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Title: Flavor Characterization of Selected Acidulants in Model and Food

Systems

Abstract approved by: _____

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Acidulants can contribute considerably to the flavor of food.

However, limited research on differences in acid flavor exists. While

numerous researchers have studied acids, most have focused on sourness

exclusively. Acids have been shown previously to differ in non-sour

components, such as bitterness and astringency. A series of experiments

were conducted to determine flavor characteristics of selected acids in

different systems.

First, selected acids (lactic, malic, citric, acetic) and 2 acid blends

(lactic/acetic 1:1 and 2:1) in a model (water) system were evaluated on an

equivalent weight (0.2% w/v) and on an equivalent pH basis. Three pH

levels were explored: 3.5, 4.5 and 6.5. The technique of free-choice profiling was applied to characterize the flavor profile. Results analyzed by generalized procrustes analysis showed two significant findings: (1) acids differ in sensory character, and (2) the flavor of an acid changes with pH.

Second, selected acids (lactic, malic, citric, tartaric) were evaluated in sweetened, flavored drinks on an equivalent weight basis at two concentrations (0.4 and 0.6% w/v). Four flavors were evaluated: orange, cherry, cola and strawberry. Descriptive analysis was used, and the data was analyzed by principal component analysis. Significant differences were found on among acids and concentrations. For the orange flavor system, citric acid enhanced the orange flavor of the drink.

Third, selected acids (lactic, citric, acetic) and 2 acid blends (acetic/lactic 1:1 and 2:1) were evaluated in emulsions on an equivalent pH basis. Three pH levels were explored: 3.5, 4.5 and 5.5. These findings support the first study as differences were found among acids, and flavor changes existed when pH changed.

Flavor Characterization of Selected Acidulants in Model and Food Systems

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Flavor Characterization of Selected Acidulants in Model and Food Systems

1. INTRODUCTION

The importance of acids in the food industry is greatly underestimated (Blendford, 1986). Acids are one of the most functional groups of ingredients (Andres, 1985). They contribute a large variety of properties in food products. For example, acids can control pH, preserve food, provide leavening, aid in gel formation, prevent non-enzymatic browning, act as a synergist to antioxidants, chelate metal ions, and most importantly, add and enhance flavor in foods. Recently, acid salts are becoming popular with their use in foods as texture modifiers.

Acids are commonplace in nearly every food we consume. While they are formed naturally in fermented foods such as bread, beer, wine, sauerkraut, they are also in many formulated products, ie. jams, jellies, and preserves, bakery products, dairy products, meat products, beverages, and confectionery products. Many acidulants occur naturally in food and in cells of plants and animals. Thus, most are Generally Recognized as Safe (GRAS).

A new trend in the food industry today is the combining of more than one acid in a formulated food product. It is rare in nature to find one acidulant alone in a natural product. Thus, adding a combination of acids helps mimic the real flavor of a food. For example, in pickled products

acetic acid is being partially replaced by lactic acid in order to provide a milder, more subtle taste (Fabian & Wadsworth, 1939b).

While most acid research has focused exclusively on sourness, recent literature has indicated that acids have non-sour components as well (Rubico & McDaniel, 1992; Settle et al., 1986; Straub, 1992). Rubico & McDaniel (1993) profiled the flavor of 8 organic, 3 organic blends and 2 inorganic acids and concluded that acids have different flavor profiles. When formulating a food product, the desired pH level may have a large impact on the flavor the acid contributes.

This research was conducted to investigate the differences in the flavor profile of acids at different pH levels. By selecting pH levels currently found in food systems, the information on the differences in the flavor profiles of acids can be used in formulating acidified foods and selecting acidulants based on desired attributes in foods. While the first study represents the foundation for future work as the acids were evaluated in water (model study), the second and third studies involved evaluation of acids in more complex systems, ie. in a sweetened, flavored drink and in a protein emulsion. The objectives chosen to meet these goals were as follows:

Study 1:

•to investigate sensory characteristics of selected acids and acid blends by the use of free-choice profiling.

Study 2:

•to investigate sensory characteristics of sweetened, flavored drinks containing common food acidulants.

•to determine similarities and differences among the acids, ie. if certain acids can be substituted for each other in specific flavor systems.

•to determine how acids affect flavor in different flavor systems.

•to determine if acid concentration affects the flavor of the drinks

Study 3:

•to investigate sensory characteristics of protein emulsions containing common acidulants and acid blends.

•to determine similarities and differences among the different acid emulsions.

•to determine if the flavor of the protein emulsions change with pH.

2. LITERATURE REVIEW

2.1 Acids

Table 2.1 summarizes several key physical and chemical properties,

and Figure 2.1 shows the structures of selected acidulants.

2.1.1 Acetic

References to acetic acid, or methane carboxylic acid, can be found in

some of the earliest languages; it has been used in food products since

ancient times (Wagner, 1978). It is widely distributed in nature, and can be

found naturally in plant and animal tissues. Acetyl CoA is an important

substrate in human fatty acid and carbohydrate metabolism. Acetic acid is

GRAS as a direct food ingredient. No limit exists on the acceptable daily

intake for humans when produced with current good manufacturing

practices (GMP) (FDA, 1993a). It is commercially sold in 2 forms: as glacial

acetic acid and as vinegar (expressed as a percent acetic acid). The acetic acid

concentration of food grade vinegar found in retail stores is between 6 to 10

% (Sharrock, 1986).

Acetic acid can be produced by several methods and by a variety of

substrates. For example, acetic acid was first made by the oxidation of

Table 2.1 Key properties of acidulants

ACID	EMPERICAL FORMULA	MOLECULAR WEIGHT	EQUIVALENT WEIGHT	# OF COOH	pK_a†	IONIZATION CONSTANT*
ACETIC	$C_2H_4O_2$	60	60	1	4.75	2.0×10^{-5}[b]
CITRIC	$C_6H_8O_7$	192	64	3	3.14	82×10^{-5}[a]
					4.77	1.8×10^{-5}[a]
					6.39	0.4×10^{-5}[a]
LACTIC	$C_3H_6O_3$	90	90	1	3.08	14×10^{-5}[b]
MALIC	$C_4H_6O_5$	134	67	2	3.40	40×10^{-5}[a]
					5.11	0.9×10^{-5}[a]
TARTARIC	$C_4H_6O_6$	150	75	2	2.98	104×10^{-5}[a]
					4.34	4.6×10^{-5}[a]

†Blocher and Busta, 1983

*K_a- denotes the first dissociation constant, a measure of the extent an acid produces hydrogen ions when dissolved in water ([a]Gardner, 1966; [b]Arnold, 1975)

Figure 2.1-Chemical structures of acidulants

ACETIC

CH3-COOH

CITRIC

CH₂-COOH
|
HO-C-COOH
|
CH₂COOH

LACTIC

H
|
CH3-C-COOH
|
OH

MALIC

H
|
HO-C-COOH
|
H-C-COOH
|
H

TARTARIC

H
|
HO-C-COOH
|
HO-C-COOH
|
H
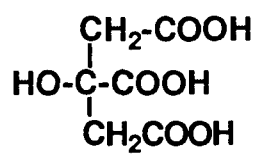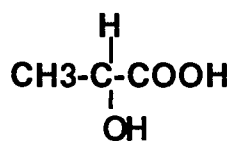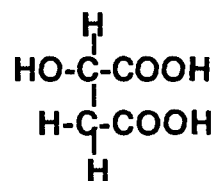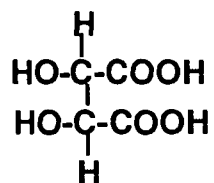
alcohol in the presence of bacterium *Acetobacter aceti* (Arnold, 1975):

$$CH_3CH_2OH + O_2 \text{ --------------> } CH_3COOH + H_2O$$
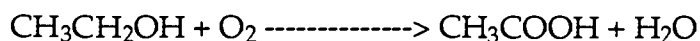
Another way acetic acid can be made is from pyroligneous acid, the aqueous fraction of the destructive distillation of wood (Arnold, 1975). Acetic acid can be synthesized from acetylene in the presence of water with a mercury catalyst to produce acetaldehyde, and then oxidation with air using a manganese catalyst to form acetic acid (Arnold, 1975):

$$CHCH + H_2O \text{ --------------> } CH_3CHO$$

$$CH_3CHO + 1/2\ O_2 \text{ --------------> } CH_3COOH$$
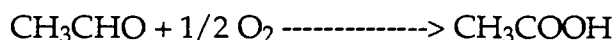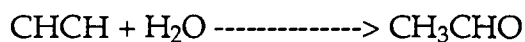
Alternately, acetic acid can be synthesized by the reaction of methanol and carbon monoxide (Arnold, 1975):

$$CH_3OH + CO \text{ --------------> } CH_3COOH$$
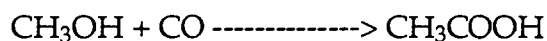
Generally, the most common method for making acetic acid and vinegar today is based on fermentation. Using specific bacterial strains of genera *Acetobacter* and *Acetomonas*, acetic acid is commercially produced from alcohol via fermentation of substrates such as grapes, grains, and apples (Dziezak, 1990).

Acetic acid performs 2 key functions in food products: as an acidifier and as a flavoring agent. Vinegar performs many more. Vinegar (with its primary component acetic acid) can serve to lower the pH, to control growth of microorganisms (specifically, bacterial and yeast growth), to

enhance flavors, as a curing and pickling agent, and as a flavoring agent and adjunct (Doores, 1990; Dziezak, 1990). Acetic acid (and/or vinegar) can be found in products such as pickles as a pickling agent (Arnold, 1975), canned vegetables as a preservative (FDA, 1993a), condiments ie., mayonnaise, catsup, and mustard to impact flavor and as a preservative (Arnold, 1975; Garibaldi, 1968; Emulsol Corp., 1937), salad dressings for flavor and as a preservative (Emulsol Corp., 1937), bread as a fungicidal agent (Wagner, 1978), marinades for meat, poultry, and fish for flavor, bakery products, stews, soups, and cheese (Dziezak, 1990). The main reasons acetic acid is so extensively used in foods as a food preservative is due to its toxic effect on microorganisms, its commercial availability, and its low cost (Levine & Fellers, 1939). Sodium diacetate (often called "salt 'n' vinegar") is widely used in crisps and starch extrusion products (Sharrock, 1986).

The aroma of acetic acid separates it from all other acids. It is a weak acid with a high boiling point (118°C), making it extremely volatile. It has a strong pungent, vinegary aroma, a burning taste, and is astringent (Arnold, 1975; Johnson & Peterson, 1974; Wagner, 1978). Acetic acid as well as its salts are very miscible in water. The safety and health issue to be concerned about with acetic acid solutions is that it can be strongly

corrosive to the skin, and cause irreparable scarring of tissues of the eyes, nose, or mouth (Wagner, 1978).

2.1.2 Citric

Citric acid, or propan-2-ol-1,2,3-tricarboxylic acid, is also very widely distributed in nature. It is a natural constituent and metabolite for animals and plants. In plants, citric acid is the predominant acid and is found in the most abundance in citrus fruits, such as lemons (4.0-8.0%), grapefruits (1.2-2.1%), tangerines (0.9-1.2%), and oranges (0.6-1.0%) (Bouchard & Merritt, 1978). Citric acid is produced during the Krebs cycle in humans. Human blood normally contains ~25 ppm of citric acid (Arnold, 1975). It is GRAS as a multipurpose food additive and as a sequestrant, and no limit exists on the acceptable daily intake for humans when produced with GMP (FDA, 1993b; FDA, 1993c). Citric acid is sold commercially in 2 forms: anhydrous and monohydrate.

Citric acid is produced generally in 2 ways by various substrates through mycological fermentation. It can be produced by the fermentation of pretreated molasses solutions with *Aspergilli niger* (Bouchard and Merritt, 1978). Molasses is the preferred source of sugar due to its relatively low cost. Alternately, it can be produced from lemons and from canned pineapple waste. The principle behind the fermentation is based on cultivating the microorganism under unfavorable conditions in order for

the Krebs cycle to be stopped and for citric acid to accumulate (Arnold, 1975).

Citric acid is the most widely used and accepted organic acid in foods (Andres, 1985; Bouchard & Merrott, 1978; Dziezak, 1990). It is often the standard for comparison to other acids in food products (Gardner, 1972; Sanders, 1966). Citric acid is also considered one of the most versatile acidulants as it can perform a wide range of functions in foods (Arnold, 1975). The bulk of citric acid is used in non-alcoholic beverages. Citric acid can also be used to cure meats (Gardner, 1972), to adjust pH, prevent cloudiness, and inhibit oxidation in wine (Amerine et al., 1965; Gardner, 1972), to control pH for optimum gel formation and serve as a flavoring agent in jams, jellies, fruit butters, and preserves (Bouchard and Merritt, 1978), to reduce the amount of inversion, control browning, prevent oxidation of ingredients, and impart a sour flavor (Porter, 1985; Stuckey, 1954), optimize stability of frozen foods by enhancing action of antioxidants and inactivating enzymes (Bouchard & Merritt, 1978), and to impart a tangy citrus flavor to carbonated and still beverages (Miles Laboratories, 1984). Citric acid is the acidulant of choice in soft drinks due to its natural tang and rapid solubility (Bouchard & Merritt, 1978). It is also a valuable acidulant for dairy products. For example, sodium citrate is used in processed cheese to prevent fat separation and impact flexibility (Bouchard

& Merritt, 1978). It is also used in whipping cream and vegetable-based dairy substances as an important stabilizer. Sodium and potassium citrates, widely used as buffers, are often added with the parent acid to protect foods from direct acidifications.

Citric acid possesses a powerful sequestering action on heavy metals. It can act as a valuable antioxidant synergist and aid in inhibiting flavor and color deterioration in a wide range of foods (Arnold, 1975). One advantage of citric acid over others is that it is not optically active. This eliminates problems with nutritional value altering with different enantiomers as with other acids. However, citric acid is very hygroscopic, which can create problems in powdered products (Doores, 1990).

The flavor profile of citric acid is different than others in that it delivers a burst, or a quick initial impact, of sourness (Dziezak, 1990; Pszczola, 1988). The tartness of citric acid has been described as clean (Johnson & Peterson, 1974). It is a relatively strong organic acid with high solubility in water, while its calcium salt is only sparingly soluble (Arnold, 1975).

2.1.3 Lactic

Lactic acid, or 2-hydroxypropanoic acid, is one of the earliest used acidulants in foods (Gardner, 1972). It is widely distributed in food in the L(+)-form from lactic acid fermentation, and was originally isolated from

sour milk and meat (Holten et al., 1971). It is typically the endproduct of anaerobic carbohydrate metabolism. Tired muscles often accumulate large quantities of this acid. Lactic acid is GRAS as a direct food ingredient, and no limit exists on the acceptable daily intake for humans when produced with GMP (FDA, 1993d). Lactic acid and its salts are completely non-toxic (Holten et al., 1971).

Lactic acid can be purchased in a range of concentrations: 50% to 90%, depending upon the manufacturer. It is commercially sold as a syrupy, colorless aqueous liquid in either a racemic mixture (DL) or L(+). Lactic acid can be produced by several methods. The most common one is by anaerobic thermophilic fermentation of a carbohydrate substrate with lactic acid bacteria. To keep the pH up chalk or limestone is added, which produces a crude crystalline calcium lactate. Sulfuric acid is then added to decompose the salt, and lactic acid is produced after a purification and concentration step (Arnold, 1975; Purac, 1989). The most common carbohydrate substrate is refined sugar, while other processes can use less expensive carbohydrates, ie., potato starch, molasses, corn sugar, or milk whey (Gardner, 1972). A synthetic method for making lactic acid is from the by-product of the manufacture of acrylonitrile, acetaldehyde cyanohydrin (lactonitrile) (Anonymous, 1964; Arnold, 1975):

$$CHCH + HCN \text{ --------------} > CH_2CHCN$$

$$CHCH + HCN + H_2O \text{ -------------> } CH_3CHOHCN$$
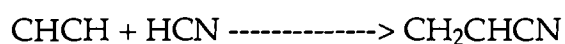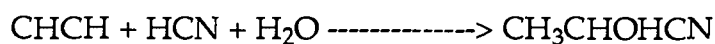
Lactic acid can also be made synthetically from propylene by ammonoxidation in which hydrogen cyanide is the by-product (Arnold, 1975):

$$CH_2CHCH_3 + NH_3 + 1\ 1/2\ O_2 \text{ -------------> } CH_2CHCN + 3H_2O$$

$$CH_2CHCN + NH_3 + 1\ 1/2\ O_2 \text{ -------------> } 2HCN + CO + 2H_2O$$
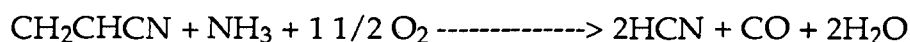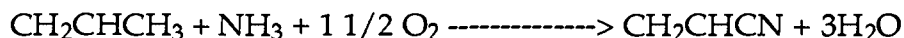
The hydrogen cyanide product from either synthesis method is then combined with acetaldehyde:

$$CH_3CHO + HCN \text{ -------------> } CH_3CHOHCN$$
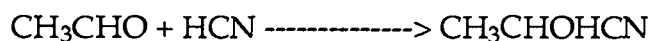
For either synthetic process in the final step the steam hydrolyses the nitrile into lactic acid:

$$CH_3CHOHCN + 2H_2O \text{ -------------> } CH_3CHOHCOOH + NH_3$$

Lactic acid is a speciality acid with some very specific markets (Arnold, 1975). It is more commonly used for its sensory qualities than its antimicrobial properties (Doores, 1990). The action of lactic acid is bacteriostatic and not generally bactercidal (Holten et al., 1971). Lactic acid is added to food products to adjust acidity and improve whipping properties of pan-dried egg whites (Holten et al., 1971; Van Ness, 1981), to provide sour flavor and to preserve in imitation dairy products (Anon., 1985), to ensure clarity of the brine in spanish-type olives (Anon., 1964; Doores, 1990), to adjust pH during mashing and wort cooking in beer

(Anon., 1985), to improve digestibility of baby food with the L-form (Purac, 1989), as a pickling agent (when used with vinegar) in sauerkraut and pickles (Van Ness, 1981), and to provide a mild, tart flavor without masking that of natural fruit in frozen dairy desserts and beverages (Purac, 1989). Lactic acid is the most widely used organic acid in meat products due to its mild acid taste and preservative effect (Purac, 1989). It is the natural sour dough acid in rye and rye-wheat breads (Anon., 1985). Lactic acid is also irreplaceable as a controller of fermentation in olive and onion treatment before fermentation (Arnold, 1978). Buffered lactic acid is used in high-boiled sweets, fruit gums and jellies to eliminate risk of sucrose inversion which causes stickiness (Vreeman, 1986). Lactic acid is more suitable in hard candies than citric acid due to its liquid form (making it easier to mix) and its lower inversion rate. Lactic acid is often added with its sodium salt for production of high boilings, without increased risk of sucrose inversion and crystal formation.

Calcium lactate is used to preserve the firmness of apple slices during processing, inhibit browning of fruits and vegetables, and as a gelling agent for pectins in jams and jellies (Purac, 1989). Sodium lactate is used in meat products, such as sausages and hams, to lower water activity, which thereby improves microbial stability (Purac, 1989). Calcium and Sodium stearoyl lactylates are used as emulsifiers and dough conditioners (protein

plasticizers) in the baking industry (Arnold, 1978; Anon., 1985).

Lactic acid has unique physical properties. It readily forms self-condensates (internal esters), even in aqueous solutions. When lactic is heated, α-hydroxyl group of one molecule and carboxyl of another dehydrate and form a series of polylactic acids, such as lactyllactic acid, linear trimer, and higher polymers. The relative amount of the different species are a function of temperature. These occur in all solutions that are more than 18% lactic acid (Gardner, 1972). Monomeric lactic acid is reformed upon dilution with water.

Lactic acid is also extremely hygroscopic, thus resulting in a liquid product. Lactic acid and its salts are completely miscible with water and alcohol. Lactic acid is also very stable to heat.

With its low volatility, the relatively weak aroma of lactic acid has been described as mild and creamy (Doores, 1990). Arnold (1975) described the aroma as slightly acrid. The flavor of lactic acid has commonly been described as mild and fruity, while at the same time not masking the aroma of weaker aromatic flavors.

2.1.4 Malic

Malic acid, or ethan-1-ol-1,2-dicarboxylic acid, is very similar to citric acid. It is widely distributed in nature, involved in the Krebs cycle, and a major fruit acidulant. It is the principal fruit acid in apples, watermelons,

plums, cherries, peaches, and bananas. Malic acid is GRAS as a direct food ingredient, and no limit exists on the acceptable daily intake for humans when produced with GMP except in baby food (FDA, 1993e). While the L-form is what nature creates, malic is commercially sold in the DL-form as a white, odorless, crystalline powder in various granular sizes.

Only one key process is commercially used for producing malic acid. The process involves heating maleic anhydride with water under pressure at around 180°C. The result is an equilibrium mixture of malic and fumaric acids. The sparingly soluble fumaric acid is filtered off and recycled, and malic acid is concentrated and cystallized (Arnold, 1975).

Malic acid is a relative newcomer in the food industry as it was commercially introduced in 1965 (Arnold, 1975). However, it is considered more versatile than citric acid (Arnold, 1975). For example, malic acid is naturally anhydrous, making it more suitable than citric acid for powder mixtures. Malic acid is added in food products to provide tartness and lower pH in carbonated and still beverages and powdered drink mixes (Berger, 1981), to provide tartness and pH control in jellies, jams preserves, canned fruits, vegetables, salad dressings, and desserts (Berger, 1981a), to sequester heavy metals, provide pH control, preserve flavor, and aid in gel structure in gelatin desserts (Andres, 1985). It has been found to be more effective than other acidulants with sweetening agents, ie. reduced amount

of aspartame needed with malic acid versus other organic acids.

Malic acid has distinct sensory properties. It has flavor-fixing qualities and the ability to retain some tastes longer. Malic acid can be described as having a smooth, tart taste which builds up and disappears gradually without a burst in flavor like citric acid (Bisle, 1977; Johnson and Peterson, 1974). While their ionization strength is similar, malic acid has a stronger apparent acidity than citric acid (Gardner, 1966). Therefore, in some products smaller amounts can be used relative to citric acid to achieve the same taste effect.

While on a microbial level malic acid doesn't perform very differently from other acids, its physical properties do differ. Malic acid has a very low melting point (130°C), which is desirable in viscous materials at high temperatures ie. hard candies (Gardner, 1966). It has low hygroscopicity, and its calcium salts are more soluble than citric or tartaric salts.

2.1.5 Tartaric

Tartaric acid, or ethan-1,2-diol-1,2-dicarboxylic acid, is rarely found in nature. It is the first acidulant to be used in significant quantities by the food industry (Sharrock, 1986). Sources are the juice of grapes and a few other fruits and plants. It is poorly (if at all) absorbed by the mammalian gut. Nature produces the dextrarotary (+) form, and the commercial

product is L (+). It racemises with great ease, so a certain amount is always present. Tartaric acid is GRAS as a direct food ingredient, and no limit exists on the acceptable daily intake when produced with GMP (FDA, 1993f). It is commercially sold as an white, crystalline, odorless powder.

Tartaric acid is produced from by-products of wine production. The waste products consist of : (1) press cakes from unfermented or partially fermented grape juice, (2) lees (dried slimy sedimentes from wine vats), and (3) argols (crystalline crusts from wine vats used in 2nd fermentation) (Berger, 1981b). While there are several ways to purify and separate the insoluble tartarates, the basic procedure includes taking the raw material and autoclaving or gently roasting to destroy interferring organic matter. Then the material is ground, slurried with water, neutralised with lime, and then treated with gypsum or calcium chloride (Arnold, 1975):

$$2KHT + Ca(OH)_2 + CaSO_4 \text{-----------} > 2CaT + K_2SO_4 + 2H_2O$$
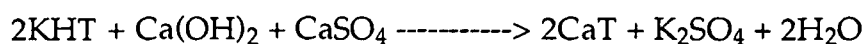
(T = tartaric acid residue)

When the mixture is cooled, it is filtered and then the filtrate is worked-up with potassium sulfate. This is then washed and decomposed with dilute sulfuric acid, and the filtered solution is evaporated and crystallized. Methods either recover the acid, cream of tartar, or Rochelle salt ($KNaC_4H4O_6 \cdot 4H_20$) (Gardner, 1972).

A synthetic method for producing tartaric acid is from maleic

anhydride, where it is oxidized in an aqueous solution by hydrogen

peroxide in the presence of a catalyst (Arnold, 1975):

```
CHCO\                          CHOHCOOH
 | |     O + H2O + H2O2 ----------> |
CHCO/                          CHOHCOOH
```
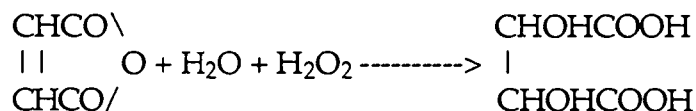
Tartaric acid is another versatile acid like citric and malic acids. It

was the dominant acidulant over a century ago. However, it has been

priced out of the market, and it has only survived in the countries where it

is produced, ie. Italy, Spain, and France (Arnold, 1975). In most countries,

it has been completely replaced by citric acid. On the other hand, cream of

tartar is still very popular in most countries.

Tartaric acid has 3 key functions: as an acidulant, a leavening agent,

and a flavor adjunct. It is seldom used as a microbial agent. Due to its

effect on flavor, tartaric acid is widely used in grape- and lime-flavored

beverages (Anon., 1962). Also, it is the acidulant of choice for grape-

flavored and for tart-tasting jams, jellies, and candies (Gardner, 1972). It

and its acidic monosodium salt, cream of tartar, are used to modify the

flow properties of candy mass as it is being cast (Dziesak, 1990). They are

also both common ingredients of baking powders and leavening systems.

Potassium acid tartrate, sodium potassium tartrate, choline bitartrate, and

diacetyl tartaric acid also serve many functions in food.

Tartaric acid is the most water-soluble of the solid acidulants. It is a strong acid, and it crystallizes anhydrous. The calcium salt is only sparingly soluble. It is moderately hygroscopic. Tartaric acid contributes a strong, tart taste which enhances fruit flavors (Dziesak, 1990; Doores, 1990).

2.1.6 Lactic/Acetic Combinations

In nature foods are not confined to any one acid. For example, oranges naturally contain citric, malic and quinic acids, and apples contain malic, quinic, lactic and succinic acids just to name a few. Another example is with fermented products, such as sauerkraut, buttermilk, genuine dill pickles. These products all contain lactic and acetic acids. It is the combination of acids (in addition to the traces of many other compounds) that is responsible for the flavor. Food companies are starting to create products today using a combination of acids to simulate the real taste of natural products, ie. using malic and citric acids in a fruit-flavored soft drink.

While lactic acid is the principal acid produced in many fermented products, Fabian and Wadsworth (1939a) found it is not suitable in making sour pickles when used alone as it does not possess sufficient germicidal value. When lactic acid was added to acetic acid in sweet pickles, relish, and processed dill pickles, Fabian and Wadsworth (1939b) found an improvement in flavor. The combination of lactic and acetic acids in

processed dill pickles produced a better flavored product than either acid
did alone. The improvement in flavor due to adding lactic acid to acetic
acid (or vinegar) was described as milder, more subtle.

From a microbial standpoint, Doores (1990) reported that lactic and
acetic acids work synergistically together in salad dressings and marinades,
especially inhibitory to outgrowth of heterofermentative lactobacilli. This
combination was more effective than either acid separately. Besides
products such as salad dressings and pickle brines, lactic and acetic acid
combinations have become popular in wine and fruit gums (Vreeman,
1986).

<u>2.2 Taste Perception</u>

Throughout history, acids have been described as predominantly
sour. Most research on acids has focused exclusively on sourness.
However, several studies have reported that acids have non-sour
components as well (Rubico & McDaniel, 1993; Settle et al., 1986; Straub,
1992). Settle et al. (1986) found that not only the intensity of the sourness
but the intensity of the saltiness and bitterness was significantly different
among 4 organic and 3 inorganic acids. Straub (1992) found significant
differences in sourness and astringency time-intensity curves for 6 organic
and 2 inorganic acids. Rubico & McDaniel (1993) reported differences in

the flavor profiles of 9 organic and 2 inorganic acids on astringency, bitterness, and citrus character. Croizer (1920) reported acids eliciting astringency at subthresholds levels for sourness. Realizing the extreme difficulty of representing the sour taste without other non-sour components present, the issue of using standards for sourness creates a difficult situation. The acid chosen for the reference standard of sourness may significantly affect the taste test results.

Another difficult problem in taste research with acids is the presence of olfactory and/or trigeminal retronasal sensations. In some situations, nasal detection may be responsible for supposed differences found in responsiveness to taste stimuli (Settle et al., 1986). For example, acetic acid is extremely volatile; therefore, comparing the flavor profile versus citric, malic, tartaric, and/or lactic acids which possess a lack of, or minimal volatility, creates a problem. Acetic acid will be identified by its intense aroma, and incredibly consistent data will result compared to other acids (CoSeteng et al., 1989). Baker et al. (1958) reported that panelists vary in their sensitivities to acids.

The four basic tastes (sweet, sour, salty and bitter) are often confused by panelists. Relatively high inaccuracies have been reported in the assignment of sour and bitter labels, and experience with different

compounds rather than feedback has improved performance (McAuliffe &
Meiselman, 1974).

2.2.1 Sourness

While the perception of acid sourness has been researched
extensively, the mechanism is still unclear. Many researchers have
theorized on the influencing factors of sourness, but none have been fully
accepted.

When acids are dissolved in water, they dissociate into ions:

$$HA <-------> H^+ + A^-$$
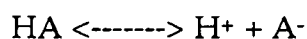
[where HA is the undissociated acid molecule, H^+ is the H ion, A^- is the
acid anion]

Organic acids, being weak acids by nature, do not completely
dissociate. The stronger the acid, the more completely it will dissociate
into its ions. Acid dissociation constants are used to measure this
relationship and are based on the following equation:

$$K_a = [H^+] [A^-] / [HA]$$

Researchers have suggested that acid sourness may be related to
physiological reactions at the receptor sites. Early work was based on cell
pentration by acids. The H ion was assumed to be responsible for eliciting
sour taste. Crozier (1920) found sourness intensity to be related to
ionization (H ion) strength of acids and cell penetration rate when

studying acetic, butyric, and hydrochloric acids. Studying relative permeability of 9 acids, Taylor et al. (1930) evaluated effect of various substituents on the acid molecule. It was assumed that only the undissociated acid molecules could pass through the membrane and physiological stimulation of sourness perception was due to the H ion. They demonstrated that all equi-sour acid solutions produce the same pH witin the interior of the cell. Ganzevles and Kroeze (1987a) suggested that the H ion receptor process is independent of the receptor process for undissociated acid. After studying 7 acids, they found that the sourness from hydrochloric acid and the sourness from weak carboxylic acids (ie. citric acid) are elicited by different receptor processes. These results were supported by Ganzevles and Kroeze (1987b).

Beidler (1971) argued that sourness is dependent upon both the H ion and the anion. The anion is hypothesized to enhance further binding of H ion by preventing membrane charging. Beatty and Cragg (1935) reported that the amount of phosphate buffer required to bring the pH of acid solutions at equi-molar concentrations to ~4.5 was proportional to the relative sourness intensity, specifically studying acetic, hydrochloric, chloroacetic, tartaric, and malic acids.

In an attempt to explain acid sourness from a different perspective, many researchers have tried to determine the chemical factors involved.

Kahlenberg (1898) and Richards (1898) believed that sourness could be explained by the H ion exclusively. Many researchers in later years have disputed this. Harvey (1920) theorized that sourness is a function of 2 independent variables: the H ion and titratable acidity (TA). Amerine et al. (1965), evaluating malic, citric, lactic, and tartaric acids, also found both pH and titratable acidity to be important in sourness perception. Disputing Harvey's findings, CoSeteng et al. (1989) found that TA and pH did not independently influence sourness. They found the chemical structure of the acid, dissociation constants, and anion concentration to play a role in sourness perception. Pangborn (1963) believed that sour taste is associated with the H ion and the degree of dissociation, after studying citric, acetic, tartaric, and lactic acids.

Theories on the perception of sourness intensity also exist. Evaluating citric, malic, and fumaric acids, Buechsenstein and Ough (1979) found approximately equi-molar acid concentrations to be equi-sour. On an equi-weight basis, the lower the molecular weight of the acid the more sour it is perceived (Buechsenstein and Ough, 1979; CoSeteng et al., 1989). Based on the number of carboxylic groups on an acid molecule, the more groups found on the acid molecule the less sour it is perceived (CoSeteng et al., 1989). Thus, monocarboxylic acids are more sour than dicarboxylic acids, which are more sour than tri-carboxylic acids.

Human saliva has been studied to further explain sourness perception. Sour taste causes the most salivation in humans (Moncrieff, 1971). Chauncey et al. (1967) believed that sour receptor stimulation was related to rate of saliva secretion, and found significantly different salivary flows for tartaric, lactic, acetic, and citric acids at equi-molar concentrations. After studying citric-tartaric and citric-fumaric, Norris et al. (1984) concluded that specific characteristics of the acid anion may influence binding and play an important role in eliciting sourness in addition to salivary flow. Noble et al. (1986) studied the relative sourness of 6 organic acid anions in 2-acid buffered solutions of equal pH and equal TA and found that perceived sourness varies with anion in systems with equal pH and equal TA.

Moskowitz (1971) studied sourness by determining the psychophysical functions of 24 carboxylic acids. A simple relationshp between sourness and physio-chemical properties of acids was not found. Straub (1992) confirmed these results. While extensive research on sourness perception has been conducted, the mechanism is still unknown.

2.2.2 Astringency

Since the beginning of time, astringency has been considered a basic taste (Bartoshuk, 1978). Others have classified astringency as a chemically

induced tactile sensation (Bate-Smith, 1954). Today it is still debated if astringency is a taste or a tactile sensation.

The word astringency is derived from the Latin words, *ad stringere,* meaning "to bind". Haslam and Lilley (1988) defined the taste of astringency as "a feeling of extreme dryness or puckeriness..not confined to a particular region of the mouth or tongue...." The American Society for Testing and Materials (ASTM) Committee on sensory evaluation of materials and products defined astringency as "the complex of sensations due to shrinking, drawing, or puckering of the epithelium as a result of exposure to substances such as alums or tannins" (ASTM, 1993).

Astringency is a desirable sensory characteristic in many foods and beverages (Bate-Smith, 1954). However, the subtle balance of just the right amount of astringency is crucial. Astringency (from tannins) is an important attribute in foods and beverages such as tea, coffee, wine, beer, apples, ciders, many berry crops, and nuts (Haslam and Lilley, 1988). Salts and multivalent cations (Al, Cr, Zn, Pb, Ca, B), mineral acids, and dehydrating agents such as alcohol and demethyl ketone have also been found to elicit the astringency sensation (Haslam and Lilley, 1988). Astringency in foods is due to the presence of specific compounds, which have the ability to combine with and precipitate proteins in the mouth (Bate-Smith, 1954). Astringent substances can cause shrinking, drawing, or

puckering. However, no previous research has attempted to break down astringency into separate sub-qualities during psychological evaluation (Lee and Lawless, 1991).

Not unlike sourness perception, the mechanism of astringency is unclear and several theories have been proposed. A popular theory proposed by Bate-Smith (1973) involves polyphenolic compounds ie. tannins. These complex with proteins and/or mucopolysaccharides of saliva, either precipitating them or causing sufficient conformational changes so that they lose their lubricating power. The result is a mouth that feels dry and rough.

Astringency is an important attribute for differentiating acids. Rubico (1993) found significant differences in astringency between 9 organic and 2 inorganic acids. Rubico and McDaniel (1992) suggested the need to study not only protein-tannin interactions but also acid-protein interactions. By modifying the pH, Guinard et al. (1986) found that acidity adjustments can have a significant effect on astringency.

There are two problems often encountered with astringency research. First, there is a lack of research studying astringency in model systems as most research evaluates it in a complex media ie. wine (Guinard et al., 1986). The second is that astringency is often confused with bitterness. Some researchers consider the two to be "twin sensations" (Lea and

Arnold, 1978). This is due to the lack of a purely astringent stimuli that are not bitter, as nearly all phenolic compounds have both characters by nature.

2.3 Descriptive Analysis

Characterization of the perceived flavor of a food is a complex task (Amerine et al., 1965). Traditionally, companies employed "expert tasters" to make decisions on the fate of products or the outcome of ingredient changes. Today, food markets are extremely diverse and complex, and new products have ~99% failure rate. With an increasing importance on sensory evaluation of products, companies are turning to taste panels for more input in order to make these decisions.

2.3.1 Flavor Profile Method

Descriptive analysis (DA) is the most sophisticated of the available sensory methodologies. A.D. Little Company developed the first technique called the "Flavor Profile Method" (FPM) in the 50's (Caul, 1957). Several attempts have been made to modify or improve this method.

The FPM is a sensory method where attributes of a food are identified, described, and quantified using human subjects who have specifically trained for this purpose (Einstein, 1991). It involves using

detailed qualitative and quantitative information about products

(Oreskovich et al., 1991). Modern methods are based on historical attempts

at odor descriptions and classifications (Piggott and Canaway, 1981).

2.3.2 New Methods

After the FPM was invented, several modifed techniques were

developed, ie. Texture Profiling (Szczesniak, 1963), Quantitative

Descriptive Analysis[tm] (Stone et al, 1974), and Spectrum[tm] (Meilgaard et al.,

1991). Regardless of the method used, all have 4 similar features: (1)

selection and training of panelists, (2) development of language, (3)

evaluation sessions and (4) data analysis and interpretation (Rubico, 1993).

Panelists work together to develop a common language to describe

perceiveable product attributes. While training helps familiarize panelists

with scoring attributes, it can't eliminate all variation. Arnold and

Williams (1986) reported 5 sources of variation among panelists that are

difficult, if possible, to eliminate: (1) panelists vary in overall level of

scoring, (2) panelists use different terms to describe the same stimulus, (3)

panelists vary in their range of scoring, (4) panelists perceive different

stimuli in the same product, and (5) panelists vary in their usage of terms

and scales between sessions. The major disadvantage of these DA methods

is that they may require considerable amounts of time and money to

recruit, screen, and train panelists in order to evaluate specific products (Oreskovich et al., 1991; Einstein, 1991).

2.3.3 Free-Choice Profiling

Free-choice profiling (FCP) is the newest technique for sensory profiling of food products. It was developed by Williams and his coworkers (Williams et al., 1981) as a solution to the problem of consumers using different terms for a given attribute (Meilgaard et al., 1991). FCP has been used to assess commercial ports (Williams and Langron, 1984), coffee (Williams and Arnold, 1985), fish (Quarmby and Ratkowsky, 1988), chocolate (McEwan et al., 1989), whiskey (Guy et al., 1989), beer (Gains and Thomson, 1990), dark rum (Piggott et al., 1992), acids (Rubico, 1993), and many other products.

FCP is similar to the other DA methods in that panelists are recruited, selected, and trained in scale usage, impartiality of judgments and consistent term usage in order to obtain reproducible results (Oreskovich et al., 1991). However, less training is needed as terms are not shared or used collectively by panel members, and terms may be mutually exclusive. Each panelist develops his/her own ballot to use. While identical terms may be used by different panelists, they may have totally different meanings (Marshall and Kirby, 1988).

FCP assumes that assessors do not differ in the way in which they perceive sensory characteristics but in the ways they describe them (Arnold and Williams, 1986). Thus, it allows panelists to invent and use as many terms as needed to describe sensory characteristics of samples. In addition, it alleviates the frustration experienced by panel leaders in forcing agreement among panelists in their use of terminology (Williams and Langron, 1983). However, one problem encountered by Piggott et al. (1990) with FCP is with too few descriptors being generated or terms being too personal that making interpretation of the results is difficult. Researchers must decide what terms mean and how to combine terms among panelists.

There are several advantages of FCP over traditional methods of DA. Since minimal training is needed for FCP, a great deal of time and money can be saved. In addition, panelists do not need to have experience with characteristics of product or definitions of vocabulary (Piggott et al., 1992). The only requirement for panelists is to be objective, capable of using the measurement scale, and capable of using their own vocabulary consistently (Jack and Piggott, 1992). Another advantage is panelists do not have to use words that are proposed by others. DA can not overcome problems with individual differences, both with how samples are perceived and with the measurement scale used to quantify these (Powers, 1984).

Williams and Arnold (1985) found FCP to give results similar to traditional DA methods. This has been confirmed by Rubico (1993).

2.4 Statistical Analysis

2.4.1 Generalized Procrustes Analysis

Generalized Procrustes Analysis (GPA) has found widespread application in sensory research. It is the only statistical method for analyzing FCP data (King and Arents, 1991). The greek work *Procrustes* means "to beat". It described an innkeeper in mythological times who seized travelers, and then he either stretched or cut off their legs to make them fit his beds (Oreskovich et al., 1991).

GPA is used to combine individual configurations into a common space. It works by allowing transformations to be performed while the shapes of the configurations are maintained. This enables researchers to compare discrimination ability independently of the descriptors' scores and the measurement scales used (Williams et al., 1981). GPA involves 3 mathematical steps: (1) geometrical transformation to a common origin to eliminate the effect of using different parts of the scale, (2) isotropic scale changes to correct for differences in scoring range used, and (3) rotation/reflection of axes to match configurations as closely as possible

(Arnold and Williams, 1986). The result from GPA is a perceptual space

for each panelist, which is matched with the other panelists. After the

consensus configuration is calculated, it can be simplified to a reduced

dimensional plot by Principal Component Analysis (PCA) (Piggott et al.,

1992).

GPA is a desirable method because it allows researchers to investigate

sample relationships, then determine how these apply for individual

panelists based on the calculated residuals (King and Arents, 1991).

2.4.2 Principal Component Analysis

The most commonly used of all the multivariate procedures is PCA

(Piggott and Sharman, 1986). It can be applied to almost any multivariate

data set to great advantage as an exploratory technique. PCA was

developed by Hotelling (1933), and has seen wide application to many

products, ie. acids (Rubico, 1993), beer (Sanchez et al., 1992a; Sanchez et al.,

1992b), carbonated water (Harper and McDaniel, 1993), wine (Guinard and

Cliff, 1987), and yogurt (Barnes et al., 1991).

PCA is a data reduction method concerned with a space that defines

total variance of variables. It has 4 specific goals: (1) to summarize patterns

of correlations among observed variables, (2) to reduce large numbers of

variables to a smaller number of factors, (3) to provide an operational

definition (regression equation) for an underlying process by using

observed variables, or (4) to test theory about nature of underlying processes (Tabachnik and Fidel, 1989). In a simpler explanation, Johnson and Wichern (1988) described the main goals of PCA to be data reduction and interpretation. The overall effect of PCA is reducing dimensionality of the sample space by finding linear combinations that can explain maximum variation. Principal Components (PC) are created and ordered by the linear combinations, and the first PC explains the most variation and the last PC explains the least.

PCA creates eigenvalues from the original data, and these represent the variation where the closer to one the value is, the stronger the correlation between the original data and the PCA transformed data. Eigenvalues are referred to as "loadings", and can be plotted as vectors on the PCs.

The output created by PCA is concerned with relationships between variables and samples. The PCs summarize the patterns of correlations in the observed correlation matrix (Tabachnik and Fidel, 1989). The idea surrounding this technique is to group entities (panelists, samples, attributes) according to the amount of variation accounted for (Powers, 1989). For example, in studying the behavior of individual panelists, PCA can be used to detect homogeneity in patterns of panelists' responses, which is fundamental in judging the value of results and efficiency of

training (Sinesio et al., 1993). By learning about independence, PCA can be used in providing feedback for future work, ie. by reducing number of attributes used and for screening panelists based on past discriminatory performance. This technique is powerful in understanding the dependencies existing among variables and in determining whether subsets of variables cluster (Timm, 1975). On a final note, PCA can be used in conjunction with GPA as they provide different information (Oreskovich et al., 1991).

3. FLAVOR CHARACTERISTICS OF LACTIC, MALIC, CITRIC, AND ACETIC ACIDS AT VARIOUS pH LEVELS

Pam Hartwig and Mina R. McDaniel

3.1 Abstract

The objective of this study was, through the use of free-choice profiling, to determine flavor characteristics of 4 acids (citric, malic, lactic, acetic) and 2 acid blends (lactic/acetic 1:1 and 2:1) at 3 different pH levels (3.5, 4.5, 6.5) and at 0.2% (w/v). Generalized Procrustes Analysis was performed on the generated free-choice profiling data, which resulted in 3 significant principal axes. The first principal axis was characterized by overall intensity and sourness. The second principal axis was characterized by vinegar and saltiness. The third principal axis was characterized by astringency.

3.2 Introduction

One of the most functional ingredients in the food industry is acids

(Andres, 1985), contributing to a wide variety of properties in foods. For

example, acids can control pH, preserve food, provide leavening, aid in gel

formation, prevent non-enzymatic browning, act as a synergist for

antioxidants, chelate metal ions, and most importantly, add and enhance

flavor in foods. Acids are found in almost every type of formulated food

product, such as jams, jellies, and preserves, bakery products, dairy

products, meat products, beverages, and confectionery products. Many

acidulants occur naturally in food and in cells of plants and animals. Thus,

most acidulants are generally recognized as safe (GRAS listed).

A current trend for use of acidulants is the combination of two or

more acids in a formulated food product. In nature one acid is rarely

found alone; hence, combining acids enables an acidified food to more

readily simulate natural flavor. A good example of 2 acids frequently

found in combination is lactic and acetic acids. In preparing pickles and

relishes, lactic acid can be added to vinegar to provide a milder, more

subtle taste sensation (Gardner, 1972; Fabian and Wadsworth, 1939). In

salad dressings and marinades, lactic/acetic blends have been found to act

synergistically; they are especially inhibitory to outgrowth of

heterofermentive lactobacilli (Doores, 1990).

Acidulants have one shared sensory characteristic: sourness. However, acids are different in their degree of sourness and in their non-sour aroma and flavor characteristics. Only a few studies have profiled acid flavor. Arnold (1975) and Pszczola (1988) proposed that intensity and duration of acidic taste differs among acids. Straub (1992) found differences in power functions and time-intensity curves for sourness and astrigency of 7 organic and 1 inorganic acids. Rubico (1993) and Rubico and McDaniel (1992) evaluated the flavor profile of 8 organic, 3 organic blends, and 2 inorganic acids and found that acids differ in sensory character. Noble et al. (1986) studied 6 organic acids in binary acid solutions and found that acids at equal pH or equal titratable acidity varied in sourness intensity. While most studies have focused on sourness, published research reports acids have non-sour characteristics, ie. bitterness and astringency (Straub, 1992; Rubico, 1993; Rubico and McDaniel, 1992).

The flavor profile of acids can vary significantly depending upon the pH of the final product. For example, astringency is often present in acid solutions that are too dilute to be sour (Crozier, 1920). In this study we sought to evaluate acids on an equal weight and equal pH basis in order to compare/contrast the resulting flavor. In addition, the flavor of a given acid can change depending upon the pH level; hence, 3 different pH levels were selected. By knowing the differences in the flavor profile of acids at a

specific pH, acidified food products can be formulated to attain the specific characteristics needed. The primary objective of this study was, through use of free-choice profiling, to determine flavor characteristics of 4 acids and 2 acid blends at 3 different pH levels and at equal % (w/v).

3.3 Materials and Methods

3.3.1 Samples

Eighteen acid solutions (4 acids and 2 blends at 3 pH levels) were evaluated. Anhydrous citric and DL-malic acids were obtained from Haarmann and Reimer (H&R) Company (Elkhart, IN), glacial acetic acid (U.S.P.-F.C.C.) from J.T. Baker Inc. (Phillipsburg, NJ), and L(+)Lactic acid (88%) from PURAC America, Inc. (Lincolnshire, IL). In addition to the 4 acids, 2 acid blends were prepared: lactic/acetic (1:1 and 2:1). Acid solutions were evaluated at 0.2% (w/v); this concentration was chosen based on preliminary sensory evaluations to attain a reasonable intensity at all pH levels tested. The acid solutions were adjusted to pH 3.5, 4.5, and 6.5. These levels were selected to investigate the acid characteristics at specific pH levels where food systems are formulated. For example, acidified food products such as salad dressings, canned vegetables, and seafood salads are examples at 3.5, 4.5 and 6.5, respectively.

In preparation of 500 mL acid solutions for each panel session, 0.2% (w/v) of acid was added to 450 mL water and then adjusted with sodium hydroxide (NaOH) to the appropriate pH level. After titration, acid solutions were filled to 500 mL to ensure equi-concentration of acid in all solutions. Acids were diluted with deionized, distilled water (Milli-Q Reagent Water System, Millipore Corporation, Bedford, MA).

3.3.2 pH Determinations

NaOH pellets (U.S.P.-F.C.C.) purchased from J.T. Baker Inc. (Phillipsburg, NJ) were used to prepare the titrating solution. A stock solution was made by stirring the pellets and deionized, distilled water [1:1 (w/w)]. After making the stock solution, one dilution step was performed to prepare a ~0.35 N NaOH solution. This solution was used to adjust the pH of the acid solutions to 3.5, 4.5, and 6.5. The pH levels of the 18 samples were measured by a pH electrode with a microprocessor pH/mV meter (Corning Model 125, Medfield, MA) equipped with a combination pH electrode (Sensorex Model S200C, Stanton, CA).

3.3.3 Panelists

Seven male and 5 female students and staff from the Department of Food Science and Technology at Oregon State University served as panel members. The majority of the individuals had previous panel experience as trained panel members.

3.3.4 Presentation of Samples

Samples were presented at room temperature in coded 2 oz. plastic cups. Each cup contained ~30 mL of sample. All training and testing sessions were limited to a single pH level. This was done in order to maximize acid comparisons within each pH level, rather than measuring differences due to pH changes. Due to similar intensities of the acid solutions within each pH level, character differences were easier to observe by this presentation. At each session 4 acids and 2 blends at a given pH level were randomly presented and evaluated. One replication (18 samples) was completed within one week (3 sessions per week). Three replications were completed.

3.3.5 Training

The concept of free-choice profiling (FCP) was introduced to the panelists on the second of 8 practice sessions. During the first 2 sessions, different concentrations of citric acid, sodium chloride, sucrose, caffeine, and alum were used for identification of, or introduction to, the 4 basic tastes (sour, salty, sweet, bitter) and astringency, respectively. They were also presented to practice rating the magnitude of different characteristics on a 16-point intensity scale (0=none, 7=moderate, 15=extreme), which was used throughout the study. For the first few practice sessions, panelists were asked to list all sensory characteristics which described the perceived

attributes of the samples. Later, these terms were written down in order to develop a ballot for use in subsequent testing. To ease in interpretation, panelists were asked to define their own terms. For the testing sessions, panelists were seated in separate, well ventilated booths with incandescent lighting.

When evaluating the acid samples, the 'sip-and-spit' method was applied. Panelists were instructed to sip the sample and manipulate in the mouth for 5 seconds, then expectorate. Panelists rated their individual descriptors on the intensity perceived while in the mouth, and then rated descriptors again after expectoration.

3.3.6 Statistical Analysis

These generated data were analyzed by generalized procrustes analysis (GPA) using Procrustes-PC Version 2.0 (Dijksterhuis and van Buuren, 1989) and by Statistical Analysis System for Personal Computer (SAS, 1987, Cary, NC). For the FCP analysis, the data from each of the 12 panelists were assembled into matrices of 54 rows (6 samples for 3 pH levels assessed over 3 replications) by n columns where n represented the number of attributes for each panelist. Using these matrices GPA was performed. Analysis of variance (ANOVA) on the principal axis scores was used to determine significant differences among samples and, where appropriate, least significant differences.

3.4 Results and Discussion

3.4.1 Sensory Results

FCP of the samples generated between 10 (Panelist 1) and 17 (Panelist 2) descriptors with an average of 13 descriptors per panelist (Table 3.1). Panelists used 10 descriptors in common: Overall Intensity (OI), Sour, Astringency (Astr), Salty, Bitter, Sweet, OI*, Sour*, Astr*, Salty* [* denotes after expectoration]. Vinegar (7), Vinegar* (5), Citrus (5), and Salty*(6) were common additional terms used by several panelists (number of panelists noted in parentheses).

ANOVA on the consensus scores from GPA determined that the first 3 Principal Axes (PA) were significant. Results are displayed in Tables 3.2, 3.3, and 3.4 for the first, second, and third PAs, respectively. GPA results are graphically presented in Figures 3.1 and 3.2 for the first PA vs. the second PA and the first PA vs. the third PA, respectively. The triangles represent the 3 replications, where the size of the triangles illustrates the replication variability found with each sample. Thus, the smaller the area of the triangle, the better the panelists' ability to replicate and discriminate the sample. Table 3.5 shows the combination of descriptors for each panelist that characterize the axes, where the most important descriptors have the highest loadings.

Table 3.1-Terms generated by each panelist in the free-choice profiling of acids

	Pan 1	Pan 2	Pan 3	Pan 4	Pan 5	Pan 6	Pan 7	Pan 8	Pan 9	Pan 10	Pan 11	Pan 12
1.	OI	OI	OI	OI	OI	OI	OI	OI	OI	OI	OI	OI
2.	Sour	Sour	Sour	Sour	Sour	Sour	Sour	Sour	Sour	Sour	Sour	Sour
3.	Astrin.	Astrin.	Astrin.	Astrin.	Astrin.	Astrin.	Astrin.	Astrin.	Astrin.	Astrin.	Astrin.	Astrin.
4.	Salty	Salty	Salty	Salty	Salty	Salty	Salty	Salty	Salty	Salty	Salty	Salty
5.	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter
6.	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
7.	OI*	Metallic	Vinegar	Citrus	Metallic	Vinegar	Soapy	Vinegar	Citrus	OI*	Tart	Vinegar
8.	Sour*	Soapy	OI*	Vinegar	Fruity	OI*	OI*	OI*	Soapy	Sour*	Lime	Lemon
9.	Astrin.*	Citrus	Sour*	OI*	Vinegar	Sour*	Sour*	Sour*	Vinegar	Astrin.*	Soapy	Dirty
10.	Bitter*	Vinegar	Astrin.*	Sour*	OI*	Astrin.*	Astrin.*	Astrin.*	OI*	Bitter*	OI*	Tart
11.		OI*	Bitter*	Astrin.	Sour*	Bitter*	Bitter*	Bitter*	Sour*	Soapy*	Sour*	OI*
12.		Sour*		Bitter*	Astrin.*		Vinegar*	Astrin.*		Astrin.*	Sour*	
13.		Astrin.*		Salty*	Bitter*		Salty*	Bitter*		Bitter*	Astrin.*	
14.		Bitter*			Salty*			Salty*		Tart*	Bitter*	
15.		Salty*			Soapy*			Soapy*			Vinegar*	
16.		Soapy*			Vinegar*			Vinegar*			Salty*	
17.		Vinegar*										

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* denotes after expectoration

Table 3.2-Principal axis 1: mean scores for acids at 3 pH levels

Acid/ Acid Blend[†]	pH Level	Mean Score[*] (Principal Axis 1)
Lactic	6.5	0.377[a]
Malic	6.5	0.367[a]
Lactic/Acetic (1:1)	6.5	0.350[a]
Lactic/Acetic (2:1)	6.5	0.347[a]
Citric	6.5	0.340[a]
Acetic	6.5	0.310[ab]
Lactic	4.5	0.260[b1]
2Citric	4.5	0.110[c]
Malic	4.5	0.013[d]
Lactic/Acetic (2:1)	4.5	-0.047[d]
Citric	3.5	-0.127[e]
Lactic/Acetic (1:1)	4.5	-0.167[ef]
Lactic	3.5	-0.267[fg]
Malic	3.5	-0.260[g]
Acetic	4.5	-0.273[g]
Lactic/Acetic (2:1)	3.5	-0.370[h]
Lactic/Acetic (1:1)	3.5	-0.447[i]
Acetic	3.5	-0.563[j]

[†]All acids at 0.2% (w/v)

[*] Different letter subscripts indicate significant differences at $p < 0.05$ for each column separated by Least Significant Difference (LSD)

Table 3.3-Principal axis 2: mean scores for acids at 3 pH levels

Acid/ Acid Blend[t]	pH Level	Mean Score* (Principal Axis 2)
Acetic	4.5	0.127[a]
Acetic	3.5	0.090[ab]
Lactic/Acetic (1:1)	4.5	0.080[ab]
Acetic	6.5	0.053[bcd]
Lactic/Acetic (1:1)	3.5	0.040[cd]
Lactic/Acetic (1:1)	6.5	0.033[cde]
Lactic/Acetic (2:1)	6.5	0.033[cde]
Malic	6.5	0.021[def]
Citric	6.5	0.010[defg]
Malic	4.5	-0.013[efg]
Lactic/Acetic (2:1)	4.5	-0.014[efg]
Lactic	6.5	-0.019[fg]
Lactic	4.5	-0.027[fg]
Lactic/Acetic (2:1)	3.5	-0.030[g]
Citric	4.5	-0.033[g]
Malic	3.5	-0.096[h]
Citric	3.5	-0.103[h]
Lactic	3.5	-0.153[i]

Table 3.4-Principal axis 3: mean scores for acids at 3 pH levels

Acid/ Acid Blend†	pH Level	Mean Score* (Principal Axis 3)
Acetic	4.5	0.063a
Lactic/Acetic (1:1)	4.5	0.057a
Malic	4.5	0.053ab
Malic	3.5	0.050abc
Citric	4.5	0.047abcd
Citric	3.5	0.040abcde
Lactic/Acetic (2:1)	4.5	0.022abcdef
Lactic	4.5	-0.005bcdefg
Lactic	6.5	-0.014bcdefg
Acetic	6.5	-0.014bcdefgh
Lactic/Acetic (1:1)	6.5	-0.016cdefgh
Acetic	3.5	-0.019cdefgh
Malic	6.5	-0.020defgh
Lactic/Acetic (2:1)	6.5	-0.020defgh
Citric	6.5	-0.027efgh
Lactic/Acetic (2:1)	3.5	-0.046fgh
Lactic	3.5	-0.060gh
Lactic/Acetic (1:1)	3.5	-0.083h
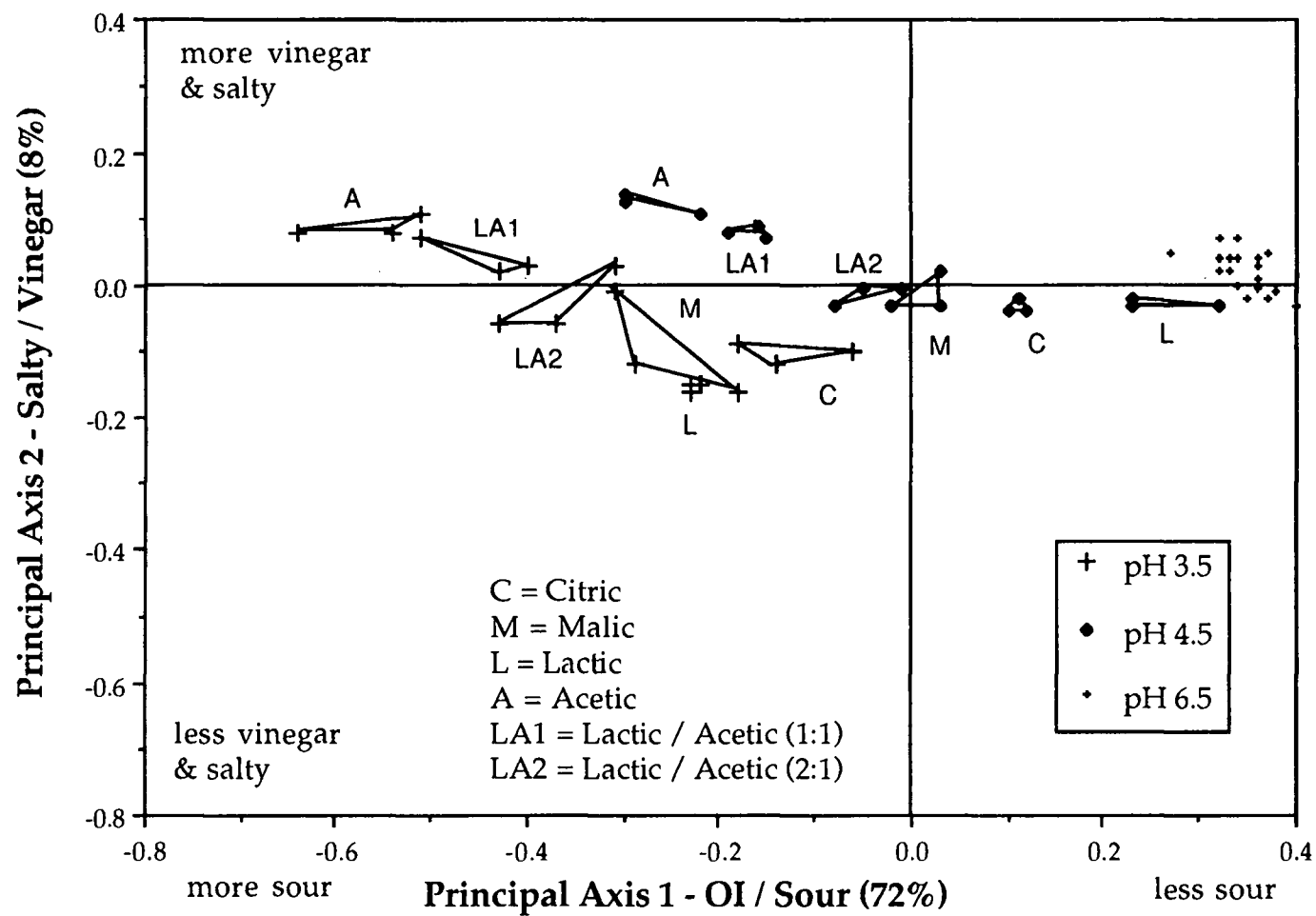
Figure 3.1-Sample consensus plot for free-choice profiling of acids following generalized procrustes analysis: principal axes 1 vs. 2.
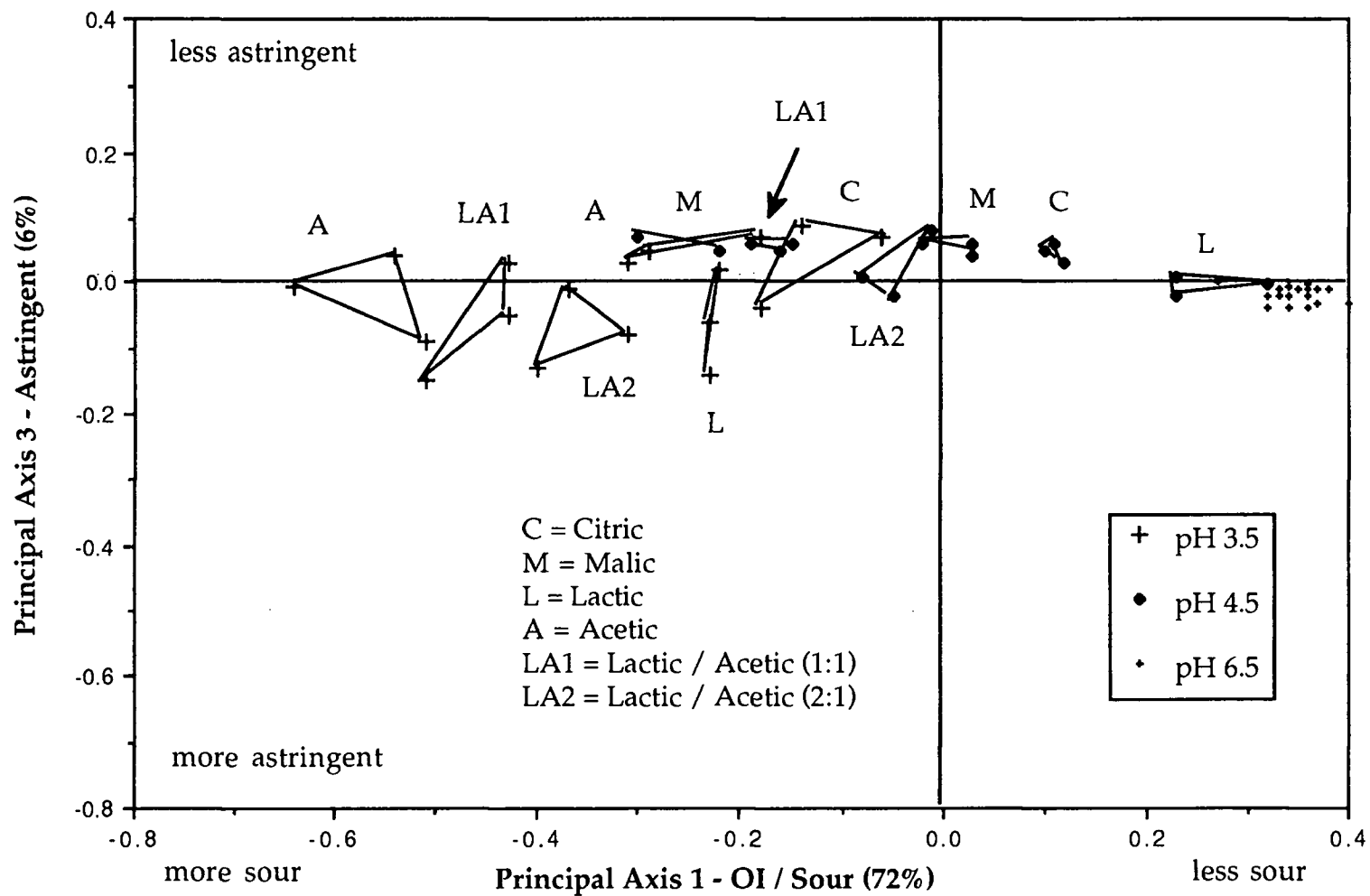
Figure 3.2-Sample consensus plot for free-choice profiling of acids following generalized procrustes analysis: principal axes 1 vs. 3.

Table 3.5-Loadings* of descriptors for the first three principal axes following free-choice profiling of acids at 3 pH levels

Pane-list #	Principal Axis 1	Principal Axis 2	Principal Axis 3
1	-OI(0.59) -Sour(0.56) -OI*(0.38) -Sour*(0.30)	OI(0.36) +Salty(0.63) +Sweet(0.43) -Astrin*(0.35)	-Astrin(0.74) -Astrin*(0.55)
2	-OI(0.55) -Sour(0.59)	OI(0.34) -Astrin(0.33) +Salty(0.55) +Vinegar(0.34)	-Astrin(0.65) -Salty(0.35) -Astrin*(0.32)
3	-OI(0.61) -Sour(0.63)	-Astrin(0.48) -Sweet(0.37) +Vinegar(0.67) -Astrin*(0.33)	-Astrin(0.49) -Sweet(0.65) -Astrin*(0.41)
4	-OI(0.54) -Sour(0.55) -Astrin(0.33) -OI*(0.32)	Salty(0.36) +Vinegar(0.73) -Citrus(0.46)	Sour(0.41) -Astrin(0.43) -Bitter(0.30)+Vinegar(0.40) +Citrus(0.40) -Bitter*(0.32)
5	-OI(0.56) -Sour(0.66)	-Astrin(0.33) +Vinegar(0.63) -Fruity(0.54)	-Astrin(0.66) -Bitter(0.34) +Sweet(0.39)
6	-OI(0.43) -Sour(0.47) -Astrin(0.43) -OI*(0.30) -Sour*(0.32) -Astrin*(0.30)	Salty(0.67) -Sweet(0.36) +Vinegar(0.56)	Sour(0.38) -Astrin(0.50) +Salty(0.45) -Vinegar(0.39) -Bitter*(0.33)
7	-OI(0.51) -Sour(0.72) -Astrin(0.31)	-Astrin(0.53) -Bitter(0.33) +Sour*(0.49) -Astrin*(0.36)	-Bitter(0.32) -OI*(0.45) -Sour*(0.49) -Astrin*(0.46) -Bitter*(0.31)
8	-OI(0.49) -Sour(0.42) -Astrin(0.40) -Vinegar(0.49)	Salty(0.79)	Sour(0.37) -Astrin(0.53) +Sour*(0.67)
9	-OI(0.43) -Sour(0.55) -Astrin(0.30) -Sour*(0.34) -Vinegar*(0.35)	Vinegar(0.55) -Sour*(0.39) +Vinegar*(0.58)	Sour(0.48) -Astrin(0.77) -Vinegar(0.31)
10	-OI(0.66) -Sour(0.70)	Salty(0.35) +Bitter(0.57) -Sour*(0.33) -Astrin*(0.58)	-Astrin(0.86)
11	-OI(0.55) -Sour(0.56)	Salty(0.90)	-Astrin(0.52) +Sour*(0.42) -Astrin*(0.64)
12	-OI(0.54) -Sour(0.52) -Astrin(0.43) -Sour*(0.32)	-Astrin(0.52) +Vinegar(-0.58) -Astrin*(0.33) +Vinegar*(0.49)	-OI(0.35) -Vinegar(0.31) -Lemon(0.38) +OI*(0.49) +Sour*(0.49)

*Descriptors with loadings <0.30 were not included in the table.

The first 3 PAs explained nearly 86% of the variation as 72%, 8%, and 6% were explained by the 1st, 2nd and 3rd PAs, respectively. The high percentage explained is due to large and obvious differences resulting when evaluating acids at different pH levels. The first PA was characterized by overall intensity and sourness as these descriptors created high loadings for every panelist (Table 3.5). Astringency was also important on this PA for half of the panel. At all pH levels, acetic acid was the most sour and intense; lactic and citric acids were the least (Figure 3.1). These findings support research by Pangborn (1963) and CoSeteng et al. (1989). They found sourness intensity to be a function of chemical structure. CoSeteng et al. (1989) found the number of carboxylic groups to be related to sourness intensity (ie. in decreasing order of sourness: mono-carboxylic acids (CA)>>di-CAs>> tri-CAs). In addition, they reported molecular weight and polarity to be important factors in sourness perception, demonstrating that increasing the molecular weight and hydrophobicity of an acid molecule increases sourness intensity.

With few exceptions, the relative order of the acids remained constant at each pH level. For example, acetic acid and then the lactic/acetic blends had the lowest PA scores for every pH level as PA 1 descriptors with the highest loadings were on the negative side of the axis.

Overlap existed between pH levels as acetic acid at pH 4.5 was significantly more sour than citric acid at pH 3.5.

The size of the triangles (showing the 3 replications) provides useful information. For example, at pH 3.5 the relatively large areas of citric and malic acids illustrate the difficulty that panelists had in finding differences between these 2 and/or the inability to replicate the perceived differences. In contrast, the close proximity of lactic acid at pH 3.5 exemplified good consensus among panelists on this sample. This finding contradicts previous research reports that found lactic acid relatively difficult to discriminate due to large replication variation (CoSeteng et al, 1989; Straub, 1992).

Flavor intensity played a large role in sample discrimination. Acids at pH 3.5 were all very intense; thus, these samples were the easiest to separate. However, as pH increased, the intensity generally decreased as did the intersample distances. As a result, at pH 6.5 the intensities of all samples were very weak and some were even barely perceivable. Thus, all were contained in one general area (Figure 3.1).

The second PA was characterized by vinegar and saltiness. For the 7 panelists who used the term vinegar, this descriptor received high loadings for their second PA. Saltiness also received high loadings from over half of the panelists (1, 2, 4, 6, 8, 10, and 11) [Table 3.5]. At pH 6.5, sourness was

diminished, leaving only minor qualities such as saltiness. This was

expected since the NaOH used for titration was at the highest concentration

in the acid solutions at the highest pH level. Table 3.6 shows the amounts

of NaOH added (in mmoles) to the acid solutions to achieve the desired pH

levels. Acid salt formation occurs when acids dissociate into protons and

acid anions ([HA] ---->[H+] + [A-]) when dissolved in water. When NaOH is

added, it readily dissociates also ([NaOH] <----> [Na+] + [OH-]. Therefore,

the following reaction occurs:

[H +] + [A-] + [Na+] + [OH-] ----> [H$_2$O] + [NaA]

Most of the acetic acid and lactic/acetic blends were on the positive

side of the axis, as were acetic, malic and citric acids at pH 6.5 (Table 3.3).

Lactic acid was the least salty/vinegar at all pH levels, and the least amount

of NaOH was added to achieve the 3 levels relative to the other acids (Table

3.6). The good replication of acetic and the lactic/acetic blends was due to

the distinct vinegar character from acetic acid, which characterized the 2nd

axis.

The third PA was characterized by astringency. Separation on this

axis was by pH, as acids at pH 4.5 were less astringent than acids at pH

levels 3.5 and 6.5. At pH 3.5, lactic acid was the most astringent,

significantly more so than citric or malic acids. At pH 4.5, lactic acid was

Table 3.6-Means[1] and standard deviations[2] of NaOH added in mmoles to acids to achieve 3 pH levels

	pH levels		
Acid	3.5	4.5	6.5
Citric	4.81[1]	8.52	15.63
	(0.32)[2]	(0.36)	(0.50)
Malic	3.75	9.22	15.23
	(0.38)	(0.27)	(0.97)
Lactic	2.64	7.74	9.09
	(0.34)	(0.65)	(0.15)
Acetic	0.37	6.08	17.10
	(0.12)	(0.85)	(0.76)
L/A (1:1)	1.27	6.38	12.71
	(0.14)	(0.17)	(0.41)
L/A (2:1)	1.71	6.38	11.04
	(0.25)	(0.43)	(0.37)

also the most astringent, significantly more so than all the others. At pH 6.5, none of the acids elicited significantly different astringent sensations.

The ASTM Committee on Sensory Evaluation of Materials and Products has defined astringency as "the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins (American Society for Testing and Materials, 1993). While historically it has been considered of equal importance to the 4 basic tastes (Bartoshuk, 1978), the chemical reactions promoting the astringency sensation in acids are still poorly understood. Rubico and McDaniel (1992) and Straub (1992) found astringency to vary markedly among organic and inorganic acids.

3.4.2 Panelist and Sample Variation

Panelists' individual performance was examined by the percentage residual variation data from GPA. None of the panelists had residuals that were significantly greater than the others; therefore, residual data were not presented. The percentage consensus and percentage within (residual) is presented in Figure 3.3, which displays the acids grouped by pH level. The lower, darker portion of the histogram represents the percentage consensus variation, and the upper, lighter portion is the within (residual) variation for the various acids. The extent of discrimination among the samples was related to intensity. For example, panelists had the greatest consensus for
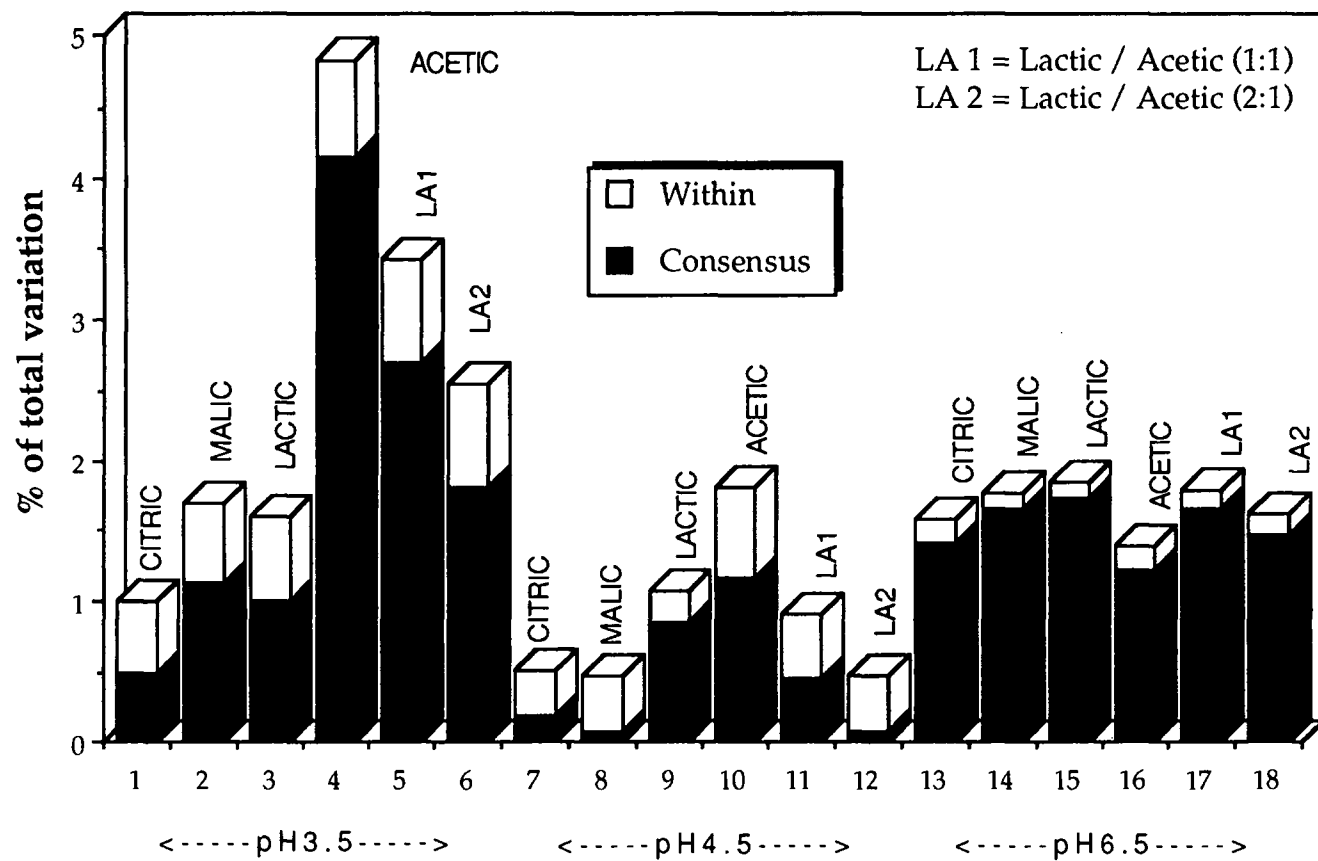
Figure 3.3-Percentages consensus and within (residual) variation distributed over 18 samples.

acetic and lactic/acetic blends at pH 3.5. This supports CoSeteng et al. (1989)

findings that acetic acid produced the most consistent results with varying

pH levels. Acids at pH 3.5 were the most intense, making differences

between samples more apparent. At pH 4.5, the lowest consensus scores

for all acids were found. Generally, these acids were slight to moderate

intensity. The low consensus scores at this level indicated that these

samples were the most difficult to evaluate. At pH 6.5, the acid intensities

were none to slight; thus, the most consistent agreement was found due to

the similar, weak to nonexistent flavor profiles.

3.5 Conclusions

This research clearly demonstrates that various food acids at a given

pH level have significantly different flavor profiles. Our observations also

show that the flavor profile of a given acid changes with pH level.

Awareness of these differences in the flavor of acidulants can be used to

formulate acidified food products with specific sensory characteristics. For

example, acids at pH 4.5 vary the most in sourness and OI. However, at pH

6.5 little difference exists between acid flavor profiles. This indicates that if

a given acidified food product were formulated at pH 4.5, then the

acidulant chosen could have a substantial impact on the overall flavor of

the product. By contrast, if an acidified food product were formulated at pH

6.5, then the food scientist could select an acidulant based on other properties or economic considerations, since differences in the flavor would not be an issue.

Note: This paper was presented at the 1994 Annual Meeting of the Institute of Food Techologists, Atlanta, GA (paper #69-8).

3.6 References

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4. FLAVOR CHARACTERISTICS OF LACTIC, MALIC, CITRIC,

AND TARTARIC ACIDS IN SWEETENED, FLAVORED DRINKS

Pam Hartwig and Mina R. McDaniel

4.1 Abstract

Four common food acidulants (lactic, malic, citric and tartaric) were added to sweetened (10% sucrose), flavored drinks at two concentrations (0.4% and 0.6% w/v). Overall intensity, sweet, sour, flavor (dependent on flavor system, eg. cherry), and astringent were the descriptors evaluated by traditional descriptive analysis. Four flavors were evaluated: orange, cherry, cola and strawberry. Significant differences were found among acids and acid concentrations. Generally for all flavors, tartaric and malic acid drinks were similar in overall intensity, sourness and astringency while lactic and citric acid drinks were similar in the three attributes but lower in intensity.

4.2 Introduction

Acids (or acidulants) are vital to the food we consume. They are in nearly all foods we eat. Many natural foods are strongly acidic, such as oranges, tomatoes, cheese and yogurt. With the growth of processed foods, there is a increased need for acidulants in food. The two primary uses of acids as food additives are to enhance and modify flavor and to aid in preservation (Blendford, 1986).

In addition to sourness, acids differ in sensory character, ie. astringency and bitterness (Rubico, 1993; Rubico and McDaniel, 1992; Straub, 1992). Settle et al. (1986) evaluated 4 organic and 3 inorganic acids across a wide range of concentrations and found perceived taste qualities to be relatively stable with concentration. They also reported that the proportion of non-sour character (saltiness and bitterness) differs significantly among acids in addition to changes in sourness intensity.

The objectives of this study were to (1) investigate the sensory characteristics of sweetened, flavored drinks, containing common food acidulants, (2) determine the similarities and/or differences among the acids, ie. if certain acids can be substituted for each other in specific flavor systems, (3) determine how acids affect flavor in different flavor systems, and (4) determine if acid concentration affects the flavor of the drinks.

4.3 Materials and Methods

4.3.1 Samples

The four flavors selected were orange, cherry, cola and strawberry. Selection of flavors was based on their popularity in beverages. For each flavor system, panelists evaluated eight acid solutions (four acids at two concentrations). C. Melchers and Co. (San Francisco, CA) supplied the flavors. Four acids were selected for their commonality in beverages: citric, malic, tartaric and lactic. Anhydrous citric and DL-malic acids were obtained from Haarmann and Reimer Corp. (Elkhart, IN), L-tartaric acid (U.S.P.-F.C.C.) from J.T. Baker Inc. (Phillipsburg, NJ), and L (+) lactic acid (88%) from PURAC America, Inc. (Lincolnshire, IL). Deionized, distilled water (Milli-Q Reagent Water System, Millipore Corporation, Bedford, MA) and sucrose (C & H granulated sugar, Concord, CA) were used to formulate the beverages.

Acid solutions contained 10% (w/v) sugar and 0.01% flavor. They were prepared at two acid concentrations (0.4% and 0.6 % (w/v)). Two concentrations were selected in order to investigate the change in flavor at different levels of acid. Acid and sucrose levels chosen correspond to levels used in commercially available beverages.

In preparation of 500 mL solutions for each panel session, the acids and sucrose were weighed or pipetted volumetrically and transferred into volumetric flasks. The water and flavor were then added, and the solutions were mixed throughly on a magnetic stir plate for several minutes.

4.3.2 Panelists

Five male and four female students and staff from the Department of Food Science and Technology at Oregon State University served on the panel. All panelists had previous panel experience as trained panel members.

4.3.3 Presentation of Samples

Acid solutions were presented at 22°C in three-digit coded 2 oz. plastic cups. Each cup contained ~30 mL of sample. Training and testing were conducted one flavor at a time. In addition, the solutions were divided and presented by concentration in order to maximize acid comparisons within each acid concentration, rather than measuring differences due to concentration. At each panel session, solutions of the four acids at the lower concentration for one flavor were presented in random order for evaluation. After a five minute rest period, solutions of the four acids at the higher concentration for the same flavor were presented in random

order for evaluation. For each flavor, one replication (eight samples) was completed each session (three sessions per week). Three replications were completed.

4.3.4 Training

Traditional descriptive analysis (DA) was the sensory method selected for this study. During the first two sessions, different concentrations of citric acid, sodium chloride, sucrose, caffeine, and alum were presented to the panelists for refamilarization with the four basic tastes and astringency, and for panelists to practice rating the magnitude of different characteristics on a 16-point intensity scale (0=none, 7=moderate, 15=extreme). During the first few practice sessions, panelists listed all sensory terms which described the acid solutions. A consensus among panelists was achieved and a ballot was developed. For the testing sessions, panelists were seated in separate, well ventilated booths with incandescent lighting.

When evaluating the acid solutions, the 'sip-and-spit' method was applied. Panelists were instructed to sip the sample and manipulate in mouth for five seconds, then expectorate.

4.3.5 Statistical Analysis

Four-way analysis of variance (ANOVA) on the generated DA data was performed to determine significant differences among acid solutions

and, where appropriate, least significant differences (LSD). Panelists, acid, concentration, and replication were the sources of variation. A randomized complete block design was used as the model, and all interactions among the 4 factors were tested. Each flavor system was analyzed as a separate data set. For each flavor evaluated, descriptor means for the acid by concentration interaction were calculated across nine panelists.

Generated DA data were also analyzed by Principal Component Analysis (PCA) using Statistical Analysis System for Personal Computer (SAS, 1987, Cary, NC). ANOVA was performed on the principal axes scores generated from PCA to determine the number of significant axes. Acid, concentration, and replication were the sources of variation for both PCA and the ANOVA on principal axes scores.

4.4 Results

Five descriptors were generated by DA and used for all four flavor systems: overall intensity (OI), flavor (dependent upon flavor system ie. cherry, cola, orange, strawberry), sweet, sour, and astringent (astr). The pH of the acid drinks were 2.15 and 2.03 for tartaric, 2.28 and 2.18 for citric, 2.34 and 2.23 for malic, and 2.46 and 2.34 for lactic, for the 0.4% and 0.6% acid drinks, respectively.

Interactions of the four factors (panelist, acid, concentration, and replication) were tested by ANOVA; the panelist by treatment (concentration) interaction was significant for every flavor system. Differences in panelist sensitivity, scale usage, and a few non-discriminators on some descriptors were responsible for this interaction. The main effects of acid and concentration were highly significant for every descriptor and for every flavor system (p<0.001). For PCA, the main effects of acid and concentration were highly significant for every flavor on the first principal axis.

4.4.1 Orange

ANOVA on consensus scores from PCA determined that the first two principal axes (PA) were significant, explaining 80% and 8% for the first and second PAs, respectively. Mean scores for the first two PAs are listed in Table 4.1 to show significance differences, and are graphically presented in Figure 4.1 to show spatial relationships. The triangles represent the three replications.

On the first PA, primary separation was by acid concentration, as drinks at the higher concentration (level 2) were located further to the right on the graph (Figure 4.1). Within both acid concentrations, tartaric acid drinks were to the far right, and lactic and citric acid drinks were to the

Table 4.1-Principal axis mean scores
for orange flavored drinks

Principal Axis 1

Acid	Level	Mean Score*
Tartaric	0.6%	3.34a
Malic	0.6%	2.11b
Citric	0.6%	0.80c
Lactic	0.6%	0.07cd
Tartaric	0.4%	-0.44de
Malic	0.4%	-1.23ef
Lactic	0.4%	-2.25fg
Citric	0.4%	-2.39g
	LSD	1.03

Principal Axis 2

Acid	Mean Score*
Citric	0.61a
Malic	0.03b
Tartaric	-0.23b
Lactic	-0.40b
LSD	0.57

*Different letter subscripts indicate
significant differences at p<0.05.
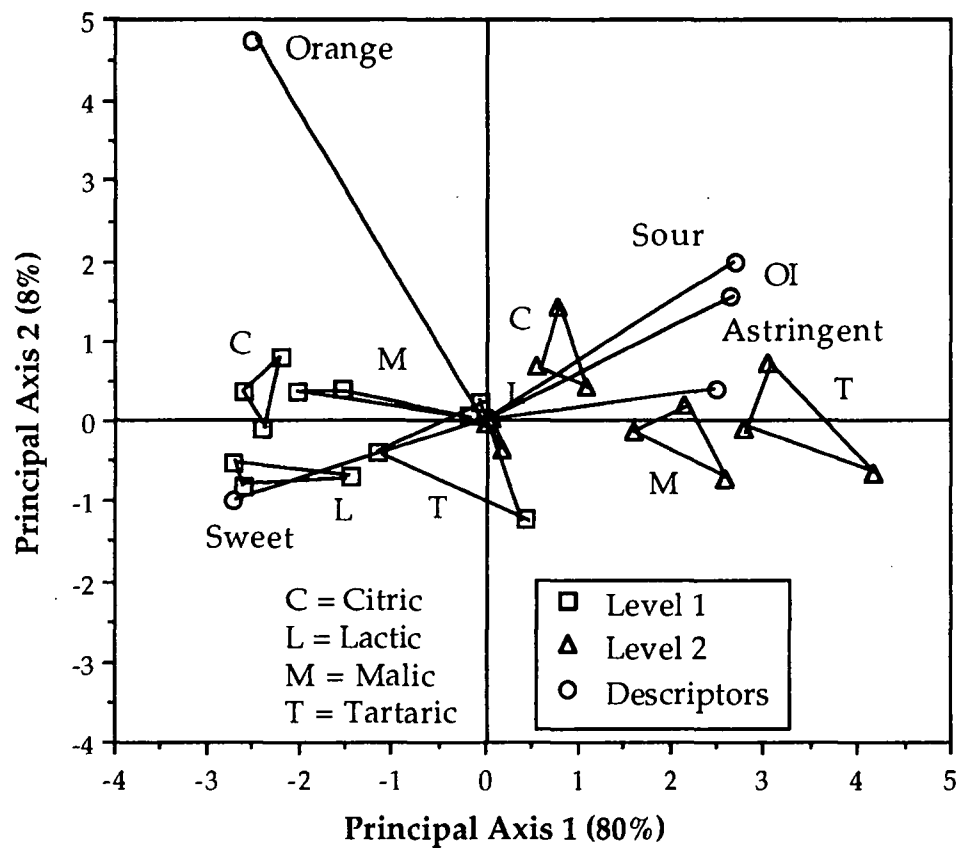
Figure 4.1-Sample consensus plot for the orange flavored drinks following principal component analysis of descriptive analysis data: principal axes 1 vs. 2. Level 1 is 0.4%, and level 2 is 0.6% w/v.

far left on the graph. Larger differences were found among the level 2 solutions. This was due to the higher acid concentration, which made differences more apparent. The descriptors OI, sour and astringent appear to be related due to their close proximity, while orange and sweet were found together on the opposite side (Figure 4.1). As expected, level 2 drinks were higher in OI, sourness and astringency due to the higher concentration of acid present. The lower concentration (level 1) drinks were higher in orange and sweetness.

On PA 2, orange drinks were only significantly different by acid, not by concentration. Citric acid drinks were significantly higher in orange flavor; the other acid drinks were more sweet.

Table 4.2 presents the mean intensity ratings and LSD values for the acid by concentration interaction on the five descriptors; figure 4.2 displays the mean intensity ratings graphically by descriptor. Tartaric acid drinks received the highest intensity scores on OI, sourness, and astringency for both concentrations; tartaric acid drinks were not significantly different from malic acid drinks (Table 4.2). Lactic acid drinks received the highest sweetness ratings. Lactic and citric acid drinks were not significantly different on any attribute at the lower acid concentration; the citric acid drink was significantly higher in OI and sourness at the higher level.

Table 4.2-Mean intensity ratings* for acid by concentration interaction on orange flavored drinks

Acid	Level	OI	Sweet	Sour	Orange	Astr
Tartaric	0.6%	11.13a	4.77d	10.07a	4.90c	5.87a
		(1.59)	(1.65)	(1.66)	(1.69)	(2.44)
Malic	0.6%	10.77a	5.37c	9.37a	4.97c	5.47ab
		(1.89)	(1.63)	(1.92)	(1.50)	(2.15)
Citric	0.6%	10.03b	5.47bc	8.87b	5.53ab	5.23abc
		(1.86)	(1.68)	(1.87)	(1.22)	(1.63)
Lactic	0.6%	9.37c	5.83abc	7.63c	5.33bc	5.23abc
		(1.90)	(1.82)	(2.22)	(1.21)	(2.62)
Tartaric	0.4%	8.57d	5.63bc	6.87d	5.33bc	4.93bcd
		(1.41)	(1.69)	(1.96)	(1.30)	(1.93)
Malic	0.4%	8.20de	5.60bc	6.63de	5.70ab	4.60cde
		(1.03)	(1.52)	(1.77)	(0.95)	(2.13)
Citric	0.4%	7.97de	5.93ab	6.00ef	5.87a	3.97e
		(1.03)	(1.70)	(1.64)	(1.25)	(2.01)
Lactic	0.4%	7.87e	6.20a	5.47f	5.50ab	4.27de
		(1.17)	(1.83)	(2.05)	(1.14)	(2.21)
	LSD	0.63	0.53	0.71	0.50	0.78

*Different letter subscripts indicate significant differences at $p < 0.05$.

Figure 4.2-Mean intensity ratings for acids on 5 descriptors at 2
concentrations for orange flavored drinks. The descriptors rated were
overall intensity, sour, astringency, sweet, and orange. Level 1 is 0.4%,
and level 2 is 0.6% w/v.

4.4.2 Cherry

ANOVA on consensus scores from PCA determined that only the first PA was significant, explaining 87% of the variation. PA mean scores are listed in Table 4.3, and are graphically presented in Figure 4.3.

Within a level, two groups of acid drinks were generally formed; tartaric and malic acid drinks were always further right on the graph than lactic and citric acid drinks. The same descriptor groupings were found as with the orange flavored drinks; OI, sour and astringent were in one cluster, and cherry and sweet were in another. Tartaric and malic acid drinks, to the far right on the graph, were higher in OI, sourness and astringency (Figure 4.3). Table 4.4 presents the mean intensity ratings and LSD values for the acid by concentration interaction on the five descriptors; figure 4.4 displays the mean intensity ratings graphically by descriptor. Within a level, the acids within each group were not significantly different on any attribute, ie. the lactic acid drink was not different from the citric acid drink. Tartaric acid drinks received the highest scores in OI, sourness, and astringency on both levels; tartaric acid drinks were not significantly diffferent from malic acid drinks.

4.4.3 Cola

ANOVA on consensus scores from PCA determined that the first two PAs were significant, explaining 90% and 6% of the variation for the

Table 4.3-Principal axis mean scores
for cherry flavored drinks

Principal Axis 1

Acid	Level	Mean Score*
Tartaric	0.6%	3.02[a]
Malic	0.6%	2.61[a]
Lactic	0.6%	0.99[b]
Citric	0.6%	0.50[b]
Tartaric	0.4%	-0.85[c]
Malic	0.4%	-1.45[cd]
Citric	0.4%	-1.97[d]
Lactic	0.4%	-2.84[e]
	LSD	0.86

*Different letter subscripts indicate
significant differences at $p < 0.05$.
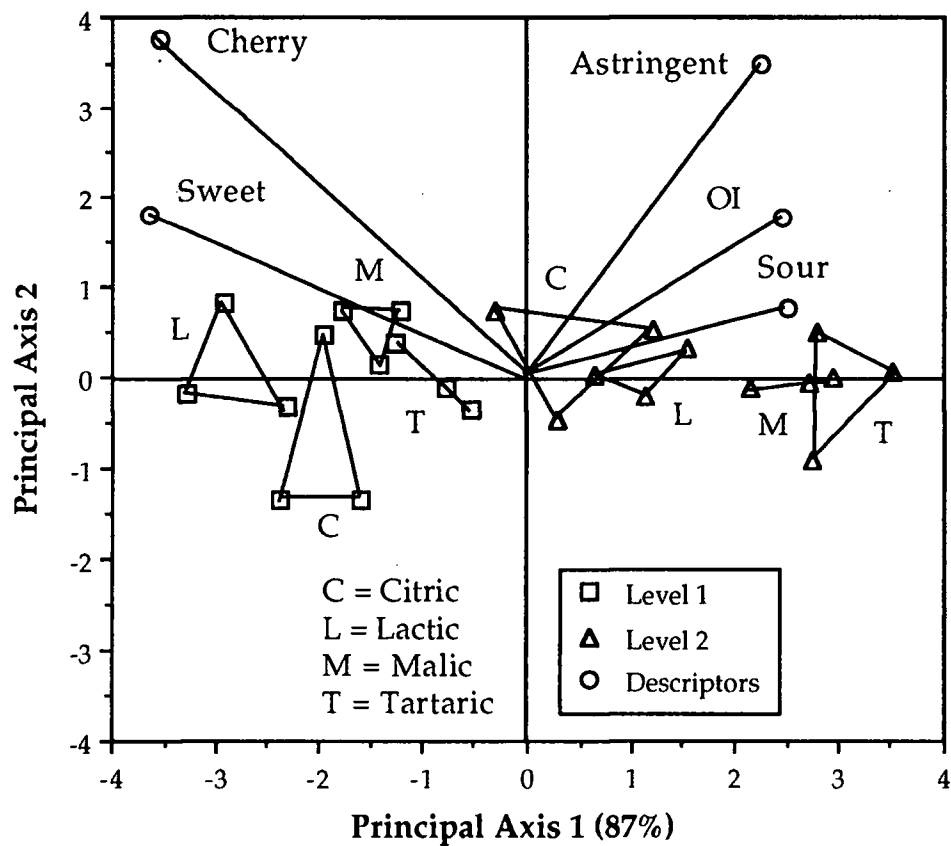
Figure 4.3-Sample consensus plot for the cherry flavored drinks following principal component analysis of descriptive analysis data: principal axes 1 vs. 2. Level 1 is 0.4%, and level 2 is 0.6% w/v.

Table 4.4-Mean intensity ratings* for acid by concentration interaction on cherry flavored drinks

<u>Acid</u>	<u>Level</u>	<u>OI</u>	<u>Sweet</u>	<u>Sour</u>	<u>Cherry</u>	<u>Astr</u>
Tartaric	0.6%	11.22[a]	4.30[d]	10.37[a]	5.00[e]	6.59[a]
		(2.03)	(1.46)	(1.94)	(1.69)	(2.91)
Malic	0.6%	11.11[a]	4.44[d]	10.07[a]	5.11[de]	6.37[ab]
		(1.55)	(1.37)	(1.66)	(1.60)	(2.68)
Citric	0.6%	9.96[b]	5.37[bc]	8.85[b]	5.56[cd]	5.52[bc]
		(1.56)	(1.47)	(1.77)	(1.34)	(2.36)
Lactic	0.6%	10.04[b]	5.04[c]	8.78[b]	5.44[cde]	5.88[abc]
		(1.79)	(1.51)	(1.99)	(1.53)	(2.72)
Tartaric	0.4%	8.44[cd]	5.48[bc]	7.00[c]	5.74[bc]	5.30[c]
		(1.72)	(1.50)	(1.88)	(1.43)	(2.57)
Malic	0.4%	8.59[c]	5.78[ab]	6.93[c]	6.11[ab]	5.07[cd]
		(1.25)	(1.58)	(1.75)	(1.50)	(2.16)
Citric	0.4%	7.70[e]	5.81[ab]	6.07[d]	5.70[bc]	4.30[de]
		(1.56)	(1.27)	(1.88)	(1.27)	(2.40)
Lactic	0.4%	7.93[de]	6.11[a]	5.48[d]	6.26[a]	4.11[e]
		(1.33)	(1.95)	(1.95)	(1.20)	(2.15)
	LSD	0.65	0.54	0.73	0.52	0.94

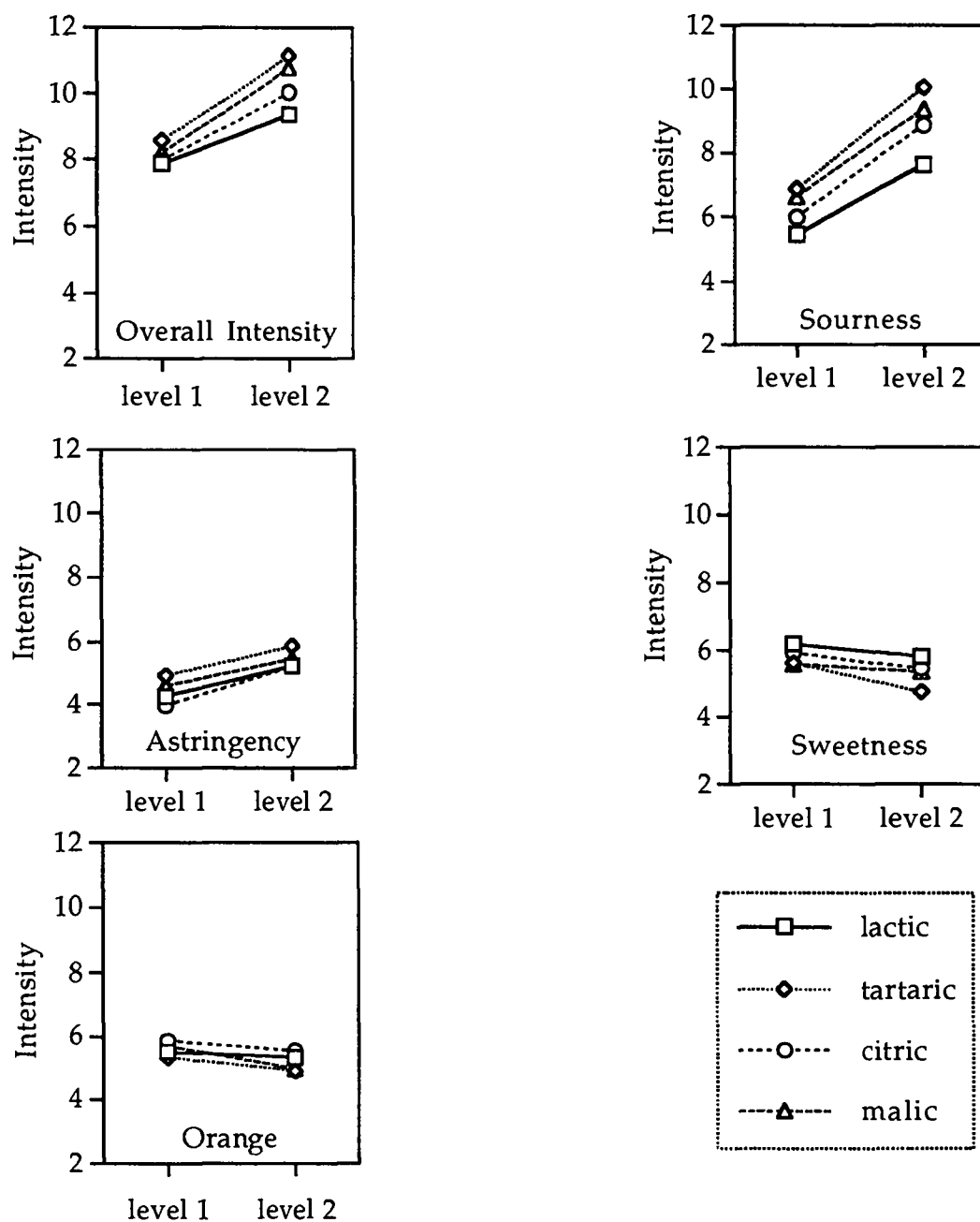*Different letter subscripts indicate significant differences at p<0.05.
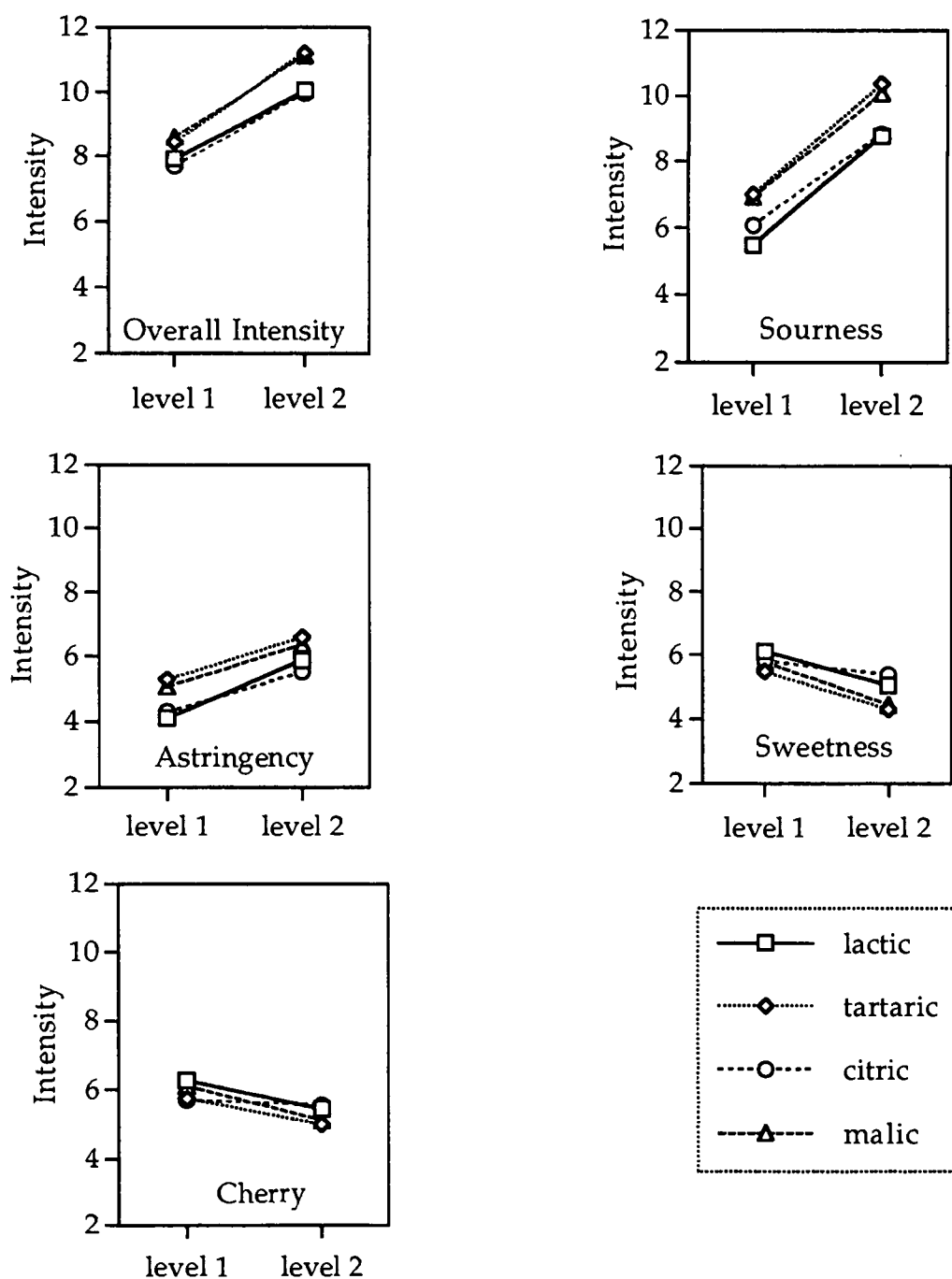
Figure 4.4-Mean intensity ratings for acids on 5 descriptors at 2 concentrations for cherry flavored drinks. The descriptors rated were overall intensity, sour, astringency, sweet, and cherry. Level 1 is 0.4%, and level 2 is 0.6% w/v.

first and second PAs, respectively. PA mean scores for the first and second PAs are listed in Table 4.5, and are graphically presented in Figure 4.5.

Within concentration, the same two groups of acid drinks (tartaric and malic acid drinks and lactic and citric acid drinks) generally clustered together as with cherry flavored drinks. On PA 1, the most variability of the four flavors was found due to large intrasample distances (Figure 4.5). We see the same trends as with the cherry flavored drinks; tartaric and malic acid drinks received higher scores than citric and lactic acid drinks on OI, sourness and astringency.

PA 2 was only significant by concentration, so comparisons among acid drinks cannot be made. On the second PA, level 2 drinks were higher in OI, sourness, and astringency. While level 2 drinks also appear to be higher in cola and sweet as well, examination of Table 4.6 shows this is not the case. Table 4.6 presents the mean intensity ratings and LSD values for the acid by concentration interaction on the five descriptors; figure 4.6 displays the mean intensity ratings graphically by descriptor. No significant differences within each group of acids (tartaric and malic acid drinks and citric and lactic acid drinks) on any attribute at either concentration were found, except for the citric acid drink at level 2 receiving a higher sourness rating than the lactic acid drink.

Table 4.5-Principal axis mean scores
for cola flavored drinks

Principal Axis 1

Acid	Level	Mean Score*
Tartaric	0.6%	3.06[a]
Malic	0.6%	2.63[a]
Citric	0.6%	0.90[b]
Lactic	0.6%	0.17[bc]
Tartaric	0.4%	-0.84[cd]
Malic	0.4%	-1.15[cde]
Citric	0.4%	-2.17[de]
Lactic	0.4%	-2.60[e]
	LSD	1.40

Principal Axis 2

Level	Mean Score*
0.6%	0.24[a]
0.4%	-0.24[b]
LSD	0.33

*Different letter subscripts indicate
significant differences at $p < 0.05$.
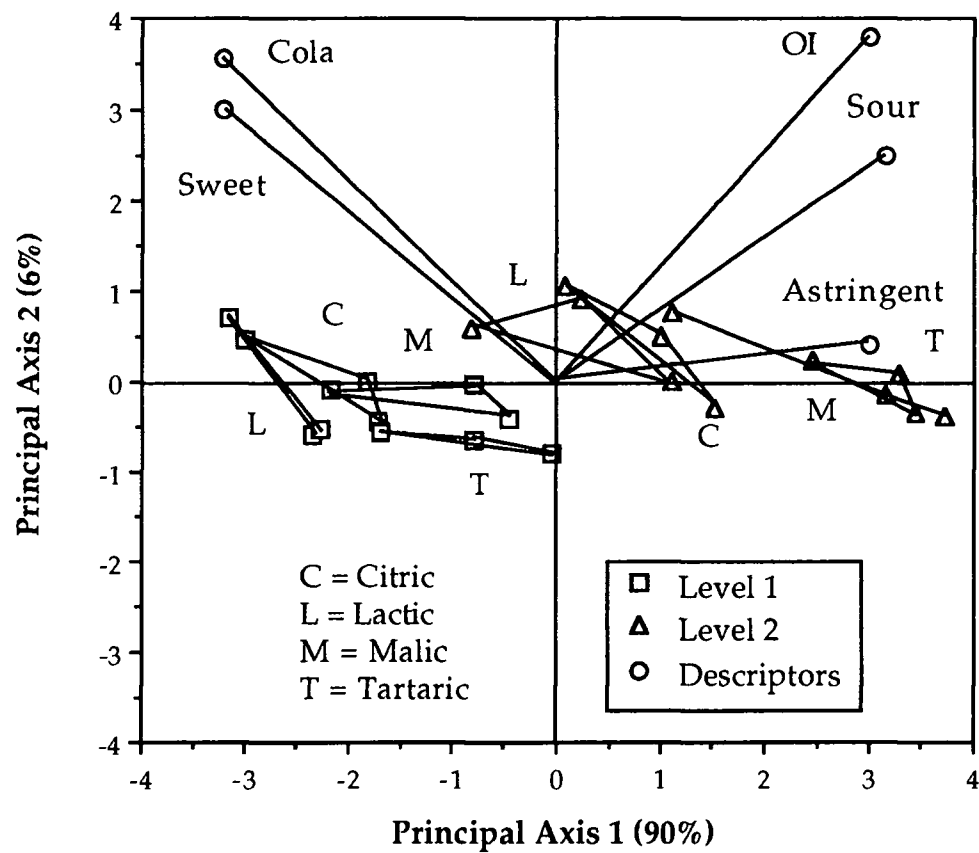
Figure 4.5-Sample consensus plot for the cola flavored drinks following principal component analysis of descriptive analysis data: principal axes 1 vs. 2. Level 1 is 0.4%, and level 2 is 0.6% w/v.

Table 4.6-Mean intensity ratings* for acid by concentration interaction on cola flavored drinks

Acid	Level	OI	Sweet	Sour	Cola	Astr
Tartaric	0.6%	11.85a	4.37d	10.59a	4.78d	6.67a
		(1.70)	(1.46)	(1.76)	(1.93)	(3.33)
Malic	0.6%	11.74a	4.44d	10.22a	4.85d	6.30a
		(1.38)	(1.31)	(1.85)	(1.56)	(3.41)
Citric	0.6%	10.70b	5.15c	9.37b	5.33c	5.81ab
		(1.61)	(1.54)	(1.84)	(1.44)	(2.36)
Lactic	0.6%	10.22b	5.44bc	8.52c	5.44bc	5.74ab
		(1.74)	(1.51)	(1.93)	(1.48)	(3.17)
Tartaric	0.4%	8.48c	5.26bc	7.26d	5.41bc	4.96bc
		(1.37)	(1.46)	(1.63)	(1.39)	(2.14)
Malic	0.4%	8.52c	5.44bc	7.19d	5.67abc	5.15bc
		(1.48)	(1.60)	(1.64)	(1.21)	(1.77)
Citric	0.4%	8.19cd	5.63ab	6.59de	5.93a	4.33c
		(1.47)	(1.64)	(2.14)	(1.24)	(2.09)
Lactic	0.4%	7.74d	5.93a	5.96e	5.89ab	4.44c
		(1.32)	(1.69)	(1.79)	(1.31)	(1.93)
	LSD	0.68	0.47	0.74	0.48	0.99

*Different letter subscripts indicate significant differences at $p < 0.05$.
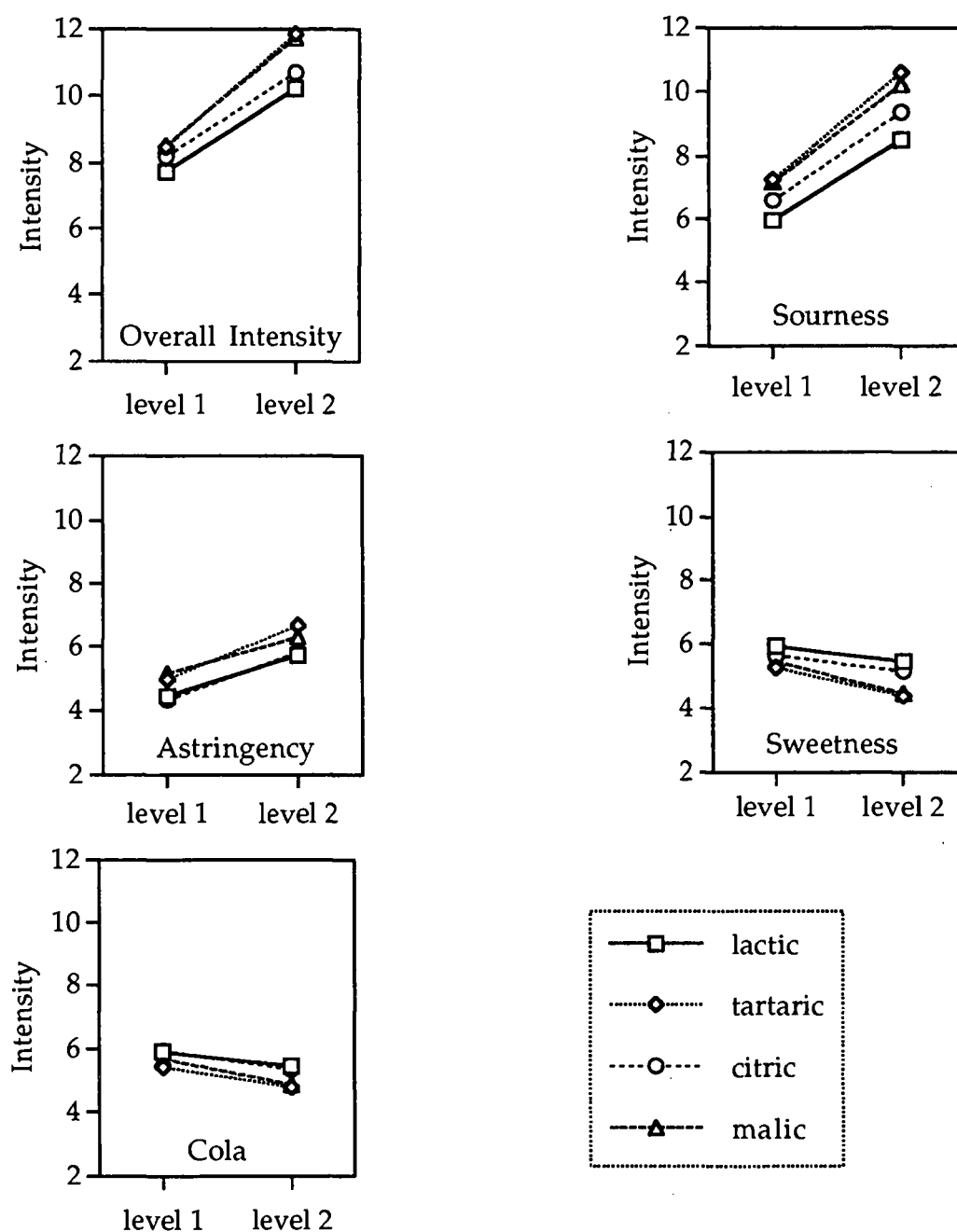
Figure 4.6-Mean intensity ratings for acids on 5 descriptors at 2 concentrations for cola flavored drinks. The descriptors rated were overall intensity, sour, astringency, sweet, and cola. Level 1 is 0.4%, and level 2 is 0.6% w/v.

4.4.4 Strawberry

ANOVA on consensus scores from PCA determined that only the first PA was significant, explaining 86% of the variation. PA mean scores are listed in Table 4.7, and are graphically presented in Figure 4.7.

Like the cherry and cola flavored drinks, within a level the same two groups of acid drinks were found (tartaric and malic acid drinks and lactic and citric acid drinks). For the descriptors, the same descriptor groupings were found; OI, sour and astringent were in one cluster, and strawberry and sweet in the other.

Table 4.8 presents the mean intensity ratings and LSD values for the acid by concentration interaction on the five descriptors; figure 4.8 displays the mean intensity ratings graphically by descriptor. Fewer differences were found among the strawberry drinks. For example, within level 1 no significant differences were found in OI and strawberry.

4.5 Discussion

4.5.1 Sourness and Overall Intensity

When rating overall intensity, sourness appeared to drive the intensity rating. The correlation of overall intensity and sourness is 0.98, which is a nearly perfect relationship. McBride and Johnson (1987) studied

Table 4.7-Principal axis mean scores
for strawberry flavored drinks

Principal Axis 1

Acid	Level	Mean Score*
Tartaric	0.6%	3.18a
Malic	0.6%	2.35a
Citric	0.6%	1.20bc
Lactic	0.6%	0.35c
Tartaric	0.4%	-1.14de
Malic	0.4%	-1.21ef
Citric	0.4%	-2.17fg
Lactic	0.4%	-2.55g
	LSD	0.96

*Different letter subscripts indicate
significant differences at $p < 0.05$.
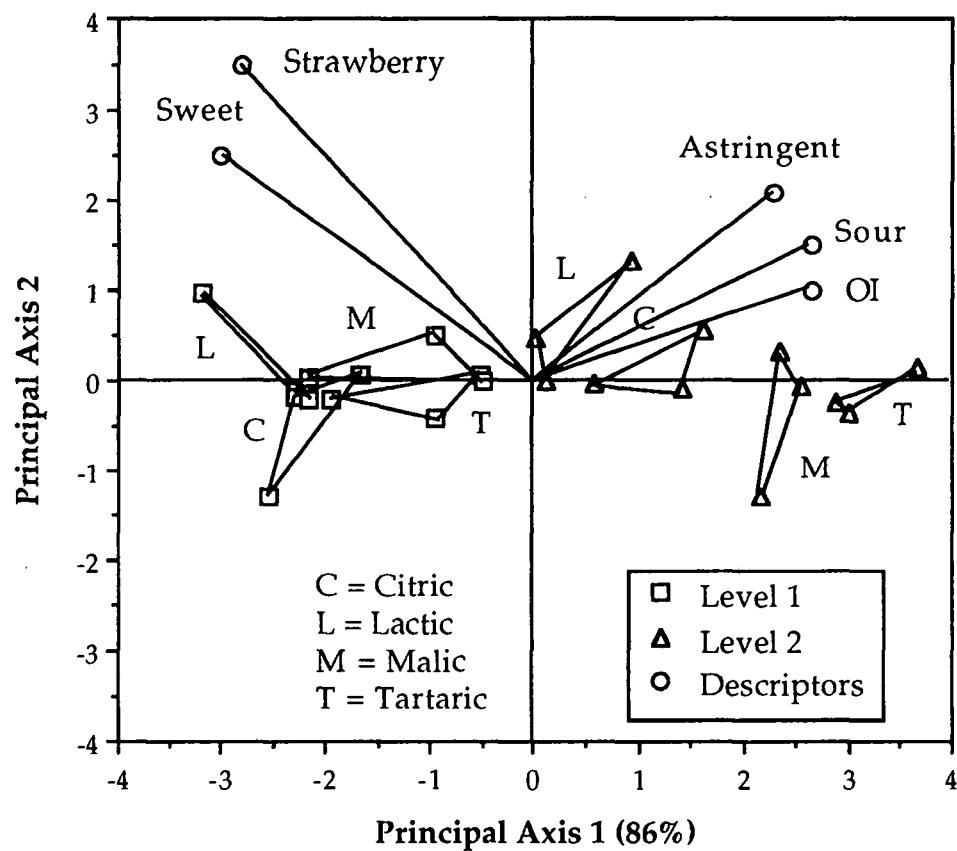
Figure 4.7-Sample consensus plot for the strawberry flavored drinks following principal component analysis of descriptive analysis data: principal axes 1 vs. 2. Level 1 is 0.4%, and level 2 is 0.6% w/v.

Table 4.8-Mean intensity ratings* for acid by concentration interaction on strawberry flavored drinks

Acid	Level	OI	Sweet	Sour	Strawberry	Astr
Tartaric	0.6%	11.11a	4.15c	10.19a	4.48d	6.33a
		(1.95)	(1.72)	(1.80)	(1.67)	(2.70)
Malic	0.6%	10.70ab	4.33c	9.85ab	4.62c	5.59ab
		(1.75)	(1.59)	(1.81)	(1.50)	(2.65)
Citric	0.6%	10.26b	5.00b	9.22b	4.93c	5.30bcd
		(1.99)	(1.59)	(2.17)	(1.59)	(2.91)
Lactic	0.6%	9.56c	5.22b	8.44c	5.41b	5.48bc
		(1.40)	(1.37)	(1.60)	(1.65)	(1.95)
Tartaric	0.4%	8.04d	5.26b	6.74de	5.59ab	4.74cde
		(1.40)	(1.53)	(1.63)	(1.37)	(2.07)
Malic	0.4%	8.30d	5.33b	7.14d	5.81ab	4.62de
		(1.59)	(1.49)	(1.46)	(1.52)	(1.71)
Citric	0.4%	7.74d	5.44ab	6.19ef	5.81ab	3.74f
		(1.68)	(1.50)	(1.94)	(1.27)	(1.77)
Lactic	0.4%	7.74d	5.92a	5.70f	5.96a	4.15ef
		(1.48)	(1.62)	(1.79)	(1.22)	(2.20)
	LSD	0.68	0.57	0.72	0.45	0.78

*Different letter subscripts indicate significant differences at $p < 0.05$.
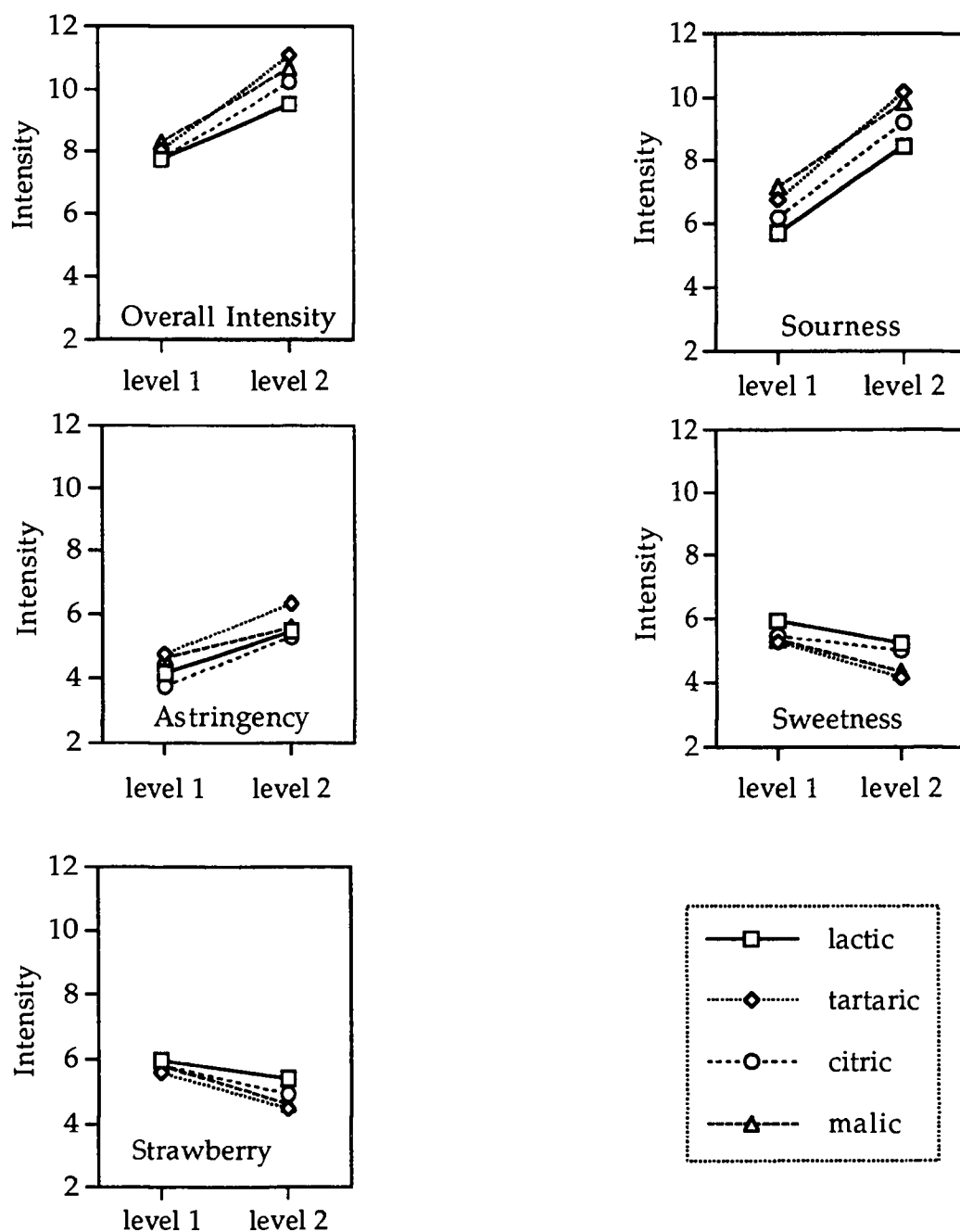
Figure 4.8-Mean intensity ratings for acids on 5 descriptors at 2 concentrations for strawberry flavored drinks. The descriptors rated were overall intensity, sour, astringency, sweet, and strawberry. Level 1 is 0.4%, and level 2 is 0.6% w/v.

sugar-citric acid mixtures in a lemon juice drink at four citric acid concentrations (0.45, 0.75, 1.11, and 1.82% w/v) and found that sourness contributed more than sweetness to overall intensity.

The primary rationale for this study was to investigate how the flavor of different acids compares in sweetened, flavored drinks in order to determine if specific acids can be used interchangedly. If certain acids can be used interchangedly, then decisions on acidulant selection can be based on other considerations. For example, in general no differences were found in sourness between citric and lactic acid drinks. This finding disputes research by Pangborn (1963), CoSeteng et al. (1989), and Hartwig and McDaniel (1994) and supports research by Rubico and McDaniel (1992). Pangborn (1963) evaluated acetic, citric, lactic, and tartaric acids at threshold and at suprathreshold concentrations and found citric acid to be the least sour, significantly less than the other three. CoSeteng et al. (1989) evaluated acetic, citric, lactic, malic, and tartaric acids in imitation apple juice; citric acid was the least sour and acetic acid the most sour on an equal w/v basis. Evaluating acetic, citric, lactic, and malic acids on an equivalent w/v and pH basis, Hartwig and McDaniel (1994) found citric acid less sour at pH 3.5 and more sour at 4.5 than lactic acid. These pH levels differed considerably from the ones in this study (pH 2.10 to 2.48). They concluded that acid flavor varies significantly, depending upon the pH level. Rubico

and McDaniel (1992) studied eight organic acids, three organic acid mixtures, and two inorganic acids on an equal w/v basis, and found citric and lactic acids were not significantly different in sourness. While each study varies in the specific acids tested, a critical factor in comparing acid sourness is the system in which evaluating the acids are evaluated, ie. water (model system) vs. a complex system such as wine. In this study, lactic and citric acid drinks were similar in sourness and could be used interchangedly, whereas citric and tartaric acids could not without significant sourness differences. However, in the study by Hartwig and McDaniel (1994) where pH is adjusted, citric and lactic acids could not be used interchangedly. This indicates that pH, in addition to the food or beverage system used, has a substantial influence on sourness and how acids compare relative to each other.

4.5.2 Sweetness

Increasing acid concentration suppressed the sweetness of sucrose. These results were found with all four acids tested, and supports most published research on the phenomena of citric acid suppressing the sweetness of sucrose (Pangborn, 1960; Pangborn, 1961; Gordon, 1965). Contradicting results, where citric acid was found to enhance the sweetness of sucrose, have also been reported (Fabian and Blum, 1943; Kamen et al., 1961).

Sweetness and sourness had an inverse relationship; as sourness increased, sweetness decreased. The correlation from PCA for sweet and sour ranged from -0.81 for orange drinks to -0.88 for cola drinks. While sourness generally increased three intensity points from 0.4% to 0.6% acid, sweetness only dropped approximately one intensity point. This indicates that only a minor suppression of sweetness was found.

4.5.3 Astringency

From the mean intensity scores (Figures 4.2, 4.4, 4.6, and 4.8), astringency appeared to be strongly related to sourness. While the astringency of the acid drinks increased as acid concentration increased, astringency was not as sensitive to concentration increases as sourness; the increase in astringency intensity was much lower than with sourness. The correlation for astringency and sourness ranged from 0.77 for the orange drinks to 0.90 for the cola drinks. Tartaric acid received the highest astringency ratings at both concentration levels in every flavor system, confirming the findings of Rubico and McDaniel (1992). However, these differences were not significant among acids at the same concentration. In a model system, Hartwig and McDaniel (1994) found lactic acid more astringent than citric and malic acids at pH levels 3.5 and 4.5. This was not found in this study. Citric and lactic acid drinks for all flavors and at both levels were not found different in astringency intensity. Differences in pH

and the presence of sugar and flavor could be responsible for the disparity found with the two studies.

4.5.4 Flavor

For the orange flavor system, citric acid drinks were significantly higher in orange flavor than the other acid drinks. No other interactions between acid and flavor were noted. This enhancement of flavor by citric acid has been reported by Haarmann and Reimer (1994). While citric acid manufacturers have claimed this enhancement for years, little evidence can be found in the scientific literature. When flavoring a citrus product, citric acid would be the logical acidulant selected, providing an additional boost in citrus flavor not found with other acids. However, for a non-citrus flavored beverage, any of the four acids profiled could be appropriate, depending upon the particular application.

For all four flavor systems, flavor intensity of the drinks was lower at the higher acid concentration (Figures 4.2, 4.4, 4.6, and 4.8). This decrease in flavor was significant in many cases, however it spanned only approximately a one intensity point difference. In contrast, the sourness intensity increase was approximately 3 points on a 16 point intensity scale. While some acid drinks were statistically different in flavor intensity, on a practical level little difference exists among acids. Both acid concentrations evaluated were at levels comparable to similar products on the market.

Thus, by increasing acid concentration, more flavoring will be needed to achieve the same flavor intensity, which means increased costs to the manufacturer.

4.6 Conclusions

This research demonstrates that acidulant selection in food has a substantial impact on the overall flavor of drinks. Furthermore, in specific flavor systems, some acids can be substituted for each other in terms of flavor. This enables selection of acids to be based on chemical properties and/or cost. For example, malic and tartaric acid drinks and lactic and citric acid drinks were not different on any attribute in three of the four flavor systems tested. However, if an orange flavored drink is being developed, selecting citric acid over the other three could have a significant effect on orange flavor intensity.

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5. FLAVOR CHARACTERISTICS OF EMULSIONS CONTAINING
LACTIC, CITRIC, AND ACETIC ACIDS

Pam Hartwig and Mina R. McDaniel

5.1 Abstract

Emulsions were titrated with three common food acidulants (lactic, citric, acetic) and two acid blends (acetic/lactic 1:1, 2:1) to achieve three pH levels (3.5, 4.5, 5.5). Using traditional descriptive analysis, seven descriptors were generated: overall intensity, sour, sweet, eggy/mayonnaise, vinegar, nutty/malty, and citrus. Significant differences were found among acids and pH levels on all attributes. The acetic acid emulsion at pH 3.5 received the highest ratings in overall intensity, sour and vinegar. No differences were found among emulsions at pH 5.5. The lactic and citric acid emulsions were not significantly different on any attribute for any pH level.

5.2 Introduction

Acids are essential constituents of most foods. Whether naturally

present, added during processing, or produced by fermentation, acids

impart flavor that enhances and modifies the overall flavor of food

(Blendford, 1986). Acids differ in sensory character (Hartwig and McDaniel,

1994a; Hartwig and McDaniel, 1994b; Rubico and McDaniel, 1992; Straub,

1992). Thus, acid selection is a critical determinant of the overall flavor in

formulated food products.

Unlike food processors, nature is not confined to any one acid in

preservation. One acid is rarely found alone; hence, combining acids may

more naturally mimic flavor in foods. A good example of two acids

frequently found in combination is lactic and acetic acids. When lactic acid

is added to vinegar in sweet pickles, dill pickles, and relish, the right

proportion can produce an improvement in flavor and a milder, more

subtle taste sensation (Fabian and Wadsworth, 1939; Wadsworth and

Fabian, 1939; Gardner, 1972). In salad dressings and marinades, acetic/lactic

combinations have been found to act synergistically against outgrowth of

heterofermentive lactobacilli (Doores, 1990). In a model (water) system,

Hartwig and McDaniel (1994b) found acetic/lactic combinations lower in

overall intensity and sourness than acetic acid alone at pH levels of 3.5

and 4.5.

While the model (water) system served as a benchmark for evaluating acid flavor changes with pH, this study was conducted to further explore how acid flavor changes with pH adjustment in emulsions. The system selected is representative of food products such as salad dressings, mayonnaise and sauces. The objectives were to (1) investigate the sensory characteristics of emulsions, containing common food acidulants and acid blends, (2) determine similarities and/or differences among emulsions containing different acids, ie. if certain acids can be substituted for each other, and (3) determine if the flavor of the emulsion changes with pH.

5.3 Materials and Methods

5.3.1 Samples

Oil-in-water emulsions containing three acids (citric, acetic, lactic) and two acid blends (acetic/lactic 1:1, 2:1) at three pH levels (3.5, 4.5, 5.5) were evaluated. Anhydrous citric acid was obtained from Haarmann and Reimer (H & R) Company (Elkhart, IN), glacial acetic acid (U.S.P-F.C.C.) from J.T. Baker Inc. (Phillipsburg, NJ), and L-lactic acid (88%) from PURAC America, Inc. (Lincolnshire, IL). These acids were chosen due to their commonality in food. In addition to the three acids, two acid blends were

prepared: acetic/lactic (1:1 and 2:1). Selection was based on their frequent combination in pickled products. The three pH levels were selected as they are common levels at which food systems are formulated. Acidified food products such as salad dressings, canned vegetables, and hot dogs are examples at 3.5, 4.5 and 5.5, respectively. Emulsions consisted of 40% w/v sunflower oil (Hunt-Wesson, Fullerton, CA), 0.8% mayodan M612 (Grindsted, Brabrand, Denmark) as an emulsifier, 1.4% egg yolk powder and 2.5% egg white powder (Sam Wylde Flour Co., Inc., Seattle, WA), 5.0% sugar (C&H, Concord, CA). The remaining portion was comprised of acid (0.02% to 2.4%) and deionized, distilled water (47.9% to 50.3%) (Milli-Q Reagent Water System, Millipore Corp., Bedford, MA).

5.3.2 Preparation of Samples

In preparation of 500 g samples for each panel session, mayodan and the egg powders were weighed and mixed thoroughly in a 400 mL beaker. The oil was weighed, added to the dry mixture and mixed by hand. The mixture was then transferred into a 3.3 L mixing bowl. A Euro nine speed Mixmaster mixer was used for the electronic mixing (Model #2361, Sunbeam Appliance Co., Milwaukee, Wisconsin). While mixing at speed 4, the sugar and most of the water were gradually added. The mixture was titrated to desired pH level with acid solutions while mixing, then weighed

and brought to final weight with water. The emulsions were stored in plastic containers overnight at 0°C.

Ten percent acid solutions were used for titration. In addition, for the emulsions containing acetic acid at pH 3.5, 30% solutions were used due to the large quantity of acetic acid required.

5.3.3 pH Determinations

The pH levels of the 15 samples were measured with a microprocessor pH/mV meter (Corning Model 320, Medfield, MA) equipped with a combination pH electrode (Sensorex Model S200C, Stanton, CA).

5.3.4 Panelists

Six male and four female students and staff from the Department of Food Science and Technology at Oregon State University served on the panel. All panelists had previous panel experience as trained panel members.

5.3.5 Presentation of Samples

Samples were presented at 22°C in three-digit coded 2 oz. plastic cups. Each cup contained ~30 mL of sample. Training and testing sessions were limited to a single pH level. This was done in order to maximize acid comparisons within each pH level, rather than measuring differences due to pH changes. Due to the similar intensities of the samples within each

pH level, character differences were easier to observe by this presentation.

At each session five emulsions (containing each of the three acids and two

acid blends) were randomly presented for evaluation. One replication

(fifteen samples) was completed within one week (three sessions per

week). Three replications were completed.

5.3.6 Training

Traditional descriptive analysis (DA) was the sensory method selected

for this study. During the first session, different concentrations of citric

acid, sodium chloride, sucrose, caffeine, and alum were presented to the

panelists for refamilarization with the four basic tastes and astringency,

and for the panelists to practice rating the magnitude of different

characteristics on a 16-point intensity scale (0=none, 7=moderate,

15=extreme). During the first few practice sessions panelists listed all

sensory terms which described the emulsions. A consensus among

panelists was achieved, and a ballot was developed. For the testing

sessions, panelists were seated in separate, well ventilated booths with

incandescent lighting.

Panelists were instructed to manipulate the sample in the mouth for

five seconds, then expectorate. In between samples panelists rinsed with a

dilute lemon juice solution to facilitate removal of the oily mouth-coating.

Panelists then rinsed with water before evaluating the next emulsion.

5.3.7 Statistical Analysis

Four-way analysis of variance (ANOVA) on the generated DA data was performed to determine significant differences among emulsions and, where appropriate, least significant differences (LSD). Panelists, acid, pH, and replication were the sources of variation. A randomized complete block design was used, and all interactions among the 4 factors were tested. For each flavor evaluated, descriptor means for each acid were calculated across the ten panelists.

Generated DA data were also analyzed by Principal Component Analysis (PCA) using Statistical Analysis System for Personal Computer (SAS, 1987). ANOVA was performed on the principal axes scores generated from PCA to determine the number of significant axes. For PCA the fifteen emulsions were analyzed as independent samples (not five acids at three pH levels) due to the considerable differences among emulsions at different pH levels.

5.4 Results and Discussion

ANOVA on consensus scores from PCA determined that the first two principal axes (PA) were significant, explaining 83% and 13% for the first and second PAs, respectively. Mean scores for the first two PAs are listed

in Table 5.1, and are graphically presented in Figure 5.1. The triangles

represent the three replications.

On the first PA, primary separation was by pH level, as emulsions at

pH 3.5 were all significantly different from the ones at pH 4.5. Within a pH

level, the largest differences were found among emulsions at pH 3.5

(Figure 5.1). This was due to the amount of acid needed to achieve the

lowest pH level tested, leading to more apparent differences among

samples. For example, emulsions containing acetic acid required a

significantly larger quantity of acid to achieve pH 3.5 (Table 5.2).

Of the acids studied, acetic acid has the highest pK_a at 4.75. Therefore,

lowering the pH of this emulsion to 3.5, which is more than one pH unit

away from the pK_a, required a substantial volume of acid titrant. As a

result, these emulsions are separated on the PCA plot from all other

samples. At pH 5.5 emulsions were not significantly different from each

other; hence, all were contained in a small cluster.

The lactic and citric acid emulsions were not significantly different

from each other at any pH level on any PA. This has been reported

previously by Hartwig and McDaniel (1994a) and Rubico (1993). However,

research by Hartwig and McDaniel (1994b) found lactic acid more sour than

citric acid at pH 3.5 and less sour at pH 4.5. This indicates that the

Table 5.1-Principal axis mean scores for emulsions

Principal Axis 1		
Acid	pH	Mean Score*
Acetic	3.5	5.31[a]
A/L (2:1)	3.5	4.27[b]
A/L (1:1)	3.5	2.99[c]
Lactic	3.5	1.00[d]
Citric	3.5	0.99[d]
Acetic	4.5	-0.41[e]
A/L (2:1)	4.5	-0.82[ef]
A/L (1:1)	4.5	-0.95[efg]
Citric	4.5	-1.18[fg]
Lactic	4.5	-1.46[gh]
Citric	5.5	-1.83[h]
A/L (2:1)	5.5	-1.89[h]
Lactic	5.5	-1.96[h]
Acetic	5.5	-1.99[h]
A/L (1:1)	5.5	-2.07[h]
	LSD	0.63

Principal Axis 2		
Acid	pH	Mean Score*
Citric	3.5	1.79[a]
Lactic	3.5	1.33[ab]
Acetic	4.5	0.89[bc]
A/L (1:1)	4.5	0.65[bcd]
Citric	4.5	0.43[cde]
A/L (2:1)	4.5	0.19[cdef]
Lactic	4.5	-0.01[defg]
A/L (1:1)	3.5	-0.18[efg]
A/L (2:1)	3.5	-0.57[fgh]
Lactic	5.5	-0.63[gh]
A/L (2:1)	5.5	-0.64[gh]
Acetic	5.5	-0.66[gh]
A/L (1:1)	5.5	-0.67[gh]
Citric	5.5	-0.73[gh]
Acetic	3.5	-1.17[h]
	LSD	0.81

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*Different letter subscripts indicate
significant differences at $p < 0.05$.
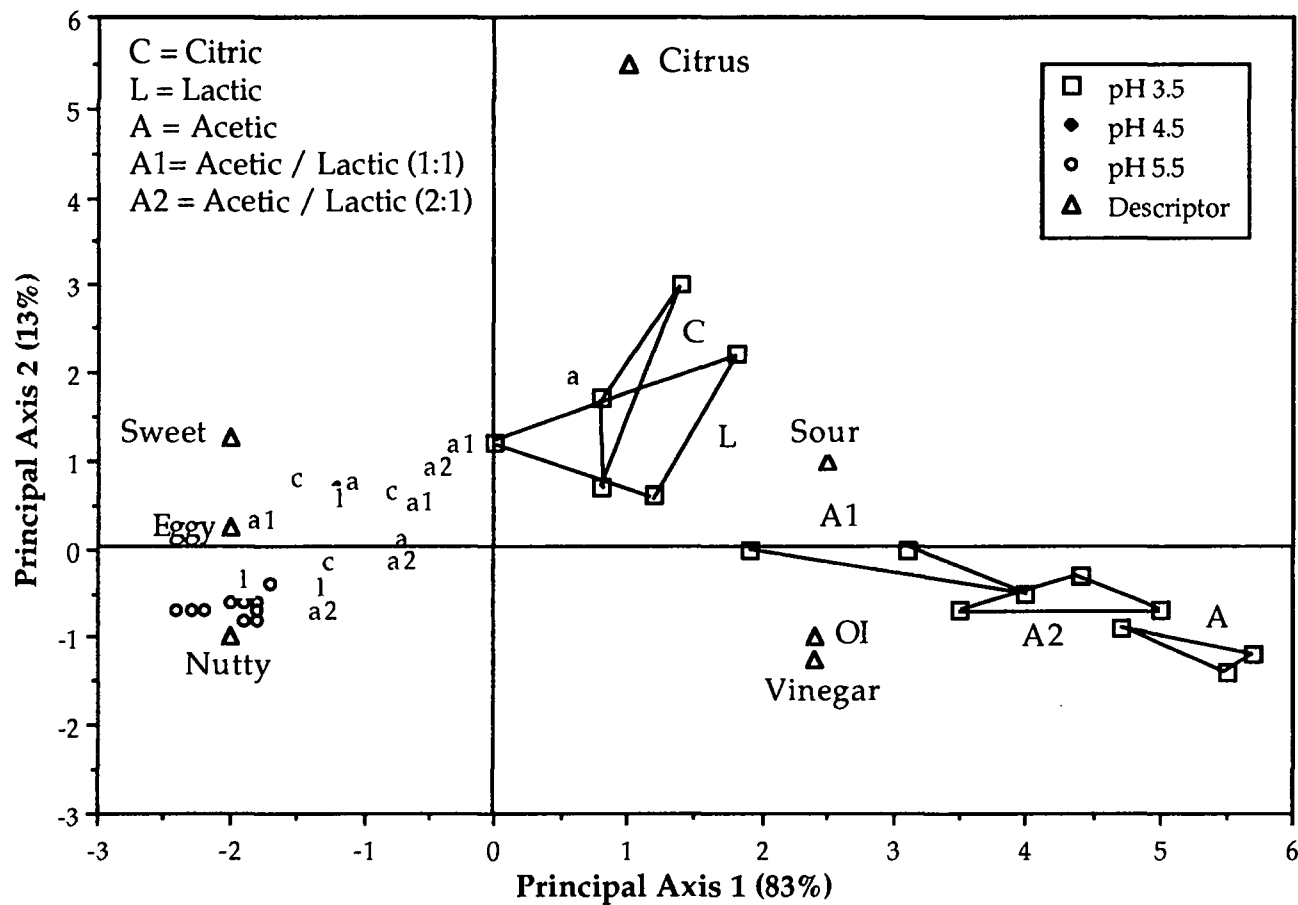
Figure 5.1-Sample consensus plot for emulsions following principal component analysis of descriptive analysis data: principal axes 1 vs. 2.

Table 5.2-Means[1] and standard deviations[2] of acid
added to emulsions in % (w/w) to achieve 3 pH levels

Acid	pH levels		
	3.5	4.5	5.5
Citric	0.66[1]	0.12	0.02
	(0.05)[2]	(0.02)	(0.004)
Lactic	0.69	0.15	0.03
	(0.11)	(0.01)	(0.004)
Acetic	2.40	0.14	0.02
	(1.07)	(0.03)	(0.002)
A/L (1:1)	1.26	0.13	0.02
	(0.58)	(0.02)	(0.005)
A/L (2:1)	1.86	0.13	0.02
	(0.41)	(0.02)	(0.004)

emulsion system affected acid flavor, and diluted the differences found in the model system by Hartwig and McDaniel (1994b).

Seven descriptors were generated from DA: overall intensity (OI), sour, sweet, eggy/mayonnaise (eggy), vinegar, nutty/malty (nutty), and citrus. Sour, OI and vinegar drove the first PA to the right, and sweet, eggy and nutty drove PA 1 to the left. Emulsions at pH 3.5 were highest in sourness, OI and vinegar, and emulsions at pH 5.5 were highest in sweetness, eggy and nutty. Correlations between OI and vinegar, OI and sour, and vinegar and sour were 0.99, 0.96 and 0.94, respectively. This illustrates that vinegar was the largest contributor to OI. The relationship between OI and sour with acids has been reported by Hartwig and McDaniel (1994b) and McBride and Johnson (1987).

The second PA was characterized by citrus (positive axis), as the citric acid emulsion at pH 3.5 was the furthest up on the graph, followed closely by the lactic acid emulsion (Figure 5.1). At pH 3.5, the citric acid emulsion was significantly different from all acetic acid samples. Citrus is a flavor reported to be imparted by citric acid (H & R, 1994). Hartwig and McDaniel (1994a) reported an enhancement of orange flavor by citric acid in orange drinks, which was not found with the other organic acids evaluated. Hartwig and McDaniel (1994b) found only a few panelists using the descriptor citrus to discriminate among acids.

PA 2 was also characterized by vinegar (negative axis) (Figure 5.1). While citric acid inherently possesses a citrus flavor, the separation of citric acid from acetic acid and its blends was also influenced by vinegar character. Lactic acid was not expected to be similar to citric acid in citrus flavor. However, lactic acid was grouped with citric acid, probably due to its lack of vinegar character.

ANOVA on DA data for each emulsion determined that all main effects (panelists, acids, pH levels, replications) were significant ($p<0.001$). For mean separation LSD values were calculated; significant differences were found among emulsions on every attribute. Interactions among the 4 factors were also highly significant, and are a result of differences in panelist sensitivity and scale usage. Table 5.3 displays the mean intensity ratings and LSD values for each emulsion across the seven descriptors. Emulsions containing acetic acid received the highest ratings in OI, sourness and vinegar at pH levels 3.5 and 4.5. At pH 5.5 no significant differences among emulsions were found on any descriptor.

At pH 4.5 the acetic acid emulsion was highest in OI, significantly higher than the citric and lactic acid emulsions. It was also highest in sourness and vinegar, significantly higher than all others except for the acetic/lactic 2:1 emulsion. No significant differences were found among emulsions in sweetness, eggy and nutty at pH 4.5.

Table 5.3-Mean intensity ratings for emulsions

Acid	pH	OI	Sour	Sweet	Eggy	Vinegar	Nutty	Citrus
Citric	3.5	7.97^d	6.93^d	4.37^{bc}	3.07^e	2.33^d	2.17^d	2.20^a
		(2.01)	(2.00)	(1.19)	(1.55)	(2.66)	(1.62)	(2.11)
Lactic	3.5	8.07^d	6.67^d	3.80^c	3.33^{de}	2.07^{de}	2.07^{de}	1.90^{ab}
		(2.16)	(2.67)	(1.54)	(1.94)	(2.68)	(1.86)	(2.17)
Acetic	3.5	13.37^a	12.50^a	1.47^e	1.20^g	9.67^a	0.67^f	0.70^d
		(2.11)	(2.33)	(1.63)	(1.63)	(4.25)	(1.40)	(1.60)
A/L(1:1)	3.5	10.57^c	9.70^c	2.80^d	1.97^f	5.43^c	1.60^e	1.03^{cd}
		(2.30)	(2.52)	(2.14)	(1.69)	(3.65)	(1.63)	(1.65)
A/L (2:1)	3.5	11.80^b	11.00^b	1.93^e	1.70^{fg}	8.00^b	1.00^f	1.00^{cd}
		(2.41)	(2.53)	(1.66)	(1.66)	(4.15)	(1.78)	(1.89)
Citric	4.5	6.07^{fg}	2.63^g	4.87^{ab}	3.70^{bcd}	0.33^{gh}	3.37^{bc}	0.90^{cd}
		(1.51)	(1.88)	(1.41)	(1.78)	(0.80)	(1.77)	(1.56)
Lactic	4.5	6.17^{fg}	2.43^g	4.87^{ab}	3.93^{abc}	0.17^h	3.57^{abc}	0.53^{de}
		(1.66)	(1.76)	(1.41)	(1.87)	(0.65)	(1.94)	(1.17)
Acetic	4.5	7.07^e	4.50^e	4.63^{ab}	3.57^{cde}	1.40^{ef}	3.30^{bc}	1.40^{bc}
		(1.72)	(2.18)	(1.25)	(1.74)	(2.04)	(1.84)	(2.18)
A/L (1:1)	4.5	6.53^{ef}	3.60^f	5.13^a	3.70^{bcd}	0.47^{gh}	3.10^c	1.03^{cd}
		(1.59)	(1.71)	(1.41)	(1.68)	(1.07)	(1.73)	(1.88)
A/L (2:1)	4.5	6.80^e	3.93^{ef}	4.63^{ab}	3.80^{abcd}	0.90^{fg}	3.30^{bc}	0.77^d
		(1.73)	(2.27)	(1.40)	(1.65)	(1.37)	(1.73)	(1.68)
Citric	5.5	6.07^{fg}	0.80^h	4.60^{ab}	4.13^{ab}	0.00^h	3.73^{ab}	0.00^f
		(1.64)	(1.00)	(1.99)	(2.01)	(0.00)	(1.84)	(0.00)
Lactic	5.5	5.87^g	0.60^h	4.63^{ab}	4.33^a	0.00^h	3.73^{ab}	0.07^{ef}
		(1.61)	(1.00)	(1.50)	(1.95)	(0.00)	(2.08)	(0.37)
Acetic	5.5	6.03^{fg}	0.70^h	4.90^{ab}	4.20^{ab}	0.00^h	3.77^{ab}	0.00^f
		(1.59)	(0.99)	(1.40)	(2.01)	(0.00)	(2.01)	(0.00)
A/L (1:1)	5.5	6.00^{fg}	0.70^h	4.90^{ab}	4.23^{ab}	0.00^h	3.93^a	0.00^f
		(1.49)	(0.99)	(1.45)	(2.00)	(0.00)	(2.08)	(0.00)
A/L (2:1)	5.5	6.07^{fg}	0.77^h	4.63^{ab}	4.30^a	0.03^h	3.73^{ab}	0.07^{ef}
		(1.46)	(1.04)	(1.52)	(1.84)	(0.18)	(2.03)	(0.25)
	LSD	0.59	0.65	0.62	0.55	0.73	0.56	0.53

5.5 Conclusions

When formulating a food product, acidulant selection has a considerable effect on the overall flavor of a food. While some acids such as lactic and citric can be substituted for each other, citric and acetic acids would not be equivalent. From a practical standpoint, the citric acid emulsions required the least amount of acid to achieve the three pH levels (Table 5.2). Therefore, it would be more economical to use citric acid in affecting acid flavor. Considerable differences were found among comparison to the other acids. Also, it would not be sensible to use acetic acid for a product at pH 3.5 or lower due to the large quantity of acid required. Another important findings is that the pH of the final product is a critical factor emulsions at pH 3.5, while no notable differences were present at pH 5.5. Thus, at pH 5.5 selection of acids can be based on other factors such as pKa, cost or solubility, since flavor considerations are not an issue.

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6. OVERALL CONCLUSIONS

When formulating an acidified food product, how do you know which acid is best suited for this application? In some instances acid selection may not be critical, while in others selecting one acid over another can have a considerable effect on the flavor of food. Awareness of differences in acid flavor could be beneficial to the food technologist as acidified food products can be formulated with desired characteristics.

This research demonstrates that acids contribute significantly to the flavor of food. In addition, this research illustrates that acids differ considerably in sensory character at equivalent pH levels and % w/v. While it is no secret that acids are sour, that is just part of the story in describing acid flavor. Acids can also differ in sweet, bitter, astringent, citrus, and vinegar character. Thus, acidulant selection is an important consideration in formulating food.

In specific food systems, acids can be used interchangedly. For example, in a non-citrus flavored drink, lactic and citric acids give similar taste sensations and could be used interchangedly, whereas citric and tartaric acids would not be equivalent.

The flavor of an acid also changes with pH. For example, in the model study at pH 6.5 and in the emulsion study at pH 5.5, samples were

not significantly different on any attribute. In this case, acidulant selection could be based on other factors, ie. chemical properties, cost. However, in both systems at pH 3.5, large flavor differences were found among acids. Thus, the pH of the final product is an important factor in acidulant selection.

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