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Title: Changes in Fruit Flavor Intensity in Sucrose Based Model Systems: Effects of pH Level, Acid Type, and Sourness

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Abstract approved ______________________
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Acidulants are an important functional ingredient group in the food industry, providing sourness, lowering pH, ensuring safe processing, and extending shelf life. Hence, understanding the effect of acids on sensory characteristics of foods is critical. Of the many topics dealing with acidulants, sourness is a topic where considerable research has been done. To a lesser extent researchers have examined how acidulants, and sourness generated from the hydrogen ion or the acid anion, effect the perception of fruit flavor. A series of experiments was conducted to determine how selected acid types, pH, and sourness effect the perception of selected fruit flavors in model systems.

First, selected organic acids (citric, lactic, malic) were evaluated on an equivalent weight (0.4%w/v) at 5 fixed pH levels (2.6, 3.0, 3.4, 3.8, and 4.2), in a solution of water, sugar (10% w/v), and fruit flavor (lemon, and strawberry) to determine if acid type and pH level effect fruit flavor perception. Generalized

descriptive analysis was used to characterize the differences between the treatments for the descriptors of fruit flavor, sweetness, sourness, and astringency. The pH influenced fruit flavor intensity more than acid. Lemon flavor increased with decreasing pH, while strawberry flavor decreased with increasing pH. These findings suggest that pH effects the perception of fruit flavor differently depending on the system. The results described for the lemon and strawberry flavored experiments are part of a larger study involving phosphoric acid and blends of citric, lactic, and malic acid–each paired in equal proportions–in apple, forest berry, orange and tropical flavored systems.

In the second, three equi-sourness levels of selected acids (citric, lactic, malic) were generated at pH 3.0, in a solution of water, sugar (10% w/v), and selected fruit flavor (lemon, strawberry) to determine if sourness from selected acidulants effect fruit flavor perception differently. Increasing sourness intensity increased lemon and strawberry flavor. In the strawberry experiment malic acid provided more strawberry flavor than citric and lactic acid at the low sourness level only. These findings suggest that sourness effects flavor more than acid type.

Changes in Fruit Flavor Intensity in Sucrose Based Model Systems: Effects of pH Level, Acid Type, and Sourness

By
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John D. Cowden, Author

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CONTRIBUTION OF AUTHORS

Dr. Alix Gitelman contributed with the analysis and interpretation of data for chapter three.

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1. INTRODUCTION

Acidulants are an important functional ingredient group in the food industry. Acidulants contribute a considerable variety of properties to food products. Not only do acids provide sourness to food, they also decrease pH, helping to ensure safe processing, and extending shelf life. Additionally acidulants help ensure food quality, help prevent non-enzymatic browning, act as synergists to antioxidants, and can enhance flavor in foods.

Acids are widespread in food products occurring naturally in the cells of plants and animals. Acids are found in fermented food products such as pickles, cheese, and wine. Additionally acids are used in formulated products such as salad dressings, marinades, meat processing, beverages, and candies. Because of the importance of acids, understanding their sensory characteristics is critical.

Of the many topics dealing with acidulants, sourness is an area where considerable research has been done. Several researchers have shown that at equal weight, or equal molar concentrations acids are not equally sour (Noble and others 1986; Straub 1989; Watine 1995; Berry 2001). Research has also shown that sourness varies with pH (Richards 1898) and with increasing acid concentration (Harvey 1920). Additionally research has shown that acids can differ in non-sour components such as saltiness and bitterness (Settle and others

1986; Rubico and McDaniel 1992; Hartwig and McDaniel 1995). These studies examined the differences between acidulants in a simple water and acid system.

In more complex model fruit flavored beverage systems, increasing sourness from citric acid enhanced the perception of citrus and overall flavor (McBride and Johnson 1987; Bonnans and Noble 1993; Stampanoni 1993; Hartwig 1994).

The present study was conducted to evaluate how acid type, pH, and sourness, effect the perception of fruit flavor. Previous studies were conducted at room temperature; these studies were conducted at 4°C in order to more closely represent real differences in cold in beverages. To examine these effects the research was divided into two studies as follows:

Study 1

- Acid type and pH were examined in a model solution of water, sugar, and a fruit flavoring to determine if particular acids and pH levels effect fruit flavor perception.

Study 2

- Acid type and sourness level were examined at fixed pH in a model solution of water, sugar, and a fruit flavoring to determine if acid type and sourness effects fruit flavor perception.

2. LITERATURE REVIEW

2.1. THE ACIDS

Table 2.1 summarizes several physical and chemical properties, and chemical structures of the acidulants used.

2.1.1. Citric Acid

Citric acid is widely distributed in nature in both plants and animals as an important part of the Krebs Cycle, and is generally thought to be an important fruit acid. In fact, citric acid (2-hydroxy-1,2,3-propane tricarboxylic acid) derives its name from the Latin citrus, the citron tree, a fruit that resembles a lemon, and was first isolated from lemon juice in 1784 by Carl Scheele (Mattey and Kristiansen 1999). In addition to being the major acid in lemons and limes (4-8%), it is also found in grapefruit (1.2-2.1%), oranges (0.6-0.8%), Black Currants (1.5-3%), strawberries (0.6-0.8%), gooseberries (1%), raspberries (1.0-1.3%), apple (0.008%), pineapple, tamarind, potatoes (0.3-0.5%), tomatoes (0.05-1.1%), peas and eggplant (Berry 2001). Citric acid was first produced commercially from lemons and later pineapple waste (Gardener 1972; Berry 2001). Though citric acid is still obtained from lemons and pineapple waste, more efficient and economical methods have been developed.

Table 2.1 -- Chemical Properties of Acidulants

Acid	Empirical Formula	Chemical Structure	Molecular Weight	Equivalent Weight	pK_a*
Citric	$C_6H_8O_7$	CH_2-COOH | HO-C-COOH | CH_2-COOH	192.12	64.04	3.14 [a] 4.77 [a] 6.39 [a]
Lactic	$C_3H_6O_3$	H | CH3-C-COOH | OH	90.08	90.08	3.08 [a]
Malic	$C_4H_6O_5$	H | HO-C-COOH | H-C-COOH | H	134.09	67.05	3.40 [a] 5.11 [a]
Phosphoric	H_3PO_4	OH | OH-P=O | OH	98.00	49.00	2.14 [b] 6.86 [b] 12.4 [b]

[a](Blocher and Busta 1983); [b](Mathews and Van Holde 1995)

In 1919 an industrial process to produce citric acid using *Aspergillus niger* was developed and is still widely used today (Mattey and Kristiansen 1999; Wolschek and Kubicek 1999; Berry 2001). Though several methods of citric acid production have been developed, only the surface method and the submerged process method are viable (Mattey and Kristiansen 1999). There are several substrates used in the production of citric acid, including refined or raw sucrose, syrups, starch, hydrol, alkanes, oils and fats, cellulose, and molasses (Lesniak 1999). Despite the variety of available substrates, most methods generally use molasses as the preferred source of sugar because of its availability and cost. In the 1960's and 1970's methods of citric acid production were developed employing *Candida spp.* yeast using both carbohydrates and *n*-alkanes as substrate; these methods are gaining in popularity (Mattey and Kristiansen 1999). No matter which technique is used to produce citric acid, the basic objective is to cultivate the microorganism under unfavorable conditions so that the Krebs Cycle is disrupted allowing citric acid to be accumulated instead of being metabolized (Arnold 1975).

Citric acid is a very important and widely used acidulant in the food industry, partly because of its cost and solubility. Citric acid is considered to be one of the most versatile acidulants, and can perform a wide range of functions in foods; it is often considered the standard for comparison to other acids in food products (Sanders 1966; Gardener 1972; Arnold 1975). It is classified as GRAS as a multipurpose food additive and sequestrant, and no limit exists on the acceptable daily intake for humans (Food and Drug Administration 2001a). Citric acid is used in the canning of vegetables to lower pH and inhibit off flavors (Gardener 1972). Citric acid and its salts, sodium and calcium citrate, are also widely used in the dairy industry as a flavoring agent and antimicrobial agent in cottage cheese, buttermilk, and other dairy products (Gardener 1972; Dziezak 1990). In jams and jellies, citric acid is used to control pH to allow for optimum gel formation as well as contributing to flavor (Gardener 1972; Bouchard and Merrit 1978; Berry 2001). In candies and fondants citric acid is used to enhance fruit flavor, to prevent the crystallization of sugar, and to prevent oxidation of ingredients like nuts (Gardener 1972; Berry 2001).

Citric acid is also widely used in both carbonated and non-carbonated beverages. It is added to carbonated beverages to bring out the flavor and impart sourness, to act as a preservative, and to chelate trace metals that cause haze and deterioration of color and flavor (Gardener 1972; Bouchard and Merrit 1978; Berry 2001). In still or non-carbonated artificially flavored beverages and beverage powders, citric acid is one of the most popular acidulants used because it provides sourness and complements fruit and berry flavors (Gardener 1972; Berry 2001). However using citric acid in powdered formulations can be disadvantageous, as citric acid readily complexes with water; in these applications an anhydrous acid is preferred (Arnold 1975; Doores 1990).

Citric acid is a powerful sequestering agent for heavy metals giving it great value as an antioxidant synergist for inhibiting rancidity, flavor, and color deterioration in a wide range of foods containing fats and oils (Gardener 1972; Arnold 1975; Mattey and Kristiansen 1999). In addition to the food industry, citric acid is commonly used in the pharmaceutical and industrial industries (Mattey and Kristiansen 1999).

2.1.2. Lactic Acid

Lactic acid (2-hydroxypropanoic acid) occurs in small amounts in most living matter, as it is typically the end product of anaerobic carbohydrate metabolism (Arnold 1975). Scheele first identified lactic acid in milk in 1780. It was given the Latin name for milk as it is the acid formed in the souring of milk (Van Velthuijsen 1994). Lactic acid, unlike citric acid is optically active. In animal organisms it is always L-lactic, but during production of lactic acid through fermentation it occurs in both the D and L forms (Arnold 1975). Lactic acid is widely found in many foods, both naturally, or as a product of microbial fermentation (Berry 2001). Industrial manufacture of lactic acid was begun in 1883 in Boston Massachusetts.

Today, lactic acid is produced either by carbohydrate metabolism, or by chemical synthesis (Berry 2001). To produce lactic acid through fermentation, substrates of sucrose, dextrose, glucose syrups, molasses and whey are used (Van Velthuijsen 1994). Common microorganisms used in the production of lactic acid are: *Lactobacillus casei, L. helveticus, L. acidophilus, L. Delbrueckii,* and *Bacillus coagulans* (Van Velthuijsen 1994). The synthetic manufacture of lactic acid is a complex process. It is a by-product of the manufacture of acrylonitrile, acetaldehyde cyanohdrin (lactonitrile), or from propylene by ammonoxidation giving hydrogen cyanide as a byproduct (Arnold 1975). Regardless of production method used, it is purified and concentrated to an aqueous solution between 50 and 90% acid (Gardener 1972; Berry 2001). One of the unusual chemical properties of lactic acid is that it readily undergoes self-esterification. When heated in aqueous solutions containing more than 18 percent lactic acid, polylactic acids are formed (Gardener 1972). Hydrolysis of the lactic acid polymers occurs upon dilution with water (Gardener 1972).

Lactic acid is a very important acid and widely used, as well as being one of the earliest acids used in the food industry. Functions of lactic acid in food products are for flavoring, pH regulation, and preservation, however it is more commonly used for its sensory qualities (Doores 1990; Van Velthuijsen 1994; Berry 2001). Though Doores (1990) indicates that lactic acid has a mild, creamy odor with a pleasant sour taste, the odor is most likely coming from impurities in the production process and not from the acid, which is not volatile. It is classified as GRAS when used as a direct food ingredient and no limit exists on the acceptable daily intake for humans (Food and Drug Administration 2001b). Lactic acid is used in dairy products such as cottage cheese, imitation Mozzarella cheese, butter, and yogurt powders; calcium lactate is used in pudding powders and powdered milk (Gardener 1972; Van Velthuijsen 1994). Calcium lactate is also used to preserve the firmness of apple slices during processing and to inhibit the discoloration of fruits and vegetables (Gardener 1972).

Lactic acid is also used in bakery products such as hard type biscuits, cake mixes, rye bread, and artificial sourdough bread (Gardener 1972; Berry 2001). It is also used in the production of emulsifiers – calcium and sodium stearoyl lactates – which function as dough conditioners in many baked goods (Dziezak 1990). Lactic acid is also commonly used in beverages to control pH and add flavor (Gardener 1972; Dziezak 1990; Van Velthuijsen 1994; Berry 2001). Additionally lactic acid is used in the confectionary industry for high sugar sweets to eliminate the risk of sucrose inversion (Vreeman 1986; Dziezak 1990; Van Velthuijsen 1994; Berry 2001). For the packing and preserving of Spanish type olives and pickles, lactic acid is used because it ensures clarity of the brine and inhibits further fermentation (Gardener 1972; Berry 2001). Additionally, lactic acid is employed in jams, jellies, mincemeat, and mayonnaise for solubilizing pepper oleoresin (Gardener 1972). Lactic acid is also used in meat products such as salami to ensure microbial safety and provide sourness (Berry 2001).

2.1.3. Malic Acid

Malic acid (ethan-1-ol-1,2-dicarboxylic acid), like citric acid, is very widely distributed in nature, and is also an important part in the Krebs Cycle. Malic acid is a very important fruit acid; as it is also known as pomalous or apple acid (Berry 2001). Malic acid is the major acid in apples (70-95% of total acids), pears (85-90%), quince (100%), cherry (95%), plum (75-100%), apricots (25-70%), peaches (95%), grapes (40-60%), gooseberries (45%), watermelons (100%), bananas (50%), and rhubarb (35-75%) (Arnold 1975). Additionally malic acid is the second largest acid in citrus, figs, berries, beans and tomatoes (Berry 2001). Despite its occurrence in fruit, the production of malic acid from fruit juice has not been developed, nor are fermentation methods utilized in production (Arnold 1975). Instead, malic acid is commercially produced through the recovery of the equilibrium product mixture of the catalytic hydration of maleic and fumaric acids (Arnold 1975; Berry 2001). Despite the extreme corrosiveness of the reaction, and the residual maleic acid that needs to be removed, the production of malic acid is thought to be cheaper than citric (Arnold 1975). Malic acid, is different from citric acid, but similar to lactic acid in that it is optically active. In nature malic acid occurs in its L-form, however the synthesized product is a racemic mixture of D-and L-isomers (Dziezak 1990).

Malic acid is a very important acid in the food industry. Considered a relative newcomer to the food industry in 1965, it is considered to be similar to citric acid (Arnold 1975; Dziezak 1990). It is widely used as a flavor enhancer, flavoring agent, and for pH control in foods and beverages (Berry 2001). Malic acid is described as having a clean tart taste that lingers, without imparting a "burst" in flavor (Gardener 1972; Dziezak 1990; Berry 2001). It works well in powder mixtures because it comes in a powder form and is naturally anhydrous, unlike citric or lactic acid (Arnold 1975). Malic acid has a stronger apparent acidity than citric acid, which enables smaller amounts of it to be used in certain applications (10-20% by weight less than citric acid) for the same taste effect (Gardener 1972; Dziezak 1990). Malic acid is GRAS as a direct food ingredient, and no limit exists on the acceptable daily intake with the exception of baby foods (Food and Drug Administration 2001c). Malic acid is used in both liquid and powdered beverage mixes. In addition to being used as an acidifier and flavor enhancer, it is claimed that malic acid suppresses the bitter after-taste in low calorie drink formulations (Berry 2001). Additionally malic acid is used in juice beverages to help stabilize the color (Gardener 1972). Malic acid is used in many of the same applications that citric is used. Additionally it is used in candies because of its low melting point and high solubility (Gardener 1972; Berry 2001). Malic acid is also used in the production of sour dough breads and in the synthesis of emulsifying agents (Gardener 1972).

2.1.4. Phosphoric Acid

Orthophosphoric (phosphoric) acid is the simplest oxy acid of phosphorus. It is manufactured in two ways, a more common wet process, or by thermal processing. The commercial wet process consists essentially of grinding phosphate rock (remnants of prehistoric bone), reacting it with sulfuric acid, and then separating calcium sulphate by filtration (Toy and Walsh 1987; Corbridge 1995a). The remaining acid is then purified by liquid-liquid extraction with organic solvents (Corbridge 1995a). Despite the purification procedures in the wet process, there are still impurities such as fluorine, sulphate, arsenic, and calcium, iron, aluminum, and silicon oxides (Toy and Walsh 1995; Corbridge 1995a).

One factor involved in the success of the wet process over the thermal process is the relative price of sulphuric acid compared to that of electricity (Gardener 1972; Corbridge 1995a). Another factor that favors the wet production is that it yields a valuable by-product, gypsum (calcium sulfate), which is used in the construction industry (Corbridge 1995a).

Phosphoric acid is also produced through the reaction of phosphorus vapor with steam, known as the thermal process. This process produces high yields at temperatures 650-800°C using catalysts; because of energy costs it cannot compete with the wet process (Corbridge 1995a).

Despite, the fact that phosphoric acid has three dissociation constants, the third dissociation constant is so high, that it offers no acidic properties and is considered to be a dibasic acid (Arnold 1975). Phosphoric acid is a clear syrupy liquid that is available at various concentrations up to 75% w/v (Berry 2001).

Phosphoric acid is the only inorganic acid extensively employed as a food or beverage acidulants (Gardener 1972). Phosphoric acid accounts for about 25% of the weight of all the acids used in food and beverages. Phosphoric acid is used extensively as an acidulant, flavoring agent, pH control, and buffering agent (Gardener 1972; Berry 2001). Phosphoric acid is the least expensive of all food grade acidulants; it is also the strongest giving the lowest attainable pH (Gardener 1972). Phosphoric acid and a number of its salts are listed as GRAS by the FDA (Food and Drug Administration 2001d). The majority of phosphoric acid is used in cola, root beer, and similar carbonated beverages in amounts ranging from 0.013 to 0.084% with a pH around 2.3 to 2.5 (Gardener 1972; Toy and Walsh 1995). It is also used as a general protein acidulant and coagulant in cheese production (Arnold 1975; Berry 2001). Phosphoric acid is also used to neutralize the caustic used to peel fruit, and to clarify and acidify collagen in the production of gelatin (Gardener 1972). Sodium and calcium phosphates are also used as leavening agents (Corbridge 1995b). Additionally, phosphoric acid is used in jams and jellies to ensure proper jelling and to chelate metal cations (Dziezak 1990). Trisodium phosphate and phosphoric acid are also used in the cleaning of dairy and brewing equipment (Gardener 1972). A number of salts of phosphoric acid are also used as emulsifying agents (Gardener 1972).

Phosphoric acid has several other non-food uses. The applications of phosphoric acid include fertilizers, detergents, metal surface treatment, glasses, cements, dental and medical materials, animal foodstuffs, pesticides, synthetic polymers, and fire retardants (Corbridge 1995b).

2.2. TASTE AND FLAVOR COMPONENTS OF ACIDS

2.2.1. Sourness

There are several questions of interest involved in the understanding of the sensory characteristics of acids. One question of interest is the mechanism involved in eliciting the sensation of sourness. Several researchers have theorized the physical factors contributing to sourness, however none have been fully accepted. Acids dissolved in water disassociate into ions:

$$HA \leftrightarrow H^+ + A^-$$

Where the undissociated acid molecule is HA, H^+ is the hydrogen ion, and A^- is the acid anion. Acid strength plays a role in the molecules' ability to disassociate. A strong acid such as HCl will completely disassociate, while organic acids, being weak acids, will not completely disassociate. Acid dissociation constants are used to measure this relationship and are based on the relationship $K_a = [H^+][A^-]/[HA]$.

Early researchers theorized that sourness could be explained by the H ion exclusively (Kahlenberg 1898; Richards 1898), but this idea has been challenged. Later it was hypothesized that the hydrogen ion content, and titratable acidity are two independent variables important to sourness perception (Harvey 1920; Amerine and others 1965). Other researchers have shown that titratable acidity, and pH does not independently influence sourness as Harvey (1920) suggested (Pangborn 1963; Furukawa and others 1969; CoSeteng and others 1989).

Furukawa and others (1969) found a high correlation between acid taste and the dissociation constant in equi-molar concentrations of organic acids (butyric, fumaric, acetic, malic, lactic, ascorbic, and acetic). Other researchers have shown the chemical structure of the acid, anion concentration, and dissociation constants are also factors that influence sourness perception (Pangborn 1963; CoSeteng and others 1989).

In an attempt to isolate factors that contribute to sourness, Noble and others (1986) studied the sourness of six organic acids in binary systems. They found that in systems at equal pH and equal titratable acidity, sourness was related to some extent to the number of protons an acid has (the fewer the protons the more sour the acid)(Noble and others 1986). Because of the differences between acids in sourness at equal pH and equal titratable acidity, Noble and the others (1986) determined that the anion was the major cause of the perception of sourness.

More recently Solwasky and Noble (1998) studied how chemical qualities of organic acids contribute to perceived sourness and astringency. They found perception of sourness increased at fixed pH by increasing the normality of the solution. The effect of the anion was also examined. The only significant difference Solwalsky and Noble (1998) found when examining acid anions was that the solution with lactic acid as the dominant anion was more sour than the one where citric was the dominant ion. Solwalsky and Noble (1998) concluded the physical factors that affect the perception of sourness of acids are the hydrogen ion, the concentration of the acid, and the anion.

Understanding that acids have several physical factors that contribute to sourness (the concentration of the acid, concentration of the hydrogen ion, and the acid ion itself), acids have been compared both by weight, and molarity to evaluate their relative sourness, and to determine if they can be equally sour. In a study examining citric, malic, and fumaric acid it was shown that the lower the molecular weight of the acid, the more sour it is perceived (Buechsenstein and Ough 1979). Research has shown that acids with more carboxylic groups are less sour (Noble and others 1986; CoSeteng and others 1989).

Straub (1989) demonstrated that acids could be equally sour by varying molar concentrations. Using power functions it was shown that based on molar concentrations tartaric, citric, malic, and fumaric acids were similar in sourness, but that more lactic acid was required to create similar sourness perception (Straub 1989). The work done by Straub (1989) supported earlier research that determined the psychophysical functions of 24 carboxylic acids, failing to find a relationship between the physio-chemical properties of the acidulants and sourness (Moskowitz 1977).

2.2.2. Sensations of acids in addition to sourness

In addition to sourness, acids have other taste and flavor components that distinguish them from each other. Through descriptive analysis, organic acids and blends of organic acids have been shown to differ greatly in sensory characteristics. Settle and others (1986) examined the chemosensory properties of sour tastants. They examined citric, hydrochloric, lactic, malic, phosphoric, sulfuric, and tartaric acid in water. This study also found that acids have flavor components other than sourness including bitterness and saltiness, and that flavor components of the individual acids were relatively stable at various concentrations.

A similar study to Settle and the others (1986) found that acids not only differ in sourness, bitterness, and saltiness, but also astringency when compared on an equal molar basis (Rubico and McDaniel 1992). For example, hydrochloric acid and phosphoric acid were rated to be more astringent than citric, lactic and malic acids. Hartwig and McDaniel (1995) addressed more questions about the sensory characteristics of acids at equal concentration, but reduced one variable previously not accounted for, pH. To do this citric, malic, acetic, and lactic acid, and lactic and acetic blends were examined at fixed concentrations and three fixed pHs levels (Hartwig and McDaniel 1995). At pH 6.5 the acids were not considered different; at pH 4.5 the lactic and acetic blend was the most sour, followed by malic, and citric, with lactic being the least sour; at pH 3.5 acetic was the most sour, followed by the lactic and acetic blends, malic, lactic, with citric being the most sour (Hartwig and McDaniel 1995). Descriptors used in this study in addition to sourness were sweet, bitter, astringent and salty.

Though literature on acids has indicated that citric, lactic, and malic acids have odors to them (Doores 1990; Berry 2001), these acids are not volatile acids. The odors mentioned in literature are probably due to trace impurities generated during processing. Additionally Berry (2001) indicated that phosphoric acid had a taste resembling the taste of acetic and citric acids but without any fruity flavors, however no literature was found to indicate that citric and acetic acids have fruity flavors.

2.2.3. How acidulants behave in complex systems

The previous studies mentioned examined the flavor components of various organic acidulants in a solution containing only water, and did not attempt to examine how the acids interact with other ingredients, such as sugar, or flavorings, as would occur in real food systems.

Several studies have examined how acids behave in more complex systems. Bonnans and Noble (1993) examined how acids suppress sweetness in sugar-acid mixtures. They evaluated 18 orange flavored solutions with three levels of citric acid, and three equi-sweet levels of either sucrose or aspartame. They found that in binary mixtures of sweetness and sourness, both tastants were suppressed. However, sweetness was suppressed more by sourness than sourness was suppressed by sweetness. In addition to the limited ability of sweetness to suppress sourness, Savant (2001) examined the ability of salts, and sugar salt combinations for their efficacy of suppressing sourness. This study found that suppression of sourness was not mediated by sugar molarity or weight, but was significantly influenced by its perceived sweetness intensity (Savant 2001). Savant (2001) also found that in tertiary mixtures that a two-component masker of sweetness and saltiness was more effective than each component alone, indicating that the more complex a system, the more suppression occurs.

2.3. TASTE AND FLAVOR INTERACTIONS

2.3.1. Sourness and flavor interactions

In beverage systems, there have been several studies that suggest sourness affects the perception of flavors. One study examined the effects of sourness by using various concentrations of citric acid (0.45, 0.75, 1.11, and 1.82%) and various concentrations of sucrose (2, 4, 8, and 16%) on the overall flavor strength of acid removed lemon juice (McBride and Johnson 1987). This study found that the perceived overall flavor of the lemon juice increased with increasing citric acid concentrations. The perceived overall flavor of the lemon juice also increased with increasing sucrose concentrations, but to a lesser extent indicating that sourness coming from increasing citric acid concentration increases lemon flavor perception. Stampanoni (1993) further examined how increasing sourness, through increasing citric acid concentration, affected lemon and orange flavor perception. To do this orange and lemon flavored sherbets and beverages were evaluated with increasing concentrations of citric acid (1.5 g/L to 4 g/L)

(Stampanoni 1993). Stampanoni (1993) found that as citric acid concentration increased fruit flavor perception increased for both flavor systems, indicating that sourness coming from increasing citric acid concentration increases citrus flavor.

Another study examined the ability of sweeteners in addition to the ability of sourness generated from citric acid to enhance fruit flavor (Bonnans and Noble 1993). To do this, levels of sweetener (sucrose vs. aspartame) and citric acid were varied and the intensities of sweetness, sourness and fruitiness were evaluated (Bonnans and Noble 1993). Bonnans and Noble (1993) found that fruitiness intensity of orange flavor was enhanced primarily by sourness; sweetness enhanced the flavor to a lesser extent.

In addition to citric acid, Hartwig (1994) examined the effects of sourness generated from lactic, malic, and tartaric acids on the perception of flavor. This study examined fruit flavors outside of the citrus realm, examining orange, cherry, strawberry, and cola flavored systems (Hartwig 1994). Within a flavor system, differences in flavor were found between acids at the same concentration, and between concentrations of the same acid. Hartwig (1994) concluded that the differences between acids, and between concentrations were due to the inherent properties of the acid such as pH, buffering capacity, and chemical structure.

More recently a very complex study examined several characteristics affecting the perception of citrus drink, including sourness, sweetness, naturalness of fruit flavor, and color (King and Duineveld 1998). King and Duineveld (1998) varied the sucrose, citric acid, flavoring, quinine HCl, and coloring (Cochineal

Red). Through increasing sucrose concentration, or increasing the sweetness to sourness ratio, the intensity of natural orange flavor decreased (King and Duineveld 1998). Another finding was that as sourness increased, or increasing the sourness to sweetness ratio, more fruit flavor was perceived, and it was perceived as being more natural (King and Duineveld 1998). Thus, at least for citrus flavored systems sourness increases the perception of fruit flavor.

2.3.2. Taste and flavor interactions with non-sour tastants

There have been studies that examined interactions between sweetness and saltiness with aroma compounds, but not sourness with aroma compounds. One study examined the effects of citral mixed with either various concentrations of sugar or NaCl (Murphy and Cain 1980). Murphy and Cain (1980) found incomplete additivity (90% for NaCl and citral, 85% for sucrose and citral) in perceived overall intensity, regardless of there being harmony with the tastant and odorant or not.

A more recent study by Hornung and Enns (1986), examined the amount that aroma (ethyl butyrate) and taste (sucrose) contribute to overall intensity. This study also found incomplete addititvity (the overall intensity of the ethyl butyrate and sucrose solutions equaled 68% of the sum of the two) (Hornung and Enns 1986). Hornung and Enns (1986) also found that ethyl butyrate raised sweetness intensity, and sucrose raised odor intensity, when only one attribute was rated.

Frank, and Byram (1988) further examined the relationship between odor and tastant interactions. Various levels of strawberry, and peanut odorant were added to whipped cream with either various levels of sucrose or NaCl (Frank and Byram 1988). Instead of examining overall intensity they focused on sweetness and saltiness of samples and found that odorants can modify taste perception, as the strawberry odorant increased the perception of sweetness in samples containing sweeteners. Strawberry flavor was not found to increase the perception of saltiness in samples containing salt. Additionally they found that peanut odorant increased the perception of saltiness, but not sweetness. This indicates that when evaluating individual attributes, additivity of flavor intensity only occurs when harmony exists between taste and aroma compounds.

Frank and others (1990) examined in more detail how aromas can enhance taste components. To do this subjects evaluated samples with various concentrations of sucrose and strawberry flavor, and rated the samples for sweet, salty, sour bitter, and other tastes; then they evaluated the same samples just for sweetness (Frank and others 1990). When several descriptors were evaluated, strawberry flavor did not significantly effect sweetness intensity, however when only sweetness was evaluated, strawberry flavor significantly increased the perception of sweetness (Frank and others 1990). This study suggests that odor enhancing taste components is more of a contextual effect instead of an additive effect as previously suggested.

Another study examined the possibility of taste components to enhance aroma components. Wiseman (1991), created fruity flavor power functions with iso-sweet concentrations of aspartame, acesulfame-K, blended aspartame/acesufame-K and sucrose. This study found that sweeteners enhanced the perception of both strawberry and orange flavor. Additionally aspartame solutions gave higher fruity flavor perception than sucrose, however the slope for fruitiness was less with aspartame than sucrose (Wiseman 1991). This study however only evaluated the fruitiness descriptor; it is possible that the flavor effect was contextual as suggested by Frank and others (1990).

2.4. SENSORY TECHNIQUES

2.4.1. Scaling techniques

One important element in sensory analysis is quantifying the qualitative differences between samples. There are several ways to measure the intensity of qualitative attributes of foods. One method is the use of category scales, which are limited sets of words or numbers that have equal intervals between categories (Meilgaard and others 1991). Category scales were first introduced into the sensory arena in 1947 by the Quartermaster Food and Container Institute, which used a 7-point hedonic scale to determine soldier preferences of foods (Peryam and Pilgrim 1957). The purpose of this type of scale was to allow statistical analysis of subject responses. The 9-point Hedonic scale, which has nine phrases in a single continuum ranging from like extremely to dislike extremely, was developed further and first published by Peryam and Girardot (1952) and has been modified little since.

Another example of a category scale is the 16-point intensity scale used in the Spectrum® technique (Meilgaard and others 1991). This intensity scale is grounded at several points, 0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme, with corresponding universal intensity standards (Meilgaard and others 1991). The category scale for the Spectrum® technique was developed as a universally usable and understandable scale so information can be compared at a later date, and between labs.

Another scaling technique that is employed in sensory analysis is magnitude estimation. Magnitude estimation is a scaling technique that gives subjects the freedom to assign numbers to the perceived magnitude of a given stimulus. Subjects are instructed to assign a number relating to the intensity of the sample compared to either a reference modulus, or to the first sample they evaluated. Data from magnitude estimation is normalized by dividing individual panelist responses by their geometric means, this reduces panel variance. The normalized data are then log transformed and regressed on the log of the stimulus concentration to yield power functions (McDaniel and Sawyer 1981; Rubico 1993). From power functions, perceptual matches of taste qualities such as sourness can be determined creating equi-sour concentrations of various acids. In terms of estimating psychophysical functions such as power functions, magnitude estimation has been shown to yield better results than category scaling (Stevens 1957). Additionally category scaling has been criticized because it has finite end points, and distance between points are not physiologically equal (Stevens 1975; Moskowitz 1977).

Magnitude estimation scales and category scales have also been compared in terms of discrimination power. McDaniel (1974) compared the nine point hedonic scale to a magnitude estimation scale for preference and found the magnitude estimation scale to be more sensitive. A more recent study however did not find a difference in discrimination power between category scales and magnitude estimation scales (Lawless and Malone 1986). Lawless and Malone (1986) were examining the sensitivity of scaling techniques for descriptive analysis, where McDaniel (1974) was comparing the techniques on hedonic information. It has been suggested that magnitude estimation has an advantage over category scaling in that it is indefinite, and the data can be converted to percentages that can be compared among tests (Giovanni and Pangborn 1983). However Lawless and Malone (1986) found that category scales were slightly lower in variability, higher in reliability, and more user friendly. Ultimately which scale is used depends on the task at hand. For determining power functions, magnitude estimation seems a better scale to use; for descriptive analysis, category scaling appears to be more user friendly.

Regardless of which scaling technique is used, scale data can be further analyzed because of their numerical nature. Scale data can be graphically examined and analyzed using multivariate Analysis of Variance, and univariate Analysis of Variance, followed by examining correlation matrices, and appropriate multiple comparison tests such as Tukey HSD. If data sets are large, appropriate data reduction techniques such as Principle Components Analysis can be employed.

2.4.2. Descriptive Analysis

Descriptive analysis is a powerful sensory tool that is used to identify and quantify the aroma and flavor characteristics of food and non-food products. Descriptive techniques are often used to monitor competitors' offerings, and can indicate in sensory dimensions how similar products differ (Lawless and Heymann 1999). Descriptive techniques are also ideal for shelf life testing to determine when a product significantly changes (Lawless and Heymann 1999). Another use for descriptive techniques is in product development to determine how close your product is to its target (Lawless and Heymann 1999).

Descriptive analysis, because of its precision, requires highly trained panels. Descriptive analysis is generally not used on a day-to-day basis to monitor the quality of products, it is more used as a refined tool, as it can take months to train a panel to agree on terms and scale usage. In selecting panelists for a descriptive panel, one should consider the panelists' ability to carry out sensory tests reliably and consistently (Piggot and Hunter 1999). In addition, panelists should be screened to determine sensory impairment, to determine acuity and discriminating ability (Piggot and Hunter 1999).

Descriptive analysis can be used to describe qualitative aspects of products. The qualitative aspects are: appearance such as color, texture, size and shape; aroma, through olfactory smells and nasal feelings (pungent); flavor characteristics such as taste, oral feelings, and olfactory smells; oral textures such as mechanical, geometrical, and fat and moisture parameters; and skin feel and texture (Meilgaard and others 1991). These qualitative characteristics as described by the panelists, are related to the chemical and physical properties of the product being described. Qualitative aspects should also be chosen based on several criteria. The most important criterion is their ability to discriminate between the various food samples (Lawless and Heymann 1999). It is also important for terms to be non-redundant, relate to consumer acceptance, correspond to instrumental measurements, to be unambiguous, and easy to attain references for (Lawless and Heymann 1999).

2.4.2.1. Flavor Profile

The first attempt at descriptive analysis was with the invention of the Flavor Profile Method, developed by Arthur D. Little and Co. in the late 1940's (Caul 1957; Lawless and Heymann 1999). It lead the way to the research and development of all different aspects of sensory evaluation (Stone and Sidel 1993). In the Flavor Profile the description of the character of the product – aroma, flavor, and aftertaste – is developed identifying the separate characteristics that make up the overall impression. The intensity of the characteristics is then determined. In the original method a panel of 4-6 were trained over a several week period to use common descriptors (Lawless and Heymann 1999). In Flavor Profile the group evaluates the products on a 5-point or 7-point intensity scale through general discussion, and consensus opinion to profile samples. The panel leader then summarizes the data in a report. In the Flavor Profile it is important that the panel leader and panel work as a team to reach consensus decisions (Meilgaard and others 1991). The leader is responsible for directing the conversation and for providing a consensus conclusion for the test. This method does not analyze the results using statistics.

2.4.2.2. Texture Profile®

The Texture Profile® is another descriptive analysis technique. The Texture Profile® was developed by scientists working for General Foods in the 1960's to predominantly describe the textural attributes of any product (Brandt and others 1963). Texture Profile® was defined by Brandt and others (1963) as "the sensory analysis of the texture complex of a food in terms of its mechanical, geometrical, fat and moisture characteristics, the degree of each present and the order in which they appear from first bite through complete mastication." The objective of this method was to allow direct comparison of results with known material, and to provide a relationship with instrumental data. In order to meet these criterion, Texture Profile® uses standardized terminology, which is grounded with set standards. It is important that all panelists receive the same training, including proper bite, chewing and swallowing techniques (Lawless and Heymann 1999). Originally the 5-point scale from the Flavor Profile® was used to evaluate descriptors. This scale was later expanded to a 13-point scale, and more recently, category, line and magnitude estimation scales have been used (Meilgaard and others 1991). Statistical analysis can be performed when line or category scales are used, in other cases consensus values from the whole panel are determined. This method works well for describing mechanical, geometrical and other textural characteristics, but does little for flavor and aroma attributes of foods (Szczesniak 1963).

2.4.2.3. Quantitative Descriptive Analysis®

The Quantitative Descriptive Analysis Method (QDA®) was developed by Stone and others (1974), because of dissatisfaction among sensory analysts with the lack of statistical analysis employed by the Flavor Profile®. The QDA® method relies heavily on statistics to help ensure the results are actionable. The panelists (10 to 12) are selected according to their ability to discriminate sensory properties between samples. The panel leader acts as a facilitator, encouraging communication among subjects to develop consistent terminology. It is the subjects' responsibility to develop descriptors through consensus, after being exposed to a wide variety of samples (Stone and Sidel 1993). Subjects then evaluate samples individually in booths using a line scale isolated from one another. Data are then analyzed statistically using ANOVA and MANOVA (Lawless and Heymann 1999). QDA® as a descriptive analysis technique seems to be slightly stronger than the Flavor Profile® because of the statistical power involved in the results.

2.4.2.4. Sensory Spectrum®

Sensory Spectrum® is a descriptive technique developed by Gail Civille utilizing a customized approach to panel development, selection, training, and maintenance. Subjects are trained to use a standardized lexicon of terms to describe products. A standardized 16-point intensity scale is also used, and is anchored with multiple reference points (Meilgaard and others 1991; Lawless and Heymann 1999). By using standardized terms and a standardized scale, in theory different studies on the same material should provide comparable results. The intensity scale is considered to be an absolute, and a 5 of sweetness equals a 5 on the salty scales (Lawless and Heymann 1999). Because of the strict guidelines, panel training for Spectrum is very extensive.

2.4.2.5. Free-Choice Profiling

If descriptive analysis is needed, but time is short, Free-Choice Profiling would be the technique of choice. Free-Choice Profiling was developed by Williams and Arnold at the Agricultural and Food Council in the U.K. (Meilgaard and others 1991). With Free-Choice Profiling, subject training is greatly reduced, as individuals invent and use as many terms they need to consistently describe the sensory characteristics of the samples (Lawless and Heymann 1999). Each person has an individual ballot, which may or may not have terms in common with other panelists. The data from Free-Choice Profiling are analyzed using the multivariate technique of Generalized Procrusties analysis, which adjusts for use of different parts of the scale and different terms used by different subjects (Meilgaard and others 1991). One drawback to Free-Choice Profiling is that the analysis is fairly complicated and takes considerable time to interpret.

2.4.2.6. Generic Descriptive Analysis

Even though there are several trademark descriptive techniques, it is possible to combine various aspects of several different methods to design a technique that will and answer the questions in which a researcher is interested.

3. Perceptions of Fruity Flavor Intensity in Sucrose-Based Model Systems: Manipulation of Acid Types and pH Level

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3.1. ABSTRACT

Citric, lactic and malic acids (0.4%w/v) were examined at 5 fixed pH levels (2.6, 3.0, 3.4, 3.8, and 4.2), in a solution of water, sugar (10% w/v), and lemon or strawberry flavor to determine if acid type and pH level effect fruit flavor perception. Malic acid provided more lemon flavor than citric and lactic acids because of the correlation to sourness. The pH also effected fruit flavor intensity. Lemon flavor increased with decreasing pH, and strawberry flavor decreased with decreasing pH. These findings suggest that pH effects the perception of fruit flavor differently depending on the system.

Keywords: acids, pH, sourness, fruit flavor, beverage

3.2. INTRODUCTION

Acidulants constitute an important functional ingredient group in the food industry, providing sourness, lowering pH, ensuring safe processing, and extending shelf life. As such, understanding the effect of acids on sensory characteristics of foods and beverages is critical. To advance this understanding under realistic circumstances requires evaluating the affect these acids have on flavor perceptions in complex food and beverage systems. This paper reports results of a sensory evaluation study in which panelists evaluated lemon and strawberry flavor intensity in solutions of water, sugar, and organic acids (citric, lactic, and malic) in fixed concentrations at five fixed pH levels.

Previous work has shown that at equi-molar concentrations, tartaric, citric, malic, and fumaric acids are comparable in sourness, but that lactic acid requires higher concentrations to elicit an equivalent sourness intensity rating (Straub 1989). Others have shown that organic acids and blends of organic acids differ quite a bit in sourness as well as saltiness, bitterness, and astringency when compared at equi-molar concentrations and equal pH levels (Settle and others 1986; Rubico and McDaniel 1992; Hartwig and McDaniel 1995). Specifically, sourness and astringency decrease with increasing pH, but only sourness increases when normality is increased at a fixed pH (Solwalsky and Noble 1998).

The aforementioned studies describe the flavor components of various organic acidulants in solutions containing only water. Other work has examined these flavor components in more complicated systems (McBride and Johnson 1987; Bonnans and Noble 1993; Stampanoni 1993; Hartwig 1994; Savant 2001), though none have involved the mixtures that are the focus of the present study. Increasing *sourness* in fruit flavored beverage systems enhances the perception of citrus and overall flavor (McBride and Johnson 1987; Bonnans and Noble 1993; Stampanoni 1993; Hartwig 1994), although the relationship between sourness and acid type in these systems is not well understood. In studies with tomato juice and wine, subjects could not detect a difference between organic acids in taste or flavor (Dalmasso and Wiese 1991; Kallithraka and others 1997), but those results cannot necessarily be transferred to lemon and strawberry flavor systems.

To understand the interrelationships between acids, pH and flavor a semi-trained panel used descriptive analysis to evaluate various samples of lemon and strawberry beverage systems. Previous work on the sensory characteristics of acids used samples that were served at room temperature, where differences in flavor characteristics may be more apparent than at refrigeration temperatures where the vapor pressure of aromatics is reduced. For the present study samples were served at 4° C to represent the temperature at which fruity beverages are generally consumed.

The results described herein for the strawberry and lemon flavored experiments are part of a larger study involving phosphoric acid and blends of citric, lactic, and malic acid—each paired in equal proportions—in apple, forest berry, orange, and tropical flavor systems. Results from the larger study are given in Appendix A.

3.3. MATERIALS AND METHODS

3.3.1. Samples

Citric, lactic, and malic acid at 0.4% w/v concentration were evaluated with lemon and strawberry flavorings in a 10 % w/v sucrose solution. C & H pure cane sugar (Crockett, CA) was used for the sucrose solution. The liquid lactic acid (90% w/v) was obtained from Purac (Gorinchem, the Netherlands), citric acid from Tate and Lyle, (Decatur, Illinois), and malic acid, from Bartek (Ontario, Canada). Acid solutions were buffered to five pH levels with NaOH, (98.8% Pellets, J.T. Baker Phillipsburg, New Jersey; see Table 3.1). Lemon (0.3 ml/L) and strawberry (0.15ml/L) flavors were obtained from Givaudan (The Netherlands; code numbers 513137H and 77880-33, respectively). Recommended dosage levels for the flavorings were evaluated and adjusted when necessary. Sugar, acid, and flavorings were mixed with bottled water (Sierra Springs, Portland Oregon). Samples (30 ml) labeled with three digit random codes were presented to panelists at 4^{0}C in 60 ml portion cups with lids (Sweetheart Cup Co., Owings Mills MD). The order in which panelists received the samples was randomized.

Table 3.1--Average (Standard Error) percent (w/v) NaOH added to adjust each acid to desired pH

	Acid		
pH	citric	lactic	malic
2.6	0.005 (0.002)	0.006 (0.000)	0.007 (0.003)
3.0	0.037 (0.004)	0.028 (0.004)	0.042 (0.007)
3.4	0.053 (0.006)	0.056 (0.001)	0.062 (0.005)
3.8	0.083 (0.003)	0.084 (0.001)	0.101 (0.004)
4.2	0.113 (0.002)	0.123 (0.002)	0.128 (0.003)

3.3.2. Subject Screening and Selection

Eighteen volunteer subjects from the population of students and employees of Oregon State University were screened for their sensitivity to differences in sourness. Screening involved ranking samples for sourness. Solutions of 0.3% lactic acid with 10% sucrose—adjusted with NaOH to pH levels 2.6, 3.0, 3.4, 3.8, and 4.2—were presented to the subjects. Subjects who had no more than one reversal of pH order between two trials were selected for use on the panel. Four males and seven females made up the final panel—all but two subjects had previous trained panel experience.

3.3.3. Training

Panelist training was aimed at familiarizing subjects with basic tastes, a 16-point intensity scale, and the fruit flavors. This training also served to develop standards and a tasting protocol. Subjects were trained to use a 16-point scale with universal intensity standards (Meilgaard and others 1991). They were introduced to sweetness (5, 10, and 15% w/v sucrose solution), sourness (0.06, and 0.3% w/v citric acid solution), and astringency (0.025% w/v alum solution). Using these solutions, subjects developed intensity standards for sweetness (intensity ratings of 5, 9 and 13 for the three concentrations of sucrose), sourness (intensity ratings of 3 and 12 for the two concentrations of citric acid), and astringency (intensity rating of 4 for alum). The newly developed intensity standards were confirmed through blind presentation of solutions to subjects.

Each model flavor system was treated as a separate test; subjects had two training days focused on each fruit flavor system, and testing was completed on one flavor system before training began for the second flavor system. Fruit flavor intensity standards for lemon and strawberry flavors were determined by group consensus. The following tasting protocol was developed to ensure subject consistency:

1. Take a small amount of sample (5-8 ml) into mouth, evaluate for fruit flavor, sweetness, and sourness;
2. Expectorate;
3. Wait ten seconds;
4. Evaluate for astringency.

This protocol was used in training for each fruit flavor and with solutions of various acid and pH combinations, and the same protocol was followed during the experiments themselves.

3.3.4. Design

Panelists evaluated all three acids (citric, lactic, malic) at five pH levels (2.6, 3.0, 3.4, 3.8, and 4.2) in duplicate for each flavor under a completely randomized block design, where the subjects were treated as blocks. All treatment combinations (15 in all) were randomly assigned within each flavor.

3.3.5. Testing

For each fruit flavor subjects assessed each sample for intensity of flavoring, sweetness, sourness and astringency, using the 16-point intensity scale and the standards developed in training as references. That is, during the testing subjects were permitted to reference the quality and intensity standards. Subjects evaluated each sample following the protocol developed during training, and they evaluated all samples in duplicate over a two-day period. They were presented with five samples at a setting, with a two-minute break between settings. Samples were prepared 3 days before testing; poured into sample cups; and stored and served at 4°C.

3.3.6. Statistical Analysis

Statistical analysis was performed using SPSS® (Chicago), and separate analyses were used for each flavor system. Due to the inherent multivariate nature of sensory data—outcomes from one sample consist of multiple descriptors of that sample—multivariate statistical procedures are needed for the analysis. If differences are detected across the treatments using a multivariate analysis of variance (MANOVA), plots of the univariate components of the outcomes are assessed to help explain those differences. In addition, a correlation analysis of the univariate components of outcomes further sheds light on their interrelationship.

3.4. RESULTS AND DISCUSSION

Table 3.2 describes effects that are tested for both flavor systems using the MANOVA procedure. In what follows, results for the lemon flavor experiment are presented and then results for the strawberry flavor experiment.

3.4.1. MANOVA

MANOVA results were significant for all sources of variation except for the subject by pH by acid interaction term for the strawberry experiment (p-value ≤ 0.050). As expected, subject by pH and subject main effect terms were significant. A significant subject by pH interaction occurs when not all subjects perceived differences in the same direction or with the same intensity. A significant subject main effect indicates that subjects used different portions of the scale. Upon data inspection these effects were found to be minor compared to the acid and pH effects.

The significant acid by pH interaction indicates relationships between the acids were different through the pH range. Additionally the main effects of acid and pH are of interest.

Table 3.2 -- Sources of variation in the multivariate analysis of variance model for analyzing fruit flavorings.

Source	ANOVA Variable Type	Mean Square Terms	MANOVA and ANOVA Test For:
Subject	Random	Subject*Acid + Subject*pH - Subject*Acid*pH	Used to see if at least two of the subjects uses the intensity scale differently than others for all descriptors
Acid	Fixed	Subject*Acid	Used to see if there is a difference in average perceived intensity between at least two acids
pH	Fixed	Subject*pH	Used to see if there is a difference in average perceived intensity for at least two pH levels
Acid*pH	Fixed	Error	Used to test the interaction of acid and pH to determine if at least one of the acids changed differently at various levels of pH when compared to other acids across the same pH
Subject*Acid	Random	Subject*Acid*pH	Used to test the interaction of subject and acid to determine if at least one of the subjects rated the perceived intensity of at least one of the acids differently than the other subjects
Subject*pH	Random	Subject*Acid*pH	Used to test the interaction of subject and pH to determine if at least one of the subjects rated the perceived intensity of at least one of the pHs differently than the other subjects
Subject*Acid*pH	Random	Error	Used to test the interaction of acid, pH, and subject to determine if at least one of the subjects evaluated the acid*pH interaction differently than the other subjects

3.4.2. Correlations

Descriptors used in the lemon and strawberry experiments were correlated (Table 3.3). For the lemon experiment all correlations except lemon and sweetness were significant (p-value ≤ 0.05). For the strawberry experiment all of the descriptors used were related to one another, but not always in the same way as with the lemon experiment.

There was a positive correlation between lemon and sourness of 0.347, while a negative correlation of –0.349 existed between strawberry and sourness. Previous research has shown that sourness increases with decreasing pH (Solwalsky and Noble 1998), so it was expected that as pH increased perceived lemon flavor decreased. The positive correlation between lemon flavor and sourness is supported in the literature (McBride and Johnson 1987; Bonnans and Noble 1993; Stampanoni 1993; King and Duineveld 1998). The negative correlation between sourness and strawberry flavor was not expected. Strawberry flavor, instead of being enhanced by the addition of acid, was significantly suppressed. There was a strong correlation (0.413) between strawberry flavor and sweetness. A negative correlation between sweetness and sourness for both the lemon and strawberry experiments was found (Table 3.3) indicating that as pH increased sourness decreased and sweetness increased. This sweetness and sourness relationship is supported in past studies where sourness was suppressed by sweetness (Bonnans and Noble 1993; Savant 2001).

Table 3.3 -- Correlation matrix of descriptors for separate experiments of a. lemon flavored and b. strawberry flavored systems.

a. Lemon

	lemon	sweetness	sourness	astringency
lemon	1	-0.087*	0.347	0.29
sweetness	--	1	-0.29	-0.231
sourness	--	--	1	0.527
astringency	--	--	--	1

b. Strawberry

	strawberry	sweetness	sourness	astringency
strawberry	1	0.413	-0.349	-0.272
sweetness	--	1	-0.623	-0.483
sourness	--	--	1	0.604
astringency	--	--	--	1

* Indicates non-significant correlation (p-value > 0.05)

3.4.3. Lemon Flavor Experiment

The major objective of this study was to examine the effect of acid and pH on the perception of fruit flavor. Lemon flavor intensity was significantly different between pH levels across all acids through univariate ANOVA (Figure 3.1, Tukey HSD, p-value ≤ 0.05). Lemon flavor was perceived highest at pH 3.0 (with an average intensity score of 6.92 across acids) though it was not significantly different from either pH 2.6 (with an average intensity score of 6.61 across acids) or pH 3.4 (with an average intensity score of 6.45 across acids)(Tukey HSD, p-value > 0.05). The only significant difference was for pH 4.2, (with an average intensity score of 5.83 across acids) which was significantly lower in lemon flavor than pH2.6 and 3.0 (Tukey HSD, p-value > 0.05).

The acid by pH interaction for the lemon-flavored system was not significantly different. Malic acid samples, averaged across pH levels, were perceived significantly higher in lemon flavor compared to the averaged citric and lactic acid samples (Figure 3.2, Tukey HSD, p-value ≤ 0.05). Malic acid was also rated the most sour acid in the lemon experiment, which is supported by Watine (1995); the correlation between lemon flavor and sourness is 0.347. Sausville and Carr (1965) hypothesized that malic acid has a special enhancement effect to fruit

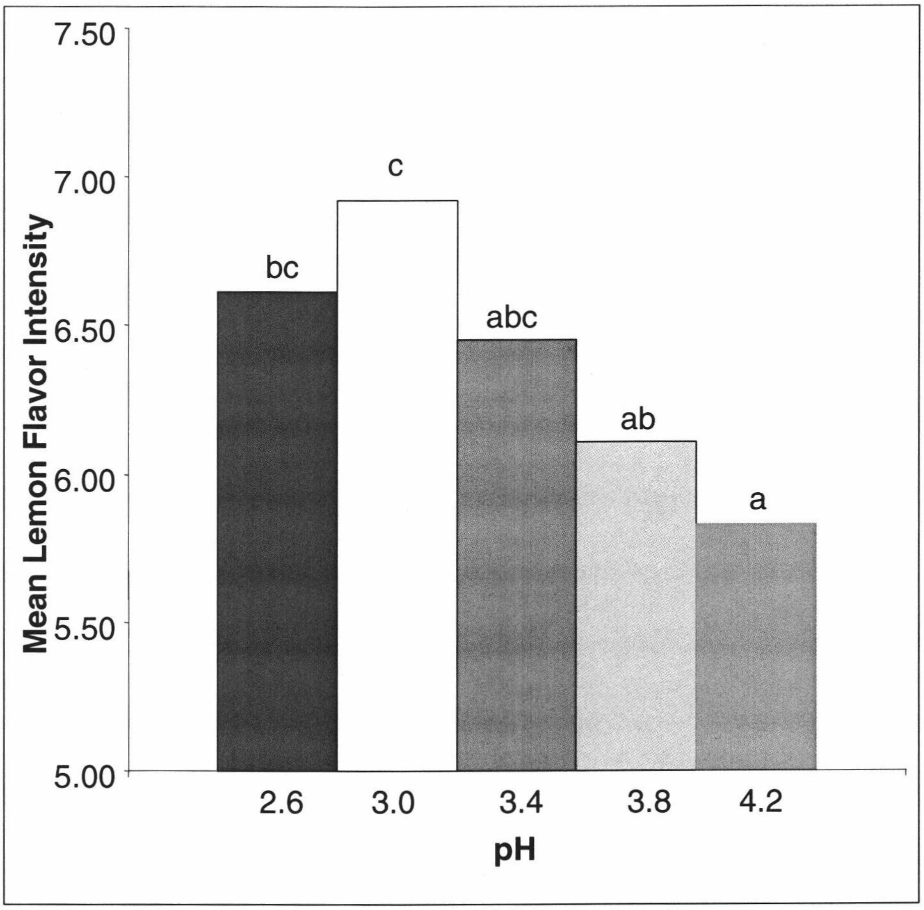

Figure 3.1-- Lemon flavor intensity responses at various pH's averaged across acids. 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Means with the same letter are not significantly different $\alpha \leq 0.05$.

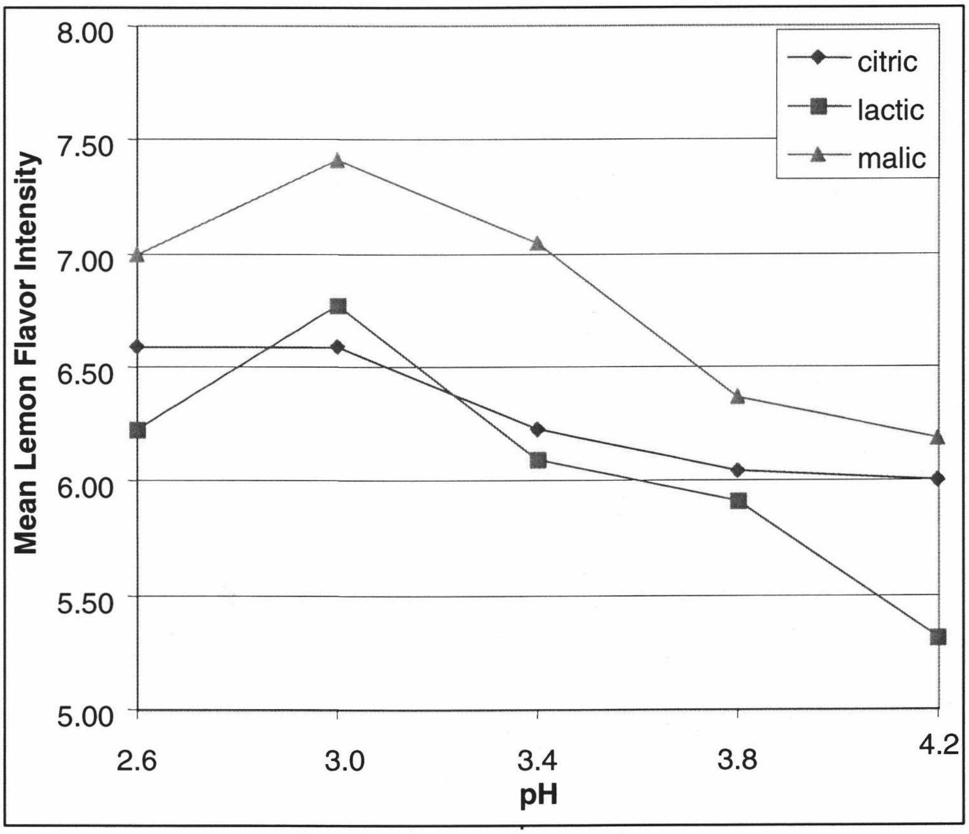

Figure 3.2 -- Mean responses for lemon flavor intensity averaged across subjects. Subjects used a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Differences between acids within a pH not significant $\alpha \leq 0.05$.

flavors containing citrus oils. Some studies suggest sourness is tied to flavor perception, (Bonnans and Noble 1993; Stampanoni 1993). It is reasonable to conclude from current findings that the perception of lemon flavor is highest with malic acid because it is the most sour acid, not because of any enhancement effect. Lactic, malic, and citric acid would be expected to give the same lemon flavor perception at equal sourness intensities.

3.4.4. Strawberry Flavor Experiment

Strawberry flavor intensity increased with increased pH. This differs from the relationship with lemon flavor and pH found in the lemon experiment. The trends in strawberry flavor for malic acid appear to be different from citric and lactic acids (Figure 3.3). However, the strawberry flavor intensities between acids were not significantly different within each pH level. Differences between the acids are more apparent when examining individual acids across pH levels (Figure 3.3). Lactic acid samples did not differ in their intensity of strawberry flavor; citric and malic acid samples were perceived differently at different pH levels. Lactic and citric acid samples followed a similar trend across pH levels. Citric acid samples separated into a low strawberry flavor intensity group (pH 2.6, mean intensity rating 7.26), and a high strawberry flavor intensity group (pH3.4, 3.8, and 4.2, with mean intensity ratings 8.86, 8.32, and 8.41, respectively) with pH 3.0 falling into both groups (mean intensity rating 8.23)(Tukey HSD, p-value ≤ 0.05). Malic acid samples separated into low, (pH 2.6 mean intensity rating

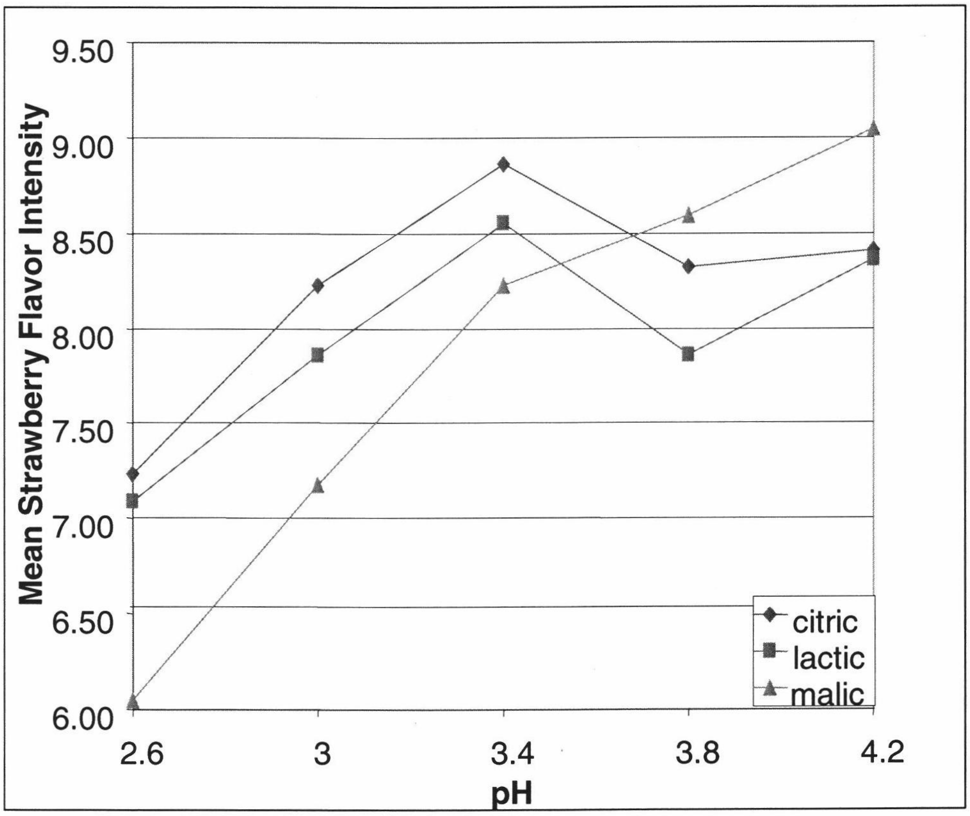

Figure 3.3--Mean responses for each acid of strawberry flavor intensity averaged across subjects. Subjects used a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Using Tukey HSD, differences less than 1.33 are not significant (p-value = 0.05, MSE 1.842).

6.05) medium, (pH 3.0 mean intensity rating 7.18) and high (pH 3.8 and 4.2, mean intensity ratings 8.89 and 9.05, respectively) strawberry flavor intensity groups, with pH 3.4 falling into both the medium and high group (mean intensity rating 8.23)(Tukey HSD, p-value ≤ 0.05).

In both the strawberry and lemon flavored systems there were no differences in fruit flavor intensity between acids within a pH level which agrees with past research (Dalmasso and Wiese 1991; Kallithraka and others 1997). In this study the largest differences in fruit flavor occurred between pH levels, which also caused differences in sourness. The malic acid system was rated higher in lemon flavor intensity compared to other acids, probably because malic acid is more sour than citric and lactic acids. It is hypothesized that malic acid provided more lemon flavor because of the correlation between sourness and fruit flavor, and not because it enhances lemon flavor. Therefore it is expected that citric, lactic, and malic acids at equal sourness levels would yield the same flavor intensity.

3.4.5. Sweetness, Sourness and Astringency Descriptors From Both Experiments

Because of the similarity between experiments for sweetness, sourness, and astringency intensity ratings for all flavor experiments, these will be examined as a group. As pH increased sourness decreased for both lemon and strawberry experiments (Figure 3.4). Malic acid was highest in sourness for both experiments, and was significantly higher than citric acid at pH 3.0 and 3.4 and lactic acid at pH 3.4 for the lemon experiment (Tukey HSD, p-value ≤ 0.05). Citric acid was the least sour for the strawberry experiment except at pH 4.2, while lactic acid was the least sour for the lemon experiment except at pH 3.0. Sweetness ratings (Figure 3.5) unlike sourness, increased as pH increased because sourness and sweetness were negatively correlated (–0.29 and –0.623 for the lemon and strawberry experiments, respectively). Malic acid samples, which were highest in sourness, were least sweet for the lemon and strawberry experiment, except at pH 3.8 in the lemon experiment. Citric acid samples were rated as sweetest in both experiments except at pH 3.8 in the lemon experiment and pH 4.2 in the strawberry experiment. Additionally, citric acid was significantly sweeter than malic acid at pH 3.0 and 3.4, and lactic acid at pH 3.0 (Tukey HSD, p-value ≤ 0.05). Astringency followed similar trends to sourness as positive correlation between sourness and astringency exists (0.527 and 0.604 for lemon and strawberry experiments, respectively). The relationship between sourness and astringency is supported in past research (Rubico and McDaniel 1992; Solwalsky and Noble 1998).

By adjusting pH, significant differences were found in both lemon and strawberry flavor intensities, and descriptors sweetness, sourness, and astringency. Changes in fruit flavor intensity were dependant on flavoring chosen as lemon flavor increased with decreasing pH and strawberry flavor increased

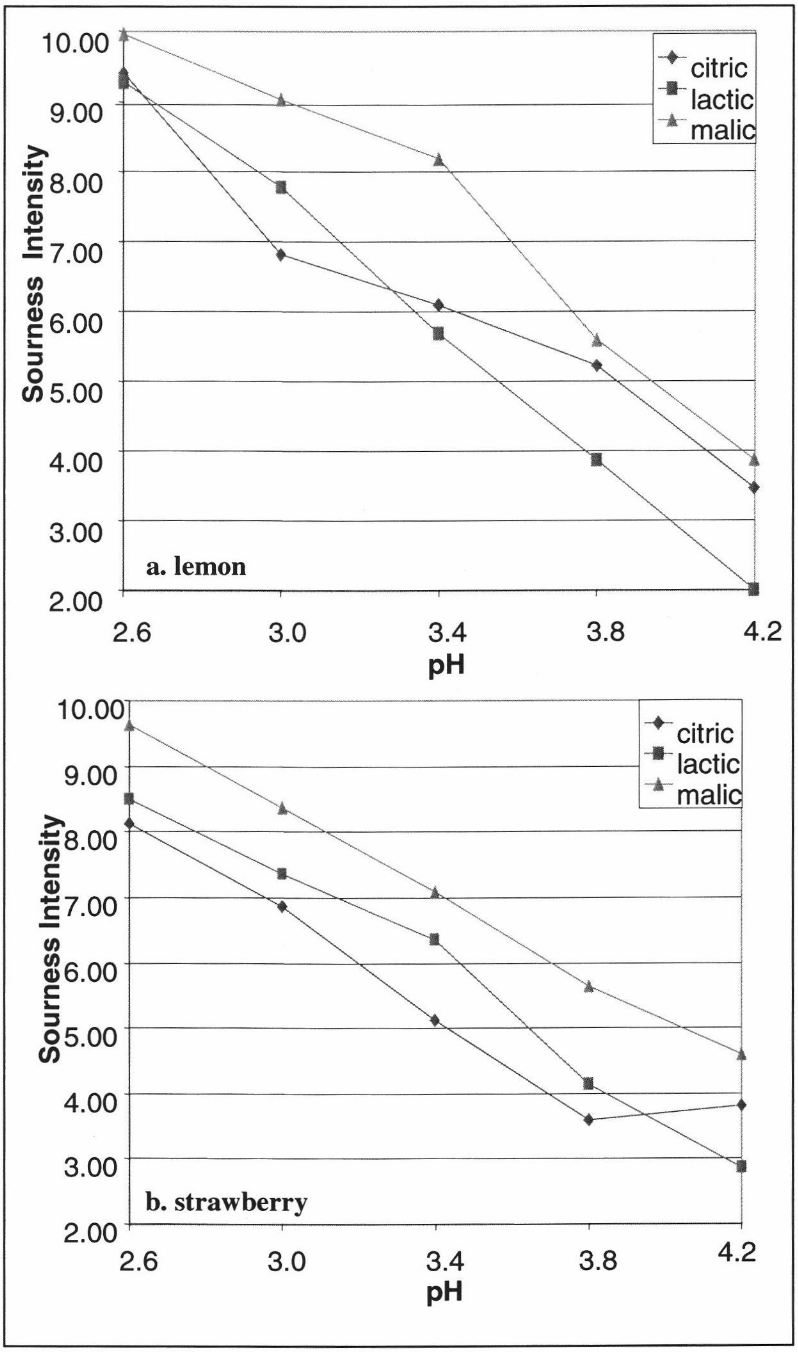

Figure 3.4-- Mean responses for sourness for a. lemon experiment, and b. strawberry experiment. Subjects used 16-point intensity scale, (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Using Tukey HSD, differences less than 1.95, and 1.44 not significant at $\alpha = 0.05$ for lemon and strawberry experiments, respectively.

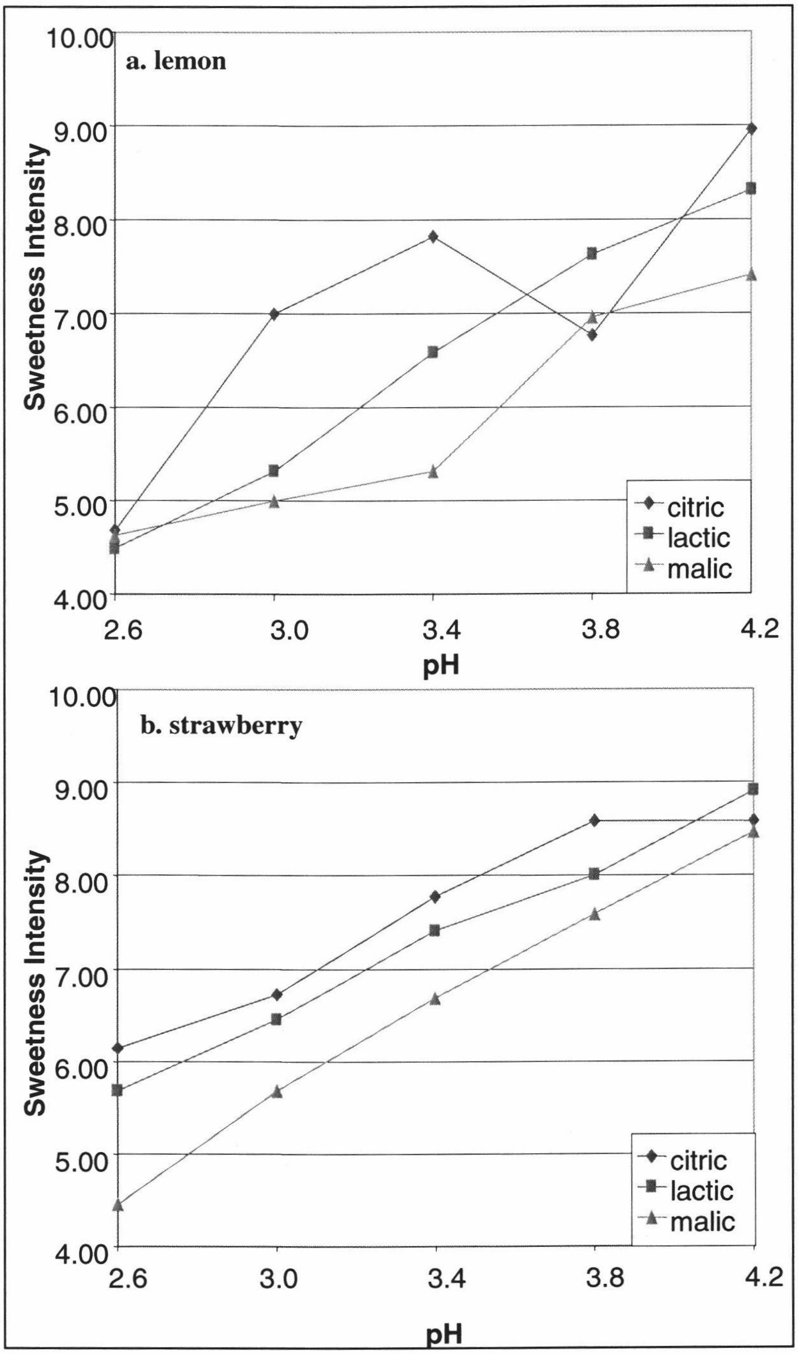

Figure 3.5 -- Mean responses for sweetness for a. lemon experiment, and b. strawberry experiment. Subjects used 16-point intensity scale, (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Using Tukey HSD, differences less than 1.47, and 1.87 not significant at α = 0.05 for lemon and strawberry experiments, respectively.

with increasing pH. These findings are similar to results from previous studies where it is suggested that harmony between taste and aroma compounds are required to have an enhancing effect, and that dissimilar aroma taste pairs such as sweet and peanut do not exhibit an enhancing effect (Frank and Byram 1988). Frank and Byram (1988) were, however, looking at the effects of aroma on taste while this study was looking at the effect of taste on flavor.

Lemon flavor increasing with decreasing pH or increasing sourness is supported by previous research in citrus systems (McBride and Johnson 1987; Bonnans and Noble 1993; Stampanoni 1993; King and Duineveld 1998). Trends in strawberry intensity are in agreement with similar findings for perceived forest berry and tropical flavor where flavor increased with increasing pH and decreasing sourness (Appendix A). Results from previous studies that examined the effect of sourness on fruit flavor in citrus flavored systems indicate that perhaps citrus flavors are more harmonious with sourness than other fruit flavors such as strawberry. Therefore the effect of pH and sourness on fruit flavor intensity is flavor specific.

3.5. CONCLUSION

In this study, pH had a larger effect on flavor perception than chemical structure of the acid anion, as differences in fruit flavor perception between the acids within a pH were not significant. In the lemon experiment malic acid was perceived highest in lemon flavor and sourness when averaged across pH levels.

It is hypothesized that malic acid provided more lemon flavor because of the correlation between sourness and fruit flavor, and not because it enhances lemon flavor. Therefore it is expected that citric, lactic, and malic acids at equal sourness levels would yield the same flavor intensity. When formulating fruit flavored beverages, identifying the appropriate pH to provide maximum flavor intensity is more important than acid used, as lactic, citric, or malic acid would provide similar flavor intensity within a given pH.

Additionally the sourness generated from varying the hydrogen ion concentration effects fruit flavors differently, increasing the perception of lemon flavor, while decreasing the perception of strawberry flavor. The results from this finding suggest that if one were interested in formulating a lemon flavored beverage with a relatively high pH, finding a way to increase sourness would be critical to increase flavor perception. On the other hand, if one were interested in formulating a strawberry flavored beverage at a low pH, finding a way to minimize sourness to increase flavor perception would be critical.

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4. Changes in Fruit Flavor Intensity in Sucrose Based Model Systems: manipulation of acid types, acid anion concentration, and sourness levels at pH 3.0

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4.1. ABSTRACT

Three equi-sourness levels of citric, lactic, and malic acids were generated at pH 3.0, in a solution of water, sugar (10% w/v), and lemon or strawberry flavor at 4°C to determine if acid type and sourness level effect fruit flavor perception. Increasing sourness intensity increased lemon and strawberry flavor. At the low sourness intensity malic acid provided more strawberry flavor than citric or lactic acid. These findings suggest that sourness effects flavor more than acid type.

Keywords: acids, sourness, fruit flavor, beverage, sensory

4.2. INTRODUCTION

Considerable research has been done on the sourness of acidulants. To a lesser extent researchers have examined how acidulants, specifically sourness intensity, effect the perception of fruit flavor in beverage systems at room temperature. This paper reports the findings of a sensory evaluation experiment that examined the perception of fruit flavor (lemon and strawberry) intensity as affected by acid type (citric, lactic and malic acid) and sourness intensity at pH 3.0. To determine if sourness alone affects fruit flavor perception, three equi-sour levels for each acid type were generated by adjusting anion concentration to pH 3.0, in a model solution of water, sugar, and a fruit flavoring. All testing was conducted with samples at 4°C to emulate common consumption conditions.

Many beverage systems contain acidulants added to provide sourness. Research on fruit flavored systems has shown that fruitiness is enhanced in the presence of acidulants. Increasing sourness resulted in enhanced perception of citrus and overall flavor in studies by McBride and Johnson (1987), Bonnans and Noble (1993), Stampanoni (1993), and Hartwig and McDaniel (1995). Cowden and co-workers (2002) found that pH or resulting sourness level, affected flavor perception more than acid type. They evaluated samples in a sweetened beverage system and tested at 4°C to better resemble a true consuming situation. Their results for citrus flavored systems agreed with earlier studies; citrus flavors increased with increased sourness. However for flavors that are associated more with sweetness, such as strawberry and tropical flavors, fruit flavor intensity decreased with increasing sourness.

Sourness generated from acids has been studied for a considerable period of time (Kahlenberg 1898; Richards 1898). It is complicated to compare studies, as some researchers controlled pH, molarity, weight, and/or sourness. Early researchers theorized that sourness could be explained by the hydrogen ion alone (Kahlenberg 1898; Richards 1898). Other researchers have shown that the chemical structure of the acid, anion concentration, and dissociation constants are also factors that influence sourness perception (Pangborn 1963; CoSeteng and others 1989). In paired tests Noble and others (1986) found that at equal pH and titratable acidity lactic acid was significantly more sour than citric and fumaric acids, and that citric acid was significantly less sour than tartaric, malic, succinic, fumaric or lactic acid. Using power functions, Straub (1989) found that based on equi-molar concentrations, tartaric, citric, malic, and fumaric acids are similar in sourness, while more lactic acid is required to create an equivalent sourness intensity (Straub 1989). To provide equal sourness of an established concentration of citric acid, Berry (2001) suggested to substitute 50-60% phosphoric acid, 67-72% fumaric acid, 78-94% malic acid, or 80-85% tartaric acid. The slight conflict in sourness intensities of acids between researchers is likely due to the concentrations (equi-normal, equi-molar, or equi-weight) at which the acids were examined. Recently Solwalsky and Noble (1998) performed a study to understand what qualities of organic acids contribute to sourness. They found that sourness and astringency decrease with increasing pH, but only sourness increases when normality is increased at a fixed pH.

To understand the interrelationships between acids, sourness, and flavor a semi-trained panel used descriptive analysis to evaluate various samples of lemon and strawberry flavored beverage systems. With the exception of Cowden (2002), previous studies examined acids at room temperature where differences would be more apparent; samples here were served at 4°C to identify what realistic differences might exist

4.3. METHODS

4.3.1. Samples

Citric, lactic, and malic acids were evaluated with strawberry and lemon flavorings in a 10 % w/v sucrose solution at pH 3.0. The liquid lactic acid (90% w/v) was obtained from Purac (Gorinchem, the Netherlands), citric acid from Tate and Lyle, (Decatur, Illinois), and malic acid, from Bartek (Ontario, Canada). Acid solutions were buffered to pH 3.0 with NaOH, (98.8% Pellets, J.T. Baker Phillipsburg, New Jersey). Lemon (0.3 ml/L) and strawberry (0.15ml/L) flavors were obtained from Givaudan (The Netherlands)(code numbers513137H and 77880-33, respectively). To determine the amount of flavoring to use, recommended dosage levels were evaluated and then adjusted if necessary. Sugar, acid, and flavorings were mixed with bottled water (Sierra Springs, Portland Oregon). Samples (30 ml), labeled with three digit random codes, were presented at 4^0C in 60 ml portion cups with lids (Sweetheart Cup Co., Owings Mills MD). Sample order was randomized.

4.3.2. Subject Screening and Selection

Twenty-one subjects from the population of students and employees of Oregon State University were screened for sensitivity to differences in sourness. Screening involved ranking samples for sourness. Solutions of 0.1, 0.2, 0.4, 0.8, and 1.6% lactic acid with 10% sucrose, adjusted to pH 3.0, with NaOH were presented to subjects. Subjects that had no more than one reversal of concentration were selected for use on the panel. Fifteen panelists, three males and twelve females, made up the final panel, eight of whom had previous trained panel experience.

4.3.3. Determination of Equi-sour Levels and Descriptive Testing

Each fruit flavor constituted a separate experiment. Three equi-sour concentrations in the fruit flavored system were established for citric, lactic and malic acids using magnitude estimation, power function calculations, and difference from control techniques (Stevens 1953; Stevens 1956; Stevens and Galanter 1957; Moskowitz and Sidel 1971; Rubico and McDaniel 1992). Subjects used magnitude estimation to evaluate samples containing an acid, sugar, and fruit flavoring using 0.4% citric acid for a reference with a sourness rating of 100. Organic acids and the five concentrations (g/l) used to determine power functions are listed in Table 4.1. Panelists rated sourness intensities for the five concentrations of each acid, power functions were generated, and equi-sourness levels were calculated. The same panel confirmed the equi-sourness levels using the difference from control method with 0.2, 0.4, or 0.8% citric acid as the reference.

After equi-sour levels were determined (Table 4.2), subjects were trained in descriptive analysis techniques; training consisted of three training sessions for scaling, and six sessions to familiarize subjects with each flavor. During training, subjects learned the 16-point intensity scale, using the universal intensity standards cited in Meilgaard and others (1991). Subjects developed intensity standards for sweetness (5, 10, and 15% w/v sucrose with intensity 4, 8, and 13, respectively) saltiness (0.25% w/v NaCl with intensity 6), and astringency (0.025% w/v alum with intensity 5). For the lemon-flavored experiment, a lemon flavor standard was developed through round table discussion (0.3 ml/L lemon flavor, 0.4%citric acid and 10% w/v sucrose with intensities of 8 for lemon, 6 for sweetness, 0 for salty, and 4 for astringency). A strawberry flavor standard was also developed (0.15 ml/L strawberry flavor, 0.4% w/v citric and 10% w/v sucrose with intensities of 8 for strawberry, 6 for sweetness, 0 for salty, and 3 for astringency).

Table 4.1 – Molarity and Normailty of acid concentrations used to generate power functions

Acid	Molecular (Equivalent) Weight	Concentration %(w/v) 0.1	0.2	0.4	0.8	1.6
Citric	192.12	0.0052 [a]	0.0104	0.0208	0.0416	0.0833
	(64.04)	(0.0156) [b]	(0.0312)	(0.0625)	(0.1249)	(0.2498)
Lactic	90.08	0.0111	0.0222	0.0444	0.0888	0.1776
	(90.08)	(0.0111)	(0.0222)	(0.0444)	(0.0888)	(0.1776)
Malic	134.09	0.0075	0.0149	0.0298	0.0597	0.1193
	(67.05)	(0.0149)	(0.0298)	(0.0597)	(0.1193)	(0.2386)

[a] = Molarity, [b] = Normality

Table 4.2 -- Molar concentrations of citric, lactic, and malic acids at three equi-sour levels for each flavor.

Flavor	Sourness Level	citric	lactic	malic
Lemon	low	0.0104	0.0217	0.0105
		(0.0077) [a]	(0.0084)	(0.0084)
	medium	0.0208	0.0444	0.0216
		(0.0189)	(0.0182)	(0.0189)
	high	0.0416	0.0887	0.0445
		(0.0378)	(0.0357)	(0.0336)
Strawberry	low	0.0104	0.0218	0.0122
		(0.0144)	(0.0126)	(0.0126)
	medium	0.0208	0.0419	0.0236
		(0.0266)	(0.0245)	(0.0224)
	high	0.0416	0.0806	0.0454
		(0.0539)	(0.0448)	(0.0434)

[a] = moles NaOH required to adjust solution to pH 3.0

Another objective of training was to develop a sample evaluation protocol. Tasting protocol involved taking a small portion of sample into the mouth (5-8 ml), rating it for fruit flavor, sweetness, and saltiness, expectorating, waiting 10 seconds, and then rating astringency. Subjects rinsed their mouths with bottled water (Sierra Springs, Portland Oregon) between samples. During testing subjects were allowed to reference standards between samples.

4.3.4. Design

Subjects tested citric, lactic, and malic acid at the three equi-sour levels (nine samples total) in triplicate in a randomized complete block design. Subjects acted as blocks since the within individual variability was expected to be smaller than the variability between subjects. All treatments were randomly assigned within a flavor to each subject, and each fruit flavor was treated as a separate test.

4.3.5. Testing

Subjects were instructed to evaluate the samples according to the protocol developed during training. They evaluated all samples in triplicate, one replication per day. Sample order was randomized. Samples were prepared 3 days before testing, poured into sample cups, and stored and served at 4°C

4.3.6. Analysis

Statistical analyses were performed using SPSS®(Chicago, Illinois). Because sensory analysis employs multiple descriptors to examine samples, the descriptors are multivariate in nature; hence, multivariate statistical methods are appropriate for the analysis. Multivariate Analysis of Variance (MANOVA) using Wilks' Lambda was the first analysis performed. MANOVA identified terms in the model (Table 4.3) that were significantly different. Correlation of the components followed MANOVA, identifying the relationships between descriptors. Univariate Analysis of Variance (ANOVA) on each descriptor was then performed. ANOVA examined the trends of the individual descriptors for the terms in the model (Table 4.3) helping to identify the differences found from MANOVA.

4.4. RESULTS

4.4.1. Equi-sourness determination

Power functions of the acids in each flavor system were generated using magnitude estimation (Figure 4.1). The exponents of the power functions for the acids generated in this study were in the range of the exponents from past

Table 4.3 -- Sources of variation in the model created to analyze the effect of acid type and sourness level on the perception of fruit flavoring.

Source	Variable Type	ANOVA Mean Square Terms	MANOVA and ANOVA Test For:
Subject	Random	Subject*Acid + Subject* Sourness - Subject*Acid* Sourness	Used to see if at least two of the subjects use the intensity scale differently than others for all descriptors
Acid	Fixed	Subject*Acid	Used to see if there is a difference in average perceived intensity between at least two acids
Sourness Level	Fixed	Subject* Sourness	Used to see if there is a difference in average perceived intensity for at least two sourness level levels
Subject*Acid	Random	Subject*Acid* Sourness	Used to test the interaction of subject and acid to determine if at least one of the subjects rated the perceived intensity of at least one of the acids differently than the other subjects
Subject* Sourness Level	Random	Subject*Acid* Sourness	Used to test the interaction of subject and sourness level to determine if at least one of the subjects rated the perceived intensity of at least one of the sourness levels differently than the other subjects
Acid* Sourness Level	Fixed	Error	Used to test the interaction of acid and sourness level to determine if at least one of the acids changed differently at various sourness level when compared to other acids across the same sourness levels
Subject*Acid* Sourness Level	Random	Error	Used to test the interaction of acid, sourness, and subject to determine if at least one of the subjects evaluated the acid*sourness level interaction differently than the other subjects

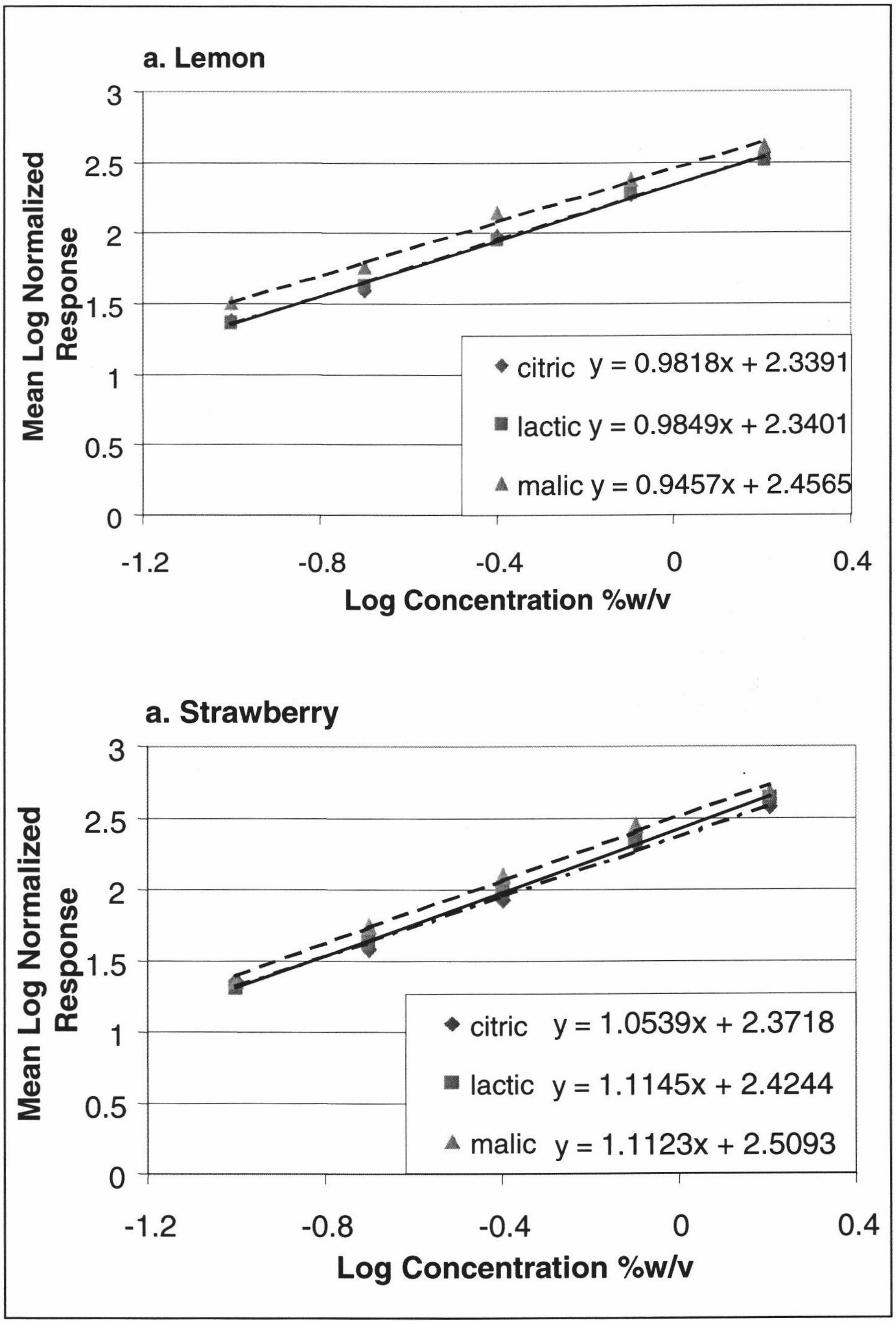

Figure 4.1-- Sourness power functions of citric, lactic, and malic acids, adjusted to pH 3.0. Used to calculate equi-sourness levels between acids.

research. Moskowitz (1971) had exponents of 0.72, 0.84, and 0.77 for citric, lactic and malic acids respectively, while Rubico (1992) had values of 1.23, 1.26, and 1.24 for citric lactic and malic acid, respectively. The exponents of the acids in this study for the lemon and strawberry experiments were, 0.98 and 1.05 for citric acid, 0.98 and 1.11 for lactic acid, and 0.95 and 1.11 for malic acid. From the power functions, concentrations of lactic and malic acid were calculated for three equi-sour levels (0.2%, 0.4%, and 0.8% citric acid) for low, medium and high sourness, respectively (Table 4.2). Sourness intensity increased by a factor of 1.97, and 2.08 for lemon and strawberry systems, respectively. For both fruit flavor systems, less malic acid was required than citric acid (30%, 27.5% and 25% w/w less acid for the lemon system, and 18%, 21%, and 24% less acid for the strawberry system, low, medium, and high sourness levels, respectively) to provide similar perceived sourness. For the lemon flavor system 2%, 5.75%, and 9.25% w/w less lactic acid for low, medium, and high sourness levels, respectively, was required to produce sourness intensity similar to citric acid. For strawberry the same concentration of lactic acid and citric acid produced similar sourness intensity.

4.4.2. Descriptive Testing

The main objective of the fruit flavored experiments was to examine how strawberry and lemon flavor were perceived. Lemon and strawberry flavor results are presented separately. Due to the similarity in the experiments the results for sweetness, sourness and astringency from both experiments are presented together.

4.4.2.1. MANOVA

MANOVA results were significant for all sources of variation except for the subject by sourness by acid interaction term, the sourness by acid interaction term, and the subject by acid interaction term for both the lemon and strawberry experiments ($p\text{-value} \leq 0.05$). Additionally the acid main effect in the lemon experiment was not significant as well ($p\text{-value} \leq 0.05$). As expected, the subject by sourness interaction term for both experiments ($p\text{-value} < 0.0001$), and subject main effect terms ($p\text{-value} < 0.0001$) were significant. A significant subject by sourness level interaction occurs when not all subjects perceived differences in the same direction or with the same intensity. A significant subject main effect indicates that subjects used different portions of the scale. Upon data inspection these effects were found to be minor compared to the acid and sourness effects.

The significant acid by sourness interaction indicates the relationship between the acids was different through the sourness levels for the strawberry test. Additionally the main effects of acid and sourness are of interest.

4.4.2.2. Correlations

Descriptors used in the lemon experiment evaluating the treatments of acid type and sourness level were correlated, as were the descriptors in the strawberry experiment (Table 4.4). Saltiness was not significantly correlated with the other lemon experiment descriptors; all other correlations were significant (p-value $\leq$ 0.05). A negative correlation between sweetness and both lemon and strawberry flavors existed. One of the objectives of this study was to examine how sourness level as a treatment effects fruit flavor perception. Because of the negative correlation between sweetness and both fruit flavors, and because an inverse relationship exists between sweetness and sourness (Bonnans and Noble 1993; Savant 2001), fruit flavor increases, with increasing sourness for both lemon and strawberry flavor. The relationship found between strawberry flavor and sweetness is not supported by past research (Cowden 2002). Cowden (2002) did not examine the fruit flavors at a fixed pH however, indicating, that sourness coming from the hydrogen ion effects the perception of fruit flavor differently than sourness coming from the acid anion. A negative correlation between sweetness and astringency for both the lemon and strawberry experiments was found (Table 4.4) indicating that as sweetness increased, astringency, which is related to sourness, decreased (Solwalsky and Noble 1998). Though significant correlations between saltiness and other descriptors exist in the strawberry flavored experiment, they are negligible.

Table 4.4 -- Correlation Matrix of descriptors for separate experiments of a. lemon flavored and b. strawberry flavored systems.

a. lemon

	lemon	sweetness	saltiness	astringency
lemon	1	-0.530	0.068 *	0.461
sweetness	--	1	0.002 *	-0.478
saltiness	--	--	1	0.037 *
astringency	--	--	--	1

b. strawberry

	strawberry	sweetness	saltiness	astringency
strawberry	1	-0.474	0.200	0.379
sweetness	--	1	-0.089	-0.208
saltiness	--	--	1	0.143
astringency	--	--	--	1

* Indicates non-significant correlation (p-value > 0.05)

4.4.2.3. Lemon Flavor Experiment

The main objective of the study was to examine the effect of acid and sourness level on the perception of fruit flavor. Lemon flavor intensity was not different between acid types within a sourness level (p-value ≤ 0.05). This indicates that lactic, citric, and malic acids provide similar flavor intensity at equal sourness levels. Lemon flavor intensity, however, significantly increased across all acids as sourness level increased indicating lemon flavor is dependant on sourness, and not acid type (Figure 4.2, Tukey HSD, p-value ≤ 0.05). Lemon flavor was perceived highest at the highest sourness level tested (9.11 averaged across acids), in the mid range at the medium sourness level (6.73 averaged across acids), and lowest at the lowest sourness level tested (4.96 averaged across acids)(Tukey HSD, p-value ≤ 0.05).

4.4.2.4. Strawberry Flavor Experiment

Perceived strawberry flavor increased significantly with each increase in sourness level (5.91, 7.34, and 9.17 strawberry intensity ratings for low, medium, and high sourness intensities averaged across acids, respectively) similar to the findings from the lemon flavor experiment (Figure 4.3)(Tukey HSD, p-value $\leq$ 0.05). The effect of acid was significant for the strawberry flavor experiment(F-stat = 6.22, with 2,28 df, p-value = 0.006). Upon inspection malic acid had more

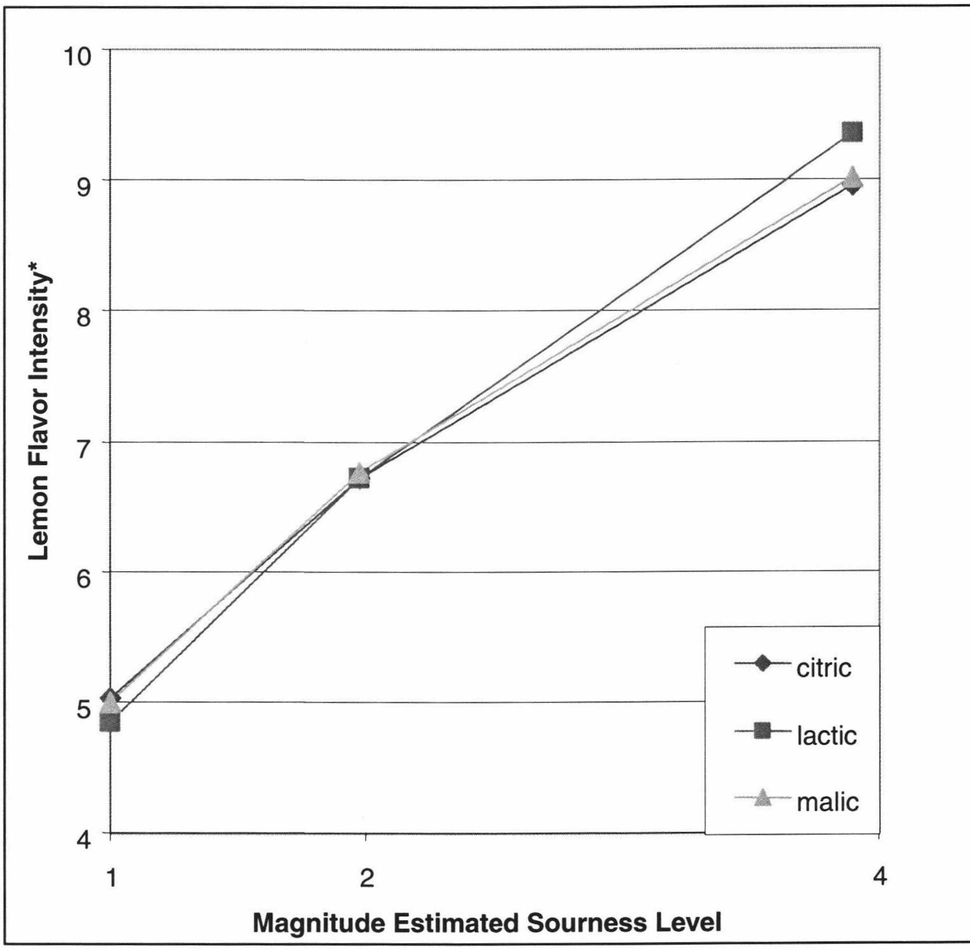

Figure 4.2 -- The relationship between three equi-sour magnitude estimated sourness levels of citric, lactic, and malic acid and their effect on the perceived flavor intensity of lemon flavor. *Subjects used a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme) for lemon flavor. Acids within a sourness level not significant, however perceived lemon flavor significantly increases with each increase in sourness level (MSE = 0.943 $\alpha \leq 0.05$).

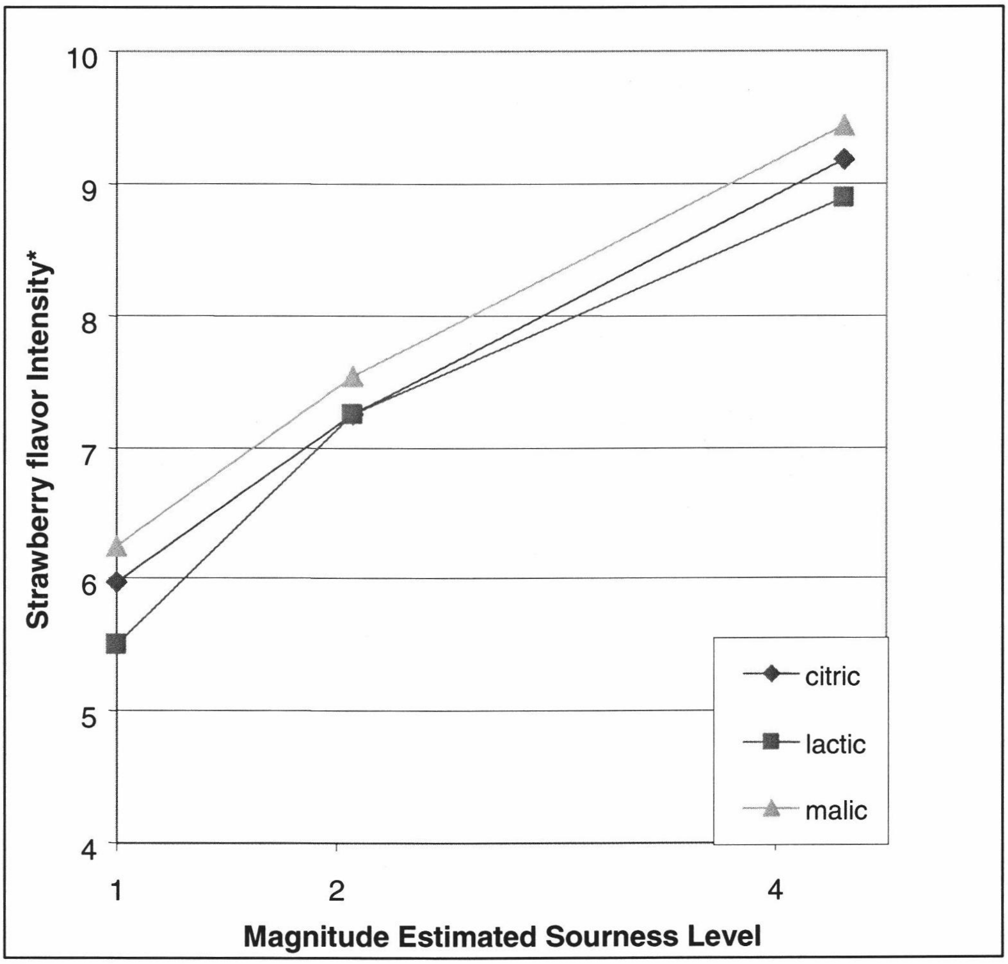

Figure 4.3 -- The relationship between three equi-sour magnitude estimated sourness levels of citric, lactic, and malic acid and their effect on the perceived flavor intensity of strawberry flavor. *Subjects used a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme) for strawberry flavor. Perceived strawberry flavor significantly increases with each increase in sourness level (MSE = 2.156, $\alpha \leq 0.05$). Acids within a sourness level not significant, however perceived lemon flavor significantly increases with each increase in sourness level (MSE = 2.156, $\alpha \leq 0.05$).

strawberry flavor (6.24 averaged across subjects) than lactic acid (5.51 averaged across subjects) at the low sourness level only (Tukey HSD, p-value ≤ 0.05).

Equi-sourness levels were confirmed using difference from control, with citric acid as the control. Neither Lactic, nor malic acids were considered different from citric acid (Tukey HSD, p-value ≤ 0.05). It is reasonable to conclude that differences in strawberry flavor between malic and lactic acid are from slight differences in sourness not accounted for from the sourness matching exercise.

4.4.2.5. Behavior of Descriptors Sweetness, Sourness and Astringency

Because of the similarity of the results for sweetness, saltiness, and astringency descriptors for both flavor experiments, these will be examined as a group. Perceived sweetness decreased significantly with each increase in sourness level for both the lemon (6.91, 5.84, and 4.90 intensity ratings averaged across acids for low, medium and high sourness levels, respectively) and the strawberry system (7.18, 6.23, and 5.07 intensity ratings averaged across acids for low, medium and high sourness levels, respectively) (Figure 4.4, Tukey HSD, p-value ≤ 0.05). No significant differences between acids across sourness levels were found. This relationship between sweetness and sourness has been documented by other researchers (Bonnans and Noble 1993; Savant 2001).

Saltiness intensity was not found to be significantly different in the lemon experiment between acids or between sourness levels (Figure 4.5). Of 15 subjects

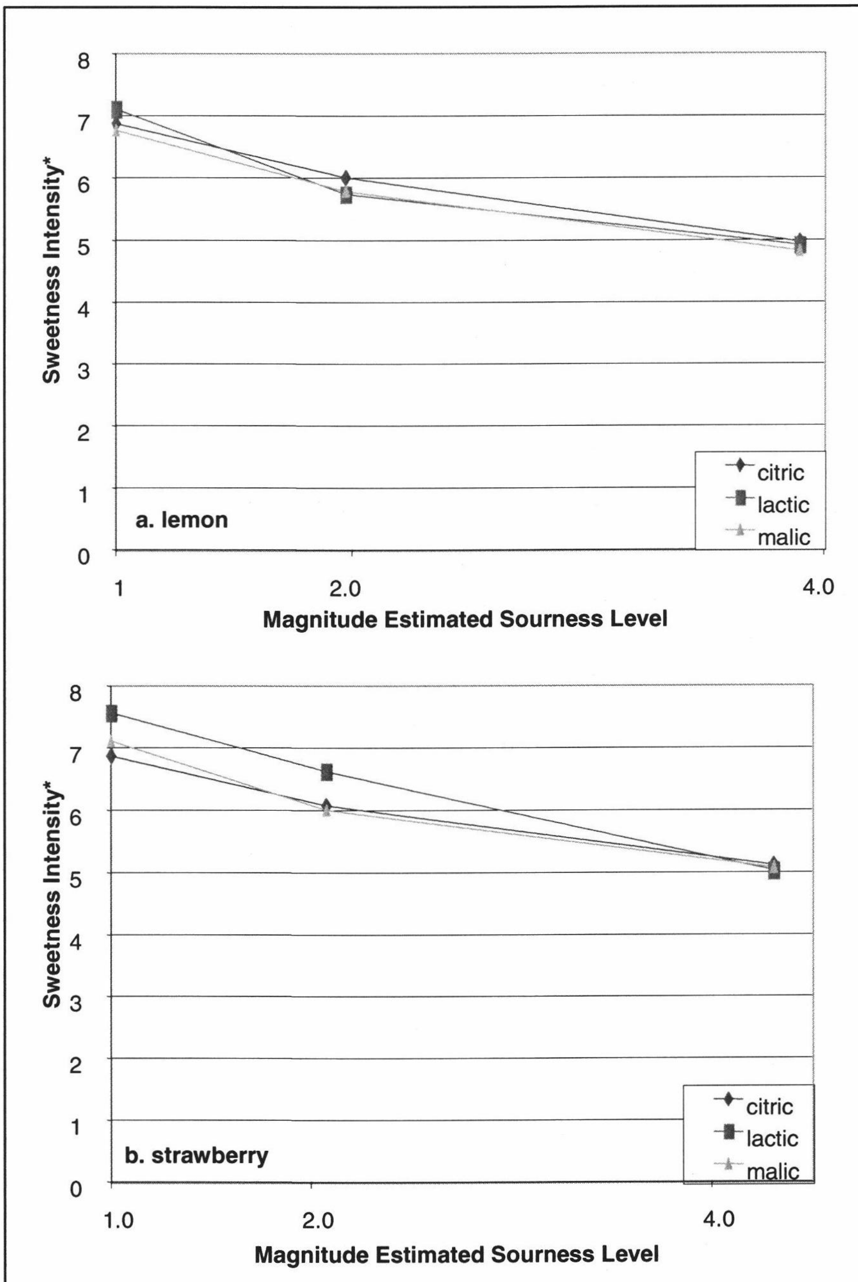

Figure 4.4 -- Mean responses for sweetness for a. lemon experiment, and b. strawberry experiment. *Subjects used a 16-point intensity scale, (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Differences between acids within a sourness level not significant. Differences between sourness intensities averaged across acids are significant (MSE = 0.834, and 1.746 for lemon and strawberry experiments, respectively, $\alpha \leq 0.05$).

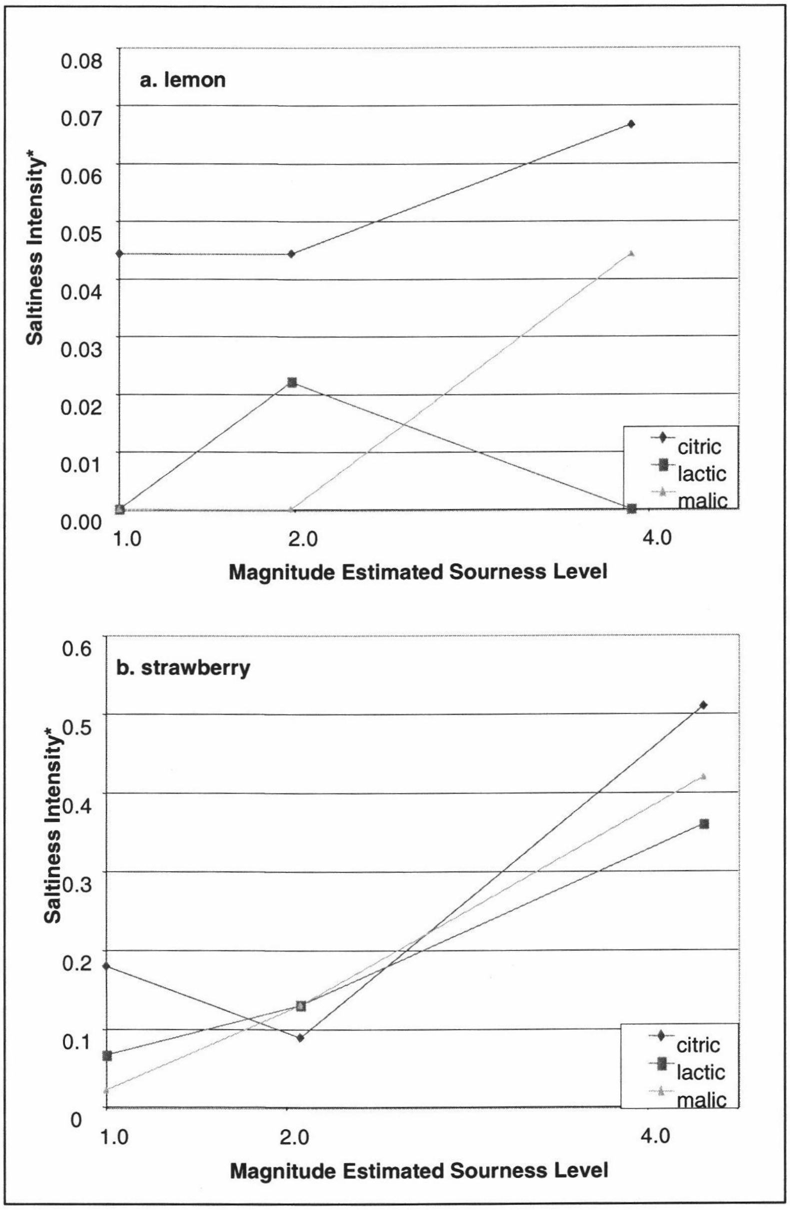

Figure 4.5 -- Mean responses for saltiness for a. lemon experiment, and b. strawberry experiment. *Subjects used a 16-point intensity scale, (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Differences between acids within a sourness level not significant. Differences between sourness intensities averaged across acids are significant for strawberry test only (MSE = 0.0371, and 0.259 for lemon and strawberry experiments, respectively, $\alpha \leq 0.05$).

only two used the saltiness descriptor, one used it four times out of 27 observations, but never for the same sample, the other subject used the descriptor twice, again not using it for the same sample. For the strawberry experiment, high sourness level samples (0.43 intensity averaged across acids) were significantly more salty than low (0.09 intensity averaged across acids) and medium sourness samples (0.12 intensity averaged across acids) (Figure 4.5, Tukey HSD, p-value ≤ 0.05). Of the 15 subjects 10 of them used the saltiness descriptor, indicating that in the strawberry system the sodium hydroxide used to buffer the solutions to pH 3.0 had a noticeable effect, at least for the high sourness level.

Perceived astringency increased significantly with each increase in sourness level for both lemon (2.30, 3.23, and 3.95 intensity ratings averaged across acids for low, medium and high sourness intensities, respectively) and strawberry (1.68, 2.56, and 3.13 intensity ratings averaged across acids for low, medium and high sourness intensities, respectively) experiments (Figure 4.6, Tukey HSD, p-value ≤ 0.05). No significant differences between acids existed for the astringency descriptor. The relationship found between sourness and astringency at a fixed pH is not supported by past research (Solwalsky and Noble 1998). Solwalsky and Noble (1998) studied acids at room temperature in a water solution, instead of at 4°C in a model beverage system, and found that astringency increased with sourness as pH decreased, but not as acid concentration increased.

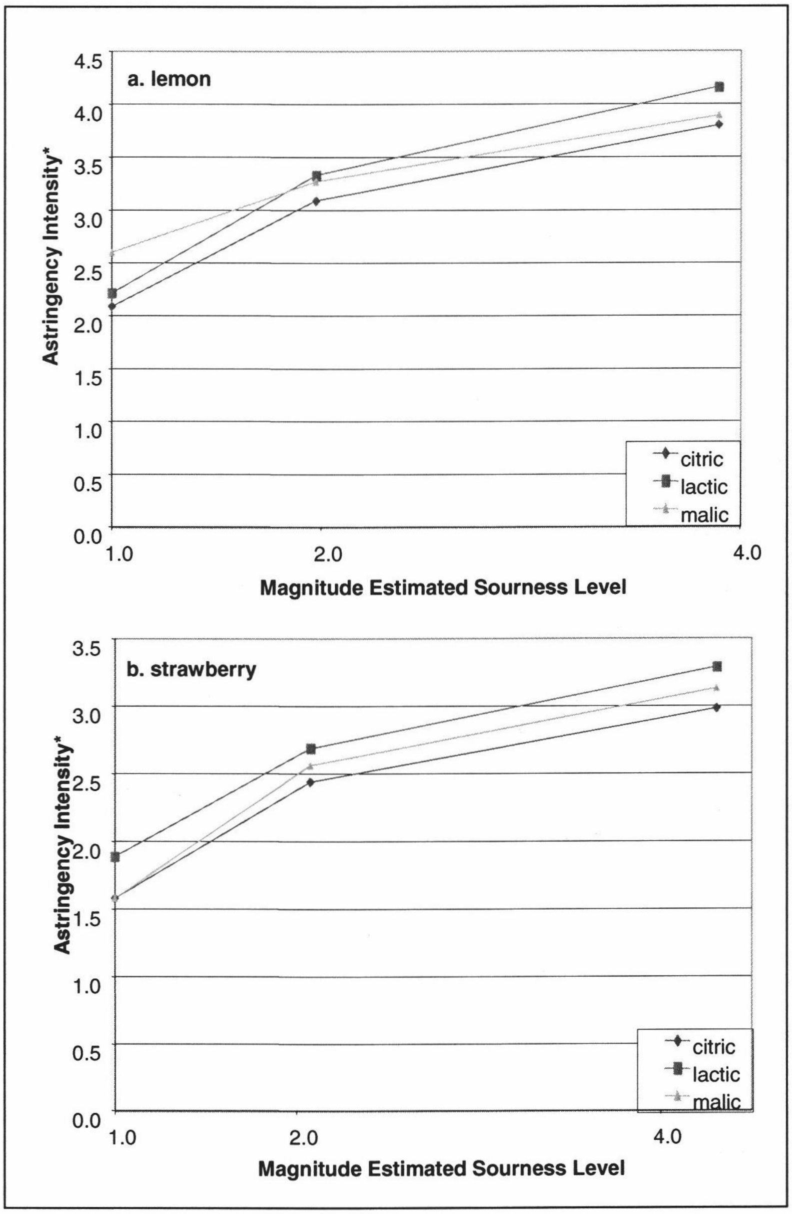

Figure 4.6 -- Mean responses for astringency for a. lemon experiment, and b. strawberry experiment. *Subjects used a 16-point intensity scale, (0 = none, 3 slight, 7 = moderate, 11 = large, 15 = extreme). Differences between acids within a sourness level not significant. Differences between sourness intensities averaged across acids are significant (MSE = 0.801, and 1.141 for lemon and strawberry experiments, respectively, $\alpha \leq 0.05$).

It is possible that in a more complex model beverage system at 4°C the perception of astringency is linked to the perception of sourness.

This study examined acids in a complex model system, at three fixed sourness levels at pH 3.0, evaluating the effect of particular acids and sourness level on fruit flavor perception. By manipulating acid concentrations at a fixed pH to obtain three equi-sour levels, the results overwhelmingly show that fruit flavor perception increases as sourness increases. These results are supported in past research on citrus flavored systems (McBride and Johnson 1987; Bonnans and Noble 1993; Stampanoni 1993; King and Duineveld 1998). The trends in perceived strawberry however are not in agreement with Cowden and others (2002), who found that as sourness decreased, the perception of strawberry flavor increased. Cowden and others (2002) examined acids at a fixed concentration while varying pH (or the sourness generated from the hydrogen ion) and found that as sourness decreased, strawberry flavor increased. The current study fixed pH while varying the acid anion concentration to create different sourness levels and found that as sourness increased strawberry flavor increased. These findings suggest that the sourness generated from the hydrogen ion and the acid anion effect the perception of fruit flavor differently.

Solwalsky and Noble (1998) found that sourness comes from both the hydrogen ion and the acid anions. Studies on taste transduction indicate that tastants can interact with either ion channels or specific receptors (Buck 2000). Several theories of sourness taste transduction have been proposed dealing with ion channels (Buck 2000). Delwiche and others (1999) have proposed that salt anions, which were once thought to only interact with ion channels, also interact with receptor sites. It has been shown that hydrogen ions and acid anions generate sourness. Thus it is possible that interactions between acid anions and taste receptors, not only ion channel diffusion, are involved in sourness perception. Therefore, changing acid anion concentrations could alter flavor perception. This could explain why fruit flavor perception decreased as sourness from pH increased, and why fruit flavor perception increased as sourness increased from increasing acid anion concentration while maintaining pH 3.0.

4.5. CONCLUSION

In this study, sourness had a larger effect on flavor perception than acid type. The results from this study suggest that if one were interested in formulating a fruit flavored beverage with specific pH requirements, such as a flavored milk, the fruit flavor intensity can be increased by adjusting the sourness component of the beverage.

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5. THESIS SUMMARY

Though past studies have found differences in sourness and the overall perception of acids, the differences found were usually in simple systems containing only water, and at ambient temperature. The experiments for this thesis were conducted at 4°C in model beverage flavored systems to better emulate common consumption situations.

When formulating beverages, selecting a pH below 4.6 to help facilitate high acid processing is critical. Once under the critical pH, how does one determine the appropriate pH and or acid type to use? When examining acids at fixed concentration while varying pH, pH had a larger effect on flavor perception than chemical structure of the acid anion, as differences in fruit flavor perception between the organic acids within a pH were not significant. This study shows when formulating fruit flavored beverages, identifying the appropriate pH to provide maximum flavor intensity is dependent on the flavor system, and should be investigated for each beverage.

When a pH parameter is fixed because of beverage properties, such as a high protein beverage, or a dairy beverage increasing fruit flavor perception by adjusting pH is not possible. Therefore a second study was developed to evaluate the effects of increasing sourness through increasing acid concentration while maintaining a constant pH.

Results from the second study in this thesis indicate that, strawberry and lemon fruit flavor can be increased by increasing sourness intensity regardless of organic acid used. These results somewhat contradict those of the first study found that sourness from pH increases the perception of lemon increased, while the perception of strawberry flavor decreased. The findings from these two studies indicate that sourness coming from the hydrogen ion, and sourness coming from the acid anion are perceived and processed differently, affecting higher level processing of fruity flavor information. Thus the mechanisms of sourness perception should be examined further.

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APPENDICES

APPENDIX A TABLES AND FIGURES FOR COMPLETE DATA SET FROM CHAPTER 3

Appendix A1 --Average (Standard Error) percent (w/v) NaOH added to adjust each acid to desired pH.

	Acid						
pH	citric	citric/lactic	citric/malic	lactic	lactic/malic	malic	phosphoric
2.6	0.005 (0.002)	0.010 (0.001)	0.007 (0.002)	0.006 (0.000)	0.006 (0.002)	0.007 (0.003)	0.032 (0.002)
3.0	0.037 (0.004)	0.044 (0.002)	0.044 (0.002)	0.028 (0.004)	0.030 (0.002)	0.042 (0.007)	0.038 (0.002)
3.4	0.053 (0.006)	0.058 (0.002)	0.071 (0.004)	0.056 (0.001)	0.068 (0.004)	0.062 (0.005)	0.041 (0.002)
3.8	0.083 (0.003)	0.086 (0.003)	0.089 (0.002)	0.084 (0.010)	0.107 (0.002)	0.101 (0.004)	0.047 (0.003)
4.2	0.113 (0.002)	0.119 (0.005)	0.114 (0.004)	0.123 (0.002)	0.136 (0.001)	0.128 (0.003)	0.058 (0.004)

Appendix A2 -- Flavor solution standards, with developed intensity rating from consensus

Flavoring	Percent Used (v/v)	Acid Used	pH	Descriptor	Intensity Given
Forest Berry	0.03	none		Forest Berry	9
				Sweetness	10
Forest Berry	0.03	lactic	2.6	Forest Berry	6
				Sweetness	7
				Sourness	11
				Astringency	4
Lemon	0.03	citric	2.6	Lemon	7
Apple	0.02	lactic	4.2	Apple	6
				Sweet	10
Apple	0.02	malic	2.6	Apple	11
				Sour	10
Orange	0.03	citric	3.4	Orange	10
				Sweetness	8
				Sourness	6
				Astringency	4
Tropical	0.015	citric	3.4	Tropical	11
				Sweetness	8
				Sourness	6
Strawberry	0.015	citric	3.4	Strawberry	10
				Sweetness	8
				Sourness	5
				Astringency	2

Appendix A3 -- MANOVA tables of varied pH experiment using Wilks' Lambda a. apple test, b. forest berry test, c. lemon test, d. orange test e. strawberry test f. tropical test

a. apple

Effect	Value	F	Hypothesis df	Error df	Sig.
Subject	0.033	53.12	40	1462	0.000
Acid	0.238	28.53	24	1344	0.000
pH	0.208	49.47	16	1177	0.000
Subject*Acid	0.284	2.38	240	1540	0.000
Subject*pH	0.265	3.80	160	1537	0.000
Acid*pH	0.456	3.48	96	1528	0.000
Subject*pH*acid	0.109	1.19	960	1543	0.001

b. forest berry

Effect	Value	F	Hypothesis df	Error df	Sig.
Subject	0.131	25.42	40	1431	0.000
Acid	0.332	20.36	24	1316	0.000
pH	0.253	40.96	16	1152	0.000
Subject*Acid	0.399	1.63	240	1508	0.000
Subject*pH	0.418	2.30	160	1505	0.000
Acid*pH	0.566	2.41	96	1496	0.000
Subject*pH*acid	0.126	1.07	956	1511	0.108

c. lemon

Effect	Value	F	Hypothesis df	Error df	Sig.
Subject	0.032	53.95	40	1450	0.000
Acid	0.216	30.67	24	1334	0.000
pH	0.183	54.24	16	1168	0.000
Subject*Acid	0.265	2.51	240	1528	0.000
Subject*pH	0.287	3.51	160	1525	0.000
Acid*pH	0.499	3.03	96	1516	0.000
Subject*pH*acid	0.109	1.18	960	1531	0.002

d. orange

Effect	Value	F	Hypothesis df	Error df	Sig.
Subject	0.097	30.90	40	1450	0.000
Acid	0.194	33.29	24	1334	0.000
pH	0.173	56.52	16	1168	0.000
Subject*Acid	0.282	2.38	240	1528	0.000
Subject*pH	0.248	3.99	160	1525	0.000
Acid*pH	0.504	2.98	96	1516	0.000
Subject*pH*acid	0.15	0.97	960	1531	0.715

e. strawberry

Effect	Value	F	Hypothesis df	Error df	Sig.
Subject	0.124	26.63	40	1450	0.000
Acid	0.211	31.27	24	1334	0.000
pH	0.225	45.92	16	1168	0.000
Subject*Acid	0.332	2.03	240	1528	0.000
Subject*pH	0.274	3.66	160	1525	0.000
Acid*pH	0.555	2.53	96	1516	0.000
Subject*pH*acid	0.114	1.15	960	1531	0.008

f. tropical

Effect	Value	F	Hypothesis df	Error df	Sig.
Subject	0.067	37.66	40	1450	0.000
Acid	0.267	25.56	24	1334	0.000
pH	0.275	38.34	16	1168	0.000
Subject*Acid	0.247	2.67	240	1528	0.000
Subject*pH	0.24	4.11	160	1525	0.000
Acid*pH	0.582	2.31	96	1516	0.000
Subject*pH*acid	0.135	1.04	960	1531	0.270

Appendix A4 -- Correlation matrix of descriptors for separate experiments of a. apple, b. forest berry, c. lemon, d. orange, e. strawberry, and f tropical flavor

a. apple

	apple	sweetness	sourness	astringency
apple	1	-0.114	0.475	0.310
sweetness	--	1	-0.432	-0.350
sourness	--	--	1	0.562
astringency	--	--	--	1

b. forest berry

	forest berry	sweetness	sourness	astringency
forest berry	1	0.360	-0.279	-0.031 *
sweetness	--	1	-0.374	-0.172
sourness	--	--	1	0.188
astringency	--	--	--	1

* Indicates non-significant correlation (p-value > 0.05)

c. lemon

	lemon	sweetness	sourness	astringency
lemon	1	-0.142	0.387	0.302
sweetness	--	1	-0.379	-0.328
sourness	--	--	1	0.577
astringency	--	--	--	1

d. orange

	orange	sweetness	sourness	astringency
orange	1	0.131	0.081	0.111
sweetness	--	1	-0.574	-0.337
sourness	--	--	1	0.597
astringency	--	--	--	1

e. strawberry

	strawberry	sweetness	sourness	astringency
strawberry	1	0.312	-0.197	-0.196
sweetness	--	1	-0.646	-0.509
sourness	--	--	1	0.645
astringency	--	--	--	1

f. tropical

	tropical	sweetness	sourness	astringency
tropical	1	0.220	-0.016 *	-0.105
sweetness	--	1	-0.573	-0.449
sourness	--	--	1	0.592
astringency	--	--	--	1

* Indicates non-significant correlation (p-value > 0.05)

Appendix A5 -- ANOVA tables of varied pH apple flavor experiment a. apple, b. sweetness descriptor, c. sourness descriptor, d. astringency descriptor

a. apple flavor

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	215.75	4	53.94	8.39	0.000
	Error	257.27	40	6.43		
Subject	Hypothesis	529.23	10	52.92	5.78	0.000
	Error	514.43	56	9.16		
Acid	Hypothesis	444.91	6	74.15	14.46	0.000
	Error	307.68	60	5.13		
Subject*pH	Hypothesis	257.27	40	6.43	2.69	0.000
	Error	575.40	240	2.40		
Acid*pH	Hypothesis	179.31	24	7.47	3.12	0.000
	Error	575.32	240	2.40		
Subject*Acid	Hypothesis	307.67	60	5.13	2.14	0.000
	Error	575.35	240	2.40		
Subject*pH*Acid	Hypothesis	574.80	240	2.40	1.10	0.215
	Error	849.00	388	2.19		

b. sweetness

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	803.78	4	200.95	17.89	0.000
	Error	449.29	40	11.23		
Subject	Hypothesis	1312.75	10	131.28	8.67	0.000
	Error	880.47	58	15.15		
Acid	Hypothesis	534.12	6	89.02	13.19	0.000
	Error	404.88	60	6.75		
Subject*pH	Hypothesis	449.30	40	11.23	3.96	0.000
	Error	680.77	240	2.83		
Acid*pH	Hypothesis	229.26	24	9.55	3.37	0.000
	Error	680.69	240	2.83		
Subject*Acid	Hypothesis	404.88	60	6.75	2.38	0.000
	Error	680.72	240	2.83		
Subject*pH*Acid	Hypothesis	680.14	240	2.83	1.18	0.079
	Error	934.83	388	2.41		

c. sourness

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	2692.47	4	673.12	99.32	0.000
	Error	271.10	40	6.78		
Subject	Hypothesis	1661.69	10	166.17	15.80	0.000
	Error	544.71	52	10.52		
Acid	Hypothesis	2558.03	6	426.34	57.11	0.000
	Error	447.94	60	7.47		
Subject*pH	Hypothesis	271.10	40	6.78	1.82	0.003
	Error	895.21	240	3.73		
Acid*pH	Hypothesis	418.71	24	17.45	4.68	0.000
	Error	895.12	240	3.73		
Subject*Acid	Hypothesis	447.94	60	7.47	2.00	0.000
	Error	895.15	240	3.73		
Subject*pH*Acid	Hypothesis	894.54	240	3.73	1.34	0.005
	Error	1076.50	388	2.77		

d. astringency

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	780.31	4	195.08	32.13	0.000
	Error	242.90	40	6.07		
Subject	Hypothesis	951.76	10	95.18	13.08	0.000
	Error	376.17	52	7.28		
Acid	Hypothesis	330.96	6	55.16	22.85	0.000
	Error	144.87	60	2.41		
Subject*pH	Hypothesis	242.91	40	6.07	5.03	0.000
	Error	290.31	240	1.21		
Acid*pH	Hypothesis	132.43	24	5.52	4.57	0.000
	Error	290.27	240	1.21		
Subject*Acid	Hypothesis	144.87	60	2.41	2.00	0.000
	Error	290.29	240	1.21		
Subject*pH*Acid	Hypothesis	289.99	240	1.21	1.05	0.326
	Error	445.33	388	1.15		

Appendix A6 -- ANOVA tables of varied pH forest berry flavor experiment a. forest berry, b. sweetness descriptor, c. sourness descriptor, d. astringency descriptor

a. forest berry flavor

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	203.90	4	50.98	6.99	0.000
	Error	292.38	40	7.30		
Subject	Hypothesis	257.92	10	25.79	3.08	0.005
	Error	365.44	44	8.38		
Acid	Hypothesis	22.59	6	3.77	0.98	0.446
	Error	231.50	60	3.84		
Subject*pH	Hypothesis	292.35	40	7.31	2.64	0.000
	Error	673.54	243	2.77		
Acid*pH	Hypothesis	173.02	24	7.21	2.61	0.000
	Error	672.51	243	2.77		
Subject*Acid	Hypothesis	230.68	60	3.85	1.39	0.044
	Error	672.86	243	2.77		
Subject*pH*Acid	Hypothesis	662.09	239	2.77	1.11	0.183
	Error	948.33	380	2.50		

b. sweetness

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	598.43	4	149.61	26.77	0.000
	Error	224.05	40	5.59		
Subject	Hypothesis	925.57	10	92.56	12.00	0.000
	Error	401.68	52	7.72		
Acid	Hypothesis	165.36	6	27.56	6.11	0.000
	Error	271.62	60	4.51		
Subject*pH	Hypothesis	223.89	40	5.60	2.34	0.000
	Error	582.71	244	2.39		
Acid*pH	Hypothesis	93.17	24	3.88	1.62	0.037
	Error	581.71	243	2.39		
Subject*Acid	Hypothesis	271.07	60	4.52	1.89	0.000
	Error	582.05	243	2.39		
Subject*pH*Acid	Hypothesis	571.61	239	2.39	1.03	0.395
	Error	882.00	380	2.32		

c. sourness

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	3102.92	4	775.73	204.43	0.000
	Error	152.58	40	3.79		
Subject	Hypothesis	1119.10	10	111.91	15.88	0.000
	Error	305.86	43	7.05		
Acid	Hypothesis	2199.59	6	366.60	55.04	0.000
	Error	401.14	60	6.66		
Subject*pH	Hypothesis	151.82	40	3.80	1.11	0.306
	Error	831.94	244	3.41		
Acid*pH	Hypothesis	367.57	24	15.32	4.49	0.000
	Error	830.41	243	3.41		
Subject*Acid	Hypothesis	400.32	60	6.67	1.96	0.000
	Error	830.94	244	3.41		
Subject*pH*Acid	Hypothesis	814.97	239	3.41	0.98	0.552
	Error	1317.00	380	3.47		

d. astringency

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	150.98	4	37.74	5.95	0.001
	Error	254.50	40	6.34		
Subject	Hypothesis	756.26	10	75.63	12.29	0.000
	Error	190.01	31	6.16		
Acid	Hypothesis	48.87	6	8.15	2.52	0.030
	Error	195.06	60	3.23		
Subject*pH	Hypothesis	254.15	40	6.35	1.86	0.003
	Error	832.98	243	3.42		
Acid*pH	Hypothesis	81.92	24	3.41	1.00	0.470
	Error	831.70	243	3.42		
Subject*Acid	Hypothesis	193.71	60	3.23	0.94	0.596
	Error	832.14	243	3.42		
Subject*pH*Acid	Hypothesis	818.76	239	3.43	1.11	0.189
	Error	1176.00	380	3.09		

Appendix A7 -- ANOVA tables of varied pH lemon flavor experiment a. lemon, b. sweetness descriptor, c. sourness descriptor, d. astringency descriptor

a. lemon flavor

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	96.59	4	24.15	3.59	0.014
	Error	269.13	40	6.73		
Subject	Hypothesis	195.82	10	19.58	2.02	0.046
	Error	591.11	61	9.68		
Acid	Hypothesis	283.53	6	47.26	9.79	0.000
	Error	289.56	60	4.83		
Subject*pH	Hypothesis	269.13	40	6.73	3.59	0.000
	Error	449.77	240	1.87		
Acid*pH	Hypothesis	42.72	24	1.78	0.95	0.534
	Error	449.77	240	1.87		
Subject*Acid	Hypothesis	289.56	60	4.83	2.58	0.000
	Error	449.77	240	1.87		
Subject*pH*Acid	Hypothesis	449.77	240	1.87	0.92	0.764
	Error	785.50	385	2.04		

b. sweetness

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	978.60	4	244.65	38.71	0.000
	Error	252.77	40	6.32		
Subject	Hypothesis	1029.78	10	102.98	8.77	0.000
	Error	785.61	67	11.74		
Acid	Hypothesis	623.26	6	103.88	13.17	0.000
	Error	473.25	60	7.89		
Subject*pH	Hypothesis	252.77	40	6.32	2.56	0.000
	Error	591.64	240	2.47		
Acid*pH	Hypothesis	203.98	24	8.50	3.45	0.000
	Error	591.64	240	2.47		
Subject*Acid	Hypothesis	473.25	60	7.89	3.20	0.000
	Error	591.64	240	2.47		
Subject*pH*Acid	Hypothesis	591.64	240	2.47	1.24	0.030
	Error	764.00	385	1.98		

c. sourness

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	2917.56	4	729.39	140.66	0.000
	Error	207.41	40	5.19		
Subject	Hypothesis	2211.09	10	221.11	26.52	0.000
	Error	394.97	47	8.34		
Acid	Hypothesis	2727.54	6	454.59	68.06	0.000
	Error	400.74	60	6.68		
Subject*pH	Hypothesis	207.41	40	5.19	1.47	0.042
	Error	846.39	240	3.53		
Acid*pH	Hypothesis	490.04	24	20.42	5.79	0.000
	Error	846.39	240	3.53		
Subject*Acid	Hypothesis	400.74	60	6.68	1.89	0.000
	Error	846.39	240	3.53		
Subject*pH*Acid	Hypothesis	846.39	240	3.53	1.14	0.120
	Error	1186.00	385	3.08		

d. astringency

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	764.14	4	191.04	33.21	0.000
	Error	230.09	40	5.75		
Subject	Hypothesis	1191.07	10	119.11	19.22	0.000
	Error	261.77	42	6.20		
Acid	Hypothesis	234.32	6	39.05	18.86	0.000
	Error	124.28	60	2.07		
Subject*pH	Hypothesis	230.09	40	5.75	3.54	0.000
	Error	390.02	240	1.63		
Acid*pH	Hypothesis	136.95	24	5.71	3.51	0.000
	Error	390.02	240	1.63		
Subject*Acid	Hypothesis	124.28	60	2.07	1.27	0.105
	Error	390.02	240	1.63		
Subject*pH*Acid	Hypothesis	390.02	240	1.63	1.52	0.000
	Error	411.00	385	1.07		

Appendix A8 -- ANOVA tables of varied pH orange flavor experiment a. orange, b. sweetness descriptor, c. sourness descriptor, d. astringency descriptor

a. orange flavor

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	93.86	4	23.47	3.79	0.011
	Error	247.82	40	6.20		
Subject	Hypothesis	119.44	10	11.94	1.38	0.210
	Error	501.46	58	8.63		
Acid	Hypothesis	251.38	6	41.90	9.76	0.000
	Error	257.51	60	4.29		
Subject*pH	Hypothesis	247.82	40	6.20	3.33	0.000
	Error	446.14	240	1.86		
Acid*pH	Hypothesis	89.97	24	3.75	2.02	0.004
	Error	446.14	240	1.86		
Subject*Acid	Hypothesis	257.51	60	4.29	2.31	0.000
	Error	446.14	240	1.86		
Subject*pH*Acid	Hypothesis	446.14	240	1.86	1.02	0.414
	Error	698.50	385	1.81		

b. sweetness

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	808.83	4	202.21	27.18	0.000
	Error	297.60	40	7.44		
Subject	Hypothesis	423.15	10	42.32	4.06	0.000
	Error	628.41	60	10.43		
Acid	Hypothesis	325.32	6	54.22	10.99	0.000
	Error	295.94	60	4.93		
Subject*pH	Hypothesis	297.60	40	7.44	3.83	0.000
	Error	466.40	240	1.94		
Acid*pH	Hypothesis	190.77	24	7.95	4.09	0.000
	Error	466.40	240	1.94		
Subject*Acid	Hypothesis	295.94	60	4.93	2.54	0.000
	Error	466.40	240	1.94		
Subject*pH*Acid	Hypothesis	466.40	240	1.94	0.99	0.521
	Error	753.50	385	1.96		

c. sourness

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	2530.01	4	632.50	91.64	0.000
	Error	276.08	40	6.90		
Subject	Hypothesis	190.87	10	19.09	1.83	0.073
	Error	653.84	63	10.40		
Acid	Hypothesis	2290.35	6	381.73	68.76	0.000
	Error	333.08	60	5.55		
Subject*pH	Hypothesis	276.08	40	6.90	3.37	0.000
	Error	491.98	240	2.05		
Acid*pH	Hypothesis	403.74	24	16.82	8.21	0.000
	Error	491.98	240	2.05		
Subject*Acid	Hypothesis	333.08	60	5.55	2.71	0.000
	Error	491.98	240	2.05		
Subject*pH*Acid	Hypothesis	491.98	240	2.05	0.98	0.560
	Error	804.00	385	2.09		

d. astringency

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	776.60	4	194.15	27.83	0.000
	Error	279.00	40	6.98		
Subject	Hypothesis	1138.98	10	113.90	12.37	0.000
	Error	558.39	61	9.21		
Acid	Hypothesis	351.59	6	58.60	17.94	0.000
	Error	196.03	60	3.27		
Subject*pH	Hypothesis	279.00	40	6.98	6.75	0.000
	Error	247.98	240	1.03		
Acid*pH	Hypothesis	80.82	24	3.37	3.26	0.000
	Error	247.98	240	1.03		
Subject*Acid	Hypothesis	196.03	60	3.27	3.16	0.000
	Error	247.98	240	1.03		
Subject*pH*Acid	Hypothesis	247.98	240	1.03	0.97	0.582
	Error	408.00	385	1.06		

Appendix A9 -- ANOVA tables of varied pH strawberry flavor experiment a. strawberry, b. sweetness descriptor, c. sourness descriptor, d. astringency descriptor

a. strawberry flavor

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	227.28	4	56.82	8.30	0.000
	Error	273.72	40	6.84		
Subject	Hypothesis	170.25	10	17.03	2.22	0.034
	Error	340.55	44	7.65		
Acid	Hypothesis	81.99	6	13.67	4.91	0.000
	Error	167.15	60	2.79		
Subject*pH	Hypothesis	273.72	40	6.84	3.46	0.000
	Error	474.15	240	1.98		
Acid*pH	Hypothesis	123.85	24	5.16	2.61	0.000
	Error	474.15	240	1.98		
Subject*Acid	Hypothesis	167.15	60	2.79	1.41	0.038
	Error	474.15	240	1.98		
Subject*pH*Acid	Hypothesis	474.15	240	1.98	1.08	0.258
	Error	706.00	385	1.83		

b. sweetness

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	751.13	4	187.78	23.91	0.000
	Error	314.13	40	7.85		
Subject	Hypothesis	310.79	10	31.08	3.23	0.003
	Error	490.95	51	9.62		
Acid	Hypothesis	463.55	6	77.26	19.83	0.000
	Error	233.73	60	3.90		
Subject*pH	Hypothesis	314.13	40	7.85	3.69	0.000
	Error	510.80	240	2.13		
Acid*pH	Hypothesis	175.34	24	7.31	3.43	0.000
	Error	510.80	240	2.13		
Subject*Acid	Hypothesis	233.73	60	3.90	1.83	0.001
	Error	510.80	240	2.13		
Subject*pH*Acid	Hypothesis	510.80	240	2.13	1.31	0.010
	Error	626.50	385	1.63		

c. sourness

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	2066.27	4	516.57	103.23	0.000
	Error	200.16	40	5.00		
Subject	Hypothesis	248.33	10	24.83	3.21	0.003
	Error	431.32	56	7.75		
Acid	Hypothesis	2075.02	6	345.84	68.20	0.000
	Error	304.24	60	5.07		
Subject*pH	Hypothesis	200.16	40	5.00	2.15	0.000
	Error	559.10	240	2.33		
Acid*pH	Hypothesis	300.07	24	12.50	5.37	0.000
	Error	559.10	240	2.33		
Subject*Acid	Hypothesis	304.24	60	5.07	2.18	0.000
	Error	559.10	240	2.33		
Subject*pH*Acid	Hypothesis	559.10	240	2.33	1.15	0.114
	Error	781.00	385	2.03		

d. astringency

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	881.77	4	220.44	36.08	0.000
	Error	244.40	40	6.11		
Subject	Hypothesis	749.95	10	75.00	10.72	0.000
	Error	332.94	48	6.99		
Acid	Hypothesis	381.40	6	63.57	27.96	0.000
	Error	136.43	60	2.27		
Subject*pH	Hypothesis	244.40	40	6.11	4.40	0.000
	Error	333.58	240	1.39		
Acid*pH	Hypothesis	83.45	24	3.48	2.50	0.000
	Error	333.58	240	1.39		
Subject*Acid	Hypothesis	136.43	60	2.27	1.64	0.005
	Error	333.58	240	1.39		
Subject*pH*Acid	Hypothesis	333.58	240	1.39	1.00	0.499
	Error	535.50	385	1.39		

Appendix A10 -- ANOVA tables of varied pH tropical flavor experiment a. tropical, b. sweetness descriptor, c. sourness descriptor, d. astringency descriptor

a. tropical flavor

Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	77.60	4	19.40	3.30	0.020
	Error	234.80	40	5.87		
Subject	Hypothesis	202.86	10	20.29	2.81	0.008
	Error	332.53	46	7.21		
Acid	Hypothesis	216.90	6	36.15	9.53	0.000
	Error	227.67	60	3.79		
Subject*pH	Hypothesis	234.80	40	5.87	2.39	0.000
	Error	589.40	240	2.46		
Acid*pH	Hypothesis	94.60	24	3.94	1.61	0.041
	Error	589.40	240	2.46		
Subject*Acid	Hypothesis	227.67	60	3.79	1.55	0.012
	Error	589.40	240	2.46		
Subject*pH*Acid	Hypothesis	589.40	240	2.46	0.93	0.720
	Error	1013.00	385	2.63		

b. sweetness

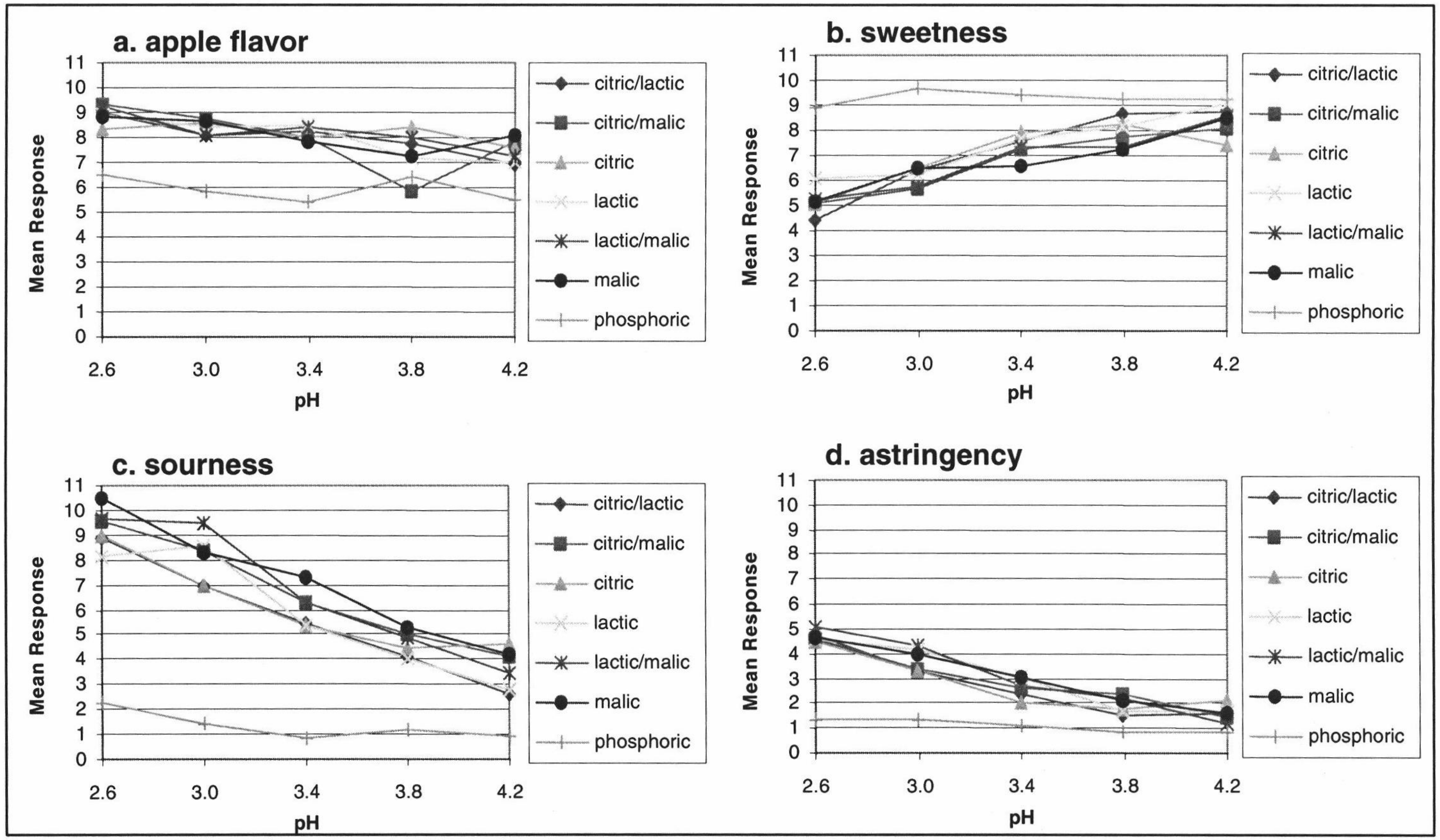
Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	705.59	4	176.40	16.55	0.000
	Error	426.26	40	10.66		
Subject	Hypothesis	762.01	10	76.20	5.45	0.000
	Error	832.24	59	13.99		
Acid	Hypothesis	389.96	6	64.99	12.67	0.000
	Error	307.66	60	5.13		
Subject*pH	Hypothesis	426.26	40	10.66	5.94	0.000
	Error	430.43	240	1.79		
Acid*pH	Hypothesis	146.52	24	6.10	3.40	0.000
	Error	430.43	240	1.79		
Subject*Acid	Hypothesis	307.66	60	5.13	2.86	0.000
	Error	430.43	240	1.79		
Subject*pH*Acid	Hypothesis	430.43	240	1.79	0.91	0.792
	Error	760.00	385	1.97		

c. sourness

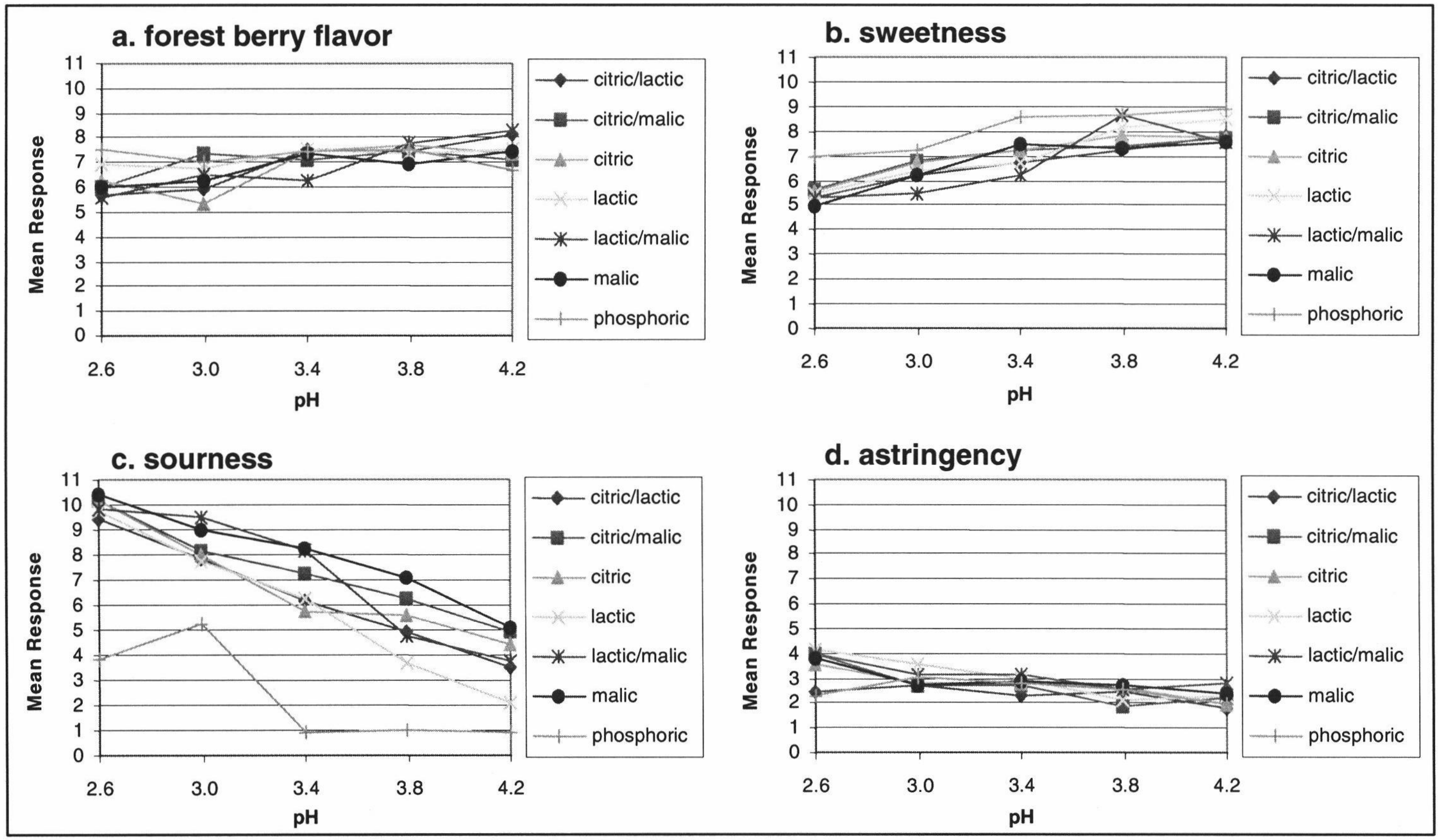
Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	1922.67	4	480.67	65.42	0.000
	Error	293.90	40	7.35		
Subject	Hypothesis	425.98	10	42.60	3.15	0.002
	Error	893.44	66	13.53		
Acid	Hypothesis	1609.23	6	268.21	29.38	0.000
	Error	547.77	60	9.13		
Subject*pH	Hypothesis	293.90	40	7.35	2.50	0.000
	Error	706.26	240	2.94		
Acid*pH	Hypothesis	314.17	24	13.09	4.45	0.000
	Error	706.26	240	2.94		
Subject*Acid	Hypothesis	547.77	60	9.13	3.10	0.000
	Error	706.26	240	2.94		
Subject*pH*Acid	Hypothesis	706.26	240	2.94	1.09	0.229
	Error	1040.50	385	2.70		

d. astringency

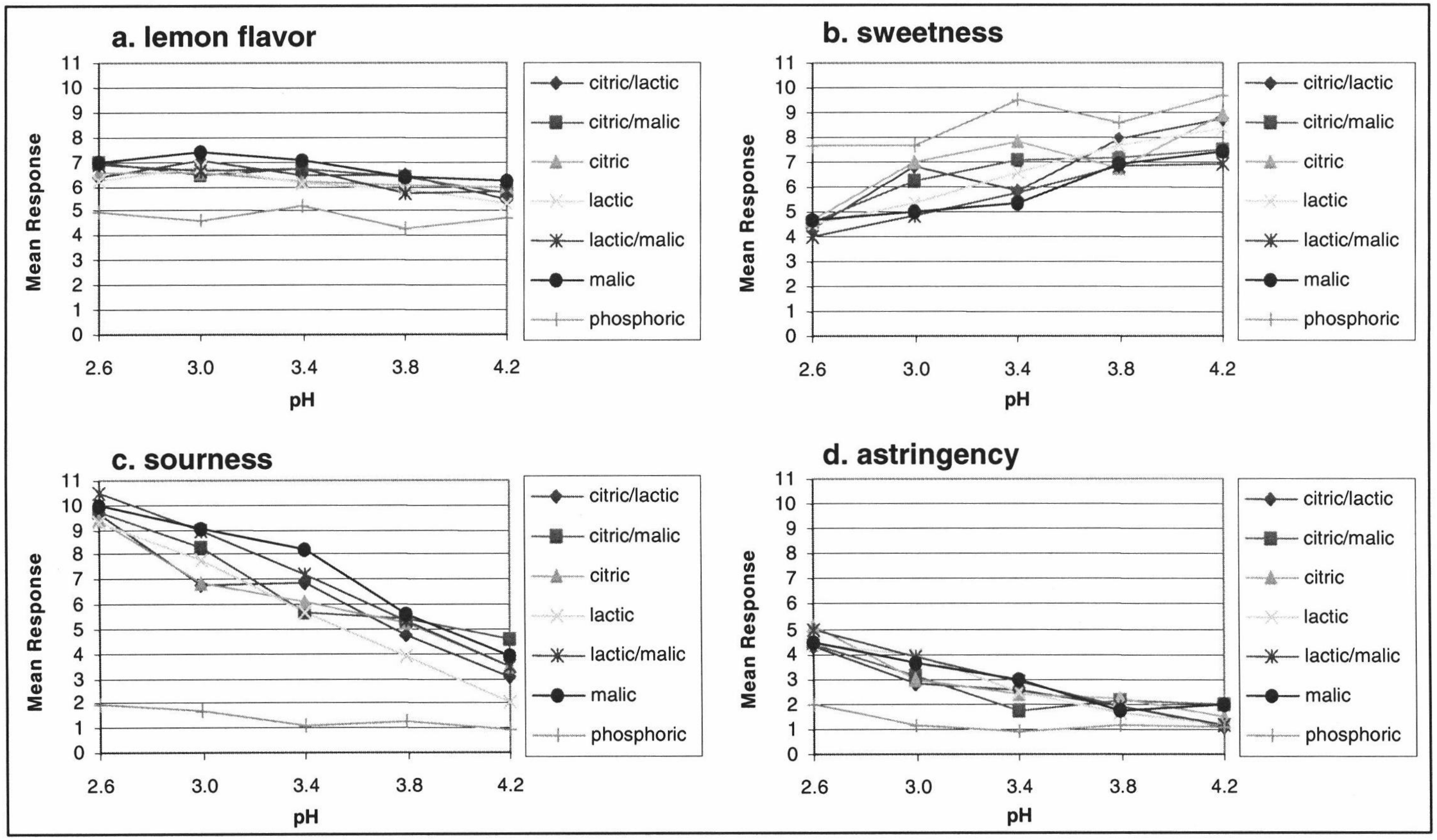
Source		Type III SS	df	MS	F	Sig.
pH	Hypothesis	653.32	4	163.33	21.77	0.000
	Error	300.08	40	7.50		
Subject	Hypothesis	1182.87	10	118.29	12.97	0.000
	Error	482.58	53	9.12		
Acid	Hypothesis	252.35	6	42.06	13.68	0.000
	Error	184.53	60	3.08		
Subject*pH	Hypothesis	300.08	40	7.50	5.16	0.000
	Error	349.06	240	1.45		
Acid*pH	Hypothesis	94.34	24	3.93	2.70	0.000
	Error	349.06	240	1.45		
Subject*Acid	Hypothesis	184.53	60	3.08	2.11	0.000
	Error	349.06	240	1.45		
Subject*pH*Acid	Hypothesis	349.06	240	1.45	1.11	0.186
	Error	505.50	385	1.31		



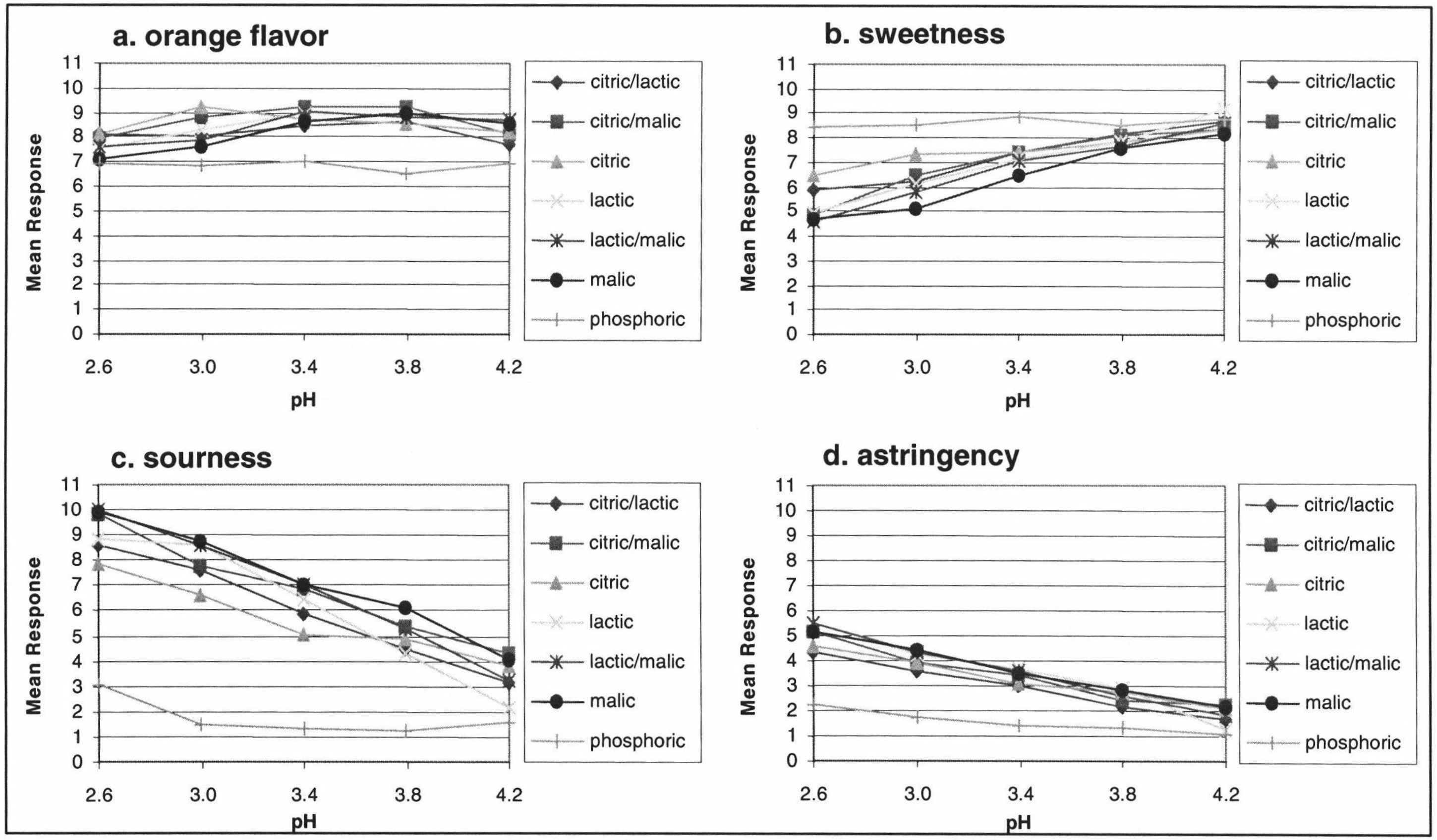
Appendix A11 -- Mean responses of a. apple flavor, b. sweetness, c. sourness, and d. astringency descriptor used in apple flavor experiment. Subjects used 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Differences less than 1.86 (MSE 2.267), 2.13 (MSE 2.572), 2.23 (MSE 3.139), and 1.43 (MSE 1.171) not significant for apple flavor, sweetness, sourness, and astringency, respectively (Tukey HSD, α = 0.05).



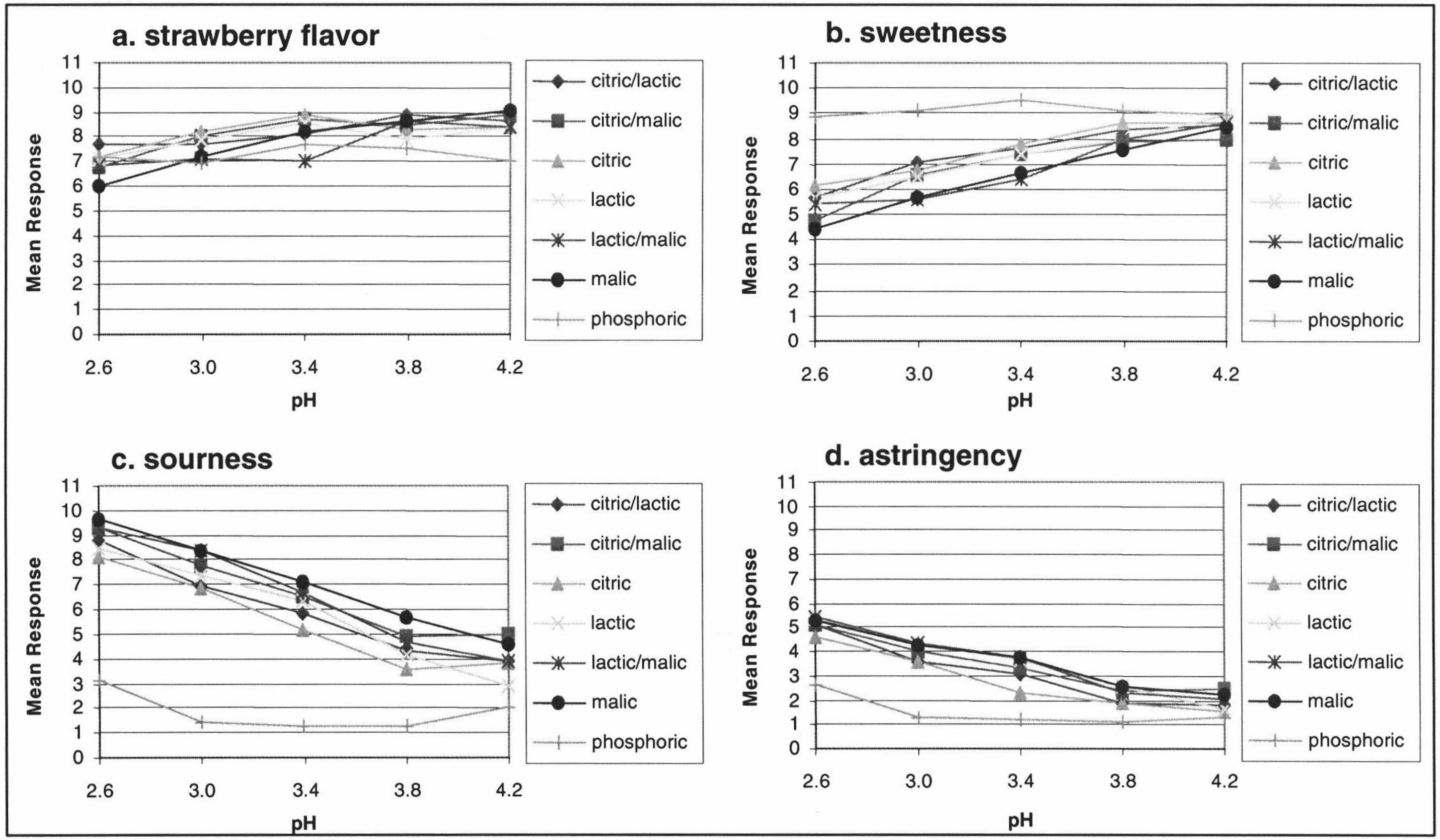
Appendix A12 -- Mean responses of a. forest berry flavor, b. sweetness, c. sourness, and d. astringency descriptor used in forest berry flavor experiment. Subjects used 16-point intensity scale . Differences less than 1.86 (MSE 2.602), 1.90 (MSE 2.348), and 2.14 (MSE 3.444), not significant for forest berry flavor, sweetness, and sourness, respectively (Tukey HSD, $\alpha = 0.05$). No significant differences found for astringency descriptor (MSE 3.223, Tukey HSD, $\alpha = 0.05$).



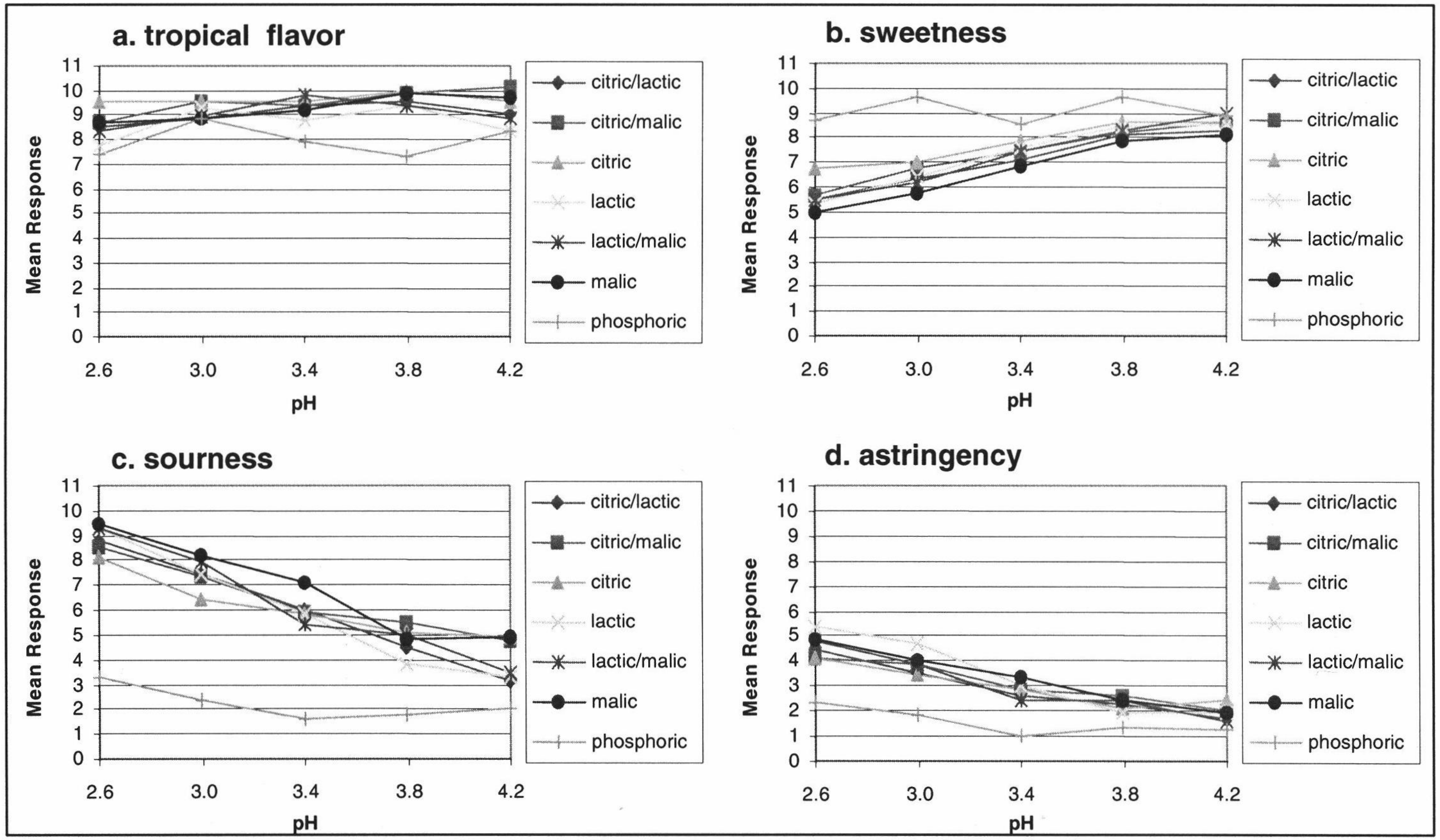
Appendix A13 -- Mean responses of a. lemon flavor, b. sweetness, c. sourness, and d. astringency descriptor used in lemon flavor experiment. Subjects used 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Differences less than 1.96 (MSE 2.080), 2.21 (MSE 3.407), and 1.46 (MSE 1.287) not significant for sweetness, sourness, and astringency, respectively (Tukey HSD, $\alpha = 0.05$). No significant differences found for lemon flavor descriptor (MSE 1.919, Tukey HSD, $\alpha = 0.05$).



Appendix A14 -- Mean responses of a. orange flavor, b. sweetness, c. sourness, and d. astringency descriptor used in orange flavor experiment. Subjects used 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Differences less than 1.75 (MSE 1.831), 1.83 (MSE 1.950), 1.88 (MSE 2.074), and 1.54 (MSE 1.050) not significant for orange flavor, sweetness, sourness, and astringency, respectively (Tukey HSD, $\alpha = 0.05$).



Appendix A15 -- Mean responses of a. strawberry flavor, b. sweetness, c. sourness, and d. astringency descriptor used in strawberry flavor experiment. Subjects used 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Differences less than 1.59 (MSE 1.888), 1.56 (MSE 1.820), 1.70 (MSE 2.144), and 1.39 (MSE 1.391) not significant for strawberry flavor, sweetness, sourness, and astringency, respectively (Tukey HSD, $\alpha = 0.05$).



Appendix A16 -- Mean responses of a. tropical flavor, b. sweetness, c. sourness, and d. astringency descriptor used in tropical flavor experiment. Subjects used 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Differences less than 1.60 (MSE 1.905), 1.93 (MSE 2.7905), and 1.35 (MSE 1.367) not significant for sweetness, sourness, and astringency, respectively (Tukey HSD, $\alpha = 0.05$). No significant differences found for tropical flavor descriptor (MSE 2.564, Tukey HSD, $\alpha = 0.05$).

Appendix A17 – Fruit flavor pH means averaged across acid for a. apple, b. forest berry, c. lemon, d. orange, e. strawberry, and f. tropical experiments

a. apple

	Descriptors			
pH	Apple	Sweetness	Sourness	Astringency
2.6	8.60 ^{c}	5.71 ^{a}	8.27 ^{e}	4.21 ^{d}
3.0	8.07 ^{b}	6.66 ^{b}	7.16 ^{d}	3.40 ^{c}
3.4	7.79 ^{b}	7.68 ^{c}	5.29 ^{c}	2.42 ^{b}
3.8	7.26 ^{a}	8.09 ^{cd}	4.14 ^{b}	1.80 ^{a}
4.2	7.17 ^{a}	8.53 ^{d}	3.24 ^{a}	1.51 ^{a}
MSE	6.457	11.224	6.781	6.081

^{abc} Means within a column with the same letter are not significanlty different at ? = 0.05, using Tukey HSD. Subjects used a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme).

b. forest berry

	Descriptors			
pH	Forest Berry	Sweetness	Sourness	Astringency
2.6	6.28 ^{a}	5.60 ^{a}	9.07 ^{e}	3.47 ^{c}
3.0	6.45 ^{a}	6.42 ^{b}	7.93 ^{d}	2.96 ^{bc}
3.4	7.24 ^{b}	7.15 ^{c}	6.12 ^{c}	2.79 ^{b}
3.8	7.46 ^{b}	7.86 ^{d}	4.74 ^{b}	2.40 ^{ab}
4.2	7.51 ^{b}	7.95 ^{d}	3.52 ^{a}	2.20 ^{a}
MSE	2.602	2.348	3.44	3.223

^{abc} Means within a column with the same letter are not significanlty different at ? = 0.05, using Tukey HSD. Subjects used a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme).

c. lemon

	Descriptors			
pH	Lemon	Sweetness	Sourness	Astringency
2.6	6.44 ^{b}	4.92 ^{a}	8.64 ^{e}	4.21 ^{e}
3.0	6.51 ^{b}	6.12 ^{b}	7.04 ^{d}	3.08 ^{d}
3.4	6.34 ^{b}	6.84 ^{c}	5.83 ^{c}	2.27 ^{c}
3.8	5.88 ^{a}	7.40 ^{d}	4.48 ^{b}	1.83 ^{b}
4.2	5.60 ^{a}	8.23 ^{e}	3.04 ^{a}	1.40 ^{a}
MSE	3.226	6.842	3.519	6.448

^{abc} Means within a column with the same letter are not significanlty different at ? = 0.05, using Tukey HSD. Subjects used a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme).

d. orange

	Descriptors			
pH	Orange	Sweetness	Sourness	Astringency
2.6	7.61 ^{a}	5.70 ^{a}	8.32 ^{e}	4.64 ^{e}
3.0	8.08 ^{b}	6.53 ^{b}	7.08 ^{d}	3.73 ^{d}
3.4	8.58 ^{d}	7.42 ^{c}	5.66 ^{c}	3.10 ^{c}
3.8	8.51 ^{cd}	7.95 ^{d}	4.54 ^{b}	2.44 ^{b}
4.2	8.10 ^{bc}	8.60 ^{e}	3.18 ^{a}	1.75 ^{a}
MSE	6.196	7.44	6.902	6.975

^{abc} Means within a column with the same letter are not significanlty different at ? = 0.05, using Tukey HSD. Subjects used a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme).

e. strawberry

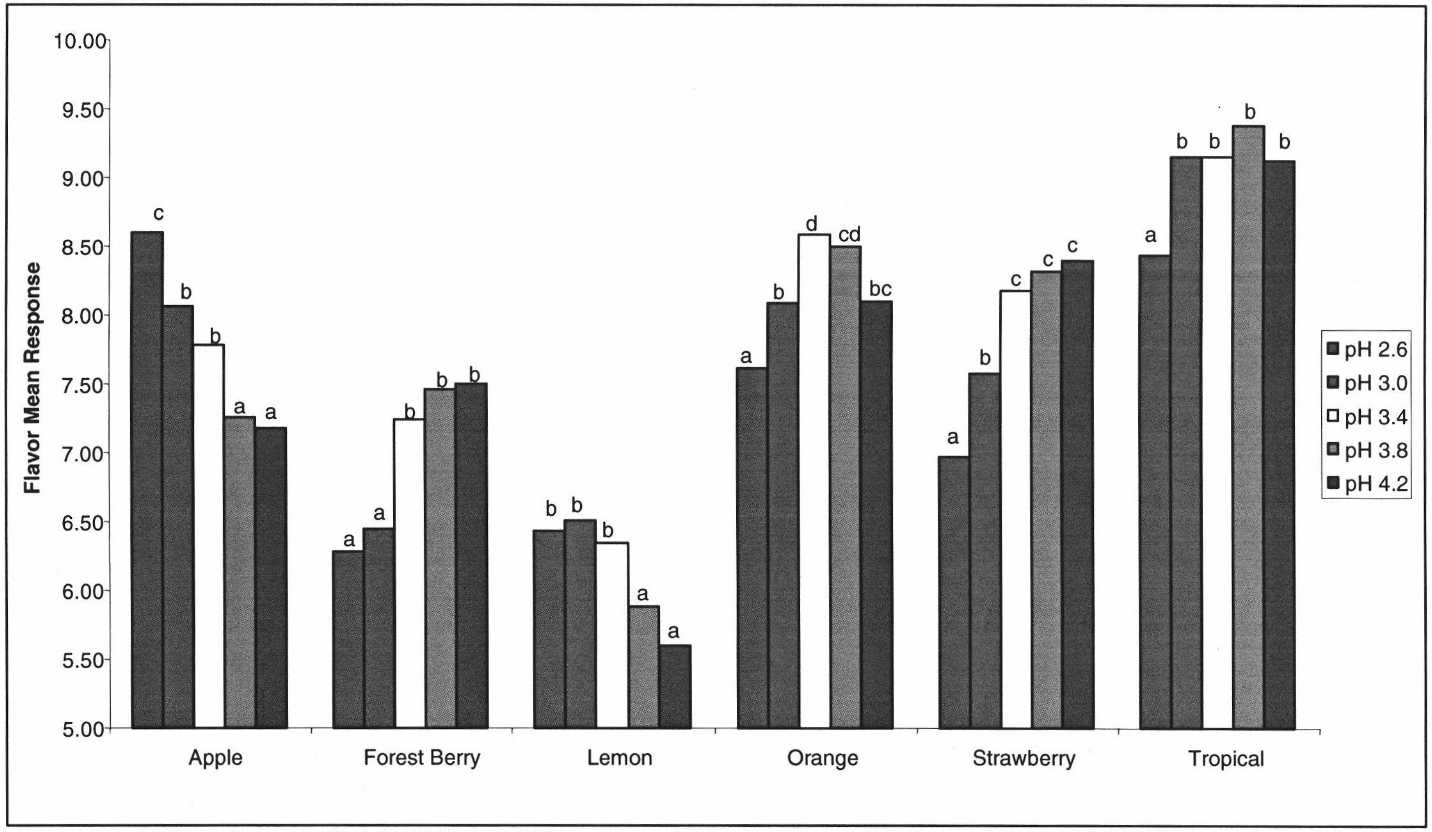
	Descriptors			
pH	Strawberry Flavor	Sweetness	Sourness	Astringency
2.6	6.97 ^{a}	5.86 ^{a}	8.11 ^{d}	4.81 ^{d}
3.0	7.58 ^{b}	6.73 ^{b}	6.73 ^{c}	3.62 ^{c}
3.4	8.18 ^{c}	7.54 ^{c}	5.55 ^{b}	3.00 ^{b}
3.8	8.32 ^{c}	8.21 ^{d}	4.08 ^{a}	2.12 ^{a}
4.2	8.40 ^{c}	8.57 ^{d}	3.72 ^{a}	1.84 ^{a}
MSE	1.888	1.820	2.144	1.391

^{abc} Means within a column with the same letter are not significanlty different at ? = 0.05, using Tukey HSD. Subjects used a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme).

f. tropical

	Descriptors			
pH	Tropical Flavor	Sweetness	Sourness	Astringency
2.6	8.42 ^{a}	6.06 ^{a}	8.12 ^{e}	4.30 ^{d}
3.0	9.15 ^{b}	6.88 ^{b}	6.73 ^{d}	3.60 ^{c}
3.4	9.16 ^{b}	7.56 ^{c}	5.40 ^{c}	2.58 ^{b}
3.8	9.34 ^{b}	8.47 ^{d}	4.35 ^{b}	2.16 ^{a}
4.2	9.13 ^{b}	8.58 ^{d}	3.79 ^{a}	1.83 ^{a}
MSE	2.564	1.905	2.795	1.367

^{abc} Means within a column with the same letter are not significanlty different at ? = 0.05, using Tukey HSD. Subjects used a 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme).



Appendix A18 -- Individual flavor pH mean responses averaged across acids using 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = large, 15 = extreme). Means within a flavor with the same letter are not significantly different $\alpha \leq 0.05$.

APPENDIX B MANOVA AND ANOVA TABLES FOR DATA SET EXAMINED IN CHAPTER 3

Appendix B1 -- MANOVA tables using Wilks' Lambda examining multivariate response for all descriptors used in a. lemon experiment, and b. strawberry experiment -- MANOVA tables using Wilks' Lambda examining multivariate response for all descriptors used in a. lemon experiment, and b. strawberry experiment

a. Lemon

Effect	Value	F	Hypothesis df	Error df	Sig.
Subject	0.028	24.321	40	616	0.000
Acid	0.604	11.619	8	324	0.000
pH	0.149	26.808	16	496	0.000
Subject * Acid	0.552	1.304	80	641	0.046
Subject * pH	0.18	2.175	160	648	0.000
Acid * pH	0.647	2.347	32	599	0.000
Acid * pH* Subject	0.162	1.17	320	650	0.049

b. strawberry

Effect	Value	F	Hypothesis df	Error df	Sig.
Subject	0.111	12.10	40	616	0.000
Acid	0.611	11.31	8	324	0.000
pH	0.168	24.52	16	496	0.000
Subject * Acid	0.553	1.30	80	641	0.048
Subject * pH	0.159	2.37	160	648	0.000
Acid * pH	0.750	1.52	32	599	0.034
Acid * pH* Subject	0.178	1.10	320	650	0.168

Appendix B2 -- ANOVA tables of lemon flavor experiment a. lemon, b. sweetness descriptor, c. sourness descriptor, d. astringency descriptor

a. lemon

Source		Type III SS	df	MS	F	Sig.
Acid	Hypothesis	31.28	2	15.64	9.64	0.00
	Error	32.46	20	1.62		
pH	Hypothesis	47.96	4	11.99	2.89	0.03
	Error	165.71	40	4.14		
Subject	Hypothesis	99.29	10	9.93	2.68	0.03
	Error	82.46	22	3.70		
Acid * pH	Hypothesis	6.66	8	0.83	0.40	0.92
	Error	165.27	80	2.07		
Subject * Acid	Hypothesis	32.46	20	1.62	0.79	0.72
	Error	165.27	80	2.07		
Subject * pH	Hypothesis	165.71	40	4.14	2.01	0.00
	Error	165.27	80	2.07		
Acid * pH* Subject	Hypothesis	165.27	80	2.07	1.12	0.28
	Error	305.50	165	1.85		

b. sweetness

Source		Type III SS	df	MS	F	Sig.
Acid	Hypothesis	76.84	2	38.42	7.662	0.00
	Error	100.29	20	5.02		
pH	Hypothesis	493.90	4	123.47	33.036	0.00
	Error	149.50	40	3.74		
Subject	Hypothesis	500.26	10	50.03	7.805	0.00
	Error	157.19	25	6.41		
Acid * pH	Hypothesis	78.79	8	9.85	4.204	0.00
	Error	187.41	80	2.34		
Subject * Acid	Hypothesis	100.29	20	5.02	2.141	0.01
	Error	187.41	80	2.34		
Subject * pH	Hypothesis	149.50	40	3.74	1.595	0.04
	Error	187.41	80	2.34		
Acid * pH* Subject	Hypothesis	187.41	80	2.34	1.259	0.11
	Error	307.00	165	1.86		

c. sourness

Source		Type III SS	df	MS	F	Sig.
Acid	Hypothesis	150.04	2	75.02	24.83	0.00
	Error	60.42	20	3.02		
pH	Hypothesis	1667.23	4	416.81	82.33	0.00
	Error	202.50	40	5.06		
Subject	Hypothesis	1143.56	10	114.36	25.25	0.00
	Error	73.99	16	4.53		
Acid * pH	Hypothesis	68.44	8	8.56	2.41	0.02
	Error	284.42	80	3.56		
Subject * Acid	Hypothesis	60.42	20	3.02	0.85	0.65
	Error	284.42	80	3.56		
Subject * pH	Hypothesis	202.50	40	5.06	1.42	0.09
	Error	284.42	80	3.56		
Acid * pH* Subject	Hypothesis	284.42	80	3.56	1.05	0.39
	Error	559.50	165	3.39		

d. astringency

Source		Type III SS	df	MS	F	Sig.
Acid	Hypothesis	2.62	2	1.31	1.06	0.37
	Error	24.78	20	1.24		
pH	Hypothesis	422.95	4	105.74	25.60	0.00
	Error	165.25	40	4.13		
Subject	Hypothesis	558.96	10	55.90	14.96	0.00
	Error	97.25	26	3.74		
Acid * pH	Hypothesis	32.96	8	4.12	2.52	0.02
	Error	130.64	80	1.63		
Subject * Acid	Hypothesis	24.78	20	1.24	0.76	0.75
	Error	130.64	80	1.63		
Subject * pH	Hypothesis	165.25	40	4.13	2.53	0.00
	Error	130.64	80	1.63		
Acid * pH* Subject	Hypothesis	130.64	80	1.63	1.22	0.15
	Error	221.00	165	1.34		

Appendix B3 -- ANOVA tables of strawberry flavor experiment a) strawberry, b) sweetness descriptor, c) sourness descriptor, d) astringency descriptor

a. strawberry

Source		Type III SS	df	MS	F	Sig.
Acid	Hypothesis	8.75	2	4.37	1.83	0.19
	Error	47.72	20	2.39		
pH	Hypothesis	149.08	4	37.27	8.76	0.00
	Error	170.26	40	4.26		
Subject	Hypothesis	94.81	10	9.48	1.97	0.08
	Error	143.44	30	4.82		
Acid * pH	Hypothesis	38.83	8	4.85	2.66	0.01
	Error	146.04	80	1.83		
Subject * Acid	Hypothesis	47.72	20	2.39	1.31	0.20
	Error	146.04	80	1.83		
Subject * pH	Hypothesis	170.26	40	4.26	2.33	0.00
	Error	146.04	80	1.83		
Acid * pH* Subject	Hypothesis	146.04	80	1.83	1.19	0.18
	Error	253.50	165	1.54		

b. sweetness

Source		Type III SS	df	MS	F	Sig.
Acid	Hypothesis	57.64	2	28.82	13.63	0.00
	Error	42.29	20	2.12		
pH	Hypothesis	450.38	4	112.60	24.936	0.00
	Error	180.62	40	4.52		
Subject	Hypothesis	179.47	10	17.95	4.071	0.00
	Error	107.79	24	4.41		
Acid * pH	Hypothesis	15.69	8	1.96	0.883	0.54
	Error	177.71	80	2.22		
Subject * Acid	Hypothesis	42.29	20	2.12	0.952	0.53
	Error	177.71	80	2.22		
Subject * pH	Hypothesis	180.62	40	4.52	2.033	0.00
	Error	177.71	80	2.22		
Acid * pH* Subject	Hypothesis	177.71	80	2.22	1.576	0.01
	Error	232.50	165	1.41		

c. sourness

Source		Type III SS	df	MS	F	Sig.
Acid	Hypothesis	147.17	2	73.59	29.11	0.00
	Error	50.56	20	2.53		
pH	Hypothesis	1142.10	4	285.53	72.00	0.00
	Error	158.63	40	3.97		
Subject	Hypothesis	133.86	10	13.39	2.94	0.01
	Error	123.86	27	4.55		
Acid * pH	Hypothesis	30.68	8	3.84	1.97	0.06
	Error	155.59	80	1.95		
Subject * Acid	Hypothesis	50.56	20	2.53	1.30	0.20
	Error	155.59	80	1.95		
Subject * pH	Hypothesis	158.63	40	3.97	2.04	0.00
	Error	155.59	80	1.95		
Acid * pH* Subject	Hypothesis	155.59	80	1.95	0.95	0.59
	Error	337.00	165	2.04		

d. astringency

Source		Type III SS	df	MS	F	Sig.
Acid	Hypothesis	45.48	2	22.74	16.37	0.00
	Error	27.79	20	1.39		
pH	Hypothesis	459.61	4	114.90	38.76	0.00
	Error	118.59	40	2.97		
Subject	Hypothesis	299.83	10	29.98	9.90	0.00
	Error	82.22	27	3.03		
Acid * pH	Hypothesis	10.43	8	1.30	0.98	0.46
	Error	105.97	80	1.33		
Subject * Acid	Hypothesis	27.79	20	1.39	1.05	0.42
	Error	105.97	80	1.33		
Subject * pH	Hypothesis	118.59	40	2.97	2.24	0.00
	Error	105.97	80	1.33		
Acid * pH* Subject	Hypothesis	105.97	80	1.33	0.71	0.96
	Error	308.00	165	1.87		

APPENDIX C MANOVA AND ANOVA TABLES FOR CHAPTER 4

Appendix C1 -- MANOVA tables for equi-sourness experiment using Wilks' Lambda examining multivariate response for all descriptors used in a. lemon experiment, and b. strawberry experiment

a. lemon

Effect	Value	F	Hypothesis	Error	Sig.
Subject	0.254	7.84	56	1041	0.000
Acid	0.95	1.75	8	534	0.085
Sourness Intensity	0.153	104.02	8	534	0.000
Subject*Acid	0.624	1.20	112	1063	0.090
Subject*Sourness	0.332	3.04	112	1063	0.000
Acid*Sourness Intensity	0.946	0.94	16	816	0.522
Subject*Sourness	0.564	0.74	224	1069	0.998

b. strawberry

Effect	Value	F	Hypothesis	Error	Sig.
Subject	0.224	8.72	56	1041	0.000
Acid	0.913	3.12	8	534	0.002
Sourness Intensity	0.375	42.32	8	534	0.000
Subject*Acid	0.658	1.06	112	1063	0.334
Subject*Sourness	0.341574	2.95	112	1063	0.000
Acid*Sourness Intensity	0.958368	0.72	16	816	0.780
Subject*Sourness	0.510728	0.87	224	1069	0.894

Appendix C2 -- ANOVA tables of equi-sourness lemon flavor experiment a. lemon, b. sweetness descriptor, c. saltiness descriptor, and d. astringency descriptor

a. lemon flavor

Source		Type III SS	df	MS	F	Sig.
Sourness Intensity	Hypothesis	1174.14	2	587.07	209.33	0.000
	Error	78.53	28	2.81		
Subject	Hypothesis	55.84	14	3.99	1.37	0.233
	Error	77.97	27	2.91		
Acid	Hypothesis	0.38	2	0.19	0.23	0.799
	Error	23.18	28	0.83		
Subject*Sourness Intensity	Hypothesis	78.53	28	2.81	3.862	0.000
	Error	40.67	56	0.73		
Acid*Sourness Intensity	Hypothesis	4.66	4	1.17	1.605	0.186
	Error	40.67	56	0.73		
Subject*Acid	Hypothesis	23.18	28	0.83	1.14	0.331
	Error	40.67	56	0.73		
Subject*Sourness Intensity*Acid	Hypothesis	40.67	56	0.73	0.735	0.917
	Error	266.67	270	0.99		

b. sweetness

Source		Type III SS	df	MS	F
Sourness Intensity	Hypothesis	274.42	2	137.21	51.74
	Error	74.25	28	2.65	
Subject	Hypothesis	125.87	14	8.99	3.10
	Error	86.66	30	2.90	
Acid	Hypothesis	1.91	2	0.96	1.18
	Error	22.76	28	0.81	
Subject*Sourness Intensity	Hypothesis	74.25	28	2.65	4.671
	Error	31.79	56	0.57	
Acid*Sourness Intensity	Hypothesis	3.32	4	0.83	1.461
	Error	31.79	56	0.57	
Subject*Acid	Hypothesis	22.76	28	0.81	1.432
	Error	31.79	56	0.57	
Subject*Sourness Intensity*Acid	Hypothesis	31.79	56	0.57	0.639
	Error	240.00	270	0.89	

c. saltiness

Source		Type III SS	df	MS	F	Sig.
Sourness Intensity	Hypothesis	0.03	2	0.02	0.24	0.790
	Error	2.04	28	0.07		
Subject	Hypothesis	1.90	14	0.14	1.36	0.228
	Error	3.34	33	0.10		
Acid	Hypothesis	0.15	2	0.08	1.45	0.251
	Error	1.48	28	0.05		
Subject*Sourness Intensity	Hypothesis	2.04	28	0.07	2.858	0.000
	Error	1.43	56	0.03		
Acid*Sourness Intensity	Hypothesis	0.05	4	0.01	0.533	0.712
	Error	1.43	56	0.03		
Subject*Acid	Hypothesis	1.48	28	0.05	2.069	0.010
	Error	1.43	56	0.03		
Subject*Sourness Intensity*Acid	Hypothesis	1.43	56	0.03	0.645	0.975
	Error	10.67	270	0.04		

d. astringency

Source		Type III SS	df	MS	F
Sourness Intensity	Hypothesis	183.50	2	91.75	26.141
	Error	98.28	28	3.51	
Subject	Hypothesis	186.12	14	13.30	3.116
	Error	148.10	35	4.27	
Acid	Hypothesis	5.72	2	2.86	1.968
	Error	40.72	28	1.45	
Subject*Sourness Intensity	Hypothesis	98.28	28	3.51	5.027
	Error	39.10	56	0.70	
Acid*Sourness Intensity	Hypothesis	5.12	4	1.28	1.834
	Error	39.10	56	0.70	
Subject*Acid	Hypothesis	40.72	28	1.45	2.083
	Error	39.10	56	0.70	
Subject*Sourness Intensity*Acid	Hypothesis	39.10	56	0.70	0.849
	Error	222.00	270	0.82	

Appendix C3 -- ANOVA tables of equi-sourness strawberry flavor experiment a. strawberry, b. sweetness descriptor, c. saltiness descriptor, and d. astringency descriptor

a. strawberry flavor

Source		Type III SS	df	MS	F	Sig.
Sourness Intensity	Hypothesis	720.64	2	360.32	45.11	0.000
	Error	223.66	28	7.99		
Subject	Hypothesis	152.02	14	10.86	1.35	0.244
	Error	217.51	27	8.05		
Acid	Hypothesis	18.68	2	9.34	6.22	0.006
	Error	42.06	28	1.50		
Subject*Sourness Intensity	Hypothesis	223.66	28	7.99	5.539	0.000
	Error	80.76	56	1.44		
Acid*Sourness Intensity	Hypothesis	3.17	4	0.79	0.55	0.700
	Error	80.76	56	1.44		
Subject*Acid	Hypothesis	42.06	28	1.50	1.042	0.436
	Error	80.76	56	1.44		
Subject*Sourness	Hypothesis	80.76	56	1.44	0.626	0.982
Intensity*Acid	Error	622.00	270	2.30		

b. sweetness

Source		Type III SS	df	MS	F
Sourness Intensity	Hypothesis	301.87	2	150.94	45.87
	Error	92.13	28	3.29	
Subject	Hypothesis	252.55	14	18.04	4.41
	Error	105.88	26	4.09	
Acid	Hypothesis	11.99	2	6.00	2.45
	Error	68.45	28	2.45	
Subject*Sourness Intensity	Hypothesis	92.13	28	3.29	2.005
	Error	91.89	56	1.64	
Acid*Sourness Intensity	Hypothesis	9.67	4	2.42	1.473
	Error	91.89	56	1.64	
Subject*Acid	Hypothesis	68.45	28	2.45	1.49
	Error	91.89	56	1.64	
Subject*Sourness	Hypothesis	91.89	56	1.64	0.928
Intensity*Acid	Error	477.33	270	1.77	

c. saltiness

Source		Type III SS	df	MS	F	Sig.
Sourness Intensity	Hypothesis	9.62	2	4.81	6.83	0.004
	Error	19.71	28	0.70		
Subject	Hypothesis	31.52	14	2.25	3.29	0.005
	Error	16.27	24	0.69		
Acid	Hypothesis	0.45	2	0.23	1.18	0.322
	Error	5.33	28	0.19		
Subject*Sourness Intensity	Hypothesis	19.71	28	0.70	3.367	0.000
	Error	11.71	56	0.21		
Acid*Sourness Intensity	Hypothesis	0.74	4	0.18	0.88	0.482
	Error	11.71	56	0.21		
Subject*Acid	Hypothesis	5.33	28	0.19	0.91	0.598
	Error	11.71	56	0.21		
Subject*Sourness	Hypothesis	11.71	56	0.21	0.777	0.872
Intensity*Acid	Error	72.67	270	0.27		

d. astringency

Source		Type III SS	df	MS	F
Sourness Intensity	Hypothesis	144.46	2	72.23	19.339
	Error	104.58	28	3.74	
Subject	Hypothesis	188.21	14	13.44	3.327
	Error	112.21	28	4.04	
Acid	Hypothesis	5.91	2	2.96	2.127
	Error	38.90	28	1.39	
Subject*Sourness Intensity	Hypothesis	104.58	28	3.74	3.448
	Error	60.67	56	1.08	
Acid*Sourness Intensity	Hypothesis	0.52	4	0.13	0.12
	Error	60.67	56	1.08	
Subject*Acid	Hypothesis	38.90	28	1.39	1.283
	Error	60.67	56	1.08	
Subject*Sourness	Hypothesis	60.67	56	1.08	0.94
Intensity*Acid	Error	311.33	270	1.15	