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Objective Flavor Comparison of Oregon Strawberries and those from other climatic condition

PI: Michael Qian, Oregon State University

Collaborators: Chad Finn, USDA-ARS HCRL, Corvallis, OR;

Jan-Marie Schroeder, Oregon Raspberry & Blackberry Commission

Objective:

Oregon's strawberry industry has more than a century history. Unfortunately, strawberry production has been declining drastically in the last two half decades due to declining price, rising cost of production and competition from California, Florida, Mexico and China.

Most of Oregon's strawberries were for processing markets. The combination of warm, dry days with cool nights optimizes color and flavor development. In addition, the cultivars grown in Oregon were selected with processing, not fresh, quality parameters in mind including high sugar levels, high acid levels, intense red internal and external color, low drip loss, and intense flavor. In other word, Oregon's berries are superior in quality for processing. The survival of Oregon's strawberry industry relies on consumer's recognition and willingness to pay for premium quality.

The goal of this research is to objectively compare the quality of Oregon strawberry with California's to assist marketing promotion of Oregon strawberry as "Smaller, Redder, Simply Better". In addition, the methodologies developed will be used to further evaluate and select new strawberry cultivars for Oregon strawberry industry.

Materials and Methods:

Strawberry samples: Five strawberry varieties each from Oregon and California were used for the study. The Oregon varieties were procured from different farms across the state while California varieties were obtained from Driscoll Company, California. The five varieties from Oregon were Totem, Puget Summer, Puget Reliance, Hood and Independence, while the five varieties from California were Ventana, Camarosa, Venice, 13G97 and San Miguel. The fruits were collected in May and June 2004, immediately quick frozen and stored at -10⁰F. Samples were stored about six months before analysis.

Sugar analysis: Fruit samples (200 g each) were slightly thawed at room temperature for 90 minutes and blended to a fine puree. 50 mL of boiling water was added to 100 gm puree and mixed well with a stirring rod. The puree was immediately heated in a boiling water bath for five minutes to inactivate the enzymes. The puree was then centrifuged at 3000 rpm, the supernatant was filtered and collected in a 40 mL vial for sugar and organic acid analysis.

The extract was diluted with 100% acetonitrile in 1:2 ratio (w/w), the pectin was precipitated out and supernatant was filtered and injected onto the Shimadzu HPLC. Acetonitrile: water (80:20 v/v) was used as the mobile phase at a constant flow rate of

1.2 mL/min. HPLC was equipped with a RID 6A refractive index detector, restek ultra-amino column (3 μ m, 200 X 4.6 mm) and a Shimadzu CR 510 Chromo Pac integrator. The column temperature was maintained at 30 °C and a 20 microliter of sample was injected. Calibration curves were constructed using pure standards at 0.1, 0.2, 0.4, 0.8 and 1.6% concentrations (w/w). The linear equation obtained was used to calculate the concentration of sugars in the strawberries.

Sweetness Index: Sugars have different sweetness impact. Since sucrose is 1.35 times sweeter than glucose and fructose is 2.3 times sweeter than glucose, a sweetness index concept was used to estimate the total sweetness perception. Glucose was assigned a sweetness value of one, sucrose 1.35 and fructose 2.3. Total sweetness/sweetness index was calculated based on the amount of individual sugars in strawberries. Total sweetness index = 1 glucose+ 1.35 sucrose + 2.3 fructose.

Organic Acid analysis: C₁₈ Sep-Pak Cartridges were conditioned with 10 mL each of methanol, water and 50% acetonitrile. 10 mL of air was passed through a conditioned C₁₈ cartridge to remove the excess acetonitrile. Sample was diluted with 0.005M sulfuric acid in 1:2 ratio. Six mL sample was applied onto the C₁₈ cartridge, the first 4 mL was discarded and the following 2 mL was collected and injected onto the HPLC. Sulfuric acid (0.005 M) was used as the mobile phase at a constant flow rate of 0.4 mL/min. Shimadzu UV –VIS spectrophotometric detector SPD-6AV was used at a wavelength of 210 nm. A Biorad Aminex ion exclusion column (HPX.87H, 300 X 7.8mm) was operated at 35°C. 20 microliters of sample was injected. Calibration curves were constructed using standards at 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8% concentrations (w/w) and the linear equation obtained was used to determine the amount of citric and malic acid.

Total anthocyanin content: About 200 gm of fruit were blended into a powder with liquid nitrogen in a cryogenic blender. Anthocyanins were extracted from 25 gm of the powder. The samples were successively extracted using 25 mL each of 100% acetone and 70% acetone. The extracts were combined and partitioned with 100 mL chloroform and centrifuged at 800 rpm for 30 minutes. Chloroform was discarded and aqueous portion was rotovaporated to remove the acetone. The final extract was brought to 50 mL in a volumetric flask with water. All the analyses were performed in triplicates. Anthocyanin pigments undergo reversible structural transformations with change in pH. Total anthocyanin content was determined by pH differential method (Wrolstad et.al) since the colored oxonium form predominates at pH 1.0 and the colorless hemiketal form predominated at pH 4.5. Dilution factors for the sample were determined with potassium chloride solution at pH 1, until the absorbance of the sample at the wavelength of maximum absorption was within the linear range of the spectrophotometer. Since pelargonidin-3-glucoside is the major pigment in strawberry and it has maximum absorption at 496 nm. Absorbance was measured for each dilution (pH 1.0 and pH 4.5) at 496 nm and 700nm(to correct for haze). The absorbance of the diluted sample was calculated as follows: $A = (A_{\lambda 496nm} - A_{700})_{pH 1.0} - (A_{\lambda 496nm} - A_{700})_{pH 4.5}$.

Total anthocyanin content was calculated using the equation: ACN (mg/100gm fruit) = $(A * MW * DF * 1000) / (E * 1)$

(MW of pelargonidin 3 glucoside = 433.39, E = 15,600, E-molar absorptivity)

Volatile aroma Analysis: Strawberries were thawed at room temperature for 2 hours. About 100 gm of strawberry was blended to a puree. 3-Hetptanone, 2-butenal, ethyl undecanoate, Gamma-undecalactone, heptanoic acid and decanol were added as internal standards at 0.5 ppm and blended again for 2 minutes. 10 gm of the puree was placed in a vial for volatile extraction using a Carboxen-PDMS-DVB SPME (solid phase micro extraction) fiber. The sample was equilibrated for 15 minutes in a water bath at 50°C. Extraction was performed at 50°C for 60 minutes. The fiber was desorbed at 270°C. The GC operation parameters were as follow: detector temperature of 270°C, column flow-2 mL/min, initial oven temperature of 35°C, hold time of 5 minutes, rate of temperature increase at 2°C /min, final temperature of 230°C and a final time of 20 minutes. A synthetic strawberry matrix consisted of 4 gm of pectin, 23 gm glucose, 23 gm fructose, 10 gm sucrose, 7 gm citric acid and 1 gm malic acid dissolved in one liter of water was used to develop the calibration curve. The linear regression equation obtained from the calibration curve was used for estimating the actual concentration of the volatiles in strawberries.

Vitamin C measurement: Strawberries (70-100 gm) were blended with 0.05N H₃PO₄ for 2 min. The puree was centrifuged at 5000 rpm for 15minutes. 5 gm of the supernatant was mixed with 45 gm of 0.05N H₃PO₄. The extract was purified by passing 5 mL extract through a C₁₈ Sep-Pak Cartridge, preconditioned with 10 mL each of methanol and water. 10 mL of air was passed through the cartridge to remove the excess water. The last one mL was collected and injected onto the HPLC analysis.

HPLC consisted of a LC-6A pump, a SIL-6B auto injector, a CTO-6A column oven, a SPD-6A UV-VIS spectrophotometer detector and a CR501 chromatopac integrator (Shimadzu, Japan). The ACCU ODS column (150X3.6mm, 3um, J&W Scientific) was maintained at 30°C. 2% KH₂PO₄, adjusted to pH 2.3 with H₃PO₄, was used as the mobile phase at a constant flow rate of 0.4 mL/min. The detector response was measured at 245 nm, which corresponds to the wavelength of maximum absorption of ascorbic acid. 10 µL of sample solution was injected and analyzed for 10 minutes. Calibration curve was constructed using standards at 0.1, 0.2, 0.3, 0.4 and 0.5% concentrations (w/w) and the linear equation obtained was used to determine the amount of ascorbic acid.

Preliminary sensory analysis: A preliminary sensory analysis was conducted using a panel of 10 members among which 4 were male and 6 female ranging in age from 21 to 45. All the panelists work in the flavor chemistry lab and have considerable experience with sensory analyses. The strawberries were rated on a 1 to 16 for their aroma, taste, color and overall fruit quality. Known descriptors for strawberry like caramel, pineapple, banana, green, musty, earthy and floral were used. During the course of the sensory session there was a consensus to use additional terms like sulfury (cauliflower, broccoli), waxy and smoky.

Results and Discussion:

Sugars and organic acids:

Sugar and organic acids contents vary in strawberries based on cultivar, region and agronomic practices. Recent studies in our lab showed that sugar content varied in the cultivars grown in Oregon and California (Table 1). Oregon had showed higher proportions of total sugars in general and specifically, higher fructose and glucose levels in specific while California cultivars had higher sucrose levels. 13G97 had the lowest total sugar content while Totem from the year 2003 had the highest. The average fructose, glucose and sucrose content in Oregon cultivars were 2.66 gm, 2.33 gm, and 0.77 gm per 100 gm of berries respectively while California had 1.93 gm, 1.69 gm, and 1.02 gm respectively.

Table 1 Sugar profile in Oregon and California strawberry cultivars

| | Fructose | Glucose | Sucrose | Total sugars | Brix |
|-------------------|-------------------------|-------------------------|-------------------------|------------------|-------|
| <i>Cultivar</i> | Average(gm/100gm berry) | Average(gm/100gm berry) | Average (gm/100gmberry) | (gm/100gm berry) | |
| Oregon | | | | | |
| Totem 2004 | 2.52±0.05 | 2.13±0.08 | 0.53±0.11 | 5.1935 | 8.08 |
| Totem 2003 | 3.15±0.09 | 2.90±0.07 | 0.55±0.02 | 6.6061 | 8.34 |
| Puget Summer | 2.92±0.09 | 2.51±0.04 | 1.06±0.09 | 6.5016 | 9.6 |
| Hood | 2.38±0.04 | 2.17±0.09 | 0.71±0.10 | 5.2766 | 8.86 |
| Puget Reliance | 2.87±0.17 | 2.66±0.28 | 0.83±0.03 | 6.3776 | 8.22 |
| Independence | 2.10±0.03 | 1.74±0.02 | 0.93±0.08 | 4.7773 | 11.18 |
| California | | | | | |
| Camarosa | 1.79±0.03 | 1.51±0.03 | 0.70±0.08 | 4.0079 | 8.22 |
| Ventana | 1.89±0.20 | 1.74±0.04 | 1.430.57 | 5.0654 | 8.86 |
| Venice | 2.23±0.09 | 1.93±0.13 | 0.74±0.07 | 4.9071 | 8.04 |
| 13G97 | 1.63±0.02 | 1.36±0.06 | 0.78±0.04 | 3.7774 | 10.18 |
| San Miguel | 2.11±0.00 | 1.89±0.13 | 1.44±0.11 | 5.4494 | 10.02 |

The taste perceived by the consumer not only depends on the total sugar content but also on the type and the quantity of individual sugars. A total sweetness index concept was used to assess fruit sweetness. Based on the total sweetness index, Totem 2003 had the highest sweetness while 13G97 from California had the lowest. Similar to the total sugars, Oregon cultivars had a higher sweetness index than California.

Table 2: Sweetness Index

| <i>Cultivar</i> | Sweetness from fructose | Sweetness from glucose | Sweetness from sucrose | Total sweetness Index |
|-------------------|-------------------------|------------------------|------------------------|-----------------------|
| Oregon | | | | |
| Totem 2004 | 5.036 | 2.1381 | 0.73 | 7.900 |
| Totem 2003 | 6.293 | 2.9043 | 0.75 | 9.947 |
| Puget summer | 5.847 | 2.5174 | 1.43 | 9.796 |
| Hood | 4.763 | 2.1770 | 0.97 | 7.909 |
| Puget Reliance | 5.737 | 2.6698 | 1.13 | 9.540 |
| independence | 4.205 | 1.7404 | 1.26 | 7.207 |
| California | | | | |
| Camarosa | 3.00 | 1.76 | 0.80 | 5.56 |

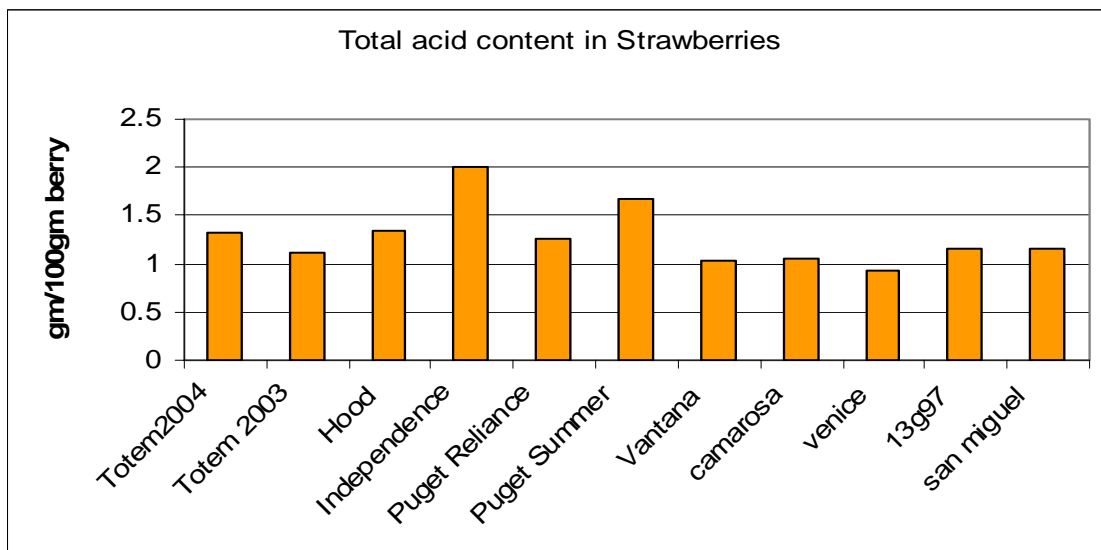
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|------------|------|------|------|------|
| Ventana | 3.17 | 1.69 | 1.62 | 6.48 |
| Venice | 3.74 | 2.11 | 0.84 | 6.69 |
| 13G97 | 2.73 | 1.48 | 0.89 | 5.10 |
| San Miguel | 3.55 | 2.01 | 1.64 | 7.20 |

Oregon cultivars varieties had higher citric acid, malic acid and total organic acid levels acids as well. California cultivars had uniform total acids content at around 1 gm/100 gm berry while there was more variation in Oregon varieties ranging from 1.1 gm to 2 gm. Independence from Oregon had the highest total acid content at 2 gm while Venice from California had the lowest content. In general, Oregon cultivars had higher citric and malic acid levels, which indicate they are better suited for producing acids, demonstrating a superior quality processed product for processing.

Table 3: Organic acid content in strawberries (%)

| | Citric acid | Malic acid | Total Acids (%) |
|-------------------|-------------|------------|-----------------|
| Oregon | | | |
| Totem2004 | 0.97 | 0.34 | 1.318 |
| Totem 2003 | 0.79 | 0.31 | 1.111 |
| Hood | 0.93 | 0.40 | 1.335 |
| Independence | 1.40 | 0.59 | 2.005 |
| Puget Reliance | 0.87 | 0.39 | 1.262 |
| Puget Summer | 1.17 | 0.50 | 1.681 |
| California | | | |
| Ventana | 0.63 | 0.38 | 1.027 |
| camarosa | 0.71 | 0.34 | 1.056 |
| venice | 0.59 | 0.33 | 0.928 |
| 13g97 | 0.78 | 0.37 | 1.161 |
| san miguel | 0.72 | 0.43 | 1.162 |

Fig. 1. Total organic acid content in strawberries



Total anthocyanin content:

Numerous researches have proved the antioxidant activity and potential health benefits of anthocyanins. Anthocyanin contents in strawberries have been reported to vary from 15 mg/100 gm of fruit to 35 mg/100 gm of fruit. Although anthocyanin levels varied dramatically from cultivar to cultivar, anthocyanin content in Oregon cultivars were much higher than those from California varieties (Table 4). Among them Totem had the highest amount (30.52 mg/100gm fruit). The average anthocyanin content for Oregon cultivars was 25.04 mg/100 gm while it was 12.28 mg/100gm fruit for those from California varieties. This result clearly showed that these Oregon cultivars are better in terms of anthocyanin content.

Table 4: Total anthocyanin content.

| Cultivar | Total anthocyanin content |
|-------------------|---------------------------|
| Oregon | |
| Totem 2004 | 30.52 ± 1.13 |
| Totem 2003 | 30.78 ± 9.01 |
| Hood | 19.24 ± 2.06 |
| Independence | 23.99 ± 2.27 |
| Puget Reliance | 20.90 ± 0.90 |
| Puget Summer | 24.83 ± 3.06 |
| California | |
| Camarosa | 15.22 ± 1.36 |
| VentanaVantana | 15.80 ± 1.86 |
| San Miguel | 12.20 ± 0.70 |
| Venice | 7.10 ± 1.10 |
| 13G97 | 11.10 ± 0.05 |

Ascorbic acid content:

Ascorbic acid content in the Oregon and California cultivars were analyzed, however, quantitative results do not show any distinct pattern. The ascorbic acid content in Totem 2003 was very low. This could be due to the fact that Totem 2003 was the only variety that was collected in the year 2003 and stored. Ascorbic acid is highly unstable and decreases over a period of time.

Table 5: Ascorbic acid content in strawberry cultivars

| Cultivar | Average (mg/gm berry) |
|----------------|-----------------------|
| Oregon | |
| Totem 2004 | 0.68±0.01 |
| Totem 2003 | 0.11±0.01 |
| Puget summer | 0.59 |
| Hood | 0.35±0.03 |
| Puget Reliance | 0.56±0.04 |
| Independence | 1.00±0.02 |

| California | |
|-------------------|-----------|
| Camarosa | 0.48±0.01 |
| Ventana | 0.62±0.02 |
| Venice | 0.57±0.01 |
| 13G97 | 0.71±0.03 |
| San Miguel | 0.72 |

Sensory Analysis:

Initial sensory results show the panelists favoring the Oregon varieties for all the attributes under study. The sweetness and sourness ratings followed their respective sugar and acid content. Totem 2004 was given the highest rating in terms of overall fruit quality followed by Puget reliance and Independence. Even in terms of overall aroma, totem 2004 had the highest rating followed by Totem 2003 and Puget reliance. In color intensity and quality Totem 2003 was given a higher rating with totem 2004, Puget reliance getting the same score. The color ratings seems to follow the overall anthocyanin content as all the Oregon varieties were rated higher for their color. Overall the Oregon strawberries were rated higher on aroma, taste and color. Among the California varieties Ventana was the best with a favorable aroma and taste. Camarosa had a good aroma but not so good overall taste.

Aroma composition:

By using gas chromatography-olfactometry (GC/O) technique, it was determined that methyl and ethyl esters of acetic, butanoic, hexanoic, hexanoic, isobutanoic and 2/3-methylbutanoic acids are primarily responsible for the fruity aroma. Hexanal, t-2-hexenal, cis-3 hexenol were the major compounds responsible for the green note. In addition to these compounds, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, 2,5-dimethyl-4-methoxy-3(2H)-furanone, gamma-decalactone and nerlidol were also considered to be extremely important to strawberry aroma. A preliminary flavor comparison was performed using strawberries from Oregon and California. Volatiles were analyzed by GC and GC-MS. A total of 69 compounds have been identified. The lactones (furanones) are the only compounds that have been described to have a strawberry like aroma.

Solid phase microextraction is an efficient technique to extract aroma compounds, especially semi-volatile compounds such as 2,5-dimethyl-4-methoxy-3(2H)-furanone. A quantitative DVB/Carboxen/PDMS SPME fiber method has developed in our lab, and the volatile aroma composition study is under progress.