2-nonen-1-ol (27); as well as noncyclic and cyclic hydrocarbons such as 4,5-dimethylthiazole (30) and isothiocyanato-cyclohexane (40) respectively. Many of these compounds have been previously reported as providing "green" aromas in foods. For example, (Z)-3-hexenal (9) was reported as providing aromas reminiscent of "green", "green leaves", and "grassy" in virgin olive oil (Morales *et al*., 1995; Aparacio *et al*., 1997). This compound was also described as providing "strong green" characteristics in green olives (Iraqi *et al*., 2005).

**Table 5.3. Volatile aromatics found in organically and conventionally grown pac choi at three stages of development**

| | | | | Treatments[1,2] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Volatile Compound | Retention Time (min) | RI[3] | O2.5 | C2.5 | O4.5 | C4.5 | O6.5 | C6.5 |
| 1 | 2-butanone | 2.5 | 585.6 | 26.7 | 25.9 | 21.2 | 18.7 | 24.2 | 20.7 |
| 2 | Methyl propionate | 2.8 | 622.2 | Trace | Trace | Trace | Trace | Trace | Trace |
| 3 | 3-methyl-2-butanone | 3.1 | 657.3 | Trace | Trace | 10.8 | Trace | 10.0 | Trace |
| 4 | 1-penten-3-ol | 3.3 | 685.1 | 13.2 | 14.8 | 25.4 | 21.2 | 20.5 | 31.8 |
| 5 | Butanoic acid, methyl ester | 3.9 | 728.1 | 44.8 | 34.9 | 41.4 | 33.8 | 36.1 | 31.6 |
| 6 | 2-methyl-3-pentanone | 4.4 | 754.1 | Trace | Trace | Trace | Trace | Trace | Trace |
| 7 | (E)-2-hepten-1-ol | 4.9 | 775.7 | 16.8 | 19.3 | 25.2 | 19.5 | 22.9 | 29.8 |
| 8 | 2-methyl-butanoic acid, methyl ester | 5.0 | 781.7 | 32.7 | 29.1 | 32.3 | 29.5 | 29.1 | 29.5 |
| 9 | (Z)-3-hexenal | 5.5 | 805.7 | 1296.1 | 1835.7 | 1773.3 | 2647.3 | 424.7 | 1058.2 |
| 10 | 2-hexyn-1-ol | 6.7 | 851.7 | 78.2 | 122.0 | 151.9 | 215.5 | 45.9 | 111.4 |
| 11 | (E)-2-hexenal | 6.9 | 857.5 | 768.6 | 974.2 | 1389.9 | 1843.1 | 596.7 | 1475.3 |
| 12 | (Z)-3-hexen-1-ol | 7.0 | 860.7 | 188.0 | 253.7 | 356.9 | 328.2 | 507.6 | 1000.0 |
| 13 | (E)-3-hepten-1-ol | 7.2 | 867.0 | 338.9 | 315.1 | 402.4 | 328.3 | 175.7 | 249.8 |
| 14 | Heptanal | 8.3 | 903.9 | Trace | Trace | Trace | Trace | Trace | Trace |
| 15 | (E,E)-2,4-hexadienal | 8.6 | 917.3 | 97.9 | 109.0 | 175.2 | 205.6 | 55.2 | 89.8 |
| 16 | 1-isothiocyanato-butane | 9.2 | 938.2 | Trace | 13.9 | 55.0 | 53.2 | 11.7 | 14.1 |
| 17 | 1-octen-3-ol | 10.7 | 984.4 | 15.1 | 11.8 | 14.9 | 11.2 | Trace | Trace |
| 18 | 4-isothiocyanato-1-butene | 10.8 | 989.0 | 31.5 | 39.6 | 13.9 | Trace | 24.8 | 11.4 |
| 19 | Octanal | 11.3 | 1004.5 | Trace | Trace | Trace | Trace | Trace | Trace |
| 20 | (E)-2-octenal | 12.1 | 1032.9 | 36.2 | 49.4 | 56.1 | 46.0 | 55.8 | 58.2 |
| 21 | Benzeneacetaldehyde | 12.6 | 1049.3 | 279.3 | 309.8 | 349.0 | 276.7 | 162.1 | 223.9 |
| 22 | 4-methyl-1-undecene | 13.3 | 1072.3 | 20.1 | 20.0 | 20.5 | 17.9 | 16.1 | 16.3 |
| 23 | 1-octanol | 13.4 | 1075.1 | 41.9 | 27.8 | 61.4 | 35.5 | 16.8 | 18.8 |
| 24 | 4-ethyl-5-methylthiazole | 13.8 | 1086.9 | 154.7 | 144.0 | 49.4 | 32.3 | 40.3 | 10.1 |
| 25 | 2-nonanone | 14.1 | 1095.7 | Trace | Trace | Trace | Trace | N.D. | N.D. |
| 26 | 3,7-dimethyl-1,6-octadien-3-ol (linalool) | 14.3 | 1100.8 | Trace | Trace | 13.1 | 11.2 | 15.1 | 15.6 |
| 27 | (E)-2-nonen-1-ol | 14.4 | 1105.7 | 204.5 | 142.4 | 192.9 | 127.2 | 25.9 | 30.6 |
| 28 | Isopinocarveol | 14.8 | 1118.4 | Trace | Trace | Trace | Trace | Trace | Trace |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 29 | 1-nonanol | 14.9 | 1124.8 | N.D. | N.D. | N.D. | N.D. | Trace | Trace |
| 30 | 4,5-dimethyl-thiazole | 15.0 | 1128.7 | 94.9 | 68.0 | 202.6 | 69.7 | 166.2 | 69.7 |
| 31 | Acetic acid, octyl ester | 15.3 | 1137.1 | N.D. | N.D. | Trace | Trace | Trace | Trace |
| 32 | Benzyl nitrile | 15.5 | 1145.2 | 22.9 | 44.5 | 21.4 | 24.1 | 14.0 | Trace |
| 33 | Camphor | 15.7 | 1151.3 | N.D. | N.D. | Trace | Trace | Trace | Trace |
| 34 | (Z)-6-nonenal | 16.1 | 1165.4 | Trace | Trace | Trace | Trace | N.D. | N.D. |
| 35 | 4-methylpentyl isothiocyanate | 16.3 | 1172.1 | Trace | Trace | Trace | Trace | Trace | Trace |
| 36 | Menthol | 16.5 | 1177.6 | 22.2 | 18.8 | 39.3 | 27.0 | 39.3 | 46.0 |
| 37 | 2-decanone | 17.0 | 1194.6 | Trace | Trace | Trace | Trace | 11.4 | Trace |
| 38 | (E)-2-decen-1-ol | 17.4 | 1208.3 | 19.6 | 20.6 | 22.8 | 18.9 | 13.7 | 16.1 |
| 39 | 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde | 17.9 | 1229.9 | 72.6 | 61.7 | 56.7 | 73.7 | 72.1 | 79.4 |
| 40 | Isothiocyanato-cyclohexane | 18.2 | 1243.5 | 102.8 | 159.9 | 202.0 | 177.3 | 214.2 | 219.3 |
| 41 | Isobornyl acetate | 19.7 | 1301.8 | Trace | Trace | Trace | Trace | 13.0 | 14.1 |
| 42 | 2-undecanone | 19.8 | 1305.5 | Trace | Trace | Trace | Trace | N.D. | N.D. |
| 43 | Dodecanal | 22.8 | 1410.8 | Trace | Trace | Trace | 10.3 | Trace | Trace |
| 44 | 6,10-dimethyl-5,9-undecadien-2-one (geranylacetone) | 24.0 | 1457.2 | Trace | 10.6 | 26.1 | 16.8 | 34.6 | 41.4 |
| 45 | 2-isothiocyanatoethyl-benzene | 24.4 | 1476.4 | 41.0 | 65.6 | 64.5 | 39.7 | 26.0 | 36.7 |
| 46 | Butylated hydroxytoluene | 25.5 | 1518.8 | Trace | Trace | Trace | Trace | Trace | Trace |
| 47 | Lilial | 25.8 | 1534.5 | Trace | Trace | Trace | Trace | Trace | Trace |
| 48 | Cedrol | 27.7 | 1615.0 | Trace | Trace | Trace | Trace | Trace | Trace |

[1] O2.5=Organic at 2.5 weeks maturity; C2.5=Conventional at 2.5 weeks maturity; O4.5=Organic at 4.5 weeks maturity; C4.5=conventional at 4.5 weeks maturity; O6.5=Organic at 6.5 weeks maturity; C6.5=Conventional at 6.5 weeks maturity.

[2] Concentration of volatile shown in pg/g of pac choi

[3] Retention index (Kovats) calculated from DB5 column.

N.D: Not detected.

Similarly, (E)-2-hexenal (11) was described as being present in blackberries providing "fruit", "orange", and "green" aroma characteristics (Klesk and Qian, 2003). The same compound was reported as present in virgin olive oil providing "bitter" characteristics (Aparicio *et al*., 1997). (Z)-3-hexen-1-ol (12) was reported as providing "green" aromas in a study focusing on describing sensory characteristics of musty compounds in foods (Chambers *et al*., 1998). Iraqi *et al*. (2005) reported that (Z)-3-hexen-1-ol (12) provided "vanilla" and "green" characteristics in green olives. (E,E)-2,4-hexadienal (15) was identified in fish oil enriched milk and reported to provide “green” and “vegetable” aromas (Venkateshwarlu *et al*., 2004). The same compound had been previously found in mayonnaise and was described as “green” and “burnt” (Hartvigsen *et al*., 2000). Other compounds found in pac choi at lower concentrations that have been previously reported as having “green” characteristics are 1-penten-3-ol (4), octanal (19), (E)-2-octenal (20), (Z)-6-nonenal (34), 2-undecanone (42), acetic acid octyl ester (octyl acetate) (31), and cedrol (48) (Chida *et al*., 2004; Buettner and Schieberle, 2001; Aparicio *et al*., 1997; Beaulieu, 2005; Klesk and Qian, 2003; Thi Minh Tu *et al*., 2002). In a study that focused on the chemicals associated with green odors and flavors in foods, several aldehydes, alcohols, ketones, azoles, and ester derivatives were reported as responsible for the green aroma in foods (Hongsoongnern and Chambers, 2008a). The same study reported that the “green” characteristics in foods can be of various types such as unripe, peapod, grassy/leafy, viney, fruity, or may appear as a combination of these. Benzeneacetaldehyde (21) was identified in extruded Amilo rye and described as “flower”, “honey”, and “bitter almond” (Heiniö *et al*., 2003). Interestingly, benzeneacetaldehyde was also described as “green” at a lower intensity in the same study. In addition, 4, 5-dimethylthiazole (30) was identified in fried beef steaks and was described as having “smoky”, “roasty”, “fragrant”, and “nutty” aroma characteristics (Specht and Baltes, 1994). Other compounds found in pac choi at low concentrations were 2-butanone (1) described as “fragrant” and “pleasant” (Morales *et al*., 1995); 1-octen-3-ol (17) and 1-butene-4-isothiocianato (18) described as “mushroom” and “sulfur” respectively (Engel *et al*., 2002); heptanal (14) and 2-nonanone (25) described as “floral” as well as 1-nonanol (29) and 2-decanone (37) which were previously described as “fatty” (Yang *et al*., 2008); camphor (33) described as “piney” and “spicy” (Mehinagic *et al*., 2006); and dodecanal (43) which was previously described as having “citrus” and “skin-like” characteristics (Hashizume and Samuta, 1997).

### *Correlating Sensory and Chemical Data*

Partial least squares regression (PLS2) was used to correlate sensory and chemical data (Figure 5.1). Only the sensory attributes and volatiles that showed significant differences (P-value ≤ 0.05) were included in this analysis. The analysis showed that 85% of the chemical data explains 86% of the sensory data.

The samples that were harvested late (at 6.5-weeks) are more correlated with attributes such as overall green, green-grassy/leafy, and salty. The volatile compounds related to these attributes are (Z)-3-hexen-1-ol (12), octyl acetate (31), 1-nonanol (29), and 2-decanone (37).

**Figure 5.1. Partial least squares regression (PLS) correlating sensory and instrumental data. O2.5=Organic at 2.5 weeks maturity; C2.5=Conventional at 2.5 weeks maturity; O4.5=Organic at 4.5 weeks maturity; C4.5=conventional at 4.5 weeks maturity; O6.5=Organic at 6.5 weeks maturity; C6.5=Conventional at 6.5 weeks maturity.**

These volatiles were present at higher concentrations in the pac choi harvested at 6.5 weeks. In fact, 1-nonanol (29) was only present in these samples and not in the samples harvested earlier. These chemicals have been associated with “bitter”, “green”, “fruity”, and “fatty” aromatics (Aparicio *et al*., 1997; Iraqi *et al*., 2005; Yang *et al*., 2008; Ti Minh Tu *et al*., 2002). Other compounds which are also closely associated with samples harvested at 6.5 weeks are 1-penten-3-ol (4), 3,7-dimethyl-1,6-octadien-3-ol (linalool) (26), camphor (33), menthol (36), isobornyl acetate (41), 6,10-dimethyl-5,9-undecadien-2-one (geranylacetone) (44), and cedrol (48). These chemicals have also been associated with “green”, “floral”, “woody”, “citrus”, and “piney” aromatics (Chida *et al*., 2004; Mehinagic *et al*., 2006). In our study, these compounds were closely related to attributes such as bitter, toothetch, soapy, cabbage, sulfur, and woody. Fiber awareness and crispness are textural attributes also related to these samples and these volatiles. This may indicate co-linear attributes that change similarly but have different etiologies.

The spinach flavor attribute was related to with dodecanal (43), an aldehyde with citrus aromatics that has also been found in cilantro and carrots in the past (Hashizume and Samuta, 1997; Fan and Sokorai, 2002; Buttery *et al*., 1968). Similarly, butylated hydroxytoluene (BHT) (46) was closely related to the spinach flavor of pac choi. Parsley flavor was correlated to butyl isothiocyanate (1-isothiocyanato-butane) (16), a derivative from glucosinolates which are frequently found in vegetables from the *Brassica* family and more specifically cabbage (Ciska and Pathak, 2004). In another study, butyl isothiocyanate (16) was also identified in cooked cauliflower and was described as having "sulfur", "green", and "pungent" aroma characteristics (Engel *et al*., 2002). Other volatile compounds closely related to the parsley flavor were 2-hexyn-1-ol (10) and (E,E)-2,4-hexadienal (15). It has been suggested that several aldehydes, alcohols, ketones or ester derivatives with 6 carbon atoms ($C_6$) in their molecules are responsible for the "green" aroma in foods (Hongsoongnern and Chambers, 2008a). (E,E)-2,4-hexadienal (15) has been previously described as having "ripe fruit", "green", and "vegetable" aroma characteristics (Aparicio *et al*., 1997; Venkateshwarlu *et al*., 2004). The pac choi samples harvested at 4.5 weeks also were correlated to the parsley and spinach flavors. This means that samples harvested at 4.5 and 6.5 weeks were usually rated at a higher intensity for these flavors compared to the samples harvested at 2.5 weeks of maturity.

Another group of volatiles is correlated with the lettuce, umami, and overall sweet attributes. This suggests that these volatiles may have "green" characteristics that are less strong compared to other chemicals more closely associated with parsley, green-grassy/leafy, and overall green attributes. The chemicals associated with lettuce, umami, and sweet flavors are (Z)-3-hexenal (9), octanal (19), benzeneacetaldehyde (21), 4-methyl-1-undecene (22), 1-octanol (23), 2-nonanone (25), (E)-2-nonen-1-ol (27), (Z)-6-nonenal (34), (E)-2-decen-1-ol (38), and 2-undecanone (42). This is in agreement with past studies in which many of these compounds have been described as having "sweet", "floral", "citrus", "fruity", and "green" characteristics. For example, (Z)-3-hexenal (9), octanal (19), and 2-undecanone (42) were described as "green" (Aparicio *et al*., 1997; Buettner and Schieberle, 2001; Klesk and Qian, 2003). At the same time, octanal (19) has also been described as having “sweet” and “citrusy” aroma characteristics (Thi Minh Tu *et al*., 2002). Benzeneacetaldehyde (21) has been reported as having “flower”, “honey”, “sweet” and “green” aroma characteristics (Heiniö *et al*., 2003). 2-nonanone (25) was described as “fruity and “floral” (Yang *et al*., 2008) and (Z)-6-nonenal (34) was reported as “citrus”, “green”, “cucumber”, and “melon-like” (Beaulieu, 2005). These compounds are more correlated to pac choi samples harvested at both 2.5 and 4.5 weeks maturity. This means that their concentration is higher at early stages and decrease as the plants get older. Another compound which is also closely related to samples at their early stage of development is benzyl nitrile (benzene acetonitrile) (32) which is another chemical formed from the degradation of glucosinolates. This compound was previously identified and quantified in turnip greens at different stages of development (Jones *et al*., 2007). Those authors found that benzene acetonitrile actually increased as the plant got older. However, the concentrations were generally small. The pac choi samples harvested at an early stage of growth (2.5 weeks) were generally associated with green-unripe, piney, and musty/earthy flavors as well as moistness.

The concentrations of many volatiles varied among maturity levels of pac choi. In addition, differences are also noted between organically and conventionally grown pac choi for a few volatiles. Analysis of variance (ANOVA) on the volatiles shows that eight volatiles found in pac choi differed between organic and conventional production. The volatiles that were generally higher for conventionally grown pac choi were (Z)-3-hexenal (9), 2-hexyn-1-ol (10), (E)-2-hexenal (11). These compounds are responsible for the “green” and “bitter” aroma in foods (Aparicio *et al*., 1997). This is in agreement with the sensory analysis of pac choi which showed that conventional pac choi had significantly higher intensities of overall green, bitter, and soapy flavors compared to organic pac choi at the earliest stage of development (2.5 weeks). However, the intensities of overall green, bitter, and soapy are similar between organic and conventional pac choi at both 4.5 and 6.5 weeks maturity levels. It may be that the introduction of other flavor volatiles such as 1-penten-3-ol (4), linalool (26), and geranylacetone (44) balanced the perception of overall green and bitter at later stages of growth. These compounds also have been associated with “green” and “floral” aromas in the past (Chida *et al*., 2004). Butanoic acid methyl ester (8) and 4,5-dimethylthiazole (30) were significantly higher in organic pac choi. However, this difference did not translate to sensory flavor differences between organic and conventional pac choi. Other compounds that were higher for organic pac choi were 2-methyl-3-

pentanone (6), camphor (33), and (Z)-6-nonenal (34). However, these chemicals were present at low concentrations.

In summary, the differences in volatile compounds among stages of growth are more substantial compared to the differences between organic and conventional production systems. In many cases, these differences in chemical composition do translate into the flavor characteristics observed in pac choi.

## Conclusions

Many more differences in sensory characteristics and chemical profile are observed among stages of growth of pac choi compared to the production method. Pac choi harvested early (2.5 weeks) is described as green-unripe, piney, musty/earthy, and moist. As the plant grows, other flavors such as lettuce, umami, and overall sweet develop. These flavors are correlated with volatiles that have been associated with “sweet”, “floral”, “citrus”, “fruity”, and “green” aromas. These volatiles are (Z)-3-hexenal (9), octanal (19), benzeneacetaldehyde (21), 4-methyl-1-undecene (22), 1-octanol (23), 2-nonanone (25), (E)-2-nonen-1-ol (27), (Z)-6-nonenal (34), (E)-2-decen-1-ol (38), and 2-undecanone (42). Lastly, when the plant reaches a mature stage at 6.5 weeks, it is perceived as having higher intensities of green, bitter, cabbage, sulfur, and woody flavors. These flavors may be associated with the presence of volatiles such as (Z)-3-hexen-1-ol (12), octyl acetate (31), 1-nonanol (29), 2-decanone (37), 1-penten-3-ol (4), linalool (26), camphor (33), menthol (36), isobornyl acetate (41), geranylacetone (44), and cedrol (48) which have been associated with “strong green”, “bitter”, “fruity”, and “fatty” odors in the past.

Finally, conventional pac choi was slightly higher in green overall, bitter, and soapy flavors compared to organic pac choi when harvested at 2.5 weeks only. This may be associated with the presence of (Z)-3-hexenal (9), 2-hexyn-1-ol (10), and (E)-2-hexenal (11). The difference in flavor between organic and conventional pac choi disappears as the plant gets older probably due to the increase of other volatile compounds also with “green”, “bitter”, and “floral” characteristics such as 1-penten-3-ol (4), linalool (26), and geranylacetone (44).

# CHAPTER 6 - Sensory and chemical properties of organically and conventionally grown pac choi (*Brassica rapa* cv. Mei Qing Choi) change little during 18 days of refrigerated storage

## Abstract

Sensory and chemical characteristics of organically and conventionally grown pac choi (often called bok choy) were identified and quantified during 18 days of shelf life storage. Sensory and instrumental data were correlated using partial least squares regression. Pac choi was grown in early autumn at the Research and Extension Center owned by Kansas State University located in Olathe, Kansas. Samples were refrigerated at 4°C and evaluated at 1, 4, 9, and 18 days after storage. Sensory analysis was conducted by a trained descriptive panel and compounds were identified and quantified using a gas chromatograph / mass spectrometer. Most of the decrease in the quality of pac choi during refrigerated storage is related to a decrease of textural attributes such as crispness and moistness as well as the increase in off-flavors such as stale/refrigerator and moldy. However, differences generally were small. Most of the flavor characteristics remained constant or varied slightly. Volatiles such as heptanal, octanal, benzeneacetaldehyde, 1-octanol, and (E)-2-nonen-1-ol generally were higher in organic pac choi, but those differences did not translate into sensory differences as none were found between the organic and conventionally grown leaves at any point in the shelf life.

## Practical Applications

The popularity of organically grown vegetables has increased the interest in understanding the sensory differences between organic and conventional vegetables. This study shows that organic and conventional pac choi have a similar a quality after storage. This may clarify previous theories regarding organic products lasting longer than conventional products.

# Introduction

Freshness, taste, and appearance are critical factors driving consumers' purchase decisions of minimally processed vegetables (Ragaert *et al.*, 2004). Because vegetables are highly perishable products, the study of their shelf life becomes important. Leafy greens are perishable mostly due to mechanical damage during post harvest handling (Mahmud *et al.*, 1999). Some of the most common defects are yellowing (Lu, 2007) caused by degradation of the chlorophyll and water-loss, which is increased by the high surface area to volume ratio of leafy vegetables (Burton, 1982). These factors are related to each other and are increased when not refrigerated (Lazan *et al.*, 1987). Lower temperatures decrease the respiration rate of vegetables and hence increase their shelf life (Geronimo and Beevers, 1964).

Some production methods are also alleged to affect the shelf life of vegetable products. For example, some studies propose that organic farming systems increase the shelf life of leafy vegetables because it causes the nitrate contents to decrease in the leaves (Rembialkowska, 2007; Bourn and Prescott, 2002). Those authors explain that this is likely due to the lower amounts of nitrogen used in organic fertilization which will generate less nitrogen for the plant to absorb. However, some studies failed to find sensory differences between organic and conventional products during storage which makes this issue controversial. For example, DeEll and Prange (1992) found no differences in firmness, sweetness, tartness, or off-flavors after storage between organically and conventionally grown apples. Nonetheless, Moreira *et al.* (2003) suggested that organically grown Swiss chard retained turgidity, color, and brightness longer than the conventionally grown chard. In contrast, Boonyakiat *et al.* (2007) suggested that cabbage and spinach grown under a conventional system had a longer shelf life than the organic vegetables. The same study did not find differences in Cos lettuce.

Sensory analysis has been used by other studies to evaluate the effects of storage. Jacobsson *et al.* (2004) evaluated samples of broccoli for aroma and flavor attributes such as freshness, cooked cabbage, sweetness, and bitterness; textural attributes including chewing resistance and crispness; as well as appearance characteristics such as freshness, greenness, compactness, brownness, evenness, and size. The shelf life of vegetables has also been evaluated from the sensory standpoint by other studies using more general terms. For example, Ares *et al.* (2008) evaluated appearance, discoloration, and off-odour of butterhead lettuce to compare the effects of different packaging during shelf life. A greater number of studies have used survival analysis to evaluate shelf life of products from the perspective of consumers rejecting the products (Hough *et al*., 2003; Giménez *et al*., 2008; Salvador *et al*., 2007).

In addition to sensory characteristics, the profile of aroma volatiles has also been studied to evaluate shelf life. For example, the volatile composition of mushrooms was generated to be used as a tool for monitoring shelf life (Dongkham and Srzednicki, 2006). Once identified and quantified, the chemical information of a vegetable can be correlated with its sensory characteristics, using a variety of multivariate statistical analyses including principal component analysis, generalized procrustes analysis, or partial least squares regression (Noble and Ebeler, 2002). This may give an idea of the relationships between aroma volatiles and sensory attributes. Echeverría *et al*. (2003) correlated volatile profiles, quality, and sensory characteristics of Fuji apples after storage under different conditions using principal component analysis. Voon *et al*. (2007) used partial least squares regression (PLS2) to correlate sensory and volatile data from durian, an exotic seasonal fruit from Southeast Asia. In Addition, Baxter *et al*. (2005) also used PLS to correlate sensory and volatile flavor data from orange juices.

The objectives of this study are (1) to evaluate the sensory characteristics and the aroma volatile content of organically and conventionally grown pac choi leaves at four times during the shelf life (1, 4, 9, and 18 days) and (2) to correlate sensory and instrumental data by means of partial least squares regression.

## Materials and Methods

### ***Samples***

Trials were conducted at the K-State Horticulture Research and Extension Center, Olathe, Kansas, on experimental plots established in 2002 for comparison of crops grown under organic and conventional production systems in high tunnels (unheated, passively ventilated greenhouses) and open field plots (Zhao *et al*., 2007a). The soil was a Kennebec silt loam. Six 9.8 m x 6.1 m high tunnels with 1.5m sidewalls (Stuppy, North Kansas City, MO) and six adjacent 9.8 m x 6.1 m field plots were used for this study. High tunnels were covered with single layer 6-mil (0.153mm) K-50 polyethylene (Klerk's Plastic Product Manufacturing, Inc., Richburg, SC). At establishment of the experimental plots, the six high tunnels were divided into three groups (blocks) and the two high tunnels in each block were randomly assigned for long-term conventional or organic management treatments. A similar set-up was used in the field plots. Organic plots were managed in compliance with USDA National Organic Program standards, and were inspected and certified in 2003, 2006, 2007 and 2008.

For this study, beginning in 2007, each high tunnel or open field plot was subdivided into three 3.2 m x 6.1 m plots to which one of three fertilizer levels were assigned (high, low, and no fertilizer) following a latin square design to avoid bias due to position effects in the high tunnels. Fertilizer rates were determined based on soil analysis at the beginning of the study in 2007, and recommendations for vegetable crops in Kansas (Marr *et al*., 1998), with compost applied to organic plots and synthetic fertilizer applied to conventional plots. Compost application rates were based on the assumption that 50% of the nitrogen from compost would be available to plants during the growing season, while 100% would be available from conventional fertilizers (Warman and Havard, 1997). Low and high fertility plots were fertilized with equal amounts of compost or synthetic fertilizer at the beginning of the growing season, and high fertility plots received additional fertilization during the growing season as described below.

Pac choi (*Brassica rapa* L. *chinensis* 'Mei Qing Choi') (Johnny's Selected Seed, Albion, ME, U.S.A.) and tomato (*Lycopersicon esculentum* 'Bush Celebrity') (Totally Tomatoes, Randolph, WI, U.S.A.) were grown in one half of each open field or high tunnel plot (6.8 m x 3 m) in 2007 and 2008, with a rotation between pac choi and tomato plots each year. In our experimental system, a spring and a fall crop of pac choi was grown each year, while a single crop of tomato was grown. Between the spring and fall pac choi crops, plots were seeded with a summer cover crop of buckwheat (*Fagopyrum sagittatum*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 134 kg/ha. In the late fall, all plots were seeded with a cover crop of annual rye (*Secale cereale*) (Albert Lea Seed, Albert Lea, MN, U.S.A.) at a rate of 229 kg/ha.

Conventional high and low fertility plots were fertilized with Jack's Professional Peat-lite $N\text{-}P_2O_5\text{-}K_2O$ 20-10-20 (Allentown, PA, U.S.A.), at a rate of 98 kg/ha. Organic plots received MicroLeverage compost $N\text{-}P_2O_5\text{-}K_2O$ 0.6-0.8-0.5 (Hughesville, MO, U.S.A) at a rate of 197 kg/ha. Starting 2 weeks after planting, high fertility plots received additional fertilization at a rate of 7.2 kg/ha. Fertilizer used on organic plots was fish hydrolyzate $N\text{-}P_2O_5\text{-}K_2O$ 2.23-4.35-0.3 (Neptune's Harvest, Gloucester, MA, U.S.A) and conventional plots received calcium nitrate and potassium nitrate at a rate calculated to apply an amount of calcium equivalent to that present in the fish hydrolyzate. The tomato crop received 6 weekly applications, for a total of 43 kg/ha, and the spring and fall pac choi crops each received three such applications. Only pac choi grown in the outside plots with high amounts of fertilizer were used for this specific study.

Pac choi transplants were started in a greenhouse in Sunshine Mix Special Blend E6340 (SunGro Horticulture, Bellevue, WA) supplemented with MicroLeverge compost. An amendment of fish hydrolysate was fertigated at a rate 18.1lbs/ hectare. Pac choi was planted on September 4, 2008 and harvested on October 6 of the same year, at approximately 4.5 wks of age, a typical time for pac choi.

## *Sample Preparation*

### *Sensory analysis*

After harvest, the plants were immediately rinsed using cold tap water to remove excess dirt and stored in a refrigerated container for transport to the Kansas State University campus located in Manhattan, KS. The samples were placed in a walk-in refrigerator for storage at 4ºC until testing at 1, 4, 9, and 18 days. This temperature was selected to reduce deterioration of the pac choi leaves while recreating normal conditions in the refrigerator of a consumer. The interval selected was based on previous research by Able *et al*. (2005). The plants were sprayed once every two days with tap water to maintain moisture. On the day of testing, plants were retrieved from the refrigerator. Random leaves of similar visual characteristics were removed from each stalk (not including the stem) and rinsed using distilled water. Excess water was eliminated with a salad spinner (Oxo International, Ltd., New York, NY). Samples were served to the panelists monadically on 6” foam plates identified with a three-digit code to eliminate potential panelist bias. One leaf was served to each panelist.

### *Volatile analysis*

Volatile analysis for each shelf life point occurred the same day as sensory testing. For solid-phase microextraction (SPME) sampling, 4 g pac choi leaves were blended with 200-mL of reverse osmosis, deionized, carbon-filtered water using an electric hand blender (Rival, Peoria, IL) for 20 s. The mixture was then filtered through a double layered cheese cloth. From the filtered solution, 1-mL was transferred to a 10-mL clear headspace vial and mixed with 0.2 g of Sodium Chloride (NaCl). Additionally, 5-µl of 0.2 ppm 1,3 dichlorobenzene in methanol (internal standard) was added. Glass vials were closed using an open-center screw cap with a 1.8 mm silicone/PTFE septum (Varian, Palo Alto, CA).

## *Panelists*

Six highly trained panelists from the Sensory Analysis Center at Kansas State University (Manhattan, KS, U.S.A.) were used for this study. The panelists had completed more than 120 hours of descriptive training, averaged more than 2000 hours of testing experience, and had prior experience testing vegetables and vegetable products.

## *Experimental procedure*

### *Sensory analysis*

The lexicon used for pac choi was created to describe the flavor of different leafy vegetables and was developed by Talavera-Bianchi *et al*. (2009) using an adaptation of the profile method (Caul, 1957; Keane, 1992), which has been used by many studies in the past to help describe a variety of products such as dairy products (Thompson *et al*., 2009; Oupadissakoon *et al*., 2009; Rètiveau *et al*., 2005; Heisserer and Chambers, 1993), green tea (Lee and Chambers, 2007) or tomatoes (Hongsoongnern and Chambers, 2008a). The previously developed lexicon consisting of thirty-two attributes with definitions and references was presented to the panelists prior to the start of testing so they could become familiar with the terminology, test procedures, and samples. Twenty-six flavor and mouth feel attributes were selected to evaluate pac choi. However, three texture attributes (i.e. crispness, moistness and fiber awareness) and three off-flavor attributes (i.e. stale/refrigerator, cardboard and moldy) were added because they were needed to describe changes in the leaves during shelf life.

The day of testing, panelists were presented with the lexicon and references determined while in orientation. Data were collected using a computerized collection system (Compusense Five version 4.6.702, Guelph, ON, Canada). Intensities for each attribute were recorded using a 0-15 point scale divided in 0.5 point increments, 0 meaning “none” and 15 meaning “extremely high”. Panelists evaluated the samples individually and followed a completely randomized block design with the shelf life stage as the blocking factor. Six samples of pac choi were evaluated in each of four 90-minute sessions at 1, 4, 9, and 18 days after storage. Reverse osmosis, deionized, carbon-filtered water and unsalted crackers were used to rinse the palate between the samples. A similar procedure has been used in the past to evaluate the sensory characteristics of four samples of calcium-biofortified lettuce (Park *et al*., 2009).

### *Gas chromatography – mass spectrometry*

Volatile compounds were separated, identified and quantified using a Varian Saturn CP-3800 Gas Chromatograph / Mass Spectrometer 2200 (Varian Inc., Walnut Creek, CA). The sample vials were equilibrated at 40°C/500 rpm for 10 min. SPME was performed using a StableFlex Divinylbenzene / Carboxen / Polydimethylsiloxane 50/30 µm fiber (Sigma Aldrich, Saint Louis, MO) for 20 min at 40°C. The agitation during extraction was at 250 rpm. The extracted compounds were thermally desorbed at 250°C for 3 min in the front injection port of the gas chromatograph. After the injection, the fiber was baked at 270°C for 30 min. An RTX®-5 Capillary Column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness; Restek U.S., Bellefonte, PA) was used to separate the volatiles desorbed from the fiber. The initial temperature of the column was set at 40°C for 2 min and then raised to 200°C, at a rate of 5°C $min^{-1,}$ and held for 1 min (total GC run time was 35 min). Varian MS Workstation software (version 6.8) was used for system control, data collection, and data processing. Compound identification was based on NIST 2005 (National Institute of Standards and Technology, U.S. Department of Commerce, Gaithersburg, MD) version 2.0 Mass Spectra library search. The final compounds concentration was based on the concentration of the internal standard. Three replications were analyzed for each treatment. Kovats retention indices were calculated to aid in the identification of the volatile compounds. A hydrocarbon (HC) mix (1 drop of HC mix in 1 ml of carbon disulfide – $CS_2$ – directly injected to the GC) was also run under the same methodology to generate the retention times of the n-alkanes ($C_6$-$C_{20}$) for calculating the Kovats indices. Comparing Kovats indices from chemicals previously identified using the same column and stationary phase under similar conditions has shown to be an accurate method of identification (Moustafa, 2008).

## *Analysis*

Analysis of variance (ANOVA) with PROC MIXED (panelist and replication as the random effects) was used to detect overall differences among treatments for individual sensory attributes. PROC GLM was used to detect differences for individual volatile compounds. ANOVA was computed in SAS® (2002, version 9.1.3; SAS Institute, Cary, NC). Partial least squares regression (PLS2) was used to correlate sensory and instrumental data. PLS is a soft modeling method which is widely used to predict a set of dependant variables (sensory attributes) from a large set of independent variables (volatile compounds) (Noble and Ebeler, 2002). This method has been previously used to correlate instrumental and sensory data in cheese (Hough *et al*., 1996), diced tomatoes (Lee *et al*., 1999) and ice cream (Chung *et al*., 2003). Even though this analysis does not determine which volatile components are actually responsible for specific sensory attributes, it does help in studying the relationship between certain volatiles and sensory characteristics (Noble and Ebeler, 2002). This analysis was performed by Unscrambler (2005, Version 9.2; Camo Process AS, Oslo, Norway).

## Results and Discussion

### *Sensory analysis*

From the 32 attributes evaluated, 25 were statistically different among days of shelf life for both organically and conventionally grown pac choi (P-value ≤ 0.05). However, differences generally were very small (Table 6.1).

Most of the green flavors did not vary throughout shelf life. Overall green, green-grassy/leafy, and green-viney flavors remained stable after 18 days of storage. The intensity of green-unripe and green-peapod attributes did show some differences during shelf life. Green-unripe was slightly present at day 1 and its intensity decreased until it practically disappeared at day 18. Green-peapod started lower and increased as the storage time progressed. Most of the time, the intensity of flavor attributes changed by less than 1 point out of 15 as the shelf life increased. Other attributes that slightly increased during shelf life were the stale/refrigerator and the moldy off-flavors. Off-flavors may be caused by the fermentative metabolism or the transfer of undesirable flavors from air, water, or packaging materials (Kader, 2008).

The intensities of other attributes such as crispness, moistness, green-unripe, spinach, water-like, overall sweet, and umami decreased as shelf life progressed. Water loss may be responsible for the reduction of textural attributes such as crispness and moistness while degradation of chlorophyll may be affecting some of the green flavor characteristics explained by the green-unripe and spinach attributes. These are common aspects associated with the decrease of quality of leafy vegetables during storage (Burton, 1982; Lu, 2007).

**Table 6.1. Analysis of variance showing significant differences between shelf life points for organically and conventionally grown pac choi[1,2]**

|  |  | Shelf Life |  |  |  |
|---|---|---|---|---|---|
| **Attributes[1]** | **Production** | **Day 1** | **Day 4** | **Day 9** | **Day 18** |
| Crispness | Organic | 4.0ab | 4.1ab | 4.4a | 3.9b |
|  | Conventional | 4.0ab | 4.4ab | 4.4a | 3.8b |
| Moistness | Organic | 4.5a | 4.6a | 4.3ab | 3.9b |
|  | Conventional | 4.7a | 4.7a | 4.0b | 3.6b |
| Fiber Awareness | Organic | 4.3b | 4.1c | 4.7a | 4.1bc |
|  | Conventional | 4.4ab | 4.1b | 4.4a | 4.2b |
| Overall Green | Organic | 7.2 | 7.0 | 7.1 | 6.9 |
|  | Conventional | 7.1 | 7.3 | 6.9 | 6.9 |
| Green-Unripe | Organic | 0.6a | 0.4ab | 0.2b | 0.1b |
|  | Conventional | 0.5a | 0.5a | 0.4a | 0.0b |
| Green-Peapod | Organic | 0.9c | 1.4b | 1.8a | 1.8a |
|  | Conventional | 1.2b | 1.3b | 1.5a | 1.7a |
| Green-Grassy/leafy | Organic | 6.4 | 6.5 | 6.5 | 6.5 |
|  | Conventional | 6.2 | 6.6 | 6.3 | 6.5 |
| Green-Viney | Organic | 2.6 | 2.5 | 2.6 | 2.4 |
|  | Conventional | 2.4 | 2.7 | 2.5 | 2.5 |
| Cabbage | Organic | 2.9 | 2.7 | 2.7 | 2.9 |
|  | Conventional | 2.9ab | 2.4c | 2.6bc | 3.0a |
| Lettuce | Organic | 1.7b | 2.0a | 1.5b | 1.6b |
|  | Conventional | 1.6 | 1.6 | 1.7 | 1.7 |
| Spinach | Organic | 2.2a | 2.1ab | 1.8bc | 1.8c |
|  | Conventional | 2.0 | 1.9 | 1.8 | 1.8 |
| Parsley | Organic | 1.4b | 1.6ab | 1.3b | 1.9a |
|  | Conventional | 1.4b | 1.4b | 1.4b | 1.8a |
| Radish | Organic | 1.6c | 1.7bc | 2.2a | 2.1ab |
|  | Conventional | 1.8ab | 1.6b | 2.0ab | 2.1a |
| Piney | Organic | 1.0b | 0.8b | 0.9b | 1.7a |
|  | Conventional | 0.9b | 0.8b | 1.0b | 1.4a |
| Woody | Organic | 1.6b | 1.6b | 1.8b | 2.3a |
|  | Conventional | 1.4c | 1.8b | 1.9ab | 2.2a |
| Water-like | Organic | 1.3b | 1.6a | 1.4ab | 1.4ab |
|  | Conventional | 1.5ab | 1.6a | 1.3b | 1.3b |
| Musty/Earthy | Organic | 2.0c | 1.9c | 2.2b | 2.8a |
|  | Conventional | 1.8c | 2.0bc | 2.0b | 2.4a |
| Sulfur | Organic | 1.5b | 1.5b | 1.7b | 2.2a |
|  | Conventional | 1.7ab | 1.5b | 1.6ab | 1.8a |
| Soapy | Organic | 0.5b | 0.7b | 0.8b | 1.3a |
|  | Conventional | 0.6b | 0.8ab | 0.9ab | 1.1a |
| Petroleum-like | Organic | 0.6b | 0.4b | 0.8b | 1.4a |
|  | Conventional | 0.3b | 0.7b | 0.6b | 1.3a |

| | | | | | |
|---|---|---|---|---|---|
| Pungent | Organic | 2.2b | 2.2b | 2.6b | 2.9a |
| | Conventional | 2.6a | 2.2b | 2.4ab | 2.7a |
| Bite | Organic | 2.5ab | 2.3b | 2.6a | 2.5ab |
| | Conventional | 2.6a | 2.2b | 2.4ab | 2.3ab |
| Toothetch | Organic | 1.8ab | 1.8b | 1.9ab | 2.0a |
| | Conventional | 1.7b | 1.8b | 1.8b | 2.1a |
| Overall Sweet | Organic | 1.3b | 1.8a | 1.3b | 1.2b |
| | Conventional | 1.3b | 1.8a | 1.3b | 1.2b |
| Sour | Organic | 1.7b | 1.7b | 1.9a | 1.8ab |
| | Conventional | 1.6b | 1.6b | 1.9a | 1.7ab |
| Bitter | Organic | 6.1b | 6.1b | 7.1a | 7.2a |
| | Conventional | 5.9b | 5.9b | 6.9a | 6.9a |
| Salty | Organic | 0.9b | 1.1ab | 1.0b | 1.2a |
| | Conventional | 0.9b | 1.0ab | 0.9b | 1.2a |
| Umami | Organic | 2.1b | 2.5a | 1.9b | 1.9b |
| | Conventional | 1.9b | 2.3a | 1.9b | 1.9b |
| Astringent | Organic | 2.0b | 1.8b | 1.9b | 2.3a |
| | Conventional | 1.8b | 1.7b | 1.8b | 2.2a |
| Stale/Refrigerator | Organic | 0.0c | 0.2c | 0.8b | 1.4a |
| | Conventional | 0.0b | 0.0b | 0.4b | 1.4a |
| Cardboard | Organic | 0.0b | 0.1b | 0.3ab | 0.4a |
| | Conventional | 0.0 | 0.0 | 0.2 | 0.3 |
| Moldy | Organic | 0.0b | 0.0b | 0.1b | 0.5a |
| | Conventional | 0.0b | 0.0b | 0.1b | 0.4a |

[1] Intensities are based on a 15-point numerical scale with 0.5 increments, where 0 means “none” and 15 means “extremely strong.”

[2] Different letters show significant different among shelf life points at 95% confidence

No differences were found between organic and conventional pac choi at any point during shelf life. This means that production system had no effect on sensory properties up to 18 days of shelf-life.

The flavor of pac choi was generally similar between days 1 and 4 with some variation for days 9 and 18. However, differences again were small. It is apparent that most of the decrease in the quality of pac choi during refrigerated storage is related to a decrease in the intensity of textural attributes such as crispness and moistness as well as the increase of off-flavors such as stale/refrigerator and moldy. Loss of crispness and moisture is likely due to water loss which has been suggested to be one of the main factors affecting quality of pac choi during shelf life (Lu, 2007). The appearance of off-flavors is also and important factor indicating the end of shelf life for leafy vegetables (Kader, 2008). Most of the flavor characteristics evaluated remained stable or varied only slightly throughout shelf life. Further flavor quality decline may be observed at a shelf life longer than 18 days.

### *Gas chromatography – mass spectrometry*

Thirty-eight flavor volatiles were identified and quantified (Table 6.2) in the pac choi leaves. Seventeen volatiles were significantly different among shelf life stages (P-value $\leq 0.05$). In all of the cases, the concentration of the volatiles decreased as the shelf life increased. These volatiles were 1-penten-3-ol (4), (E)-2-hepten-1-ol (6), 2-methyl-butanoic acid, methyl ester (7), 2-hexyn-1-ol (9), (E,E)-2,4-hexadienal (14), (E)-2-octenal (18), 4-methyl-1-undecene (20), 1-octanol (21), 3,7-dimethyl-1,6-octadien-3-ol (also known as linalool) (23), camphor (27), menthol (29), (E)-2-decen-1-ol (30), 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (31), isobornyl acetate (33), dodecanal (34), 6,10-dimethyl-5,9-undecadien-2-one (also named geranylacetone) (35), and lillial (38). Water loss, the loss of aroma volatiles, the reduction of sugars and acids, and the development of off-flavors are common factors dictating the post harvest life of vegetable products (Kader, 2008). In our study, many of the aroma volatiles that reduced at prolonged stages of shelf life have been linked to “green” aromas in the past (Table 6.3). It would be expected that the reduction of these aroma volatiles during storage may cause the reduction in the intensity of certain sensory characteristics. The flavor attributes that also showed a reduction in intensity were green-unripe, spinach, overall sweet, and umami. Other flavor attributes that also explain “green” characteristics such as overall green, green-grassy/leafy, and green-viney were not statistically different (P-value $\leq 0.05$) among the times evaluated and remained relatively stable throughout the shelf life of the pac choi.

Aroma volatiles that are present in high concentrations were statistically similar (P-value $\leq 0.05$) throughout shelf life. Some of these volatiles are (Z)-3-hexenal (8), (E)-2-hexenal (10), (Z)-3-hexen-1-ol (11), 4-isothiocyanato-1-butene (16), and 4-ethyl-5-methylthiazole (22). These volatiles have also been associated with “green” characteristics in the past (Table 6.3).

**Table 6.2. Volatile aromatics found in organically and conventionally grown pac choi during shelf life[1,2]**

| | | **Day 1** | | **Day 4** | | **Day 9** | | **Day 18** | |
|---|---|---|---|---|---|---|---|---|---|
| | | **ORG** | **CON** | **ORG** | **CON** | **ORG** | **CON** | **ORG** | **CON** |
| **1** | 2-butanone | 14.0 | 12.7 | 12.4 | 13.0 | 10.2 | Trace | 10.7 | 11.3 |
| **2** | Methyl propionate | Trace | Trace | Trace | Trace | Trace | Trace | Trace | Trace |
| **3** | 3-methyl-2-butanone | Trace | Trace | Trace | Trace | Trace | Trace | Trace | Trace |
| **4** | 1-Penten-3-ol | 53.6 | 42.0 | 43.8 | 41.8 | 36.6 | 30.1 | 24.9 | 27.8 |
| **5** | Butanoic acid, methyl ester | 20.0 | 19.0 | 18.9 | 17.9 | 15.5 | 15.3 | 21.2 | 17.8 |
| **6** | (E)-2-hepten-1-ol | 63.1 | 48.8 | 46.8 | 47.9 | 37.7 | 30.2 | 25.8 | 30.8 |
| **7** | 2-methyl-butanoic acid, methyl ester | 24.7 | 23.8 | 21.2 | 21.9 | 20.5 | 18.8 | 19.3 | 19.2 |
| **8** | (Z)-3-hexenal | 12371.3 | 13437.6 | 12037.8 | 15677.1 | 10579.0 | 10406.1 | 9445.3 | 10920.3 |
| **9** | 2-hexyn-1-ol | 1143.0 | 1042.2 | 969.5 | 1090.8 | 783.6 | 657.7 | 631.0 | 751.7 |
| **10** | (E)-2-hexenal | 14144.7 | 10304.2 | 12577.2 | 10492.9 | 10470.2 | 7636.7 | 7239.0 | 7394.7 |
| **11** | (Z)-3-hexen-1-ol | 1029.7 | 941.4 | 848.9 | 748.5 | 889.5 | 680.4 | 541.1 | 606.1 |
| **12** | (E)-3-hepten-1-ol | 470.6 | 347.0 | 395.5 | 368.9 | 315.8 | 251.8 | 238.8 | 290.1 |
| **13** | Heptanal | 10.8 | Trace | 11.7 | Trace | 12.3 | Trace | 10.4 | Trace |
| **14** | (E,E)-2,4-hexadienal | 470.6 | 512.8 | 351.6 | 459.2 | 277.2 | 269.8 | 252.7 | 335.8 |
| **15** | 1-isothiocyanato-butane | 98.9 | 265.1 | 253.1 | 388.7 | 149.6 | 219.7 | 324.4 | 129.4 |
| **16** | 4-isothiocyanato-1-butene | 12475.6 | 10540.6 | 10898.3 | 9921.3 | 11141.6 | 10826.1 | 7267.5 | 9718.9 |
| **17** | Octanal | Trace | Trace | Trace | Trace | Trace | Trace | Trace | Trace |
| **18** | (E)-2-octenal | 72.1 | 74.4 | 46.1 | 38.0 | 48.5 | 45.6 | 18.8 | 22.0 |
| **19** | Benzeneacetaldehyde | 356.2 | 247.4 | 361.8 | 270.7 | 300.9 | 226.3 | 245.2 | 216.8 |
| **20** | 4-methyl-1-undecene | 25.0 | 24.7 | 21.8 | 21.5 | 18.9 | 17.6 | 15.9 | 17.3 |
| **21** | 1-octanol | 76.9 | 58.0 | 64.7 | 42.5 | 56.7 | 39.2 | 31.9 | 34.5 |
| **22** | 4-ethyl-5-methylthiazole | 14432.5 | 12443.5 | 14440.6 | 12882.2 | 14022.2 | 13621.4 | 9610.7 | 12896.5 |
| **23** | 3,7-dimethyl-1,6-octadien-3-ol (linalool) | 30.4 | 22.7 | 28.1 | 20.1 | 24.3 | 22.0 | 16.6 | 17.3 |
| **24** | (E)-2-nonen-1-ol | 138.0 | 59.8 | 108.4 | 63.9 | 110.2 | 45.5 | 70.8 | 47.4 |
| **25** | 4,5-dimethyl-thiazole | 284.6 | 185.5 | 165.5 | 234.6 | 198.0 | 165.3 | 117.3 | 189.8 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **26** | Benzyl nitrile | Trace | Trace | Trace | 28.4 | Trace | Trace | Trace | Trace |
| **27** | Camphor | Trace | Trace | Trace | Trace | Trace | Trace | Trace | Trace |
| **28** | 4-Methylpentyl isothiocyanate | 179.4 | 98.6 | 161.9 | 104.4 | 121.1 | 136.5 | 100.6 | 117.4 |
| **29** | Menthol | 26.1 | 29.3 | 19.3 | 16.8 | 15.0 | 17.5 | 11.5 | 17.7 |
| **30** | (E)-2-decen-1-ol | 38.7 | 27.7 | 38.9 | 33.9 | 28.1 | 21.1 | 24.5 | 27.4 |
| **31** | 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde | 375.7 | 290.1 | 277.1 | 242.3 | 240.5 | 211.8 | 173.6 | 192.8 |
| **32** | Isothiocyanato-cyclohexane | 266.6 | 252.5 | 221.2 | 229.8 | 212.0 | 199.2 | 179.8 | 211.9 |
| **33** | Isobornyl acetate | Trace | Trace | Trace | Trace | Trace | Trace | Trace | Trace |
| **34** | Dodecanal | Trace | Trace | Trace | Trace | Trace | Trace | Trace | Trace |
| **35** | 6,10-dimethyl-5,9-undecadien-2-one (Geranylacetone) | 31.0 | 24.2 | 30.5 | 25.3 | 22.8 | 24.6 | 11.9 | 12.1 |
| **36** | 2-isothiocyanatoethyl-benzene | 3995.0 | 2556.9 | 4646.2 | 3032.2 | 3521.3 | 2746.2 | 2370.7 | 2899.2 |
| **37** | Butylated hydroxytoluene | Trace | Trace | Trace | Trace | Trace | Trace | Trace | Trace |
| **38** | Lilial | Trace | Trace | Trace | Trace | N.D. | N.D. | N.D. | N.D. |

[1] ORG=Organic; CON=Conventional

[2] Concentration of volatile shown in pg/g of pac choi

N.D: Not Detected

**Table 6.3. Volatile aromatics found in organically and conventionally grown pac choi during shelf life including retention index and expected aroma characteristics**

| | | **RI[1]** | **Literature Retention Index** | **Expected Aroma** |
|---|---|---|---|---|
| **1** | 2-butanone | **585.6** | **580**<br>Baloga *et al.*, 1990 | Fragrant, pleasant<br>Morales *et al.*, 1995 |
| **2** | Methyl propionate | **622.2** | **621**<br>Beaulieu and Grimm, 2001 | |
| **3** | 3-methyl-2-butanone | **657.3** | **654**<br>Larsen and Frisvad, 1995 | |
| **4** | 1-Penten-3-ol | **685.1** | **680**<br>Meynier *et al.*, 1999 | Powerful grassy green aroma<br>Chida *et al.*, 2004 |
| **5** | Butanoic acid, methyl ester | **728.1** | **717**<br>Beaulieu and Grimm, 2001 | |
| **6** | (E)-2-hepten-1-ol | **775.7** | **970**<br>Rembold *et al.*, 1989[2] | |
| **7** | 2-methyl-butanoic acid, methyl ester | **781.7** | **772**<br>Beaulieu and Grimm, 2001 | |
| **8** | (Z)-3-hexenal | **805.7** | **796**<br>Beaulieu and Grimm, 2001 | Strong green, green leaves, grassy<br>Morales *et al.*, 1995<br>Iraqi *et al.*, 2005 |
| **9** | 2-hexyn-1-ol | **851.7** | **847**<br>Lee and Kim, 2002 | |
| **10** | (E)-2-hexenal | **857.5** | **848**<br>Gómez *et al.*, 1993 | Green, green-fruity, bitter<br>Morales *et al.*, 1995<br>Klesk and Kian 2003 |
| **11** | (Z)-3-hexen-1-ol | **860.7** | **849**<br>Gómez *et al.*, 1993 | Green, banana-like<br>Morales *et al.*, 1995<br>Chambers *et al.*, 1998 |
| **12** | (E)-3-hepten-1-ol | **867.0** | **968**<br>NIST[3] | |
| **13** | Heptanal | **903.9** | **898**<br>Gómez *et al.*, 1993 | Floral<br>Yang, Shewfelt, Lee, & Kays, 2008 |
| **14** | (E,E)-2,4-hexadienal | **917.3** | **911**<br>Flamini *et al.*, 2003 | Green, vegetable, burnt<br>Venkateshwarlu *et al.*, 2004<br>Hartvigsen *et al.*, 2000 |

| | | | | |
|---|---|---|---|---|
| **15** | 1-isothiocyanato-butane | **938.2** | **943**<br>Afsharypuor *et al.*, 1998 | |
| **16** | 4-isothiocyanato-1-butene | **989.0** | **978**<br>Engel *et al.*, 2002 | Sulfur<br>Engel *et al.*, 2002 |
| **17** | Octanal | **1004.5** | **1003**<br>Flamini *et al.*, 2003 | Green, citrus-like<br>Buettner and Schieberle, 2001<br>Iraqi *et al.*, 2005 |
| **18** | (E)-2-octenal | **1032.9** | **1057**<br>Beaulieu and Grimm, 2001 | Green<br>Aparicio *et al.*, 1997 |
| **19** | Benzeneacetaldehyde | **1049.3** | **1045**<br>Gómez *et al.*, 1993 | Green, flower, honey, bitteralmond<br>Heiniö *et al.*, 2003 |
| **20** | 4-methyl-1-undecene | **1072.3** | **1085**<br>Timón *et al.*, 1998 | |
| **21** | 1-octanol | **1075.1** | **1070**<br>Beaulieu and Grimm, 2001 | Grass, pepper<br>Mehinagic *et al.*, 2006 |
| **22** | 4-ethyl-5-methylthiazole | **1086.9** | **1068**<br>Mottram and Whitfield, 1995[4] | |
| **23** | 3,7-dimethyl-1,6-octadien-3-ol (linalool) | **1100.8** | **1090**<br>Senatore *et al.*, 1997 | Sweet, floral, citrus, woody<br>Thi Minh Tu *et al.*, 2002<br>Chida *et al.*, 2004 |
| **24** | (E)-2-nonen-1-ol | **1105.7** | **1149**<br>Flath *et al.*, 1983[5] | |
| **25** | 4,5-dimethyl-thiazole | **1128.7** | **945**<br>Parker *et al.*, 2000 | Smoky, roasty, fragrant, nutty<br>Specht and Baltes, 1994 |
| **26** | Benzyl nitrile | **1145.2** | **1142**<br>Tellez *et al.*, 2002 | |
| **27** | Camphor | **1151.3** | **1144**<br>Sagrero-Nieves *et al.*, 1997 | Piney, spicy<br>Mehinagic *et al.*, 2006 |
| **28** | 4-Methylpentyl isothiocyanate | **1172.1** | **1166**<br>Afsharypuor and Suleimany, 2002[6] | |
| **29** | Menthol | **1177.6** | **1171**<br>Egolf and Jurs, 1993[7] | Refreshing, light, sweet, pungent<br>Chida *et al.*, 2004 |

| | | | | |
|---|---|---|---|---|
| **30** | (E)-2-decen-1-ol | **1208.3** | **1254**<br>Smallfield *et al.*, 1994[8] | |
| **31** | 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde | **1229.9** | **1223**<br>Bader *et al.*, 2003 | |
| **32** | Isothiocyanato-cyclohexane | **1243.5** | **1231**<br>Rapior *et al.*, 2003 | |
| **33** | Isobornyl acetate | **1301.8** | **1317**<br>Porta *et al.*, 1999 | |
| **34** | Dodecanal | **1410.8** | **1411**<br>Högnadóttir and Rouseff, 2003 | Green, sour, citrus<br>Thi Minh Tu *et al.*, 2002<br>Hashizume and Samuta, 1997 |
| **35** | 6,10-dimethyl-5,9-undecadien-2-one (Geranylacetone) | **1457.2** | **1448**<br>Beaulieu and Grimm, 2001 | Pungent, floral, sweet, slightly green, magnolia-like<br>Chida *et al.*, 2004 |
| **36** | 2-isothiocyanatoethyl-benzene | **1476.4** | **1465**<br>Afsharypuor and Suleimany, 2002[9] | |
| **37** | Butylated hydroxytoluene | **1518.8** | **1504**<br>Gómez *et al.*, 1993 | |
| **38** | Lilial | **1534.5** | **1543**<br>NIST[3] | |

[1] Retention index (Kovats) calculated from DB5, 30m column; [2] Calculated with a SE-54, 30m column; [3] Column used unknown; [4] Calculated with a DB5, 50m column; [5] Calculated with a DB1, 60m column; [6] Calculated with a HP5MS, 30m column; [7] Calculated with an OV101, 50m column; [8] Calculated with a DB1, 9.5m column; [9] Calculated with a HP5MS, 30m column.

The lack of change in those compounds may relate to the relative stability of the overall flavor of pac choi throughout its shelf life. Some sensory attributes increased in intensity but the differences generally were very small.

Heptanal (13), octanal (17), benzeneacetaldehyde (19), 1-octanol (21), and (E)-2-nonen-1-ol (24) volatiles were generally higher for organic pac choi throughout shelf life. Possibly because these chemicals were present at relatively low concentrations, these differences did not appear to translate into sensory differences. Organic and conventionally grown pac choi were not different from the sensory standpoint throughout shelf life.

### *Relationship between sensory and chemical data*

Partial least squares regression (PLS) was performed to further assess the relationships between sensory and chemical data for the samples evaluated (Figure 6.1). Results indicate that 83% of the chemical data explains 61% of the sensory data. Aroma volatiles and sensory attributes that were not found to be significant (P-value $\leq 0.10$) in the analysis of variance were not included in PLS analysis. Results show that both organic and conventional samples evaluated at days 1 and 4 are more similar to each other compared to the samples evaluated at days 9 and 18. This shows that during the first 4 days of storage, the quality of pac choi remains stable.

All the aroma volatiles were more related to samples evaluated at days 1 and 4 compared to samples evaluated at days 9 and 18. This means that the concentration of the chemicals started higher in most of the cases and then declined at longer stages of shelf life. The concentration of aroma volatiles is expected to decline during storage (Kader, 2008; Dongkham and Srzednicki 2006). These changes in volatile composition may affect the sensory characteristics of the vegetables. For example, it was reported that the loss of certain chemicals such as 1-octen-3-ol and 3-octanone was correlated to the loss of freshness and aroma of mushrooms during storage (Dongkham and Srzednicki 2006). In our study, the loss of volatile concentration is correlated with the reduction of attributes such as spinach, green-unripe, umami, overall sweet, water-like, moistness, and crispness. Other attributes such as cabbage, sulfur, woody, or bitter increased slightly towards the last days of shelf day evaluated. However, the variation was very small.

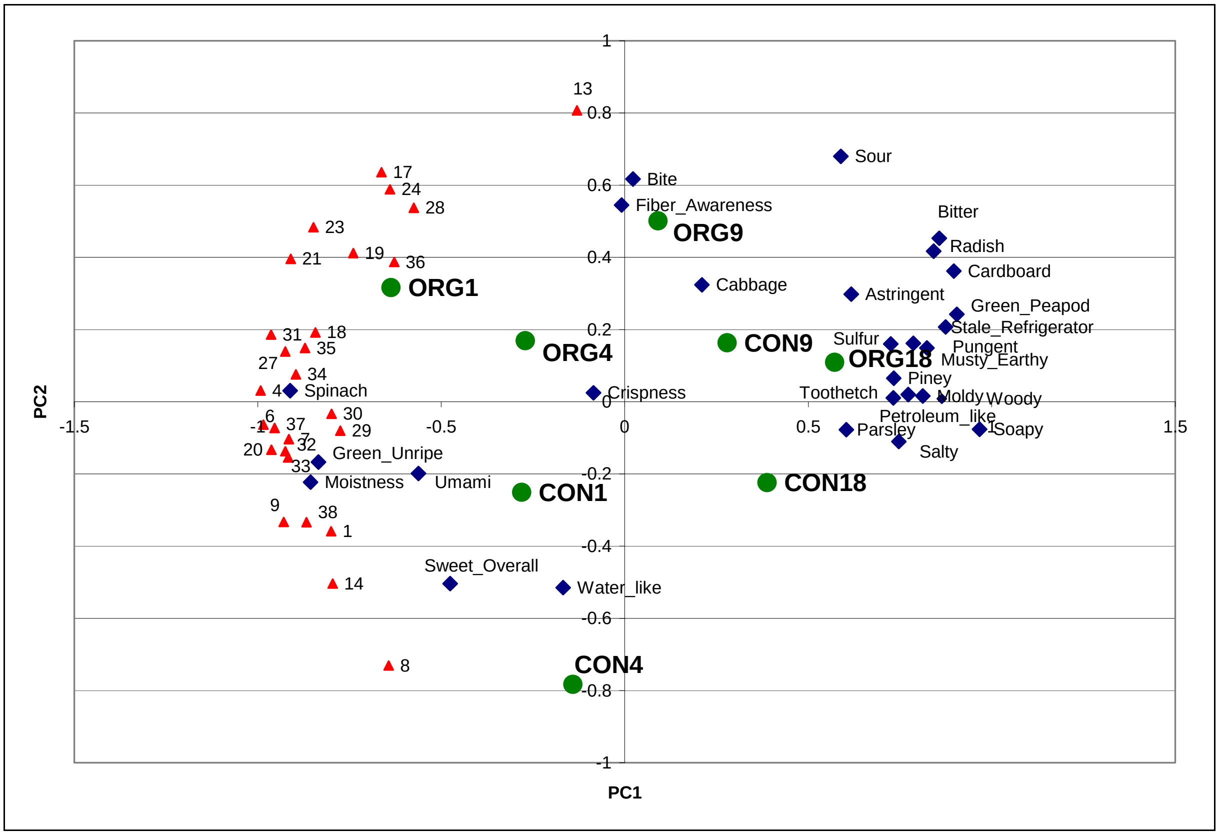

**Figure 6.1. Partial least squares regression (PLS) correlating sensory and instrumental data. CON1=Conventional Day 1; ORG1=Organic Day 1; CON4=Conventional Day 4; ORG4=Organic Day 4; CON9=Conventional Day 9; ORG9=Organic Day 9; CON18=Conventional Day 18; ORG18=Organic Day 18.**

This study suggests that certain volatiles may have higher concentrations in organic samples. This is the case of heptanal (13), octanal (17), benzeneacetaldehyde (19), octanol (21), linalool (23), (E)-2-nonen-1-ol (24), Methylpentyl isothiocyanate (28), and 2-isothiocyanatoethyl-benzene (36). Conversely, other volatiles have higher concentrations in conventional samples. This is the case of (Z)-3-hexenal and (E,E)-2,4-hexadienal which have been associated with “green” aromas in the past (Iraqi *et al*., 2005; Venkateshwarlu *et al*., 2004).

The analysis confirms that the few differences in volatile concentration that were found among shelf life points and production systems did not translate into sensory differences probably because the volatiles with highest concentration  such as (E)-2-hexenal (10) and 4-isothiocyanato-1-butene (16) remained relatively stable throughout shelf life. These chemicals have been previously associated with “green” and “sulfur” aromas respectively (Klesk and Kian, 2003; Engel *et al*., 2002), key characteristics of pac choi and other plants from the *Brassica* family.

## Conclusions

The sensory characteristics of pac choi grown under organic and conventional system remained similar throughout the 18-day storage period. This means that the vegetable grown under a certain production system did not outlast the other from a sensory perspective. From the chemical perspective a few differences are noted. Aroma volatiles such as heptanal (13), octanal (17), benzeneacetaldehyde (19), 1-octanol (21), and (E)-2-nonen-1-ol (24) were generally higher in organic pac choi. However, these differences did not translate into sensory differences. PLS analysis suggests that little change occurred in the pac choi in the first 4 days of shelf life. It is apparent that most of the decrease in the quality of pac choi during refrigerated storage is related to a decrease in the intensity of textural attributes such as crispness and moistness as well as the increase of off-flavors such as stale/refrigerator and moldy, however those changes were small. Most of the flavor characteristics evaluated remained constant or varied slightly throughout shelf life. This may be because major volatile compounds such as (E)-2-hexenal (10) and 4-isothiocyanato-1-butene (16) remained relatively stable throughout shelf life.

# CHAPTER 7 - References

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# Appendix A - SAS® Program Code used for analysis of leafy vegetables

## Principal Component Analysis

```
data leafy;
input sample$ Green_Overall Green_Unripe Green_Peapod Green_Grassy_Leafy
Green_Viney Cabbage Celery Lettuce Spinach Parsley Beet Radish Citrus Piney
Woody Water_like Musty_Earthy Floral Sulfur Metallic Soapy Petroleum_like
Pungent Bite Toothetch Heatburn Sweet_Overall Sour Bitter Salty Umami
Astringent
;
cards;
***DATA DELETED***
;
proc factor data=leafy method=principal msa rotate=varimax scree;
proc print;
run;
```

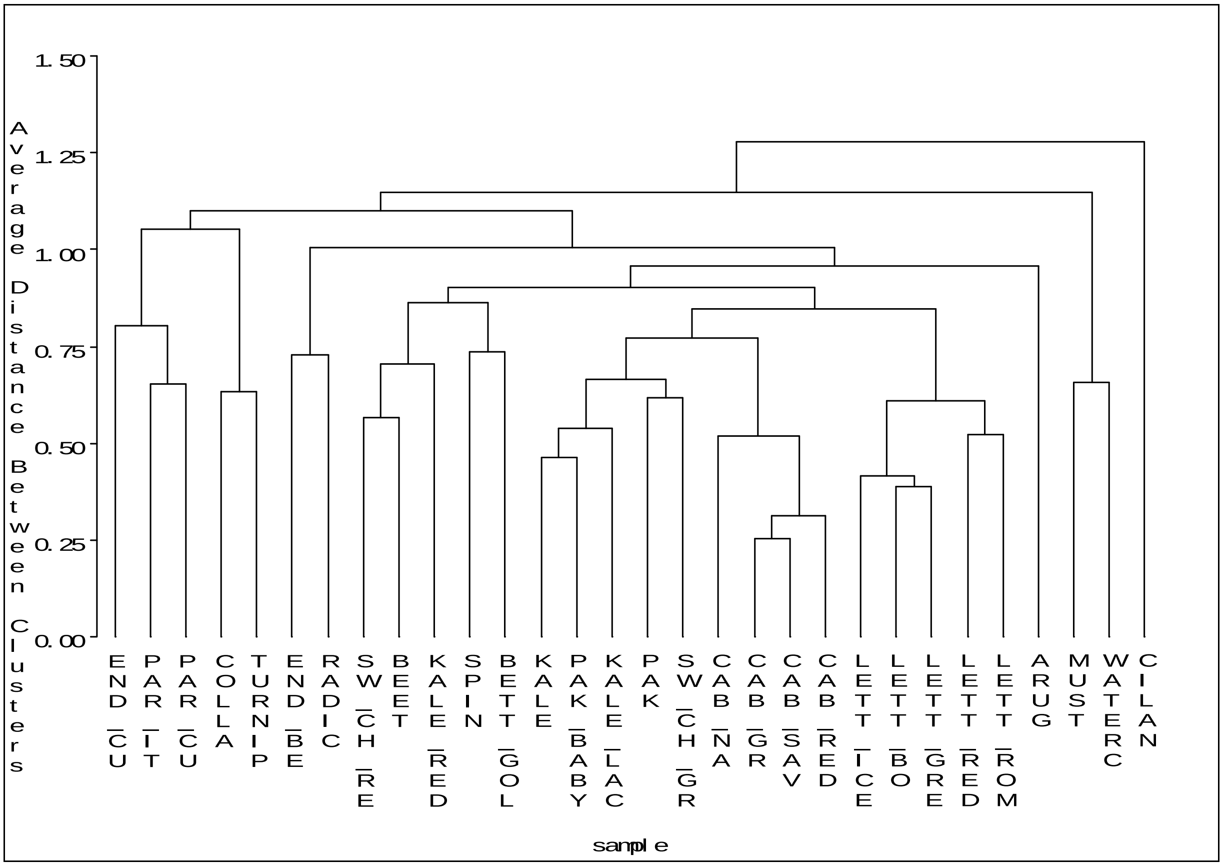
## Cluster Analysis

```
data leafy;
input sample$ Green_Overall   Green_Unripe Green_Peapod Green_Grassy_Leafy
Green_Viney Cabbage Celery Lettuce Spinach Parsley Beet Radish Citrus Piney
Woody Water_like Musty_Earthy Floral Sulfur Metallic Soapy
Petroleum_likePungent Bite Toothetch Heatburn Sweet_Overall Sour Bitter Salty
Umami Astringent
;
cards;
***DATA DELETED***
;
proc cluster standard method=average ccc pseudo outtree=tree;
var Green_Overall -- Astringent;
ID Sample;
run;
Proc tree data=tree nclusters=9;
copy Green_Overall -- Astringent;
ID sample;
run;
proc print data=tree;
run;
```

# Appendix B - Factor loadings of flavor attributes of green leafy vegetables

| | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 | Factor 7 | Factor 8 | Factor 9 |
|---|---|---|---|---|---|---|---|---|---|
| Green, Unripe | **0.90** | -0.06 | -0.04 | -0.03 | 0.10 | -0.09 | -0.21 | 0.08 | -0.05 |
| Bitter | **0.87** | 0.39 | -0.03 | 0.13 | 0.08 | -0.01 | 0.02 | -0.05 | 0.08 |
| Astringent | **0.64** | 0.11 | 0.24 | -0.07 | 0.11 | -0.05 | 0.10 | 0.53 | 0.19 |
| Green, Viney | **0.61** | 0.51 | -0.20 | 0.05 | 0.32 | -0.09 | 0.17 | 0.14 | 0.14 |
| Water-like | **-0.51** | -0.02 | -0.30 | -0.28 | -0.24 | -0.23 | -0.43 | -0.14 | -0.35 |
| Sweet, Overall | **-0.72** | -0.08 | -0.09 | -0.12 | 0.10 | -0.44 | -0.02 | 0.00 | -0.16 |
| Pungent | 0.12 | **0.96** | 0.04 | -0.04 | 0.02 | -0.02 | -0.02 | 0.01 | 0.02 |
| Heatburn | -0.10 | **0.93** | -0.08 | -0.01 | 0.04 | -0.01 | 0.12 | 0.00 | 0.06 |
| Bite | 0.27 | **0.90** | 0.17 | 0.08 | 0.03 | -0.04 | -0.05 | -0.02 | 0.14 |
| Radish | 0.11 | **0.84** | 0.40 | -0.04 | 0.13 | -0.09 | -0.05 | 0.06 | -0.09 |
| Cabbage | 0.07 | 0.26 | **0.87** | -0.13 | -0.07 | -0.07 | -0.22 | -0.07 | 0.20 |
| Green, Peapod | -0.25 | -0.02 | **0.80** | -0.14 | 0.07 | 0.00 | -0.10 | 0.12 | 0.02 |
| Sulfur | 0.23 | 0.19 | **0.60** | 0.16 | -0.24 | -0.09 | -0.21 | -0.20 | -0.13 |
| Celery | -0.04 | 0.05 | **-0.57** | 0.32 | 0.33 | -0.19 | 0.10 | -0.23 | 0.25 |
| Lettuce | -0.24 | -0.11 | **-0.65** | -0.34 | 0.08 | -0.19 | -0.40 | 0.00 | -0.21 |
| Citrus | -0.12 | -0.03 | -0.12 | **0.87** | -0.13 | 0.05 | -0.11 | 0.13 | 0.21 |
| Floral | -0.04 | -0.11 | -0.11 | **0.86** | 0.07 | -0.05 | 0.19 | -0.17 | 0.15 |
| Petroleum-like | 0.44 | 0.16 | 0.06 | **0.76** | 0.10 | -0.02 | 0.09 | 0.17 | -0.27 |
| Piney | 0.42 | 0.17 | 0.12 | **0.59** | 0.31 | 0.03 | 0.17 | 0.28 | -0.17 |
| Green, Grassy/Leafy | 0.14 | -0.01 | -0.45 | 0.13 | **0.82** | -0.04 | 0.01 | 0.03 | -0.06 |
| Green, Overall | 0.31 | 0.21 | -0.21 | 0.15 | **0.82** | -0.07 | 0.04 | 0.20 | 0.08 |
| Woody | 0.15 | 0.12 | 0.11 | 0.07 | **0.71** | 0.55 | 0.11 | 0.06 | 0.09 |
| Parsley | 0.26 | -0.09 | -0.12 | 0.47 | **0.51** | -0.12 | 0.36 | -0.36 | -0.05 |
| Metallic | 0.31 | -0.07 | -0.23 | 0.30 | **-0.75** | -0.13 | -0.02 | -0.05 | 0.11 |
| Musty/Earthy | 0.11 | -0.04 | 0.05 | 0.08 | 0.17 | **0.93** | 0.07 | 0.18 | 0.09 |
| Beet | -0.10 | -0.14 | -0.07 | -0.14 | -0.08 | **0.90** | 0.17 | 0.02 | -0.08 |
| Salty | -0.04 | -0.04 | -0.29 | -0.13 | -0.03 | 0.09 | **0.76** | 0.36 | 0.29 |
| Umami | -0.38 | 0.13 | -0.07 | 0.16 | 0.15 | 0.12 | **0.74** | 0.06 | -0.01 |
| Soapy | 0.45 | -0.06 | -0.19 | 0.22 | 0.07 | 0.18 | **0.68** | 0.07 | -0.14 |
| Spinach | -0.08 | -0.02 | -0.10 | -0.01 | 0.15 | 0.06 | 0.11 | **0.86** | 0.19 |
| Tooth-etch | 0.34 | 0.02 | 0.09 | 0.16 | -0.01 | 0.17 | 0.15 | **0.78** | -0.05 |
| Sour | 0.29 | 0.24 | 0.14 | 0.19 | -0.04 | 0.05 | 0.12 | 0.32 | **0.76** |

# Appendix C - Cluster analysis of green leafy vegetables



# Appendix D - SAS® Program Code used for analysis of pac choi in different environments

## Field

```
Data opt;
input lscolumn    lsrow Pa M$ L$ F$ Rep Crispness Moistness Fiber_Awareness
Green_Overall Green_Unripe Green_Peapod Green_Grassy_Leafy Green_Viney
Cabbage Lettuce Spinach Parsley Radish Piney Woody Water_like Musty_Earthy
Sulfur Soapy Petroleum_like Pungent Bite Toothetch Sweet_Overall Sour
BitterSalty Umami Astringent;
cards;
***DATA DELETED***
;
%macro mix(y);
proc mixed;
class M F lscolumn lsrow Pa;
model &y=M|F/ddfm=satterth;
random Pa lscolumn lsrow lscolumn*M Pa*lscolumn*M;
lsmeans M|F/pdiff;
run;
%mend mix;
%mix (Crispness);
%mix (Moistness);
%mix (Fiber_Awareness);
%mix (Green_Overall);
%mix (Green_Unripe);
%mix (Green_Peapod);
%mix (Green_Grassy_Leafy);
%mix (Green_Viney);
%mix (Cabbage);
%mix (Lettuce);
%mix (Spinach);
%mix (Parsley);
%mix (Radish);
%mix (Piney);
%mix (Woody);
%mix (Water_like);
%mix (Musty_Earthy);
%mix (Sulfur);
%mix (Soapy);
%mix (Petroleum_like);
%mix (Pungent);
%mix (Bite);
%mix (Toothetch);
%mix (Sweet_Overall);
%mix (Sour);
%mix (Bitter);
%mix (Salty);
%mix (Umami);
%mix (Astringent);
```

# High Tunnel

```
Data opt;
input lscolumn    lsrow Pa M$ L$ F$ Rep Crispness Moistness Fiber_Awareness
Green_Overall Green_Unripe Green_Peapod Green_Grassy_Leafy Green_VineyCabbage
Lettuce Spinach Parsley Radish Piney Woody Water_like Musty_Earthy Sulfur
Soapy Petroleum_like Pungent Bite Toothetch Sweet_Overall Sour Bitter Salty
Umami Astringent;
cards;
***DATA DELETED***
;
%macro mix(y);
proc mixed;
class M F lscolumn lsrow Pa;
model &y=M|F/ddfm=satterth;
random Pa lscolumn lsrow lscolumn*M Pa*lscolumn*M;
lsmeans M|F/pdiff;
run;
%mend mix;
%mix (Crispness);
%mix (Moistness);
%mix (Fiber_Awareness);
%mix (Green_Overall);
%mix (Green_Unripe);
%mix (Green_Peapod);
%mix (Green_Grassy_Leafy);
%mix (Green_Viney);
%mix (Cabbage);
%mix (Lettuce);
%mix (Spinach);
%mix (Parsley);
%mix (Radish);
%mix (Piney);
%mix (Woody);
%mix (Water_like);
%mix (Musty_Earthy);
%mix (Sulfur);
%mix (Soapy);
%mix (Petroleum_like);
%mix (Pungent);
%mix (Bite);
%mix (Toothetch);
%mix (Sweet_Overall);
%mix (Sour);
%mix (Bitter);
%mix (Salty);
%mix (Umami);
%mix (Astringent);
```

# Principal Component Analysis

```
data assign2;
input Judge$ Sample$ Rep$ Crispness Moistness Fiber_Awareness Green_Overall
Green_Unripe Green_Peapod Green_Grassy_Leafy Green_Viney Cabbage Lettuce
Spinach Parsley Radish Piney Woody Water_like Musty_Earthy Sulfur Soapy
Petroleum_like Pungent Bite Toothetch Sweet_Overall Sour Bitter Salty
UmamiAstringent;
datalines;
***DATA DELETED***
;
ods rtf;
proc sort;
by sample rep judge;
proc means mean noprint;
by sample;
var Crispness Moistness Fiber_Awareness Green_Overall Green_Unripe
Green_Peapod Green_Grassy_Leafy Green_Viney Cabbage Lettuce Spinach Parsley
Radish Piney Woody Water_like Musty_Earthy Sulfur Soapy Petroleum_like
Pungent Bite Toothetch Sweet_Overall Sour Bitter Salty Umami Astringent;
output out = meancheese mean = mCrispness mMoistness mFiber_Awareness
mGreen_Overall mGreen_Unripe mGreen_Peapod mGreen_Grassy_Leafy
mGreen_VineymCabbage mLettuce mSpinach mParsley mRadish mPiney mWoody
mWater_like mMusty_Earthy mSulfur mSoapy mPetroleum_like mPungent mBite
mToothetch mSweet_Overall mSour mBitter mSalty mUmami mAstringent;
proc factor data = meancheese scree score cov outstat = cheese rotate =
varimax method = prin mineigen = 0.05;
var mCrispness mMoistness mFiber_Awareness mGreen_Overall mGreen_Unripe
mGreen_Peapod mGreen_Grassy_Leafy mGreen_VineymCabbage mLettuce mSpinach
mParsley    mRadish mPiney mWoody mWater_like mMusty_Earthy mSulfur mSoapy
mPetroleum_like mPungent mBite mToothetch mSweet_Overall mSour mBitter mSalty
mUmami      mAstringent;
proc score data = meancheese scores = cheese out = sccheese;
var mCrispness mMoistness mFiber_Awareness mGreen_Overall mGreen_Unripe
mGreen_Peapod mGreen_Grassy_Leafy mGreen_VineymCabbage mLettuce mSpinach
mParsley    mRadish mPiney mWoody mWater_like mMusty_Earthy mSulfur mSoapy
mPetroleum_like mPungent mBite mToothetch mSweet_Overall mSour mBitter mSalty
mUmami      mAstringent;
proc print data = sccheese;
run;
ods rtf close; quit;
```

# Cluster Analysis

```
data assign2;
input Sample$ Crispness Moistness Fiber_Awareness Green_Overall Green_Unripe
Green_Peapod Green_Grassy_Leafy Green_Viney Cabbage Lettuce Spinach Parsley
Radish Piney Woody Water_like Musty_Earthy Sulfur Soapy Petroleum_like
Pungent Bite Toothetch Sweet_Overall Sour Bitter Salty Umami;
datalines;
***DATA DELETED***
;
proc cluster data = assign2 outtree=treesl method=ward ccc;
id sample; run;
title2 'Ward-tree';
proc tree;
id sample; run;
proc cluster data = assign2 outtree=treesl method=average ccc;
id sample; run;
title2 'Average-tree';
proc tree;
id sample; run;
```

# Appendix E - SAS® Program Code used for analysis of tomato in different environments

## Field

```
Data tomato;
input lscolumn lsrow Pa M$ L$ F$ Rep Tomato_ID_AR Green_Viney_AROver_Ripe_AR
Cardboard_AR Color Uniformity_Color Juiciness Mealy Skin_Awareness
Seed_Awareness Fiber_Awareness Tomato_ID Ripeness Green_Viney Umami Fruity
Cardboard Fermented Musty_Earthy Overall_Sweet Sweet Sour Salt Bitter
Astringent Metallic Chemical
;
cards;
***DATA DELETED***
;
%macro mix(y);
proc mixed;
class M F lscolumn lsrow Pa;
model &y=M|F/ddfm=satterth;
random Pa lscolumn lsrow lscolumn*M Pa*lscolumn*M;
lsmeans M|F/pdiff;
run;
%mend mix;
%mix (Tomato_ID_AR);
%mix (Green_Viney_AR);
%mix (Over_Ripe_AR);
%mix (Cardboard_AR);
%mix (Color);
%mix (Uniformity_Color);
%mix (Juiciness);
%mix (Mealy);
%mix (Skin_Awareness);
%mix (Seed_Awareness);
%mix (Fiber_Awareness);
%mix (Tomato_ID);
%mix (Ripeness);
%mix (Green_Viney);
%mix (Umami);
%mix (Fruity);
%mix (Cardboard);
%mix (Fermented);
%mix (Musty_Earthy);
%mix (Overall_Sweet);
%mix (Sweet);
%mix (Sour);
%mix (Salt);
%mix (Bitter);
%mix (Astringent);
%mix (Metallic);
%mix (Chemical);
```

# High Tunnel

```
Data tomato;
input lscolumn lsrow Pa M$ L$ F$ Rep Tomato_ID_AR Green_Viney_AR Over_Ripe_AR
Cardboard_AR Color Uniformity_Color Juiciness Mealy Skin_Awareness
Seed_Awareness Fiber_Awareness Tomato_ID Ripeness Green_Viney Umami Fruity
Cardboard Fermented Musty_Earthy Overall_Sweet Sweet Sour Salt Bitter
Astringent Metallic Chemical
;
cards;
***DATA DELETED***
;
%macro mix(y);
proc mixed;
class M F lscolumn lsrow Pa;
model &y=M|F/ddfm=satterth;
random Pa lscolumn lsrow lscolumn*M Pa*lscolumn*M;
lsmeans M|F/pdiff;
run;
%mend mix;
%mix (Tomato_ID_AR);
%mix (Green_Viney_AR);
%mix (Over_Ripe_AR);
%mix (Cardboard_AR);
%mix (Color);
%mix (Uniformity_Color);
%mix (Juiciness);
%mix (Mealy);
%mix (Skin_Awareness);
%mix (Seed_Awareness);
%mix (Fiber_Awareness);
%mix (Tomato_ID);
%mix (Ripeness);
%mix (Green_Viney);
%mix (Umami);
%mix (Fruity);
%mix (Cardboard);
%mix (Fermented);
%mix (Musty_Earthy);
%mix (Overall_Sweet);
%mix (Sweet);
%mix (Sour);
%mix (Salt);
%mix (Bitter);
%mix (Astringent);
%mix (Metallic);
%mix (Chemical);
```

# Principal Component Analysis

```
data assign2;
input Judge$ Sample$ Rep$ Tomato_ID_AR Green_Viney_AR Over_Ripe_AR Color
Uniformity_Color Juiciness Mealy Skin_Awareness Seed_Awareness iber_Awareness
Tomato_ID Ripeness Green_Viney Umami Fruity Fermented Musty_Earthy
Overall_Sweet Sweet Sour Salt Bitter Astringent Metallic;
datalines;
***DATA DELETED***
;
ods rtf;
proc sort;
by sample rep judge;
proc means mean noprint;
by sample;
var Tomato_ID_AR  Green_Viney_AR Over_Ripe_AR Color Uniformity_Color
Juiciness Mealy Skin_Awareness Seed_Awareness Fiber_Awareness Tomato_ID
Ripeness Green_Viney Umami Fruity Fermented Musty_Earthy Overall_Sweet Sweet
Sour Salt Bitter Astringent Metallic;
output out = meancheese mean = mTomato_ID_AR mGreen_Viney_AR mOver_Ripe_AR
mColor mUniformity_Color mJuiciness mMealy mSkin_Awareness mSeed_Awareness
mFiber_Awareness mTomato_ID mRipeness mGreen_Viney mUmami mFruity mFermented
mMusty_Earthy mOverall_Sweet mSweet mSour mSalt mBitter mAstringent
mMetallic;
proc factor data = meancheese scree score cov outstat = cheese rotate =
varimax method = prin mineigen = 0.05;
var mTomato_ID_AR mGreen_Viney_AR mOver_Ripe_AR mColor mUniformity_Color
mJuiciness mMealy mSkin_Awareness mSeed_Awareness mFiber_Awareness mTomato_ID
mRipeness mGreen_Viney mUmami mFruity mFermented mMusty_Earthy mOverall_Sweet
mSweet mSour mSalt mBitter mAstringent mMetallic;
proc score data = meancheese scores = cheese out = sccheese;
var mTomato_ID_AR mGreen_Viney_AR mOver_Ripe_AR mColor mUniformity_Color
mJuiciness mMealy mSkin_Awareness mSeed_Awareness mFiber_Awareness mTomato_ID
mRipeness mGreen_Viney mUmami mFruity mFermented mMusty_Earthy mOverall_Sweet
mSweet mSour mSalt mBitter mAstringent mMetallic;
proc print data = sccheese;
run;
ods rtf close; quit;
```

# Cluster Analysis

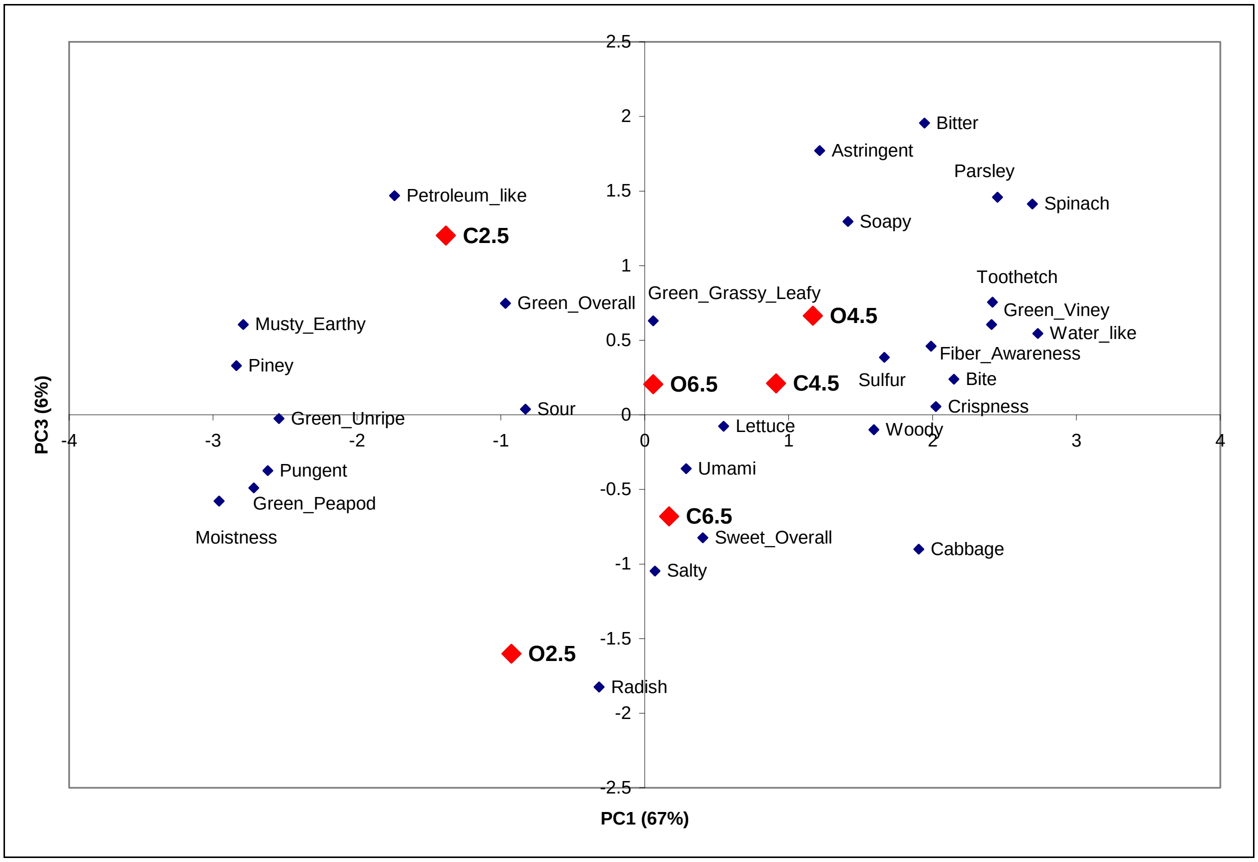
```
data assign2;
input Sample$ Tomato_ID_AR Green_Viney_AR Over_Ripe_AR Color Uniformity_Color
Juiciness Mealy Skin_Awareness Seed_Awareness Fiber_Awareness Tomato_ID
Ripeness Green_Viney Umami Fruity Fermented Musty_Earthy Overall_Sweet Sweet
Sour Salt Bitter Astringent Metallic;
datalines;
***DATA DELETED***
;
proc cluster data = assign2 outtree=treesl method=ward ccc;
id sample; run;
title2 'Ward-tree';
proc tree;
id sample; run;
proc cluster data = assign2 outtree=treesl method=average ccc;
id sample; run;
title2 'Average-tree';
proc tree;
id sample; run;
```

# Appendix F - SAS® Program Code used for ANOVA analysis of sensory characteristics of pac choi at different stages of development

```
data stage;
input Pa T$ M$ Rep Crispness Moistness Fiber_Awareness Green_Overall
Green_Unripe Green_Peapod Green_Grassy_Leafy Green_Viney Cabbage Lettuce
Spinach Parsley Radish Piney Woody Water_like Musty_Earthy Sulfur Soapy
Petroleum_like Pungent Bite Toothetch Sweet_Overall Sour Bitter Salty
UmamiAstringent;
cards;
***DATA DELETED***
;
%macro mix(y);
PROC mixed covtest cl;
class pa rep M T;
model &y=T|M/ddfm=satterth;
random rep rep*M Rep*M*T Pa*Rep*M;
lsmeans T|M/pdiff cl;
run;
%mend mix;
%mix (Crispness);
%mix (Moistness);
%mix (Fiber_Awareness);
%mix (Green_Overall);
%mix (Green_Unripe);
%mix (Green_Peapod);
%mix (Green_Grassy_Leafy);
%mix (Green_Viney);
%mix (Cabbage);
%mix (Lettuce);
%mix (Spinach);
%mix (Parsley);
%mix (Radish);
%mix (Piney);
%mix (Woody);
%mix (Water_like);
%mix (Musty_Earthy);
%mix (Sulfur);
%mix (Soapy);
%mix (Petroleum_like);
%mix (Pungent);
%mix (Bite);
%mix (Toothetch);
%mix (Sweet_Overall);
%mix (Sour);
%mix (Bitter);
%mix (Salty);
%mix (Umami);
%mix (Astringent);
```

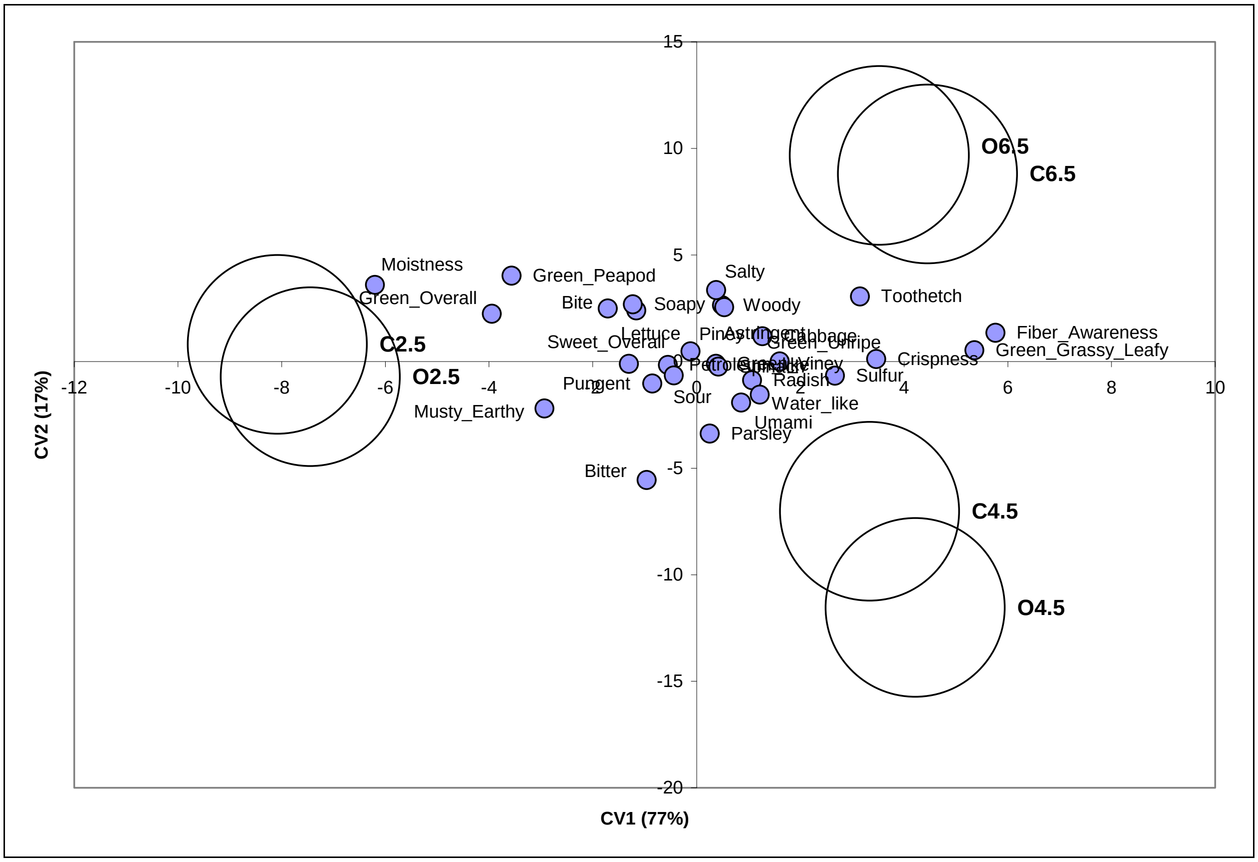
# Appendix G - Principal component analysis of pac choi at different stages of development

## Dimensions 1 vs. 3

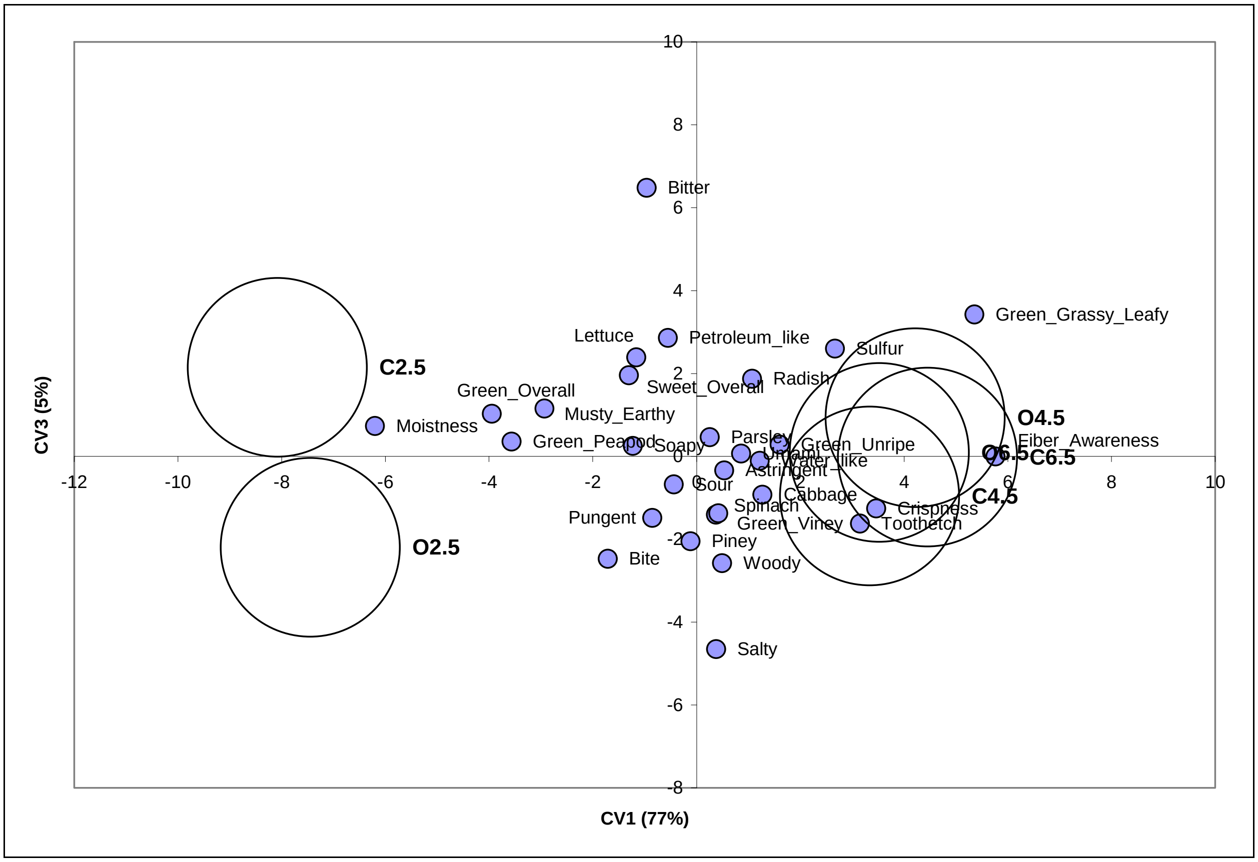


# Appendix H - Canonical variate analysis of pac choi at different stages of development

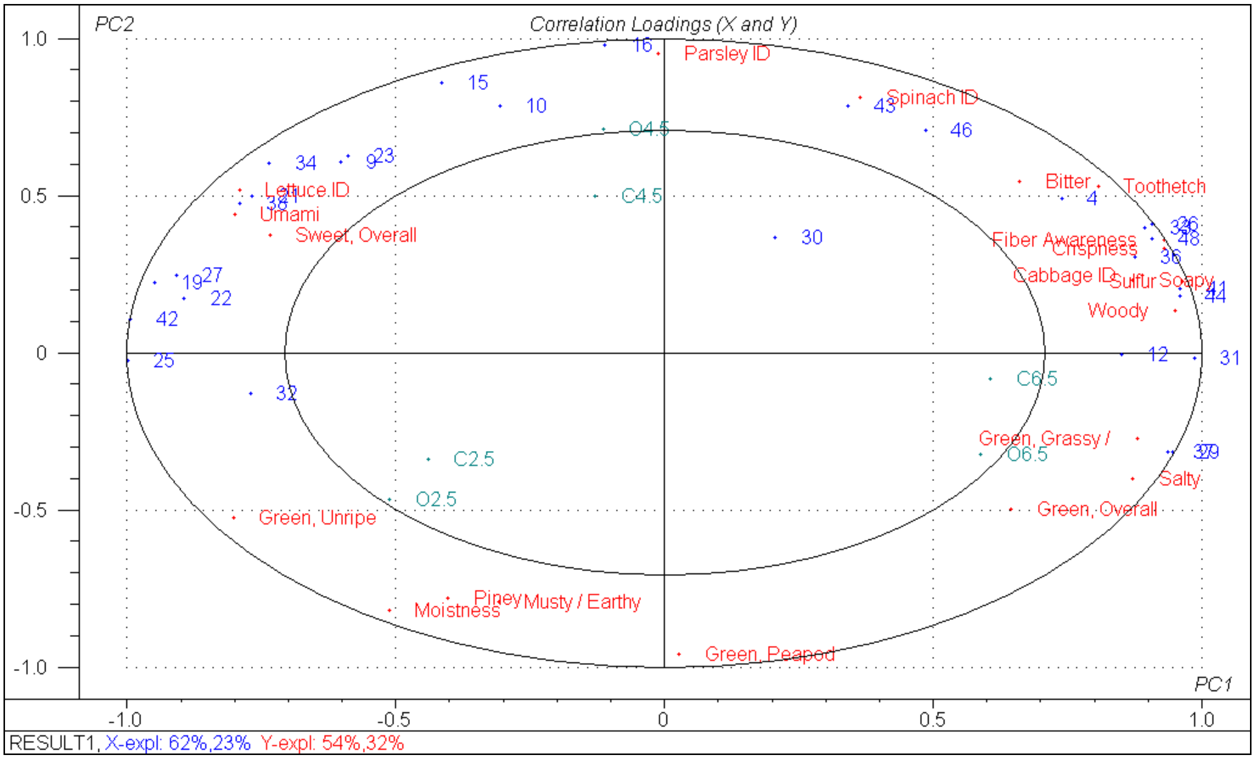
## Dimensions 1 vs. 2



# Dimensions 1 vs. 3



# Appendix I - The Unscrambler® output of partial least squares regression of pac choi at different stages of development including sensory and instrumental data

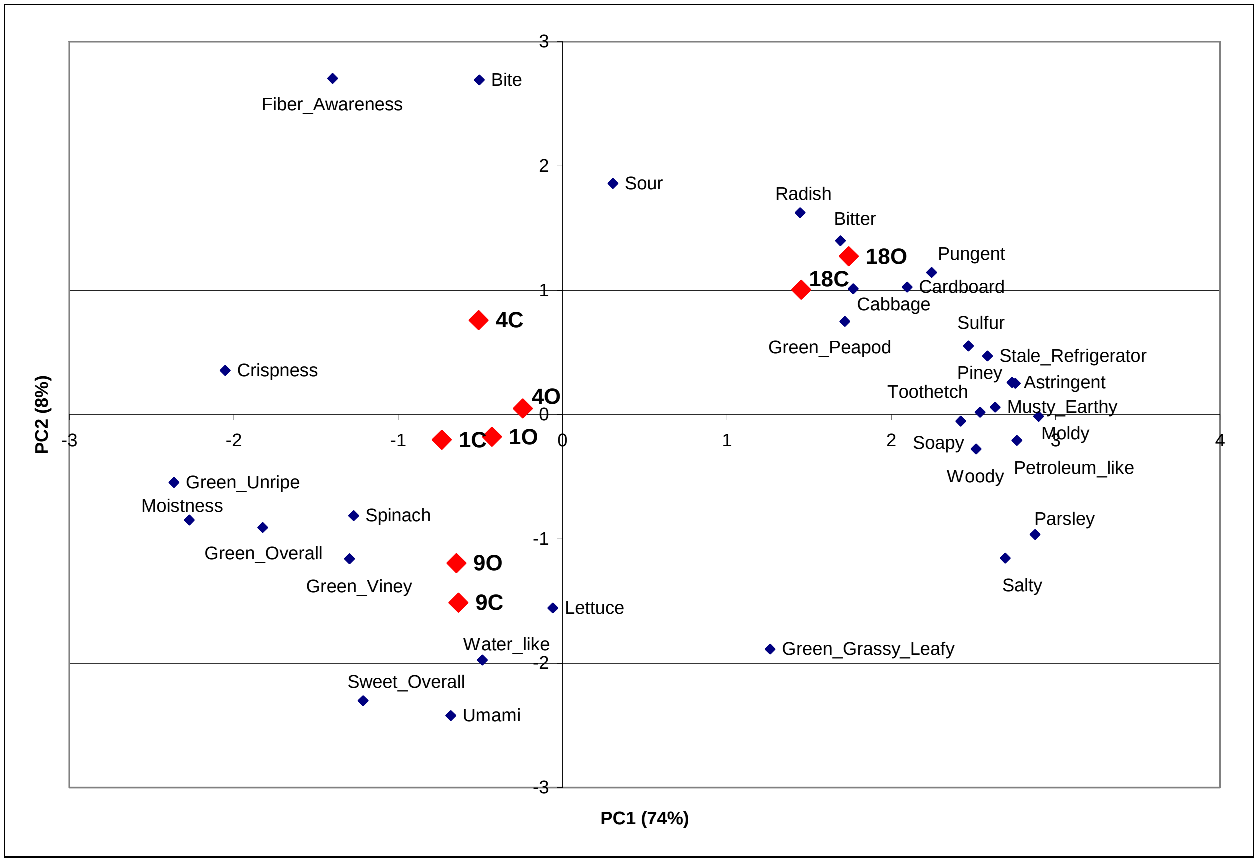


# Appendix J - SAS® Program Code used for ANOVA analysis of sensory characteristics of pac choi at different stages of shelf life

```
data stage;
input Pa T$ M$ Rep Crispness Moistness Fiber_Awareness Green_Overall
Green_Unripe Green_Peapod Green_Grassy_Leafy Green_Viney Cabbage Lettuce
Spinach Parsley Radish Piney Woody Water_like Musty_Earthy Sulfur Soapy
Petroleum_like Pungent Bite Toothetch Sweet_Overall Sour Bitter Salty
UmamiAstringent Stale_Refrigerator Cardboard Moldy
;
cards;
***DATA DELETED***
;
%macro mix(y);
PROC mixed covtest cl;
class pa rep M T;
model &y=T|M/ddfm=satterth;
random rep rep*M Rep*M*T Pa*Rep*M;
lsmeans T|M/pdiff cl;
run;
%mend mix;
%mix (Crispness);
%mix (Moistness);
%mix (Fiber_Awareness);
%mix (Green_Overall);
%mix (Green_Unripe);
%mix (Green_Peapod);
%mix (Green_Grassy_Leafy);
%mix (Green_Viney);
%mix (Cabbage);
%mix (Lettuce);
%mix (Spinach);
%mix (Parsley);
%mix (Radish);
%mix (Piney);
%mix (Woody);
%mix (Water_like);
%mix (Musty_Earthy);
%mix (Sulfur);
%mix (Soapy);
%mix (Petroleum_like);
%mix (Pungent);
%mix (Bite);
%mix (Toothetch);
%mix (Sweet_Overall);
%mix (Sour);
%mix (Bitter);
%mix (Salty);
%mix (Umami);
%mix (Astringent);
%mix (Stale_Refrigerator);
%mix (Cardboard);
%mix (Moldy);
```

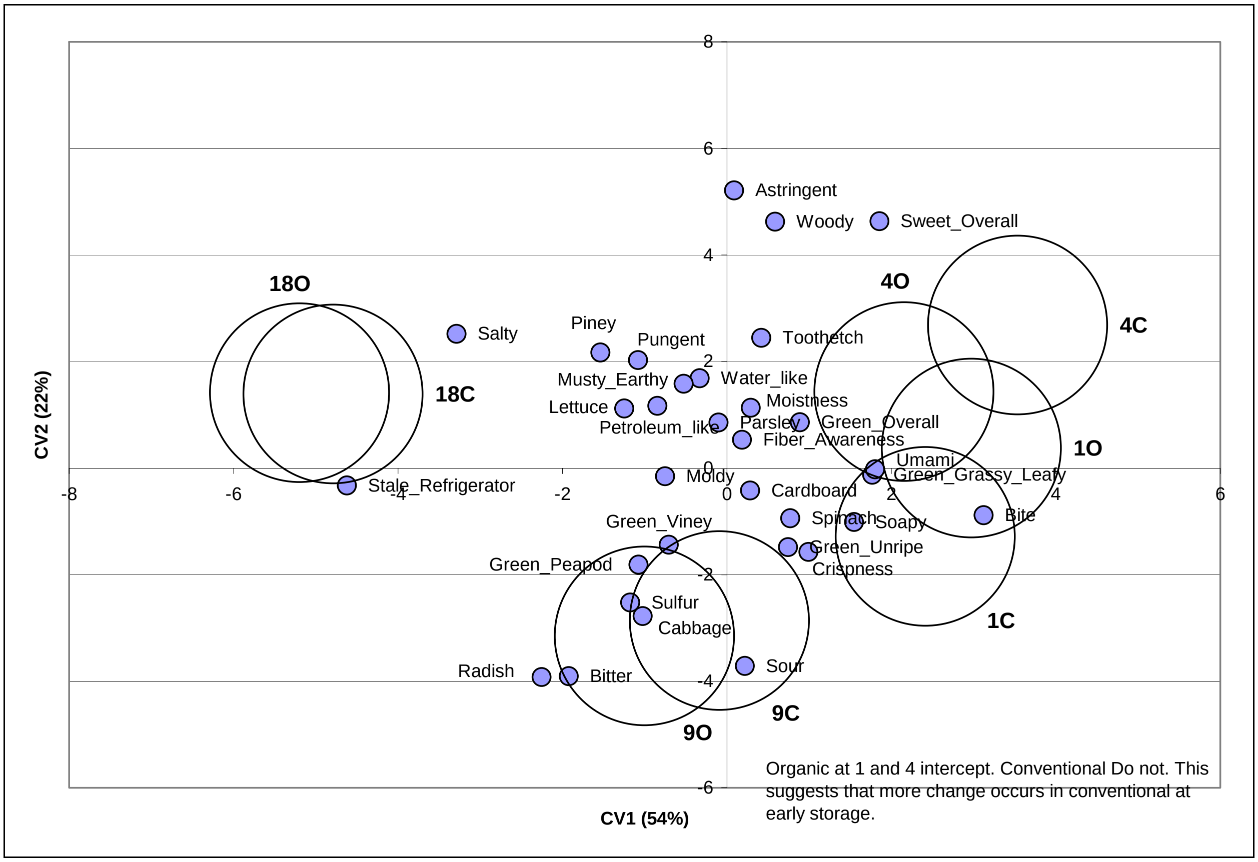
# Appendix K - Principal component analysis of pac choi at different stages of shelf life

## Dimensions 1 vs. 3



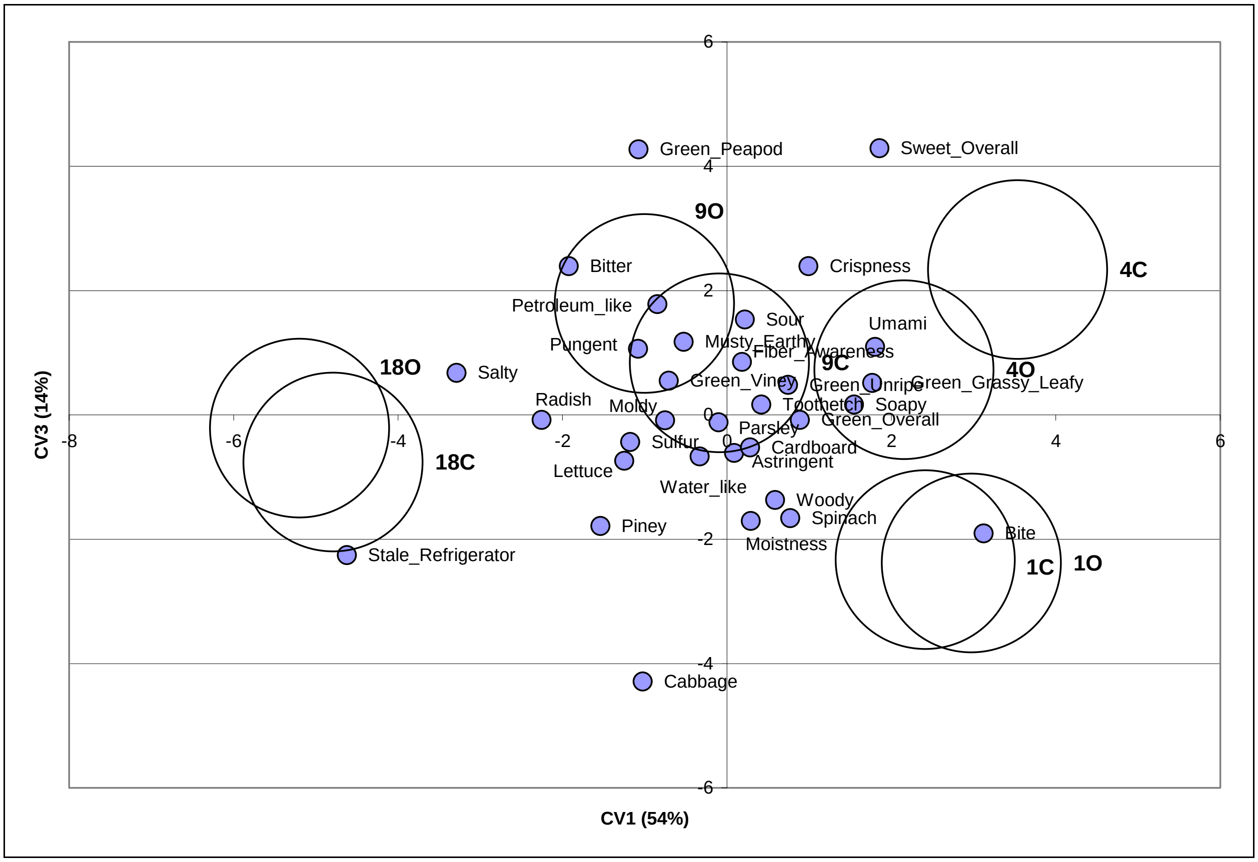
# Appendix L - Canonical variate analysis of pac choi at different stages of shelf life

## Dimensions 1 vs. 2

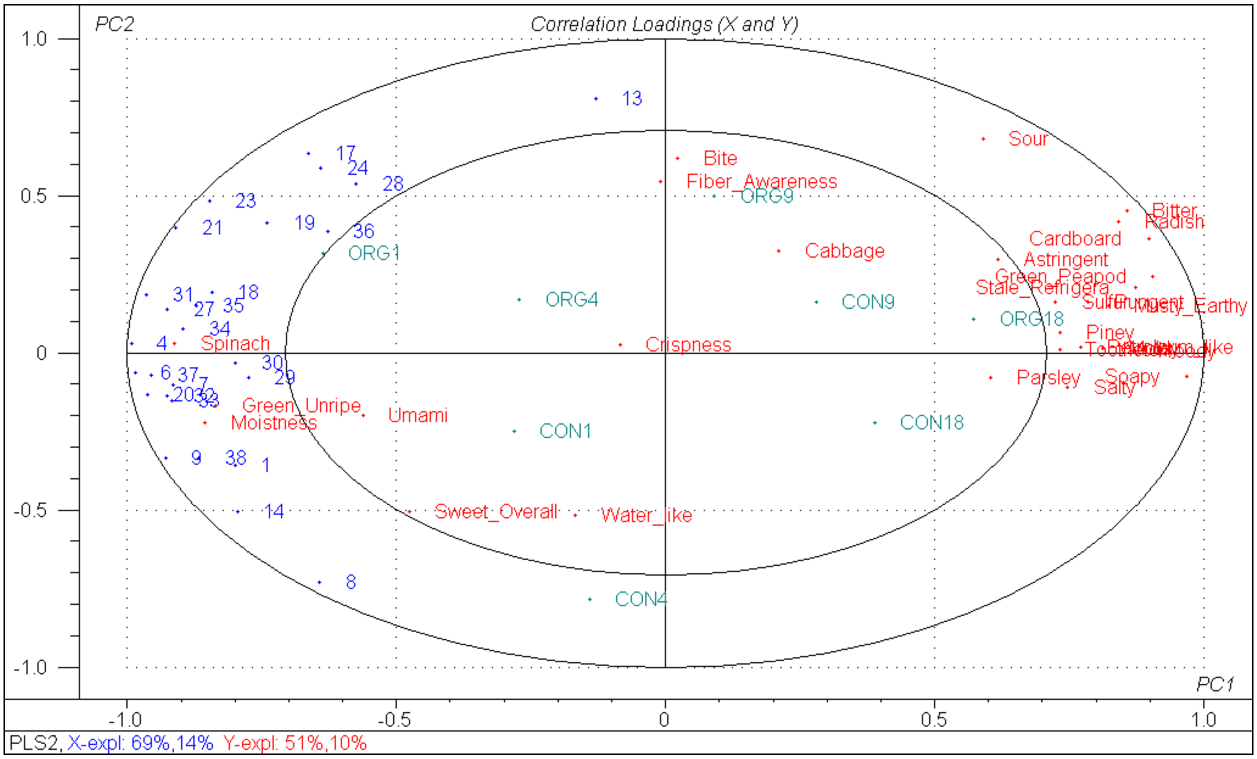


Organic at 1 and 4 intercept. Conventional Do not. This suggests that more change occurs in conventional at early storage.

# Dimensions 1 vs. 3



# Appendix M - The Unscrambler® output of partial least squares regression of pac choi at different stages of shelf life including sensory and instrumental data



# Appendix N - Main flavor and texture changes in pac choi after 18 days of refrigerated storage