A Chemical Basis for Sour Taste Perception of Acid Solutions and Fresh-Pack Dill Pickles

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ABSTRACT: Sour taste is influenced by pH and acids present in foods. It is not currently possible, however, to accurately predict and modify sour taste intensity in foods containing organic acids. The objective of this study was to investigate the roles of protonated (undissociated) organic acid species and hydrogen ions in evoking sour taste. Sour taste intensity increased linearly with hydrogen ion concentration ($R^2 = 0.995$), and with the concentration of protonated organic acid species at pH 3.5 ($R^2 = 0.884$), 4.0 ($R^2 = 0.929$), and 4.5 ($R^2 = 0.975$). The structures of organic acid molecules did not affect sour taste after adjusting for the effects of protonated organic acid species and hydrogen ions. Sour taste intensity was also linearly related to the total concentration of protonated organic acid species in fresh-pack dill pickles ($R^2 = 0.957$). This study showed that the sour taste of organic acids was directly related to the number of molecules with at least 1 protonated carboxyl group plus the hydrogen ions in solution. Conclusions from these results are that all protonated organic acids are equally sour on a molar basis, that all protonated species of a given organic acid are equally sour, and that hydrogen ions and protonated organic acids are approximately equal in sour taste on a molar basis. This study provides a new understanding of the chemical species that are able to elicit sour taste and reveals a basis for predicting sour taste intensity in the formulation of acidified foods.

Keywords: cucumber, flavor, organic acid, pH, protonated acid, undissociated acid

Introduction

Sour taste is the aspect of flavor most commonly associated with acids, although other taste characteristics such as bitterness, saltiness, and astringency may coexist (Meiselman and Dzendolet 1967; McBurney and Shick 1971; Settle and others 1986; Rubico and McDaniel 1992; Hartwig and McDaniel 1995; Siebert 1999). Many studies have sought to identify the chemical basis for sour taste perception (Neta and others 2007). The property shared by all acids is the dissociation of protons when 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 (Richards 1898; Pfaffmann 1959; Koyama and Kurihara 1972; Ganzevles and Kroeze 1987a; Ugawa and others 1998; Ogiso and others 2000; Lyall and others 2001; Richter and others 2003; Lugaz and others 2005). Furthermore, the pH of various organic acid solutions is considerably different at their observed threshold concentrations (Liljestrand 1922; Berg and others 1955; Amerine and others 1965). These studies have shown 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 influencing 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." Several studies have reported that sour taste intensity increases with increasing titratable acidity at equivalent pH (Makhlouf and Blum 1972; Plane and others 1980; Lugaz and others 2005). Early studies conducted by 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, later studies by Ganzevles and Kroeze (1987b) showed that the titration volume rank order at pH 4.4 for several acids differed from the sour response rank order. Furthermore, Norris and others (1984) showed that binary acid solutions with equivalent pH and titratable acidity evoked significantly different sour taste responses. 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 clear relationship between pH, titratable acidity, and relative sour taste intensity.

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 into the taste cell membrane was enhanced as nonpolar 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 the sour taste response. Johanningsmeier and others (2005) proposed that the intensity of sour taste is directly related to 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 objective of this study was to investigate the roles of protonated organic acid species and hydrogen ions in sour taste of aqueous solutions and fresh-pack dill pickles.

Materials and Methods

Subjects

Nonsmoking female students and staff from the Dept. of Food Science at North Carolina State Univ. (Raleigh, N.C., U.S.A.) between the ages of 22 and 49 y served as panel members. The panel comprised 9 subjects, who were selected based on availability and ability to distinguish and scale the basic tastes (Meilgaard and others 1999).

Training

The descriptive panel was trained for 40 h in the Spectrum™ Method (Meilgaard and others 1999) to evaluate several attributes of aqueous solutions on a 15-point universal intensity scale. An additional 15 h of training was 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. Concentrations for reference samples were selected from the Spectrum Method (Meilgaard and others 1999) 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. During training sessions, panelists were first presented with the reference solutions labeled as such followed by presentation of these solutions with 3-digit codes to familiarize the panelists with recognition of the particular stimulus and determination of stimulus intensity. Following consistent identification of these samples by the panel, training sessions then consisted of additional solutions (different acids, acid combinations, or dill pickles). Panelists were encouraged to discuss and further clarify sensations and practiced scoring these solutions consistently. Analysis of variance of results from training sessions was used to determine that the panel could consistently detect and quantify the selected attributes in the solutions and dill pickles prior to formal experiments.

Procedure

Samples were presented at room temperature in 2 oz plastic cups labeled with a 3-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 nonanticipated off-note was observed.

Subjects evaluated no more than 6 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 5 s, and expectorate it into a waste cup. Reference solutions for sour taste, astringency, and vinegar flavor were provided in each session for panelists' calibration. For dill pickles, no more than 5 samples were tested in each session. A pickle reference with a sour taste intensity of 8 was provided for panelists' calibration. Panelists had the option to either swallow or expectorate samples during evaluation of pickle products. Each panelist evaluated each sample in triplicate.

Water and crackers were provided as palate cleansers between samples. For aqueous solutions, 5.5% carboxymethylcellulose (CMC; Aqualon, Wilmington, Del., U.S.A.) and/or Muenster cheese were supplied to counteract the drying and puckering sensations of astringency. CMC has been previously shown to act as an effective interstimulus rinse for astringency in model solutions (Brannan and others 2001).

Sour taste of acid solutions

Food-grade acid solutions were prepared with purified water (Rainbow Water Service, Durham, N.C., U.S.A.) 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 Inc. (St. Louis, Mo., U.S.A.). Citric acid, hydrochloric acid, and sodium hydroxide were obtained from Spectrum Chemical & Laboratories Products (Gardena, Calif., U.S.A.). pH adjustments were done using either 2 N sodium hydroxide or 2 N hydrochloric acid.

Sour taste of organic acids. The sour taste of protonated organic acid species was evaluated using mixtures of 3 different organic acids, chosen from a group of 8 organic acids. For each set of solutions, total molar acid concentration and pH were held constant. The concentration of protonated organic acid species 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 concentrations (Table 1).

Sour taste comparisons between single and mixed organic acid solutions. The sour taste of single organic acid solutions was compared to the sour taste of solutions containing mixtures of 3 organic acids 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 8 organic acids used in this study. Total acid concentration was allowed to vary (Table 2).

Relative sour taste intensity of hydrogen ions and organic acids. Sour taste potencies of hydrogen ions and protonated organic acid species were compared using 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 (pH 2.10 to 3.32) in solutions having mixtures of 3 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 3 organic acids at a constant hydrogen ion concentration (2.51 mM, pH 2.6). The compositions of these solutions are given in Table 3.

Sour taste of organic acids in 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, Ohio, U.S.A.), and packed into 720 mL (24 oz) jars. The jars were filled with cucumber slices and appropriate cover brine solutions (described below) to give a 60/40 (cucumbers/brine) pack-out ratio, pasteurized at 75 °C (center of the jar) for 15 min in a water bath, and cooled to room temperature. The pasteurized jars were stored for at least 8 wk to allow equilibration of flavor prior to sensory evaluation.

The reference pickle brine was prepared with NaCl (final concentration of 2% after equilibration), sodium benzoate, vinegar containing 13% acetic acid, calcium chloride dihydrate, FD&C yellow 5 food coloring, and dill spice concentrate. Brines for the exper-

imental treatments were prepared in the same way as the reference formula, except that vinegar was replaced by different blends of organic acids. Each treatment consisted of mixtures of 3 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. This experiment was divided into 2 sets of samples. For the 1st one, total molar concentration of organic acids was held constant and the concentration of protonated organic acid species was varied by altering the acid blends. For the 2nd set, total molar concentration was allowed to vary while a target level of protonated acid species was attained (Table 4).

Table 1 — Stimulus concentrations of organic acids in aqueous solutions

Organic acid concentration (mM)								Total protonated organic acid species (mM)	Total acid (mM)	pH
Acetic	Adipic	Citric	Fumaric	Lactic	Malic	Succinic	Tartaric			
			1	12			2	10.6	15	3.5
	1		8				6	11.5	15	3.5
		3		4			8	12.3	15	3.5
				2	1		12	13.3	15	3.5
2			9		4			14.0	15	3.5
			1	22.5			1.5	16.8	25	3.5
	2.5			18			4.5	18.2	25	3.5
1			9				15	21.7	25	3.5
					7	6	12	23.2	25	3.5
15		4				6		24.1	25	3.5
		23			1	1		25.0	25	3.5
				3	1		6	5.8	10	4.0
	4		3	3				7.3	10	4.0
8	1					1		8.7	10	4.0
		5			3	2		9.8	10	4.0
			1	27			2	11.4	30	4.0
	3			16			11	15.1	30	4.0
2	1		27					20.1	30	4.0
					10	8	12	23.7	30	4.0
20		5				5		26.3	30	4.0
		25			3	2		29.6	30	4.0
			3	23			4	5.7	30	4.5
	5			15			10	9.5	30	4.5
3.5	6.5		20					15.2	30	4.5
				20	5	5		19.3	30	4.5
11		14				5		24.4	30	4.5
		20			2	8		28.4	30	4.5

Table 2 — Factorial treatment design for comparing sour taste intensity of single acid solutions and sour taste intensity of solutions containing mixtures of 3 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	

The pH was adjusted to 4.0 in all samples by addition of 6 N sodium hydroxide. For each treatment, a mixture of 120 g cucumber slurry and 80 g cover brine was titrated with 6 N sodium hydroxide. The amount of 6 N sodium hydroxide required to attain the target pH in 24 oz jars after equilibration was calculated by multiplying the titration volume by the appropriate factor.

Calculation of organic acid species concentrations and pH measurements

The distribution of organic acid species was calculated using $_{pH}$Tools, a modeling program implemented in MATLAB^{TM} developed in this laboratory by Dougherty and others (2006). This software adjusts the published pK_a values of 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 in 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 titration curve. In the case of dill pickle products, a 60/40 blend of cucumber slurry/brine (no added acid) was titrated with 2 N hydrochloric acid from an initial pH 5.5 to a final pH 2.0. The titration curve was then used as a complex buffer in $_{pH}$Tools to adjust the pK_a values of acids for ionic strength in order to determine the distribution of organic acid species in pickle products. The pH was measured using a pH electrode with a microprocessor pH/mV meter (Accumet AR25) equipped with an AcuFet solid-state electrode (Fisher Scientific, Atlanta, Ga., U.S.A.).

Data analysis

Acid solutions and dill pickle samples were evaluated in a randomized complete block design with 3 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 Inst. Inc., Cary, N.C., U.S.A.).

Results and Discussion

Sour taste of hydrogen ions

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. We found a linear relationship ($R^2 = 0.995$) between hydrogen ion concentration and the intensity of sour taste from 0.31 mM to 10 mM (Figure 1). Curtis and others (1984) used magnitude estimation to evaluate the sour taste intensity of mixed acid solutions. The authors reported magnitude estimates for the sour taste intensity of pure HCl solutions ranging from 0.53 to 8.48 mM. Linear regression of their sour taste intensity values with hydrogen ion concentration also resulted in a strong correlation ($R^2 = 0.99$). In the present study, the sour taste intensity of 1.0 mM hydrogen ions (pH 3.0) was only about 1 unit on a 15-point scale, and the sour taste of 0.31 mM hydrogen ions (pH 3.5) was barely detectable. Since the

Table 3 – Stimulus concentrations for comparing sour taste potencies of protonated organic acid species and hydrogen ions

Organic acid concentration (mM)							[H⁺] (mM)	Total protonated organic acid species (mM)
Adipic	Citric	Fumaric	Lactic	Malic	Succinic	Tartaric	[H^+] (mM)	
							0.31	0
							1.00	0
							1.77	0
							3.16	0
							5.62	0
							10	0
4			0.5		0.5		0.47	5
4	0.5			0.5			0.74	5
3				0.5	1.5		1.00	5
	1		3	1			1.78	5
3	1				1		2.00	5
3	1			1			3.16	5
		1.5	2	1.5			3.98	5
	3	1				1	5.62	5
2		1.5				1.5	6.03	5
	2			1.5	1.5		7.94	5
							2.51	0
0.67			0.67	0.67			2.51	2
	1.33		1.33			1.33	2.51	4
2			2	2			2.51	6
	2.67	2.67				2.67	2.51	8

Table 4 – Stimulus concentrations of organic acids for fresh-pack dill pickles at pH 4.0

Organic acid concentration (mM)							Total protonated organic acid species (mM)	Total acid (mM)
Acetic	Adipic	Citric	Fumaric	Lactic	Malic	Succinic		
			10	120	10		51.7	90
		5		80	5		58.7	90
	10			85	45		74.1	90
	21				21	21	60	63
	35			35	35		75	105
43	43			43			90	129

pH of most acid or acidified foods is above 3.0, the contribution of hydrogen ions to sour taste intensity in foods may be considered negligible. Therefore, the organic acids, either added or naturally present, would be expected to be primarily responsible for the sour taste in foods.

Sour taste of organic acids

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 the concentration of protonated acid molecules in solution are inextricably related to the dissociation constant(s) of the carboxyl group(s) present in any single 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 challenge was dealt with by using groups of 3 organic acids in solutions selected from a larger group of 8 food-grade organic acids. The 8 acids from which the sets of 3 acids were selected were monocarboxylic, dicarboxylic, and tricarboxylic organic acids with a range of pK_a values from 2.98 to 6.39. 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 5). These acids also had a range of flavor characteristics other than sour taste. Solutions made with mixtures of these acids made it possible to prepare groups of solutions with constant pH and constant total molar concentrations of acids, but with variable molar concentrations of acid molecules with one or more protonated carboxyl groups (Table 1).

Sour taste intensity increased linearly with the total molar concentration of all organic acid species having at least 1 protonated carboxyl group at pH 3.5 ($R^2 = 0.884$), 4.0 ($R^2 = 0.929$), and 4.5 ($R^2 = 0.975$) (Figure 2), regardless of the type of acids present in the mixtures. These results are consistent with the hypothesis that sour taste intensity is linearly related to the concentration of organic acid species with at least 1 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 was negligible. The main function of pH was to determine the degree of protonation of the organic acids in solution.
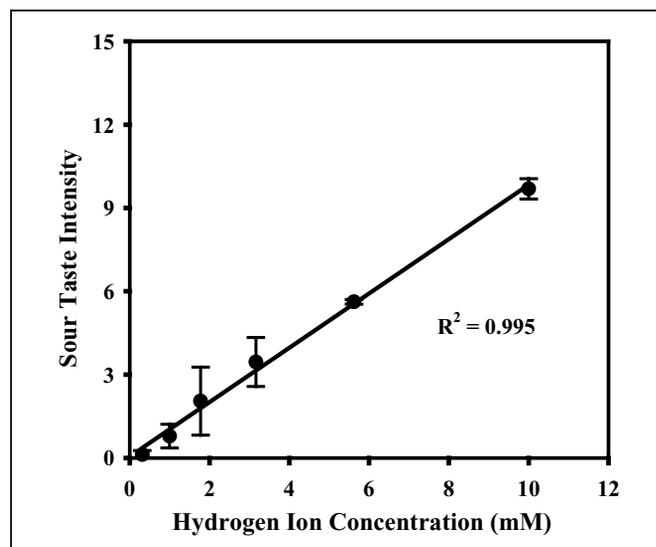
The linear relationship observed between the sour taste intensity of mixtures containing 8 different acids that were partially and fully protonated could occur only if the following were true. First, on a molar basis, all protonated organic acids must be perceived as equally sour. For instance, molecules of citric, malic, or lactic acid will produce the same sour taste response provided at least 1 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 responses.

Sour taste comparison between single and mixed organic acid solutions

As verification of the equality of sour taste intensity for different acids and all their protonated forms, the sour taste intensity of single organic acid solutions was compared to the sour taste intensity of solutions containing mixtures of 3 organic acids. There was no significant difference ($P < 0.05$) between the sour taste of a single acid and the sour taste of mixtures of 3 different acids for 8 out of 9 pairs of solutions (Table 6), which gives validity to the conclusion that for a given pH, all organic acid molecules are perceived to be equally sour provided that at least 1 carboxyl group is protonated. Notice that 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 any pH, acids with high pK_a provided higher concentrations of protonated organic acid species in solution than acids having low pK_a, and thus required 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 concentration of protonated organic acid species and hydrogen ion concentration (pH).

Relative sour taste intensity of hydrogen ions and organic acids

Since organic acids, due to their chemical nature, contribute both hydrogen ions and protonated acid species to an aqueous solution or food product, we further investigated the sour taste of solutions with sufficiently low pH so that both hydrogen ions and protonated organic acids would contribute substantially to the total sour taste intensity. In order to describe the individual and/or combined effects of hydrogen ions and protonated organic acid species on sour taste, 3 sets of solutions were necessary. 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, and also in the presence of a constant concentration (5 mM) of protonated organic acid species.

Figure 1 — Relationship between sour taste intensity and hydrogen ion concentration

Table 5 — Dissociation constants (pK_a) and hydrophobicities (log P) for organic acids used for sour taste evaluations

Acid	pK_{a1}^a	pK_{a2}^a	pK_{a3}^a	log P[b]
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

[a]Gardner (1977).
[b]Gardner (1980).

The statistical fit of these data to 3 different mathematical models was compared. Model 1 assumed that hydrogen ions and protonated organic acid species have different abilities to elicit sour taste and that there is interaction between these factors.

$$Y = \beta_0 + \beta_1 X + \beta_2 Z + \beta_3 XZ \tag{1}$$

where Y represents sour taste intensity, X is the concentration of protonated organic acid species, Z is the concentration of hydrogen ions, and βs 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 these factors.

$$Y = \beta_0 + \beta_1 X + \beta_2 Z \tag{2}$$

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

Model 3 considered total additivity between protonated organic acid species and hydrogen ions, meaning that they elicit approximately equal sour taste responses.

$$Y = \beta_0 + \beta_1 (X + Z) \tag{3}$$

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

Analysis of variance showed that the interaction term in Model 1 was not significantly different than zero ($P = 0.20$), indicating that no significant evidence of interaction between hydrogen ions and protonated acid molecules was found regarding sour taste perception. In addition, the slopes of the linear regression for hydrogen ions and protonated acid molecules in Model 2 were not found to be significantly different from one another ($P = 0.18$). Therefore, Model 3, the simplest model, sufficiently describes the data.

Figure 3 shows the fit of sour taste intensities to Model 3 ($\beta_0 = -0.63$, $P = 0.19$; $\beta_1 = 1.02$, $P < 0.0001$), which assumes that protonated organic acids and hydrogen ions elicit equal sour intensities on a molar basis. This model provided a very strong correlation between sour taste intensity and the molar concentration of all organic acid species that have one or more protonated carboxyl groups plus the molar concentration of hydrogen ions ($R^2 = 0.932$, $P < 0.0001$), which confirms the hypothesis of Johanningsmeier and others (2005).

Frijters and Ophuis (1983) suggested that 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. These studies support our findings that stimulus additivity between protonated organic acid species and hydrogen ions may 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 in agreement with our findings, 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 states that acid molecules must penetrate the cell membrane and release hydrogen ions inside the cell to produce a response (Taylor and others 1930; Gardner 1980; Ogiso and others 2000; Lyall and others 2001). If that were the case, sour taste intensity and the concentration of fully protonated molecules would be highly correlated because only uncharged molecules would be expected to diffuse through the cell membrane. However, Figure 4 shows that sour taste intensity was not correlated with the concentration of fully protonated organic acid species ($R^2 = 0.017$, 0.097, and 0.001 for pH 3.5, 4.0, and 4.5, respectively). The linear dependence of sour taste on the concentration of all protonated organic acid species (Figure 2) suggests that physicochemical interaction at the receptor level is the most likely trigger in the sour taste transduction mechanism for organic acids.

Our findings are at variance with the proposal of Shallenberger (1996) that sour taste is a function of potential hydrogen ion concentration. According to his hypothesis, organic acids would have the same sour taste potential at equal normal concentration rather than at equal molar concentrations assuming they are protonated. Makhlouf and Blum (1972) have associated sour taste with the capacity of the acid to release hydrogen ions in solution and suggested that strong acids (low pK_a, high capacity to dissociate) are more sour than weak acids (high pK_a, low capacity to dissociate) on a molar basis. Based upon our results, however, weaker organic acids are more sour at a given pH than a stronger organic acid because a larger fraction of the carboxyl groups are protonated. A possible explanation for this discrepancy is the different basis of comparison between the 2 studies. Makhlouf and Blum (1972) made their conclusions
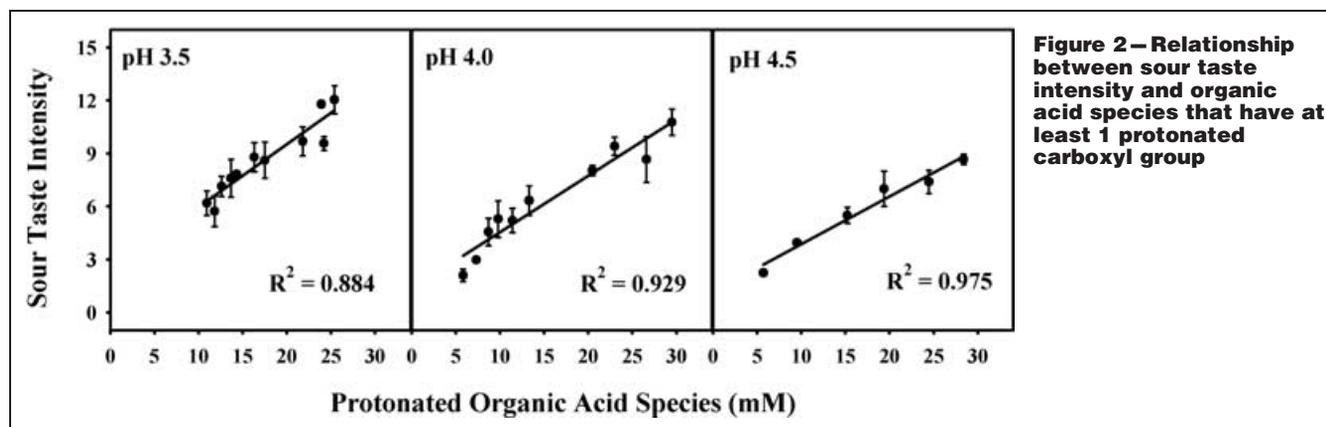

Figure 2—Relationship between sour taste intensity and organic acid species that have at least 1 protonated carboxyl group

on the basis of total molar concentration, while the present study considered protonated organic acid species.

Conflicting results for the sour taste potency of citric, lactic, tartaric, and acetic acids based on molar concentration have been reported (Ganzevles and Kroeze 1987b; CoSeteng and others 1989; Lugaz and others 2005). These studies used different ranges of molar concentration, which resulted in solutions with different pH ranges among the investigations. These factors may very well lead to conflicting and confusing conclusions unless the results of different investigations are compared with all variables considered. Unfortunately, in a number of cases sufficient data have not been provided in publications such that it is possible to re-evaluate the results.

Table 6 – Comparison between sour taste intensity of single acid solutions and sour taste intensity of solutions containing mixtures of 3 acids

Total protonated organic acid species (mM)	pH	Sour taste intensity		P value (mixed compared with single acids)
		Mixed acids	Single acids	
8	4.5	1.9 ± 0.1	2.2 ± 0.4	0.329[(NS)]
8	4.0	4.3 ± 0.7	2.5 ± 0.7	0.036[a]
8	3.5	4.8 ± 0.5	5.0 ± 07	0.671[(NS)]
16	4.5	5.6 ± 0.6	6.5 ± 0.5	0.132[(NS)]
16	4.0	7.0 ± 1.0	6.5 ± 0.6	0.462[(NS)]
16	3.5	9.9 ± 0.3	11.0 ± 1.1	0.175[(NS)]
25	4.5	9.2 ± 1.2	8.2 ± 0.6	0.263[(NS)]
25	4.0	10.7 ± 0.7	11.5 ± 1.9	0.533[(NS)]
25	3.5	12.2 ± 1.1	13.3 ± 0.1	0.162[(NS)]

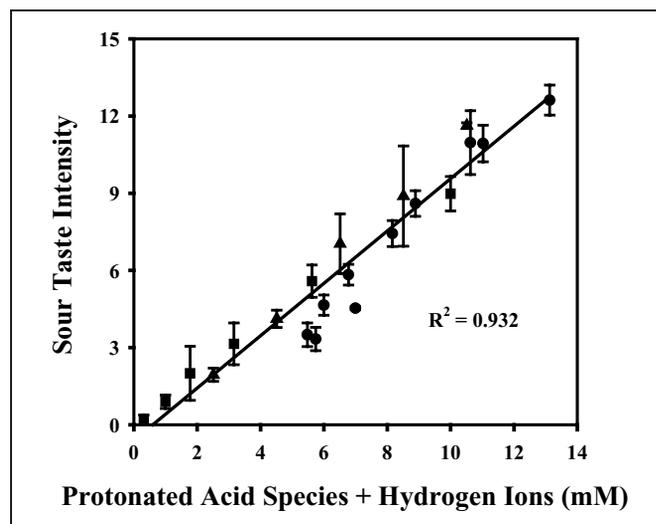[a]Significantly different if $P < 0.05$.

Figure 3 – Relationship between sour taste intensity and the sum of the concentrations of protonated organic acid species and hydrogen ions. Triangles represent hydrogen ion concentrations in the absence of protonated organic acid species (0 mM), circles indicate solutions with variable hydrogen ion concentrations and a constant protonated organic acid species concentration (5 mM), and squares indicate solutions with variable protonated organic acid species at a constant hydrogen ion concentration (2.51 mM).

Sour taste of organic acids in dill pickles

Only a limited number of sensory studies have attempted to investigate mechanisms of sour taste perception in food systems (Amerine and others 1965; Plane and others 1980; CoSeteng and others 1989). Stimuli for sour taste have been mostly examined in aqueous solutions. In order to demonstrate that the same relationships found in this study in aqueous solutions hold true in a more complex matrix, the sour taste of protonated organic acids was investigated in dill pickles. The sour taste intensity of fresh-pack dill pickles increased linearly with the concentration of protonated organic acid species ($R^2 = 0.957$) (Figure 5), giving further evidence that sour taste is elicited by molecules containing a protonated carboxyl group. This result agrees with Johanningsmeier and others (2005), who reported a linear relationship between sour taste intensity and protonated organic acid species concentration in dill pickles containing lactic, acetic, and malic acids.

The concentration of organic acid species required to attain a comparable sour taste response relative to aqueous solutions was about 4 times higher for dill pickle products. This may be explained by the complex flavor interactions that occur in food systems such as suppressive interactions between sour and salty tastes. Physiological studies have led to the suggestion that amiloride-sensitive Na^+ channels may mediate both salt and sour taste responses in mammals (Lindemann 1996; Stewart and others 1997), providing a potential mechanism for competitive inhibition. Ogawa (1969) reported that rats' neural responses to NaCl solutions were reduced by lowering pH, which also suggests interaction between salty and sour taste modalities.

Conclusion

Sour taste intensity was linearly related to the molar concentration of hydrogen ions and the molar concentration of all organic acid species that had at least 1 protonated carboxyl group. All of the organic acids in each of their protonated forms were equivalent in their ability to elicit sour taste. Protonated organic acid species and hydrogen ions were found to have approximately equal sour taste responses on a molar basis. Thus, the sour taste intensity of a mixture of protonated organic acid species and hydrogen ions was
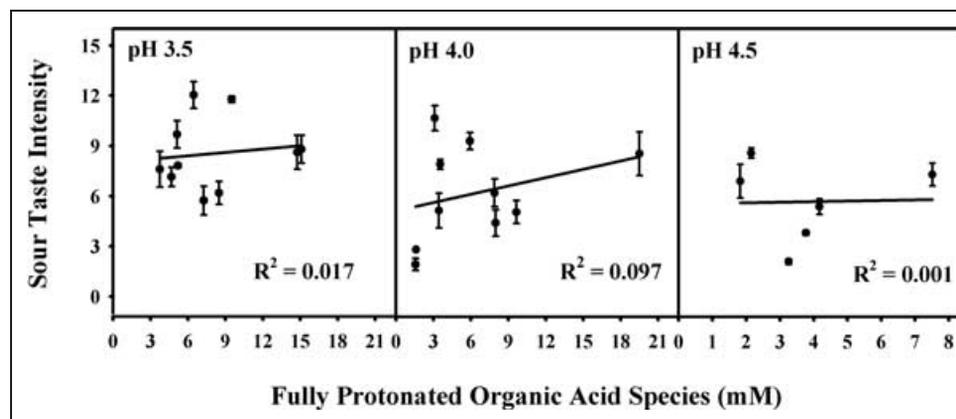

Figure 4 – Relationship between sour taste intensity and organic acid species that have all carboxyl groups in protonated form (uncharged molecules)
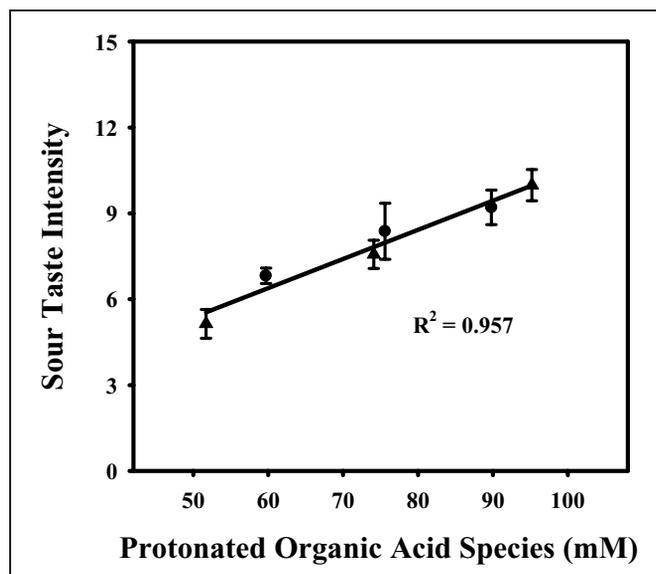
Figure 5 — Relationship between sour taste intensity and organic acid species that have at least 1 protonated carboxyl group in fresh-pack dill pickles. Samples represented by triangles and circles had the total molar concentration of organic acid constant and variable, respectively.

determined by the sum of the concentrations of individual components calculated on a molar basis. Furthermore, the linear relationship between sour taste intensity and protonated organic acid species was shown to occur both in acid solutions and in dill pickles. This study provides a new understanding of the chemical species that are able to elicit sour taste and reveals a basis for predicting sour taste intensity in the formulation of acidified foods.

Acknowledgments

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