AN ABSTRACT OF THE THESIS OF

Rajesh R Jetti for the degree of Master of Science in Food Science and Technology presented on June 17, 2005

Title: Fruit Quality Evaluation Of Strawberries (*Fragaria ananassa*) Grown In California And Oregon

Abstract approved:

Redacted for privacy

Michael C. Qian

Five genotypes from California (Ventana, Camarosa, San Miguel, 13G97, and Venice) and Oregon (Totem, Puget Reliance, Puget Summer, Hood and Independence) were evaluated for quality. The volatile compounds were analyzed using head space solid phase micro extraction-gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Odor activity values of the compounds were calculated to understand the contribution of the individual compounds to the overall aroma. Ethyl butanoate, furaneol, ethyl hexanoate, ethyl acetate, ethyl isovalerate, hexyl acetate and gamma dodecalactone have the highest odor activity values. The chemical results were correlated with the sensory data obtained from a trained panel of 10 members. Correlation was achieved for the floral, pineapple, banana, peach and caramel notes. The green notes did not correlate with the concentration and odor activity values of the corresponding compounds. This variation could be due to the very high odor activity of other compounds resulting in the masking of the green notes.

The sugar, organic acid and ascorbic acid composition was investigated using HPLC. Anthocyanin content was determined by pH differential method. The color properties were studied by measuring tri-stimulus measurements. The average fructose, glucose, sucrose and the total sugar content in the ten cultivars were 2.24, 1.96, 0.92 and 5.13 g/100 g respectively. Total acid content ranged from 0.93 g/100 g to 2.01 g/100 g. Puget Summer had the highest total sugars and Independence had the highest acids and ascorbic acid content. The anthocyanin content varied from 7.1 mg/100 g fruit to 30.52 mg/100 g fruit. The sensory evaluation of the genotypes in terms of sweetness and sourness could be partially explained by sugar/acid ratio. A consumer preference test showed that 'Totem' and 'Puget Reliance' were preferred over 'Camarosa' and 'Ventana' for their aroma.

Fruit Quality Evaluation Of Strawberries (*Fragaria ananassa*) Grown In California And Oregon

by
Rajesh R Jetti

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented June 17, 2005
Commencement June 2006

Master of Science thesis of Rajesh R Jetti
presented on June 17, 2005.

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Rajesh R Jetti, Author

ACKNOWLEDGEMENTS

I sincerely thank **Dr. Michael C Qian,** for his valuable guidance, and intellectual stimulation during my stay at Oregon State University.

I thank **Dr. Robert McGorrin,** Head of the dept, FST, and **Dr. Alan Bakalinsky,** Head of the Graduate Committee for providing an opportunity to pursue Masters in Food Science at OSU and for their continuous encouragement.

I would like to thank **Dr. Chad Finn** for encouraging me with his significant inputs during the various stages of the research project.

I would also like to thank **Dr. Philip Brownell** for giving me the privilege of having him on my graduate committee.

The HPLC work could not have been successful without the advice and support of **Erning Yang**.

I thank Cindy Lederer and Astrid Kurniana for assisting me with the design and execution of the sensory part of my research.

My special thanks go to **Helen Burbank, Sarah Mahler, Pedro Vazquez, Sandy Wang** and **Fang Yu** for their assistance and inputs during various stages of my research work.

My days in Corvallis have been memorable due to the friends I had in **Duangdao Masrungson, Fu Yao,** and **Shilpa Mahajan.**

I appreciate the enthusiasm showed by all the **instructors** at OSU which made learning a wonderful, joyful experience. I would like to thank them for their continuous support and encouragement in all my endeavors.

I would also like to thank the **Office Staff** for their wonderful support during my stay here.

Lastly and most importantly, I thank my **family** including the newest member **Lalita**. They have given me the strength and encouragement to follow my dreams and have always supported me in my endeavors.

Rajesh R Jetti.

CONTRIBUTION OF AUTHORS

Dr. Chad Finn, with his immense experience and understanding of the berries assisted us in the design of the project. His inputs regarding the differences in the profile of cultivars from different regions have been immensely helpful in the conceptualization of the research project. Erning Yang assisted development of methodology in sugar and organic acids analysis by HPLC, developing calibration curves in volatile analysis. Dr. Michael C Qian, my advisor has been instrumental in the designing of the project, arranging the necessary funding, supervision and assistance at every stage of the project.

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CHAPTER 1

INTRODUCTION

BACKGROUND

Flavor quality of fruits and vegetables is a combination of an impression on the tongue (taste), an impression on the nose (aroma), and the mouth feel impression. The volatile compounds in the food are usually responsible for the aroma (*1*). The aroma of a food may be the result of a complex combination of volatiles, or it may be the result of relatively few compounds. The compounds that have a greater impact on the perceived aroma are usually referred to as the character impact compounds (*1*). Some of the examples of character impact compounds are 4-(p-hydroxyphenyl)-2-butanone in raspberry, (Z)-3-hexenal in fresh tomato, 2-furfurylthiol in coffee etc.(*2*)

Strawberries (*Fragaria ananassa*) are highly valued for their unique flavor and pleasant color, and are one of the most frequently consumed fruits. The soluble sugars present in the strawberry impart the sweetness while the organic acids, e.g. citric and malic acids, contribute to the tartness. Volatiles present in the strawberry, prominent esters and furanones, are responsible for its characteristic aroma. The aroma, together with the sugars and acids, give strawberry its characteristic flavor which has made it a popular fruit among the consumers.

The main objective of this research was to map the fruit quality of Totem and compare it with other genotypes grown in California and Oregon by instrumental analysis and sensory evaluation.

LITERATURE REVIEW

VOLATILE COMPOUNDS IN FOODS AND THEIR ORIGIN

"Flavor is the sensation produced by a material taken in the mouth, perceived principally by the senses of taste and smell, and also by the general pain, tactile, and temperature receptors in the mouth. Flavor also denotes the sum of the characteristics of the material which produces that sensation" (*3*).

The smell of food is composed of "aroma", olfactory sensations from sniffed volatiles, and "odor", olfactory sensations retronasally obtained from volatiles released in the mouth (*4, 5*). The aroma of the food material is derived from the volatiles present in the food.

A large numbers of volatiles are present in fruits and vegetables that differ from each other in terms of their chemical properties. Many different foods have volatile compounds in common. Although these compounds are common, they occur in varying proportions and concentrations in different foods giving the food its characteristic aroma profile.

Some volatile compounds provide greater contributions to the overall aroma of the fruit than the others. This could be due to either their higher concentrations in the food or their lower thresholds or a combination of both. These compounds are responsible for the characteristic aroma of the food, and are called character impact compounds. Table 1.1 provides a list of some of the fruits and vegetables that have the character impact compounds identified in them.

Table 1.1: Important character impact compounds in fruits and vegetables (*6*)

Compounds	Food
Cis-2,6-nonadienal	Cucumber
Cis-3-hexenal	Tomato
2-Isobutyl-3-methoxypyrazine	Bell pepper
Diallyl disulfide	Garlic
Dipropyl disulfide	Onion (cooked)
Geranial (citral)	Lemon
Methyl anthranilate	Grape
1-(p-Hydroxyphenyl)-butan-3-one	Raspberry
Ethyl 2-methylbutanoate	Apple
Ethyl (E,Z)-2,4- decadienoate	Pear
3-Methylbutyl acetate	Banana
γ-Decalactone	Peach

Many volatile compounds are present in intact fruits, that are produced by the normal metabolism of the plant. In banana, orange, pear, carrots and bell peppers, the aroma can be perceived in the intact fruit or vegetable. While some volatiles are produced only when the fruit or vegetable is broken by cutting, chewing or blending, such as in cucumber, melons, and tomatoes, the main characteristic aroma is only produced when the enzymes are allowed to act when the tissue is broken. The aroma compounds are typically generated from fatty acids, amino acids, carbohydrates and other precursors.

Fatty acids

One of the most common ways of volatile formation in foods is the enzyme induced oxidative breakdown of unsaturated fatty acids (*7*). Beta-oxidation is thought to produce "primary aromas". Fatty acid acyl-CoA derivatives are metabolized to shorter chain acyl-CoAs with various chain lengths. These acyl-CoAs are converted into esters via alcohol acyltransferase (*8*).

Lipoxygenases and isomerases are the main enzymes involved in lipoxygenase pathway (LOX) that produces a variety of C_6 and C_9 aroma compounds, that are responsible for the "secondary aromas". Many fruit acids, alcohols, aldehydes, and esters are derived from lipoxygenase degradation of linoleic and linolenic acids. Acylhydrolases free polyunsaturated fatty acids from glycolipids, phospholipids and triacylglycerols, thus initiating the enzymatic oxidative degradation. Linoleic and linolenic acids are degraded by LOX and LOX isozymes to produce fatty acid hydroperoxides. Hydroperoxide lyase converts fatty acid hydroperoxides to aldehydes and oxoacids. There are three classes of hydroperoxide lyases based on substrate specificity- C_9, C_{13} and non-specific hydroperoxide lyases. Hydroperoxide lyase cleaves 13-hydroperoxylinoleic acid to 12-oxo-(9Z)-dodecenoic acid and hexanal, 13-hydroperoxylinolenic acid to 12-oxo-(9Z)-dodecenoic acid and (3Z)-hexenal. When the substrates are 9-hydroperoxides of linoleic and linolenic acids, the products are 9-oxononanoic acid and (3Z)-nonenal or (3Z, 6Z)-nonadienal respectively (Sanz, et.al., 1997), Isomerization of the (3Z)-enal to (2E)-enal form is carried out by (3Z,2E)-enal isomerase in most plants. Alcohol hydrogenase acts on these unsaturated carbonyls to produce the corresponding alcohols which are converted to esters by alcohol acyltransferase.

Each plant has its own set of enzymes and other inherent matrix differences that can lead to the different aroma products, starting with the same unsaturated fatty acids which are principally linoleic and oleic acids. Many aldehyes, ketones and alcohols can be formed via fatty acid metabolism. Hexanol, 2-hexenal, hexanal and cis-3-hexenal all of which contribute to the "green odors" are formed through enzymatic oxidation of linoleic and linolenic acids (*6*). Cis-3-hexenol and cis-3-hexenal have freshly cut grass aroma and have been reported by various authors (*9*). Trans-2-hexenal is important to the aroma of apples (*6*), trans-2-hexenal and cis-2-hexenol are important in blueberries (*10*) while n-hexanal and trans-2-hexenal (Dirinck et al., 1981) are important in strawberries. 2, 6-Nonadienal formed from the breakdown of linolenic acids is the main character impact compound in cucumbers (*11*).

Besides enzyme-catalyzed oxidative breakdown, unsaturated fatty acids also undergo oxidative breakdown during cooking (*6*). Trans-2-Nonenal occurs in small amounts in raw carrots, while its concentration increases considerably in cooked carrots (*12*) and similarly 1-Octen-3-one in cooked mushrooms.

Amino Acids

Amino acid metabolism generates the aliphatic and branch chain alcohols, acids, carbonyls and esters. Action of enzyme systems on amino acids when the tissue of the food material is damaged produces certain volatile compounds. For example when onion is cut, amino acids such as S-alkyl-L-cysteine breaksdown to give compounds like dipropyl sulfide, that has a characteristic aroma of onions (*13*). Also alliin in garlic breaks down into allicin which is further transformed into diallyl disulphide, responsible for the characteristic garlic odor (*14*).

Thermal degradation of amino acids and sugar degradation always occur together. The reaction of amino acids with sugars (Maillard reaction) and sugar degradation is probably the main source of volatile compounds in baked and roasted foods (*14*). Some of the most commonly encountered Strecker degradation of amino acids also produces important aroma compounds such as 2-methylpropanal (from valine), 3-methylbutananl (from leucine), phenylacetaldehyde (from phenyl alanine) and methional (from methionine) (*13*).

Carbohydrates

Relatively few aroma compounds are derived from carbohydrates. Terpenes are produced from carbohydrates through the isoprenoid pathway (*7*) and furanones are derived from carbohydrates through Maillard reaction, the browning reaction of reducing sugars with amine acids (*7, 15*).

Despite its importance as a character impact compound, little is known about the biosynthesis and metabolism of furaneol in plants. Quantification of furaneol, methoxyfuraneol, and furaneol glucoside during fruit ripening indicates conversion of furaneol into mesifurane and furaneol glucoside (*16, 17*). Recently, a study of the metabolism of furaneol in detached ripening strawberry fruits demonstrated the incorporation of *S*-(*methyl*-14C)-adenosyl-L-methionine (SAM) and (14C) furaneol into methoxyfuraneol (*18, 19*). This observation indicates the putative role of a methyltransferase enzyme, able to transfer the methyl group from SAM to furaneol. Methyltransferases are ubiquitous enzymes that catalyze the transfer of a methyl group from SAM to an acceptor substrate, generating O-, *N*-, *S*-, and C-methyl derivatives and *S*-adenosylhomocysteine (*20, 21*). During the process of fruit maturation, furaneol can be

rapidly converted into mesifurane and its glucoside (*16, 17*). Zabetakis and Holden (*22*) suggested that the total amount and the ratio of furaneol and mesifurane determine the different taste of wild strawberry in contrast to that of cultivated varieties.

D-fructose-6-phosphate could be the precursor of furaneol in strawberries (*23*), contrary to what was found by Hecquet et al. (1996) in yeast. This furaneol arising from D-fructose 6-phosphate would be rapidly converted into mesifurane and furaneol glucoside (*18*). The physiological source of D-fructose 6-phosphate in strawberry could be the pentose phosphate (*24, 25*).

Carotenoids

Coupled oxidative reactions of carotenoids with linoleic acid and lipoxydase result in bleaching of the carotenoids (*13*). The oxidative breakdown of carotenoids and the types of compounds formed are similar to the oxidative breakdown of unsaturated fatty acids. In tomato, the volatiles 6-methylhept-5-en-2-one, geranyl acetone, and farnesylacetone result from the oxidative cleavage of lycopene, phytofluene, phytoene, and others (*26*). Similarly, α-and β-ionone probably result from the oxidative breakdown of α-and β-carotenes. Geranial and geraniol arise from breakdown of lycopene (*13*). Terpenoids other than carotenoids, such as terpene and sesquiterpene hydrocarbons also undergo oxidative attack. 4-Terpineneol and α-terpineol are result from oxidation of terpinolene (*12*).

Lignin

Some of the volatile compounds like p-vinylguaiacol, guaiacol, eugenol, vanillin, myrsiticn, apiole and elemicin are formed from intermediate compounds in the plant

mechanism which produces lignin (*6*). Vanillin is the major flavor component of vanilla. Eugenol is the aroma component of cloves and contributes to the aroma of many foods. Guaiacol and p-vinylguaiacol are related to the smoky aroma in smoked foods (*6*).

Glucosinates

Glucosinates present in fruits and vegetables readily split to isothiocyanates and cyanides by enzyme action when the vegetable or fruit tissue is damaged (*27*). The major components formed are allyl isothiocyanate, 3-butenyl isothiocyanate, and the corresponding cyanides (*28-31*). Other vegetables in which volatiles are similarly formed are radish (4-methylthio-3-butenyl isothiocyanate), rutabaga (2-phenylethyl isothiocyanate), and watercress (2-phenylethyl isothiocyanate) (*32*).

Sample Preparation and Extraction

Efforts have been made by researchers for the past several decades to identify the chemical compounds responsible for the flavors of many foods. Aroma compounds responsible for the flavor of a fruit or vegetable are present in extremely small quantities. Hence, sample preparation is an important aspect for flavor. Enzymatic actions alter the actual aroma profile and cause artifact formation (*33, 34*). High concentrations of salts like saturated $CaCl_2$, have shown to deactivate fruit enzymes (*35-37*). All the known methods of volatile extraction alters to some extent the overall volatile composition obtained from the fruit (*22*). The proper technique can be chosen based on the type of food, the objective of the study, and the resources available. Some of the most important methods of aroma extraction are solvent extraction, distillation and headspace analysis.

Solvent Extraction

Organic solvents are often used to extract the volatiles from fruit matrices. Generally the fruit is blended and then either batch or continuous extraction is performed with the solvent of choice. The solvent is selected based on the extraction time and the nature of the volatiles to be extracted. Some volatiles differ in their polarity and solubility. Non-polar solvents like pentane and hexane are effective in selectively eliminating water and low boiling alcohols, and thus are useful in isolating volatiles from alcoholic beverages. A polar solvent like ethyl ether is extensively used because its extraction efficiency is high (*38*). Ethyl ether will extract more water, methanol, and ethanol (*34*). Diethyl ether and pentane can be used together to extract the polar and non-polar compounds. Solvent extractions has been used in mapping the aroma profile of strawberries as well (*39*) .

Solvent also extracts lipids and other nonvolatiles that need to be removed before GC analysis. This is done typically by vacuum distillation. More recently, a compact and versatile distillation unit called solvent assisted flavor extraction (SAFE) was developed for the fast and careful isolation of volatiles from complex food matrixes (*40*). SAFE is type of vacuum distillation. Vacuum distillation is a technique that uses vacuum to extract analytes from a sample. A cryogenically cooled trap is used to condense the compounds of interest. The sample is evacuated at low pressure vaporizing volatile compounds, including water. The condenser (column or a flask) retains vaporized water and those vaporized compounds that are not condensed in the column are collected in the cryotrap. The extract retained in the cryotrap are then transferred back

into the condesor. SAFE allows the isolation of volatiles from either solvent extracts, aqueous foods such as milk or beer, for aqueous. food suspensions such as fruit pulps (like strawberry-(*39, 41-43*) or even matrixes with high oil content (*40*).

Distillation

Distillation is the method of choice for low volatile matrices (*38*). Simultaneous steam distillation-extraction (SDE) is the most commonly used distillation method (*22, 34, 40*). If volatile constituents can be steam distilled without degradation of volatiles, then SDE using a Likens-Nickerson apparatus is possible (*44*). The apparatus provides for simultaneous condensation of steam distillate and an immiscible extracting solvent, and can also be operated under reduced pressure (*38*). Both the aqueous fruit sample and the solvent are heated in their respective flasks, which are connected by a central condenser. Water vapor, volatiles in the fruit matrix and the solvent vapors move up into the central condenser where liquid-liquid extraction of volatiles into the solvent occurs. The liquids collect in their respective side arms, and density differences between solvent and water phases affect the siphoning of solvent with volatiles and water back into their respective flasks. The extract is then dried and concentrated. SDE can be run for hours with little or no solvent loss (*34*) and small volumes of solvents can be used. One of the disadvantages of SDE is that it discriminates against very water soluble volatiles such as 2,5-dimethyl-4-hydroxy-3-(2H)-furanone (furaneol) (*40*).

Headspace Analysis

Headspace techniques, originally developed in the late 1960's have evolved into a major analytical procedures for determining the composition of volatiles in a wide range

of materials (*45*). It is being widely used in food and flavor industries for the analysis of fruits, vegetables, beverages etc. Headspace analysis is one of the best techniques for the analysis of highly volatile compounds.

Headspace analysis involves the direct analysis of volatiles in the gas phase above a sample. It is a simple technique which has numerous advantages over more conventional techniques like extraction and distillation. It is simple, fast, and eliminates column degradation due to non-volatile residues. As headspace analysis is a solventless technique, it allows for the analysis of highly volatile components which would otherwise be obscured by the solvent peaks. Headspace analysis can be divided into two types-static and dynamic headspace analyses.

Static headspace analysis

In static headspace analysis, the volatiles are equilibrated between the gas phase and liquid phases present in the sample. At equilibrium, the volatiles evaporate into the gas phase at the same rate as the volatiles condense into the liquid phase producing a partial pressure. This creates a relationship between the gas phase concentration (or partial pressure) and the concentration of the volatile in the liquid which is expressed as a partition coefficient. Partition coefficients are typically determined from the ratio of the concentration of the component of interest in the gas phase compared to its concentration in solution. Equilibrium headspace samples are limited to the fixed volume of gas above the sample. However this produces two problems. Since the volume is fixed and the total amount of material in the vapor phase is generally small, it is difficult to detect low concentration, highly potent aroma components. To overcome this obstacle sample concentration can be performed by using cold trapping/cryogenic focusing. The

major limitation is that water is usually the major component in food-derived headspace samples. Ice will form and block gas flow if a large headspace sample is introduced (*46*). Even so static headspace has been successfully used in strawberry by various researchers (*3, 47*).

Dynamic Headspace/Purge and Trap

In dynamic headspace analyses the carrier gas is either swept over the sample surface or bubbled through the sample, depending on foaming tendencies and samples can be concentrated using cold traps, solvent traps or solid adsorbents. Tennax is widely used to concentrate headspace volatiles.

Dynamic headspace techniques has been the headspace procedure of choice because of its efficiency in terms of minimum analysis time, minimum sample size and maximum number of volatile components obtained. It has been used to determine the aroma components in many food and beverages including : milk (*48-50*), cheese (*51-53*), bread (*54*), grapes (*55*), wine (*56*), apples (*57*), citrus (*58*), vegetable oils (*59*), meat (*60*), seafood, (*61*) coffee (*62*), cocoa (*63*) and strawberry (*64, 65*).

Sstatic and dynamic headspace techniques accentuate the more volatile components at the expense of the less volatile. This distortion is most severe with dynamic headspace analysis (non-equilibrium). The most volatile components will preferentially evaporate into the sweep gas with essentially none of the higher boiling point components volatilized. Hence dynamic headspace analysis should be employed only when highly volatile components are to be analyzed.

Volatile extraction by SPME

Solid-phase microextraction (SPME), a simple, effective, adsorption/desorption technique, eliminates the need for solvents or complicated apparatus for concentrating volatile compounds in liquid samples or headspace. SPME is compatible with analyte separation/detection by gas chromatography and provides reproducible results for wide concentrations of analytes. By controlling the polarity and thickness of the coating on the SPME fiber, maintaining consistent sampling time, and adjusting several other extraction parameters, an analyst can ensure highly consistent, quantifiable results from low concentration of analytes.

The SPME device consists of a fused silica fiber, coated on the outer surface with a stationary phase and bonded to a stainless steel plunger. The unit works by drawing the fiber into a protective needle, passing the needle through the septum that seals the sample vial, and then extending the fiber into the headspace above the sample. Where volatiles then absorb to the coating on the fiber. The volatiles are subsequently desorbed into the injection port of a GC.

In SPME, equilibria are established among the concentrations of an analyte in the sample, in the headspace above the sample, and in the polymer coating on the fused silica fiber. The amount of analyte adsorbed by the fiber depends on the thickness of the polymer coating and the distribution constant for the analyte. Extraction time is determined by the length of time required to obtain precise extractions for the analytes with the highest distribution constants. The distribution constant generally increases with increasing molecular weight and boiling point of the analyte. Selectivity can be altered by changing the type of polymer coating on the fiber, or the coating thickness, to match the

characteristics of the analytes of interest. In general, highly volatile compounds require a thick coating, and a thin coating is most effective for adsorbing/desorbing semivolatile analytes.

The polarity and thickness of the coating on the fiber, the sampling method (headspace sampling or fiber immersion), pH, and salt content of the sample, sample agitation, and other factors affect results from SPME. Consistent sampling time, temperature, and fiber immersion depth are critical in getting consistent results. Nonpolar analytes are most effectively extracted with a nonpolar fiber coating and polar analytes are most effectively extracted with a polar coating. The polymer increases the available surface area and thus improves extraction of small polar molecules. A thick fiber coating will extract more of a given analyte than will a thin coating and will effectively remove high boiling compounds from the sample matrix, but the desorption rate will be prolonged, and analytes will be carried over to the next extraction.. A thin coating ensures fast diffusion and release of higher boiling compounds during thermal desorption.

Sample agitation enhances extraction and reduces time, especially for high molecular weight analytes with high diffusion coefficients. Sonication promotes analyte adsorption, but can add heat to the sample. This might be beneficial for vaporizing the analytes for headspace extraction.

Adding 25-30% sodium chloride to the sample prior to extraction can increase the ionic strength of the solution and, in turn, reduce the solubility of some analytes. Acidic and basic compounds are more effectively extracted at acidic and basic pH, respectively.

The fiber coating composition is important to the various classed of volatiles absorbed. A divinyl benzene-polydimethyl siloxane Carboxen (DVB-PDMS-Carboxen) fiber used in SPME has the capability to extract both the polar and non polar compounds and it is better suited to the analyses of volatiles in fruits (*66*).

Volatiles in strawberries have been extracted by many different methods, including SPME. Even though selectivity is a major concern in SPME, it has been successively used by several researchers to extract the major compounds of interest in strawberry (*67-71*).

Strawberries

Strawberries are highly valued for their sweetness, flavor and attractive color. Strawberries belong to the genus *Fragaria* that includes 40 species belonging to the family Rosaceae. *Fragaria ananassa* is the species cultivated variety around the world. Strawberries are cultivated in all arable regions of the globe. The berries are consumed as fresh fruits and are also used as frozen berries or for juices, liquors, jams, sorbets, ice creams, yogurts, syrups, and concentrated aroma preparations. The USA is the leading strawberry producing nation, with approximately 20% of the world's crop, followed by Spain, Japan, the Korean Republic and Poland (*72*). US production averages nearly 1,000,000 tonnes. Total strawberry acreage in USA during the year 2004 was 53,300 acres, with most acerage being found in California at about 33,200 acres, followed by Florida at 7,100 acres and Oregon at approximately 3500 acres. (*72*). California accounts for 88.5% of strawberry production followed by Florida (7.5%), Oregon (1.5%) and Washington (0.5%) (USDA 2004) (*72*).

Strawberry Aroma

Strawberry aroma is the result of a complex multicomponent relationship among many aromatic constituents (*73*).The first report on strawberry aroma was published in 1939 by Coppens and Hoejenbos (*74*). Since then, numerous studies have been carried out to identify the compounds responsible for the strawberry aroma and so far 360 compounds (*75*) have been conclusively identified. These compounds have been identified using sophisticated instrumental techniques such as headspace analysis, GC-MS and selected ion monitoring. Agronomic and seasonal variations have been found to cause variation in the quantitative profile of strawberry volatiles (*22*). Genotype and growing conditions have been shown to cause changes to the key aroma compounds (*76*). Further non volatiles like sugars and acids can also influence the flavor perception in terms of intensity and fruitiness (*77, 78*). Hence cultivars with the same volatile content can be perceived differently by the consumers if they differ in the sugar and acid content.

Relative importance of Chemical Classes

Esters are the most important class of components in strawberries (*3*). Ethyl esters were found to be the major volatiles compounds with ethyl butanoate and ethyl hexanoate being the two main esters identified (*65*). These esters are thought to be formed from even numbered carbon volatile acids and alcohols, catalyzed by alcohol acyltransferase.

Strawberry aroma is mainly determined by a complex mixture of esters, aldehydes, alchohols, sulfur compounds and lactones which have been extensively studied (*3, 79*). Strawberry aroma was described as resulting from a mixture of caramel, jam, fruity, floral

and to a lesser extent, "green notes". The chemical classes that compose this aroma can be considered according to their flavor impact. Esters (fruity and floral notes) largely dominate the other classes, both qualitatively and quantitatively (*3*). Ethyl and methyl esters are considered to be the most significant components of strawberry aroma (*65*). The ratio of ethyl and methyl esters depends on the variety (*3, 42, 65*) and year (*80*) dependant. Ethyl esters namely, ethyl butanoate, ethyl hexanoate, are the major volatile components at all ripening stages of strawberry (*65*). Butanoates and hexanoates account for 50-80% of total volatiles in fresh strawberries (*80, 81*). Formation of esters has been reported to occur only in the mature stage because of lack of ester forming enzyme activity at immature stages (*82*). Volatile formation is supposed to peak during 36-41 days after blooming (*65*). Ethyl ester formation has also been found to be variety dependent (*83*).

Hexanal and trans-2-hexenal contribute to the green notes of the strawberries. These aldehydes are formed enzymatically during maceration from the unsaturated linoleic and linolenic acids. The concentration of the carobonyl compounds depends mainly on the stage of ripeness and also on varietal considerations.

Except for linalool, which contributes to the intense and pleasant character of 'Senga Sengana' , alcohols (56 compounds), even if numerous, are not considered to contribute significantly to strawberry flavor.

Acetic acid, butanoic acid and hexanoic acids are the major acids found in strawberries (*39*). Carboxylic acids have a small impact because their concentrations remain largely below their threshold values. However 2-methylbutanoic acid was found to have a characteristic strawberry flavor (*39*).

One of the most important flavor compounds in strawberries is 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (furaneol). Furaneol was identified for the first time as a natural aroma component in pineapples (*84*). It has since been detected in several fruits such as strawberry (*39, 43, 69, 85*), raspberry (*86*), mango (*87*), and tomato (*88*) and many other fruits. Mesifurane is also one of the major character impact aroma compounds in strawberry fruits (*43, 86*). However these two compounds have not been found in all cultivated strawberry cultivars (*43*).

Several odor descriptors were assigned to furaneol: caramel-like-note (*89*); sweet, floral, and fruity(*90*); and caramel-like, becoming fruity and strawberry-like at low concentrations(*91*). Because of its lability in aqueous solutions at 25°C (*92*), furaneol is difficult to detect and quantify. Recovery (*93*) and detection limits of furaneol were shown to be affected to a large extent by the conditions of isolation and GC procedures, respectively. Its degradation depends on pH and temperature. It is more stable at 5°C and pH 4 (*94, 95*).

Mesifurane is a major volatile of strawberry aroma and has a greater stability than furaneol (*95*). Although it is considered to be typical of wild strawberry aroma, it is found in much greater amounts in cultivated varieties (*87*), when compared with wild varieties. Mesifurane and furaneol content reaches the highest concentration at the overripe stage (*17*).

Sulfur compounds usually represent a small proportion of total volatiles, but they are extremely potent and are character impact compounds in fruits such as durian (*96*), grapefruit (*97*), melon (*35, 98*) and tomato (*35*). Sulfur compounds have been identified in strawberry (*3, 99, 100*) and are supposed to be important in some of the cultivars for their

characteristic aroma (*3*). So far hydrogen sulphide, methanethiol, dimethyl disulfide(*3, 99, 100*), methyl thioacetate, methyl thiobutyrate (*3, 99*), dimethyl sulfide, dimethyl trisulfide, carbon disulfide and sulphur dioxide (*99*) were identified in strawberry.

Methanethiol is highly unstable and is rapidly converted to other sulfur compounds such as dimethyl disulfide and hence an accurate determination of the concentration of the same is difficult(*99*). But because of its lower threshold concentration and high volatility it is considered an important aroma component. Methanethiol is considered to be an important aroma-active compound in strawberries. The "rotten" note of some varieties is caused by methanethiol (*3*). It is formed enzymatically (thioesterases) from indigenous thiol esters during maceration.

The concentration of dimethyl disulfide detected in strawberries was lower than its threshold (13 ng/ml), hence may not contribute much to the aroma of strawberry (*99*). Similarly carbon disulfide may not contribute much to the aroma of strawberries because of its high threshold concentration (*99*). Two thiol esters, methyl thioacetate and methyl thiobutyrate were detected among which, methyl thioacetate is considered more important than methyl thiobutyrate. Accurate quantification of sulphur volatiles can be achieved by using a pulse flame photometric detector (*99*) instead of a flame ionization detector which has lower sensitivity (*3*).

Sugars, Organic acids and Vitamin C in Strawberries

The main sugars found in strawberries are fructose, glucose and sucrose, while citric acid and malic acid are the important organic acids. Strawberries also contain significant amount of vitamin C. The average content of these quality parameters and the

variability found in the fruits depending on the variety and growing conditions are given in the following table.

Table 1.2: Average sugar and organic acids content in strawberries (*101-103*)

	Average	Range
	g/100 g fruit	
Sugars		
Fructose	2.30	2.13-2.40
Glucose	2.17	1.90-2.33
Sucrose	1.00	0.08-1.45
Organic acids		
Citric acid	0.75	0.67-0.94
Malic acid	0.30	0.09-0.34
Vitamins		
Ascorbic acid	64 mg/100 g	45-94 mg/100 g

Anthocyanins

Anthocyanins are water soluble pigments that are located in the vacuoles and confer a range of colors, from orange to purple.

Acetone extraction of anthocyanins (*104*)

In this method, acetone extracts the anthocyanins from the plant material (fruit), and chloroform partitioning further isolates and partially purifies the pigments. The addition of chloroform results in separation between the aqueous portion (which contains the anthocyanin, phenolics, sugars, organic acids, and other water soluble compounds) and the bulk phase (which contains the immiscible organic solvents, lipids, carotenoids, chlorophyll pigments, and other nonpolar compounds). This method has the advantage

of producing an extract with no lipophilic contaminants. The absence of a concentration step minimizes the risk of acid-dependent pigment degradation.

Estimation of Total Anthocyanin content (*105*)

Pelargonidin-3-glucoside is the major pigment of strawberries. It undergoes structural formations with change in pH. At pH 1.0, anthocyanins exist in the highly colored oxonium or flavilium form and at pH 4.5; they are predominately in the colorless carbinol form. The quantitative procedure for determining anthocyanin content is based on this fact. One aliquot of an aqueous anthocyanin solution (extract) is adjusted to pH 1.0 and another to pH 4.5. The difference in absorbance at the wavelength of maximum absorption will be proportional to anthoycanin content.

Color Measurement

Color is an indication of taste and flavor quality (e.g., freshness, over-ripeness or under-ripeness) in strawberries and it is usually the primary attribute consumers consider in making purchasing decisions. Color measurements are useful for grading strawberries. The human eye has its limitations as a color-differentiation device (*106*). Eye fatigue, color blindness and viewing conditions are examples of limitations of the human eye. Furthermore, the eye does a poor job in differentiating three important characteristics that account for how we perceive and judge color acceptability: (1) hue-which is a measure of red, green and blue (2) chroma or saturation; and (3) lightness/darkness. On average, the human eye perceives hue differences first, chroma or saturation differences second, and lightness/darkness last. To determine if a color is acceptable, colorimeter readings are converted into acceptability values. The internationally accepted method for measuring color was developed by the Commission International de l'Eclairage (CIE) and

is known as L*a*b* color measurement (also called CIELAB). These three-dimensional scales are based on the opponent-colors theory that states that the red, green and blue human eye cone receptor signals are re-mixed into black-white, red-green, and yellow-blue opponent colors as the signals move from the eye to the brain.

L, a b type of scales simulate this as:

- L (lightness) axis— 0 is black, 100 is white;
- a (red-green) axis— positive values are red; negative values are green and 0 is neutral; and
- b (yellow-blue) axis— positive values are yellow; negative values are blue and 0 is neutral.

All colors that can be perceived visually can be measured in any L, a, b scale. A tristimulus colorimeter can quantify the 10 million shades of colors human eye can detect. The numbers obtained from a colorimeter can be used for comparing samples (strawberries).

Sensory Evaluation

Sensory evaluation involves the testing of food material for the attributes under study. These attributes are appearance, odor (aroma or fragrance), consistency and texture, and also flavor. Sensory analysis of food relies upon evaluation through the use of our senses (odor, taste, tactile,). The primary function of sensory analyses is to conduct valid and reliable tests that provide data on which sound decisions can be made. Even though sophisticated and highly sensitive measuring instruments such as gas chromatographs, mass spectrometers, nuclear magnetic resonance spectrometers, IR and UV spectrophotometers and so on are now available, the importance of sensory analysis has

grown even further. It must be realized that only through the coordination of instrumental and sensory analysis can optimal information be obtained. Even at the limit of instrument sensitivity, our "biological detector" (our senses) may still perceive an odor, taste, etc. Additionally, the instruments will only analyze single components, whereas our senses give us an overall impression of aroma, taste, temperature and tactile components.

Descriptive analysis

Descriptive analysis involves the detection (discrimination) and the description of both the qualitative and quantitative sensory aspects of a product by a trained panel of 5-100 judges, depending on the type of product. Panelists must be able to detect and describe the perceived qualitative sensory attributes of a sample. These qualitative aspects of a product combine to define the product and include all of the appearance, aroma, flavor or texture which differentiates it from others. In addition, panelists must learn to differentiate and rate the quantitative or intensity aspects of a sample and to define to what degree each characteristic or qualitative note is present in that sample. Two products may contain the same qualitative descriptors, but they may differ markedly in intensity of each, thus resulting in quite different and easily distinctive sensory profiles or pictures of each product.

Panelists are selected based on their ability to discriminate differences in sensory properties among samples. The panel leader acts as a facilitator and assists the panel in developing a consistent terminology. The training of the panel is done by using reference standards and the samples to be tested. The panelists evaluate products one at a time in separate booths to reduce distraction and panelist interaction. The results of the test are analyzed statistically.

Consumer Tests

The primary purpose of affective tests (consumer tests) is to assess the personal response (preference/or acceptance) by current or potential customers of a product, a product idea, or specific product characteristics. The most effective tests for preference or acceptance are based on carefully designed test protocols run among carefully selected subjects with representative products. The choice of test protocol and subjects is based on the project objective. Potential respondents are screened based on a questionnaire and those selected and willing are given the products to be evaluated with a scorecard requesting their preference and reasons therefore along with past buying habits and various demographic questions such as age, income, employment, ethnic background, etc. Results are calculated in the form of overall preference scores and for various subgroups.

When a product researcher needs to determine the "affective status" of a product, i.e., how well it is liked by consumers, an acceptance test is used. From relative acceptance scores one can infer preference, the sample with the higher score is preferred. As part of a consumer test, the reasons for any preference or rejection by asking additional questions about the sensory attributes (appearance, aroma/fragrance, flavor, texture, mouth feel etc).

Relating Sensory Evaluation to Analytical Measurements

Comparisons between instrumental and sensory measurements provide a means of testing the reliability of instruments to provide sensory information about samples. Depending on the type of the analytical data collected, the matrices of the different analytical measurement can be compared to the sensory measurements in one of two

ways: the consensus configurations from the analytical and sensory can be performed, or the matrix of the analytical data can be analyzed with the sensory data to obtain a single consensus configuration. Analytical data can also be analyzed by a separate method and compared to the principal axes of the sensory consensus plot.

Relating consumer data and descriptive data

The trained descriptive or expert panel provides a thumbprint or spectrum of a product's sensory properties. This sensory documentation constitutes a list of a real attribute characteristics or differences among products which can be used both to design relevant questionnaires and to interpret the resulting consumer data after the test is completed. By associating consumer data with panel data and when possible with ingredient and processing variables, or with instrumental or chemical analyses, the researcher can discover the relationships between the product's attributes and the ultimate bottom line, consumer acceptance.

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Chapter 2.

Quantification of Strawberry aroma using headspace solid- phase microextraction gas chromatography and correlation with sensory descriptive analysis

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ABSTRACT

Aroma compounds in strawberries were quantified using headspace solid-phase microextraction/gas chromatography-flame ionization detection (GC-FID). Ten strawberry cultivars grown in California and Oregon were studied. The standard curves were built in a synthetic matrix and quantification was achieved using multiple internal standards. High correlation coefficient ($R^2 > 0.97$) was obtained for all the standard curves. Furaneol was found in only five of the varieties under study. Odor activity values (OAV) of aroma compounds were calculated to understand the contribution of the individual compound to the overall aroma. Although OAV varied based on the cultivars, in general, ethyl butanoate, mesifurane, furaneol, ethyl hexanoate, ethyl 3-methylbutanoate, hexyl acetate and γ-dodecalactone had the highest odor activity values. Descriptive sensory analysis was performed by a trained panel of 10 members. A PCA plot was built to understand the principal components of strawberry aroma. The chemical results were compared with sensory data. Correlation was achieved for the floral, pineapple, banana, peach and caramel notes. The green notes did not correlate with the concentration and odor activity values of the corresponding compounds in the varieties under study. This variation could be due to the very high odor activity of other compounds resulting in the masking of the green notes.

(Key words: strawberry, SPME, odor activity value, quantification)

Abbreviation Key: GC = gas chromatography, FID = Flame ionization detector, MS = mass spectrometry, SPME = Solid phase micro extraction, OAV = odor activity value,

PCA = principal component analysis, DVB-PDMS = divinylbenzene-polydimethyl siloxane

INTRODUCTION

Strawberries (Fragaria X *ananassa*) are highly valued for their sweetness, flavor and attractive color. Strawberries belong to the genus *Fragaria* and are cultivated in all arable regions of the globe. The USA is the leading strawberry producing nation, with approximately 20% of the world's crop. US production averages nearly 1,004,118 tons (*1*). Total strawberry acreage in USA during the year 2004 was 53,300 acres, with most hectarage in California at about 33,200 acres, followed by Florida at 7,100 acres and Oregon at approximately 3500 acres (*1*). California accounts for 88.5% of strawberry production followed by Florida (7.5%) and Oregon (1.5%) (*1*). 'Totem' is the most widely grown cultivar in Oregon.

The volatile compounds in strawberry have been studied extensively. The first report on strawberry volatiles was published in 1939 by Coppens and Hoejenbos (*2*). Since then, numerous studies have been carried out and 360 compounds have been conclusively identified (*3*). Strawberry aroma is a complex mixture of esters, aldehydes, alcohols, and sulfur compounds which have been extensively studied (*4-7*). 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (*8*) and 2,5-dimethyl-4-methoxy-3(2H)-furanone (*5*) are considered the two most important character impact compounds in strawberry, even though these two compounds have not been found in all the cultivated varieties (*9*).

All the studies have concluded that esters are the most important class of compounds among the aroma compounds in strawberry. Ethyl and methyl esters are

considered to be the most siginificant components of strawberry aroma (*4*). Butanoates and hexanoates account for 50-80% of total volatiles in fresh strawberries (*10, 11*). Ethyl esters are the major volatile compounds at all ripening stages (*4*) and its concentration has also been found to be cultivar dependent (*12*).

n-Hexanal and trans-2-hexenal contribute to the green notes of the strawberries. While alchohols are numerous, except for linalool, which is present in large amounts and contributes to the intense and pleasant character of some cultivars, alcohols are not considered to contribute significantly to strawberry flavor. Acetic , butanoic and hexanoic acids are the major acids found in strawberries (*5*). Carboxylic acids have a small impact because their concentrations remain largely below their threshold values. However 2-methylbutanoic acid was found to have a characteristic strawberry flavor.

Even though present at very low concentrations, sulfur compounds have been identified in strawberry (*6, 13, 14*) and are supposed to be important in some of the cultivars for their characteristic aroma (*6*). So far hydrogen sulphide, methanethiol, dimethyl disulfide(*6, 13, 14*), methyl thioacetate, methyl thiobutanoate (*6, 13*), dimethyl sulfide, dimethyl trisulfide, carbon disulfide and sulphur dioxide (*13*) were identified in strawberry.

The aroma compounds in strawberries can be divided into three groups (*15*), based on their contribution to the overall aroma. The first group comprises esters which includes methyl butanoate, ethyl butanoate, butyl acetate, methyl hexanoate and many other esters. They are responsible for the fruity odor notes. Esters encompass 25% to 90% of the total volatiles in ripe strawberry fruit (*11*).

The second group consists of mesifurane and furaneol which give the characteristic sweet odor notes. Mesifurane (*8*) and furaneol (*5*) are considered to be among the most important aroma compounds in wild strawberries (Fragaria X *vesca*), although these two compounds have not been found in all the cultivated strawberries (*9*) . Because of its lability in aqueous solutions at 25°C (*16*), furaneol is difficult to detect and quantify. Recovery (*17*) and detection limits of furaneol were shown to be affected to a large extent by the conditions of isolation and GC procedures, respectively. Its degradation depends on pH and temperature. It is more stable at 5°C and at pH 4 (*18, 19*).

The third group contributes to the green notes and consists of hexanal, trans-2-hexenal and cis-3-hexenol. The concentration of these compounds depends on stage of the ripeness of the fruit and also on the variety.

The relationship between the aroma and strawberry cultivars has been the subject of numerous investigations. Genotypic, agronomic and seasonal variations have been found to cause variation in the quantitative profile of strawberry aroma (*20*).

Several extraction methods have been used for strawberry aroma analysis. Among them, solid phase micro extraction (SPME) has been proved as a rapid, inexpensive and efficient technique that can be used to provide the aroma fingerprints of strawberries (*21*). A divinyl benzene-polydimethyl siloxane Carboxen (DVB-PDMS-Carboxen) fiber has the capability to extract both the polar and non polar compounds and thus it is suited to the aroma analysis. (*22*). Since the SPME fiber has different selectivity towards different classes of compounds, multiple internal standards with properties similar to that

of corresponding compounds can be used to further improve quantification accuracy (*23*).

Quantitative flavor profiling (*24*) is a sensory descriptive technique that has been developed to obtain accurate sensory data. A group of experienced panelists generate descriptive terminology before the evaluation of the samples. These terms as well as the aroma intensity were used to describe the test samples. Principle Component Analysis (PCA) can be used to look at samples in a descriptive space.

The aim of this study is to quantify and map the aroma profile of 'Totem' and other most widely grown strawberry cultivars in Oregon and California using SPME-gas chromatography and odor activity value, and correlate the analytical data with sensory results.

MATERIALS AND METHODS

Strawberries

Ventana, Camarosa, 13G97, San Miguel and Venice strawberry genotypes were procured from Driscoll Strawberry Associates Inc. (Salinas, CA). Totem, Hood, Puget Reliance, Puget Summer and Independence were obtained from Norpac Foods (Stayton, OR) and Townsend Farms (Fairview, Oregon). All the berries were collected in summer 2004, individually quick frozen at -34°F and stored at -10°F. Samples were analyzed within 9 months.

Chemicals

Methyl butanoate, methyl hexanoate, ethyl butanoate, ethyl hexanoate, ethyl 3-methylbutanoate (ethyl isovalerate), butyl acetate, 3-methylbutyl acetate (isoamyl acetate),

octyl acetate, butyl butanoate, trans-2-hexenyl acetate, hexyl hexanaote, trans-2-hexenal, hexanal, nonanal, 4-methoxy-2,5-dimethyl-3(2H)-furanone (mesifurane), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol), γ-octalactone, δ-decalactone, γ-dodecalactone, 3,7-dimethyl-1,6-octadien-3-ol (linalool), 2-decanol, benzothiazole, 2-methylbutanoic acid and 6E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (nerolidol) were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). Hexyl acetate, 3-heptanone and γ-undecalactone were purchased from K & K Laboratories (Jamaica, NY). Calcium chloride and sodium chloride were obtained from Fisher Chemical Company (Fairlawn, NJ). Glucose and methanol were obtained from EMD chemicals (Gibbstown, NJ), fructose and sucrose were obtained from J.T. Baker (Phillipsburg, NJ).

Sample Preparation and SPME extraction

Strawberries were thawed at room temperature for 90 minutes. Calcium chloride (1%) and distilled water (10%) were added, and the berries were blended to a fine puree with distilled water . Internal standards at 0.5 ppm concentration and 2 g sodium chloride were added to 10 g of puree in a 40 mL amber glass vials (I-Chem, New Castle, DE) with polytetrafluoroethylene needle-pierceable septum plastic screw caps. A stirring bar was placed in the vial and the samples were equilibrated for 15 minutes prior to extraction in a circulating water bath at 50°C. A Stableflex 50/30 μm DVB-Carboxen-PDMS fiber (Supelco, Bellefonte, PA) was used in this study. Prior to use, the fiber was conditioned at 270 °C for 4 hours. After equilibration, the SPME fiber was exposed to the headspace for 1 hour at the same agitation speed and temperature. After extraction, the SPME fiber

was introduced in the injector of the GC for desorption at 250°C for 3 min in the splitless mode.

Gas Chromatography (GC-FID)

The volatiles were chromatographed with a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector and a HP-wax column (30 m x 0.25 mm ID x 0.50 μm film thickness). Injection port was maintained at 250°C and the detector at 270°C. Carrier gas (nitrogen) flow rate was 2 ml/min at 35°C and 1:1 split condition was used under constant pressure. The oven temperature was programmed at 35°C to hold for 5 minutes, increased at a rate of 2°C to 230°C and held at 230°C for 5 minutes. Hydrocarbon standards (C_8 to C_{40}) were injected using the same temperature program to determine the retention indices of the individual compounds using modified Kovats method (*25*).

Gas chromatography-mass spectrometry (GC-MS)

Volatile identification was performed using an Agilent 6890 gas chromatography equipped with an Agilent 5973 mass selective detector. Enhanced chemstation software, G1701CA v.C.00.01.08 (Agilent Technologies, Inc., Wilmington, DE) was used to acquire and manage the data. A ZB-wax column (30 m × 0.25 mm ID × 0.25 μm film thickness, Phenomenex, Torrance, CA) was used for the separation of the volatiles with same oven temperature program at constant flow. Electron impact mass spectrometric data from m/z 35 to 300 was collected using a scan rate of 5.27/s, with an ionization voltage of 70 eV. The volatile compounds were identified by comparing the mass spectral data with the Wiley 275.L (G1035) Database (Agilent) and retention indices.

Quantification

A synthetic matrix was developed using 4 g pectin, 23 g glucose, 23 g fructose, 10 g sucrose, 7 g citric acid and 1g malic acid dissolved in one liter of millipore water according to literature (*26, 27*) . An internal standard solution in methanol containing 50 ppm each of 3-heptanone, 2-decanol, and γ-undecalactone was prepared by diluting a 500 ppm internal standard stock solution. An aliquot (0.1 g) of the internal standard solution (50 ppm) was then added to 10 gm of the synthetic matrix to yield a final concentration of 0.5 ppm. Three standard stock solutions of 1000 ppm each containing 8 internal standards were prepared in HPLC grade methanol. The stock solutions were further diluted with methanol to get a final concentration of 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm, 100 ppm, 200 ppm and 400 ppm. Standard solution at 0.1 g was added to 10 g of the matrix in a vial to yield a final concentrations of 0.0625 ppm, 0.125 ppm, 0.25 ppm, 0.5 ppm. 1 ppm, 2 ppm and 4 ppm respectively. Standards were extracted using the same SPME fiber under same conditions and injected on to the GC-FID. The GC running conditions were the same as used for strawberry samples. Calibration curves were built for methyl butanoate, methyl hexanoate, ethyl butanoate, ethyl hexanoate, ethyl isovalerate, butyl acetate, isoamylacetate, octyl acetate, butyl butanoate, hexyl acetate, trans-2-hexenyl acetate, trans-2-hexenyl acetate, trans-2-hexenal, hexanal, nonanal, mesifurane, furaneol, γ-octalactone, δ-decalactone, γ-dodecalactone, linalool, benzothiazole, trans-2-methyl-butanoic acid, and nerolidol.. The calibration curves of ethyl butyrate, hexanal, γ-dodecalactone, linalool and trans-2-methyl butanoic acid were used to quantify the esters, carbonyl compounds, lactones, alcohols and acid volatiles for which pure standards were not available.

Sensory Evaluation

Ten experienced panelists, 5 men and 5 women between the ages of 21 and 43 years were chosen for the sensory study. A total of six one-hour training sessions and six one-hour testing sessions were conducted. During the first training session, all the test samples were provided to develop the descriptive terminology. The flavor descriptors floral, caramel, pineapple, peach, banana, and green were selected from literature (*28*) and musty, waxy, sulfur and citrus terms were identified by the panelists among the samples under study. In the subsequent training sessions the panelists were trained to rate the intensity on a 0-15 scale. Vegetable oil (Wesson oil, Conagra Foods, Los Angeles, CA), Hi-C Orange lavaburst (Minute Maid, Houston, TX) juice, grape juice (Welch's, Concord, MA), and cinnamon gum (Trident, Cadbury Adams, Parsippany, NJ) were used as aroma intensity standards for 3, 7, 11 and 15 respectively (*29*). The standards and the samples (30 ml puree) were provided in an 8-ounce wine glass and the glass was covered with a plastic (non odorous) lid ((Solo cup company, Urbana, IL). Testing took place in individual testing booths under red lighting to mask the color differences among the samples. A randomized complete block design was used where each panelist received each sample three times (3 replications). Samples were coded with 3-digit random numbers.

Statistical Analysis

Analysis of variance and principal component analyses among the cultivars for sensory analyses was done using SPSS statistical package (SPSS, Chicago, IL).

The standard curves (Figure 2) built using pure internal standards showed a high linear correlation coefficient (R^2 >0.97). Standard deviation between the replicates was very low (<10%) for all the compounds except octyl butanoate (38 %) and octyl 2 methyl butanaote (19 %).

The concentrations of the compounds identified in the different strawberry cultivars are given in Table 2.1 and 2.2. The results show a high degree of consistency among the replicates with a standard deviation of less than 10% for majority of the compounds. Esters account for a major portion of the volatiles in all the ten cultivars. Prominent among them are ethyl acetate, methyl butanoate, ethyl butanaote, ethyl isovalerate, methyl hexanoate and ethyl hexanaote. These esters contribute to the fruity and floral notes of the strawberry aroma. The cultivar Venice has a higher concentration of ethyl acetate (10.28 ppm), methyl butanoate (1.84 ppm) and ethyl hexanoate (2.08 ppm). Ethyl butanoate is highest in Puget Reliance (3.33 ppm) and Totem (2.61 ppm), while ethyl isovalerate is present at similar levels in all the other varieties. Hood and Independence have slightly higher levels of methyl hexanoate (0.32 ppm, 0.20 ppm respectively).

Hexanal, trans-2-hexenal, hexanol and cis-3-hexen-1-ol, which have been previously described to contribute to the green notes have been found at different concentrations in the varieties under study. Mesifurane, one of the two compounds to be reported to have a strawberry like aroma has been found in eight of the ten varieties under study with a concentration ranging from 0.01 to 1.19 ppm. Furaneol, which has also been described having a strawberry or caramel aroma has been found in only five of the ten varieties under study.

Figure 1: Chromatogram showing the aroma profile of the genotype Totem.

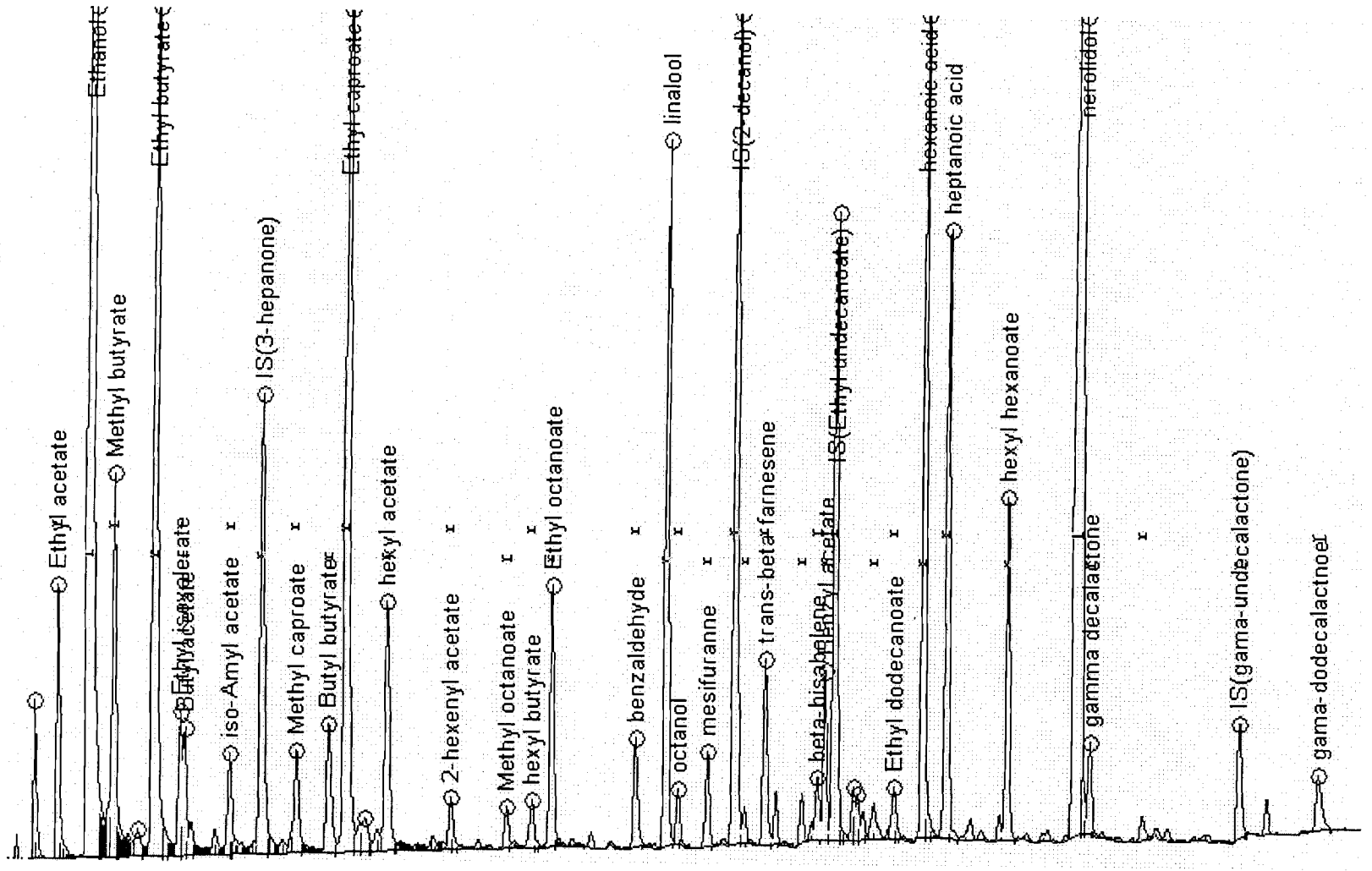
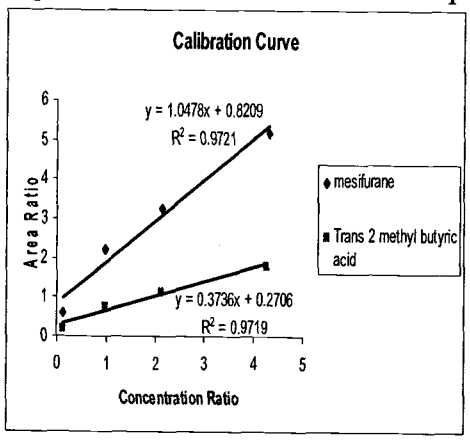
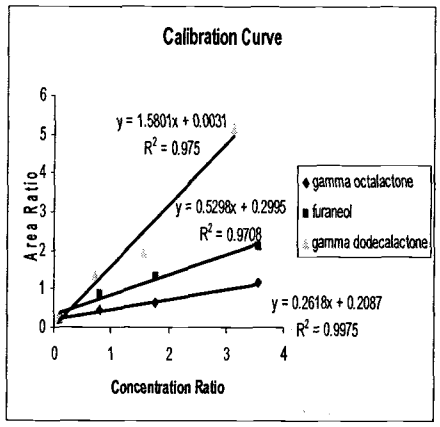
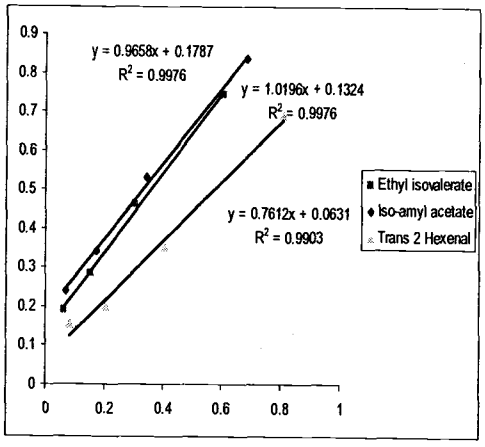
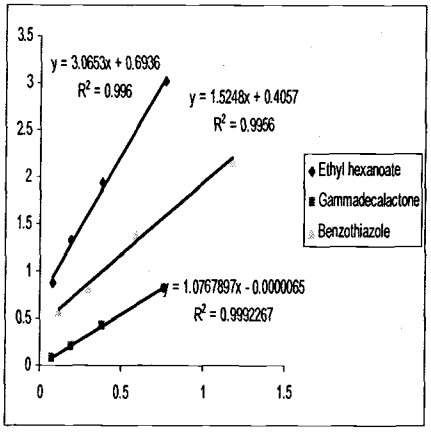


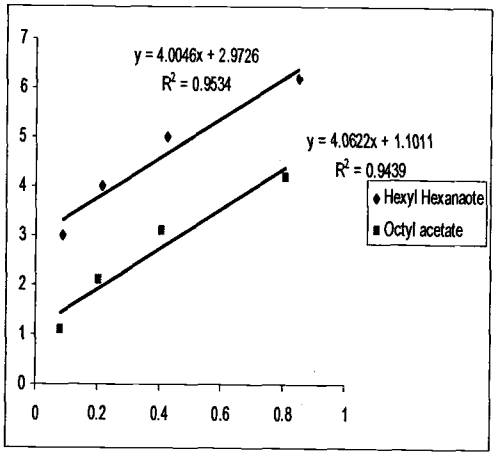
Figure 2- Calibration curves for important compounds detected in strawberry.











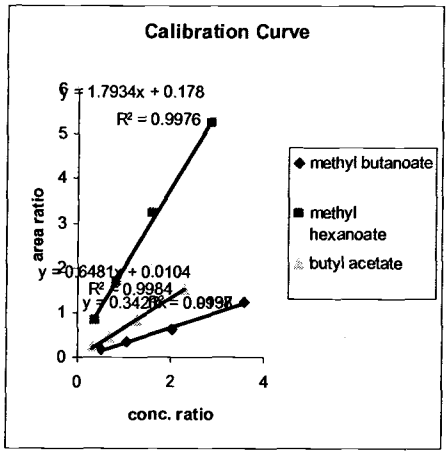


Table 2.1- Regression equations for major compounds identified in strawberry

Compound	Internal Standard	Regression Equation	R^2
Methyl butanoate	3-Heptanone	Y = 0.32x - 0.01	0.9937
Ethyl butanoate		Y = 0.49x + 0.20	0.9777
Ethyl isovalerate		Y = 1.01x + 0.13	0.9976
Hexanal		Y = 0.54x + 0.27	0.9829
Butyl Acetate		Y = 1.46x + 0.18	0.9923
Isoamyl acetate		Y = 0.96x + 0.17	0.9976
Methyl hexanoate		Y = 1.79x + 0.17	0.9976
Trans-2-hexenal		Y = 0.76x + 0.03	0.9903
Ethyl hexanoate		Y = 3.06x + 0.69	0.9956
Hexyl Acetate		Y = 1.67x + 0.89	0.9724
Octyl Acetate	2-Decanol	Y = 0.84x + 1.31	0.9916
Trans-2-hexenyl acetate		Y = 3.26x + 1.64	0.9788
Linalool		Y = 2.11x + 0.49	0.9765
Nonanal		Y = 13.95x -3.59	0.9861
Mesifurane		Y = 1.04x + 0.82	0.9721
Furaneol		Y = 0.52x + 0.29	0.9708
Nerolidol	γ-Undecalactone	Y = 16.11x + 3.27	0.9723
δ-decalactone		Y = 1.07x	0.9940
γ-dodecalactone		Y = 1.58x	0.9975

Table 2.2- Volatile compounds and their concentration (in mg/kg) in the strawberry genotypes from Oregon

		Concentration (mg/kg)				
Compound	Retention Index	Totem	Puget Reliance	Puget Summer	Hood	Independence
Methyl acetate	851	ND	0.04[a]	0.02[a]	ND	0.05 ± 0.02
Ethyl acetate	903	0.44 ± 0.02	0.60 ± 0.03	0.06[a]	ND	0.02[a]
Methyl butanoate	1001	0.97 ± 0.07	0.95 ± 0.02	0.82 ± 0.07	0.17 ± 0.04	0.56 ± 0.14
Ethyl butanoate	1055	2.61 ± 0.01	3.33 ± 0.06	1.95[b]	0.32 ± 0.02	0.02[a]
Ethyl isovalerate	1085	0.11 ± 0.01	0.12[a]	0.01[a]	ND	0.03 ± 0.01
Hexanal	1093	ND	0.05[a]	0.06[a]	0.09 ± 0.01	0.09[a]
Butyl acetate	1117	0.15 ± 0.01	0.14[a]	0.06[a]	ND	ND
Isoamyl acetate	1133	0.10 ± 0.01	0.01[a]	0.02[a]	0.01[a]	0.03 ± 0.01
Methyl hexanoate	1197	0.06[a]	0.07[a]	0.01 ± 0.00	0.20 ± 0.10	0.32 ± 0.01
Trans-2-hexenal	1228	ND	0.16 ± 0.03	0.15 ± 0.01	0.11 ± 0.03	0.28 ± 0.05
Butyl butanoate	1229	0.04[b]	ND	ND	ND	ND
Ethyl hexanoate	1245	0.40 ± 0.02	0.68 ± 0.05	0.06[b]	0.07 ± 0.05	0.07 ± 0.04
Hexyl acetate	1284	0.06 ± 0.01	0.06 ± 0.01	0.05[b]	0.08 ± 0.01	0.06 ± 0.01
Octanal	1298	ND	0.01[a]	ND	0.03[a]	ND
Cis-3-hexen-1-ol acetate	1314	ND	0.04 ± 0.01	0.02[b]	ND	ND
Trans-2-hexenyl acetate	1347	0.01[a]	0.01[a]	0.06[b]	0.21 ± 0.04	0.02[b]
Nonanal	1374	ND	ND	ND	0.01[a]	0.01[a]
1-Hexanol	1374	ND	0.01[a]	<0.01	ND	ND
Cis-3-hexen-1-ol	1401	ND	0.01[a]	<0.01	ND	0.01[a]
Hexyl butanoate	1426	0.13 ± 0.02	0.15 ± 0.02	0.14 ± 0.1	0.04 ± 0.01	0.01[a]
Trans-2-octenal	1442	ND	0.43 ± 0.01	0.05 ± 0.01	0.02[b]	0.01[a]
Epoxydihydrolinalool (Linalool oxide)	1467	ND	0.01[a]	<0.01	ND	0.01[a]
Octyl acetate	1487	ND	<0.01	<0.01	0.13 ± 0.01	0.03 ± 0.01
Benzaldehyde	1533	0.11 ± 0.02	0.16 ± 0.02	0.07 ± 0.01	0.03 ± 0.01	ND
Linalool	1565	0.21 ± 0.02	0.19[a]	0.12[a]	0.71 ± 0.04	0.64 ± 0.12
2-Methylpropanoic acid	1578	ND	ND	ND	ND	0.09 ± 0.02
1-Octanol	1578	0.02 ± 0.00	0.01[a]	0.01[a]	0.21 ± 0.01	ND
Heptyl acetate	1590	ND	ND	ND	ND	0.02[a]
Mesifurane	1601	0.09[a]	0.09 ± 0.01	0.04[a]	ND	0.38 ± 0.09
Hexyl hexanoate	1629	nf	0.03[a]	0.03[a]	1.55 ± 0.13	0.08[a]
Trans-2-hexenyl hexanoate	1685	0.17 ± 0.01	ND	ND	ND	0.88 ± 0.07
2-Methylbutanoic acid	1691	ND	1.52 ± 0.30	1.06 ± 0.14	ND	6.41 ± 0.68
Decyl acetate	1701	ND	0.02[a]	0.01[a]	0.19 ± 0.05	ND
α-Terpineol	1715	0.01a	0.01a	<0.01	0.33 ± 0.02	0.38 ± 0.03
Benzyl acetate	1743	1.34 ± 0.10	1.76 ± 0.32	0.53 ± 0.04	0.06 ± 0.01	0.68 ± 0.07
Linalyl formate	1775	ND	0.49 ± 0.10	0.53 ± 0.02	0.37 ± 0.12	0.13 ± 0.01

Pentyl hexanoate	1810	ND	0.09 ± 0.02	0.32 ± 0.05	0.15 ± 0.03	ND
Decyl 3-methylbutanoate	1822	ND	0.32 ± 0.11	0.24 ± 0.01	0.66 ± 0.12	0.12 ± 0.01
Ethyl dodecanoate	1827	0.24 ± 0.02	ND	ND	ND	ND
rans-2-geraniol	1834	ND	0.01[a]	0.03[a]	0.14 ± 0.09	0.02[a]
Hexanoic acid	1862	ND	18.33 ± 3.57	8.22 ± 0.72	10.10 ± 2.35	3.12 ± 0.42
Benzene methanol	1892	ND	ND	ND	ND	1.42 ± 0.01
Furaneol	2020	ND	0.05 ± 0.01	ND	nf	0.03[a]
Nerolidol	2063	1.07 ± 0.01	0.97 ± 0.20	0.07[a]	1.69 ± 0.13	3.41 ± 0.81
γ-decalactone	2083	ND	0.30 ± 0.03	ND	0.66 ± 0.06	0.25 ± 0.06
Ethyl cinnamate	2160	0.33 ± 0.02	0.41 ± 0.02	0.07[a]	ND	0.12 ± 0.03
3-Phenylallyl acetate (Cinnamyl acetate)	2192	ND	0.24 ± 0.02	0.13 ± 0.02	ND	0.12 ± 0.02
δ-decalactone	2234	ND	ND	ND	0.66 ± 0.06	0.33 ± 0.03
Jasmolactone	2249	ND	<0.01	<0.01	ND	0.02[b]
γ-dodecalactone	2422	0.20 ± 0.03	0.19[a]	0.17 ± 0.03	0.71 ± 0.04	0.80 ± 0.32
a- S.D ± 0.01, ND = Not Detected						

Table 2.3- Volatile compounds and their concentration (in mg/kg) in the five California strawberry genotypes

		Concentration (mg/kg)				
Compound	Retention Index	Ventana	Camarosa	San Miguel	Venice	13G97
Methyl acetate	851	0.02[a]	0.02[a]	0.10[a]	0.39[a]	0.02[a]
Ethyl acetate	903	0.05 ± 0.01	0.03[a]	0.12[a]	10.28 ± 0.06	0.03[a]
Methyl butanoate	1001	0.21 ±0.02	0.36 ± 0.02	0.50[a]	1.84[a]	0.39 0.05
Methyl pentanoate	1051	ND	ND	ND	2.30[a]	ND
Ethyl butanoate	1055	0.27 ±0.02	0.01[a]	0.28[a]	0.55 ± 0.01	0.07 0.01
Ethyl isovalerate	1085	0.01[a]	0.03[a]	0.01[a]	0.05[a]	0.02 ± 0.01
Butyl acetate	1117	<0.01	ND	ND	0.10[a]	0.01[a]
Hexanal	1093	0.03[a]	0.03[a]	0.52[a]	ND	0.05 ± 0.01
Isoamyl acetate	1133	<0.01	0.01[a]	<0.01	0.08[a]	0.01[a]
2-Pentenal	1139	ND	ND	<0.01	ND	ND
Ethyl pentanoate	1156	ND	ND	ND	1.76 ± 0.58	0.02[a]
Methyl hexanoate	1197	0.02b	0.02[a]	0.03[a]	0.11[a]	0.08 ± 0.04
Trans-2-hexenal	1228	0.01[b]	0.26[a]	0.02[a]	0.06[a]	0.09 ± 0.03
Butyl butanoate	1229	ND	ND	ND	ND	ND
Ethyl hexanoate	1245	0.08 ± 0.01	0.06[a]	0.05[a]	2.08 ± 0.23	0.25 ± 0.20
Hexyl acetate	1284	<0.01	0.02[a]	<0.01	0.05 ± 0.01	0.04 ± 0.03
Octanal	1298	ND	<0.01	<0.01	ND	ND
3-Hexen-1-ol-Acetate	1314	0.01[b]	ND	0.01[a]	ND	ND
2-hexenyl acetate	1347	<0.01	0.01[a]	<0.01	0.01[a]	<0.01
Nonanal	1374	ND	<0.01	ND	ND	0.01[a]
1-Hexanol	1374	<0.01	ND	<0.01	0.01[a]	ND
3-hexen-1-ol	1401	<0.01	<0.01	<0.01	ND	ND
Hexyl butanoate	1426	0.02[a]	0.22 ± 0.01	0.01[a]	ND	0.02[a]
2-Octenal	1442	0.01[a]	0.01[a]	<0.01	ND	0.01[a]
epoxydihydrolinalool (Linalool oxide)	1467	<0.01	<0.01	<0.01	0.02 ± 0.01	0.02 ± 0.01
Trans-2-4-heptadienal	1477	ND	ND	<0.01	ND	ND
Octyl acetate	1487	<0.01	<0.0	<0.01	0.42 ± 0.01	0.03 ± 0.02
Acetic acid	1506	0.03[b]	0.04[a]	0.01[a]	ND	ND
Benzaldehyde	1533	0.11[a]	0.06[a]	0.06[a]	0.18[a]	0.08 ± 0.04
Linalool	1565	0.06 ± 0.01	0.03[a]	0.03[a]	0.27[a]	0.22 ± 0.15
2-Methyl propionic acid	1578	ND	ND	ND	ND	ND
1-octanol	1578	0.01[b]	0.04[a]	0.02[b]	0.07 ± 0.01	0.07 ± 0.04

Heptyl acetate	1590	ND	ND	ND	ND	ND
Mesifurane	1601	ND	0.04[a]	0.01[a]	1.19 ± 0.01	0.08 ± 0.03
Hexyl hexanoate	1629	0.03[a]	0.02[a]	0.03[a]	ND	1.55 ± 0.63
Octyl butanoate	1629	ND	ND	ND	0.12[b]	0.45 ± 0.13
Octyl 2 methyl butanoate	1651	0.11 ± 0.04	0.56 ± 0.07	0.18[b]	0.24[a]	0.16 ± 0.03
2-Hexenyl hexanoate	1685	ND	0.52 ± 0.06	<0.01	<0.01	0.13 ± 0.34
2-Methyl butyric acid	1691	0.36 ± 0.01	0.17[a]	ND	ND	ND
Decyl acetate	1701	ND	0.02[a]	ND	0.17[a]	ND
Alpha terpineol	1715	ND	<0.01	<0.01	0.01[a]	0.17 ± 0.08
Benzyl acetate	1743	0.23 ± 0.06	0.11 ± 0.03	0.05[a]	0.71 ± 0.02	0.41 ± 0.14
Linalyl formate	1775	0.99 ± 0.04	0.31 ± 0.05	0.15[b]	0.61 ± 0.02	ND
Pentyl hexanoate	1810	0.21 ± 0.04	0.10 ± 0.02	0.07[b]	0.14[b]	ND
Ethyl nicotinate	1789	ND	ND	ND	ND	0.12 ± 0.03
Decyl isobutanoate	1822	0.12 ± 0.02	0.91 ± 0.05	0.06[a]	0.23[a]	0.07 ± 0.02
Ethyl dodecanoate	1827	ND	ND	ND	ND	ND
Trans-2-geraniol	1834	<0.01	0.04 ± 0.01	ND	ND	ND
Hexanoic acid	1862	3.05 ± 0.10	2.21 ± 0.04	0.83[a]	0.04[a]	3.74 ± 0.60
Benzene methanol	1892	ND	ND	ND	ND	ND
Furaneol	2020	0.03 ± 0.01	nf	<0.01		<0.01
Nerolidol	2063	0.10[b]	0.05[b]	<0.01	0.03 ± 0.03	0.89 ± 0.77
γ-decalactone	2083	0.11[b]	0.23 ± 0.01	ND	0.14 ± 0.01	0.73 ± 0.55
Ethyl cinnamate	2160	0.55 ± 0.01	0.61 ± 0.15	0.45 ± 0.03	2.73 ± 0.05	0.36 ± 0.08
3-Phenylallyl acetate (Cinnamyl acetate)	2192	0.19 ± 0.01	0.20 ± 0.01	0.13 ± 0.01	0.19[b]	0.19 ± 0.02
δ-decalactone	2234	ND	0.78 ± 0.01	0.03[b]	ND	0.01[b]
Jasmolactone	2249	<0.01	<0.01	<0.01	ND	ND
γ-dodecalactone	2422	0.09 ± 0.02	0.22 ± 0.02	<0.01	0.20 ± 0.02	0.20 ± 0.01
a- S.D ±0.01, ND = Not Detected						

Table 2.4- Odor activity values (OAV's) and the threshold levels of volatiles identified in Oregon Strawberries.

		Odor Activity Value (OAV)				
Compound	Threshold	Totem	Puget Reliance	Puget Summer	Hood	Independence
Ethyl acetate	0.025	17.8	24.1	2.5	ND	0.7
Methyl butanoate	0.06	16.3	15.9	13.7	2.8	9.4
Ethyl butanoate	0.001	2610	3331.3	1956.5	324.5	15.8
Ethyl isovalerate	0.002	72.5	85.7	8.7	ND	21.2
Butyl acetate	0.066	2.3	2.1	0.8	ND	ND
Hexanal	0.054	ND	1.2	1.5	2	2
Isoamyl acetate	0.02	5	4.9	1.4	0.7	1.6
Trans-2-hexenal	0.017	ND	9.5	9.2	6.7	16.7
Butyl butanoate	100	<0.1	ND	ND	ND	ND
Ethyl hexanoate	0.001	400	685	63.5	74.7	66.7
Hexyl acetate	0.002	32.9	31.9	26.1	42.3	32
Octanal	0.001	ND	14.7	ND	36	ND
Nonanal	0.001	ND	ND	ND	13.7	10.5
1-Hexanol	2.5	ND	<0.1	2.5	ND	ND
3-Hexen-1-ol	0.07	nf	0.21	0.05	ND	ND
Hexyl butanoate	0.25	0.53	0.61	0.56	0.16	0.05
2-Octenal	0.436	ND	ND	16.81	7.12	2.77
Octyl acetate	0.001	ND	0.06	0.22	10.74	2.63
Acetic acid	60	ND	<0.1	<0.1	ND	<0.01
Benzaldehyde	0.35	0.3	0.4	0.2	<0.1	nf
Linalool	0.01	20.7	19.4	11.8	71.7	64.81
1-Octanol	0.11	0.1	0.1	0.1	1.9	ND
2-Methyl butyric acid	0.25	ND	ND	4.25	ND	25.65
Mesifurane	0.00003	3000	3000	1333.3	ND	12666.6
Benzyl acetate	0.75	1.8	23.4	7.1	0.7	9.1
Trans 2 geraniol	0.009	ND	1.5	3.3	15.6	1.7
Hexanoic acid	3	ND	6.1	2.7	3.3	1
Furaneol	0.00004	ND	1392.2	ND	ND	837.4
γ-decalactone	0.011	ND	27.4	ND	60.4	22.9
δ-decalactone	0.336	ND	ND	ND	ND	3.36
Jasmolactone	0.006	ND	<0.1	<0.1	ND	0.01
γ-dodecalactone	0.007	28.2	27.7	24.9	101.7	114.7

ND = Not Detected. Threshold levels of compounds (in water) were obtained from the flavor base of Leffingwell & Associates(37),.

Table 2.5- Odor activity values (OAV's) and the threshold levels of volatiles identified in California Strawberries.

		Odor Activity Value (OAV)				
Compound	Threshold	Ventana	Camarosa	San Miguel	Venice	13G97
Acetaldehyde	0.015	0.7	ND	0.9	4.43	ND
Acetone	0.013	1.8	0.8	1.6	17	2.2
Ethyl acetate	0.025	1.9	1.1	6	411.5	1.2
Methyl butanoate	0.06	3.5	6.1	8.3	30.7	6.6
Ethyl butanoate	0.001	273.6	13.8	280.4	550	69.6
Ethyl isovalerate	0.0015	7.7	20.5	8.3	36.3	17.7
Butyl acetate	0.066	0.1	0.7	ND	1.5	0.2
Hexanal	0.045	0.7	ND	1.1	ND	0.7
Isoamyl acetate	0.02	0.2	0.7	0.2	3.9	0.5
2-Pentenal	1.5	ND	ND	<0.1	ND	ND
Trans 2 hexenal	0.017	1	15.3	1.3	3.4	5.4
Ethyl hexanoate	0.001	86.5	65	48.9	2079.9	257
Hexyl acetate	0.002	2.1	10.3	2.1	27.8	19.5
Octanal	0.0007	ND	ND	4.9	ND	ND
2-Heptenal	0.013	ND	ND	0.1	ND	ND
Nonanal	0.001	ND	ND	ND	ND	12
1-Hexanol	2.5	<0.1	ND	<0.1	<0.1	ND
3-hexen-1-ol	0.07	<0.1	<0.1	<0.1	ND	ND
Hexyl butanoate	0.25	<0.1	0.9	<0.1	ND	0.1
2-Octenal	0.003	4.7	5	1.3	48.7	3.7
Octyl acetate	0.012	<0.1	0.4	<0.1	35.2	2.5
Acetic acid	60	<0.1	<0.1	<0.1	ND	ND
Benzaldehyde	0.35	0.3	0.18	0.18	0.51	0.24
Linalool	0.01	6.2	3.7	3.4	27.1	22.8
1-octanol	0.11	0.1	0.4	0.1	0.6	0.6
Mesifurane	0.00003	ND	1333.3	333.3	39666.6	2666.6
Benzyl acetate	0.075	3.1	1.4	0.6	9.4	5.5
Trans-2-geraniol	0.009	0.6	5	ND	ND	ND
Hexanoic acid	3	1	0.7	0.2	<0.1	1.2
Furaneol	0.00004	742.5	ND	163.8	ND	176.8
γ-decalactone	0.011	187.4	21.7	3.5	13.1	66.3
δ-decalactone	0.1	ND	7.8	0.2	ND	0.1
Jasmolactone	2	<0.1	<0.1	<0.1	ND	ND
γ-dodecalactone	0.007	12.8	32.1	0.7	29.3	28.8
ND = Not Detected						

RESULTS AND DISCUSSION

SPME extraction parameters

The sensitivity and accuracy of aroma analysis depend on employing the optimum SPME extraction conditions. Extraction was evaluated for varying periods of time (30min, 45 minutes, 60 minutes and 90 minutes) at different temperatures (30°C, 40°C, and 50°C) and at different dilutions. The concentration of the volatiles was highest at 60 minutes with no significant difference between 60 and 90 minutes. Extraction at 50°C yielded a higher amount of higher boiling volatile compounds when compared with 30°C and 40°C. 5 g, 10 g, 15 g and 20 g of sample was used for extraction. Maximum yield was obtained at 10 gm sample size. Some of the cultivars had a very high viscosity and hence distilled water was added at varying amounts (10%, 20%, 30%, 40% and 50%) to facilitate the action of the stir bar and subsequent release of volatiles. Based on these results, the SPME extraction conditions selected for all the aroma analyses are 10% distilled water addition, 15 minutes of equilibration time and 60 minutes extraction time at 50°C.

Even though ethyl undecanoate and heptanoic acid were also used as internal standards along with 3-heptanone, 2-decanol, and γ-undecalactone, these standards were coeluting with other peaks in some of the samples. Hence quantification was performed with 3-heptanone, 2-decanol, and γ-undecalactone.

Aroma Analysis

A total of 66 compounds had been identified in the ten cultivars. Twenty six esters, six alcohols, five lactones and five aldehydes which are important in strawberry were identified. A typical chromatogram of genotype totem is shown in figure 1.

The standard curves (Figure 2) built using pure internal standards showed a high linear correlation coefficient (R^2 >0.97). Standard deviation between the replicates was very low (<10%) for all the compounds except octyl butanoate (38 %) and octyl 2 methyl butanaote (19 %).

The concentrations of the compounds identified in the different strawberry cultivars are given in Table 2.1 and 2.2. The results show a high degree of consistency among the replicates with a standard deviation of less than 10% for majority of the compounds. Esters account for a major portion of the volatiles in all the ten cultivars. Prominent among them are ethyl acetate, methyl butanoate, ethyl butanaote, ethyl isovalerate, methyl hexanoate and ethyl hexanaote. These esters contribute to the fruity and floral notes of the strawberry aroma. The cultivar Venice has a higher concentration of ethyl acetate (10.28 ppm), methyl butanoate (1.84 ppm) and ethyl hexanoate (2.08 ppm). Ethyl butanoate is highest in Puget Reliance (3.33 ppm) and Totem (2.61 ppm), while ethyl isovalerate is present at similar levels in all the other varieties. Hood and Independence have slightly higher levels of methyl hexanoate (0.32 ppm, 0.20 ppm respectively).

Hexanal, trans-2-hexenal, hexanol and cis-3-hexen-1-ol, which have been previously described to contribute to the green notes have been found at different concentrations in the varieties under study. Mesifurane, one of the two compounds to be reported to have a strawberry like aroma has been found in eight of the ten varieties under study with a concentration ranging from 0.01 to 1.19 ppm. Furaneol, which has also been described having a strawberry or caramel aroma has been found in only five of the ten varieties under study.

Even though carboxylic acids like acetic acid, butanoic acid, hexanoic acid were found in certain genotypes, quantification was not performed due to SPME fiber's selectivity towards carboxylic acids.

Since some compounds have greater impacts on the overall aroma than others due to the odor sensory threshold difference, odor activity values (OAV = ratio of concentration of compound to its threshold value) have been used to get a better understanding of the contribution of each compound to the overall aroma. Table 2.3 and 2.4 summarize the odor activity values calculated based on the threshold concentrations from literature. Among the major 37 aroma compounds identified in the study, six compounds have an odor activity value higher than 100, two compounds between 50-100, eleven compounds between 10-50, eight compounds had OAV's in the range 1-10 and ten compounds less than 1. Although OAV varied based on the cultivars, in general, ethyl butanoate, mesifurane, furaneol, ethyl hexanoate, ethyl 3-methylbutanoate, hexyl acetate and γ-dodecalactone had the highest odor activity values. Even though some of the compounds like hexanoic acid, 2-methylbutanoic acid and acetic acid had higher concentrations than other compounds, they have lower odor activity because of their higher threshold concentrations.

Venice cultivar has the highest combined total OAV's (42,997) for all the compounds while Hood has the lowest (777). The combined OAV's for Totem, Puget Reliance, Puget Summer, Hood, Independence, Ventana, Camarosa San Miguel and 13G97 are 6208, 8712, 3497,13,938, 1339, 1547, 872 and 3368 respectively. In the varieties Totem, Puget Reliance, Puget Summer and Venice both ester and lactones (mesifurane and furaneol) contribute to the odor activity while in Independence, Ventana, Camarosa, San Miguel and 13G97 lactones are primarily responsible for the odor activity. In the variety Hood, most of

the odor activity is contributed by the esters as both mesifurane and furaneol were not detected.

Even though SPME has been successfully used in the identification of key aroma compounds in strawberry by several authors (*21, 32*) there is no single study that attempted to quantify all the aroma compounds using SPME. In this study, even though SPME has been successfully used to extract all the major aroma compounds reported in literature and to quantify the key esters and lactones, it was less sensitive for acids, alcohols and furaneol. Acetic acid was found in only six of the varieties studied and the concentration is much lower than reported earlier. Propanoic and butanoic acids were not found in any of the varieties studied, even though they were reported by several authors in previous studies (*21, 33*). In strawberry 26 alcohols have been identified so far, and only seven of them were found in this study. However it has to be noted that not all volatile compounds have been identified in all the strawberry cultivars, because of variations in genetic base, cultivation practices and climatic conditions. Since most of alcohols and acids have very high sensory thresholds, they contribute very little to the overall aroma of strawberry, the quantification data obtained by using SPME method in this study still provides useful information about aroma profile of strawberry cultivars grown in Oregon and California.

Sensory Analysis

Principal component analysis was used to describe the sensory profile of the ten strawberry cultivars. In the PCA plot, principal component one accounted for 37% of total variation, while principal component two (PC2), three (PC3) and four (PC4) accounted for 19%, 11% and 9.37% respectively. PC1 differentiated the samples based on the floral, pineapple, caramel, peach, banana, green, waxy, sulfur, and musty notes. PC2 grouped the samples based on the peach note, PC3 on citrus and overall aroma, while PC4 categorized the

samples based on the banana note. Totem, Puget Reliance, Puget Summer and Venice had higher amounts of floral, caramel, pineapple, peach and banana notes. Hood, Independence, Ventana, Camarosa, San Miguel and 13G97 had higher amounts of green, sulfur, musty and waxy notes (Figure 3). Puget Reliance was significantly different from Hood, Camarosa and Ventana on PC1. Puget Reliance had higher fruity and floral notes while Hood, Camarosa and Ventana had higher green, sulfur, waxy and musty notes. Puget Reliance, Independence and 13G97 were significantly different from each other on PC2 (Figure 3) which explained the variation in peach note. Totem, Camarosa, Puget Reliance, San Miguel and Venice had higher citrus and overall aroma (Figure 4). There was no significant difference between any of the varieties with regards to citrus and overall aroma (same subsets). Independence, San Miguel, Totem and Venice had higher amounts of banana notes than the rest of the cultivars on PC4 (Figure 5). Venice and Camarosa differed significantly from one another in terms of the amount of banana note in them.

Correlation of analytical data with sensory data

Totem, Puget Reliance, Puget Summer and Venice have higher amounts (Figure 2) of fruity aroma (pineapple, banana, peach). The sensory characteristics of a product generally result from many chemical compounds acting in concert (*34*). Methyl butanoate, ethyl acetate, ethyl butanaoate, ethyl hexanoate, ethyl isovalerate and isoamyl acetate contribute to the fruity aroma notes in strawberry. The combined OAV's of these

Figure 3- Principal component plot-principal component one showing the descriptive profile for the attributes-floral, pine apple, caramel, peach, banana, green, waxy, sulfur and musty

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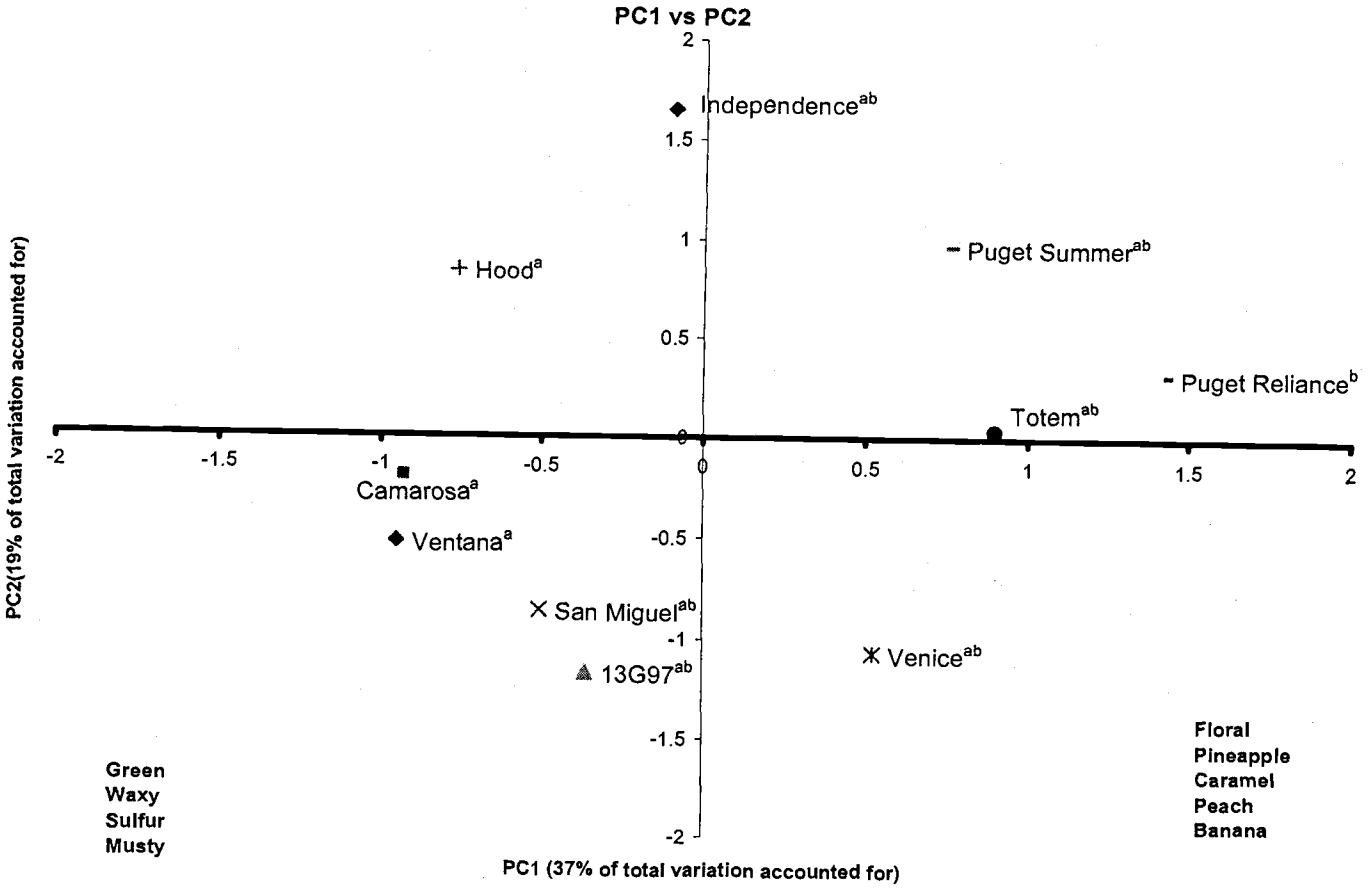


Figure 4- Principal component plot-principal component two showing the descriptive profile of strawberries for the attribute peach.

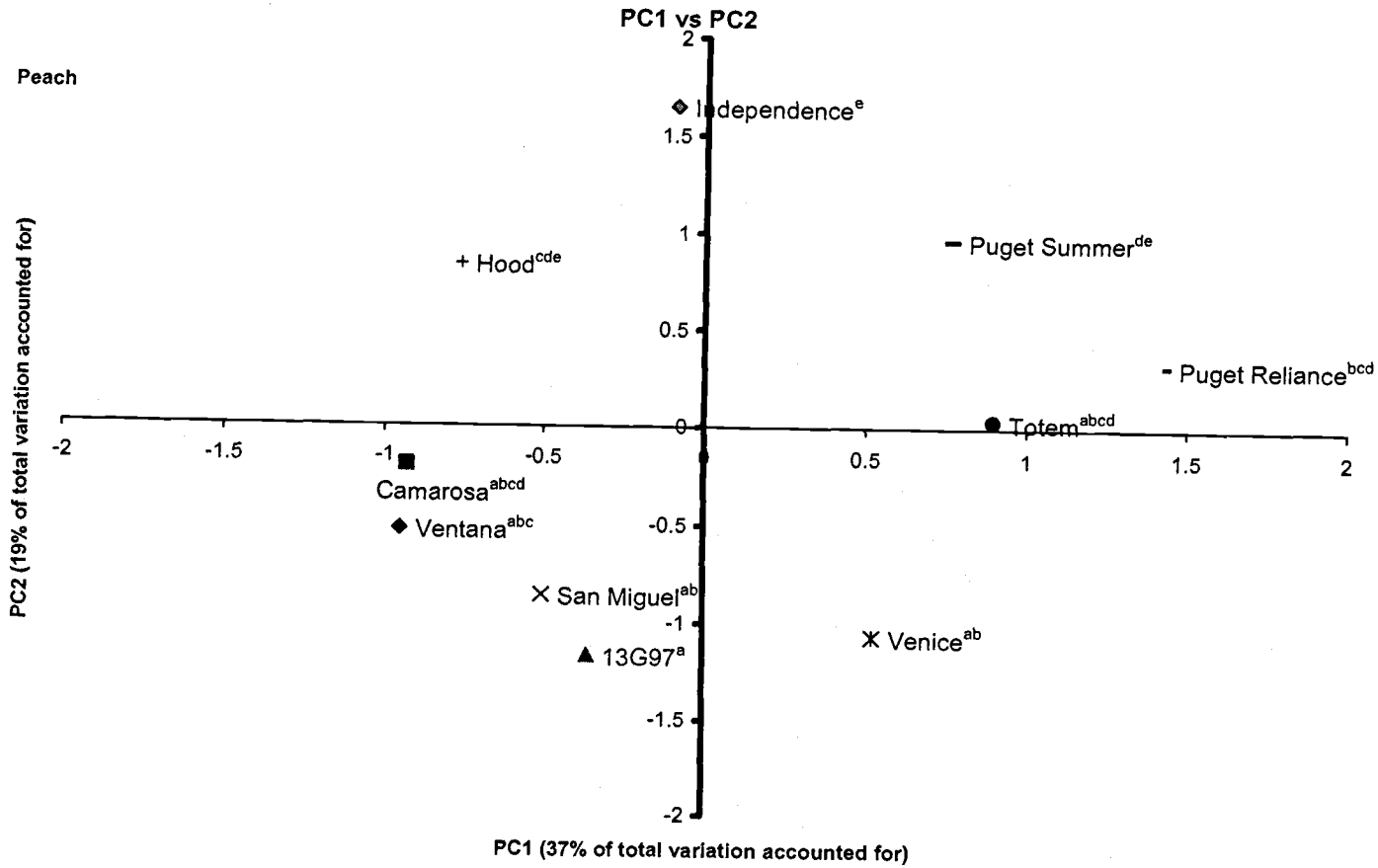


Figure 5- Principal component plot-principal component three showing the descriptive profile of strawberries for the attribute citrus and overall aroma.

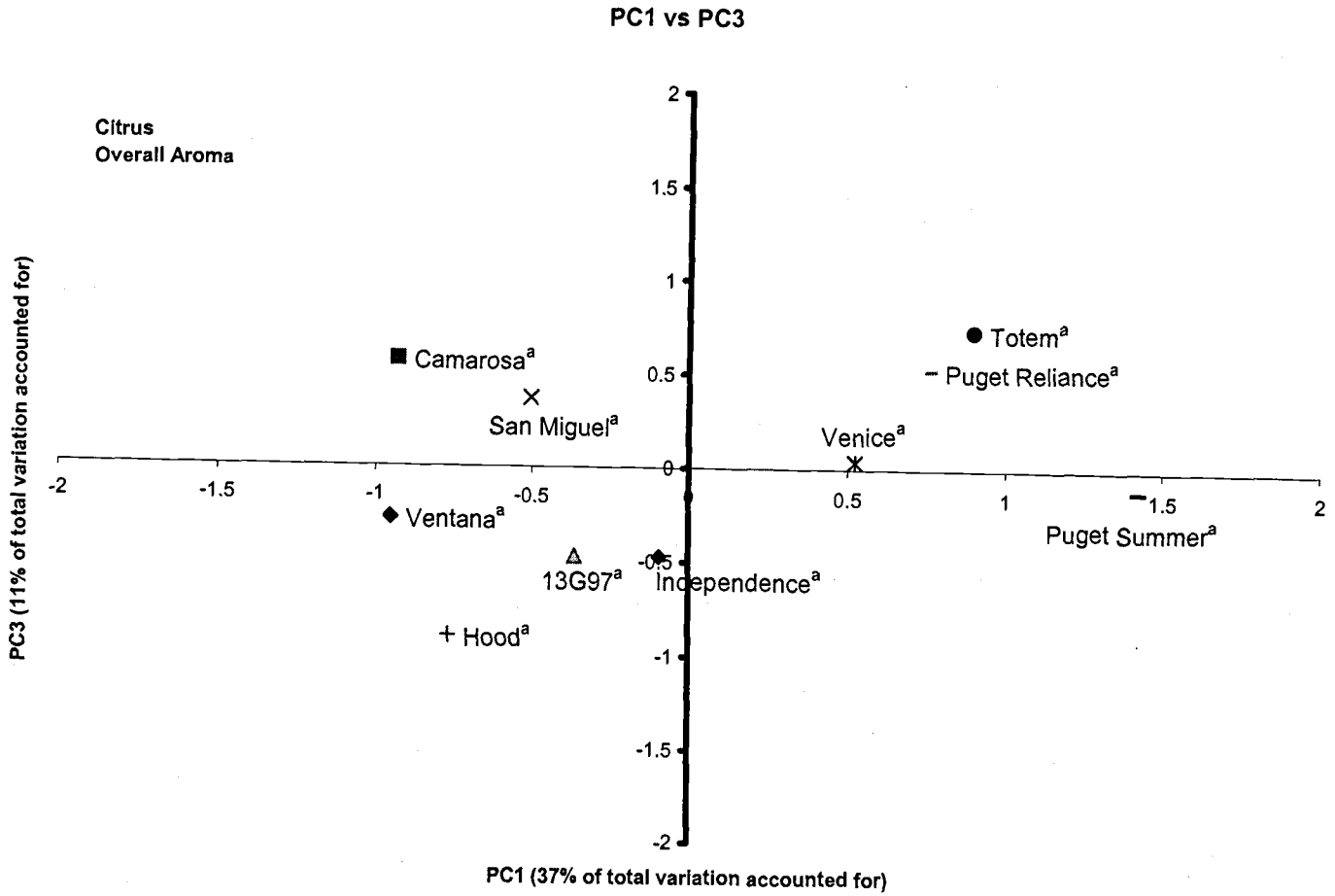
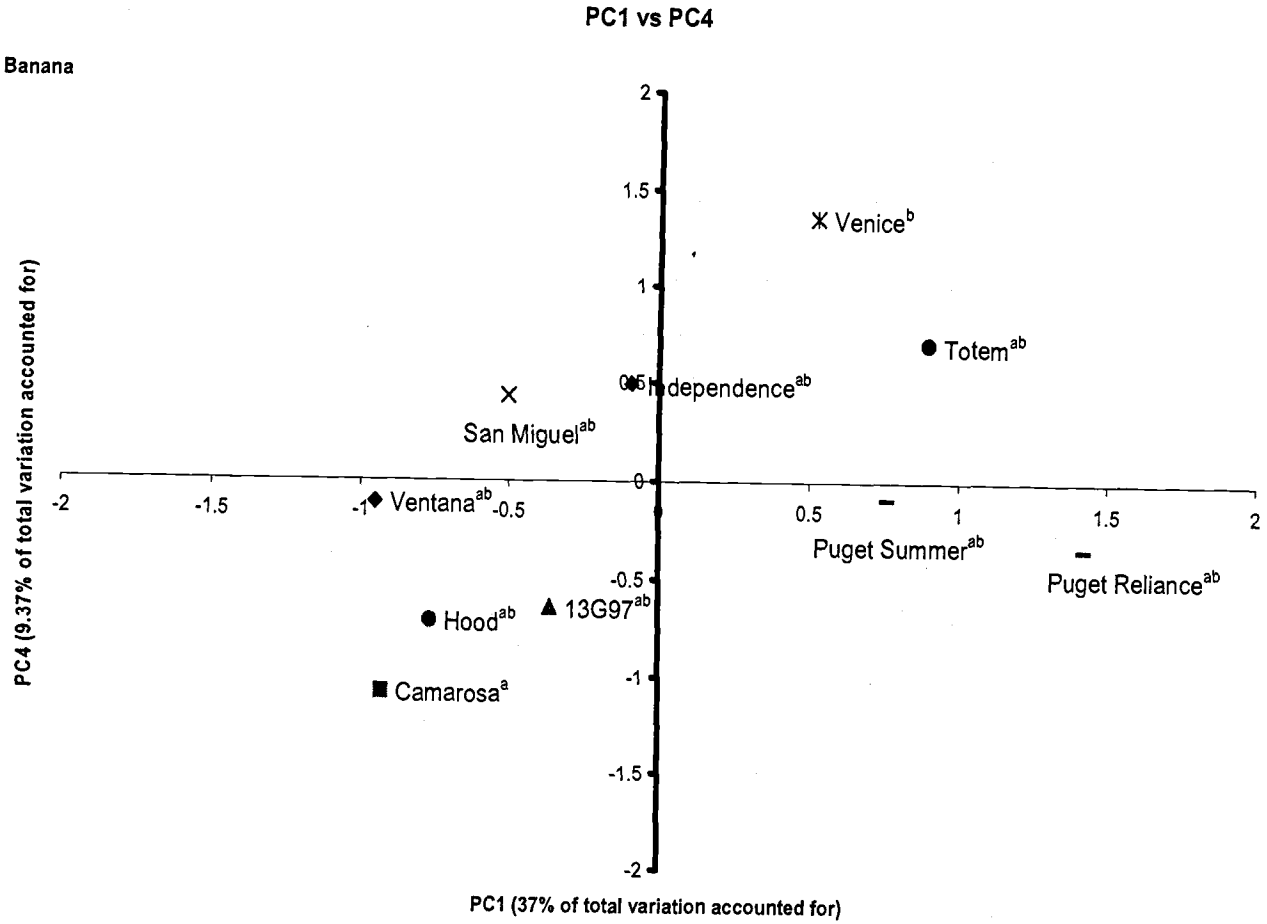


Figure 6- Principal component plot-principal component four showing the descriptive profile of strawberries for the attribute banana.



compounds for Totem, Puget Reliance, Puget Summer and Venice are 3053, 4037, 2045 and 3111 respectively, while it is 372, 106, 351, 352, 402, and 114 for Ventana, Cam San Miguel, 13G97, Hood and Independence respectively, which were perceived as having lower fruity notes by the panelists.

The caramel note in strawberries is contributed mainly by mesifurane and furaneol. Totem, Puget Reliance, Puget Summer and Venice have higher caramel notes. Mesifurane was found in 8 cultivars and furaneol in just five cultivars. The total OAV of mesifurane and furaneol were 3000, 3000, 1333 and 39666 for Totem, Puget Reliance, Puget Summer and Venice respectively while Ventana, Camarosa, San Miguel, 13G97, Hood and Independence have 0, 1333, 333, 2666, 0 and 12,666 respectively. The discrepancy in the cultivars Independence and 13G97 could be explained by aroma interaction. But it has to be kept in mind that recovery of furaneol was low for the SPME method, and correlation of this attribute is only possible when a more accurate method is deployed to quantify the actual concentration of furaneol.

In general, hexanal, trans-2-hexenal, cis-3-hexen-1-ol, octanal, nonanal, trans-2-otenal are responsible for the green notes in strawberry. Ventana, Camarosa, 13G97, San Miguel, Hood and Independence have higher amounts of green notes. But the OAV's do not convey the same information. The sensory impact of some compounds may be masked by other compounds (*35*), or enhanced by other compounds (*36*). The OAV's of the fruity notes are significantly higher than the green notes. The OAV's of all the fruity notes put together range from 100- 3000, while the combined green notes range from 5-20. Hence it is possible that the very strong fruity notes are able to suppress the green notes.

Musty and sulfur notes could not be correlated with any of the compounds identified in strawberry. All the samples tended to lose the musty note in 30-45 minutes after the samples were blended. Many sulfur compounds like hydrogen sulphide, methanethiol, dimethyl sulfide, dimethyl disulfide, methyl thioacetate and methyl thiobutanoate have been identified in strawberry (*6, 13*) using different extraction techniques and a sulfur specific detector. Sulfur compounds were not quantified in this study, and there was no other compounds identified to be responsible for the perceived sulfur odor by the panelists.

Conclusion

The present study provides a better understanding of the aroma profiles of strawberries grown in California and Oregon. This data can be used in the breeding programs to develop varieties that have a desirable aroma in addition to other quality parameters. Quantification of volatiles using multiple internal standards and calibration curves provides reliable and accurate information about the aroma compounds in strawberries. Correlation of the sensory data with the instrumental data gives an accurate interpretation of the importance of individual compounds in the overall aroma of strawberry. Methyl butanoate, ethyl acetate, ethyl butanoate, ethyl hexanoate and isoamyl acetate contribute to the fruity notes in strawberry while mesifurane and furaneol contribute to the caramel note. The stronger fruity notes in some of the cultivars may mask the green notes due to their higher odor activity values. Even though sulfur compounds are present in minor proportions, they impart significant aroma character to the fruits. Quantification of the sulfur compounds and relating it to the sensory data could help explain the sulfur notes found in the varieties under study.

Acknowledgements

The authors would like to thank Astrid Kurniana for assisting in conducting the sensory tasting and statistical analyses and all the dedicated panelists for their contribution in the sensory evaluation. The authors also thank for the financial support provided by Oregon Strawberry Commission.

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Chapter 3.
Quality Evaluation Of Some New Promising Strawberry Varieties and Comparison With Major Cultivars Grown In California and Oregon

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ABSTRACT

The chemical composition of ten strawberry cultivars five developed in California and five developed in the pacific northwest (U.S.A) was determined to evaluate the quality. The sugar, organic acid and ascorbic acid composition was investigated using HPLC. Anthoycanin content was determined by pH differential method. The color properties were studied by measuring tristimulus measurements. A trained sensory panel evaluated the taste of the strawberries. The average fructose, glucose, sucrose and the total sugar content in the ten cultivars were 2.24, 1.96, 0.92 and 5.13 g/100 g respectively. Total acid content ranged from 0.93 g/100 g to 2.01 g/100 g. 'Puget Summer' had the highest total sugars and Independence had the highest acids and ascorbic acid content. The anthocyanin content varied from 7.10 mg/100 g fruit to 30.52 mg/100 g fruit. To account for the difference in sweetness among the individual sugars, a sweetness index was calculated. The sensory evaluation of the strawberries in terms of sweetness and sourness could be partially explained by sugar/acid ratio. A consumer preference test for Totem, Puget Reliance, Camarosa and Ventana showed that the strawberries differed in overall aroma and strawberry aroma.

(Key words: strawberry, SPME, sugars, acid, ascorbic acid, anthocyanins, color)

INTRODUCTION

Strawberries (Fragaria X ananassa) are cultivated in nearly all countries of the world and are one of the most popular fruits that are consumed as fresh or conserved (*1*). Besides its attractive color and taste, strawberry is also a good source of carbohydrates, vitamin C, and antioxidant compounds, such as anthoycyanins (*2*).

An understanding of the qualitative and quantitative distribution of the characteristic sugars and organic acids in fruits is important in evaluating fruit quality (*3*). Glucose, fructose, and sucrose are the major sugars present in strawberry. Glucose and fructose are predominant over sucrose, and the total sugar content can change during the growing period (*4, 5*). Research conducted with commercial strawberries detected variations in total sugar content due to stage of ripeness, production year, and cultivar (*6-9*). Like sugars, organic acids are important for flavor, and the sugar/acid ratio is calculated to determine the optimum time for strawberry harvesting, because it is considered an index of quality (*10*).

Ascorbic acid (Vitamin C) is considered very important due to its nutritional implications and is included among the quality parameters evaluated for fruits and fruit products (*11-13*). The content of ascorbic acid in fruits and vegetables depends on various factors such as genotypic differences, preharvest climatic conditions, and postharvest handling procedures (*14*). Unlike other organic acids and sugars, ascorbic acid is highly unstable, due to the activity of ascorbic acid oxidase and photo-oxidation (*15, 16*) and thus it is taken as an indicator of the freshness of the fruit.

Anthocyanins exist widely in fruits, flowers, and vegetables and are responsible for their bright colors such as orange, red and blue (*17*). Recent and renewed interest in

anthocyanins is not only due to their use as natural colorants (*18, 19*) but also their potential health benefits as antioxidants and anti-inflammatory agents (*20-23*). The amount of anthocyanins present in the fruit is important for the maturity evaluation of strawberries. The index of maturity used for harvesting is the red color from anthocyanin synthesis on half to three-fourths of the fruit (*24*). The main anthocyanin found in strawberries is pelargonidin 3-glucoside, with cyanidin 3-glucoside and pelargonidin 3-rutinoside present as minor constituents (*25*).

Color is used by horticulturists as a major criterion for determining quality of fruit. Visual expression of color is a cultivar characteristic affected by climate and environment.

This study aims at mapping the quality profile of some promising new varieties and compare with Ventana and Camarosa from California and Totem from Oregon. It includes qualitative and quantitative measurements of sugars, organic acids, ascorbic acid, anthoycanins, and color measurement.

MATERIALS AND METHODS

Strawberries

'Ventana', 'Camarosa', '13G97', 'San Miguel' and 'Venice' varieties were procured from Driscoll Company, California. 'Totem', 'Hood', 'Puget Reliance', 'Puget Summer' and 'Independence' were obtained Norpac Foods (Stayton, OR) and Townsend Farms (Fairview, OR). All the berries were collected in summer 2004, individually quick frozen at -34°F and stored at -10°F. All the analytical measurements and sensory evaluation were performed in triplicates within nine months.

Chemicals

Glucose, methanol and acetonitrile were obtained from EMD chemicals (Gibbstown, NJ). Fructose, Sucrose and ascorbic acid were obtained from J.T Baker (Phillipsburg, NJ). Citric acid and malic acid were obtained from Fisher Chemical Company (Fairlawn, NJ)

Sugar Analysis

Fruit samples (200 g each) were slightly thawed at room temperature for 90 min and blended to a fine puree. Fifty mL of boiling water was added to 100 g puree and mixed well with a stirring rod. The puree was immediately heated in a boiling water bath for 5 min 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 the supernatant filtered and injected onto the Shimadzu HPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a RID 6A refractive index detector, Restek ultra-amino column (3μm, 200 mm x 4.6 mm, Bellefonte, PA) and a Shimadzu CR 510 Chromo Pac integrator. The column temperature was maintained at 30 ^{0}C and 20 μL of sample was injected. Acetonitrile: water (80:20 v/v) was used as the mobile phase at a constant flow rate of 1.2 mL/min. 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 (*26-29*), 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

C_{18} Sep-Pak Cartridges (Waters Corporation Milford, MA) were conditioned with 10 mL each of methanol, water and 50% acetonitrile. Ten mL of air was passed through a conditioned C_{18} 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 C_{18} 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 (HP X.87H, 300 mm x 7.8 mm, Richmond, CA) was operated at 35°C. 20 μL 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

200 g 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 min. 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. Total anthcyanin content was determined by pH differential method [45]. 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 700 nm (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/100 gm fruit) = (A * MW * DF * 1000)/ (ϵ * 1)

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

Ascorbic acid

Strawberries (70-100 g) were blended with 0.05 N H_3PO_4 for 2 min. The puree was centrifuged at 5000 rpm for 15 min. 5 g of the supernatant was mixed with 45 g of 0.05N H_3PO_4. The extract was purified by passing 5 mL extract through a C_{18} Sep-Pak

Cartridge preconditioned with 10 mL each of methanol and water. Ten 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 (150 X 3.6 mm, 3 μm, J&W Scientific) was maintained at 30°C. 2% KH_2PO_4, adjusted to pH 2.3 with H_3PO_4, 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. Ten μL of sample solution was injected and analyzed for 10 min. 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.

Color measurement

200 g of strawberry was thawed and blended to a puree. The color properties (CIEL*a*b*) were measured using a Hunter lab color quest colorimeter (Hunter Associates Laboratory Inc., Reston, Virginia) in 45/0 sphere mode.

Statistical Analysis

Analysis of variance and principal component analyses among the cultivars for sensory analyses was done using SPSS statistical package (SPSS, Chicago, IL). Statistical significance was accepted if the P value was less than or equal to 0.05.

Sensory Analysis

Descriptive Analysis

Ten experienced panelists, five men and five women between the ages of 21 and 43 years were chosen for the sensory study. The panelists rated the samples in terms of the degree of sweetness and sourness. The panelists were trained to rate the intensity on a 1-16 scale. The standards and the samples (30ml puree) were provided in an 8-ounce wine glass and the glass was covered with a plastic (non odorous) lid (Solo Cup co, Urbana, IL). Testing took place in individual testing booths under red lighting to mask the color differences among the samples. A randomized complete block design was used where each panelist received each sample 3 times (3 replications). Samples were coded with 3-digit random numbers.

Consumer Test

Seventy-nine consumers were recruited from the Corvallis community. Prospective consumers were screened based on if they ate fresh or frozen strawberries. Consumers were asked to answer a demographic questionnaire at the end of testing. The following questions were asked: gender, age, out-of-season purchasing, association of red color with quality, ranking of strawberry attributes, and importance of berry size.

Preparation of strawberry samples for consumer test

Samples were thawed at room temperature for 90 min and then blended to a fine puree in a Waring™ commercial blender. 500 g of berries were blended at a time for 30 seconds in the "on" position (no speed settings). 1000 g of puree was required for 36 panelists for each two hour session. Total consumer testing time was 4 and 1/2 hours

and new samples were prepared every two hours. Pureed samples were poured into a 2 L beaker and covered with aluminum foil until used for sensory testing. Sample temperatures at serving ranged from 6°C - 12°C (42.8°F - 53.6°F). Samples were served in the same way as in descriptive analysis. within 2 hr after pureeing. Six consumers were seated in the individual booth testing areas under red lighting. Each testing sessions lasted 20 min. Samples were presented in monadic order. Consumers were instructed to drink spring water to cleanse their palates between samples.

Sensory Design and Statistical Analysis

Acceptance testing was used to determine how much each sample is liked based on a 9-point hedonic scale for a set of attributes: Overall Aroma Liking, Overall Liking (includes aroma and flavor), Fresh Strawberry Flavor, Sweetness, and Sourness where 9=like extremely and 1=dislike extremely. For Overall Liking, consumers were offered open-comment sections on the ballot where they could comment on what they liked and disliked about the sample. Just about right testing was used to determine how sweet consumers thought the strawberry purees were based on a 5-point JAR scale where 5=much too sweet, 4=somewhat too sweet, 3=just about sweetness, 2=not quite sweet enough, and 1=not nearly sweet enough. For Sweetness intensity, a 9-point intensity scale was used where 9=extremely strong and 1=none.

A balanced complete block design was used to provide a minimum of 79 observations on each sample. Each consumer received a total of four samples, served in monadic order. Serving order was balanced across testing sessions so that each sample

was seen in each serving position (first, second, third, and fourth) an approximately equal number of times.

Analysis of variance was conducted on the strawberry puree ratings for Overall Aroma Liking, Fresh Strawberry Aroma Strength, Overall Liking, Flavor liking, Fresh Strawberry Flavor Liking, Sweetness "Just about Right" rating, Sweetness Liking, and Sourness Liking (Compusense 5.0®, version 4.6, Guleph, Canada). The ANOVA model comprised two main effects: consumer and sample. Consumer was treated as a random effect and sample was treated as a fixed effect. Significant differences detected by ANOVA were subjected to post-hoc Tukey HSD multiple comparison to test of sample means at the 95% confidence interval ($P \leq 0.05$).

RESULTS AND DISCUSSION

Sugars and organic acids

Fructose, glucose and sucrose were the major soluble sugars found in the cultivars under study. The fructose content reported in literature (*30, 31*) varied from 1.3 g/100 g to 3.05 g/100 g, while glucose, sucrose and total sugar content ranged from 0.72-2.60 g/100 g,0.61-2.14 g/100 g and 2.66-6.93 g/100 g respectively. The average fructose, glucose, sucrose and the total sugar content in the ten genotypes are 2.24, 1.96, 0.92 and 5.13 g/100 g respectively (Table 3.1).

The total sugar content varied from 3.78 g/100 g fruit (13G97) to 6.50 g/100 g fruit (Puget Summer) with an average of 5.13 g/100 g fruit. Puget Summer, 'Puget Reliance', 'San Miguel', 'Hood' and 'Totem' had the higher total sugar content than the

average among the genotypes studied, while '13G97', 'Camarosa', 'Independence', 'Venice' and 'Ventana' had lower than average total sugar content.

The fructose content ranged from 1.88 g/100 g (Ventana) to 2.92 g/100 g (Puget Summer) berry. The average fructose content in all the genotype was 2.24 g/100 g fruit, while it was 1.93 g/100 g in the genotypes from California and 2.66 g/100 g in varieties from Oregon.

Glucose content varied from 1.36 g/100 g (13G97) to 2.66 g/100 g fruit ('Puget Reliance') with an average of 1.96 g/100 g fruit among all the varieties studied. 'Totem', 'Hood', 'Puget Reliance' and 'Puget Summer' had a higher average fructose and glucose content while the genotypes 'Ventana', 'Camarosa', 'Venice', '13G97', 'San Miguel' and 'Independence' had lower average fructose and glucose content among the varieties studied. California strawberries had an average glucose content of 1.69 g/100 g while the north-west berries had 2.33 g/100 g. The sucrose content varied considerably among the genotpyes studied with an average sucrose content of 0.91 g /100 g fruit. 'Ventana', 'San Miguel', 'Independence' and 'Puget Summer' had higher than average sucrose content. 'Ventana' had the highest sucrose content at 1.43 g/100 g berries while Totem had the lowest at 0.53 g/100 g berry. California cultivars had an average sucrose content of 1.02 g/100 g while north-west cultivars had 0.77 g/100 g.

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. The sweetness index varied from 5.79 (13G97) to 9.93 (Puget Summer) with an average sweetness index of 7.82. Pacific north-

west cultivars had a higher sweetness index than California cultivars. Citric acid and malic acid were the major organic acids present (Table 3.1) in the varieties under study. The total organic acid content varied from 0.93 g/100 g (Independence) to 2.01 gm/100 gm (Venice) with an average of 1.29 g/100 g fruit. Independence had the highest citric acid and (1.41 g/100 g) malic acid content (0.6 g/100 g) while Venice had the lowest (0.6, 0.33 g/ 100 g) amounts of the same.

The sugar-acid ratio (Table 3.1) varied from 3.25 to 5.28. In the sensory study the cultivars 'Totem', 'Puget Reliance', 'Puget Summer', 'Hood' and 'Independence' were rated as being sourer and less sweet than 'Camarosa', 'Ventana', '13G97', 'Venice' and 'San Miguel'. This can be partially explained by the sugar/acid ratio. The sensory panelist's conclusion is in agreement with the sugar/acid ratio of seven of the ten cultivars. Camarosa, 13G97 were rated as sweeter even though they have a lower sugar/acid ratio (3.79,3.25 respectively) while Puget Reliance was rated sour in spite of a higher sugar acid ratio (5.05). It could be due to the reason that Puget Reliance has higher citric acid content than Camarosa and 13G97. In general, citric acid is more acidic than malic acid. . However it has to be noted that taste is a complex phenomenon, which depends on sugars, acids, volatiles, food matrix, and it may not be possible to establish a simple relationship between taste perception and sugar/acid ratio.

Anthocyanins

Anthocyanin contents in strawberries have been reported to vary from 15 mg/100 g of fruit to 35 mg/100 g [45] of fruit. Among the genotypes studied the anthocyanin content varied from 7.1 mg/100 g fruit to 30.52mg/100 g (Table 3.2). The

Table 3.1: Sugar and Organic acids content among ten strawberry genotypes.

	g/100 g fruit (%)									
Cultivar	Fructose	Glucose	Sucrose	Total sugars	Sweetness Index	Citric Acid*	Malic Acid*	Total Acid content*	Sugar/Acid Ratio	Ascorbic Acid*
Ventana	1.89±0.20	1.74±0.04	1.43±0.57	5.07	7.40	0.64	0.39	1.03	4.93	0.62
Camarosa	1.79±0.03	1.51±0.03	0.70±0.08	4.01	6.29	0.71	0.34	1.06	3.80	0.48
Venice	2.23±0.09	1.93±0.13	0.74±0.07	4.91	7.57	0.60	0.33	0.93	5.27	0.57
13G97	1.63±0.02	1.36±0.06	0.78±0.04	3.78	5.79	0.78	0.38	1.16	3.25	0.71
San Miguel	2.11±0.00	1.89±0.13	1.44±0.11	5.45	8.19	0.72	0.44	1.16	4.69	0.72
Totem	2.52±0.05	2.13±0.08	0.53±0.11	5.19	7.99	0.98	0.34	1.32	3.94	0.68
Hood	2.38±0.04	2.17±0.09	0.71±0.10	5.28	8.20	0.93	0.40	1.34	3.95	0.35
Independence	2.10±0.03	1.74±0.02	0.93±0.08	4.78	7.41	1.41	0.60	2.01	2.38	1
Puget Reliance	2.87±0.17	2.66±0.28	0.83±0.03	6.36	9.48	0.87	0.39	1.26	5.04	0.56
Puget Summer	2.92±0.09	2.51±0.04	1.06±0.09	6.59	9.93	1.18	0.50	1.68	3.87	0.59

*-Standard deviation less than 1/100.

Table 3.2: Anthocyanin content in mg/100 gm and the CIEL*a*b* values.

	Anthocyanin Content	Color Measurement				
Genotype	mg/100 g fruit	L*	a*	b*	Hue	Chroma
Ventana	15.80 ± 1.86	33.78	38.46	21.85	29.60	44.23
Camarosa	15.22 ± 1.36	34.62	37.48	20.52	28.70	42.72
Venice	7.10 ± 1.10	46.82	25.03	12.14	25.68	27.81
13G97	11.10 ± 0.05	42.11	36.44	17.6	25.80	40.46
San Miguel	12.20 ± 0.70	40.01	37.38	18.05	25.77	41.50
Totem	30.52 ± 1.13	29.1	35.73	19.69	28.85	40.79
Hood	19.24 ± 2.06	30.24	38.29	22.8	30.71	44.56
Independence	23.99 ± 2.27	35.15	41.3	25.3	31.49	48.42
Puget Reliance	20.90 ± 0.90	33.93	39.67	25.65	32.88	47.24
Puget Summer	24.83 ± 3.06	30.66	41.01	26.55	32.91	48.85

average anthocyanin content was 18.09 mg/100 g fruit. Totem had the highest total anthocyanin content (30.52 mg/100 g fruit) and Venice had the lowest anthocyanin content (7.1 mg/100 g fruit). The average anthocyanin content in north-west cultivars was 25.04 mg/100 g while it was 12.28 mg/100 g fruit for the varieties from California.

Ascorbic acid

Strawberries contain an average ascorbic content of 0.60 g/100 g fruit (*30*). The average ascorbic acid content in the genotypes studied was 0.62 g/100 g fruit (Table 3.1). 'Independence' had 1g/100 g of ascorbic acid while Camarosa had less than half of it at 0.48 g/100 g fruit. It has to be noted that, Independence which has the highest amount of ascorbic acid also has the highest amount of total organic acids (2.01 g/100 g) as well.

Color Measurement

A higher L* indicates a lighter sample and lower L* indicates darker sample. Similarly a higher a* indicates a more red sample and a* indicates the amount of green color in the sample, while higher b* indicates yellowness and a lower b* indicates blueness. Strawberries are known for their attractive red color and a shining surface texture. L* and a* are important in the color evaluation of strawberries.

The average L* and a* among the varieties studied are 35.64 and 37.07 respectively. Venice, San Miguel and 13G97 had higher than average L* while the remaining varieties had less than average L* values. 'Ventana', 'Camarosa', 'Hood', 'Independence', 'Puget Reliance' and 'Puget Summer' had higher than average a* values while 'Venice', 'San Miguel', '13G97' and 'Totem' had lower than average a* values. The cultivars from California differed in L* value (Table 3.2) from the north-west cultivars while there was

no difference with regards to a* values. Pacific north-west cultivars have an average a* value (redness) of 38.68 while California genotypes have a* value of 34.95. California cultivars are lighter in color with an average of 39.46 while Oregon varieties are darker with an average value of 31.81. Anthocyanins are responsible for the color of strawberries. Oregon strawberries have a higher anthocyanin content which is responsible for a lower L* value, while California varieties because of the lower anthocyanin content have a higher L* value.

Sensory Results

Trained Panel Results

A principal component plot was built to understanding the profile of the genotypes student. Venice, 13G97, San Miguel, Ventana, and Camarosa were perceived as being more sweeter while Puget Reliance, Totem, Puget Summer, Independence and Hood were rated to be more sourer among the varieties investigated.

Consumer test

Overall aroma liking ($P < 0.001$) and fresh strawberry aroma intensity ($P < 0.001$) had significant sample effects. Consumers did not find significant differences for the remaining flavor attributes of overall liking (includes aroma and flavor), overall flavor liking, fresh strawberry flavor liking, sweetness liking, sweetness "Just about Right" rating, and Sourness Liking ($P > 0.05$) (Table 3.3).

Consumers liked the aroma of Totem and Puget Reliance significantly more than they liked the aroma of Camarosa and Ventana. In addition, consumers rated the strength of fresh strawberry aroma higher in the Totem and Puget Reliance compared to Ventana

and Camarosa (Table 3.3). For Overall Aroma Liking ($P < 0.001$), consumers preferred the overall aroma of Totem and Puget Reliance. significantly more than Camarosa and Ventana (Table 3.3). For Fresh Strawberry Aroma intensity ($P < 0.001$), consumers rated fresh strawberry aroma intensities in Totem and Puget Reliance significantly higher than Camarosa (Table 3.3). Totem was rated significantly higher in fresh strawberry aroma (Table 3.3).

Table 3.3 Strawberry Puree Sample Means[ab], Standard Deviations, and Significance (Tukey's HSD $P \leq 0.05$ level)

Attribute	*p value*	*Totem*	*Puget Reliance*	*Camarosa*	*Ventana*
Overall aroma* **	0.000	$6.4^{a} \pm 2.0$	$6.1^{a} \pm 2.0$	$4.9^{b} \pm 2.2$	$5.0^{b} \pm 2.2$
Fresh strawberry aroma* **	0.000	$5.3^{a} \pm 2.1$	$4.9^{ab} \pm 2.1$	$4.0^{c} \pm 2.3$	$4.2^{bc} \pm 2.0$
Overall liking[ns]	0.405	5.2 ± 1.9	5.0 ± 2.2	4.7 ± 2.1	4.9 ± 1.9
Overall flavor[ns]	0.531	5.2 ± 1.9	4.9 ± 2.3	4.8 ± 2.1	4.8 ± 2.1
Fresh strawberry flavor[ns]	0.369	5.2 ± 2.0	5.1 ± 2.3	4.8 ± 2.2	4.8 ± 2.1
Sweetness liking[ns]	0.287	4.6 ± 2.0	4.4 ± 2.2	4.9 ± 2.0	4.4 ± 1.9
Sweetness rating[ns]	0.897	2.1 ± 0.9	2.0 ± 0.8	2.0 ± 0.7	2.1 ± 0.8
Sourness liking[ns]	0.322	4.9 ± 1.7	4.5 ± 1.9	4.8 ± 1.8	4.9 ± 1.7

For the Just-About-Right **Sweetness rating** the scale is 5=much too sweet, 4=somewhat too sweet, 3=just about right, 2=not quite sweet enough and 1=not nearly sweet enough.

For **Fresh Strawberry Aroma** Intensity the scale is 9=extremely strong, 7=very strong, 5=moderately strong, 3=slightly strong, 0=none

For the remaining **LIKING** questions the scale is 9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, 3=dislike extremely.

***Attributes are significant at $P \leq 0.001$.

[a,b,c]Attribute means with different superscript letters were rated significantly different from one another (Tukey's HSD $P \leq 0.05$ level). [ns]Attributes are not significant at $P > 0.05$.

than Camarosa and Ventana. Puget Reliance was rated higher than Camarosa in the fresh strawberry aroma but it was not rated significantly different from Ventana or Totem Consumers did not show a significant flavor preference among the four strawberries for overall liking (includes aroma and flavor), flavor liking, fresh strawberry flavor liking, sweetness liking, sweetness "Just About Right" rating, and Sourness Liking ($P \geq 0.05$) (Table 3.3). Mean consumer ratings for overall liking, overall flavor liking, and fresh strawberry flavor liking were similar across these attributes and across samples. These flavor attribute means ranged from 4.8 to 5.1. A five on the scale is "neither like nor dislike" ("neutral middle"). For "Just About Right" sweetness, consumers generally thought all four samples were not sweet enough with "just about right" ratings equaling "2" or "not quite sweet enough". Correspondingly the "Sweetness Liking" mean ratings ranged from 4.4 to 4.9 (dislike slightly to neither like nor dislike).

The most useful information is gained by examining the percentages of responses for each scale point (Table 3.4). Analysis of the just-about-right scale can pose some problems. For example, statistically comparing the means across puree samples for sweetness if the means are based on skewed ratings. If one sample had the mean based

Table 3.4. Percentage cross tabulation for Sweetness Just About Right Question (n=79)

Sample	*5 [Much too sweet]*	*4 [Somewhat too sweet]*	*3 [Just about right in sweetness]*	*2 [Not Quite sweet enough]*	*1 [Not nearly sweet enough]*	*Total*
Totem	1	23	25	23	28	100
Camarosa			24	53	23	100
Ventana	1		25	53	20	100
Puget Reliance		4	23	47	27	100

on a large spread of ratings over the entire scale whereas another sample had the same mean but this mean was based on a tight grouping of ratings across only one end of

the scale. It is possible to miss some important information by only looking at the means. For example, there might be two segments of consumers: one group that prefers a stronger level of the attribute and another group that prefers less. Examining only the mean from such opinions could lead to a false impression that the product was at or near optimum level. However this is not the case with the four strawberry puree samples: For sweetness "just about right" frequencies, the majority of consumers scored on the lower end of the scale across all samples (1=not nearly sweet enough to 3=just about right). Therefore the distribution of ratings on the scale for all samples is primarily with the 1 to 3 scale-range and that indicates that the consumer groups agreed that the samples were on the "not sweet enough" side.

Conclusion

The strawberries from California and Oregon differed in many of the quality parameters under study. The strawberries from California had more sucrose content while the cultivars from Oregon had higher fructose, glucose, citric acid, total acid and anthocyanin content. Consumer test conducted on four genotypes showed that the consumer preference can be explained partially by the sugar/acid ratio. A combination of aroma studies and all other quality parameters like sugars, acids and color might be able to predict the consumer preference of the strawberry varieties studied.

Acknowledgements

The authors would like to thank Astrid Kurniana and Cindy Lederer for assisting in conducting the sensory tasting and statistical analyses and all the dedicated panelists for their contribution in the sensory evaluation. The authors also thank the Oregon Strawberry Commission for providing the necessary financial assistance.

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CONCLUSION

Solid phase micro extraction technique of volatile extraction was highly reproducible in addition to being simple and fast. Standard curves built in a synthetic matrix provide more accurate quantitative results. The varieties Totem, Puget Reliance, Puget Summer and Venice have higher amounts of fruity aroma. This is due to the presence of higher concentration of ethyl acetate, ethyl butyrate, isoamyl acetate, ethyl hexanoate and methyl butyrate. These varieties have higher amounts of caramel note, which is mainly contributed by mesifurane and furaneol. Furaneol which is considered as an important character impact compound has been found in only five of the ten varieties studied. Ventana, Camarosa, 13G97, San Miguel, Hood and Independence have higher amounts of green notes even though hexanal, trans-2-hexenal, cis-3-hexen-1-ol, and cis-3-hexenyl acetate which contribute to the green notes are at lower concentrations. Ethyl butyrate is present in considerably higher amounts in the other varieties which could be masking the green notes in them, while is not so here.

The sugar and acids content varied among the varieties studied. Oregon varieties had higher fructose, glucose, total sugars, citric acid and malic acid while California strawberries had higher sucrose content. There was no difference in the ascorbic acid content among the California and Oregon strawberries. Puget Summer had the highest total sugar content while 13G97 had the lowest. Independence had the highest. The sweetness index calculated gives a better understanding of the perceived sweetness by the consumer.

Anthoycanin content in California strawberries differed significantly from the anthocyanin content of Oregon strawberries. 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. There was no significant difference between the California and Oregon strawberries in the ascorbic acid content. The average ascorbic acid content of California and Oregon Strawberries are 0.62 gm/100gm and 0.63gm/100gm. California varieties are darker in color with an average o L^* 39.46 while Oregon varieties are lighter with an average L^* value of 31.81.

This study provides an in-depth understanding of the profile of diverse strawberry cultivars grown in California and Oregon. It also provides the consumers more information about the choices they have to aid in their buying decision. It guides the processors in choosing the right varieties for their various needs. Further it also provides a complete picture to the breeders about the quality of the strawberries from both the regions. This information can be used by them to breed better varieties by incorporating the desirable characters from the studied varieties.

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