A Hypothesis for the Chemical Basis for Perception of Sour Taste

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ABSTRACT: Sour taste has been considered the simplest of the basic tastes because it is elicited only by hydrogen ions. However, there is not a sufficiently clear understanding of that relationship to allow sour taste intensity to be predicted and rationally modified in foods. On the basis of analysis of sensory data from our laboratory and reanalysis of previously published data, we propose a new hypothesis for the chemical basis for sour taste perception: The intensity of sour taste perception in acid solutions or acidified foods is linearly related to the molar concentration of all organic acid species with at least 1 protonated carboxyl group plus the molar concentration of free hydrogen ions. This hypothesis implies that, on a molar basis, different organic acids will be equally sour, provided at least 1 carboxyl group is protonated. The major effect of pH on sour taste will be to determine the degree of protonation of organic acids. If this hypothesis is confirmed, it will provide a new understanding of the chemical basis for this basic taste perception and have broad usefulness in the formulation of products in which sour taste is an important component of flavor.

Keywords: undissociated, organic, acids, protonated, sour taste

Introduction

Chemistry and sensory characteristics of sour taste perception

Shallenberger (1996) considered sourness to be the simplest taste because the hydrogen ion is the only chemical species to cause a sour taste. He proposed that "sour taste is entirely a function of the potential hydrogen ion concentration and not the hydrogen ion (pH) per se." He also suggested that the degree of sour taste from different acids should be equal. However, sensory studies give rather complex and sometimes confusing conclusions regarding the sour taste of acids. It was clear as early as 1920 (Harvey 1920) that the concentration of hydrogen ion alone was not a good predictor of sour taste and that titratable acidity also has an effect. Pangborn (1963) stated there was no relation between pH, total acidity, and relative sour taste of citric, lactic, tartaric, and acetic acids. A study to establish an acidity index for the taste of wines resulted in an empirical relationship, which the authors pointed out implied that the taste impact of a fully dissociated anion should be at least 10-fold greater than the taste impact of an undissociated (protonated) acid molecule (Plane and others 1980). Gardner (1980) analyzed several sets of published data and suggested that the hydrophobicity of the acid anion correlated with the sour taste of acids. However, Noble and others (1986) pointed out that the sour taste of several sets of binary acid mixtures of equal pH and titratable acidity did not correlate with the degree of hydrophobicity. More recently, Hartwig and McDaniel (1995) indicated that, while all acids give a sour taste response, acids are different in their degree of sour taste, as well as non-sour aroma and flavor characteristics. They found that acetic acid was the most sour and lactic and citric acids the least sour of a group of acids evaluated in water solutions at equivalent pH. Additionally, lowering the pH while holding the concentration of acid constant resulted in significant increases in sour taste intensity for several organic acids tested (Hartwig and McDaniel 1995; Sowalsky and Noble 1998). CoSeteng and others (1989) concluded that citric, malic, tartaric, and acetic acids gave sour taste responses that could not be explained by a single factor. They attributed sour taste to the unique chemical structure and properties of each acid and to the interaction of pH and titratable acidity.

Astringency of acids

Food acids elicit chemical feeling factors in addition to sourness. Astringency is a mouthfeel perception that commonly occurs with acid solutions in addition to the sour taste perception. Rubico and McDaniel (1992) found that acids differed in their astringency, with inorganic acids perceived as being more astringent than organic acids. Succinic acid was unusual in that it had intense bitterness, rather than astringency. Corrigan-Thomas and Lawless (1995) also found inorganic acids to be particularly astringent, even though they lacked the chemical characteristics, namely adjacent hydroxyl groups, generally associated with astringency. They suggested that the perception of astringency may be caused by a pH-dependent effect. Later, Lawless and others (1996) concluded that pH was the major factor that gave acids astringent properties. Sowalsky and Noble (1998) concluded that, unlike sour taste, astringency of acids was directly related to pH and not to the anion species.

Physiology of taste perception

Research into the physiological mechanisms of taste perception has been advancing rapidly due to the increasing ability to carry out analysis of genomic data and the ability to do genetic experiments in mice (Bachmanov and Beauchamp 2002; Margolskee 2002). Particular progress has been made on understanding sweetness, bitterness, and umami tastes (Li and others 2001; Montmayeur and others 2001; Nelson and others 2001; Ruiz and others 2001; Li and

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others 2002; Zhang and others 2003; Zhao and others 2003). Understanding the perception of sour taste has received less attention than sweetness and bitterness, particularly for mammals (Stewart and others 1997). Multiple mechanisms have been proposed to explain how hydrogen ions interact with taste receptor cells to cause a response (Herness and Gilbertson 1999; DeSimone and others 2001). Although it has been widely accepted that the hydrogen ion is the chemical entity responsible for sour taste, many physiological studies have indicated the involvement of protonated organic acids as a stimulus for sour taste as well. Of particular interest are recent studies with the chorda tympani nerve responses of intact rat tongue exposed to acids with pH adjusted from 3.0 to 7.0. Ogiso and others (2000) found that the response magnitude from hydrogen ions alone was significantly less than that of equi-pH acetic acid and concluded that protonated acids can stimulate taste receptor cells. Their results supported the suggestions of Gardner (1980) and Ganzevles and Kroeze (1987), based upon human psychophysical data, that protonated acids may enter the taste receptor cells. With use of fluorescent dyes, Lyall and others (2001) have measured the lowering of internal pH of single taste receptor cells exposed to organic acid solution and correlated this with a nerve response. Lyall and others (2001) also obtained evidence to indicate that free hydrogen ion and dissolved CO_2 have special mechanisms that affect sour taste perception separate from that of organic acids, further supporting the suggestion of Ganzevles and Kroeze (1987) that the sour taste of hydrogen ions and protonated acids are elicited by different and independent receptor processes. In addition to this evidence for an important role for the lowering of internal pH in sour taste transduction, there is also good evidence to support a role for acid-sensing ion channels (ASICs) for sour taste transduction in rat taste receptor cells (Lin and others 2002). However, elucidation of the complete mechanisms involved in sour taste transduction is complicated by wide species variability and potentially different mechanisms for strong and weak acids (DeSimone and others 2001).

During the evaluation of pickled cucumber products using mixtures of lactic and acetic acids, it was observed that products with the same pH and total molar acid concentration were more sour as the proportion of acetic acid in the mixtures increased (Johanningsmeier and others 2003). Subsequent analysis of the relationship between sour taste intensity and concentrations of the protonated forms of organic acids in pickle products, sauerkraut samples, and solutions of organic acids resulted in the formulation of a new sour taste hypothesis. Our hypothesis is that the intensity of sour taste perception in acid solutions or acidified foods is linearly related to the molar concentration of all organic acid species with at least 1 protonated carboxyl group plus the molar concentration of free hydrogen ions. The analysis of data that led us to propose this hypothesis is presented.

Methods

Cucumber pickle samples

Preparation, chemical analysis, and descriptive sensory analysis of fresh-pack cucumber pickles acidified with mixtures of vinegar (acetic acid) and filtered fermentation brine (lactic acid) is described by Johanningsmeier and others (2003). Sourness intensity, pH, and acid concentration data were further analyzed to look for possible correlations between sour taste perception and chemical composition.

Sauerkraut samples

Shredded cabbage (1240 g/jar) was fermented at 18 °C in 1380-mL jars by the natural microflora or inoculated with *Leuconostoc mesenteroides*, with 0.5% or 2.0% NaCl, dry salted or brined (60% cabbage/40% cover brine) resulting in sauerkraut samples with variable acid levels and ratios of lactic acid and acetic acids. Commercial canned sauerkraut was also evaluated. Acid concentrations were determined by high-performance liquid chromatography (McFeeters and others 1993). A trained descriptive panel of 12 individuals used 15-point category scales (0 = not detectable to 14 = very strong) for several flavor and basic taste attributes, including sour taste (Johanningsmeier and others, submitted 2004).

Published data sets

Two published data sets were found in which sensory sour taste intensity scores were determined for organic acid solutions along with sufficient information on the preparation and composition of the acid solutions so that the concentrations of the protonated acid species could be calculated. Hartwig and McDaniel (1995) used a free-choice profiling method and principal component analysis to evaluate sensory properties of solutions of malic acid, lactic acid, citric acid, and acetic acid at pH 3.5, 4.5, and 6.5 plus 2 lactic acid/acetic acid mixtures. Sowalsky and Noble (1998) used 10.2-cm line scales to rate sour taste and astringency intensities of a series of acid pair solutions including lactic/malic, lactic/tartaric, lactic/citric, malic/tartaric, malic/citric, and tartaric/citric. Binary acid solutions matched for both pH and titratable acidity were tested for each of the acid combinations.

Calculation of protonated acid species

Calculations of the concentrations of protonated acid species were done with $_{pH}$Tools, (http://www.mathworks.com/matlabcentral/fileexchange), a Matlab™-based application developed in this laboratory (Dougherty 2002). Published pK_a values for acids, generally given at 25 °C and zero ionic strength, were adjusted for the temperature and ionic strength. Adjustments of pK_a values can be calculated up to an ionic strength of 1.2 with a modification of the Davies equation developed by Samson and others (1999).

Results

Sourness of dill pickles with different proportions of lactic and acetic acids

Work in this laboratory to evaluate the flavor properties of lactic acid in pickle products showed that increasing the substitution of lactic acid for acetic acid reduced the sour taste of dill pickles (Johanningsmeier and others 2003). The sour taste intensities of the products, as determined by a trained descriptive sensory panel, did not correlate well with pH ($R^2 = 0.013$) or total molar acid concentration ($R^2 = 0.379$). The ability to calculate the concentrations of the protonated species of lactic acid, acetic acid, and malic acid in the pickled cucumbers with $_{pH}$Tools gave us the ability to relate concentrations of these species to sour taste intensity scores. Figure 1 shows that they were linearly correlated with an $R^2 = 0.825$ ($P < 0.0001$). This result led us to look in the literature for other data sets that could be analyzed in this way.

Analysis of the data of Hartwig and McDaniel (1995)

Hartwig and McDaniel (1995) used free-choice profiling to evaluate solutions of citric, malic, lactic, and acetic acids at pH 3.5, 4.5, and 6.5. These data were particularly interesting because, in contrast to our data on pickle samples, the triprotic acid, citric acid, along with diprotic malic acid were evaluated. A plot of the 1st principal component mean scores, characterized primarily by sour taste and overall intensity, as a function of total acid concentration gave a very poor correlation (Figure 2). Principal component 1 mean scores gave a good correlation ($R^2 = 0.784$, $P < 0.0001$) with the concentrations of fully protonated acid species in the solution (Figure 3). However, the linear relationship was further improved ($R^2 = 0.931$, $P < 0.0001$) by plotting the principal component 1 mean scores (sour taste and overall intensity) against the concentration of all acid species that had at least 1 protonated carboxyl group (Figure 4). For citric acid, this included the mono-, di- and tri-protonated forms of the acid. Because protons themselves give a sour taste response, the molar concentration of hydrogen ions in the solutions was added to the concentration of protonated acids in Figure 4. Due to the low concentration of free hydrogen ions relative to the concentration of partially and fully protonated acids, the addition of free hydrogen ions has a very minor effect on the relationship.

Hartwig and McDaniel (1995) analyzed results based upon equal weights of acids. They found that at all pH levels, acetic acid was the most sour and lactic and citric acids were the least sour. However, at equal weight the molar concentration of acetic acid is more than triple that of citric acid and more than double the concentration of lactic acid. The analysis in Figure 4 indicates that, on a molar basis, all protonated acids are about equally sour. This is consistent with the model proposed by Shallenberger (1996) and suggests that the structure of the acid would not be a major factor in determining the sour taste intensity. Also, at equal weights in solution, fully protonated citric acid would have about an equal number of protonated carboxyl groups in a solution as fully protonated acetic acid. The fact that it is perceived as less sour suggests the intensity of the sour taste perception is based on the number of protonated molecules, not on the protons per molecule.

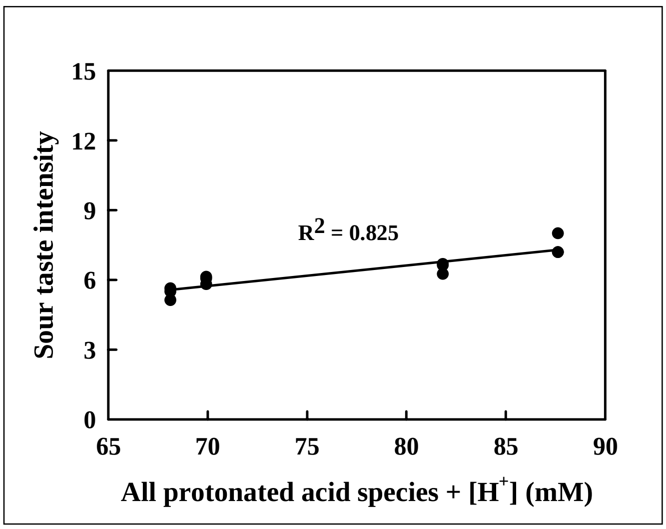

Figure 1—Relationship between sour taste intensity and the concentration of all protonated species of lactic acid, acetic acid, and malic acid in dill pickles

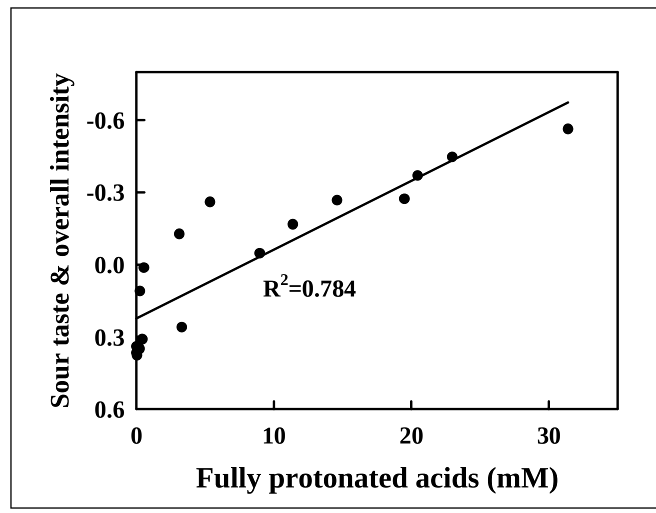

Figure 3—Relationship between sour taste and overall intensity (principal component 1) and the concentration of the fully protonated species of lactic acid, acetic acid, malic acid, and citric acid at pH 3.5, 4.5, and 6.5 (Hartwig and McDaniel 1995; negative values indicate high sour taste intensity)

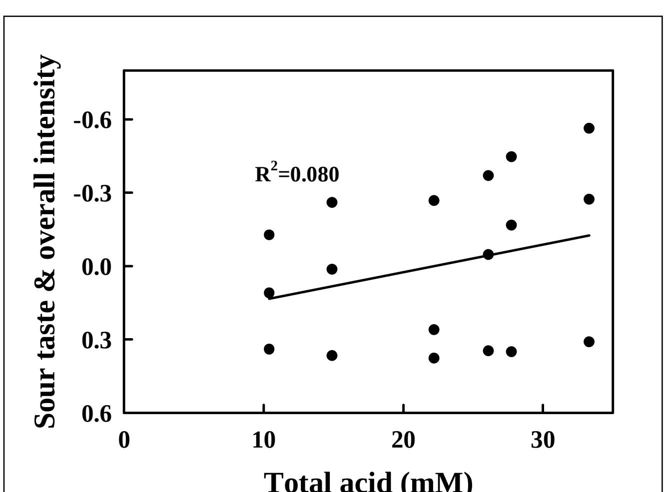

Figure 2—Relationship between sour taste and overall intensity (principal component 1) and the total concentration of all acid species in solutions of lactic acid, acetic acid, malic acid, and citric acid at pH 3.5, 4.5, and 6.5 (Hartwig and McDaniel 1995; negative values indicate high sour taste intensity).

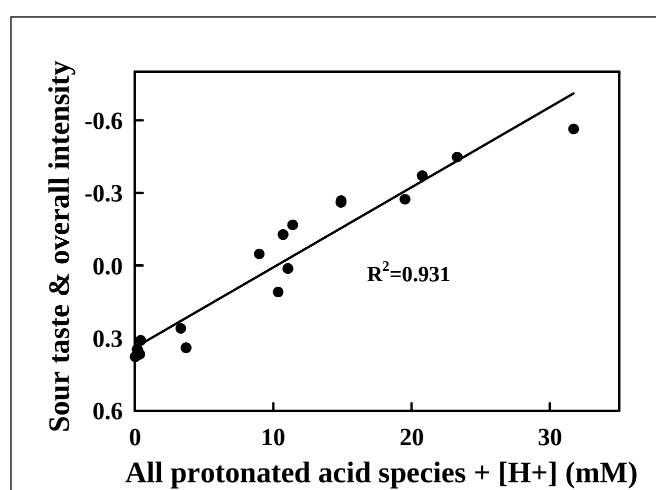

Figure 4—Relationship between sour taste and overall intensity (principal component 1) and the concentration of all acid species with at least 1 protonated carboxyl group plus the hydrogen ion concentration at pH 3.5, 4.5, and 6.5 (Hartwig and McDaniel 1995; negative values indicate high sour taste intensity)

Analysis of the data of Sowalsky and Noble (1998)

Acid pair solutions matched for pH and titratable acidity were evaluated for sour taste and astringency by Sowalsky and Noble (1998). Resulting data for solution composition and sour taste ratings was analyzed to calculate the total molar concentration of protonated acid species for each of the solutions. Again, there was a strong linear relationship ($R^2 = 0.811$, $P < 0.0001$) between the sour taste scores and the total concentration of all protonated acid species plus the hydrogen ion concentration (Figure 5). The sour taste range in these samples was narrow, so the coefficient of determination (R^2) was somewhat less than for the acid solutions prepared by Hartwig and McDaniel (1995).

Sourness of sauerkraut

As a secoond example of the application of this hypothesis for sour taste to a food, acid flavor intensity data, previously obtained by descriptive analysis of sauerkraut, were analyzed. The sauerkraut produced by varying the conditions of fermentation had a rather wide range of acid concentrations and sour taste intensities. Again there was a very high linear correlation ($R^2 = 0.912$, $P = 0.0002$) between sour taste perception and the concentration of all protonated acid species, including the concentration of hydrogen ion (Figure 6). Thus, we have 4 independent sets of sensory data that were created for objectives unrelated to the current hypothesis. Although different sensory techniques were used on different types of samples, all of them show a significant linear relationship between perceived sour taste and the molar concentrations of organic acids with at least 1 protonated carboxyl group. Although these examples do not prove the hypothesis, the correlations we see with both food samples and acid solutions lead us to conclude that this hypothesis deserves critical examination.

Proposed hypothesis testing

Mixtures of organic acids in solution will be used to vary the total protonated acid concentration while holding the pH and total acid concentration constant. This will be accomplished using several weak organic acids that have variable pKa's. It is important to hold both pH and molar acid concentration constant to properly test this hypothesis and avoid autocorrelations that may occur due to these factors. A trained descriptive sensory panel will use a modified Spectrum™ method for evaluating the acids in solution. Several sets of solutions at differing pH levels will be evaluated to test the relationship of sour taste to protonated acid concentration. $_{pH}$Tools software will be used to calculate concentrations of protonated acid species. The hypothesis will 1st be tested in aqueous solutions as described previously, and then experiments in an acidified food product will be conducted to test the hypothesis in a more complex matrix. Although sour taste is the attribute of interest, complete descriptive analysis of solutions and products will be obtained to account for other flavor attributes and interactions.

Summary

The analysis of sensory data on pickle and sauerkraut products, along with our analysis of published data on the sour taste of organic acid solutions, have led us to make the following proposal for the prediction of perceived sour taste intensity: Sour taste intensity is linearly related to the summation of the molar concentrations of organic acid species that contain at least 1 protonated carboxyl group plus the concentration of free hydrogen ions. This hypothesis has the advantage that it suggests a simple model to explain and predict sour taste intensity, provided the complex calculations required to properly estimate the concentrations of protonated acid species in complex chemical environments are done. The $_{pH}$Tools software provides the means to do these calculations. This hypothesis, if proven, would provide a basis to quantitatively predict how changes in acid types, acid concentrations, and pH would affect the intensity of sour taste. This would represent a considerable improvement over the current understanding of the ability of organic acids to elicit sour taste in foods.

This hypothesis can be considered at variance with the proposal of Shallenberger (1996) that sour taste is dependent upon the concentration of potential hydrogen ions in a solution. However, it is in agreement with Shallenberger's proposal that organic acids are equally sour. Given the apparent multiplicity of physiological mechanisms that may be involved in sour taste transduction, it is uncertain how this hypothesis might relate to current understanding of the physiology of sour taste perception.

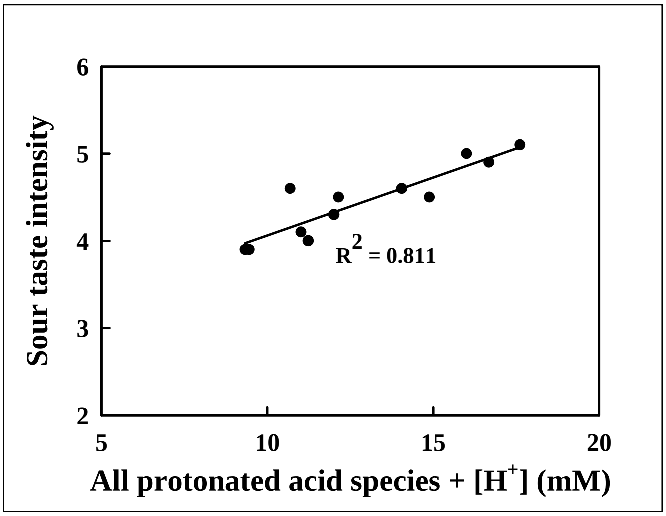

Figure 5—Relationship between sour taste and the concentration of all acid species with at least 1 protonated carboxyl group plus the hydrogen ion concentration (Sowalsky and Noble 1998)

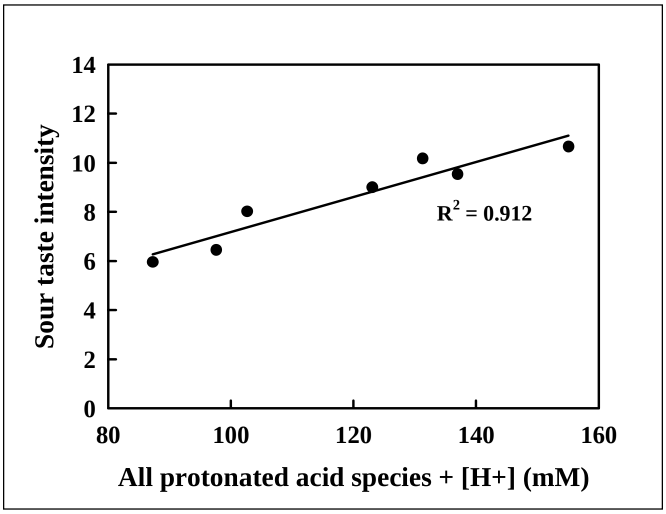

Figure 6—Relationship between sour taste intensity and the concentration of all protonated species of lactic acid, acetic acid, malic acid, and succinic acid in sauerkraut

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References

Bachmanov A, Beauchamp GK. 2002. Genetic dissection of sweet taste. American Chemical Society, Washington, D.C. In: 224th American Chemical Society Natl. Meeting; Boston; 2002 Aug 18–22. Abstr. nr 6.

Corrigan-Thomas CJ, Lawless HT. 1995. Astringent subqualities in acids. Chem Senses 20:593–600.

CoSeteng MY, McLellan MR, Downing DL. 1989. Influence of titratable acidity and pH on intensity of sourness of citric, malic, tartaric, lactic and acetic acid solutions and on the overall acceptability of imitation apple juice. Can Inst Food Sci Technol J 22:46–51.

DeSimone JA, Lyall V, Heck GL, Feldman GM. 2001. Acid detection by taste receptor cells. Resp Physiol 129:231–45.

Dougherty DP. 2002. Deterministic and semi-mechanistic approaches in predictive fermentation microbiology [DPhil dissertation]. Raleigh, N.C.: N.C. State Univ.

Ganzevles PGJ, Kroeze JHA. 1987. The sour taste of acids. The hydrogen ion and the undissociated acid as sour agents. Chem Senses 12:563–76.

Gardner RJ. 1980. Lipid solubility and the sourness of acids: implications for models of the acid taste receptor. Chem Senses Flav 5:185–94.

Hartwig P, McDaniel MR. 1995. Flavor characteristics of lactic, malic, citric and acetic acids at various pH levels. J Food Sci 60(2):384–8.

Harvey RB. 1920. The relation between the total acidity, the concentration of the hydrogen ion, and the taste of acid solutions. J Am Chem Soc 42:712–4.

Herness MS, Gilbertson TA. 1999. Cellular mechanisms of taste transduction. Ann Rev Physiol 61:873–900.

Johanningsmeier SD, Fleming HP, Thompson RL, McFeeters RF. Chemical and sensory properties of sauerkraut produced with *Leuconostoc mesenteroides* starter cultures of differing malolactic phenotypes. Submitted 2004.

Johanningsmeier SD, Thompson RL, Fleming, HP. 2003. Bag-in-box technology: sensory quality of pickles produced from process-ready, fermented cucumbers. Pickle Pak Sci 8(1):26–33.

Lawless HT, Horne J, Giasi P. 1996. Astringency of organic acids is related to pH. Chem Senses 21:397–403.

Li X, Inoue M, Reed DR, Huque T, Puchalski RB, Tordoff MG, Ninomiya Y, Beauchamp GK, Bachmanov AA. 2001. High resolution genetic mapping of the saccharin preference locus (Sac) and putative sweet taste receptor (T1R1) gene (Gpr70) to mouse distal chromosome 4. Mam Gen 12:13–6.

Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E. 2002. Human receptors for sweet and umami taste. Proc Nat Acad Sci USA 99:4692–6.

Lin W, Ogura T, Kinnamon SC. 2002. Acid-activated cation currents in rat vallate taste receptor cells. J Neurophysiol 88:133–41.

Lyall V, Rammy IA, Phan DQ, Ereso GL, Phan THT, Malik SA, Montrose MH, Chu S, Heck GL, Feldman GM, DeSimone JA. 2001. Decrease in rat taste receptor cell intracellular pH is the proximate stimulus in sour taste transduction. Am J Physiol Cell Physiol 281:C1005–13.

Margolskee RF. 2002. Molecular mechanisms of better and sweet taste transduction. J Biol Chem 277:1–4.

McFeeters RF. 1993. Single-injection HPLC analysis of acids, sugars, and alcohols in cucumber fermentations. J Agric Food Chem 41(9):1439–43.

Montmayeur JP, Liberles SD, Matsunami H, Buck LB. 2001. A candidate taste receptor gene near a sweet taste locus. Nat Neurosci 4:492–8.

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS. 2001. Mammalian sweet taste receptors. Cell 106(3):381–90.

Noble AC, Philbrick KC, Boulton RB. 1986. Comparison of sourness of organic acid anions at equal pH and equal titratable acidity. J Sens Stud 1:1–8.

Ogiso K, Shimizu Y, Watanabe K, Tonosaki K. 2000. Possible involvement of undissociated acid molecules in the acid response of the chorda tympani nerve of the rat. J Neurophysiol 83:2776–9.

Pangborn RM. 1963. Relative taste intensities of selected sugars and organic acids. J Food Sci 28:726–33.

Plane RA, Mattick LR, LaVerne DW. 1980. An acidity index for the taste of wines. Am J Enol Vitic 31:265–8.

Richter TA, Caicedo A, Roper SD. 2003. Sour taste stimuli evoke Ca^{2+} and pH responses in mouse taste cells. Journal of Physiology 547(2):475–83.

Rubico SM, McDaniel MR. 1992. Sensory evaluation of acids by free-choice profiling. Chem Sen 17:273–89.

Ruiz AL, Wong GT, Damak S, Margolskee RF. 2001. Dominant loss of responsiveness to sweet and bitter compounds caused by a single mutation in alpha-gusducin. Proc Nat Acad Sci USA 98:8868–73.

Samson E, Lemaire G, Marchand J, Beaudoin J. 1999. Modeling chemical activity effects in strong ionic solutions. Comp Mater Sci 15:285–94.

Shallenberger RS. 1996. The AH,B glycophore and general taste chemistry. Food Chem 56:209–14.

Sowalsky RA, Noble AC. 1998. Comparison of the effects of concentration, pH and anion species on astringency and sourness of organic acids. Chem Sen 23:343–9.

Stewart RE, DeSimone JA, Hill DL. 1997. New perspectives in gustatory physiology: transduction, development, and plasticity. Am J Physiol Cell Physiol 272:C1–C26.

Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba JP. 2003. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112(3):293–301.

Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJP, Zuker CS. 2003. The receptors for mammalian sweet and umami taste. Cell 115(3):255–66.