

Abstract

NETA, EDITH RAMOS DA CONCEICAO. A Chemical Basis for Sour Taste Perception in Aqueous Solutions and Fresh Pack Dill Pickles. (Under the direction of Roger F. McFeeters.)

Sour taste is related to pH and acids present in foods. It is not currently possible, however, to accurately predict and modify sour taste intensity simply by knowing the pH and the type and amount of acids present. The main objective of this study was to investigate the roles of protonated organic acid species and hydrogen ions in evoking sour taste perception. In order to test whether the intensity of sour taste was a linear function of the molar concentration of protonated organic acid species plus the molar concentration of hydrogen ions, pH of solutions was adjusted with sodium hydroxide resulting in other uncontrolled variables, such as organic anions and sodium ions. A secondary objective was to investigate the roles organic anions and sodium ions in suppressing sour taste intensity of acid solutions. Sour taste intensity and other sensory attributes were measured using the Spectrum MethodTM. The distribution of organic acid species was calculated using pHToolsTM, a modeling program implemented in MATLABTM. Mixtures of three acids, chosen from a group of eight organic acids, were used for testing the effect of protonated organic acid species, and hydrochloric acid was used for testing the effect of hydrogen ions. The effect of organic anions and sodium ions in suppressing sour taste intensity were tested at constant concentrations of protonated acid species and hydrogen ions. The effect of sodium ions was further investigated by adding NaCl to acid solutions in the absence of organic anion species. Sour taste increased linearly with hydrogen ion concentration ($R^2 = 0.995$), as well as with the molar

concentration of protonated organic acid species at pH 3.5 ($R^2 = 0.949$), 4.0 ($R^2 = 0.952$), and 4.5 ($R^2 = 0.975$). Type of acid was not a determinant factor for sour taste intensity after adjusting for the effects of protonated organic acid species and hydrogen ion concentration. Protonated organic acid species and hydrogen ions were shown to be additive factors and produce approximately the same sour taste response on a molar basis. Furthermore, sour taste intensity was linearly related to the total concentration of protonated organic acid species in fresh pack dill pickles at pH 3.5 and 4.0 ($R^2 = 0.999$, 0.999 , respectively). Suppression of sour taste in pH adjusted solutions was shown to be only partially explained by sodium ions and organic anions, although both seem to have an effect. This study reveals a simple chemical basis for the sour taste of organic acids and may allow for better control of flavor in the formulation of acidified foods.

**A Chemical Basis for Sour Taste Perception in Aqueous
Solutions and Fresh Pack Dill Pickles**

by

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Dedication

For my parents, who are my reason and inspiration to succeed.

Biography

Edith Ramos da Conceicao Neta was born on February 28, 1979 in Brazil. She was raised in a medium-size city with her parents, sister and brother. She then moved to Vicosa to start her undergraduate education. Edith obtained a Bachelor's degree in Food Engineering at Federal University of Vicosa, Brazil in December 2003. As an undergraduate, she completed internships at two fruit pulp processing centers and also gained experience in a dairy product manufacturing facility. In her senior year, Edith worked as an exchange student in the USDA ARS Food Science Research Unit at North Carolina State University for six months. The objective of her research was to validate a mathematical model that predicted chemical parameters related to pH and buffers in biological systems. After finishing her internship, Edith returned to Brazil to complete the last semester of her undergraduate program. She then headed back to NC State to pursue a Master's degree in Food Science under the direction of Dr. Roger McFeeters. During her time at NC State, she participated in the PepsiCo internship program in the summer of 2005. Edith considers living in the United States an amazing experience, and she has really enjoyed living in a new culture, learning a new language and making new friends.

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CHAPTER 1

Literature Review

1.1 Chemistry of Sour Taste

The chemistry of sour taste appears to be relatively simple in that it has been associated only with acids. In 1898, sour taste was first linked to hydrogen ions (Richards, 1898). However, as early as 1920 it was recognized that sour taste could not be explained solely on the basis of hydrogen ions. It was clear that organic acids could also stimulate a sour taste response. Organic acids all have one or more carboxyl groups, but other than carboxyl groups they have a variety of structural features. The carboxyl groups have hydrogen ions that dissociate depending upon the strength of the acid. For an acid with a single carboxyl group this dissociation is determined by the dissociation constant $K_a = \frac{[H^+][A^-]}{[HA]}$, where $[H^+]$ is the concentration of hydrogen ions in solution, $[A^-]$ is the concentration of the acid in which the hydrogen ion has dissociated from the carboxyl group, and $[HA]$ is the concentration of the acid in which a hydrogen ion remains bound to the carboxyl group. There has been a considerable amount of research that has attempted in various ways to relate the intensity of sour taste perception to properties of organic acids and their ability to bind or release hydrogen ions into water solution.

1.1.1 Hydrogen Ions, Protonated Acid Species, and Titratable Acidity

For convenience, pH ($-\log [H^+]$) is generally used to indicate hydrogen ion concentration in acid solutions and foods. The property shared by all acids is the dissociation of protons ($[H^+]$) when they are dissolved in water, thus one might expect a direct relationship between sour taste and pH. However, human psychophysical and animal physiological studies have shown that organic acids such as acetic and citric acids are more sour than hydrochloric acid at the same pH (Richards, 1898; Pfaffmann, 1959; Koyama and Kurihara, 1972; Lyall and others, 2001; Ganzevles and Kroeze, 1987a; Ogiso and others, 2000; Ugawa and others, 1998; Richter and others, 2003; Lugaz et al, 2005). Beidler (1967) showed that solutions of 20 organic and inorganic acids that produced an equivalent neural response to 5 mM HCl in rats had pH values ranging from 2.11 to 3.14. Likewise, Makhoulf and Blum (1972) found a poor correlation between sour taste of organic acids and stimulus pH using acid-induced salivary secretion as an index of response. Moreover, the pH of various organic acid solutions is considerably different at their observed threshold concentrations (Berg and others, 1955; Amerine and others, 1965). Altogether, these results indicate that, in addition to hydrogen ions, anions and/or protonated (undissociated) acid species play a role in determining sour taste intensity of organic acids.

Titrateable acidity (also known as total acidity) is a measure of both bound and free hydrogen ions in solution. It is experimentally determined by measuring the amount of sodium hydroxide required to raise the pH to 8.2 where phenolphthalein indicator dye turns pink. In 1920, Harvey (1920) raised the question: ‘What do we taste in acid solutions, hydrogen ion concentration or total acidity?’ Many studies have reported that sour taste

intensity increases with increasing titratable acidity at a given pH (Makhlouf and Blum, 1972; Norris and others, 1984; Lugaz and others, 2005; Plane and others, 1980). Shallenberger (1996) proposed that sour taste intensity is entirely a function of potential hydrogen ion concentration. The implication of his hypothesis is that acid solutions of equal normality have equal sour taste intensities as they have equivalent titration coefficients. In summary, these studies suggest that the chemical mechanism for sour taste perception may be analogous to a titration of an acid by a base to a neutral end point, the receptor serving as the 'base'. (Norris and others, 1984; Makhlouf and Blum, 1972; Beatty and Cragg, 1935; Kenrick, 1931; Liljestrand, 1922; Shallenberger, 1996). However, Richards (1898) showed that hydrochloric acid solution was more sour than equi-normal solutions of tartaric, citric, and acetic acids. Acetic acid was perceived as the least sour among the organic acids, followed by citric and tartaric acids. Likewise, Becker and others (1907) concluded that normality was not sufficient to predict sour taste response, the ranking order being: HCl > HNO₃ > lactic > acetic > butyric acid. CoSeteng and others (1989) showed that solutions of citric, malic, tartaric, lactic and acetic acids with equivalent pH and titratable acidity gave significantly different sour taste responses. Likewise, Pangborn (1963) found no relation between pH, titratable acidity and relative sour taste intensity of several organic acids at both threshold and suprathreshold concentrations.

Buffer capacity is expressed as the molar concentration of strong acid or strong base which causes variation of the pH of a buffer solution by 1 unit. Early studies suggested that the buffer capacity of an acid solution is important to determine sensory response to sour taste. Beatty and Cragg (1935) and Kenrick (1931) reported that the volume of a phosphate

buffer required to bring the pH of various equi-molar acids to about 4.5 was proportional to the sour taste intensity. Noble and others (1986) also found a positive correlation between buffer capacity of acid solutions and sour taste in five of six paired comparisons. The exception was the contrast between malic and succinic acids as the former has greater buffer capacity at the pH tested, but was rated as less sour. However, Ganzvles and Kroeze (1987b) measured the volume of NaOH needed to bring the pH of equimolar solutions of tartaric, citric, formic, lactic, acetic, and propionic acids to 4.4 and reported a titration volume rank-order which differed from the sour taste response rank order.

1.1.2 Anions

The effect of anionic species on the transduction mechanism of sour taste is not clearly understood. Norris and others (1984) suggested that differences in the anion composition of acids in solution influence the binding of the stimuli to the receptor sites. Anions have been speculated to have a collaborative effect on sour taste response by decreasing the positive charge of the membrane and thus increasing the binding of hydrogen ions to receptor molecules (Beidler and Gross, 1971; Koyama and Kurihara, 1972, Beidler, 1978). Beidler (1971) suggested that the observation by Richards (1898) that acetic acid solution was perceived to be more sour than hydrochloric acid at the same pH could be explained by the presence of anions in the acetic acid solution. He also cited a study by Liljestrand (1922), where the pH of buffered acetic acid–sodium acetate solution was significantly higher than acetic acid solution at their observed threshold concentrations. The higher concentration of anion in the buffer solution was deemed as the major factor for the lower hydrogen ion concentration required to produce an equivalent taste response. Similarly, Beidler (1967) tested the effect of anions on sour taste response by comparing the neural response of rats to acetic acid solution and to a buffered acetic – sodium acetate solution. Although the neural responses were comparable, the pH of the buffered solution was considerably higher (lower hydrogen ion concentration) as compared to the acetic acid solution. He explained the results by suggesting that in the buffered solution a lower concentration of hydrogen ion is needed because acetate ions augment binding of hydrogen ions to the receptor sites.

On the other hand, many studies have theorized that sodium salts of organic acids may inhibit the interaction between taste receptor cells and protonated (undissociated) acids (Ganzevles and Kroeze, 1987a; DeSimone and Price, 1976). Makhoulf and Blum (1972) reported that sodium acetate showed characteristics of competitive inhibition with acetic acid in sour taste perception. Additionally, Lawless (1991) suggested that some large anions decrease sour taste intensity through contributing tastes of their own, which act as masking stimuli.

1.1.3 Molar Concentration

Makhlouf and Blum (1972) reported that sour taste intensity and molar concentration of an acid are described by an equation in the form used for enzyme kinetics and that the order of molar potency was citric > tartaric > succinic > lactic > acetic > propionic. Ganzvles and Kroeze (1987b) by means of a filter-paper method found the molar rank-order for sourness to be HCl > tartaric > citric > formic > acetic > lactic > propionic acid. Lugaz and others (2005) evaluated the time-intensity of sour taste of various organic acids at equal molar concentrations and reported the order of potency to be citric, malic, lactic, and acetic acid from the most to the least sour. Ough (1963) reported that, when citric, tartaric, fumaric, and adipic acids were added to wine in equimolar concentrations, citric acid was judged as the most sour, followed by tartaric and fumaric acids, which produced equally sour taste response, and by adipic acid, which was judged as the least sour.

From these studies, it is clear that the molar concentration of acids is not the only governing factor in determining sour taste response. The diversity of results in attempting to rank the sour taste of acids based on molar concentration may be explained by differences in the range of molarity and pH tested.

1.1.4 Physical and Chemical Properties of Organic Acids

Sour taste intensity has been associated with the capacity of the acid to dissociate, which is dependent upon the pK_a values of the acids. Makhoulf and Blum (1972) found that the molar potency of several organic acids was inversely related to the first pK_a . They concluded that acids having a higher capacity to dissociate (small pK_a) are able to elicit higher sour taste response. In contrast, Hartwig and McDaniel (1995) reported that organic acids with higher pK_a values (low capacity to dissociate) were perceived as more sour than those having lower pK_a values. However, the comparisons were made on a weight percentage basis rather than a molar basis, so comparisons were made with unequal numbers of acid molecules.

A common hypothesis for sour taste perception is that an acid compound must penetrate the cell membrane and release hydrogen ions inside the cell in order to elicit a sour taste response (Taylor and others, 1930; Gardner, 1980). This hypothesis is supported by the strong correlation found between hydrophobicity of a fatty acid and its ability to elicit sour taste perception (Taylor and others, 1930; Chauncey and others, 1963; Greenberg, 1980). Gardner (1980) showed that the ease of an acid to penetrate the membrane was enhanced as non-polar groups were introduced into the acid structure and reduced with addition of polar groups. Chauncey and others (1963) and CoSeteng and others (1989) assessed the effect of physical and chemical properties of several organic acids on sour taste perception. They found that hydrophobicity was an important factor in sour taste response, and that the intensity of sour taste decreased as the number of carboxyl groups in the molecule increased. CoSeteng and others (1989) reported that sour taste intensity of organic acids increased with

molecular weight. Chauncey and others (1963) found that introduction of polar groups on acid molecules led to decreased sour taste response. In contrast, Noble and others (1986) reported that the sour taste of several binary solutions at a constant pH and titratable acidity did not correlate with the degree of hydrophobicity. Likewise, Norris and others (1984) reported that hydrophobicity was not sufficient to predict the relative sour taste response of citric, fumaric, and tartaric acids.

Siebert (1999) applied principal component analysis to 11 properties of 17 organic acids and reported that threshold concentrations could be well predicted in solutions by the number of polar groups, the number of double bonds, molecular size, and the solubility of the acids in non-polar solvents. Moskowitz (1971) assessed the relationship between sour taste and molar acid concentration as well as sour taste and pH for 24 organic acids in a psychophysical function study. The author reported that the relative sour taste intensity of acids differed across a range of molar concentrations and pH and concluded that no simple relationship existed between sour taste intensity and physical-chemical properties of the acids, such as molecular weight, polarity, and optical form.

Although significant efforts have been made to understand the chemistry of sour taste, it is not currently possible to accurately predict and modify sour taste intensity in foods by knowing the acids' concentration and pH. Our laboratory recently developed a new hypothesis for the chemical basis of sour taste of organic acids (Johanningsmeier and others, 2005), which proposes that sour taste intensity is a linear function of the total molar concentration of all organic acid species that have one or more protonated carboxyl groups

plus the concentration of free hydrogen ions. This hypothesis was based upon correlations between sour taste intensity and the molar concentrations of protonated organic acid species and hydrogen ions in dill pickles, sauerkraut, and acid solutions analyzed from published data sets (Hartwig and McDaniel, 1995; Sowalsky and Noble, 1998).

1.2 Physiology of Sour Taste

1.2.1 Anatomy of the Taste System

A taste bud contains a group of 30-100 taste cells and is located on special folds and protrusions of the lingual epithelium called papillae (Stewart and others, 1997). There are three types of gustative papilli: fungiform, foliate and vallate. Fungiform papilli are mushroom-shape structures located at the front of the tongue. They typically consist of 3-5 taste buds. Foliate and vallate papilli contain hundreds of taste buds and are found, respectively, at the sides and rear of the tongue (Gilbertson and others, 2000).

Taste receptors are bipolar cells having a short lifetime, approximately 10 days (Beidler, 1971). They occur either lightly or densely packed in taste buds. Taste receptor cells are oriented perpendicularly to the surface in contact with the stimuli in a roughly parallel arrangement with apical and basolateral regions. The apical region is composed of microvilli, which have contact with the oral cavity, while the basolateral region contains synapses with the sensory nerve fibers (Lindemann, 1996; Lindemann, 2001). Taste buds are innervated by the glossopharyngeal nerve (posterior tongue), the chorda tympani branch of the facial nerve (anterior tongue) and the vagal nerve (laryngeal buds) (Gilbertson and others, 2000). Taste perception is initiated by the interaction of the chemical stimulus with receptor sites located on microvilli. The stimuli may bind to a membrane receptor or protein, it may pass through a channel or it may activate or block an ion channel (Lindemann, 1996). The receptors then activate the synapses by releasing neuro-transmitters, which cause excitation of the nerve fibers. The signals are carried to the brain stem where the central nervous system processes the information resulting in taste perception (Lindemann, 2001).

1.2.2 Sour Taste Transduction

Significant efforts have been put forth to determine the transduction mechanisms underlying sour taste perception. Numerous animal species such as frog, rat, hamster, mudpuppy, mouse, and chimpanzee have been used in physiological studies of sour taste. However, there are several limitations to development of a single model for sour taste transduction. Differences in chemical sensitivity of taste receptors occur across animal species (Lyall and others, 2001; Beidler and Gross, 1971). Thus, one should be cautious when extrapolating conclusions from one species to another. The existence of multiple transduction pathways within a given species also represents a challenge (Lindemann, 1996). Another difficulty is the fact that lowering the pH may affect several ion channels and transport systems (Liu and Simon, 2001). Furthermore, it is crucial to consider the influence of adjacent structures such as nonsensory epithelial tissue and paracellular diffusional pathways in determining sour transduction mechanisms (Lindemann, 1996).

It is generally accepted that activation of sour taste receptors causes depolarization of acid-sensitive taste receptor cells (Lyall and others, 2001). Each cell of the body has an electrical potential difference across its plasma membrane due to differences in ion concentrations between the inside and outside of the cell (Beidler, 1978). In nerve cells, this difference is mainly determined by the membrane permeability of sodium, potassium and chloride ions (Beidler, 1967; Myamoto and others, 1998). At resting potential, the cell is negatively charged inside relative to the outside. As the cell is stimulated, an electrical gradient is established across the membrane, causing cell depolarization. Thus, the cell becomes positive inside relative to the outside. (Beidler, 1967; Lindemann, 1996). The

activation of receptors by acidic stimuli is usually accompanied by decreased intracellular pH and release of transmitters, which initiate excitation of afferent nerve fibers to the brain cortex, ultimately leading to sour taste perception (Lyall and others, 2001).

A number of methods, including electrophysiological measurements, have been applied to investigate the cellular mechanisms of sour taste transduction in animal models. A common approach has been the use of microelectrodes to record the cell depolarizing potential caused by acids (Kinnamon and others, 1988; Myamoto and others, 1988). Electrical recordings from the human chorda tympani nerve have been shown to correlate well with subjective intensity of sour taste (Borg and others, 1967). Loose-patch recording and micromethods such as patch clamping and microscopic fluorescence imaging have also been applied (Lindemann, 1996; Kinnamon and others, 1988; Miyamoto, 1998). In addition, measuring the intracellular pH has been a valuable technique (Lyall, 2001). Recent studies have benefited from genetic and molecular methodologies, which have provided advanced tools for elucidating the molecular interactions governing the transduction of sour taste stimuli (Ugawa and others, 1998).

Acid stimuli primarily elicit a sour taste response at low concentrations, which is transduced by the chorda tympani. However, at high concentrations, acids may also evoke an irritation sensation, which is transduced by the trigeminal nerve (Nagy and others, 1982; Yamasaki and others, 1984). Two general hypotheses exist as to the mechanism of sour taste perception. The first one assumes extra-cellular adsorption of acidic stimuli, while the second one presumes penetration of sour taste stimuli into the cell. Early studies proposed that sour

taste perception is induced by binding of hydrogen ions to a phosphate group on phospholipids in the gustatory receptor membrane (Koyama and Kurihara, 1972). It has been hypothesized that the chemical nature of the sour taste receptor is essentially like a lipid rather than a protein since lengthening the alkyl chain structure of an acid favors its diffusion through the membrane, while introducing polar groups decreases the acid's permeability (Taylor, 1927; Taylor, 1930; Taylor and others, 1930). In contrast, Beidler (1971) suggested that proteins are the most likely candidates for taste receptors and proposed that carboxyl groups are the specific receptor sites for sour stimuli.

It has also been speculated that protonated organic acid molecules induce sour taste perception by entering the apical membrane of taste receptor cells, dissociating inside the cell, and thus causing cytoplasmic acidification (Lyll and others, 2001; Gardner, 1980; Ogiso and others, 2000; Taylor and others, 1930). This hypothesis is supported by the evidence that weak acids acidify taste cells to a greater extent than do strong acids at a fixed pH (Koyama and Kurihara, 1972; Lyll and others, 2001; Ogiso and others, 2000; Ugawa and others, 1998; Richter and others, 2003). Lyll and others (2001) studied the effect of weak and strong acids on intracellular pH and chorda tympani nerve stimulation in rats. They found that nerve responses were positively correlated with a decrease in intracellular pH and that weak acids were more effective than strong acids in acidifying the cell. They suggested that undissociated (fully protonated) acids passively diffuse into taste receptor cells across the membrane.

Different receptor systems are possibly implicated for sour taste transduction of hydrogen ions and protonated acid species (Ganzevles and Kroeze, 1987a; Ganzevles and Kroeze, 1987b). Studies of self- and cross-adaptation of weak and strong acids have attempted to confirm this hypothesis. It is generally believed that if adaptation to one stimulus does not reduce the response to another stimulus, the mechanisms responsible for transducing the two stimuli are different (McBurney and others, 1972). Ganzevles and Kroeze (1987a) proposed that the mechanisms involved in sour taste perception from protonated organic acid species and hydrogen ions are different because acetic and hydrochloric acid behave differently in self and cross-adaptation studies.

Recently, it has been established that cell depolarization caused by the interaction between protons and ion channels mediates sour taste transduction (Herness and Gilbertson, 1999; Ugawa and others, 1998; Miyamoto and others, 1999; Stevens and others, 2001; Richter and others, 2003). Many studies have attempted to identify the specific channel molecules that function as receptors for sour tastants. In the mudpuppy, blockage of voltage-sensitive K^+ channels located in the apical membrane has been suggested to mediate sour taste transduction (Kinnamon and others, 1988; Cummings and Kinnamon, 1992; Kinnamon and Roper, 1988). In the mouse, basolaterally located chloride channels as well as apically located proton-activated cation channels have been associated with acid-induced cell depolarization (Miyamoto and others, 1998; Miyamoto and others, 2000). In the hamster, amiloride-blockable epithelial sodium channels (EnaC) also engaged in the taste of organic salts, have been proposed to contribute to sour taste transduction (Gilbertson and others, 1992). In frogs, depolarization occurs through a proton-gated cation channel and an apical

proton pump (Myamoto and others, 1988; Lauger, 1991). Ugawa and others (1998) and Lin and others (2002) proposed that acid-sensing ion channels (ASICs) located on the apical and basolateral membranes are involved in sour taste transduction in rats. Stevens and others (2001) proposed that hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are gated by extracellular protons and may act as receptors for sour taste stimuli in rats. In addition, proton movement through paracellular pathways may contribute to sour taste transduction (Herness and Gilbertson, 1999; DeSimone and others, 1995; Lyall and others, 2001). It has been proposed that tight junctions in the basolateral membrane are permeable to hydrogen ions, which may cause a decrease in the intracellular pH, contributing to sour taste transduction (Lindemann, 2001; DeSimone and others, 1995). In humans, there is evidence that neither apical epithelial Na^+ channels nor an apical Na^+/H^+ exchangers are involved in sour taste transduction (DeSimone and others, 2001).

The physiology of sour taste perception remains controversial and it is clear that significant diversity among species exists with regard to cellular schemes used for detection of stimuli. The variety of mechanisms proposed, even within individual species, highlights the complexity of elucidating sour taste transduction. Current knowledge does not provide guidelines to suggest what receptors or transduction mechanisms govern the intensity of sour taste in aqueous solutions or foods.

1.3 Descriptive Sensory Analysis

Descriptive sensory analysis makes use of trained panelists to discriminate and describe components of a product in both quantitative and qualitative terms (Meilgaard and others, 1991). Descriptive methods have been extensively used for quality control, product matching, sensory mapping of food products, and for understanding consumer responses in relation to product sensory attributes (Gacula, 1997). They have also been used to track changes during shelf-life as well as to evaluate the effect of packaging, ingredients and processing on the final sensory quality of food products (Murray and others, 2001).

There are several techniques for descriptive sensory analysis, which differ in sensory philosophy, length of training, presentation of results, and sensory scales (Gacula, 1997). The most common methods are The Flavour Profile MethodTM, Texture Profile MethodTM, Quantitative Descriptive AnalysisTM, Quantitative Flavour ProfilingTM, Free-Choice ProfilingTM, and SpectrumTM method (Murray and others, 2001). The use of these methods in both academic research and the industrial environment is well documented. A detailed description of the SpectrumTM method is presented in the following section.

1.3.1 The SpectrumTM method

The SpectrumTM method is based on sensory evaluation of the complete ‘spectrum’ of the product attributes by a trained panel. Panelists may be trained to either evaluate a few attributes or profile the product for all sensory modalities (Murray and others, 2001). In order to evaluate each attribute, a 15-point numeric scale is usually applied, which is standardized and anchored with multiple reference points to decrease panelist variability. Scales are created to have equal intensity across attributes. Reference points are chosen to represent different intensities on each attribute spectrum (Lawless and Heymann, 1998). An extensive training schedule is required to enable the panel to fully define and describe the selected sensory modalities (Meilgaard and others, 1991). The panelists are trained to use the scale identically, which makes the data values absolute and permits the use of standard statistical procedures (Lawless and Heymann, 1998). Therefore, the SpectrumTM method is designed to be suitable for cross-laboratory and across time comparisons as well as for comparison with instrumental analysis. Moreover, it has been successfully applied in routine quality assurance operations as the panel mean score can be used to determine product quality specifications (Murray and others, 2001; Lawless and Heymann, 1998). The SpectrumTM method has been widely used to assess the appearance, flavor and aroma of several food products, such as meat (Johnsen and Civille, 1986), peanut (Johnsen and others, 1998) and catfish (Johnsen and Kelly, 1990; Johnsen and others, 1987).

1.4 pH Tools Software

The pH Tools toolbox is a mathematical modeling program implemented in MATLABTM, which was developed and validated in this laboratory (Dougherty, 2002; Neta and others, 2005). The core software routines allow prediction of buffer capacity, ionic strength and titration curves of buffer systems, including foods. The distribution of various species of an acid in aqueous solution can be readily calculated. For complex systems, such as food extracts or slurries, ionizable groups of unknown identity or concentration can be titrated with an acid or base and then treated mathematically as complex buffers. The buffering influence of complex or undefined components are modeled by local polynomial regression (Fan and Gijbells, 1996), while defined or measurable components are modeled using expressions based on extensions of the standard acid-base theory (Butler and Cogley, 1998). Thus, if one or more components of a food extract are identified and their concentration measured, the contribution of these components can be subtracted from the experimental titration curve, and the residual titration curve is calculated. Published pK_a values for acids, generally given at 25°C and zero ionic strength, are adjusted for the experimental temperature, ionic strength, and if known, the dielectric constant of the medium. Reliable adjustments of pK_a values can be calculated to an ionic strength up to 1.2 with a modification of the Davies equation developed by Samson and others (1999).

In this study, pH Tools was a critical tool to calculate the distribution of acid species. The capability to readily do these complex calculations for food systems is essential both to carry out the experiments and for the practical prediction or targeting of protonated organic acid species concentrations to control sour taste of foods.

1.5 Food Acidulants

Acidulants are commonly used as food ingredients due to their functional properties and distinct applications. They are found in a wide variety of food products such as baked goods, beverages, confections, gelatin desserts, jams, jellies, dairy products, processed meats, fats and oils (Jonhson and Peterson, 1974; Hartwig and McDaniel, 1995).

The main application of acids is to provide and enhance flavor of foods and beverages (Hartwig and McDaniel, 1995). For instance, tartaric acid is very suitable when combined with grape flavor while phosphoric acid is appropriate for use in cola beverages (Jonhson and Peterson, 1974). In addition to contributing to flavor, acidulants serve numerous purposes in food processing. They are commonly used for pH control to inhibit microbial growth of food products and aid in gelling properties of gelatin desserts, jams, jellies and jellied candies (Jonhson and Peterson, 1974). Many acidulants also have the ability to chelate trace metal ions and act as a synergist with antioxidants (Jonhson and Peterson, 1974). Additionally, food acids have been used to prevent non-enzymatic browning, modify viscosity and melting properties, provide leavening, and act as curing agent (Hartwig and McDaniel, 1995; Gardner, 1977). There are many factors that influence the selection of an acidulant for a particular application. Acids should be able to blend well with other flavors and have high solubility. Physical form, and hygroscopicity must also be taken into consideration when selecting an acidulant (Jonhson and Peterson, 1974). Table 1.1 shows the main physical and chemical properties of several organic acids commercially used as food ingredients.

1.5.1 Organic Acids of Importance to the Food Industry

Acetic acid is one of the earliest used flavoring agents, since it is the major component of vinegars (Gardner, 1977). Vinegars are widely used as ingredients in several food products, such as salad dressing, mayonnaise, canned vegetables, sour and sweet pickles, sauces, and catsups.

Adipic acid is a non-hygroscopic acid, naturally occurring in beets (Ito and others, 1979). It has a slow developing taste profile, being described as smooth and mild (Doores, 1990). Aqueous solutions of adipic acid impart strong buffering at pH 2.5-3.0 and have been reported as the least sour among the common food acidulants (Gardner, 1977). Adipic acid is employed in many processed foods, including fruit-flavored beverages, jams and jellies, gelatin desserts, canned vegetables, fat, oils, flavor extracts, and confectionary products (Andres, 1995; Dziezaz, 1990). The low hygroscopic characteristic of adipic acid makes it suitable for use in powdered food mixtures, especially when a delicate, no tang flavor profile is desired (Gardner, 1977; Dziezaz, 1990).

Citric acid is a naturally occurring food acid and is the most commonly used organic acid in the food industry. It is found in high concentration in certain fruits such as lemon (4.0-8.0%), tangerines (0.9-1.2%), grapefruit (1.2-2.1%), and oranges (0.6-1.0%) (Bouchard and Merritt, 1979). The pleasant tart taste of citric acid makes it suitable for use in citrus-flavored and carbonated beverages. Furthermore, it is employed in canned fruits and vegetables, dairy products such as cheese and buttermilk, salad dressing, and mayonnaise. Citric acid is also added in candies to enhance flavor, invert sucrose, and prevent sugar

crystallization (Gardner, 1977). However, its hygroscopic characteristic often limits its application in powdered products (Doores, 1990)

Fumaric acid naturally occurs in sugar cane, mushrooms, plant leaves, and rice (Dziezaz, 1990). It is one of the most economical of the food acids, having a low cost and high strength in imparting sour taste on an equal weight basis (Gardner, 1966). However, its low solubility in water and slow rate of dissolution often limits its application (Gardner, 1977). Fumaric acid may be blended with other food acids without giving a 'burst' to the taste (Gardner, 1977). It has been described as having a metallic note (Jonhson and Peterson, 1974). Fumaric acid is used in fruit-juice drinks, gelatin desserts, pie fillings, wines, maraschino cherries, and biscuit dough (Dziezaz, 1990). It is also used as a chelating agent to prevent oxidation in lard, butter, powdered milk, cheese, potato chips, sausages, and roasted nuts (Supran and others, 1966).

Lactic acid is widely distributed in nature and is one of the earliest acidulants used in the food industry (Gardner, 1977). It can be found in bread, cheese, meat, beer and wine, where it is formed as an end product of anaerobic carbohydrate metabolism (Andres, 1995). Lactic acid is a viscous, nonvolatile liquid freely soluble in water (Gardner, 1977). Lactic acid has a mild, creamy odor with a pleasant sour taste (Doores, 1990). It also has a mild fruity flavor, predominantly ascribed to yeast, lemons, and sour apples (Rubico, 1993). Lactic has been employed as a flavoring agent in the manufacture of cheese, fruit juices, pickles, and relishes. Lactic acid has also been used in frozen desserts, jams, jellies, and in the manufacture of beer (Gardner, 1977).

Malic acid is widely distributed in natural sources, such as fruits and vegetables. It represents 100% of the acid present in watermelons and quinces, 99% in plums, 97% in apples, 96% in cherries and peaches, 92% in bananas, 87% in pears, and 80% in orange peel (Andres, 1995). It is the second predominant acid in citrus fruits, figs, tomatoes, beans, and most berries (Rubico, 1993). Malic acid is a highly soluble general-purpose acidulant, with taste that resembles citric acid. It imparts a smooth tart taste, without adding the 'burst' sensation to the flavor (Gardner, 1977; Doores, 1990). Because of its ideal blending properties, malic acid is useful in a variety of products including fruit-flavored foods and drinks, syrups, essences, and ginger (Gardner, 1977). Malic acid has also been used as an emulsifying agent as well as a synergist with antioxidants to prevent rancidity in fat and oil products (Gardner, 1977).

Succinic acid is one of the natural acids found in beets, rhubarb, asparagus, broccoli, sauerkraut, cheese, and fresh meat extracts. It is also found at a very high concentration in shellfish (Rubico, 1993). Succinic acid is a non-hygroscopic acid, having a slow buildup taste profile. Succinic acid is odorless and has a sour, acid taste. It has also been described as slightly bitter (Doores, 1990; Rubico and McDaniel, 1992; Gardner, 1966). Because succinic acid is more soluble in water at room temperature than other non-hygroscopic acids, it has been a valuable acid in formulating powdered food products. It has also been used to modify the plasticity of bread dough by combining with proteins and to increase the shelf life of dessert powders (Gardner, 1977). Other applications include preparing artificial flavorings, seasonings or condiment mixtures, and for imparting a meaty flavor in food products (Velisek and others, 1978).

Tartaric acid is the predominant acid in grapes and tamarinds (Rubico, 1993). It is the most soluble among the solid acidulants and has a strong, tart taste profile (Gardner, 1977). Tartaric acid is widely used in fruit-flavored and tart-tasting beverages, jams, jellies, and candies. Mixtures of tartaric and citric acids are commonly used in hard candies to obtain the sour-apple, wild-cherry and other especially tart-tasting varieties (Gardner, 1977). Tartaric acid is also employed as leavening agent as well as synergist with antioxidants in preventing rancidity and discoloration of cheese (Gardner, 1977).

1.5.2 Flavor Profiles of Food Acidulants

Sour taste is the aspect of flavor most commonly associated with acids. However, acidulants are also able to elicit non-sour taste characteristics such as bitterness, saltiness, and astringency (Rubico and McDaniel, 1992; Siebert, 1999; McBurney and Shick, 1971; Meiselman and Dzendolet, 1967; Settle and others, 1986). The nature and proportion of non-sour taste qualities are mainly dependent on the type and concentration of the acid as well as the pH. Settle and others (1986) reported that bitterness is the most perceived non-sour taste component of organic acids in a moderate to strong taste intensity range. Hydrochloric acid has been described as having a 'faintly bitter taste' (Harvey, 1920). Hartwig and McDaniel (1995) used the free-choice profiling method to show that common food acidulants differ widely in sensory qualities at different pHs. Using the same method, Rubico and McDaniel (1992) reported that succinic acid has a bitter note in addition to sour taste. They also observed that Oriental panelists described succinic acid as having a monosodium glutamate taste while Caucasians used the term 'savory' and 'brothy'.

In addition to other taste qualities, acids can impart olfactory and tactile sensations. It has been shown that solutions of lactic, citric, malic, tartaric, sulfuric, hydrochloric, and phosphoric acids at high concentrations can be detected by nasal inhalation, supposedly by the trigeminal nerve (Settle and others, 1986). Hartwig and McDaniel (1995) reported that acetic acid has a distinct vinegar flavor character. Meilgaard (1975) described several odors associated with organic acids such as 'cheesy', 'sweaty', 'vegetable oil', 'citrus', and 'tallowy'. Inorganic acids such as hydrochloric and phosphoric acids have been reported to be more astringent than sour (Corrigan and Lawless, 1995; Rubico and McDaniel, 1992).

Fumaric and adipic acids have been described as metallic and chalky, respectively (Jonhson and Peterson, 1974). Gardner (1977) described the sour taste of several acids as follows: malic (green), citric (fresh), succinic (salty and bitter notes), lactic (sourish but tart), and tartaric (hard).

Lugaz and others (2005) investigated the effect of chemical properties of acids and subject salivary flow rate on the temporal response of sour taste. They reported that pH, type of acid, and group of subjects (high versus low salivary flow rate) had a significant effect on the time-intensity profiles of acidulants. Likewise, Arnold (1975) proposed that the intensity and duration of sour taste differed among acids.

Astringency is an important sensory attribute of both organic and inorganic acids. Astringency is developed over time and grows in intensity with repeated stimulation (Joslyn and Goldsten, 1964; Guinard and others, 1986). It is strongly dependent on pH with the intensity of perception increasing with decreasing pH (Sowalsky and Noble, 1998; Lawless and others, 1996). Sierbert and Chassy (2003) proposed that acids evoke the astringency sensation by intensifying the interaction between salivary proteins and polyphenols already present in the saliva. Another hypothesis suggests that salivary proteins are denatured by acids due to low pH exposure, causing loss of their lubricating and protective functions (Lawless and others, 1996).

Table 1.1: Chemical and physical properties of organic acids

Acid	MW	pK_a's	Solubility (g/100mL water)	Physical Form	Hygroscopicity	Sensory Qualities
Acetic	60.05	4.75	Very Soluble (Miscible?)	Clear, colorless liquid	Not applicable	Tart and Sour
Adipic	146.14	4.43 5.41	1.9 g at 20°C 83 g 90°C	Crystalline powder	Low level of hygroscopicity	Smooth lingering tartness
Citric	192.12	3.14 4.77 6.39	Anhydrous: 181 g at 25°C Monohydrate: 208 g 25°C	Crystalline powder	Moderately hygroscopic	Tart; delivers a 'burst' of tartness
Fumaric	116.7	3.03 4.44	0.5 g at 20°C 9.8 g 100°C	White granules of crystalline powder	Nonhygroscopic	Tart; affinity for grape flavors
Lactic	90.08	3.86	Very Soluble	Liquid, also available in dry form	Not applicable	Acrid
Malic	134.09	3.40 5.11	62 g at 25°C	Crystalline powder	Nonhygroscopic	Smooth tartness
Succinic	118.09	4.19 5.50	7.8g in cold water 100g at 100°C	White minute monoclinic prisms	Nonhygroscopic	Tart; slightly bitter in aqueous solutions
Tartaric	150.09	2.98 4.34	147 g at 25°C	Crystalline powder	Nonhygroscopic	Extremely tart; augments fruit flavors

Adapted from Gardner (1977)

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CHAPTER 2

A Chemical Basis for Sour Taste Perception in Aqueous Solutions and Fresh Pack Dill Pickles

2.1 Abstract

Sour taste is related to pH and acids present in foods. It is not currently possible, however, to accurately predict and modify sour taste intensity simply by knowing the pH along with the type and concentration of acids. The main objective of this study was to investigate the roles of protonated organic acid species and hydrogen ions in evoking sour taste perception. Sour taste intensity and other sensory attributes were measured using the Spectrum MethodTM. The distribution of organic acid species was calculated using pHToolsTM, a modeling program implemented in MATLABTM. Mixtures of three acids, chosen from a group of eight organic acids, were used for testing the effect of protonated organic acid species, and hydrochloric acid was used for testing the effect of hydrogen ions. Sour taste increased linearly with hydrogen ion concentration ($R^2= 0.995$), as well as with the molar concentration of protonated organic acid species at pH 3.5 ($R^2= 0.949$), 4.0 ($R^2= 0.952$), and 4.5 ($R^2= 0.975$). Type of acid was not a determinant factor for sour taste intensity after adjusting for the effects of protonated organic acid species and hydrogen ion concentration. Protonated organic acid species and hydrogen ions produced approximately the same sour taste response on a molar basis. Sour taste intensity was linearly related to the total concentration of protonated organic acid species in fresh pack dill pickles at pH 3.5 and 4.0 ($R = 0.999, 0.999$, respectively). This study reveals a simple chemical basis for the sour taste of organic acids and may allow for better control of flavor in the formulation of acidified foods.

2.2 Introduction

Sour taste is the aspect of flavor most commonly associated with acids, although non-sour taste characteristics such as bitterness, saltiness, and astringency may coexist (Rubico and McDaniel 1992; Siebert 1999; McBurney and Shick 1971; Meiselman and Dzenolet 1967; Settle and others 1986). Many studies have sought to identify the chemical basis for sour taste perception. The property shared by all acids is the dissociation of protons when they are dissolved in water. However, sour taste intensity of organic acid solutions bears no simple relationship with hydrogen ion concentration. Human psychophysical and animal physiological studies have shown that solutions of acetic acid produce higher sour taste responses than hydrochloric acid at the same pH (Pfaffmann, 1959; Koyama and Kurihara, 1972; Lyall and others, 2001; Ganzevles and Kroeze, 1987a; Ogiso and others, 2000; Ugawa and others, 1998; Richter and others, 2003; Lugaz and others, 2005). Furthermore, the pH of various organic acid solutions is considerably different at their observed threshold concentrations (Berg and others, 1955; Amerine and others, 1965). These studies indicate that, in addition to hydrogen ions, anions and/or protonated (undissociated) acid species must play a role in determining sour taste intensity of organic acids.

Previous investigations into the factors eliciting sour taste of organic acids have produced some conflicting results. Shallenberger (1996) hypothesized that sour taste is a function of potential hydrogen ion concentration. The author suggests that perception of sour taste involves a titration-like process, where the receptor site in the cell membrane serves as the 'base'. Likewise, several studies have reported that sour taste intensity increases with increasing titratable acidity at equivalent pH (Makhlouf and Blum, 1972; Lugaz and others,

2005; Plane and others, 1980). However, Norris and others (1984) showed that binary acid solutions with equivalent pH and titratable acidity evoked significantly different sour taste response. By varying the dominant acid in the mixtures, they concluded that sour taste is dependent on the specific anionic composition of the acid. Pangborn (1963) also found no relationship between pH, titratable acidity and relative sour taste of several organic acids at both threshold and suprathreshold concentrations.

Early studies associated sour taste with the buffer capacity of the acid. Beatty and Cragg (1935) showed that equi-sour solutions of various acids required the same amount of a phosphate buffer to bring a unit volume to a pH of 4.5. However, Ganzvles and Kroeze (1987b) found that the titration volume rank-order at pH 4.4 for several acids differed from the sour response rank order.

The sour taste of organic acids has also been investigated in terms of their relative hydrophobicities. Gardner (1980) suggested that absorption of an acid into the taste cell membrane plays an important role in the mechanism of sour taste perception. He found that the ease of acid penetration in the taste cell membrane was enhanced as non-polar groups were introduced into the molecule and reduced with the addition of polar groups. Similarly, Chauncey and others (1963) showed that the presence of polar groups on the acid structure had an inhibitory effect on sour taste response. In contrast, Noble and others (1986) reported that the sour taste of several binary solutions at a constant pH and titratable acidity did not correlate with the degree of hydrophobicity. Similar results were reported by Norris and

others (1984), who found no relation between the sour taste of fumaric, citric, and tartaric acids and their relative hydrophobicities.

Despite the extensive amount of work done over the past century, it is not currently possible to accurately predict or modify sour taste intensity in foods simply by knowing the pH and the type and amount of acids present. Our laboratory recently developed a hypothesis for the chemical basis of sour taste, which may provide a better understanding of the factors determining the ability of acids to evoke sour taste response. Johanningsmeier and others (2005) proposed that the intensity of sour taste is a linear function of the total molar concentration of all organic acid species that have one or more protonated carboxyl groups (protonated organic acid species) plus the molar concentration of hydrogen ions. The main objective of this study was to investigate the roles of protonated organic acid species and hydrogen ions in evoking sour taste perception in aqueous solutions and fresh pack dill pickles.

2.3 Materials and Methods

2.3.1 Sensory Evaluation

Panelists

Non-smoking female students and staff from the Department of Food Science at North Carolina State University (Raleigh, NC), between the ages of 22 and 49, served as panel members. The panel was comprised of nine subjects, who were selected based on availability and ability to distinguish and scale the basic tastes.

Training

The descriptive sensory panel was trained for 40 hours in the Spectrum™ Method (Meilgaard and others, 1991) to evaluate several attributes of aqueous solutions on a 15-point scale. An additional 15 hours of training were completed with dill pickle products to enable the panel to define and describe the selected sensory attributes in a more complex matrix.

Aqueous solutions of citric acid, sucrose, NaCl, caffeine, alum, and acetic acid were used as reference stimuli for sour, sweet, salty, and bitter tastes, astringency, and vinegar flavor, respectively. Reference concentrations were selected from the Spectrum™ Method (Meilgaard and others, 1991) with the exception of astringency and vinegar flavor, which were developed during training. Alum has been shown to be an appropriate astringency reference standard for descriptive analysis (Wismer and Goonewardene, 2003). Concentrations of 0.25, 0.56, 1.1, and 1.5 g/L alum represented 2, 5, 10, and, 15 intensities on the astringency scale, respectively. Concentrations of 4.2, 8.8, and 38.5 mM acetic acid anchored the intensities 2, 5, and 10 for vinegar flavor, respectively.

2.3.2 Sample Presentation

Samples were presented at room temperature in 2 oz. plastic cups labeled with a three-digit code. Each sample was evaluated for sour, salty, bitter and sweet tastes, astringency, vinegar flavor, and 'other'. The 'other' category was an open scale with space allotted for a write-in descriptor to be used when a non-anticipated off-note was observed. Subjects evaluated five or six samples of acid solutions per session using the sip-and-spit method. Panelists were instructed to take a comfortable amount of sample into their mouth, swish it around for approximately five seconds and expectorate it into a waste cup. Reference solutions for sour taste, astringency and vinegar flavor were provided in every session for panelists' calibration. For dill pickle evaluations, four samples were tested in each session. A pickle reference with a sour taste intensity of 8 was provided for panelists' calibration. Dill pickle samples were evaluated for the same sensory attributes tested in aqueous solutions, in addition to dill pickle flavor. Panelists had the option to either swallow or expectorate pickle samples during evaluation.

Water and crackers were provided as palate cleansers between samples. For aqueous solutions, 5.5% carboxymethylcellulose (CMC) (Aqualon, Wilmington, DE) and/or Münster cheese were supplied to counteract the drying and puckering sensations of astringency. CMC has been previously shown to act as an effective inter-stimulus rinse for astringency in model solutions (Brannan G.D. et. al, 1999).

2.3.3 Sample Preparation

a) Aqueous Solutions

Food grade acid solutions were prepared with purified water at the beginning of each week of testing and refrigerated between sensory evaluations. Acetic, lactic, adipic, malic, tartaric, succinic, and fumaric acids were obtained from Sigma Aldrich (St. Louis, MO). Citric acid, hydrochloric acid, and sodium hydroxide were obtained from Spectrum (Spectrum Chemical & Laboratories Products, Gardena, CA). pH adjustments were done using either 2N sodium hydroxide or 2N hydrochloric acid.

Sour taste of hydrogen ion

The sour taste of hydrogen ion was evaluated using hydrochloric acid solutions from 0.31mM (pH=3.5) to 10 mM (pH=2.0).

Sour taste of organic acid mixtures

The sour taste of protonated organic acid species was evaluated using mixtures of three different organic acids in each solution. For each set of acid solutions, total molar acid concentration and pH were held constant. The concentration of protonated organic acid species in these solutions was varied by altering the acid blends. The acids were assigned to the mixtures according to their pK_a to provide the widest possible range of protonated organic acid species concentration (Tables 2.1 to 2.3).

Comparison of single acid solutions to mixed acid solutions

The sour taste of single organic acid solutions was compared to the sour taste of mixtures of three organic acids solutions in a factorial treatment arrangement of protonated organic acid species concentrations (8, 16 and 25 mM) and pH (3.5, 4.0 and 4.5). The acids in each solution were randomly chosen from the group of eight food grade organic acids used in this study. Total acid concentration was allowed to vary (Table 2.4).

Relative sour taste intensity of hydrogen ions and organic acids

Sour taste potencies of hydrogen ions and protonated organic acid species were investigated by comparing the relationship between a set of solutions with constant concentrations of protonated organic species and variable hydrogen ion concentration and a set of solutions with a constant hydrogen ion concentration and variable concentrations of protonated organic acid species. Hydrogen ion concentration was varied from 0.31 to 10 mM (pH 2.0 to 3.5) in the absence of protonated organic acid species. Hydrogen ion concentration was varied between 0.47 and 7.94 mM (2.10 to pH 3.32) in solutions having mixtures of three organic acids at a constant protonated organic acid concentration (5 mM). Protonated organic acid species concentration was varied between 0 and 8 mM using mixtures of three organic acids at a constant hydrogen ion concentration (2.51mM, pH 2.6). The compositions of these solutions are given in Table 2.5.

b) Fresh Pack Dill Pickles

Fresh size 2B (3.5 to 3.8 cm diameter) pickling cucumbers obtained from a local processor were washed, sliced to approximately 6 mm thickness using a food processor (Hobart, Model FP150, Troy, OH), and packed into 720 ml (24 oz) jars. The jars were filled with cucumber slices and appropriate cover brine solutions to give a 60/40 (cucumbers/brine) pack out ratio, pasteurized at 75°C (center of the jar) for 15 minutes in a water bath, and then cooled to room temperature. The pasteurized jars were stored for at least 8 weeks at 6°C to allow equilibration of flavor prior to sensory evaluation.

A commercial dill pickle brine formula was used to make the reference pickle product. Brine was prepared with NaCl (final concentration of 2% after equilibration), sodium benzoate, vinegar containing 13% acetic, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, FD&C No.5 yellow food coloring, and dill spice concentrate. Brine for the experimental treatments was prepared in the same way as the reference formula, except that vinegar was replaced by different blends of organic acids (Tables 2.6 and 2.7). Each treatment consisted of mixtures of three organic acids, which were chosen from the same group of organic acids used for aqueous solutions. Tartaric acid was not included because it crystallized in the brine solutions. For each set of four samples, total molar concentration of organic acids and pH were held constant, while protonated organic acid species concentration varied by altering the acid blends.

The pH was adjusted to target levels by addition of 6N sodium hydroxide. For each treatment, a mixture of 120 g cucumber slurry and 80 g cover brine was titrated with sodium

hydroxide. The amount of sodium hydroxide required to attain target pHs in 24 oz jars after equilibration was calculated by multiplying the titration volume by the appropriate factor.

2.3.4 Calculation of Organic Acid Species Concentration and pH Measurements:

The distribution of organic acid species was calculated using pHTools , a modeling program implemented in MATLABTM developed in this laboratory by Dougherty (2002) and validated by Neta and others (2005). This software adjusts the published pK_a values of the organic acids for the ionic strength and temperature of solutions using a modification of the Davies equation (Davies and Tso, 1982; Samson and others, 1999). The adjusted pK_a is used to calculate the concentrations of all species of each organic acid added to a solution. For complex systems, such as foods, ionizable groups of unknown identity or concentration can be titrated with a base or acid to generate a calibration curve. In the case of dill pickle products, a 60:40 blend of cucumber slurry: brine (no added acid) was titrated with 2N hydrochloric acid from an initial pH 5.5 to a final pH 2.0. The calibration curve was then used as a complex buffer in pHTools to adjust the pK_a values of acids for ionic strength in order to determine the distribution of organic acid species.

The pH was measured using a pH electrode with a microprocessor pH/mV meter (Accumet Research AR25) equipped with an AcuFet solid-state electrode (Fisher Scientific, Atlanta, GA).

2.3.5 Statistical Analysis

Acid solutions and dill pickle products were evaluated in a randomized complete block design with three sensory replications. Linear regression and analysis of variance ($p \leq 0.05$) were performed on treatment means using the 8.2 version of SAS[®] software (SAS institute, Inc., 1987, Cary, NC).

2.4 Results and Discussion

2.4.1 Aqueous Solutions

Sour taste of hydrogen ion

Since the beginning of efforts to understand the chemical basis for sour taste, it has been recognized that hydrogen ions in aqueous solutions are perceived to be sour. However, the relationship between the intensity of sour taste and the concentration of hydrogen ions has not been described. This was determined by evaluation of sour taste intensity as a function of hydrogen ion concentration for solutions of HCl, where hydrogen ions and chloride ions are completely dissociated. Figure 2.1 shows a linear relationship ($R^2= 0.995$) between the intensity of sour taste and hydrogen ion concentration up to 10 mM. Curtis and others (1984) used magnitude estimation to evaluate the sour taste intensity of solutions of mixed acids. The authors reported magnitude estimates for the sour taste intensity of HCl solutions ranging from 0.53 to 8.48 mM. Re-analysis of their data also showed a linear relationship between sour taste intensity and hydrogen ion concentration ($R^2= 0.99$).

In the present study, an important point relevant to acid and acidified foods was that on a 0 to 15 scale the sour taste intensity at pH 3.0 ($[H^+] = 1.0$ mM) was only about 1 and at pH 3.5 ($[H^+] = 0.31$ mM) the sour taste intensity was barely detectable. Since the pH of most acid or acidified foods is above 3.0, this result suggests that the hydrogen ion concentration may have little or no direct contribution to sour taste intensity in foods. Therefore, the concentration of organic acids, either added or naturally present in foods, would be expected to be primarily responsible for the intensity of sour taste in foods.

Sour taste of organic acid mixtures

The hypothesis of Johanningsmeier and others (2005) proposed that the critical factor determining the sour taste intensity of organic acids is the molar concentration of acid molecules in solution that have one or more protonated carboxyl groups. Experimentally, this hypothesis is difficult to test because pH and concentration of protonated acid molecules in solution are inextricably related to the dissociation constant(s) of the carboxyl group(s) present in any organic acid. Therefore, it is not possible in solutions of single acids to keep pH and total acid concentration constant while at the same time varying the concentration of protonated organic acid molecules. This experimental problem was dealt with by using groups of three organic acids in solutions selected from a larger group of eight food grade organic acids. The eight acids from which the sets of three acids were selected were mono-carboxylic, di-carboxylic, and tri-carboxylic organic acids with a range of pK_a values from 2.98 to 6.39 (Table 2.9). These acids had a 128-fold range of hydrophobicities, based upon a range of log P values (P= octanol/water distribution coefficients) from -1.84 to +0.27 (Table 2.9). These acids also had a range of taste characteristics other than sour taste. Solutions made with mixtures of these acids made it possible to prepare groups of solutions with constant pH, constant molar concentrations of acid molecules, but with variable molar concentrations of acid molecules with one or more protonated carboxyl groups (Tables 2.1 to 2.3). If multiple mixtures of acids were found to have sour taste intensities that linearly correlated with the molar concentration of acid molecules with one or more protonated carboxyl groups, the proposed hypothesis (Johanningsmeier and others 2005) would be strongly supported.

Figures 2.2 – 2.4 show that sour taste intensity increased linearly with the molar concentration of all organic acid species having at least one protonated carboxyl group at pH 3.5 ($R^2= 0.949$), 4.0 ($R^2= 0.952$), and 4.5 ($R^2= 0.975$), regardless of the type of acids present in the mixtures. Thus, these results are consistent with the hypothesis that sour taste intensity is linearly related to the concentration of acid species with at least one protonated carboxyl group (Johanningsmeier and others, 2005). Since the lowest pH used for these solutions was 3.5, the direct sour taste contribution of hydrogen ions in these solutions would be negligible. The main function of pH was to determine the degree of protonation of the organic acids in these solutions.

We observed that, as noted by a triangle symbol in Figures 2.2 – 2.4, solutions containing acetic acid concentrations higher than 10 mM resulted in a lower than expected sour taste response, and thus they were not included on the regression models. A plausible explanation for this relies on the physiological interaction between sour taste and vinegar flavor, producing perceptual suppression of sour taste intensity. Indeed, Stevenson and others (1999) found that certain odors, such as caramel, can suppress the sour taste perception of citric acid. A second possible explanation is that the lower sour taste response observed is caused by a measurement artifact, where subjects ‘dump’ a perceptual experience to another. In this case, sour taste intensity would be expressed as an additional element of the vinegar flavor attribute.

The linear relationship observed between the sour taste intensity of mixtures containing eight different acids that were partially and fully protonated could occur only if

the following were true. First, on a molar basis, organic acids having at least one protonated carboxyl group must be perceived as equally sour. For instance, acid molecules of citric, malic, or lactic acid will produce the same sour taste response provided that at least one carboxyl group is protonated. Second, for multiprotic acids all protonated species must be perceived as equally sour. Thus, mono-, di-, or tri- protonated molecules of citric acid, for example, will elicit equivalent sour taste response.

Comparison of single acid solutions to mixed acid solutions

As a verification of the equality of sour taste intensity for different acids and all their protonated forms, the sour taste intensity of single acid solutions was compared to the sour taste intensity of solutions containing mixtures of three acids. Table 2.8 shows that there was no significant difference ($p < 0.05$) between the sour taste of a single acid and the sour taste of mixtures of three different acids for eight out of nine pairs of solutions. This result supports the conclusion that for a given pH all organic acid molecules are perceived to be equally sour provided that at least one carboxyl group is protonated. It should be noted that for a given pH, the type of acid(s) present in each solution determined the total acid concentration required to achieve a target concentration of protonated organic acid species. For instance, at any pH, acids with high pK_a values give higher concentrations of protonated organic acid species than acids having low pK_a values, and thus require a lower total molar acid concentration. However, the type of acid present in solutions showed no effect on sour taste response after adjustment for the two variables that determine sour taste intensity: protonated organic acid species and hydrogen ion concentration (pH).

Relative sour taste intensity of hydrogen ions and organic acids

The previous results showed that sour taste intensity was linearly related to both hydrogen ion concentration and the concentration of protonated organic acid species in aqueous solutions. The next step was to determine if hydrogen ions and protonated acid molecules elicit an equal sour intensity response. To make this comparison the sour taste intensity of variable concentrations of protonated acid species at a constant hydrogen ion concentration (2.51 mM) was compared to the sour taste intensity of variable concentrations of hydrogen ions in the absence of protonated acid species (0 mM), and also in the presence of a constant concentration (5 mM) of protonated organic acid species.

Three statistical models were used to compare the sour taste potencies of hydrogen ions and protonated organic acid species. Model 1 assumed that hydrogen ions and protonated organic acid species have different abilities to elicit sour taste, and that there is interaction among these factors.

$$Y = \beta_0 + \beta_1 X + \beta_2 Z + \beta_3 X * Z \quad [\text{Model 1}]$$

where Y represents sour taste intensity, X is the contribution of protonated organic acid species, Z is the effect of hydrogen ions, and β_s are constant coefficients

Model 2 evaluated the data in terms of weighted additivity between protonated organic acid species and hydrogen ions, assuming no interaction between the factors:

$$Y = \beta_0 + \beta_1 X + \beta_2 Z \quad [\text{Model 2}]$$

where Y represents sour taste intensity, X is the effect of protonated organic acid species, Z is the effect of hydrogen ions, and β_s represent the partial slopes for each factor.

Model 3 considered total additivity between protonated organic acid species and hydrogen ions, which means that they both elicit approximately equal sour taste response.

$$Y = \beta_0 + \beta_1 (X + Z) \quad [\text{Model 3}]$$

where Y represents sour taste intensity, X is the effect of protonated organic acid species, Z is the effect of hydrogen ions, β_0 represents the intercept, and β_1 the slope.

An analysis of variance showed the interaction term in Model 1 to be not significant ($p = 0.20$). This means that no significant evidence of interaction between hydrogen ion and protonated acid molecules was found regarding sour taste perception. Further, the slopes of the linear regression for hydrogen ions and protonated acid molecules in Model 2 were not significantly different from one another ($p = 0.18$). Therefore, Model 3, the simplest model, sufficiently describes the data and we can conclude that sour taste intensity is caused by an addition of the sour taste contributions from protonated organic acid species and hydrogen ions, and that the relative ability of these components to elicit sour taste response is about the same on a molar basis.

Figure 2.5 shows the partial effect of each of the two parameters implicated in sour taste perception: protonated organic acid species and hydrogen ions. Sour taste was plotted against the sum of protonated organic acid species and hydrogen ions, and the regression line

represents predicted values from the additive model (Model 3: $\beta_0 = -0.63$; $\beta_1 = 1.02$; $R^2 = 0.93$). This result confirmed the overall hypothesis of Johanningsmeier and others (2005) that in aqueous solutions the intensity of sour taste is a linear function of the molar concentration of all organic acid species that have one or more protonated carboxyl groups plus the molar concentration of hydrogen ion.

Frijters and Ophuis (1983) suggested that a simple addition of effects from different sources may be assumed if there is no cross adaptation between the components. Ganzevles and Kroeze (1987a; 1987b; 1988) reported that no cross – adaptation occurs between hydrochloric acid and organic acids. Thus, these studies support our findings that stimulus additivity between protonated organic acid species and hydrogen ions does take place regarding sour taste perception. Indeed, Curtis and others (1984) reported sour taste additivity between total molar concentration of citric acid and hydrogen ions.

Ganzevles and Kroeze (1987a) proposed that the mechanisms involved in sour taste perception from protonated (undissociated) organic acids species and hydrogen ions are different, and probably independent. This is also in agreement with our results, given that sour stimuli from different sources would not compete for the same type of receptor site, and the total response would be the sum of the individual responses.

A common hypothesis for the mechanism of sour taste perception of organic acids is that an acid compound must penetrate the cell membrane and release hydrogen ions inside the cell in order to elicit sour taste response (Taylor and others, 1930). If that is the case, the

linear relation between sour taste intensity and concentration of fully protonated organic acid species would produce high correlations, as these are the only species able to penetrate the cell membrane. However, Figures 2.6, 2.7, and 2.8 show that sour taste intensity has no correlation with the concentration of fully protonated organic acid species ($R^2= 0.005, 0.017,$ and 0.403 for pH 3.5, 4.0, and 4.5, respectively). The linear dependence of sour taste on the concentration of protonated organic acid species suggests that physicochemical interaction at the receptor level is the most likely trigger in the sour transduction mechanism of organic acids. Soluble and fibrous proteins are known to bind organic acids in both protonated and semi-protonated form, which makes them potential candidates for the receptor site of sour stimuli (Steinhardt and others, 1943).

Makhlouf and Blum (1972) have linked sour taste intensity with the capacity of the acid to dissociate. They suggested that on a molar basis acids with a low pK_a (higher capacity to dissociate) are able to elicit higher sour taste response. Nevertheless, results of the present study showed a reverse trend. For a given pH, acids having higher pK_a (low capacity to dissociate) tended to elicit higher sour taste response as they give higher concentrations of protonated organic acid species. A possible explanation for this discrepancy relies on the different basis of comparison between the two studies. Makhlouf and Blum (1972) have drawn conclusions on the basis of total molar concentration while the present study considered protonated organic acid species.

Results of the present study are also at variance with the proposal of Shallenberger (1996) that sour taste is entirely a function of potential hydrogen ion concentration.

According to his hypothesis, organic acids have the same sour taste potential at equal normal concentrations, regardless if a strong or weak acid is considered. Our results show that the capacity of the acid to dissociate at a given pH is the major factor in evoking a sour taste response, as sour taste is a function of the concentration of protonated organic acid species.

2.4.2 Fresh Pack Dill Pickles

To our knowledge only a limited number of sensory studies attempted to investigate sour taste perception in food systems (CoSeteng and others, 1989; Amerine and others, 1965; Plane and others, 1980). In general, sour taste intensity has been examined in aqueous solutions, and the assumption is made that similar results will apply for food systems. However, it is important to demonstrate that the same relationships found in aqueous solutions hold true in a more complex matrix, where many other sensory qualities coexist. Figures 2.9 and 2.10 show that for fresh pack dill pickle sour taste intensity increased linearly with the concentration of protonated organic acid species at pH 3.5 ($R^2 = 0.999$) and 4.0 ($R^2 = 0.999$), respectively. These results agree with Johanningsmeier and others (2005), who reported a linear relationship between sour taste intensity and protonated organic acid species concentration in dill pickles containing mixtures of lactic, malic, and acetic acids.

The concentration of organic acid species required to attain a comparable sour taste response relative to aqueous solutions was approximately four times higher for dill pickle products. This discrepancy may be explained by the complex flavor interactions that occur in food systems. Similarly to aqueous solutions, samples having acetic acid as the major component showed lower sour taste responses than expected (represented by triangles on Figures 2.9 and 2.10), and thus were not included on regression models. The reasons for the lower sour taste response are the same as previously discussed: the high volatile and aromatic characters of acetic acid are likely affecting the ratings for sour taste intensity. It should also be noted that tartaric acid was excluded in the food application experiment because it crystallized in the pickle brine solutions.

2.5 Conclusions

The results of the present study showed that: (1) sour taste intensity is linearly related to the molar concentration of hydrogen ions; and (2) sour taste intensity is linearly related to the molar concentration of all organic acid species that have at least one protonated carboxyl group. In order for mixtures of several acids with different chemical structures and properties to give a linear sour taste response, all of the acids and all of the protonated forms of these acids must be equivalent in their ability to elicit a sour taste response. In addition, protonated organic acid species and hydrogen ions were found to have an approximately equal ability to elicit sour taste on a molar basis. Thus, the sour taste intensity of a mixture of protonated organic acid species and hydrogen ions is closely approximated by the sum of the contribution from the individual components. The linear relationship between sour taste intensity and protonated organic acid species at a given pH was shown to occur in dill pickles as well as in simple acid solutions.

These results strongly support the hypothesis of Johanningsmeier and others (2005), that sour taste intensity is linearly related to the molar concentration organic acids with one or more protonated carboxyl groups plus the concentration of hydrogen ions. Understanding the chemical parameters underlying sour taste perception will allow for better control of flavor and may provide a basis for predicting sour taste intensity in formulation of acid and acidified foods.

Tables

Table 2.1. Stimulus concentrations of organic acid solutions at pH 3.5

Organic acid concentration (mM)								Total protonated organic acid species (mM)
Acetic	Adipic	Citric	Fumaric	Lactic	Malic	Succinic	Tartaric	
			1	12			2	10.6
	1		8				6	11.5
			3	4			8	12.3
					2	1	12	13.3
2			9		4			14.0
			1	22.5			1.5	16.8
	2.5			18			4.5	18.2
1			9				15	21.7
					7	6	12	23.2
15		4				6		24.1
		23			1	1		25.0

Total molar acid concentration was equal to 15 mM for first five solutions and 25 mM for the remaining six solutions.

Table 2.2. Stimulus concentrations of organic acid solutions at pH 4.0

Organic acid concentration (mM)								Total protonated organic acid species (mM)
Acetic	Adipic	Citric	Fumaric	Lactic	Malic	Succinic	Tartaric	
				3	1		6	5.8
		4	3	3				7.3
8	1					1		8.7
		5			3	2		9.8
			1	27			2	11.4
	3			16			11	15.1
2	1		27					20.1
					10	8	12	23.7
20		5				5		26.3
		25			3	2		29.6

Total molar acid concentration was equal to 10 mM for first 4 solutions and 30 mM for the remaining six solutions.

Table 2.3. Stimulus concentrations of organic acid solutions at pH 4.5

Organic acid concentration (mM)								Total protonated organic acid species (mM)
Acetic	Adipic	Citric	Fumaric	Lactic	Malic	Succinic	Tartaric	
			3	23			4	5.7
	5			15			10	9.5
3.5	6.5		20					15.2
					20	5	5	19.3
11		14				5		24.4
		20			2	8		28.4

Total molar acid concentration was equal to 30 mM

Table 2.4. Factorial treatment design for sour taste intensity of single acids versus sour taste intensity of mixtures of three acids

pH	Total protonated organic acid species (mM)	Organic acid concentration (mM)							
		Acetic	Adipic	Citric	Fumaric	Lactic	Malic	Succinic	Tartaric
3.5	8								9.15
3.5	8	2.8			2.8			2.8	
3.5	16		16						
3.5	16		6.0	6.0		6.0			
3.5	25							25	
3.5	25	8.5	8.5	8.5					
4.0	8				11.1				
4.0	8	3.4		3.4		3.4			
4.0	16	19.2							
4.0	16					8.7	8.7		8.7
4.0	25					75.7			
4.0	25				11.8		11.8		11.8
4.5	8						11.2		
4.5	8			5.5		5.5			5.5
4.5	16							17.5	
4.5	16	6.6	6.6					6.6	
4.5	25			25.2					
4.5	25				13.1		13.1	13.1	

Table 2.5. Stimulus concentrations for comparing sour taste potencies of protonated organic acid species and hydrogen ion concentration

Trt. no.	Organic acid concentration (mM)							[H ⁺] (mM)	Total protonated organic acid species (mM)
	Adipic	Citric	Fumaric	Lactic	Malic	Succinic	Tartaric		
1								0.31	0
2								1.00	0
3								1.77	0
4								3.16	0
5								5.62	0
6								10	0
7	4			0.5		0.5		0.47	5
8	4	0.5			0.5			0.74	5
9	3				0.5	1.5		1.00	5
10		1		3	1			1.78	5
11	3			1		1		2.00	5
12	3			1	1			3.16	5
13			1.5	2	1.5			3.98	5
14		3	1				1	5.62	5
15	2		1.5				1.5	6.03	5
16		2			1.5	1.5		7.94	5
17								2.51	0
18	0.67				0.67	0.67		2.51	2
19		1.33			1.33		1.33	2.51	4
20	2				2	2		2.51	6
21		2.67	2.67				2.67	2.51	8

The concentration of protonated organic acid species was 0 mM for treatments 1 to 6 and 5 mM for treatments 7 to 16. Hydrogen ion concentration was 2.51 mM for treatments 17 to 21 (pH= 2.6).

Table 2.6. Stimulus concentrations for dill pickles at pH 3.5

Organic acid concentration (mM)							Total protonated organic acid species (mM)
Acetic	Adipic	Citric	Fumaric	Lactic	Malic	Succinic	
			5	80	5		58.7
	8			57	25		67.4
		45	10	35			74.7
60			20		10		83.2

Total molar acid concentration was equal to 90 mM

Table 2.7. Stimulus concentrations for dill pickles at pH 4.0

Organic acid concentration (mM)							Total protonated organic acid species (mM)
Acetic	Adipic	Citric	Fumaric	Lactic	Malic	Succinic	
			10	120	10		51.7
	10			85	45		74.1
		72	10	58			95.2
90		40				10	120

Total molar acid concentration was equal to 140 mM

Table 2.8. Comparison between the sour taste intensity of single acid solutions and solutions containing mixtures of three acids.

Total protonated organic acid species (mM)	Sour taste intensity			p – value (mixed acids vs. single acids)
	pH	Mixed acids	Single acids	
8	4.5	1.9	2.2	0.329 ^(NS)
8	4.0	4.3	2.5	0.036*
8	3.5	4.8	5.0	0.671 ^(NS)
16	4.5	5.6	6.5	0.132 ^(NS)
16	4.0	7.0	6.5	0.462 ^(NS)
16	3.5	9.9	11.0	0.175 ^(NS)
25	4.5	9.2	8.2	0.263 ^(NS)
25	4.0	10.7	11.5	0.533 ^(NS)
25	3.5	12.2	13.3	0.162 ^(NS)

* Significantly different if $p < 0.05$.

Table 2.9. Dissociation constants (pK_a) and hydrophobicities ($\log P$) for organic acids used in sour taste evaluations

Acid	pK_{a1}^*	pK_{a2}^*	pK_{a3}^*	$\log P^{**}$
Acetic	4.75			- 0.17
Lactic	3.86			- 0.62
Adipic	4.43	5.41		0.08
Fumaric	3.03	4.44		0.27
Malic	3.40	5.11		- 1.26
Tartaric	2.98	4.34		- 1.84
Succinic	4.19	5.50		- 0.59
Citric	3.14	4.77	6.39	- 1.72

* Gardner (1977)

** Gardner (1980)

Figures

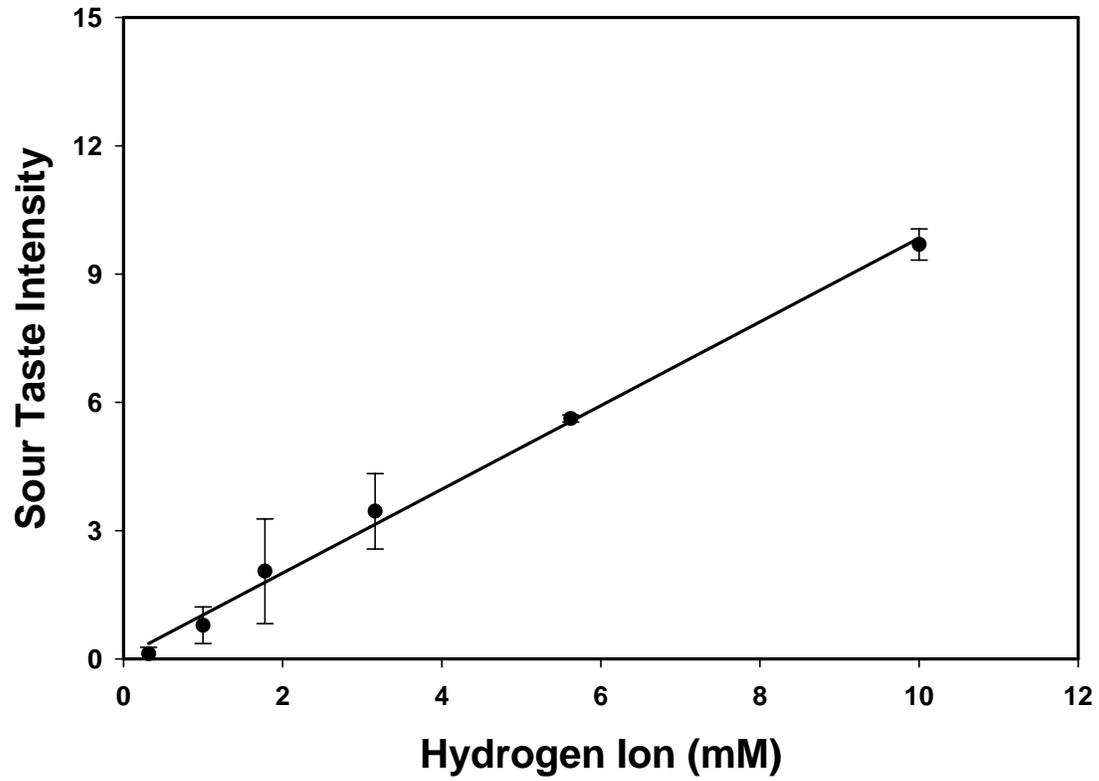


Figure 2.1. Sour taste intensity plotted as a function of hydrogen ion concentration ($R^2=0.995$). Error bars represent standard deviation from three sensory replications.

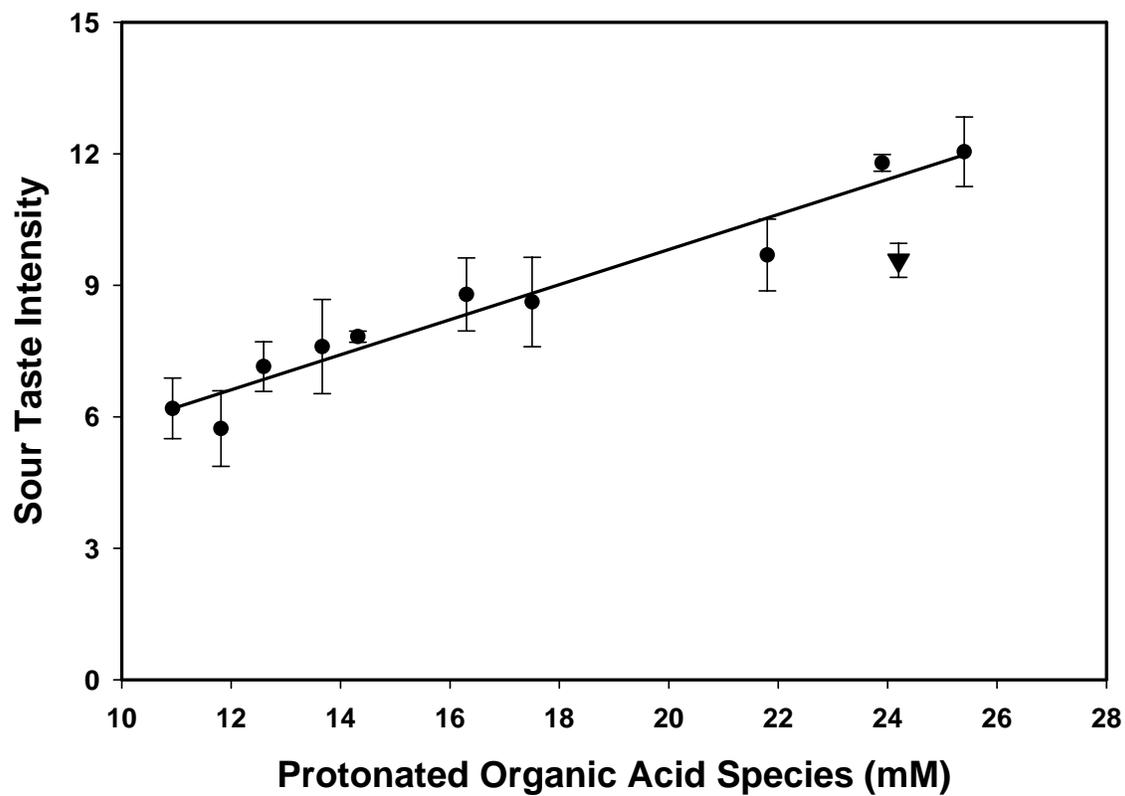


Figure 2.2. Sour taste intensity plotted as a function of organic acid species that have at least one protonated carboxyl group at pH 3.5 ($R^2 = 0.949$). ▼ represents a solution with the acetic acid concentration higher than 10 mM. Error bars represent standard deviation from three sensory replications.

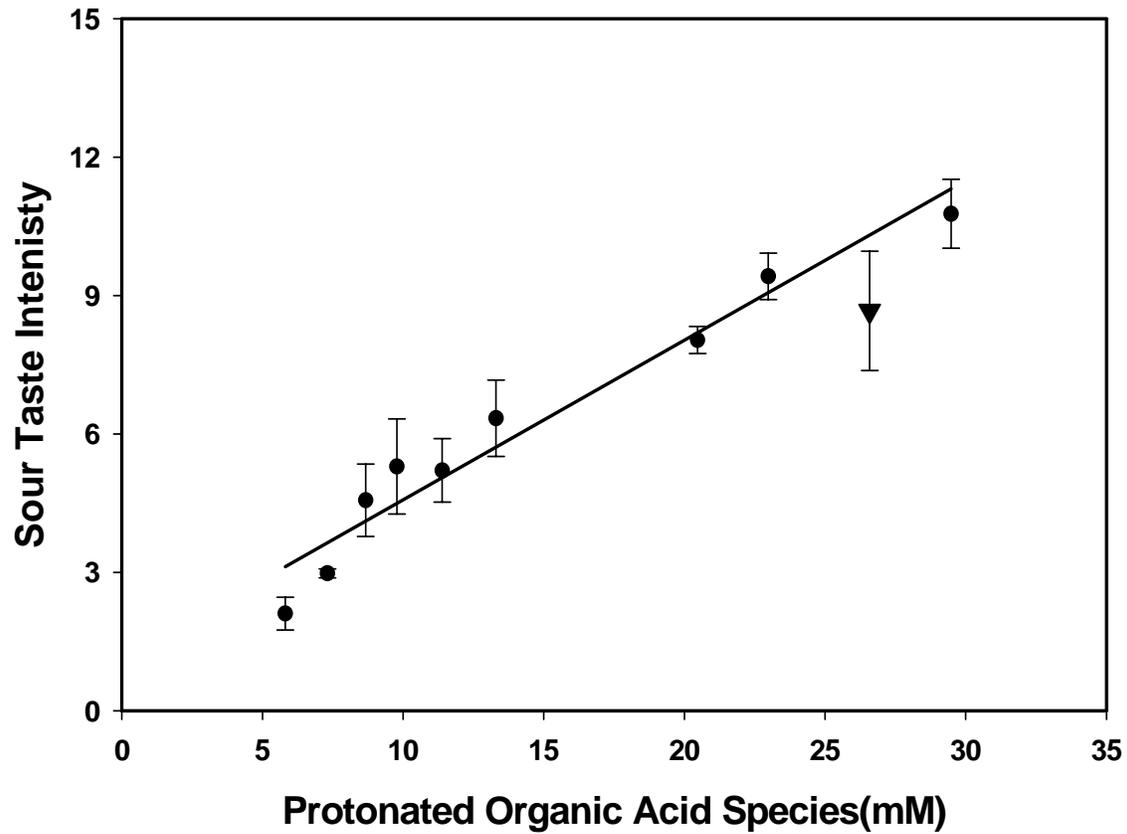


Figure 2.3. Sour taste intensity plotted as a function of organic acid species that have at least one protonated carboxyl group at pH 4.0 ($R^2 = 0.952$). ▼ represents a solution with the acetic acid concentration higher than 10 mM. Error bars represent standard deviation from three sensory replications.

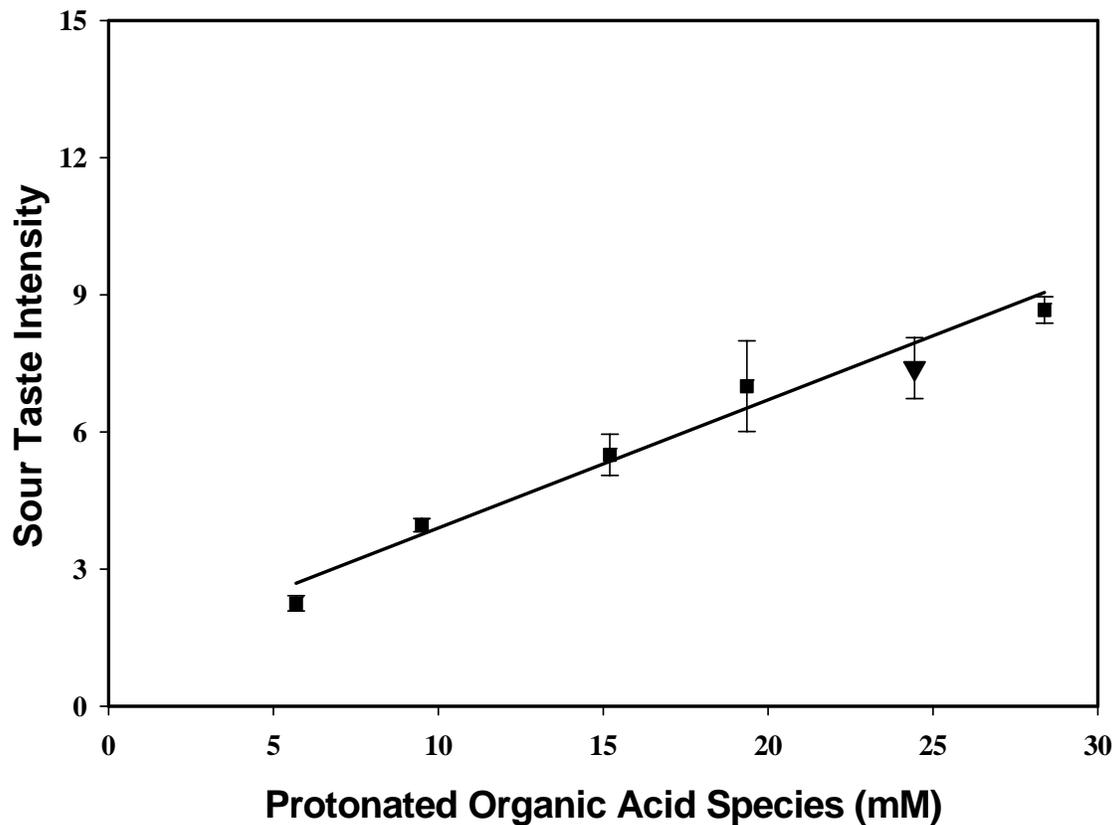


Figure 2.4. Sour taste intensity plotted as a function of organic acid species that have at least one protonated carboxyl group at pH 4.5 ($R^2= 0.975$). ▼ represents a solution with the acetic acid concentration higher than 10 mM. Error bars represent standard deviation from three sensory replications.

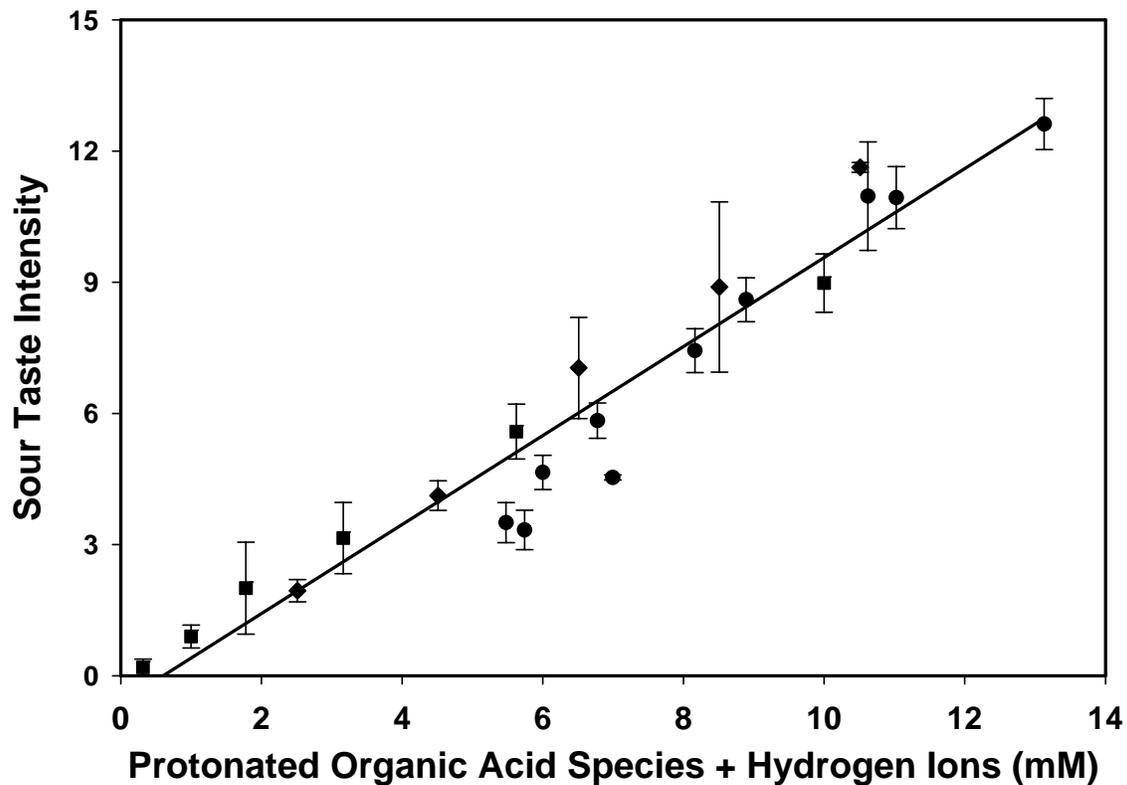


Figure 2.5. Sour taste intensity as a function of the sum of the concentrations of protonated organic acid species and hydrogen ions. ◆ represents the effect of variable hydrogen ion concentration in the absence of protonated organic acid species, ● the effect of variable hydrogen ion concentrations with a constant protonated organic acid species concentration at 5 mM. ■ represents the effect of variable protonated organic acid species at a constant hydrogen ion concentration of 2.51 mM. Error bars represent standard deviation from three sensory replications.

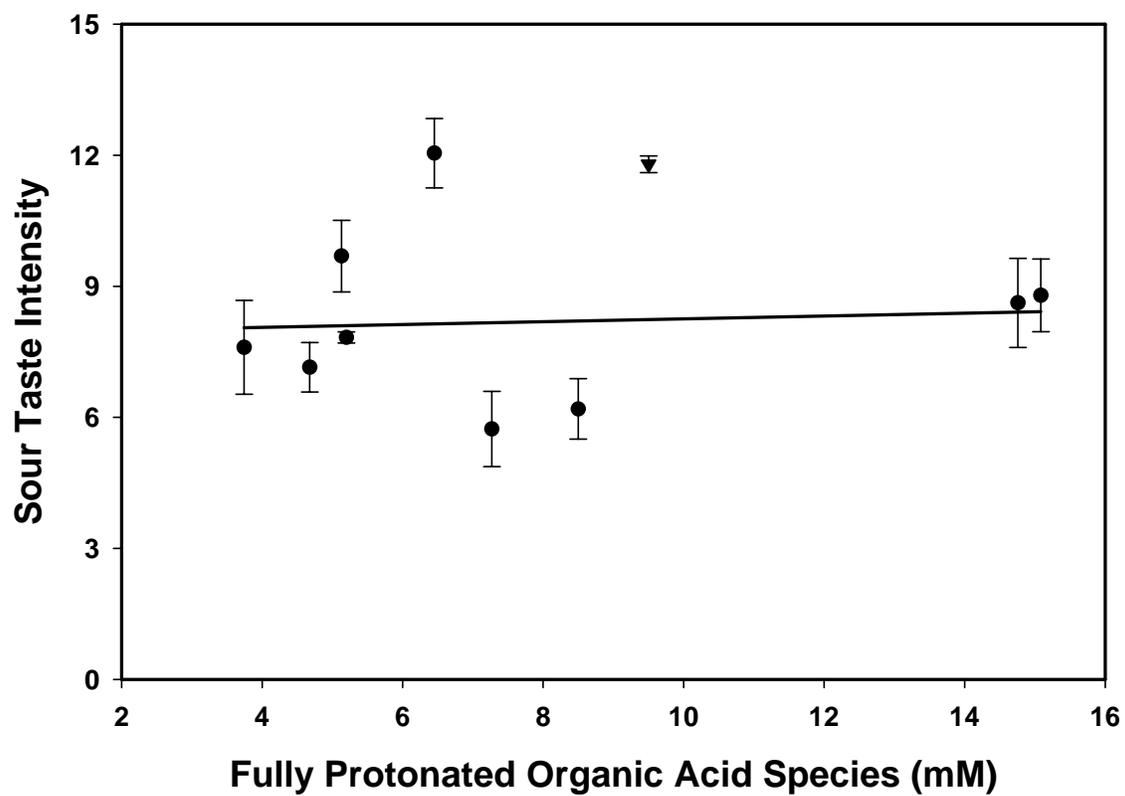


Figure 2.6. Sour taste intensity plotted as a function of organic acid species that have all carboxyl groups in a protonated form at pH 3.5 ($R^2= 0.005$). ▼ represents a solution with the acetic acid concentration higher than 10 mM. Error bars represent standard deviation from three sensory replications.

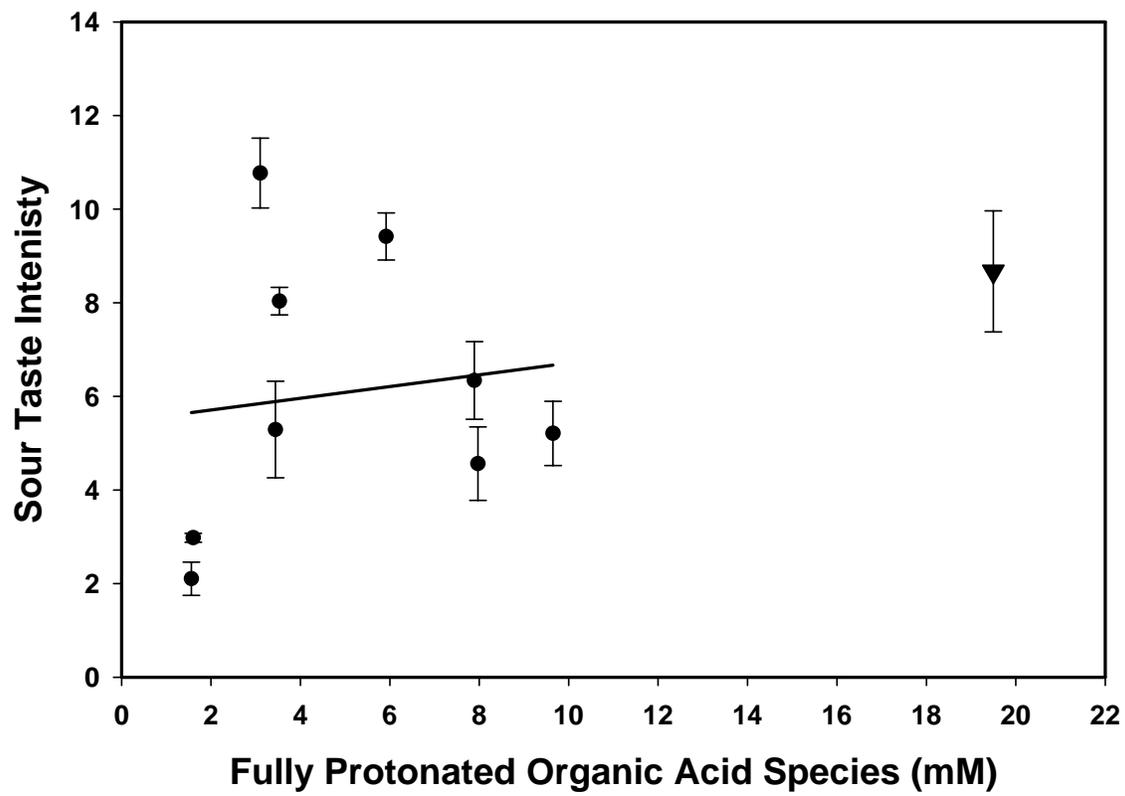


Figure 2.7. Sour taste intensity plotted as a function of organic acid species that have all carboxyl groups in a protonated form at pH 4.0 ($R^2= 0.017$). ▼ represents a solution with the acetic acid concentration higher than 10 mM. Error bars represent standard deviation from three sensory replications.

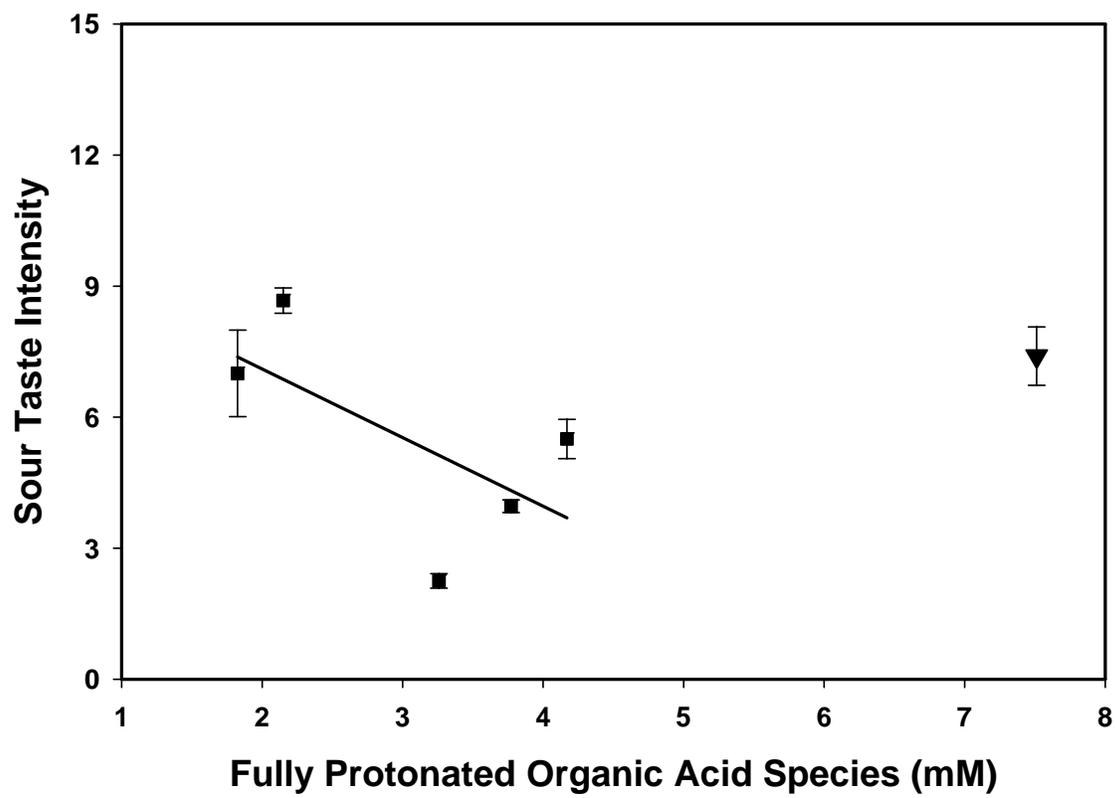


Figure 2.8. Sour taste intensity plotted as a function of organic acid species that have all carboxyl groups in a protonated form at pH 4.5 ($R^2= 0.403$). ▼ represents a solution with the acetic acid concentration higher than 10 mM. Error bars represent standard deviation from three sensory replications.

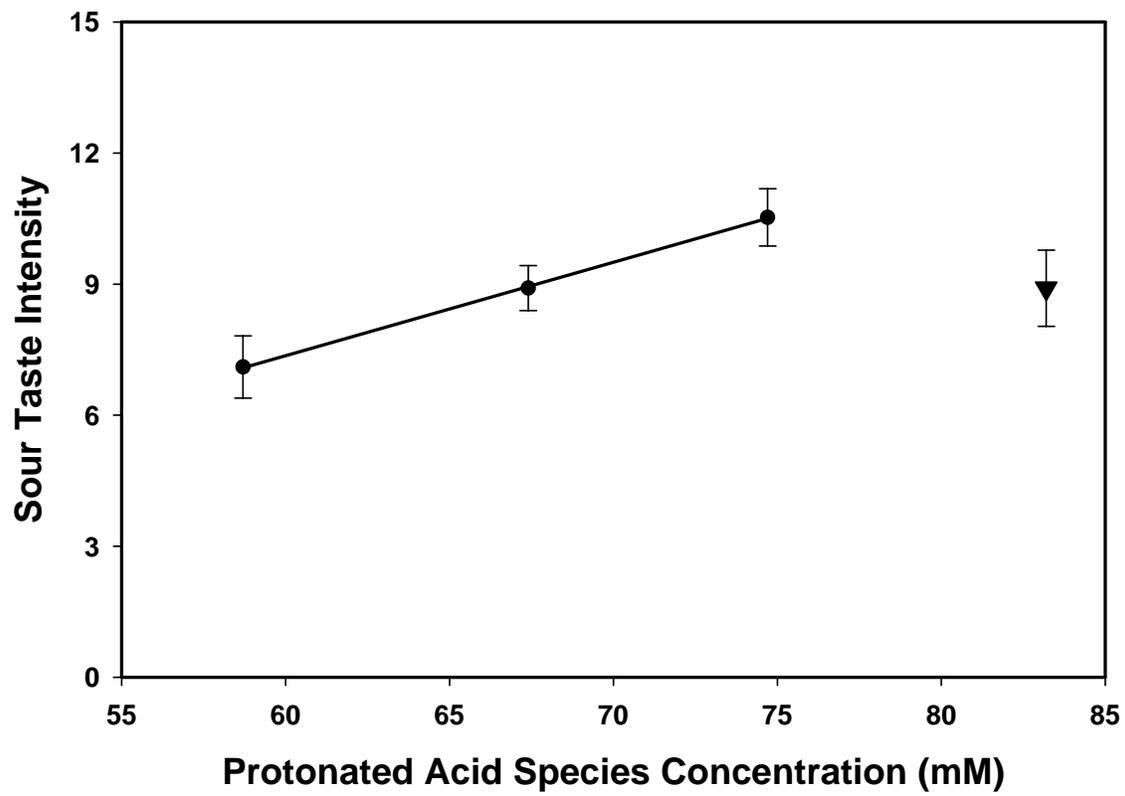


Figure 2.9. Sour taste intensity plotted as a function of protonated organic acid species at pH 3.5 in fresh pack dill pickles ($R^2= 0.999$). ▼ represents pickle samples with acetic acid as the major organic acid. Error bars represent standard deviation from three sensory replications.

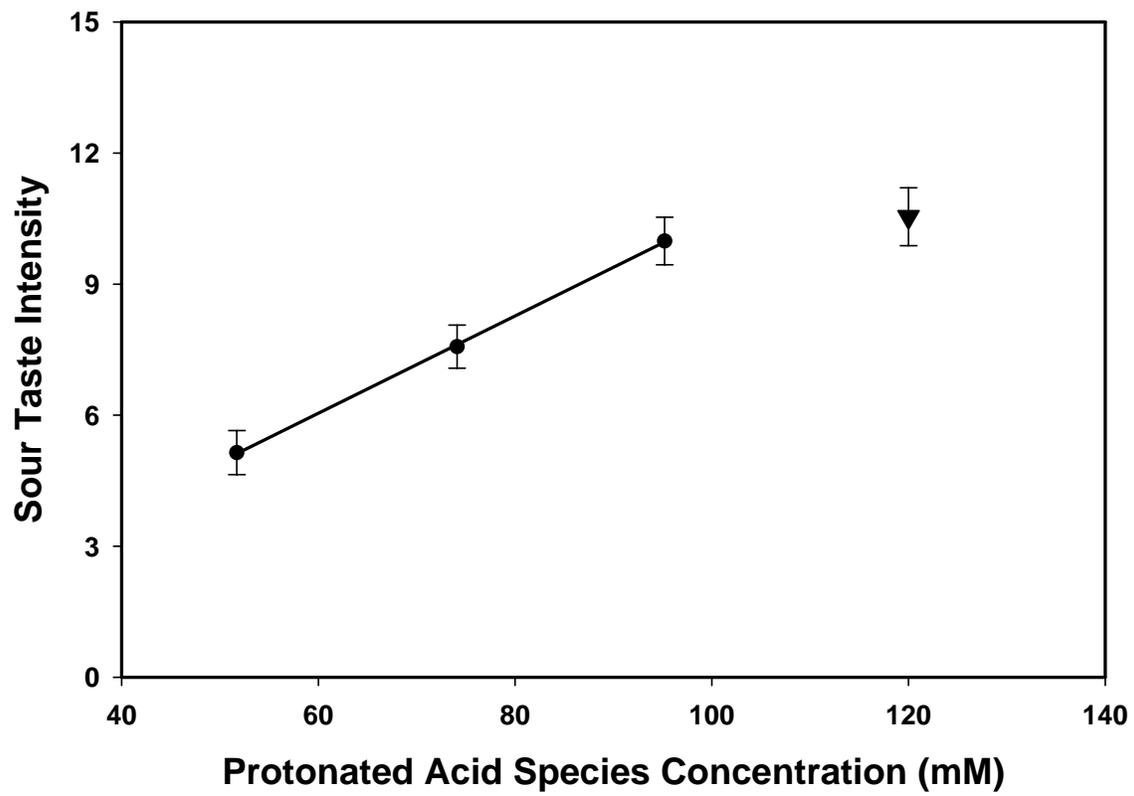


Figure 2.10. Sour taste intensity plotted as a function of protonated organic acid species at pH 4.0 in fresh pack dill pickles ($R^2 = 0.999$). ▼ represents pickle samples with acetic acid as the major organic acid. Error bars represent standard deviation from three sensory replications.

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CHAPTER 3

Effects of Organic Anions and Sodium Ions in Suppressing Sour Taste Intensity in Acid Solutions

3.1 Abstract

We have previously shown that sour taste response is elicited by two entities namely protonated organic acid species and hydrogen ions. However, in order to demonstrate that this was true, the pH of aqueous solutions was adjusted with sodium hydroxide resulting in other uncontrolled variables, such as organic anions and sodium ions. We observed that the intensity of sour taste appeared to be influenced by the presence of these species in solution. The objective of this study was to investigate the roles of organic anions and sodium ions in suppressing sour taste intensity of acid solutions. Sour taste intensity and other sensory attributes were measured using the Spectrum Method™. The distribution of organic acid species was calculated using pHTools™, a modeling program implemented in MATLAB™. The effect of organic anion species in suppressing sour taste intensity was evaluated using mixtures of three different organic acids at constant concentrations of protonated acid species and hydrogen ions. The effect of sodium ions was evaluated by adding 0 to 80 mM sodium chloride to solutions containing mixtures of three organic acids with constant concentrations of protonated organic acid species and hydrogen ions, and in the absence of anions. Sour taste decreased as the concentration of organic anion increased ($R^2 = 0.480$) but the suppression seemed to be more closely related to the amount of NaOH added ($R^2 = 0.861$). Addition of 20 mM NaCl resulted in a significant decrease in sour taste intensity of about 2 intensity units ($p = 0.016$). Sour taste was not further suppressed by higher concentrations of NaCl. Results from the present study suggest that suppression of sour taste in pH adjusted solutions is only partially explained by sodium ions and organic anions, although both seem to have an effect.

3.2 Introduction

Organic acids all have one or more carboxyl groups, but other than carboxyl groups they have a variety of structural features. The carboxyl groups have protons that dissociate depending upon the strength of the acid. For an acid with a single carboxyl group this dissociation is determined by the dissociation constant $K_a = \frac{[H^+][A^-]}{[HA]}$, where $[H^+]$ is the concentration of hydrogen ions in solution, $[A^-]$ is the concentration of the acid in which the hydrogen ion has dissociated from the carboxyl group, and $[HA]$ is the concentration of the acid in which a hydrogen ion remains bound to the carboxyl group. There has been a considerable amount of research that has attempted in various ways to relate the intensity of sour taste perception to properties of organic acids and their ability to bind hydrogen ions or release hydrogen ions into water solution. However, the influence of organic anion species $[A^-]$ on sour taste perception has not been delineated.

Norris and others (1984) suggested that differences in the anion composition of acid solutions influence the binding of the sour stimuli to the receptor sites. Anions have been hypothesized to have a collaborative effect on sour taste response by decreasing the positive charge of the membrane and thus increasing the binding of hydrogen ions to receptor molecules (Koyama and Kurihara, 1972, Beidler, 1978). Beidler (1971) suggested that the observation of Richards (1898) that acetic acid solution is perceived to be more sour than hydrochloric acid at the same pH could be explained by the presence of anions in the acetic acid solution. . He also cited a study by Liljestr and (1922), where the pH of buffered acetic acid–sodium acetate solution was significantly higher than acetic acid solution at their observed threshold concentration. The higher concentration of anion in the buffer solution

was deemed as the major factor for the lower hydrogen ion concentration required to produce an equivalent taste response. Similarly, Beidler (1967) tested the effect of anions on sour taste response by comparing the neural response of rats to acetic acid solution and to a buffered acetic – sodium acetate solution. Although the neural responses were comparable, the pH of the buffered solution was considerably higher (lower hydrogen ion concentration) as compared to the acetic acid solution. He explained the results by suggesting that in a buffered solution a lower concentration of hydrogen ion is needed because acetate ions augment binding of hydrogen ions to the receptor sites.

A number of studies have suggested that sodium salts of organic acids may inhibit the interaction between taste receptors and fully protonated (undissociated) acids, causing suppression of sour taste (Ganzevles and Kroeze, 1987; DeSimone and Price, 1976). Makhoulouf and Blum (1972) reported that sour taste intensity and molar concentration of acetic acid was described by an equation in the form of enzymatic – kinetics, and that sodium acetate showed characteristics of competitive inhibition. Lawless (1991) suggested that some large anions decrease perceived sour taste intensity through contributing tastes of their own, which act as masking stimuli.

Physiological studies have speculated that amiloride-sensitive Na^+ channels may mediate both salt and sour tastes responses in mammals (Lindemann 1996; Stewart and others 1997). In this case, sour and salty stimuli would interact with the same receptor site, providing a potential mechanism for competitive inhibition. Ogawa (1969) reported that rats'

neural responses to NaCl solutions were reduced by lowering pH, which suggests interaction between salty and sour taste modalities.

In a previous study, we showed that sour taste is caused by two entities, namely protonated organic acid species and hydrogen ions. However, in order to demonstrate that this was true, the pH of solutions was adjusted with sodium hydroxide resulting in other uncontrolled variables, such as organic anions and sodium ions. We observed that the intensity of sour taste might be influenced by the presence of these species in solution. The objective of this study was to investigate the roles of organic anions and sodium ions in suppressing sour taste intensity of acid solutions.

3.3 Materials and Methods

3.3.1 Sensory Evaluation

Panelists

Non-smoking female students and staff from the Department of Food Science at North Carolina State University (Raleigh, NC), between the ages of 22 and 49, served as panel members. The panel was comprised of nine subjects, who were selected based on availability and ability to distinguish and scale the basic tastes.

Training

The descriptive sensory panel was trained for 40 hours in the Spectrum™ Method (Meilgaard and others, 1991) to evaluate several attributes of aqueous solutions on a 15-point scale.

Aqueous solutions of citric acid, sucrose, NaCl, caffeine, alum, and acetic acid were used as reference stimuli for sour, sweet, salty, and bitter tastes, astringency sensation, and vinegar flavor, respectively. Reference concentrations were selected from the Spectrum™ Method (Meilgaard and others, 1991) with the exception of astringency and vinegar flavor, which were developed during training. Alum has been shown to be an appropriate astringency reference standard for descriptive analysis (Wismer and Goonewardene, 2003). Concentrations of 0.25, 0.56, 1.1, and 1.5 g/L alum represented 2, 5, 10, and, 15 intensities on the astringency scale, respectively. Concentrations of 4.2, 8.8, and 38.5 mM acetic acid anchored the intensities 2, 5, and 10 for vinegar flavor, respectively.

3.3.2 Sample Presentation

Samples were presented at room temperature in 2 oz. plastic cups labeled with a three-digit code. Each sample was evaluated for sour, salty, bitter and sweet tastes, astringency sensation, vinegar flavor, and 'other'. The 'other' category was an open scale with space allotted for a write-in descriptor to be used when a non-anticipated off-note was observed. Subjects evaluated five or six samples of acid solutions per session using the sip-and-spit method. Panelists were instructed to take a comfortable amount of sample into their mouth, swish it around for approximately five seconds and expectorate it into a waste cup. Reference solutions for sour taste, astringency and vinegar flavor were provided in every session for panelists' calibration.

Water and crackers were provided as palate cleansers between samples. For aqueous solutions, 5.5% carboxymethylcellulose (CMC) (Aqualon, Wilmington, DE) and/or Münster cheese were supplied to counteract the drying and puckering sensations of astringency. CMC has been previously shown to act as an effective inter-stimulus rinse for astringency in model solutions (Brannan and others, 1999).

3.3.3 Sample Preparation

Food grade acid solutions were prepared with purified water at the beginning of each week of testing and refrigerated between sensory evaluations. Acetic, lactic, adipic, malic, tartaric, succinic, and fumaric acids were obtained from Sigma Aldrich (St. Louis, MO). Citric acid, hydrochloric acid, and sodium hydroxide were obtained Spectrum (Spectrum

Chemical & Laboratories Products, Gardena, CA). pH adjustments were done using either 2N sodium hydroxide or 2N hydrochloric acid.

Aqueous solutions containing a single organic acid were evaluated at a constant concentration of protonated organic acid species concentration and varied pH (hydrogen ion concentration). Acetic and lactic acids were evaluated at 8 mM protonated organic acid species, with pH ranging from 3.0 to 5.5 and 3.0 to 4.5, respectively. Citric and malic acids were evaluated at 10 mM protonated organic acid species concentration, with pH ranging from 3.0 to 6.0 and 3.0 to 5.5, respectively.

The effect of organic anion species in suppressing sour taste intensity was evaluated using mixtures of three different organic acids in each solution. Concentrations of protonated acid species and hydrogen ion concentrations were held constant at 20 mM and 0.1 mM (pH= 4), respectively. Table 3.1 shows the stimulus concentrations used in each acid solution. It should be noted that sodium ion concentration was also a variable in this experiment, as each acid solution required a different amount of sodium hydroxide to adjust pH due to differences in buffer capacities.

The effect of sodium ion concentration in suppressing sour taste intensity was evaluated by adding 0 to 80 mM sodium chloride to acid solutions containing mixtures of three acids. Protonated organic acid species and hydrogen ion concentrations were held constant at 9 mM and 2.51 mM (pH=2.6), respectively. Table 3.2 shows the stimulus concentrations used in each solution. It should be noted that the total molar concentration

matched the concentration of protonated acid species, because the pH was significantly lower than the pK_a values of the acids. Thus, the concentration of organic anion concentration was kept near zero.

3.3.4 Calculation of Organic Acid Species Concentration and pH Measurements:

The distribution of organic acid species was calculated using $pHTools$, a modeling program implemented in MATLABTM developed in this laboratory by Dougherty (2002) and validated by Neta and others (2005). Mathematically, this software adjusts the published pK_a values of the acids for the ionic strength, temperature, and dielectric constant of the medium.

The pH was measured using a pH electrode with a microprocessor pH/mV meter (Accumet Research AR25) equipped with an AcuFet solid-state electrode (Fisher Scientific, Atlanta, GA).

3.3.5 Statistical Analysis

Aqueous solutions were evaluated in a randomized complete block design with three sensory replications. Linear regression and analysis of variance ($p \leq 0.05$) were performed on treatment means using the 8.2 version of SAS[®] software (SAS institute, Inc., 1987, Cary, NC).

3.4 Results and Discussion

In a previous study, we showed that the critical factors that determine the sour taste intensity of organic acid solutions are the molar concentration of acid molecules that have one or more protonated carboxyl group (protonated organic acid species) and the molar concentration of hydrogen ions. In addition, we showed for solutions of HCl that hydrogen ions evoke little or no sour taste response at $\text{pH} > 3.0$.

At a constant concentration of protonated organic acid species, sour taste intensity significantly decreased with increasing pH (decreasing hydrogen ion concentration) for lactic and acetic acids, as well as for citric and malic acids, respectively (Figures 3.1 and 3.2). However, hydrogen ions have little or no direct contribution to sour taste intensity in the pH range tested (between 3 and 6). Therefore, some factor(s) other than hydrogen ions must account for the decrease in sour taste intensity as pH increased.

In order to hold the concentration of protonated organic acid species constant while varying the pH, two variables were inevitably introduced. Sodium ions were incorporated into solutions as sodium hydroxide was added to increase the pH. Also, there was an increase in the organic anion concentration caused by the higher total acid concentration required to keep the concentration of protonated organic acid species constant at a higher pH. Thus, sour taste intensity may have been suppressed by the presence of organic anions and/or sodium ions in the solutions.

The effect of organic anion species in suppressing sour taste intensity was investigated in solutions containing mixtures of three acids with a constant concentration of protonated organic acid species and hydrogen ion. Figure 3.3 shows that sour taste decreased as the concentration of organic anion increased but the correlation was only 0.480. Thus, it appears that organic anion species do not play a major role in suppressing sour taste intensity in acid solutions. The effect of sodium ions in suppressing sour taste intensity could also be investigated because each acid solution had different buffer capacities, and thus required a different amount of sodium hydroxide to adjust the pH. Sour taste intensity decreased ($R^2=0.861$) as the approximate concentration of sodium ions increased from 10 to 45 mM (Figure 3.4), while salty taste intensity increased ($R^2=0.752$) (Figure 3.5). Sour taste decreased approximately 2 intensity units, while salty taste increased about 1 intensity unit. Thus, sodium ions may suppress sour taste intensity either by interaction between sour and salty tastes, or by stimuli inhibition at the receptor level.

The effect of sodium ions in suppressing sour taste intensity was further investigated by adding sodium chloride to solutions containing mixtures of three organic acids at a constant concentration of protonated organic acid species and hydrogen ion, and in the absence of organic anion species. Addition of 20 mM NaCl resulted in a significant decrease in sour taste intensity of about 2 intensity units ($p=0.016$). However, sour taste was not suppressed further by higher concentrations of NaCl (Figure 3.6). It has been proposed that amiloride-sensitive Na^+ channels in the cell membrane mediate both sour and salty responses, providing a potential mechanism for competitive inhibition (Lindemann 1996; Stewart and others 1997). However, our results conflict with the competitive inhibition

hypothesis in that no sour taste suppression was observed by increasing sodium ion concentration over 20 mM. Salty taste increased linearly with sodium ion concentration ($R^2=0.980$) with intensity scores increasing from 0 to 8 (Figure 3.7). Increasing sodium ion concentration in solution by adding sodium chloride had a higher impact on salty taste perception as compared to sodium hydroxide. This is in agreement with Van Der Klaauw and Smith (1994), who reported that organic Na salts are considerably less salty than NaCl on a molar basis. However, the magnitude of sour taste suppression was comparable when sodium ion concentration increased from 10 to 45 mM by addition of sodium hydroxide, and from 0 to 20 mM by addition of sodium chloride. Therefore, taste interaction between salty and sour modalities cannot account for the sour taste suppression caused by sodium ions in solution.

3.5 Conclusions

Organic acid anions may act as competitive inhibitors with protonated organic acids to suppress sour taste intensity. Sodium ions may also be able to suppress sour taste by competing with hydrogen ions at the receptor level or by competing at the perceptual level due to their own salty taste.

Results from the present study suggest that suppression of sour taste in aqueous solutions cannot be entirely explained by either sodium ions or organic anions. Although both seem to have an effect, their individual contributions to sour taste suppression cannot entirely explain the response that was observed. Therefore, other unknown variables must also play a role in suppressing sour taste intensity of organic acid solutions when pH is adjusted with sodium hydroxide to the range where hydrogen ions have no contribution to sour taste.

Tables

Table 3.1. Stimulus concentrations for aqueous solutions at 0.1mM hydrogen ion (pH 4.0) and 20mM protonated organic acid species.

Acids Concentration (mM)							Anion Species (mM)	Sodium Ion (mM)
Adipic	Citric	Fumaric	Lactic	Malic	Succinic	Tartaric		
10	5				5.2		0.3	11.4
10					5	8.2	3.4	17.2
	5	10	18				9.5	38.2
3			17	12.5			12.5	23.8
	5	13	18				16.0	36.0
		9.7	15			15	19.7	43.6

Table 3.2. Stimulus concentrations for aqueous solutions at 9m M protonated organic acid species, 2.51mM hydrogen ion (pH=2.6), and 0mM anion concentrations.

Acids Concentration (mM)							Sodium Ion (mM)
Adipic	Citric	Fumaric	Lactic	Malic	Succinic	Tartaric	
3		3		3			0
	4		1		4		20
3				3		3	40
3	3					3	60
			1	4	4		80

Figures

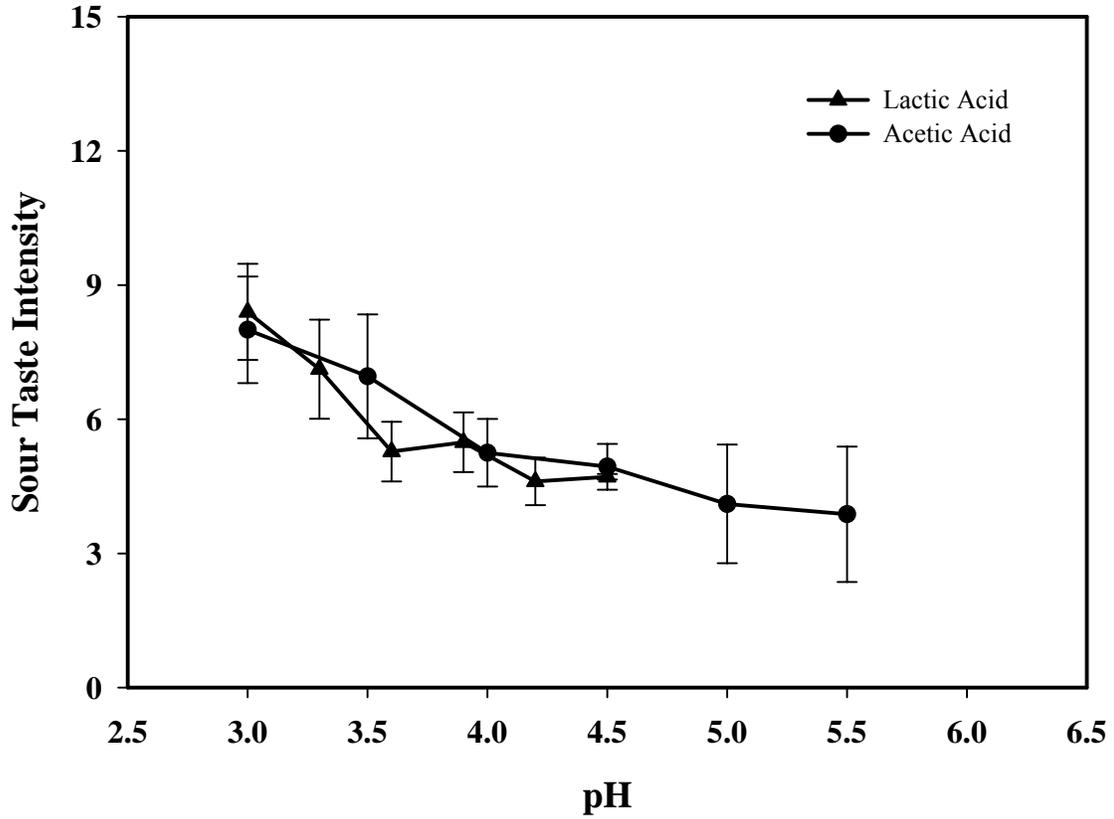


Figure 3.1. Sour taste intensity plotted as a function of pH at a constant concentration of protonated organic acid species (8 mM) for lactic and acetic acids. Error bars represent standard deviation from three sensory replications.

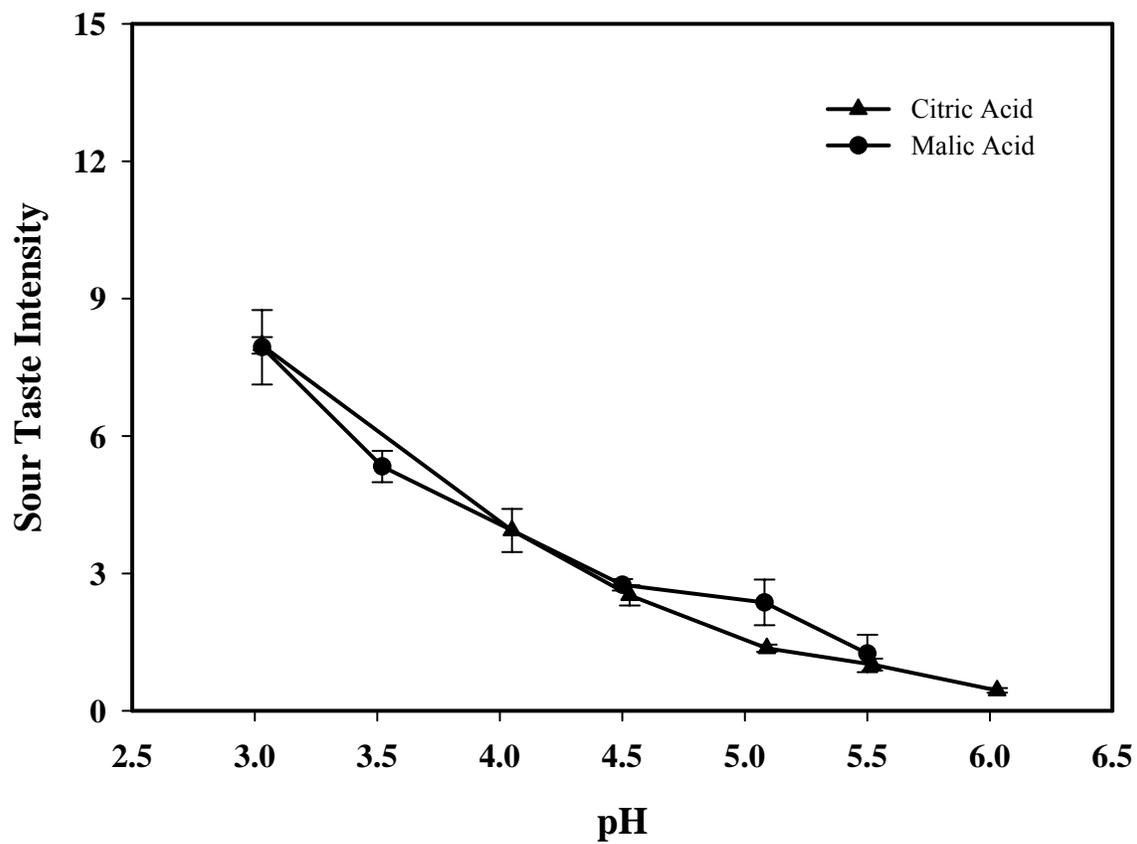


Figure 3.2. Sour taste intensity plotted as a function of pH at a constant concentration of protonated organic acid species (10 mM) for citric and malic acids. Error bars represent standard deviation from three sensory replications.

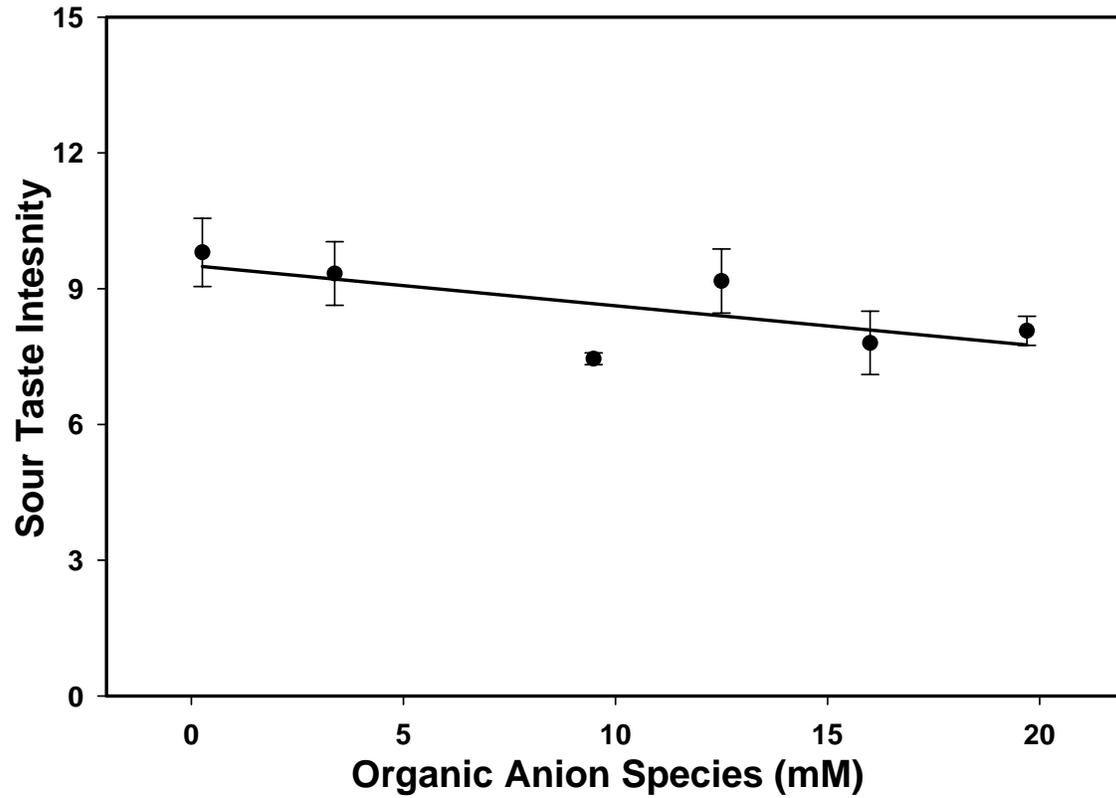


Figure 3.3. Effect of anion species in suppressing sour taste intensity ($R^2= 0.480$) in solutions containing mixtures of three acids with constant concentration of protonated organic acid species and hydrogen ion (pH). Error bars represent standard deviation from three sensory replications.

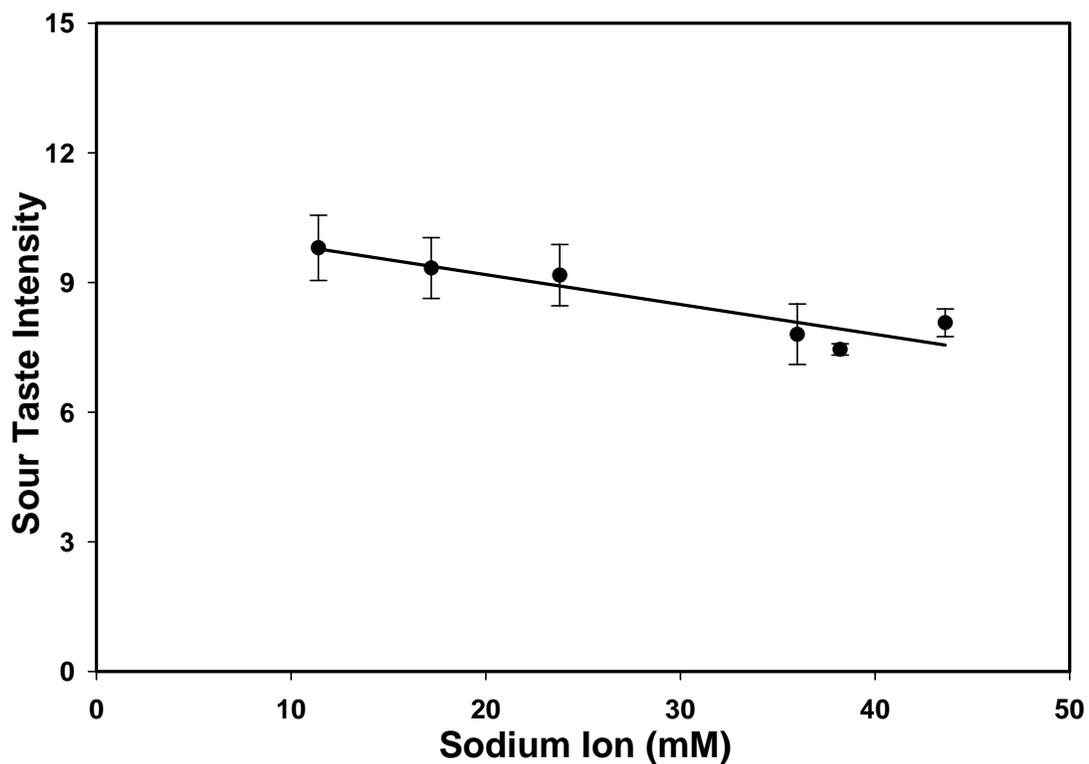


Figure 3.4. Effect of sodium ions in suppressing sour taste intensity ($R^2= 0.861$) when NaOH is added to solutions containing mixtures of three acids with constant concentration of protonated organic acid species and hydrogen ion (pH). Error bars represent standard deviation from three sensory replications.

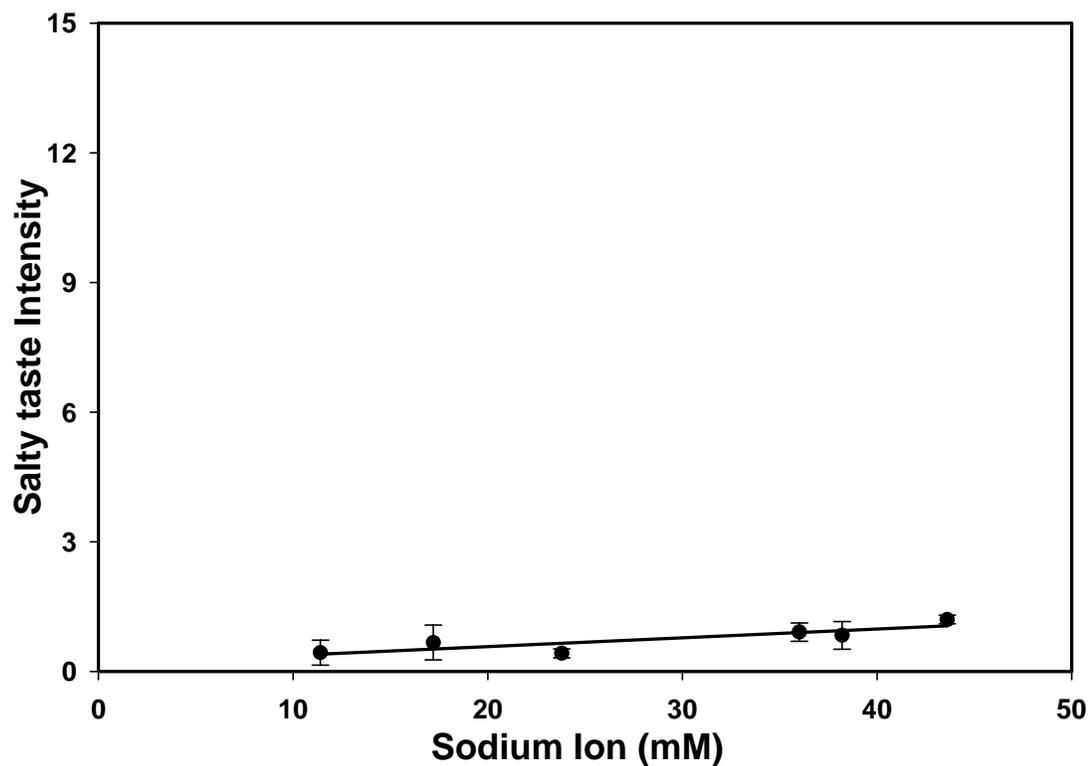


Figure 3.5. Salty taste intensity plotted as a function of sodium ion concentration ($R^2=0.752$) when NaOH is added to solutions containing mixtures of three acids with constant concentration of protonated organic acid species and hydrogen ion (pH). Error bars represent standard deviation from three sensory replications.

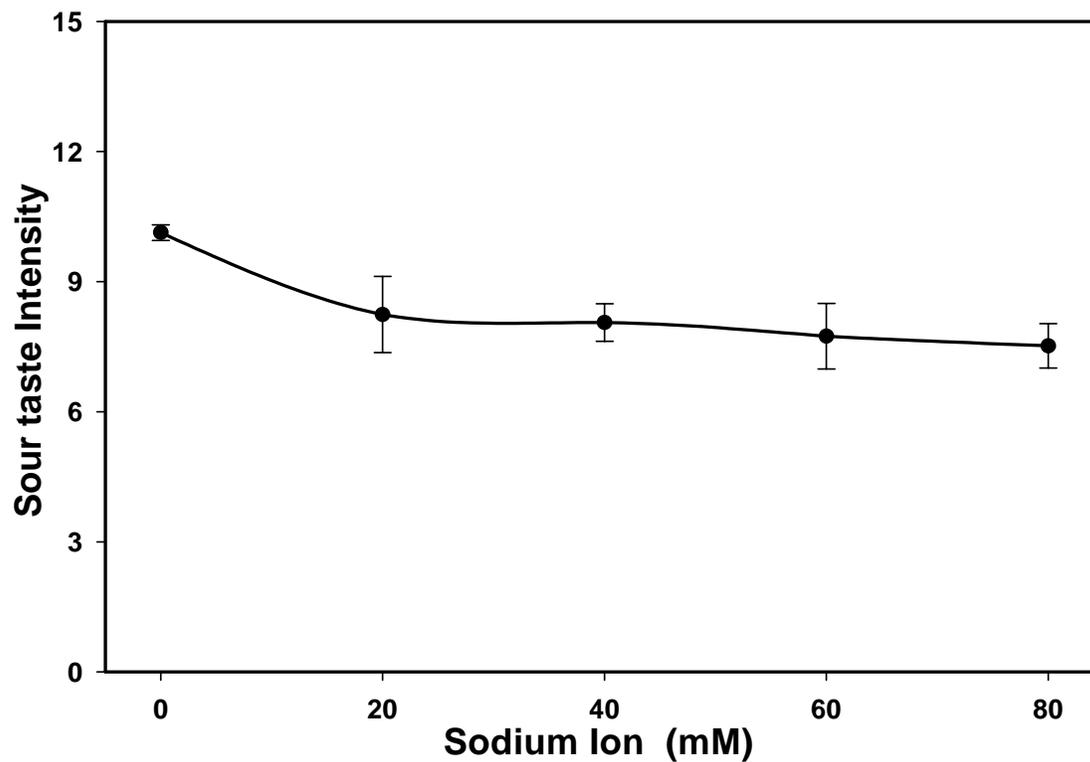


Figure 3.6. Effect of sodium ions in suppressing sour taste intensity when NaCl is added to solutions containing mixtures of three organic acids at a constant concentration of protonated organic acid species and hydrogen ion, and in the absence of organic anion species. Error bars represent standard deviation from three sensory replications.

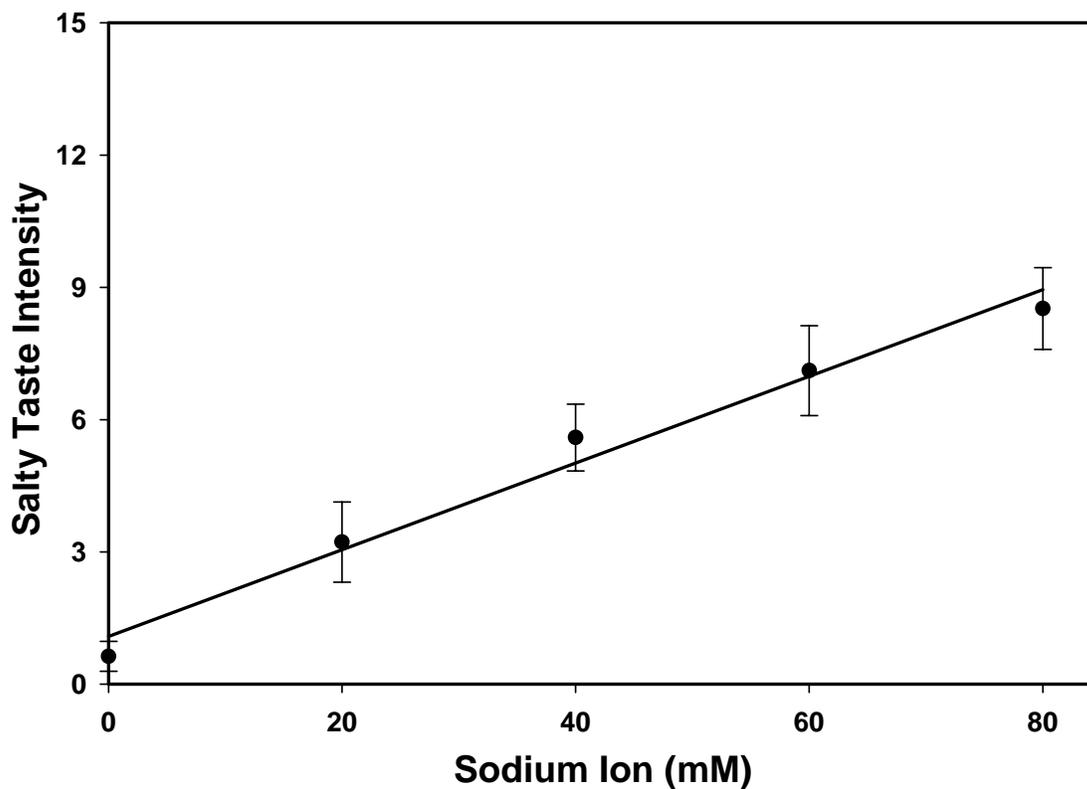


Figure 3.7. Salty taste intensity as a function of sodium ion concentration ($R^2=0.980$) when NaCl is added to solutions containing mixtures of three organic acids at a constant concentration of protonated organic acid species and hydrogen ion, and in the absence of organic anion species. Error bars represent standard deviation from three sensory replications.

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