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Addition of acids to foods allows for enhanced food safety. Acids are the primary form of defense against microbial contamination in refrigerated foods, while use of acids in conjunction with heat or high hydrostatic pressure processing lowers energy usage resulting in cost reduction. However, addition of acids to food or beverage formulations often reduces palatability due to higher sourness and this has limited the food industry's ability to better utilize them as preservatives. This study was aimed at gaining a better understanding of sourness suppression and its underlying mechanisms so that such limitations might be ultimately overcome.

This work was divided into three parts dealing with the suppression of the sourness of citric, lactic and malic acids, as perceived by a trained sensory panel in a) binary mixtures with sugars, b) binary mixtures with salts and c) tertiary mixtures. The results of the first part showed that suppression was not mediated by sugar molarity or weight, but was significantly influenced by its perceived

sweetness intensity in most cases. Sucrose and fructose were more effective than glucose in suppressing acid sourness and the data supported a separate receptor site/mechanism for glucose. Suppression was thought to have both central and peripheral components.

In binary acid-salt mixtures sodium acetate (NaAc) affected the most sourness reduction, along with the largest concurrent pH increase (above 4.4). Sodium chloride (NaCl) mixtures showed significant suppression without a pH increase. Sodium gluconate (NaGluc) mixtures showed moderate suppression with citric and malic acids with pH increases remaining below 4.4, but showed little effect on lactic acid sourness. Saltiness appeared to drive suppression only in the case of NaCl, while pH change was responsible for reduction of sourness with NaAc and NaGluc.

The tertiary trials indicated that a two-component multiple masker was more effective when its components stimulated different (as opposed to similar) receptors/receptor mechanisms in the taste system, irrespective of taste quality. Furthermore, a two-component masker was more effective than each component alone, and both components of a two-component masker did not have to be effective individually for them to function together as an effective multiple masker.

Suppression of Sourness in Binary and Tertiary Model Mixture Solutions

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Lotika Savant, Author

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I INTRODUCTION

One of the simplest ways to preserve food is to lower its pH. For heat processed foods, the lower the pH, the lower the temperature or lesser the time of heat treatment necessary for safe preservation. Thus the addition of acid allows for reduced energy use. Acid addition is employed as a processing aide even in the case of products like canned tomatoes with a naturally high acid content, in order to lower energy consumption and better preserve sensory quality due to reduced heating. With the popularity of refrigerated foods, a low pH is critical for good shelf life. Even ultra high pressure processing as a method of food preservation has only been approved for high acid foods until now. This is because while the high pressure destroys bacteria, it leaves spores untouched.

Thus acids have the ability to serve as cost-effective preservatives. Yet they have been underutilized in this capacity until now. This is mainly because the degree of acidity tolerable in foods from the standpoint of palatability has not been sufficient to ensure food sterility (Potter, 1986). Addition of acid results in a safer product, but one that is more sour and consequently less appropriate for use in food applications. And it is the sensory characteristics of food that ultimately dictate consumer choice, therefore masking of sourness is of critical interest to the food industry.

Just as the old adage - 'a spoonful of sugar makes the medicine go down' – epitomizes the use of sucrose as an aid to palatability of bitter medicine (Nicol, 1982), it is well known that the addition of sugar makes lemonade less sour. However, while bitterness and bitterness suppression have received a lot of attention in recent years, there has been little systematic work focussing on sourness suppression and its underlying mechanisms. Consequently the present research was aimed at developing knowledge and understanding in this area. The research was divided into the following three parts:

Part 1

- Suppression of sourness by selected sugars in binary mixtures with citric, lactic and malic acids, to determine the role of molarity, weight and perceived sweetness of the sugars on sourness suppression.

Part 2

- Suppression of sourness by selected salts in binary mixtures with citric, lactic and malic acids, to determine the role of molarity, weight and perceived saltiness of the salts on sourness suppression.

Part 3

- Suppression of sourness in tertiary mixtures using sugar-sugar versus sugar-salt combinations as the suppressors in order to assess the impact of taste quality on sourness suppression.

II LITERATURE REVIEW

The Acids

Food acids are a multi-functional group of ingredients. They are used for everything from sterilization and preservation to flavoring and chelating. Virtually all groups of formulated food products utilize acids. Acids obtained from fruit sources are represented in products such as jams, jellies, sodas, confectionery products, ciders, preserved fruits and vegetables. Food groups corresponding to acids produced by fermentation include dairy products, breads, sauerkraut, vinegar, olives and pickles, some types of sausages and other fish and meat products.

With respect to food preservation, acids are often employed in food processing on account of their fungicidal or bactericidal properties. In general, the mode of action of these acids is attributed to direct pH reduction, where depression of the internal pH of microbial cells occurs by ionization of the undissociated acid molecule in the cell interior. The pH reduction of a food by an acid causes an extension in the lag phase of acid sensitive microorganisms, eventually resulting in their death (Smulders et al., 1986). Alternatively, cell death could result from a disruption of substrate transport by alteration of membrane permeability due to expulsion of hydrogen ions from the cell (Green, 1999; Nykanen et al., 1998). Acids may also cause cell death by eliminating supplies of reducing agents to cellular electron transport systems (Green, 1999).

Some of the physical and chemical properties of the acids utilized in this work are listed in Table 2.1

Table 2.1 Properties of Selected Acids

Acid	Empirical Formula	Formula Weight	Ionization Constant
Citric	$C_6H_8O_7$	192.12	8.2×10^{-4} a
Lactic	$C_3H_6O_3$	90.08	1.4×10^{-4} b
Malic	$C_4H_6O_5$	134.09	4.0×10^{-4} a

a = Gardner, 1966; b = Arnold, 1975

Citric Acid

Citric acid is widely distributed in nature and is an important fruit acid as well as an important metabolite in the Kreb's cycle. It is a versatile acidulant with many food and non-food applications. Commercial citric acid production is largely through aerobic fermentation of a carbohydrate substrate like beet molasses, with a selected strain of mold such as *Aspergillus niger* (Arnold, 1975).

Its secondary property of heavy metal sequestering, gives citric acid great value as an antioxidant synergist and as an inhibitor of flavor and color deterioration in foods. As a result it has found application in patents such as formulations for prevention of color loss in foods (Obata et al., 1973), which can be applied to the food before or after processing. Citric acid is a permitted color preservative in cured pork products during storage, where a solution consisting of ≤ 30% citric acid in water can be sprayed on cured pork cuts prior to packaging (Federal Register, 1993a). Tocopherols and citric acid are also used in meat food products together, with one serving as the antioxidant and the other as an antioxidant synergist (Federal Register, 1993b). The addition of citric acid to canned liver paste alone or in combination with citrate has been shown to deliver a stable and safe product at reduced intensities of thermal processes due to the acid addition (Houben and Krol, 1991). Citric acid has also been utilized in patented acid-stabilized soup formulations as a food preservative (Hazell, 2000). The acid has also found application in solutions that serve as sweetening compositions for use in beverage mixes or in dry foods by encapsulation (Shoaf and Pischke, 1976).

Non-food patents using citric acid as an ingredient include a wash for fresh produce to increase its shelf life (Green, 1999), where the acid is a microbicide, another bactericidal wash for sterilization of food, food materials and food-processing applications (Katsuda et al., 2000), an ingredient in an animal feed composition (Ralston Purina Co., 1971), where it is a flavor adjunct and an ointment adhesive to the oral cavity (Tomoaki, 1994), where it serves as a stabilizer. A mixture of citric acid with zinc and magnesium silicate has even found use in an acid reduction promoter for fruits (Tomoaki and Masahiro, 1996).

Lactic Acid

The name lactic acid comes from the Latin word for milk since it is the acid formed in the souring of milk. Although lactic acid can be synthesized via lactonitrile, most world production is supplied by anaerobic fermentation, as is the case with citric acid. Lactic acid is the simplest hydroxy acid having an asymmetric carbon atom, and therefore exists in two optically active forms (Holten et al., 1971). The L (+)-enantiomer of lactic acid is the only one formed by metabolism in humans and animals (Van Velthuijsen, 1996).

Food applications of lactic acid can be found in baked goods, beverages, confectionery items such as high-boiled sweets, dairy products including cottage cheese, imitation mozzarella and butter, flavors and fragrances including marinade type products, preserves and sauces and even animal feed. Another valuable application of the acid is in the area of meat and fish products where it is used in sausage casings and even as a decontaminant in slaughtering lines. Lactic acid combined with sodium chloride or other ingredients such as nisin, has been found to be effective in suppressing aerobic bacteria in minced rainbow trout (Nykanen et al., 1998). Studies have also shown that at appropriate concentrations and at a suitable pH, the use of lactic acid allows effective decontamination of meat surfaces (Smulders et al., 1986). In conjunction with potassium sorbate, lactic acid has been shown to extend the shelf life of both refrigerated and vacuum-packed chicken meats (Kolsarici and Candogan, 1995). The application of lactic acid to such products is best in the form of spraying or soaking, since direct mixing into products like emulsion sausages leads to the formation of unstable emulsions (Sanguandeekul et al., 1998). The acid is also used in brines for semi-bulk storage of vegetables (Van Velthuijsen, 1996).

As an acidulant lactic acid is often used in combination with other acids as in the case of vinegar where the addition of lactic acid to acetic yields a milder and a more subtle taste. The use of lactic acid is also common in fruit-flavored beverages such as carbonated fruit juices, or products like jams, gelatins, marmalades, frozen fruit deserts etc. (Holten et al., 1971).

Non-food applications include pharmaceutical, cosmetic and industrial applications in cleaning and degreasing surfaces, as a solvent for coatings and ink, in the dyeing of textiles, etc. (Holten et al., 1971; Van Velthuijsen, 1996).

Malic Acid

Malic acid is found in various fruits and vegetables. It represents 100% of the acid found in watermelon and quince, 99% in plums, 97% in apples, 96% in cherries and peaches, 92% in bananas, 87% in pears and 80% in orange peel (Andres, 1985). It is the predominant acid in broccoli, carrots, peas, potatoes and rhubarb (Gardener, 1972). Unlike citric and lactic acids, malic acid is commercially produced by a simple chemical process from maleic anhydride by heating it with water under pressure at around 180°C (Arnold, 1975).

This highly soluble, general-purpose acidulant is used as a flavoring agent and a pH control agent (Doores, 1990). It has found application in many types of food products including sugar confectionery, soft drinks, powdered drinks, cordials, fruit squashes, jams, jellies, mousse, fruit pie fillings and frozen desert wines. As a processing aide it is used in canned products, biscuits and pet foods (Reid, 1972; Dziezak, 1990). The acid has also found use in wines and ciders, sherbets and some oils and fats (Andres, 1985). A patented beverage with post-exercise fatigue-relieving properties (Horio and Sakata, 2000) utilizes malic or citric acids with sucrose. Malic and citric acids have both also been used as components of a patented high-protein food preservative (Yoshimatsu and Okuno, 2000).

Non-food related uses of the acid include a patent for a liquid plant protecting concentrate, where a mixture of malic acid with glycine, zinc sulphate, carbonic acid and tween-80 in a spray form act as a fruit-set stimulator and fertilizer (Szarvas et al., 1987).

The Taste of Acids

The taste of acids has been studied using descriptive analysis and free-choice profiling methodologies. Such work has shown that citric, lactic and malic acids share the sensory characteristic of sourness with several other acids, but they differ in their degree of sourness and other non-sour characteristics. Lactic acid was found to have less of a salty component and be significantly more astringent than citric and malic acids (Hartwig and McDaniel, 1995). Rubico and McDaniel (1992) however, found the acids to group together with respect to astringency. Straub (1992) found the fruit acids citric, malic and tartaric to be similar in all sourness time-intensity parameters.

These studies showed that acids differ considerably at equivalent pH and % w/v and therefore cannot always be used interchangeably in food formulations. Since acids have a significant effect on food flavor, therefore acid selection by the food technologist is of critical importance in order to achieve the desired food characteristics.

The Sugars

Nutritive or carbohydrate-based sweeteners are ubiquitous in most bakery and other grain-based products, confections, jams, jellies, preserves, canned fruits and vegetables, various types of beverages, dairy based foods such as ice-creams and frozen deserts and even some soups, gravies and preserved meats. Sweeteners perform several functions in foods of which the most obvious is to provide sweetness. Sucrose, glucose, fructose and high fructose corn syrup (HFCS) are mainly used to increase the sweet flavor in a product while other sweeteners such as lower dextrose equivalent (DE) corn syrups, may be used for other functions, e.g., to increase browning or add solids to low-fat foods while contributing minimal sweetness. Other sweetener functions include development of hard, soft, chewy or other texture characters in confections and regulation of freezing point in dairy foods (Alexander, 1998).

When choosing a natural sweetener for a food formulation, the product developer must consider at least three aspects. The first of these includes the flavor contribution of the sweetener itself, above and beyond the differences in sweetness. Secondly, there is the flavor contribution of impurities, i.e. substances other than the pure sweetener, e.g. the 'treacly' flavor of pre-refinery sucrose. The third factor to be considered is the sensation of the physical character of the sweetener on the palate, e.g. the 'cooling' sensation associated with lactose. Additionally, knowledge of the metabolic differences between the natural sweeteners is essential to sweetener selection (Green, 1971).

Some properties of the selected sugars are listed in Table 2.2.

Table 2.2 Properties of Selected Sugars

Sugar	**Empirical Formula**	**Formula Weight**	**Solubility in ml H_2O to dissolve 1g sugar ***
Glucose	$C_6H_{12}O_6$	180.2	0.50
Fructose	$C_6H_{12}O_6$	180.2	0.25
Sucrose	$C_{12}H_{10}O_6$	342.3	0.50

* Shallenberger, 1993

Sucrose

The disaccharide sucrose, common table sugar, is the sugar derived from sugar cane or sugar beets. Juice extracted from the crop plant is purified and concentrated to syrup, from which sucrose is crystallized and separated. The residual, dark, syrupy material is molasses or treacle. White sugars are crystallized from the lighter colored syrups while brown sugars are formed from the darker syrups. Hydrolysis of sucrose by acids or enzymes yields invert sugar, which comprises the two monosaccharides glucose and fructose. Invert syrups could be used in beverages, canning, baking and confectionery (Clarke, 1995). Sucrose products manufactured and sold in the United States generally fall into the categories of granulated sugar, liquid sugar, brown sugars, and specialty sugars including sanding sugars, colored sugars, powdered sugars, gel grain, baker's special and others (Alexander, 1998).

Sucrose is the most common sweetener in candy making. At room temperature sucrose can be dissolved in water, giving a concentrated sugar solution of about 67%. Additional sucrose can be dissolved by raising the temperature of water, and the higher the sucrose concentration, the higher the boiling point of such solutions. Candy-makers take advantage of this relationship between boiling point and sucrose concentration to control the water content in confections (Potter and Hotchkiss, 1995).

Sugar concentration also affects freezing point depression, which is an important property in ice creams, frozen deserts, sauces and even in freeze-dried foods. Water activity is also affected by sugar concentration. The high osmotic pressure generated by sucrose in solution is an important factor in food preservation and prevention of microbial contamination, since high sugar concentrations decrease the water activity and lower the equilibrium relative humidity, leaving insufficient water available to sustain viable microorganisms. These and other properties of sucrose find applications in products ranging from breads, cakes, cookies and candy to beverages, meat and dairy products, ready-to-eat breakfast cereals and even frozen and canned vegetables (Clarke, 1995).

Fructose

Fructose or levulose is a hexose monosaccharide that is commonly found in many fruits and therefore referred to as a fruit sugar (Vacklavik and Christian, 1998). Commercially, crystalline fructose is produced from sucrose by inversion or from glucose by enzymatic isomerization. One of the main advantages of the use of crystalline fructose over sucrose is its higher relative sweetness on a weight basis, though this manifests itself depending on the tasting medium and product temperature (Hyvonen and Koivistoinen, 1982). Fructose is also more slowly absorbed than sucrose and does not increase serum triglycerides in normal subjects. Therefore it has been recommended as a sweetener for diabetics. Preliminary results of clinical dental trials also show fructose performs better than sucrose with respect to cariogenicity (Salminen and Hallikainen, 1990).

Fructose being a reducing sugar differs from the non-reducing sucrose in many of its chemical and physical properties. High solubility, hygroscopicity, sensitivity to degradation during heating and strong browning in Maillard type reactions are all properties of fructose of great importance in food systems. In cakes, biscuits and other bakery products for example, the high reactivity of fructose to the Maillard reaction necessitates lower baking temperatures or shorter baking times compared to products using sucrose (Hyvonen and Koivistoinen, 1982).

Crystalline fructose has been commercially available only since the 1980s and is therefore not so widely applied in all parts of the food industry. However its use is steadily increasing due to its application in diabetic and other special dietary foods (Salminen and Hallikainen, 1990).

Glucose

D-Glucose or dextrose is found in large quantities in grapes and hence the old-fashioned name 'grape sugar' (Green, 1971). It is by far the most widely distributed sugar in nature. It is the building block for all starch and cellulose and occurs in the sap of most plants and in the juice of several fruits. Glucose is a normal component of animal blood; it thus requires no digestion prior to absorption into the bloodstream. This most commonly occurring sugar unit is the most basic and valuable source of energy in animal metabolism (Palmer, 1971).

Glucose is commercially produced by the complete hydrolysis of starch by both acids and enzymes α-amylase and glucoamylase and the corn syrup thus obtained also contains some dextrins and maltose. The hydrolysate is further purified and then crystallized to yield dextrose monohydrate, which yields anhydrous dextrose upon further drying. The starch source for glucose production could be rice, wheat, oats or potatoes, but in the United States it is primarily cornstarch due to its relative abundance and low cost (Alexander, 1998).

Crystalline glucose is used in the manufacture of candy, chewing gum, jams, jellies, table syrups, and other foods. However, glucose syrups have found many more applications in foods. These syrups are purified concentrated aqueous solutions of nutritive saccharides, with dextrose equivalent (DE) values of 20 or more, where DE is a measure of the extent of starch hydrolysis, e.g. DE for dextrose is 100, representing 100% hydrolysis. Glucose syrups have a pleasant, sweet taste where the degree of sweetness depends on the concentration and the type of saccharides (Palmer, 1971; Alexander, 1998).

The Taste of the Sugars

The sweeteners glucose, fructose and sucrose differ with respect to their perceived sweetness at equi-weight levels. Using sucrose as a reference standard with a relative sweetness of 100, on an equi-weight basis glucose has been found to be about 64% as sweet as sucrose, while fructose is 120% as sweet (Shallenberger, 1993). With respect to side tastes, crystalline glucose at higher concentrations has been observed to have an additional taste that has been described as a burning or bitter (Alexander, 1998). This is possibly due to residual acid used in the final conversion of starch dextrins to glucose (Shallenberger, 1993).

Psychophysical studies attempting to describe the taste of sugars conducted in the seventies found sugars in solution to vary widely with respect to intensities of sweetness and bitterness, syrupy texture and aftertaste (Moskowitz, 1972; Schiffman et al., 1979), suggesting that sweet taste might be mediated by several peripheral receptor mechanisms (Schiffman et al., 1979). Further work conducted a decade later similarly found sweeteners to have other sensory characteristics besides sweetness that were important in determining their acceptability (Thomson and Tunaley, 1987; Thomson et al., 1987). Sucrose was described as being the sweetener with the most pure and clean sweetness and few perceived secondary attributes, such that no other sweetener was similar to it (Larson-Powers and Pangborn, 1978; Redlinger and Setser, 1987; Tunaley, 1989).

Table 2.3 Properties of Selected Salts

Salt	Empirical Formula	Formula weight	Appearance
Sodium Chloride	NaCl	58.44	White crystals
Sodium Acetate	$NaC_2H_3O_2^-$	82.03	White to gray granules
Sodium Gluconate	$NaC_6H_{11}O_7$	218.14	White to light amber crystals

The Salts

Salts are a class of compounds formed by the replacement of one or more hydrogen atoms of an acid with elements or groups, which are composed of anions and cations, which usually ionize in solution. They are also described as the product formed upon the neutralization of an acid by a base. Salts have several thousand known uses with the greatest single use being as feedstock for the production of chemicals (USITC, 1981). The importance of salts to mankind is evidenced by the fact that in the literature salt has been referred to as the fifth element, as vital as air, water, fire and earth (Dickinson, 1980).

Some properties of the selected salts are listed in Table 2.3.

Sodium Chloride

Sodium chloride (NaCl), the compound that constitutes table salt, is sold in several different particle sizes and forms. These include discrete crystals from rock salt used for deicing, fine granules for table salt and even finer popcorn salt, while kosher salt, pickling salt and ice cream salt are slightly coarser. Small compressed pellets are used in water softeners and large salt blocks are used as salt licks for livestock (USITC, 1981).

Three principal methods of salt production are employed in the United States. Salt mining involves sinking of a shaft to depths of 600 to 2000 feet below the earth's surface from where salt is hoisted onto conveyer belts and transported to the surface. Brine well operations involve the use of one pipe to introduce water into a salt deposit and a larger pipe around the first one to remove the brine. Lastly, solar salt is produced through a process of solar evaporation of water from natural brines such as seawater (Dickinson, 1980).

All animals, humans included, require both sodium and chloride for life and health. Since the body cannot manufacture either, they are 'essential' nutrients. While developed countries dedicate most of their NaCl to chemical production, developing countries often use most of theirs for human and animal nutrition. The addition of iodine to table salt has long been used as a preventive measure against certain kinds of goiter. Food grade NaCl is the most familiar to the general public, and besides being used at the table it is used in food processing, baking, the manufacture of cheese, the curing of meat etc. NaCl is the most common flavoring ingredient added to savory snacks and several sweet snacks as well. In many foods it is the predominant flavor note, also offering a convenient vehicle for uniform distribution of micro-ingredients such as flavors, vitamins or anti-oxidants throughout the finished product. NaCl is often applied as a topping or coating so that its taste impact is immediate and predominant (Matz, 1993).

Patents using NaCl include green vegetable pickling compositions (Kitamura, 1991), compositions for sterilizing food poisoning microorganisms (Koga and Ohashi, 1999) and even pharmaceutical formulations to compensate for electrolyte loss (Gergely et al., 1996). NaCl has also found utilization as a taste-masking agent along with organic acids, in formulations containing glucosamines, which are useful for prevention and treatment of osteoarthritis (Saito and Matahira, 2000).

Sodium Acetate

Sodium acetate (NaAcetate) is the sodium salt of acetic acid and is obtained by chemical synthesis. In food it is used as a thickening agent for cake batters, puddings, pie fillings and in buffers for controlling the pH of food during various stages of processing, as well as in the finished product as a preservative to prevent microbial growth.

A lot of research has focused on NaAcetate's preservative function in particular. In a study of the influence of certain preservatives on the interactions between various toxigenic fungi and mycotoxin production, NaAcetate was found to be inhibitory to the production of citrinin (Reddy et al., 1997). NaAcetate in conjunction with lactic acid producing bifidobacteria has been found to extend the shelf life of refrigerated catfish fillets (Kim et al., 1995). Another study on extending the shelf life of fresh camel meat with respect to the microbial and sensory quality, found that NaAcetate alone or combined with bifidobacteria exhibited an additive effect on suppression of spoilage microorganisms (Al-Sheddy et al., 1999).

Besides all the above applications, the use of NaAcetate as a flavor enhancer is also being explored and the Food Safety and Inspection Service (FSIS) is amending the Federal meat and poultry products inspection regulations to increase permissible levels of the salt as a flavor enhancer in meat and poultry products (Federal Register, 2000).

Sodium Gluconate

Sodium gluconate (NaGluconate) is the sodium salt of gluconic acid (penta-hydroxycaproic or hexanoic acid), obtained from glucose by fermentation. It forms stable complexes with calcium, aluminum, iron, zinc and other heavy metals, especially in alkaline solutions. It possesses good complexing activity in cleaning baths and is highly stable, even in concentrated alkaline solutions.

NaGluconate is physiologically compatible and non-toxic. It is readily soluble in water and yet not hygroscopic. Aqueous solutions of the salt are resistant to oxidation and reduction, even at high temperatures. NaGluconate is biodegradable and hence presents no wastewater problem. It functions as a chelating and sequestering agent in alkaline solutions, a retardant and a plasticizer. It has found application in the pharmaceutical arena in injections, in personal care products like toothpaste and as a cleaner in bottle washes. In the Food Chemicals Codex (1981) the functional food uses of NaGluconate are listed as nutrient, dietary supplement and sequestrant.

A few studies in the last decade have also explored the use of NaGluconate as a preservative. In a study on the development of microbial populations on fillets of fresh gilt-head seabream, it was found that a combination of sorbate and NaGluconate was a more effective preservative as compared to sorbate alone (Drosinos et al., 1997). A study on the microbiological safety of beef roasts investigated the potential of NaGluconate and other preservatives as inhibitors for use in microwave-ready products. It hypothesized that NaGluconate could be an effective anti-microbial compound since it bore a close molecular resemblance to sodium lactate, which has been demonstrated to possess anti-microbial properties (Stillmunkes et al., 1993).

The Taste of the Salts

NaCl, NaAcetate and NaGluconate are all sodium salts, but are quite different with respect to their saltiness. For example, at a concentration of 0.5M, NaCl has been reported to be roughly twice as salty as NaAcetate and three or four times as salty as NaGluconate (Breslin and Beauchamp, 1995; Bartoshuk, 1980). NaCl is considered the prototypically salty stimulus, while organic Na^+ salts like NaAcetate and NaGluconate are mostly salty and sour (van der Klaauw and Smith, 1995; Schiffman, 1980). Additionally, NaCl has been documented as having a sweet side taste at low concentrations (Bartoshuk et al., 1978).

The Sensation of Taste

The relation of taste to food intake is manifest to all who have observed animals eating or have reflected on their own eating habits. Sequential steps in feeding may be divided into appetitive and consummatory phases. Of these phases the former involves hunger arousal, foraging and food recognition, where taste plays little or no direct role. Consummatory activity begins with patterns that place food in the oral cavity (biting, sucking, rasping, handling, etc.), is followed by passing food into the buccal cavity or pharynx, and ceases with satiation. The sensation of taste or gustation is actively concerned with initiating and maintaining these processes. Termination occurs as a result of postingestive events in regions of the digestive tract, homeostatic conditions and neurotransmitter release. However, dimensions of complexity are added to feeding behavior in mammals on account of highly evolved neural and neurohormonal refinements and by a greatly enhanced capacity for learning. In the words of Dethier (1993), "...what began evolutionarily as a limited chemosensitivity associated with feeding, has become a refined physiological capability, and an instrument of individualistic and social activity enriched by hedonic reward and tradition".

Organization of Gustation at the Peripheral and Central Neural Level

Taste buds are peripheral gustatory organs located in tiny pits and grooves of the mouth, throat, pharynx, inside of the cheeks, soft palate and particularly the dorsal surface, or the back of the tongue (Reuter and Witt, 1993; Schiffman, 1996). Humans possess around 9,000-10,000 taste buds that are generally found in clusters lying within small elevations on the tongue called papillae. Depending on the shape and the location, four types have been identified - fungiform, foliate, circumvallate and filiform (Schiffman, 1996).

In vertebrates the presence of 'taste' buds in the oral cavity and especially on the tongue, was always taken as presumptive evidence of the ability to taste, and taste buds came to be associated with eating, food selection and palatability. However, discovery of the different functions performed by taste buds in different papillae and different parts of the oropharyngeal cavity, has led to a broader understanding of their role. For example, they are involved in visceral activities like salivation, chewing, sucking, swallowing, preabsorptive insulin release, and such protective reflexes as gagging, coughing, spitting, regurgitating and reflex apnea. Other responses (e.g. in the rat) that could be triggered by them include oral gapes and somatic motor sequences such as head shaking, chin rubbing, face washing, fluid rejection and locomotion (Dethier, 1993).

The cells in the vertebrate taste bud are continually being born, maturing, performing their gustatory functions, aging and dying. This entire cycle lasts from about 10 days to 2 weeks (Kinnamon, 1987). 55 to 75% of the taste bud cells include type I or dark cells, 15 to 30% include type II or light cells, while only 5 to 15% comprise type III cells. Type IV or basal cells are found in the basal region of the mammalian taste bud and are considered to be the stem or precursor cells to the other cells of the taste bud. Marginal cells are less specialized epithelial cells, closely associated with taste bud cells. The apical endings of type I and III cells project into the taste pit of the taste bud and only the blunt ending structures of the type III cells may penetrate the taste bud pore to be in contact with the oral cavity (Reuter and Witt, 1993).

Taste buds are associated with two classes of sensory fibers - the intragemmal and the perigemmal. The intragemmal fibers penetrate the basal lamina at the base of the taste bud and form the post-synapitc elements of synapses with chemosensory cells. The perigemmal fibers on the other hand, penetrate the basal lamina near taste buds and surround the buds en route to the epithelial surface. The sensory axons of the intragemmal fibers come from the facial, glossopharyngeal, or the vagal cranial nerves (Kinnamon, 1987). Axons of the chorda tympani, a part of the facial nerve, innervate taste buds of the fungiform papillae. The glossopharyngeal nerve bilaterally innervates circumvallate and foliate papillae, while the vagus nerve innervates taste buds of the larynx (Reuter and Witt, 1993). The trigeminal nerve fibers constitute the perigemmal fibers that serve as free nerve endings, to relay thermal, tactile, and common chemical sense information to the brain (Herness and Gilbertson, 1999).

While the precise neural pathways taken by the taste nerve fibers to the brain are not well established, it is known however that they travel from the mouth to the thalamus and from there to several cortical regions, mainly to the base of the somatosensory cortex of the parietal lobe (Schiffman, 1996).

Taste Transduction

When a taste stimulus interacts with the apical, chemosensitive tips of taste receptor cells, the chemical potential inherent in the molecules of the stimulus is converted (transduced) into a change in the membrane voltage at the apical tip of the cell (Roper, 1993). This event is termed transduction, and is a largely electrical phenomenon. This interaction involves binding of the chemical stimulus ions or non-electrolytes with chemoreceptors located within papillae and leads to the generation of receptor potential in the direction of depolarization and release of neurotransmitter from the taste cell (Kinnamon and Roper, 1988; Kurihara et al., 1981; Simon and Garvin, 1985). After the stimulus ion or molecule is absorbed, it has been suggested that there is shrinkage in the molecular volume of the receptor molecule within the membrane of the chemoreceptor cell, where the degree of shrinkage is assumed to a function of the stimulus concentration (Biedler, 1976). Furthermore, in vertebrates information about stimulus structure and concentration alters the rates of firing of the primary sensory nerve fibers (Teeter and Brand, 1987).

Like other sensory receptors, taste cells utilize apically located ion channels and receptors for transduction. But unlike most other receptor cells, taste cells use a variety of different mechanisms for the transduction of various chemical stimuli. Different mechanisms for taste transduction are necessitated by the diversity of taste stimuli - sweet, salty, sour or bitter - ranging from ions to large molecules, and widely varying pH and osmolarity in the oral environment (Kinnamon and Cummings, 1992; Avenet et al., 1993).

Sweetness Transduction

The chemical nature of sweet compounds is diverse and spans natural compounds like carbohydrates and some amino acids and artificially created compounds such as saccharins and cyclamates. Many lines of evidence suggest that a membrane-bound receptor is involved in sensing sweet stimuli. This evidence includes the observations that sweet stimuli such as carbohydrates are membrane impermeant, that certain proteolytic enzymes can specifically impair the sensation of sweetness, and that a protein extract from the Gymnemia plant can specifically block sweet responses (Herness and Gilbertson, 1999).

In animal models, the emerging picture is that sweetness transduction is coupled with guanine-nucleotide binding proteins (G-proteins) that produce a second messenger cascade of either cyclic adenosine monophosphate (cAMP), or inositol 1,4,5-triphosphate (IP_3), depending on the stimulus molecule (Eggers et al., 2000). As with other signal transduction systems that produce cAMP, the cAMP thus produced in the taste cell likely stimulates protein kinase A, which may phosphrylate ion channels, ultimately leading to cellular depolarization, an increase in intracellular calcium ion activity and release of neurotransmitter. Production of IP_3 as opposed to cAMP has been shown in the case of stimulation by non-sugar sweeteners like saccharin (Brand, 1997).

Sourness Transduction

Sourness is known to be related to the presence of hydrogen ions in solution, but a precise understanding of the relationship between hydrogen ion concentration, the type of anion, and the perceived intensity of the acid is still lacking. In simplest terms the receptor for sourness is viewed as a proton counter and at least three mechanisms have been proposed to account for sourness transduction in terms of hydrogen ion induced changes in taste cell response. The first mechanism involves a proton block of an outward-going K^+ channel, the second involves proton influx through amiloride-sensitive epithelial ion channels, similar to if not identical with channels that mediate salt taste and the third proposes that acid stimuli activate a stimulus-gated calcium ion channel that facilitates the passage of divalent and monovalent ions into the receptor cell (Brand, 1997). The action potentials elicited by acid stimuli in taste cells are thought to be concentration dependent (Kinnamon, 1993).

Owing to the fact that protons affect virtually every class of ion channel and likely have the capacity to directly affect a variety of cellular targets, acidic stimuli have the broadest effects of all gustatory stimuli in the taste system. Moreover, these stimuli alter intercellular pH, which may affect potential transduction pathways and therefore it is not surprising that many transduction mechanisms have been proposed for acids (Herness and Gilbertson, 1999).

Saltiness Transduction

Of various salts, NaCl - the prototypical salty stimulus has been most studied, and the sodium ion appears to be more important than the anion for this taste (Avenet and Lindemann, 1989).

The mechanism of Na^+ salt taste transduction is more clearly defined than other taste modalities. Saltiness is likely transduced by an epithelial-type ion channel located on the plasma membrane of the taste receptor cell (Brand, 1997). Na^+ salt-sensitive taste cells possess an amiloride-sensitive sodium selective ion channel (ASSC) and Na^+ ions on the surface of the tongue presumably pass through this channel depolarizing the cell (Akabas, 1993). Studies of ASSC physiology in mammals have shown that these channels are permeable to sodium, lithium and protons, but not potassium. Na^+ entry into taste cells has also been shown through the paracellular pathway via anion-dependent diffusion through tight junctions (Herness and Gilbertson, 1999).

Some studies have also indicated that salt deprivation or volume depletion influences the perceived intensity or the magnitude of the integrated chorda tympani nerve response to a given salt stimulus, suggesting that salt taste cells may be regulated by hormonal systems that regulate body volume status (Akabas, 1993).

Bitterness Transduction

The large number of bitter tasting compounds and the diversity of their molecular structure suggests multiple mechanisms for transduction of bitter taste stimuli (Kinnamon, 1996; Herness and Gilbertson, 1999).

For the bitter tasting agents that are membrane permeant, cellular phosphodiesterase was suggested to act as the receptor (Avenet and Lindemann, 1989). Apical K^+ conductance also appears to be involved in bitter transduction, since quinine and divalent salts such as $CaCl_2$ directly block K^+ conductance (Kinnamon, 1996). Non-permeant agents such as denatonium most likely require apical surface receptors and have been shown to be linked to G-protein coupled second messenger cascades stimulating the production of IP_3 (Kinnamon, 1996; Avenet and Lindemann, 1989).

Taste Coding Theories

The way in which the nervous system extracts and codes information about taste quality has been the subject of considerable discussion in recent years. The coding issue rests on whether the activity in a specific sensory neuron is a clear representation of the quality of the stimulus applied to its receptors or whether this activity is meaningful only in the context of activity in other afferent fibers. The labeled-line hypothesis of taste quality coding suggests that activity in a particular fiber represents a specific taste quality, whereas the across-fiber pattern theory holds that a particular pattern of activity across all afferent fibers represents a taste quality. A variant of the across-fiber pattern theory says that quality is coded by a comparison of activity across more than one fiber type (Smith and Frank, 1993).

The Role of Saliva

The major part of the aqueous oral milieu of the oral cavity is provided by the salivary glands. Saliva is a complex mixture of inorganic and organic substances secreted under parasympathetic and sympathetic nervous control (Schmale et al., 1993). Over 90% of saliva is produced by the major parotid glands that are found in front of the ear, the submaxillary glands located around the mouth under the lower jaw and the sublingual glands located beneath the tongue. However, the secretions of the lingual salivary von Ebner's glands are also important since the majority of taste buds sheltered in the epithelial folds of the circumvallate and foliate papillae in the posterior part of the tongue are in direct contact with these glands (Gurkan and Bradley, 1988).

Salivary ionic composition and flow rate have been found to vary in response to different gustatory stimuli (Dawes, 1984; Spielman, 1990). However, time-intensity parameters of gustatory stimuli have not been found to be markedly affected by salivary flow or composition. A study examining the interaction of parotid salivary flow rate with the temporal perception of sweetness, sourness and fruitiness found no significant differences between time-intensity parameters for all three attributes among salivary flow groups (Bonnans and Noble, 1995). The results of another study looking at the relation between parotid salivary flow and composition and perception of gustatory and trigeminal stimuli in foods showed no correlation between saliva composition and time intensity parameters, but indicated the possibility of a correlation between saliva flow and some texture and mouthfeel attributes (Guinard et al., 1998). Saliva does however have a baseline effect on taste perception since salivary bicarbonates buffer acid stimuli and salivary NaCl levels influence salt threshold levels such that salt taste is detected only above salivary salt concentrations (Spielman, 1990).

Mixture Suppression

Psychophysicists have long studied responses of mammalian sensory neurons to taste mixtures using sensory neurophysiological methods, involving presentation of stimuli that are mixtures of pure chemicals known to affect the taste system of the species in question (Frank, 1989). Historically, taste mixture studies were divided into those that dealt with substances having 'different' taste qualities and others that dealt with substances having 'similar' taste qualities. In this respect, stimuli that were described by the same adjective(s) were to be considered similar and vice versa (Frank, 1989). These studies were followed by others relating taste intensity to stimulus concentration via psychophysical functions. Psychophysical functions were said to show compression when successive increments in concentrations of individual substances produced progressively smaller increments in perceived intensity and to show expansion when increments in concentration yielded correspondingly larger increments in perceived intensity (Bartoshuk, 1975). Carrying the logic further to mixtures, it was found that the intensity of taste mixtures could reflect additivity - where the intensity of the mixture was equal to that of the sum of perceived intensities of its components, synergism (hyperadditivity) or suppression (hypoadditivity) (Curtis et al., 1984). It was also suggested that the suppression or synergism that taste compounds undergo in mixtures, might be predicted from the psychophysical functions produced by the unmixed components (Bartoshuk, 1974). Thus suppression might be related to compression and synergism might be related to expansion. Relating back to early concepts of similar and different taste qualities, Moskowitz hypothesized that mixtures of similar tastes tended to be associated with additivity or synergism, whereas mixtures of dissimilar tastes tended to be associated with suppression (Curtis et al., 1984).

Conditions Affecting Mixture Suppression and its Perception

Suppression depends upon the type of suppressing taste substance, e.g. sweetness is suppressed significantly more in a sucrose/quinine sulfate mixture than in a sucrose/NaCl mixture, even when the subjective intensities of all the compounds in an unmixed state are identical. The physical intensity of the suppressing component is also important in determining its effectiveness as a suppressing agent. Another factor is the number of stimuli making up the mixture, where a greater relative number of components will likely yield a greater amount of suppression in the mixture (Kroeze, 1990).

In addition to individual differences between and within subjects, the perception of mixtures is greatly affected by aging. The work of Stevens (1996) shows that with only minor exceptions, the functions of young and elderly people lie parallel to each other. This means that no matter how much mixture suppression or masking elevates thresholds, the average elderly person always ends up approximately a constant multiple higher than the average young person (Stevens, 1996).

Intensity judgments for mixtures of dissimilar-tasting substances also depend on stimulus context, e.g. in some studies the characteristics of the preceding unmixed stimuli were found to affect intensity judgments for mixed and unmixed stimuli differentially (Schifferstein, 1994). Also, these intensity judgments are influenced by conceptual factors like instructional sets that affect the subjects' ideas of attribute categories (Schifferstein, 1992).

Binary Mixtures

Suppression or partial masking may occur in binary mixtures when two or more substances of qualitatively different tastes are mixed. Thus when sucrose and NaCl are mixed, the sweetness and saltiness of the mixture may be less than when judged on the basis of equimolar unmixed components (Kroeze, 1990). The phenomenon is defined for suprathreshold concentrations (Breslin, 1996). The total perceived intensity of a mixture (two-component) is often less than the sum of the perceived intensities of the unmixed components judged separately. At the same time, the intensities of the individual components are also judged to be less in a mixture (Lawless, 1976).

The literature on binary mixtures is largely composed of the following findings. With respect to sweetness suppression, moderate and strong concentrations of NaCl have been found to suppress sucrose sweetness and also appear to suppress the sweetness of glucose and fructose. Citric acid suppresses sucrose sweetness and also that of glucose and fructose. However, the reverse suppression of citric acid sourness has also been shown (Kroeze, 1990). Strong concentrations of NaCl have been reported to enhance the sourness of citric acid in some studies, while in others they have decreased its sourness. In yet another study, mixing HCl with NaCl increased its sourness. This puzzling contradiction between acids and NaCl has been explained by assuming that 'side taste' (the sweet taste of NaCl solutions) effects may obscure suppression, or in some cases even cause enhancement (Kroeze, 1990). Frijters and Schifferstein (1994) found that NaCl suppressed the bitterness of quinine

HCl while the reverse suppression of NaCl was not observed. In a study spanning the salts of NaCl, LiCl, KCl, L-arginine:L-aspartic acid, Na-acetate and Na-gluconate, interacting with the bitter-tasting compounds of urea, quinine HCl, magnesium sulfate, KCl, amiloride HCl and caffeine (in two-component mixtures), in most cases the perceived bitterness was suppressed by salts, although the degree of suppression varied. Also, in general, bitterness suppression was not accompanied by an equivalent reciprocal suppression of saltiness. Only $MgSO_4$ and amiloride had suppressing effects on the saltiness of NaCl at intermediate concentrations and no bitter compound affected saltiness at high levels of NaCl (Breslin & Beauchamp, 1995).

Tertiary and Higher Level Mixtures

In a comparison of detection thresholds of target tastes in binary versus multi-component mixtures, Stevens and Traverzo (1997) found that while two maskers separately raised the detection threshold of the target taste by a factor of 3-4, together the maskers raised the threshold by over 9 times. This result, in their opinion, was consistent with the independence and thereby the additivity, of the two masking effects. This showed that multiple masking could be a far more efficient means of concealing a taste, whether pleasant or unpleasant.

Significance of Suppression

In terms of practical applications, if one wanted to diminish or completely mask an unwanted taste - like the bitter taste of a medicine, one could add enough sucrose to suppress it. Alternatively, one could add a multi-component masker, which might be very effective in suppression, and at the same time might significantly reduce the amounts of each of the individual maskers required to conceal the undesirable taste. In either case, partial or complete masking has great culinary and dietary significance.

In another application, suppression of the perception of sourness in products while maintaining acid levels, is not only relevant to the food industry, but could also be highly beneficial from a food safety stand point.

Sensory Methods and Data Analysis

Sensory evaluation is a scientific discipline used to evoke, measure, analyze and interpret reactions to characteristics of foods and materials as perceived by the human senses (Stone and Sidel, 1993). In order to measure reactions to characteristics of food and other materials, sensory evaluation utilizes various scaling techniques, which involve the use of numbers or words to express the intensity of a perceived attribute like sourness, sweetness, saltiness etc. Scaling is a more informative way of recording intensity of perception as compared to grading or ranking. However the validity and reliability of such measurements depends upon the selection of a broad enough scale to detect small differences in intensity and adequate panel training in the use of the scale (Meilgaard et al., 1991).

The development of scaling as a device for measuring psychological or sensory impressions can be traced to Weber's Law, developed by a German physiologist E. H. Weber in 1834. The law states that the smallest detectable increment (just noticeable difference or JND) in the intensity continuum of a stimulus is a constant proportion of the original stimulus (Schiffman, 1996). But while the law allowed the prediction of stimulus intensities that were just noticeably different from a standard, it did not directly allow the prediction of psychological or sensory magnitude. Moving toward this end Gustave Theodor Fechner developed Fechner's Psychophysical Law in 1860. In simplest terms, the law states that equal ratios in stimulus steps (i.e. equal steps on a log scale) produce equal psychological differences (or JNDs) (Riskey, 1988).

Magnitude Estimation

Magnitude estimation or free number matching is a scaling technique based on Steven's Power Law, developed by S. S. Stevens about 100 years after Fechner's work (Meilgaard et al., 1991; Schiffman, 1996). According to the law, sensory or subjective magnitude grows in proportion to the physical intensity of a stimulus raised to a power, whereby the relationship between sensation and stimulus magnitude can be plotted as a curve called a power function (Stevens, 1956; Stevens, 1957). When using magnitude estimation, the subject is typically presented with a standard stimulus of moderate intensity, called a modulus, with an assigned numerical value. Subsequent samples varying along dimensions of physical intensity are then randomly presented to the subject who assigns numerical values to them in proportion relative to the modulus or standard stimulus (Schiffman, 1996).

Magnitude estimation data are normalized (usually by a geometric mean normalization) to reduce panel variance, logged (to give log response) and then regressed on log stimulus concentration to yield power functions for each sample evaluated (McDaniel and Sawyer, 1981; Rubico, 1993). For the purposes of perceptual matching of taste qualities like sourness and sweetness, further analyses involves determination of equi-sour levels of the taste quality based on the predicted power function equations, which estimate concentration values for each sample that would yield perceptually matched responses for that taste quality.

Category Scaling

A category (or partition) scale is method of measurement in which the subject is asked to 'rate' the intensity of a particular stimulus by assigning it a value (category) on a limited, usually numerical scale (Meilgaard et al., 1991).

Direct scaling of this nature had its basis in Thurstonian scaling, developed by Thurstone, whose Law of Comparative Judgement (1959) pointed out that the degree to which the psychological impressions of any two stimuli in a set overlap (i.e. the amount of confusion between the stimuli) is enough information to infer the psychological distances among the various stimuli (Riskey, 1988). Thurstone thus demonstrated that the time-consuming process of measuring JNDs to generate psychological scales was unnecessary. Thurstonian scaling was observed to be similar to a categorization operation, essentially derived from questions like "Which is sweeter, larger or more acceptable?" Thereafter it soon became common to ask respondents for more information such as "Sort the stimuli into the following categories: very sweet, slightly sweet, and not sweet at all." Procedures were developed to turn this type of categorical data into Thurstonian scale data, but both approaches were found to produce similar results. Since the categorical approach was direct, easier and more efficient, it gained popularity leading to the use of category scale rating in its current form (Riskey, 1988).

To determine whether significant differences exist between samples evaluated, scale data can be graphically examined and analyzed using univariate and/or multivariate Analysis of Variance (ANOVA or MANOVA), followed by appropriate multiple comparison tests such as Fisher's LSD, Tukey's HSD etc.

III SUPPRESSION OF SOURNESS: A COMPARATIVE STUDY INVOLVING MIXTURES OF ORGANIC ACIDS AND SUGARS

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Abstract

This study involved the determination of the degree of sourness suppression of equisour levels of three food acids (citric, lactic, malic), by equimolar and equiweight amounts of three sugars (sucrose, fructose, glucose), in binary mixtures. The role of perceived sweetness intensity of the maskers in suppression was also observed. Acids and sweeteners were tested at two levels each, along with a no-sugar acid control. Equisour acid levels were obtained by sourness matching using magnitude estimation. Mixture intensity ratings were collected on a 16-point intensity scale using a trained discriminatory panel with 17 pre-screened panelists. The results showed that sourness suppression was not mediated by molarity or weight of the masker but was significantly influenced by its perceived intensity in most cases, suggesting that the underlying mechanisms are dominantly central. This was confirmed by testing the suppressive abilities of sweetness-matched levels of sucrose, fructose and an equi-ratio mixture of the two on citric acid sourness, where they showed no significant difference in sourness suppression. The data also supported the existence of a separate receptor site/mechanism for glucose and a small peripheral component to suppression. It remains to be determined whether these trends will be maintained in more complex systems.

Introduction

Acids have many functions in food systems including preserving, buffering, flavoring and chelating. Acids are routinely used in the food industry as a means of lowering the pH of foods and beverages in order to enhance their shelf life. Acidification of heat sensitive, low acid foods allows for the achievement of commercial sterility with milder heat treatment (Sogenfest et al., 1948), which also translates into lower energy usage and overall cost reduction. However, the addition of commonly used food grade organic acids alters the flavor of acidified foods, often rendering them unpalatable. As a result, only a few low acid foods with the ability to tolerate such acid flavors have been successful candidates for acidification (McCarthy et. al, 1991).

Suppression or partial masking of sourness regularly occurs in complex food systems. It is well known that sweeteners comprise one of the most effective masking agents for sourness. Sweeteners are combined in the formulations of acidified foods to achieve optimum taste, while maintaining the desired pH. However the most effective sweet masker(s) for a particular acid and the optimal level or range of the masker given the acid concentration, are usually ascertained by a trial-and-error approach.

The literature on binary mixture interactions is extensive, but that on sourness suppression in particular, has been limited. Studies involving interactions between sweet and sour tastants have primarily used citric acid and sucrose. Suprathreshold levels of citric acid have been shown to suppress sucrose sweetness (Pangborn, 1961). Sucrose was reported to suppress the perceived intensity of citric acid

(McBride and Johnson, 1987, McBride, 1989). Schifferstein and Frijters (1991) reported that equisweet levels of aspartame, saccharin, fructose and sucrose were equally effective in suppressing the perceived sourness of citric acid, while McBride and Finlay (1990) found that sucrose suppressed the sourness intensity of citric acid more effectively than fructose. Mutually suppressing interactions between citric acid and the sweeteners aspartame and sucrose revealed that there was a greater suppression of sweetness by acid levels than of sourness by increasing sweetener levels (Bonnans and Noble, 1993).

The phenomenon of suppression in general, depends upon three main factors - the type of suppressing taste substance, the physical intensity of the suppressing component and the relative number of components that make up the mixtures (Kroeze, 1989). With respect to the physical intensity of the suppressing component, it is not clear whether the weight, molarity or perceived intensity of the suppressor are important determinants of suppression. This study proposes to better understand the role played by each of these factors. A relationship between sourness suppression and sugar molarity would favor molecular mechanisms as being responsible for the suppression, while one with sugar weight might implicate physical effects such as viscosity. On the other hand, a correspondence with perceived sugar sweetness would be indicative of neural effects (central/peripheral). While the idea that suppression in sweet-sour mixtures is a perceptual phenomenon related to perceived intensity of the masking agent has been explored (Schifferstein and Frijters, 1991), the work was limited to sourness suppression of citric acid alone, and the effects of molarity or weight of the masker were not concurrently examined.

The objective of this study was to determine the effect of molarity, weight and perceived sweetness intensity of three sugars on the perceived sourness intensity of three food grade organic acids at perceptually iso-sour levels in a water-based model system. The results thus obtained were confirmed by testing the effect of equisweet concentrations of two of the sugars on the sourness of one acid.

Materials and Methods

Subjects

Selection of subjects was based on performance in a pre-screening exercise that tested for sensitivity and reproducibility via a ranking test. 17 panelists (12 women, 5 men) qualified for participation in the study. The mean age of the participants was 25 (range 19-30 years). Subjects included staff and students of Oregon State University and residents from the local community. All were compensated for their participation.

Stimuli

All food grade solutes (>99%pure) were purchased through Springfield Specialties, OR other than lactic acid (~60% pure), which was obtained from Purac America, Inc., IL. All solutions were made in distilled water. The acids used in the study were citric, lactic and malic. Acid levels to be used in the mixtures were selected by a sourness matching exercise to be iso-sour with 0.00625M and 0.025M citric acid, designated as low and high levels respectively. The iso-sour acid levels were obtained by training panelists in utilizing the technique of magnitude estimation (Stevens. 1956; Stevens, 1957) and testing them as described by Moskowitz and Sidel (1971), and Rubico (1993) using 0.00625M citric acid as the reference modulus. The determination of sourness-matched levels was conducted over the weeks preceding the binary acid-sugar testing. The power functions of the acids used to determine the iso-sour levels are shown in Figure 3.1. The sweeteners in the study were sucrose, fructose and glucose. Sweetener levels were also designated low and high, with the equimolar (Em) levels being 0.0625M and 0.25M, and equiweight (Ew) levels being 11.25g/L and 45g/L. The molar values were selected based on literature values and pilot testing. With respect to the weight levels, since the sugars glucose and fructose fortuitously have identical formula weights, which also correspond to their molecular weights, selection of the above mentioned weights reduced the total number of samples to be tested. Since sucrose has a different formula weight and molecular weight, for the purposes of experimental design and analysis, it was treated as two sweeteners i.e. sucrose equimolar and sucrose equiweight. A sugar-free acid control was simultaneously tested at every level of each acid. This resulted in a factorial plus control experimental design {(3 acids X 2 acid levels X 4 sweeteners X 2 sweetener levels) + acid control}. Additionally, evaluation of all '48 + control' treatment combinations was conducted in duplicate giving two replications.

For the confirmation of the results from the above work, only two sugars – sucrose and fructose, and one acid – citric, were utilized. Sweetness matching was

Figure 3.1 Power Functions of Acid Sourness{Each data point represents the mean of 51 values (17 panelists X 3 replications)}

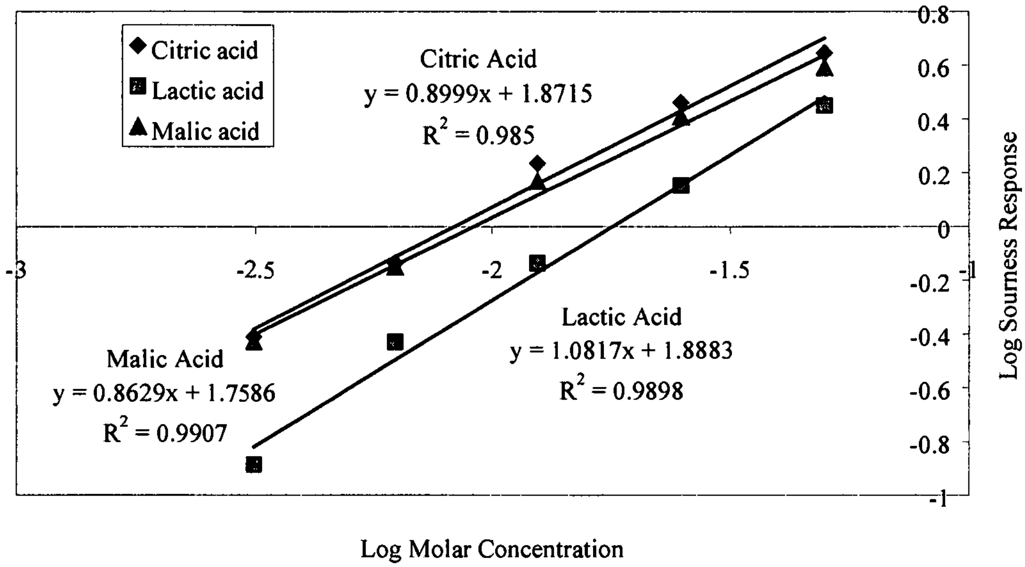
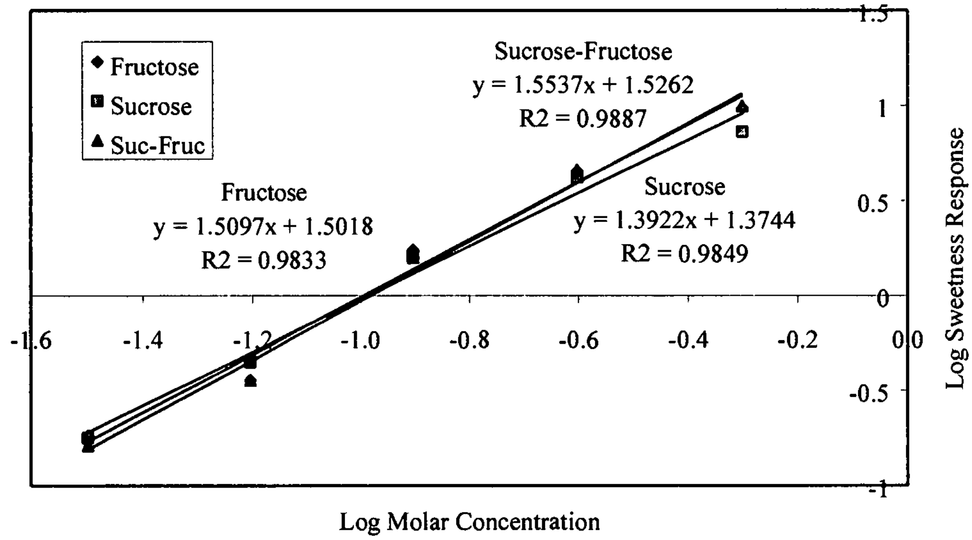


Figure 3.2 Power Functions of Sugar Sweetness {Each data point represents the mean of 51 values (17 panelists X 3 replications)}



performed on sucrose, fructose and a 1:1 equiratio mixture of the two. The preparation of the equiratio sugar mixture was conducted based on a procedure described by Frijters and Oude Ophuis, (1983). Three sweetness matched levels obtained from the sugar power functions (Figure 3.2) were used to test the effect of equisweet sugar concentrations on the suppression of citric acid sourness, which was used as a representative system. The sugar levels selected from the plots were equisweet with 0.0313M, 0.0625M and 0.125M sucrose. The concentration of citric acid in all mixtures was fixed at 0.00625M.

Procedure

Solutions were prepared 12-14 hours before testing and were stored overnight at 4°C. Prior to serving they were allowed to equilibrate to room temperature (21-22°C). Approximately 30 mL of each sample was presented in 3oz. plastic cups and samples were coded with three-digit random numbers. Up to five samples were served in a set and no more than four sets were presented a day. The serving order of samples was randomized within an acid type and level, with each sample being evaluated twice. Stimuli were sampled by the sip-and-spit method. After tasting the samples, panelists rated the sweetness and sourness intensities of the mixture solutions on a 16-point intensity scale (0=none, 3 = mild, 7 = mild to moderate, 11 = moderate, 15 = moderate to high). Panelists were trained in the use of the scale with the help of references and training exercises conducted prior to testing. The sourness references made with citric acid were as follows: sour 3 (0.00313M), sour 7 (0.00625M), sour 11 (0.0125M) and sour 15 (0.025M). The sweetness references were made with fructose and were as follows: sweet 3 (0.0625M), sweet 7 (0.125M), sweet 11 (0.25M) and sweet 15 (0.5M). Panelists were encouraged to thoroughly rinse their mouth with water between samples. Subjects were also asked to refrain from smoking, eating or drinking anything at least a half-hour before testing. The pH of all the controls and binary mixture solutions was recorded prior to testing.

Data Analysis

The data were analyzed using a mixed-model analysis of variance (ANOVA) within session with Tukey's honest significant difference (Tukey's HSD) as the post-hoc means separation procedure. Sessions were separated by acid type and level, giving a total of 6 sessions. For the confirmation, the analysis was conducted within a sweetness matched sugar level. The model looked at the effects of treatment (fixed effect), panelist (random effect) and the interaction of panelist and sample (random effect). Analyses were conducted using SPSS® v 8.0 (Chicago, IL).

Results

The Effect of Equiweight and Equimolar Sugar Concentrations and that of the Perceived Sweetness of the Sugars at Equiweight Levels

Mean sourness and sweetness intensity ratings for all mixtures are presented in Table 3.1. From here on sourness suppression or the degree of suppression will refer to a reduction in the sourness rating for a mixture solution as compared to the sourness rating of the corresponding acid-only control.

Table 3.1 Mean sourness and sweetness intensity ratings for control (no sugar) and binary acid-sugar mixtures at low and high sugar and acid levels

Acid Level	Sugar Level & Type	Citric Acid Mixtures		Lactic Acid Mixtures		Malic Acid Mixtures	
		Mean Sourness	Mean Sweetness	Mean Sourness	Mean Sweetness	Mean Sourness	Mean Sweetness
Low Acid Level	Control	8.53^{a}	0.97^{f}	7.97^{a}	0.85^{f}	8.12^{ab}	0.97^{g}
	Low Fructose	7.88^{a}	3.38^{de}	7.50^{a}	3.00^{e}	8.56^{a}	2.74^{e}
	Low Glucose	8.35^{a}	1.18^{f}	8.32^{a}	1.50^{f}	8.50^{a}	1.21^{fg}
	Low Sucrose (Em)	6.79^{ab}	4.85^{cd}	7.03^{ab}	4.32^{d}	7.06^{bc}	4.59^{d}
	Low Sucrose (Ew)	7.50^{a}	2.18^{ef}	7.38^{ab}	1.82^{f}	7.88^{ab}	2.26^{ef}
	High Fructose	5.53^{b}	10.32^{ab}	5.21^{cd}	9.26^{b}	5.38^{d}	10.21^{b}
	High Glucose	7.88^{a}	5.18^{c}	7.12^{ab}	3.26^{de}	8.06^{ab}	3.97^{d}
	High Sucrose (Em)	5.06^{b}	11.85^{a}	4.06^{d}	11.88^{a}	3.74^{e}	12.62^{a}
	High Sucrose (Ew)	5.71^{b}	9.12^{b}	6.09^{bc}	7.85^{c}	5.85^{cd}	7.44^{c}
High Acid Level	Control	12.38^{a}	0.68^{f}	11.18^{ab}	0.91^{d}	11.32^{ab}	0.82^{e}
	Low Fructose	11.97^{a}	2.15^{de}	10.53^{abcd}	1.56^{d}	11.62^{a}	1.79^{de}
	Low Glucose	11.82^{ab}	1.09^{f}	11.65^{a}	1.18^{d}	11.79^{a}	1.56^{e}
	Low Sucrose (Em)	11.32^{ab}	3.50^{c}	10.09^{bcd}	3.03^{c}	9.91^{bc}	3.74^{c}
	Low Sucrose (Ew)	11.62^{ab}	1.44^{ef}	10.97^{abc}	1.65^{d}	10.74^{ab}	2.03^{de}
	High Fructose	9.91^{c}	7.24^{b}	9.76^{cd}	6.35^{b}	8.56^{cd}	9.18^{b}
	High Glucose	11.97^{a}	3.18^{cd}	10.82^{abc}	2.68^{c}	10.50^{ab}	3.06^{cd}
	High Sucrose (Em)	9.32^{c}	9.85^{a}	7.62^{e}	10.26^{a}	7.44^{d}	11.12^{a}
	High Sucrose (Ew)	10.53^{bc}	6.88^{b}	9.38^{d}	6.38^{b}	8.82^{cd}	7.97^{b}

Means with different alphabetic superscripts within a column and acid level are significantly different at $p \leq 0.05$;
Means are across 17 panelists and 2 reps
Scale values correspond to 0=none, 3 = mild, 7 = mild to moderate, 11 = moderate and 15 = moderate to high

Trends within Acids and across Sugars

From the values in Table 3.1, it can be seen that for all the 3 acid mixtures and at low and high acid levels, sourness suppression is significant for the mixtures with fructose and Em as well as Ew sucrose at the high sugar level ($p<0.05$). Additionally, mixtures of low and high level malic acid with Em sucrose at the low sugar level were found to show significantly more sourness suppression ($p<0.05$) as compared to the low sugar level mixtures with fructose and glucose. Also, an interesting anomaly albeit non-significant ($p>0.05$) was noted in low sugar level mixtures of glucose with lactic and malic acids at both acid levels, and fructose with malic acid at both acid levels, where the sugar-acid mixture was perceived to have a higher sourness than the corresponding acid control.

The pH of all the mixture solutions was dictated by the pH of the acid type and level present in the mixture, and so the pH of the low citric acid control and corresponding mixtures was 2.63 ± 0.01 while that of the high acid control and mixtures was 2.45 ± 0.01. The pH of the lactic acid controls and mixtures at the low and high acid levels respectively was $3.45 \pm 0.01$and 3.39 ± 0.01, while that of the malic acid controls and mixtures was $2.82 \pm 0.01$and 2.54 ± 0.01 at low and high acid levels respectively.

Trends within Sugars and across Acids

Figures 3.3 a-f show degree of sourness suppression as a function of perceived sweetness of sugars at equiweight levels. From a comparison of figures 3.3 a and b showing the glucose-acid mixtures versus figures 3.3 c and d and 3.3 e and f, representing fructose and sucrose sugar-acid mixtures respectively, it appears that the glucose-acid mixtures behaved differently than the acid mixtures with the other sugars. The glucose in the mixtures while perceived to impart some sweet taste, did not show much corresponding sourness suppression (degree of suppression not exceeding 1 at the levels examined). The acid-sugar mixtures of fructose and sucrose showed marginally higher suppression in the mild sweetness intensity range and substantially higher suppression in the mild to moderate sweetness intensity range.

The Effect of Equisweet Sugar Concentrations

Figures 3.4. a-c show the perceived sourness and sweetness ratings for mixtures at three equisweet levels. Within the three perceptually sweetness-matched levels, the sweetness ratings for the sugar types were not significantly different, demonstrating the success of the sweetness matching. The corresponding sourness ratings for the mixtures were similarly not significantly different from each other, but were significantly different from the acid-only and sugar-only controls at all three equisweet levels.

Figure 3.3 Comparison of trends in suppression within sugars across acid types

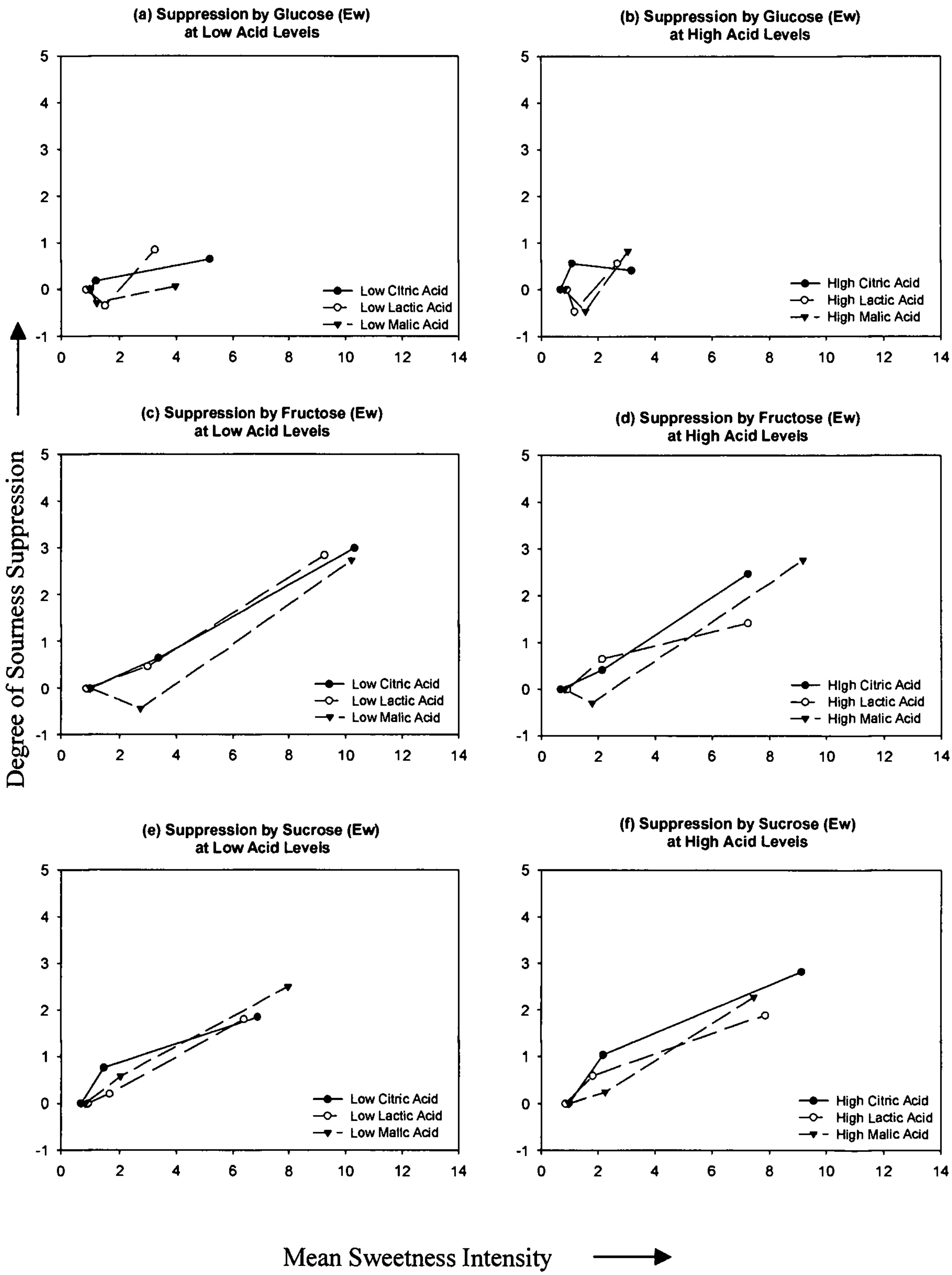
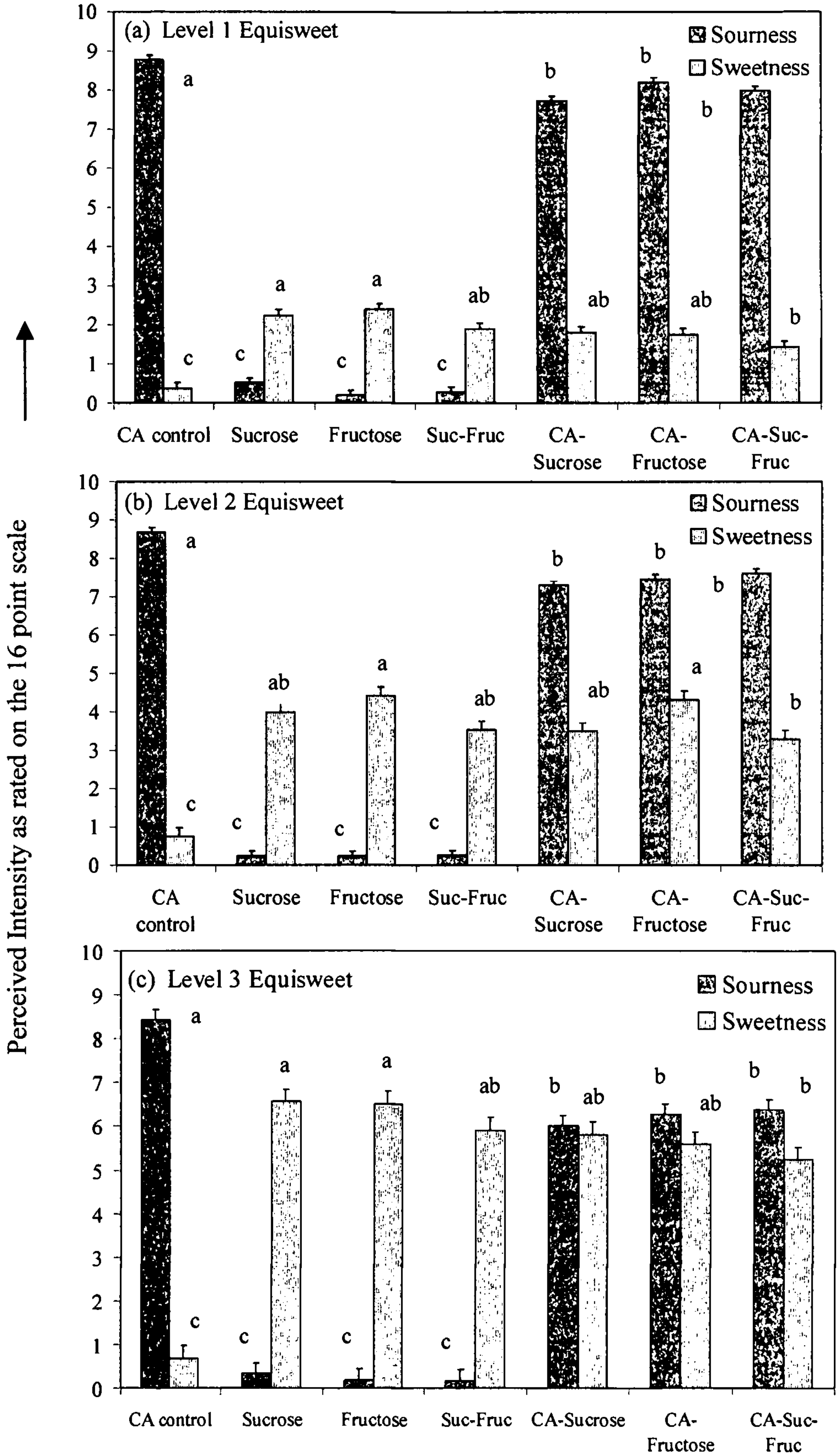


Figure 3.4 Sourness and Sweetness Ratings at Equisweet Levels 1, 2 and 3



Discussion

Across acid types and levels, it was found that equiweight and equimolar levels of the sugars affected unequal amounts of sourness suppression in terms of a decrease in perceived sourness intensity ratings. If the suppression at either the equiweight or equimolar sugar levels had been equivalent across the acids, then physical sugar parameters or molecular mechanisms would be the likely cause of such suppression. However the results of obtained imply that neither of the two completely explain the observed suppression. Though a sugar concentration effect in general is undeniable, since it is linked to sugar weight, sugar molarity and perceived sweetness, in the context of the stated objectives for this study, sugar weight and sugar molarity alone did not account for the suppression seen.

To examine the effect of the perceived intensity of the masking agent on that of the target taste, the three sugars employed in this study were selected such that they were ranked in their perceived sweetness intensity. Shallenberger (1993) averaged the findings of several taste studies to show that on a weight basis, when sucrose has a relative sweetness of 100, the average relative sweetness of D-glucose is 64 while that of D-fructose is 120.5, making fructose the sweetest of the three. On the other hand, on a molar basis sucrose is the sweetest of the three.

As seen from the mean values in Table 3.1, at equimolar sugar levels sucrose mixtures were found to be the sweetest and the glucose mixtures were the least sweet. Thus the order of perceived sweetness intensity of the individual sugars as predicted by Shallenberger (1993), was also observed in mixtures of the same sugars with the three acids at two levels each and this order of perceived sweetness was translated into a corresponding suppression of sourness with sucrose suppressing more than fructose, which in turn suppressed more than glucose.

With sugars on an equiweight basis fructose mixtures were the sweetest and the glucose mixtures were the least sweet at the low acid level as expected. But at the high acid level, there were no significant differences ($p>0.05$) between the sweetness ratings of the fructose and sucrose mixtures. These results are in accordance with Cardello et al (1979), who similarly observed that fructose, which has a sweetening advantage over sucrose in model distilled water solutions as well as in low acid media at low sugar concentrations, loses this advantage in highly acidic solutions. Thus at the high acid level, fructose and sucrose mixtures with all three acids were found to be similar in their sweetness ratings and similar in terms of the corresponding sourness ratings ($p>0.05$). Consequently, while it seemed likely that the perceived sweetness intensity of sucrose and fructose determined the extent of sourness suppression, this needed to be confirmed by directly examining the effect of equisweet sugar concentrations on suppression.

The results from the confirmatory study showed perceived sweetness intensity of sucrose and fructose to be the main determinant of sourness suppression of citric acid. At all three sweetness matched levels examined, the resulting suppression in the acid-sugar mixtures was not significantly different irrespective of sugar type i.e. sucrose, fructose or an equisweet mixture of the two (Figures 3.4 a-c). Since only perceived sweetness and not sugar concentration is equivalent at equisweet levels, and equisweet sugar levels produced equivalent suppression in sugar-citric acid mixtures, it seems that sourness suppression is mostly perceptual and a result of higher order processing at the brain level as opposed to the peripheral level. These findings demonstrate that central processing mechanisms may be largely responsible for the observed behavior.

With respect to the glucose mixtures across acid types and levels, glucose contributed sweetness mostly at the high sugar level. But the corresponding sourness ratings did not reflect this, as they remained largely unchanged from the sourness rating of the no sugar control. A comparison of the suppressive ability of the three sweeteners as a function of their perceived sweetness across acid types and levels shows that glucose (Figures 3.3 a-b), appears to behave differently than fructose (Figures 3.3 c-d) and sucrose (Figures 3.3 e-f) in the range of concentrations examined. With two out of three acids, glucose mixtures (Figures 3.3 a-b) showed a trend of initial sourness enhancement, followed by sourness suppression in mixtures. Fructose mixtures (Figures 3.3 c-d) showed initial sourness enhancement only with malic acid and suppression with citric and lactic acids. Sucrose mixtures show sourness suppression with all three acids. Based on the observed response patterns, in general glucose appears to be a less effective suppressor of sourness compared to sucrose or fructose in a low range of perceived sweetness, though more data would be needed in the region of higher perceived sweetness values for glucose, in order to confirm these trends. However, a distinct response pattern to the same acids and differential efficacy as a suppressor compared to sucrose and fructose in a similar range of perceived sweetness, points towards separate receptors/receptor mechanisms for the different sugars and thereby a peripheral component to suppression. Work with quinine-sucrose mixtures (Lawless, 1979, Lawless, 1982) has also shown the possible existence of a peripheral component to mixture suppression in addition to the central. Thus in contrast to Schifferstein and Frijter's (1991), who deemed mixture suppression to be a purely perceptual phenomenon, and found it unlikely that it could be accounted for by receptor events, we believe that suppression has both receptor-related and perceptual components. This supports Kroeze' (1989) idea that the phenomenon of mixture suppression should be viewed as a continuum with central and peripheral components, as opposed to one or the other.

In terms of conceptual process models from the realm of integration psychophysics, it has been hypothesized that since homogenous mixtures of fructose-sucrose and fructose-glucose have a sweetness that at higher concentrations exceeds that of the individual components, these mixtures follow a separate-sites model, where the separate sites model implies that the two components of the mixture are transduced independently at separate receptor sites (McBride, 1989). Further evidence to support independent receptor sites/mechanisms for the transduction of glucose and fructose comes from the isolation of fructose non-tasting and glucose non-tasting variants from natural populations of adult and larval fruitflies (*Drosophila melanogaster*), and the identification of potential human glucose non-tasters by elevated glucose thresholds (Kennedy et al, 1997). Another psychophysical study suggested different receptor cell mechanisms for furanose and pyranose monosaccharides based on human and fruitfly (*Drosophila adiastola*) response functions. The results of this study also suggested a mechanism for the pyranose-furanose disaccharide sucrose similar to that of furanose monosaccharides

(Armstrong et al, 1998). The findings from the present study on sourness suppression appear to corroborate the findings from the aforementioned studies and support the proposed existence of a separate receptor/mechanism for glucose from the receptor(s)/mechanism for fructose and sucrose.

It remains to be seen whether the findings from this work using model solutions are supported in more complex systems. In some cases, as in a study of the perception of sweet/acid mixtures in gels (Barylko-Pikielna et al., 1999), this has been supported. It was found that the general rules of behavior observed for the mixtures in water solutions were also followed in gels, with an additional effect of the nature of the gelling agent. More of such work could eventually lead us away from the current trial-and-error based approach to determining the ranges for mixture components in real products.

In conclusion, sourness suppression is not mediated by the molarity or weight of the sugar in binary mixture solutions of the sugars and acids examined here. For the sugars sucrose and fructose, the perceived intensity of the masker and thereby central neural mechanisms, appear to be largely responsible for the observed suppression. The data also support the existence of a separate receptor/mechanism for glucose from fructose and sucrose and thereby a peripheral component to suppression.

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IV SUPPRESSION OF SOURNESS: A COMPARATIVE STUDY INVOLVING MIXTURES OF ORGANIC ACIDS AND SALTS

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Abstract

This study involved the determination of the degree of sourness suppression of equisour levels of three food acids (citric, lactic, malic), by equimolar and equiweight amounts of three salts (sodium chloride - NaCl, sodium acetate - NaAc and sodium gluconate - NaGluc), in binary mixtures. The role of perceived saltiness intensity of the salty maskers in suppression was also observed. Acids and salts were tested at two levels each, in addition to an acid-only control (no added salt). Equi-sour acid levels were obtained by sourness matching using magnitude estimation. Mixture intensity ratings were collected on a 16-point intensity scale using a trained discriminatory panel with 17 pre-screened panelists. NaAc-acid mixtures showed the most sourness suppression, but also showed the largest concurrent pH increase (above 4.4). NaCl-acid mixtures showed significant sourness reduction without the associated pH increase, but were the saltiest of all mixtures at equiweight levels. NaGluc mixtures with lactic acid did not show much sourness reduction, but with citric and malic acids showed moderate suppression with pH increases remaining below 4.4. Saltiness appeared to drive suppression only in the case of NaCl, while pH change was responsible for reduction of sourness with NaAc and NaGluc. These findings were verified by testing equi-pH mixtures of all the salts with citric acid.

Introduction

Acids have a wide variety of functional uses. They serve as flavor adjuncts, buffers, chelators, prevent non-enzymatic browning, aid in gel formation and provide leavening (Hartwig and McDaniel, 1995). One of the most important roles they play is as food preservatives. Even in cases where heat is utilized as the method to extend shelf life, the addition of acids facilitates shorter sterilization times, since the resistance of most organisms to heat is reduced as pH is lowered (Gardner, 1966).

In food and beverage formulations acids interact with numerous other ingredients. These interactions often result in a suppression of acid sourness, but should not increase the pH of the system in order that the acids may continue to serve as preservatives. In previous work we examined the role of such interactions between organic acids and sugars in suppressing sourness using a model system approach (Savant and McDaniel, 1999). In continuation, this study aimed to look at the interactions of the same organic acids with sodium salts in model solutions to study sourness suppression by salts.

Of the three salts examined, sodium chloride (NaCl) is inorganic and commonly used in food formulations. Sodium acetate (NaAc) and sodium gluconate (NaGluc) are organic and while less commonly encountered, certainly have food applications and were included in the study in order to examine the effect of perceived saltiness of the salt in suppressing acid sourness, while staying within the domain of sodium salts. {At equimolar levels these three salts have been described as being ranked in their relative saltiness, with NaCl being the most and NaGluc being the least salty of the three (Breslin and Beauchamp, 1995; Bartoshuk, 1980)}.

For anions other than chloride, the perceived saltiness is attenuated or gustatory qualities other than saltiness appear (Ye et al., 1991; Schiffman, 1980). Furthermore, the anion effect is believed to be peripheral in origin, since it is seen in recordings from the chorda tympani nerve in animals (Ye et al., 1991).

The literature on salt-acid interactions at sub and supra-threshold levels is limited and has been largely restricted to the interaction of NaCl with various organic or inorganic acids (Breslin, 1996). Other mixture interactions involving NaCl and/or other sodium salts and non-sodium salts that have been studied include salt-sugar (Aroulmoji et al., 2000; Schiffman et al., 1999) and salt-bitter interactions (Breslin and Beauchamp, 1995).

The objective of this work was to study salt-acid interactions by examining the effects of molarity, weight and relative perceptual saltiness of 3 anion-substituted sodium salts on the sourness of three food grade organic acids at perceptually iso-sour levels in a water-based model system.

Materials and Methods

Subjects

Selection of subjects was based on performance in a pre-screening exercise that tested for sensitivity and reproducibility via a ranking test. 17 panelists (12 women, 5 men) with a mean age of 25, qualified for participation in the study. Subjects included staff and students of Oregon State University and residents from the local community. All were compensated for their participation.

Stimuli

All solutes were food grade (purchased from Springfield Specialties, OR, while lactic acid was obtained from Purac America, Inc., IL). Solutions were made in distilled water. The acids used in the study were citric, lactic and malic. Acid levels used in the mixtures were iso-sour with 0.00625M and 0.025M citric acid designated as low and high levels respectively, and were obtained from acid power functions developed by the same panelists prior to binary acid-salt mixture testing (Figure 3.1, Savant and McDaniel, 2001). The salts were NaCl, NaAc and NaGluc. Salt levels were also designated low and high, with the equimolar (Em) levels being 0.05M and 0.20M, and equiweight (Ew) levels being 4.1g/L and 16.4g/L. The molar values were selected based on literature values and pilot testing. For the selection of the weight levels, NaAc was used as a reference point and so the weights corresponding to 0.05 and 0.20M NaAc were used for the study. Thus NaAc was used at two levels (low and high equiweight or equimolar) in the study, while NaCl and NaGluc had four levels each (low and high equiweight, and low and high equimolar). For the purposes of experimental design and analysis, equimolar versus equiweight NaCl and NaGluc were considered 4 salts as opposed to 2. A salt-free acid control was simultaneously tested at every level of each acid. This resulted in a factorial plus control experimental design {(3 acids X 2 acid levels X 5 salts X 2 salt levels) + acid control}. Additionally, evaluation of all '60 + control' treatment combinations was conducted twice giving two replications.

Procedure

Solutions were prepared 12-14 hours before testing and were stored overnight at 4°C. Prior to serving they were allowed to equilibrate to room temperature (21-22°C). Approximately 30 mL of each sample was presented in 3oz. plastic cups and samples were coded with three-digit random numbers. Up to five samples were served in a set and no more than four sets were presented a day. The serving order of samples was randomized within an acid type and level, with each sample being evaluated twice. Stimuli were sampled by the sip-and-spit method. After tasting the sample, panelists rated the sourness and saltiness intensities of the mixture solution on a 16-point intensity scale (0=none, 3 = mild, 7 = mild to moderate, 11 = moderate, 15 = moderate to high). Panelists were trained in the use of the scale with the help of references and training exercises conducted prior to testing. The sourness references made with citric acid were as follows: sour 3 (0.00313M), sour 7 (0.00625M), sour 11 (0.0125M) and sour 15 (0.025M). The saltiness references were made with NaCl and were as follows: salt 3 (0.05M), salt 7 (0.10M), salt 11 (0.20M) and salt 15 (0.40M). Panelists were encouraged to thoroughly rinse their mouth with water between samples. Subjects were also asked to refrain from smoking, eating or drinking anything at least a half-hour before testing. The pH of all the controls and binary mixture solutions was recorded prior to testing.

Data Analysis

The data were analyzed using a mixed-model analysis of variance (ANOVA) within session with Tukey's honest significant difference (Tukey's HSD) as the post-hoc means separation procedure. Sessions were separated by acid type and level, giving a total of 6 sessions. The model looked at the effects of panelist (random effect), treatment (fixed effect) and the interaction of panelist and sample (random effect). Analyses were conducted using SPSS® v 8.0 (Chicago, IL).

Results

Mean sourness and saltiness intensity ratings for all mixtures are presented in Table 4.1. In the context of the results of this study, sourness suppression or the degree of sourness suppression refers to a reduction in the sourness rating for a mixture solution as compared to the sourness rating of the corresponding acid-only control.

The Effect of Equimolar Salt Concentrations

A comparison of intensity ratings for equimolar salt mixtures across both acid levels (Table 4.1) reveals that all the NaCl mixtures were perceived to be significantly saltier ($p<0.05$) than NaAc and NaGluc mixtures across acid and salt levels. NaGluc and NaAc mixtures were in general not significantly different in saltiness other than in mixture with citric acid at the high acid level and malic acid at the high acid, low salt level (Table 4.1), where NaAc was saltier than NaGluc ($p<0.05$). With respect to sourness, the NaAc mixtures showed the most sourness suppression with the lowest sourness ratings of the three in general, significantly lower compared to NaGluc mixtures at the high acid, high salt levels and NaCl at the

Table 4.1 Mean sourness and saltiness intensity ratings for control and binary acid-salt mixtures at low and high salt and acid levels

	Salt Level & Type	Citric Acid Mixtures		Lactic Acid Mixtures		Malic Acid Mixtures	
		Mean Sourness	Mean Saltiness	Mean Sourness	Mean Saltiness	Mean Sourness	Mean Saltiness
Low Acid Level	Control	8.52^{a}	1.06^{g}	8.34^{a}	1.63^{e}	8.28^{a}	1.41^{g}
	Low Na-Acetate	5.06^{c}	4.24^{def}	5.35^{bcd}	3.82^{cd}	4.35^{cd}	3.12^{e}
	Low Na-Chloride (Em)	6.74^{b}	5.82^{c}	6.18^{bc}	6.88^{b}	5.85^{b}	5.29^{d}
	Low Na-Gluconate (Em)	5.74^{bc}	3.44^{f}	5.65^{bc}	2.24^{de}	4.88^{bcd}	2.32^{efg}
	Low Na-Chloride (Ew)	5.44^{bc}	8.09^{b}	5.44^{bcd}	8.35^{b}	5.44^{bc}	6.97^{c}
	Low Na-Gluconate (Ew)	5.71^{bc}	2.00^{g}	6.74^{b}	1.85^{e}	5.56^{bc}	1.74^{fg}
	High Na-Acetate	3.56^{d}	4.85^{cde}	3.91^{d}	4.41^{c}	3.88^{d}	4.41^{d}
	High Na-Chloride (Em)	5.12^{c}	11.5^{a}	4.97^{cd}	11.67^{a}	5.00^{bcd}	10.71^{b}
	High Na-Gluconate (Em)	4.62^{cd}	5.29^{cd}	5.12^{cd}	4.38^{c}	4.03^{d}	4.76^{d}
	High Na-Chloride (Ew)	4.88^{cd}	12.03^{a}	5.38^{bcd}	12.56^{a}	4.47^{cd}	12.12^{a}
	High Na-Gluconate (Ew)	4.97^{c}	3.74^{ef}	5.62^{bc}	3.12^{cde}	4.79^{bcd}	2.82^{ef}
High Acid Level	Control	11.54^{a}	2.44^{e}	10.71^{a}	2.44^{e}	11.34^{a}	2.46^{f}
	Low Na-Acetate	7.59^{cde}	6.74^{c}	10.29^{a}	4.38^{d}	7.50^{def}	6.41^{cd}
	Low Na-Chloride (Em)	8.35^{bcd}	8.82^{b}	8.74^{bcd}	7.62^{c}	9.18^{bc}	7.06^{c}
	Low Na-Gluconate (Em)	8.85^{bc}	4.50^{d}	9.50^{abc}	4.26^{d}	9.06^{bcd}	3.50^{ef}
	Low Na-Chloride (Ew)	8.82^{bc}	9.76^{b}	8.65^{cde}	9.44^{b}	9.41^{bc}	9.06^{b}
	Low Na-Gluconate (Ew)	9.06^{b}	2.32^{e}	10.03^{ab}	2.50^{e}	10.12^{ab}	2.29^{f}
	High Na-Acetate	6.44^{e}	7.24^{c}	7.38^{def}	7.26^{c}	6.06^{f}	7.09^{c}
	High Na-Chloride (Em)	7.79^{bcde}	11.71^{a}	7.35^{ef}	11.94^{a}	8.15^{cde}	12.41^{a}
	High Na-Gluconate (Em)	8.59^{bc}	7.13^{c}	9.71^{abc}	5.34^{d}	7.97^{cde}	5.71^{cd}
	High Na-Chloride (Ew)	7.09^{de}	12.38^{a}	7.26^{f}	12.09^{a}	6.74^{ef}	13.35^{a}
	High Na-Gluconate (Ew)	8.38^{bcd}	4.24^{d}	10.62^{a}	4.12^{d}	9.09^{bcd}	4.79^{de}

Means with different alphabetic superscripts within a column and acid level are significantly different at $p \leq 0.05$; Means are across 17 panelists and 2 reps low acid, low salt levels. NaCl and NaGluc mixtures were not significantly different in sourness, i.e. they showed the same amount of suppression, except for the high acid, high salt level of lactic, where the NaGluc mixture was more sour than the NaCl ($p<0.05$, Table 4.1). Interestingly, the high acid level controls (no salt) were always rated saltier than the corresponding low acid level controls.

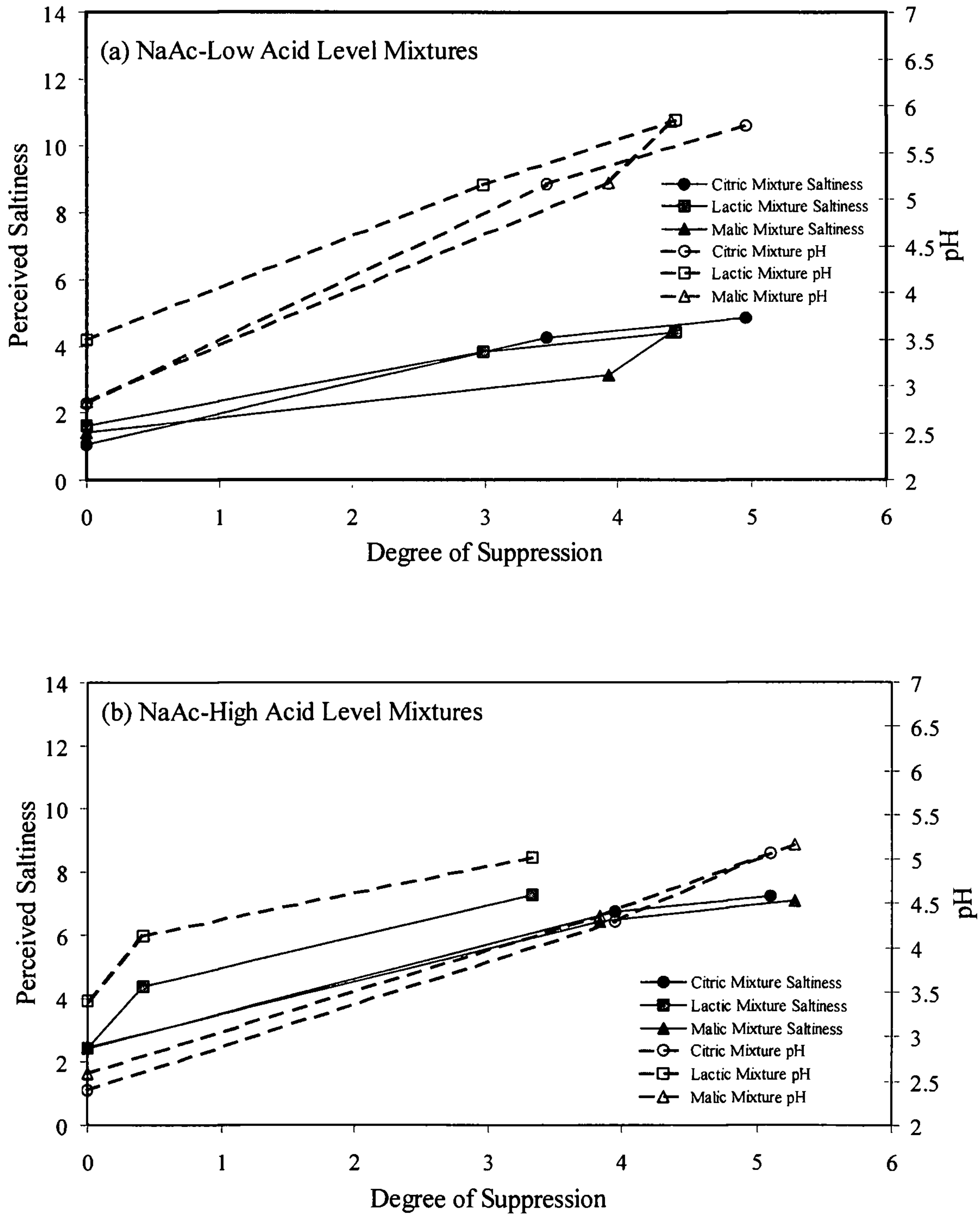
The Effect of Equiweight Salt Concentrations

At equiweight levels the NaCl mixtures were once again perceived to be significantly saltier ($p<0.05$) than NaAc and NaGluc mixtures across acid and salt levels, and NaAc mixtures were in turn significantly saltier than NaGluc mixtures ($p<0.05$, Table 4.1). In terms of sourness, the NaGluc mixtures generally had the highest mean sourness ratings and were significantly more sour than NaAc mixtures across acid and salt levels. NaGluc and NaCl mixtures were mostly not significantly different with respect to sourness. Once again the high acid controls (no salt) were rated saltier than the corresponding low acid controls.

The Role of pH

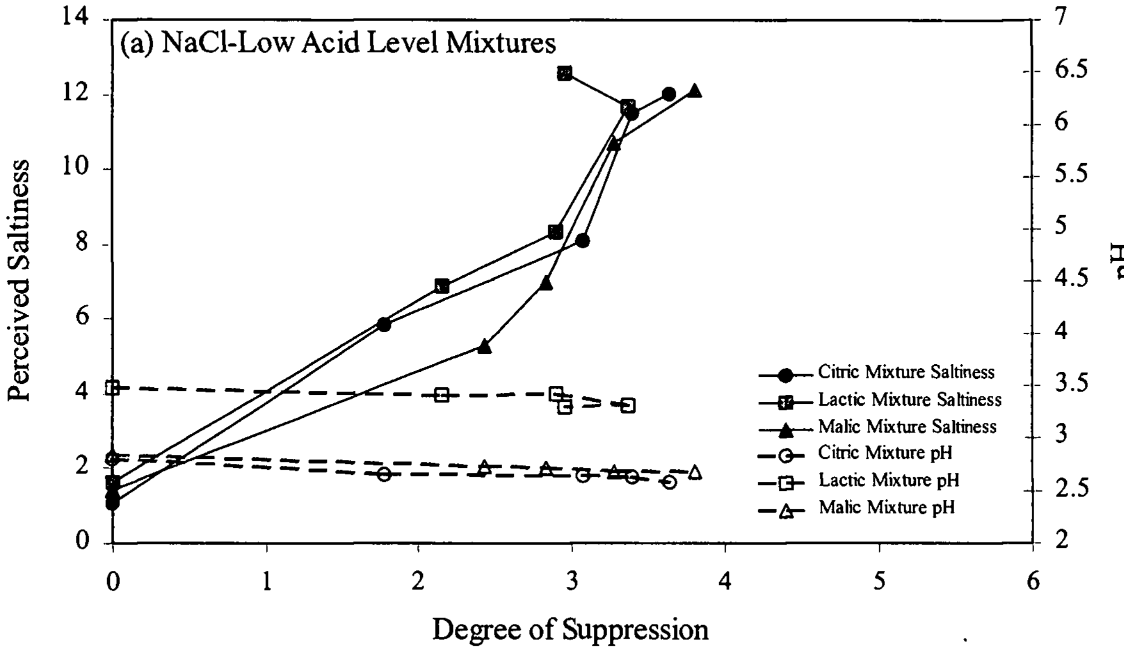
An examination of pH data showed that pH of the NaAc and NaGluc mixture solutions depended on the type and level of salt and acid present in the mixture, while in the case of NaCl mixtures, pH depended mainly on the type and level of the component acid. Figures 4.1 a-b, 4.2 a-b and 4.3 a-b illustrate the relationship between pH, perceived saltiness and degree of suppression for salts.

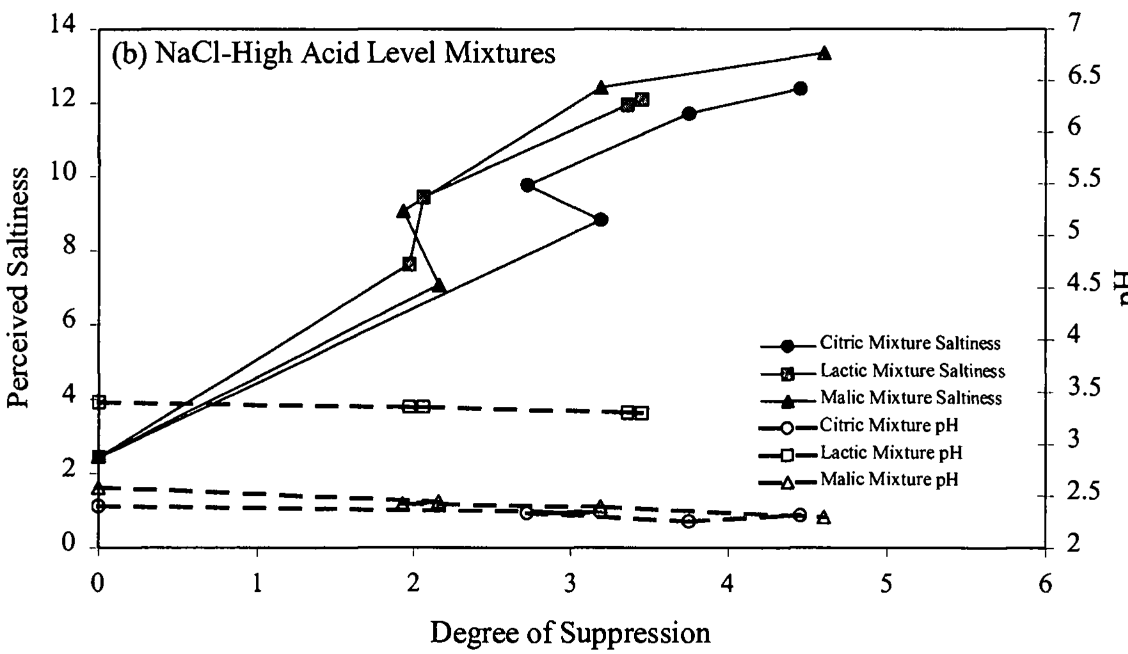
Figure 4.1 Degree of Suppression as a Function of Perceived Saltiness and pH of NaAc Mixtures at Low and High Acid Levels



Degree of Suppression refers to a reduction in the sourness rating for a mixture solution relative to the sourness rating of the corresponding acid-only control

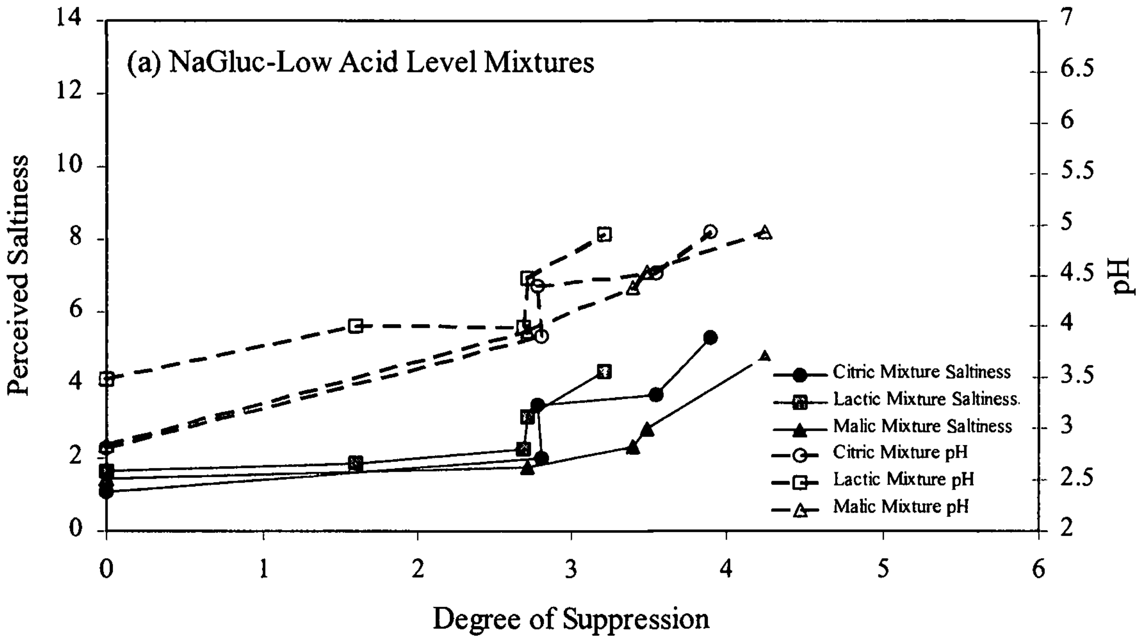
Figure 4.2 Degree of Suppression as a Function of Perceived Saltiness and pH of NaCl Mixtures at Low and High Acid Levels

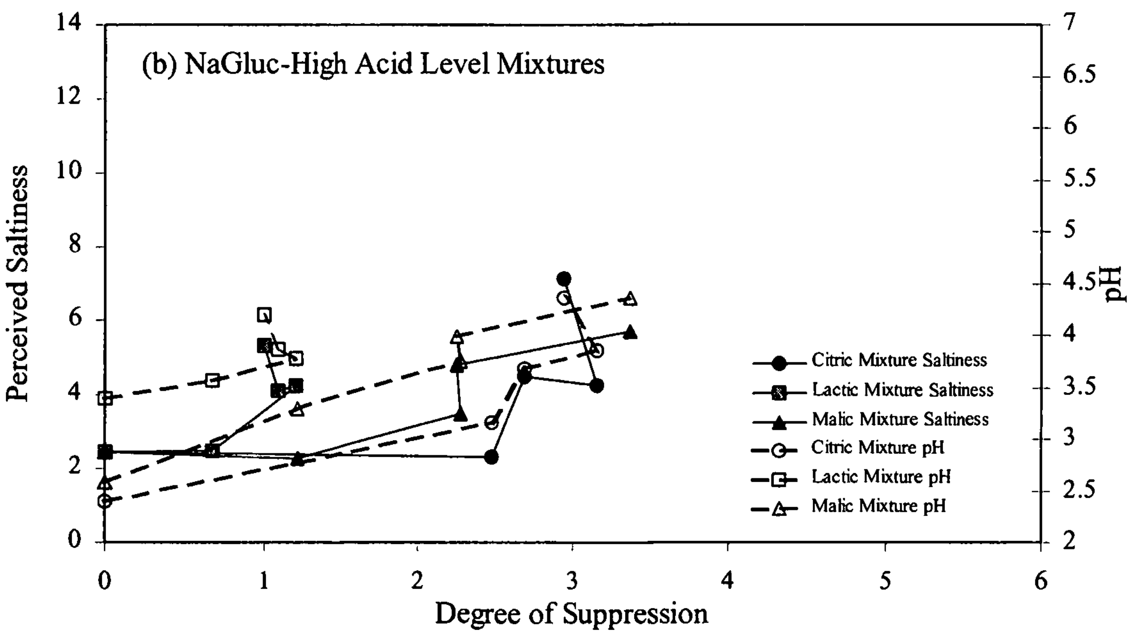




Degree of Suppression refers to a reduction in the sourness rating for a mixture solution relative to the sourness rating of the corresponding acid-only control

Figure 4.3 Degree of Suppression as a Function of Perceived Saltiness and pH of NaGluc Mixtures at Low and High Acid Levels





Degree of suppression refers to a reduction in the sourness rating for a mixture solution relative to the sourness rating of the corresponding acid-only control

pH-adjusted Equimolar or Equiweight Salt Concentration Effect

Since an examination of the effect of equimolar salt levels and equiweight salt levels from the above data was not possible without simultaneously accounting for the effect of pH, the above experiments were repeated for citric acid (CA) mixtures (low acid level only) after adjusting solution pH's to a common level. Thus the pH of the NaAc-CA and NaGluc-CA mixtures was adjusted to the pH of the corresponding level of NaCl-CA, i.e. low or high salt and equimolar or equiweight, by using 0.1N HCl (Table 4.2). The results from the pH-adjusted taste trials are shown in Figures 4.4 a-b and 4.5 a-b. From the figures it can be seen that at the pH-adjusted levels, mixtures of NaAc and NaGluc with CA were as sour as or significantly more sour than the corresponding acid-only control, effectively demonstrating no sourness suppression. Thus at equi-pH levels, only NaCl-CA mixtures showed sourness suppression compared to the control. With respect to saltiness, in general all three salt-acid mixtures were rated close in saltiness at equimolar salt levels (Figure 4.4 a-b), while NaGluc mixtures were rated significantly lower in saltiness compared to the other two, which were not significantly different at equiweight salt levels (Figure 4.5 a-b).

Discussion

There are several theories for mixture suppression and they are variously classified. One method of classification is based on the location of their proposed action in the sensory system. According to this method, the mechanism of mixture suppression could be pre-sensory, peripheral-sensory or central-sensory. A pre-

Table 4.2 Initial and Final pHs of Salt-Acid Mixtures for pH-adjusted trials

Salt -Level	**Mixtures at Equimolar Salt Concentrations**			**Mixtures at Equiweight Salt Concentrations**		
	Initial pH	Final pH	Volume of 0.1N HCl added	Initial pH	Final pH	Volume of 0.1N HCl added
NaAc - 1	3.90	2.85	4.5 mL	3.90	2.68	6 mL
NaCl - 1	2.85	2.85	-	2.68	2.68	-
NaGluc - 1	4.25	2.85	20 mL	4.20	2.68	17 mL
NaAc - 2	5.71	2.75	28 mL	5.71	2.63	30 mL
NaCl – 2	2.75	2.75	-	2.63	2.63	-
NaGluc - 2	4.76	2.75	26 mL	4.57	2.63	25 mL

Figure 4.4 Sourness and Saltiness Ratings at Equimolar Salt Levels 1 and 2, adjusted to the pH of the NaCl-CA Mixture at those molarities

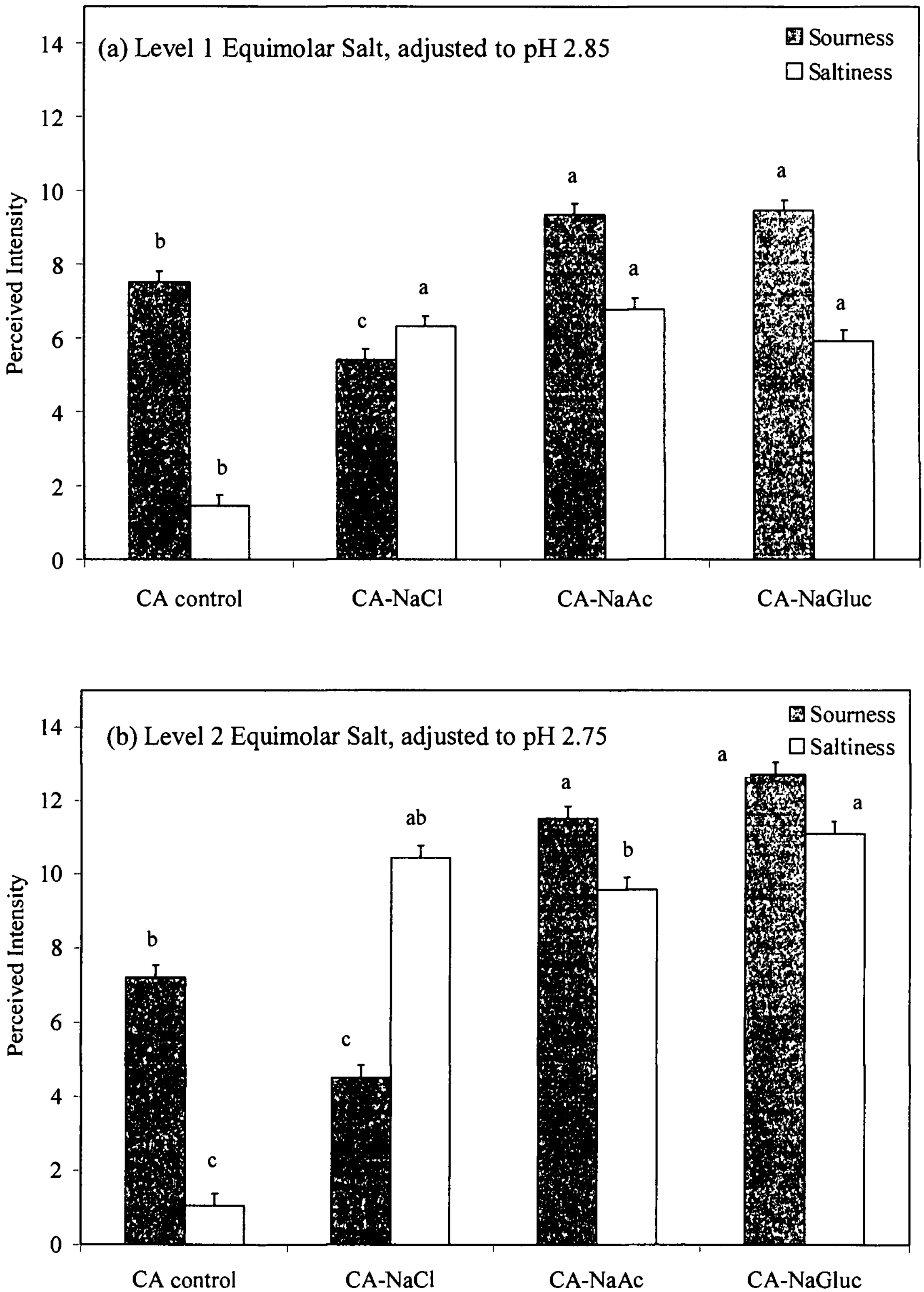
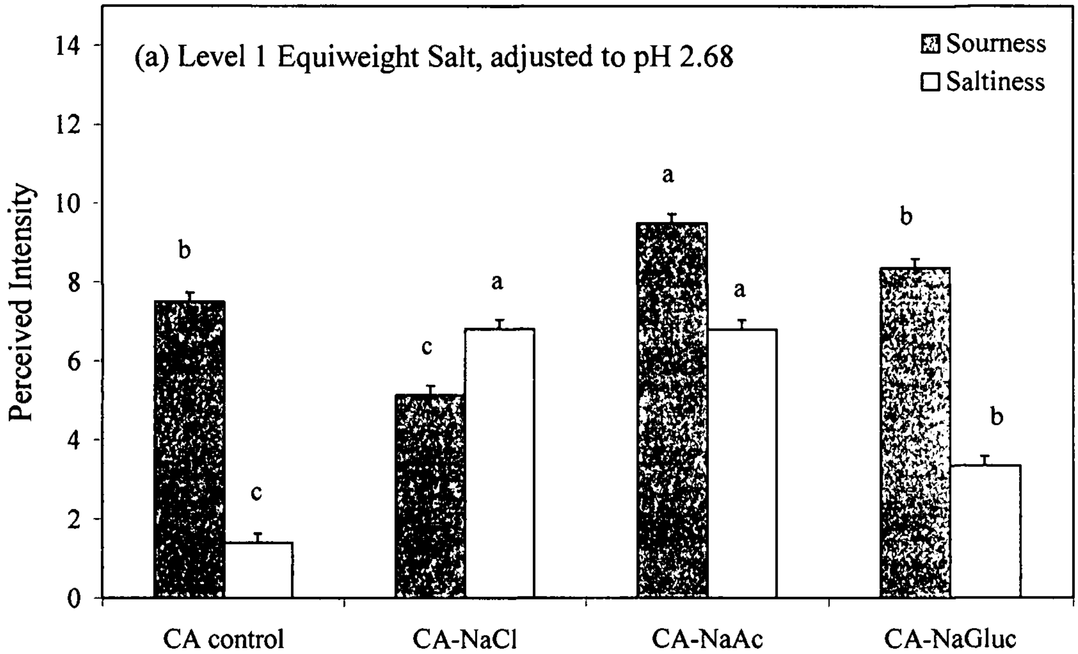
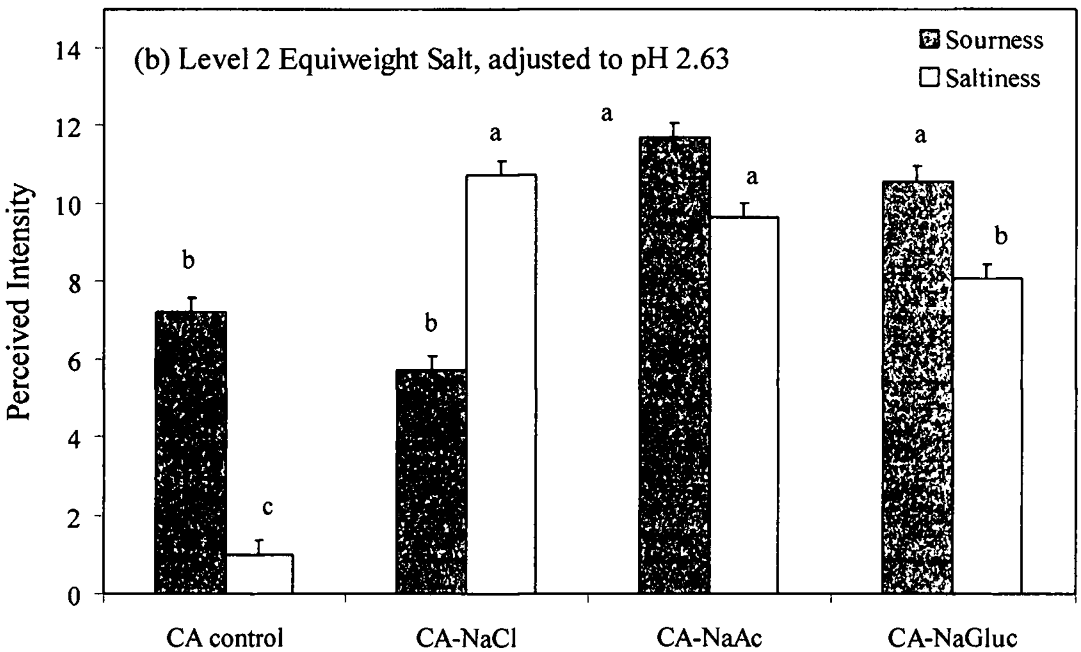


Figure 4.5 Sourness and Saltiness Ratings at Equiweight Salt Levels 1 and 2, adjusted to the pH of the NaCl-CA Mixture at those molarities





sensory mechanism of suppression would be located outside the taste system and an example of such a mechanism would include two or more chemical stimuli interacting in solution before coming in contact with the taste receptors. A peripheral sensory mechanism would be located at the peripheral level of the taste system while the mechanism of action for a central-sensory theory would be located at the central neural level (Gillian, 1982). In this study, the observed mixture suppression appears to fall mainly into the categories of pre-sensory and central-sensory mechanisms.

As can be seen from Figure 4.1 a, NaAc mixtures show increasing suppression with increasing pH, indicating a pre-sensory mechanism of action in this instance. The chemical pre-sensory interactions that could explain the increase in pH with the addition of salt would include the build up of undissociated acetic acid formed by combining acetate ions from the salt and hydrogen ions from the acid within the mixture. Such a formation of undissociated acid would reduce the number of free hydrogen ions in the system leading to an increase in pH. The relationship between degree of sourness suppression and pH in the NaAc mixtures was clear at the low acid level (Figure 4 a), but at the high acid level (Figure 4 b) there was some overlap of the pH and saltiness responses. This might have been due to the fact that the panelists tended to give higher saltiness ratings to mixtures at higher acid levels compared to equivalent salt concentrations in mixtures at low acid levels, e.g. the saltiness of low NaAc mixture with CA at the low acid level had a mean rating of 4.24, while that at the high acid level had a rating of 6.74 (Table 4.1). These higher saltiness ratings could be because of the phenomenon of salt-acid confusion (Sandick and Cardello, 1983; McCutcheon, 1986). This confusion has been hypothesized to result from acid stimuli evoking a pattern of activity in a small set of acid-sensitive receptors that is similar to the pattern evoked by a salt stimulus on a set of salt-sensitive receptors. The perceptual differences between the stimuli become more defined (less confusing) only as the size of the responding receptor sets increases and the generated neural patterns become more distinct (McCutcheon, 1986).

In the case of NaCl mixtures, the pH of the low and high salt mixtures did not change significantly from that of the acid control (without added salt) across acid types and levels, demonstrating a lack of relation between the observed sourness suppression and pH (Figures 4.2 a-b). The slight drop in pH from the low salt to high salt level mixtures within an acid level, could be due to a 'halo effect' created by the additional Na^+ and Cl^- ions in solution, that form a cloud around the dissociated acid ions – hydrogen and citrate, lactate or malate as the case might be, making the ions less available for reassociation and formation of undissociated acid, thus slightly shifting the equilibrium in favor of dissociation (Penner, 2000). Due to the lack of a relation between suppression and pH it appears unlikely that pre-sensory suppressive mechanisms are at work here. On the other hand degree of sourness suppression appeared to increase with increasing saltiness, showing a positive, linear relation between the two. As salt concentration and consequently perceived saltiness increased, suppression increased as well. Therefore sourness suppression by NaCl may be centrally mediated. At the low acid level however, an increase in saltiness was linearly related to degree of suppression only until degree of suppression reached 3 (Figure 4.2 a), after which saltiness increased without corresponding sourness reduction. At the high acid level however , the linear relation between saltiness and suppression was observed up to almost 5 on the degree of suppression scale (Figure 4.2 b). This indicates that NaCl saltiness suppresses acid sourness depending on the level of acidity, i.e. for a given acid level increasing levels of NaCl lead to higher saltiness perception and this results in increasing sourness suppression up to a point after which more salt does not give more suppression until the acid level is raised.

As in the case of NaAc mixtures above, NaGluc mixtures showed more of an association between degree of sourness suppression and pH as opposed to saltiness (Figures 4.3 a-b). As with NaAc mixtures, the increase in pH upon addition of salt was thought to be related to the formation of undissociated gluconic acid from the gluconate and hydrogen ions present in the mixture. Once again the relationship between pH and suppression was clearer at the low acid level (Figure 4.3 a) and overlaid with the saltiness response at the high acid level (Figure 4.3 b), probably due to the aforementioned phenomenon of salt-acid confusion. At the high acid level however, the lactic acid mixtures showed negligible pH or saltiness increases after reaching a value of 1 on the degree of suppression axis, and correspondingly minimal sourness suppression (Figure 4.3 b). The citric acid mixtures demonstrated sourness reduction as far as a degree of suppression value of 3, after which the trend did not seem linear. Malic mixtures showed slightly more suppression than citric and a linear trend possibly continuing beyond a 3 on the X-axis.

The effect of equimolar and equiweight salt levels was assessed independent of the pH effect in the pH-adjusted taste trials. But equimolar and equiweight levels of the three salts were found not to result in an equal degree of sourness suppression in mixtures with citric acid that had been adjusted to an equal pH (Figures 4.4 a-b and 4.5 a-b). At pH-adjusted equimolar levels the sourness ratings of the CA-NaAc and CA-NaGluc mixtures were significantly higher than that of the CA-NaCl mixture and even higher than the acid-only control (Figures 4.4 a-b). Similarly, at pH-adjusted equiweight salt levels, once again the sourness ratings of CA-NaAc and CA-NaGluc were significantly higher than those of CA-NaCl and the acid-only control (Figures 4.5 a-b). While the higher sourness values of NaAc and NaGluc mixtures might have been largely due to the added HCl, the differences observed in sourness reduction by equimolar and equiweight salt levels points away from a peripheral-sensory explanation for mixture suppression in the salt-acid mixtures studied. With respect to the effect of perceived saltiness on suppression, this could not be examined in the mixtures with all three acids since the predicted order of saltiness for the salts at equimolar levels, i.e. NaCl > NaAc > NaGluc, was not observed to occur in the mixtures (Table 4.1), and solution pH as opposed to saltiness was found to be the important factor in suppression with NaAc and NaGluc (Figures 4.1 a-b and 4.3 a-b). In the pH-adjusted trials for citric acid mixtures the role of perceived saltiness was examined, but at equimolar levels the literature-based predicted order of saltiness was once again not observed in the mixtures (Figures 4.4 a-b). In fact at equimolar levels, all three salt mixtures were found to be very close in perceived saltiness, except for NaAc-CA at the high molar level, which was slightly less salty (Figure 4.4 b).

In conclusion, with respect to the overall aim of reduction in acid sourness without increasing the pH value of the mixture over 4-4.5, NaCl was the most effective sourness suppressing salt. NaCl-acid mixtures showed significant levels of sourness suppression with no concurrent pH increase. The only potentially undesirable side effect observed in this case was the relatively large degree of saltiness of the NaCl mixtures compared to the mixtures of the other two salts, since NaCl is the saltiest of the three at equimolar levels. NaAc while being the most effective at sourness suppression, caused the largest pH increase and therefore has limited application for the purposes of this study. NaGluc caused a relatively lower amount of suppression in comparison to the other two salts and had a moderate pH increase associated with salt addition. However, in mixtures with citric and malic acids it effectively reduces sourness, does not contribute an overly salty taste and retains a pH below 4.5 at a high acid level. Thus NaGluc would be a good candidate for suppression of citric and malic acid sourness at relatively higher acid levels, while NaCl could be used with success for all the acid types and levels examined.

Sourness suppression by the three salts tested in binary salt-CA mixtures did not appear to be mediated by salt weight or molarity. Saltiness appeared to play a role in suppression only in the case of NaCl, while pre-sensory mechanisms were found to be responsible for sourness reduction in NaAc and NaGluc.

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V. SUPPRESSION OF SOURNESS IN TERTIARY MIXTURES: THE IMPACT OF TASTE QUALITY

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Abstract

The impact of taste quality on sourness suppression was examined in tertiary mixtures of citric acid with either two sugars (sucrose with fructose or glucose), or one salt and one sugar (sodium chloride with fructose or glucose) serving as maskers 1 and 2 respectively in a two-component multiple masker. Mixtures of 0.0125M of citric acid with sucrose and sodium chloride were perceptually matched for sourness. This was followed by the addition of a fixed amount of a second masker – fructose or glucose - to the equisour mixtures. The tertiary mixtures thus created were presented to a trained discriminatory panel comprising 23 pre-screened panelists, who assigned mixture intensity ratings to samples on a 16-point intensity scale. The results indicated that a two-component multiple masker was more effective when the two components stimulated different receptors/receptor mechanisms in the taste system as opposed to similar receptors/mechanisms, irrespective of whether the two components imparted the same or different taste qualities. Furthermore, a two-component masker was always more effective than either of the two components acting independently, and both components of a two-component multiple masker did not necessarily have to be effective maskers individually in order for the two of them to function together as a more effective multiple masker.

Introduction

Suppression or partial masking of a target taste can be accomplished by the use of a single masker or several maskers. Of the two, the phenomenon of suppression by more than one masker is more commonly encountered in real world foods and beverages, making the study of taste suppression in tertiary or higher level mixtures of great practical significance.

However, only a handful of studies have examined suppression in higher order mixtures. A study by Stevens and Traverzo (1997) compared the ability of sucrose and citric acid to completely mask the taste of sodium chloride in mixture with each of the maskers alone versus both of them together. Using detection thresholds as the tool for comparison of masker efficacy, the authors found that each of the maskers separately raised the detection threshold of the salt by a factor of three or four. But when used in conjunction, they raised the threshold over nine fold and in order to achieve comparable masking with either masker alone their concentrations would have had to be increased over ten times. While this study clearly showed the efficiency of multiple masking, the authors did not test or comment on how their results might have been influenced by the fact that all three components of their tertiary mixture were of a different taste quality, i.e. target = salty, masker 1 = sweet and masker 2 = sour.

In an earlier psychophysical study, McBride and Finlay (1990) looked at perceptual integration in tertiary taste mixtures of sucrose, fructose and citric acid, where mixture integration deals with ability of mixture constituents to conjointly contribute to its detectability, as opposed to mixture suppression, which focuses on the detection of one of the mixture constituents (Stevens, 1997). McBride and Finlay reconciled their results by suggesting that there might actually be two taste coding mechanisms in operation for integrative mixtures, one for taste intensity and another for taste quality. The present study was hence devised to examine the role of taste quality in mixture suppression.

From previous work with binary acid-sugar and acid-salt mixtures (Savant and McDaniel, 1999; Savant and McDaniel, 2001) it was established that among the sugars examined, sucrose and fructose were more effective sourness suppressors compared to glucose and of the salts sodium chloride was the most effective. In order to extend the findings of the prior binary studies to the present tertiary mixture trials, the same substances were used here in mixture with citric acid. Thus the objective of this study was to compare the effect of addition of a fixed amount of fructose or glucose (serving as a second masker having a sweet taste), to equisour binary mixtures of citric acid-sucrose and citric acid-sodium chloride, in order to determine the effect of a multiple masker made up of two sweet maskers (sucrose and fructose/glucose) versus another made of one salty and one sweet masker (sodium chloride and fructose/glucose) on the perceived sourness intensity of a fixed level of citric acid in tertiary mixture solutions.

Materials and Methods

Subjects

23 panelists (14 women, 9 men) were selected for participation in the study based on their performance in a pre-screening exercise that tested for sensitivity and reproducibility via a ranking test. The mean age of the participants was 27 (range 25-39 years). Subjects included students and faculty of Oregon State University and residents from the local community. All were compensated for their participation.

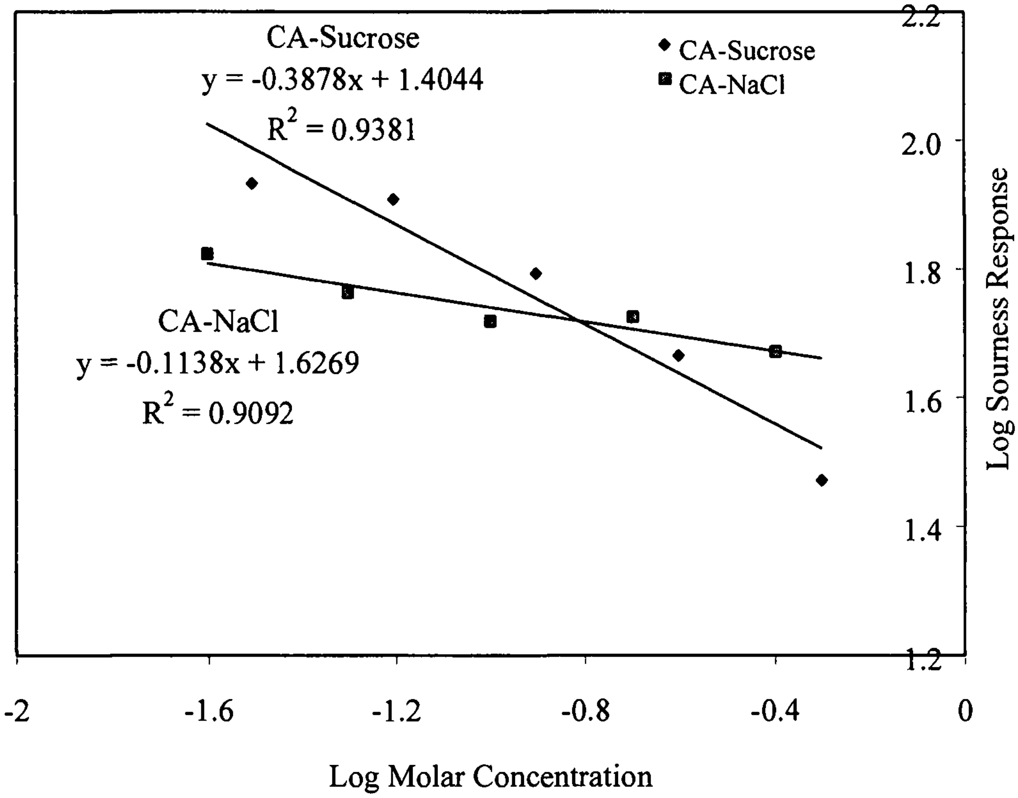
Stimuli

All solutes were food grade (purchased from Springfield Specialties, OR) and the solutions were made in distilled water. The stimuli were tertiary mixtures made up of three components including (a) citric acid (CA), (b) sucrose or sodium chloride (NaCl), and (c) fructose or glucose, all dissolved in distilled water. Binary mixture controls of CA-sucrose, CA-NaCl, CA-fructose and CA-glucose were also simultaneously presented, as was an acid-only control. The levels of sucrose and NaCl used (Table 5.1) were determined by sourness matching of mixtures of the two with CA at a fixed concentration of 0.0125M. The iso-sour acid levels were obtained by training and testing panelists using magnitude estimation as outlined by Moskowitz and Sidel (1971), and Rubico (1993). The panelists evaluated the mixture samples for sourness relative to a CA reference modulus at a concentration of 0.00625M and all samples were presented thrice for a total of three replications. The resulting power functions were in a manner of speaking a reflection of the

Table 5.1 Equi-sour Levels of CA-NaCl and CA-Sucrose Mixtures

Equisour Level	CA-NaCl Mixture		CA-Sucrose Mixture	
	CA conc. in M	NaCl conc. in M	CA conc. in M	Sucrose conc. in M
Level 1	0.0125	0.158	0.0125	0.154
Level 2	0.0125	0.063	0.0125	0.118

Figure 5.1 CA-Sucrose and CA-NaCl Mixture Power Functions of Sourness at Fixed CA Concentration of 0.0125M {Each data point represents the mean of 69 values (23 panelists X 3 replications)}



suppressive ability of sucrose and NaCl on the perceived sourness of CA. The functions of both the acid-suppressor mixtures are shown in Figure 5.1. Thus the predicted equisour level 1 and 2 concentrations of sucrose and NaCl (Table 5.1) could be described as equi-potent with respect to sourness suppression in citric acid at a concentration of 0.0125M.

The fixed level of fructose to be added as a possible third component of the tertiary mixture was taken to be equisweet to sucrose at level 1 and was determined to be 0.229M from sweetness power functions for sucrose and fructose that were established in prior work (Figure 3.2, Savant and McDaniel, 2001). The fixed level of glucose used as the alternate third component in the tertiary mixtures was taken to be 0.229M, equivalent in molarity and weight to fructose.

Procedure

Solutions were prepared 12-14 hours before testing and were stored overnight at 4°C. Prior to serving they were allowed to equilibrate to room temperature (21-22°C). Approximately 30 mL of each sample was presented in 3oz. plastic cups and samples were coded with three-digit random numbers. Seven samples were served in a set and no more than four sets were presented a day. All sample evaluations were conducted in duplicate giving two replications. Stimuli were sampled by the sip-and-spit method. After tasting the sample, panelists rated the sourness, sweetness and saltiness intensities of the mixture solution on a 16-point intensity scale (0=none, 3 = mild, 7 = mild to moderate, 11 = moderate, 15 = moderate to high). Panelists were trained in the use of the scale with the help of references and training exercises conducted prior to testing. The sourness references made with citric acid were: sour 3 (0.00313M), sour 7 (0.00625M), sour 11 (0.0125M) and sour 15 (0.025M). The sweetness references made with fructose were as follows: sweet 3 (0.0625M), sweet 7 (0.125M), sweet 11 (0.25M) and sweet 15 (0.5M). The saltiness references made with NaCl were: salt 3 (0.05M), salt 7 (0.10M), salt 11 (0.20M) and salt 15 (0.40M). Panelists were encouraged to thoroughly rinse their mouth with water between samples. Subjects were also asked to refrain from smoking, eating or drinking at least a half-hour before testing.

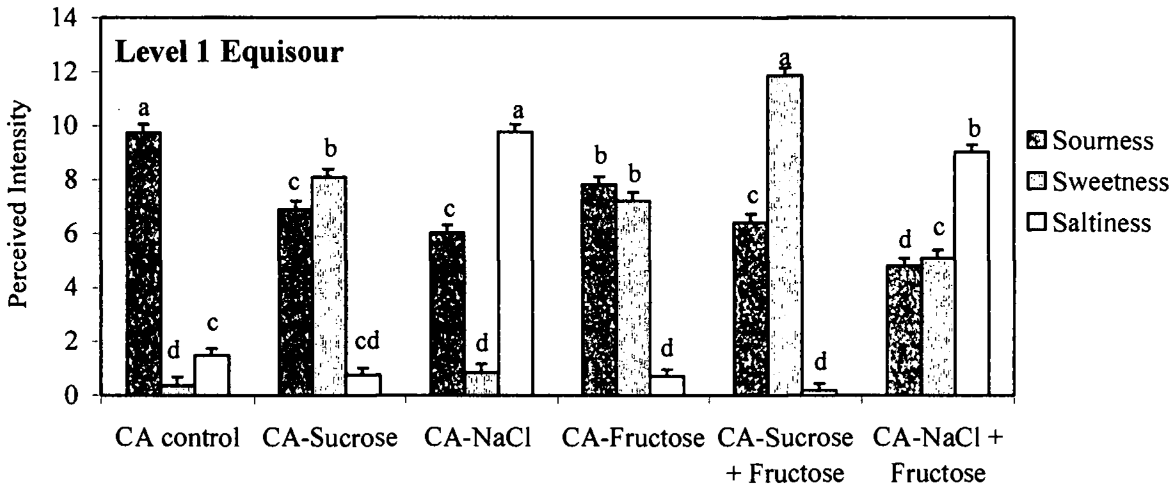
Data Analysis

The data were analyzed using multivariate analysis of variance (MANOVA) within an equisour level, with Tukey's honest significant difference (Tukey's HSD) as the post-hoc means separation procedure. The model looked at the effects of treatment (fixed effect), panelist (random effect) and the interaction of panelist and sample (random effect). Analyses were conducted using SPSS® v 10.1 (Chicago, IL).

Results

The results for the tertiary mixture experiments with the fixed amount of added fructose are shown in Figures 5.2 a-b, while the results for the corresponding mixtures with a fixed amount of added glucose are shown in Figures 5.3 a-b.

Figure 5.2 Effect of Addition of a Fixed Amount of Fructose to Equisour Mixtures of CA-Sucrose and CA-NaCl at Levels 1 and 2



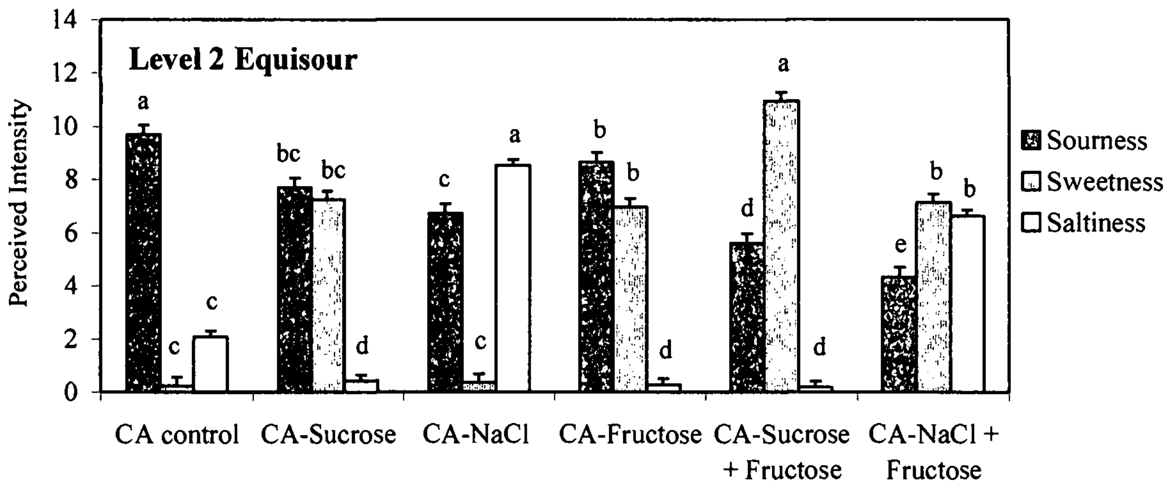
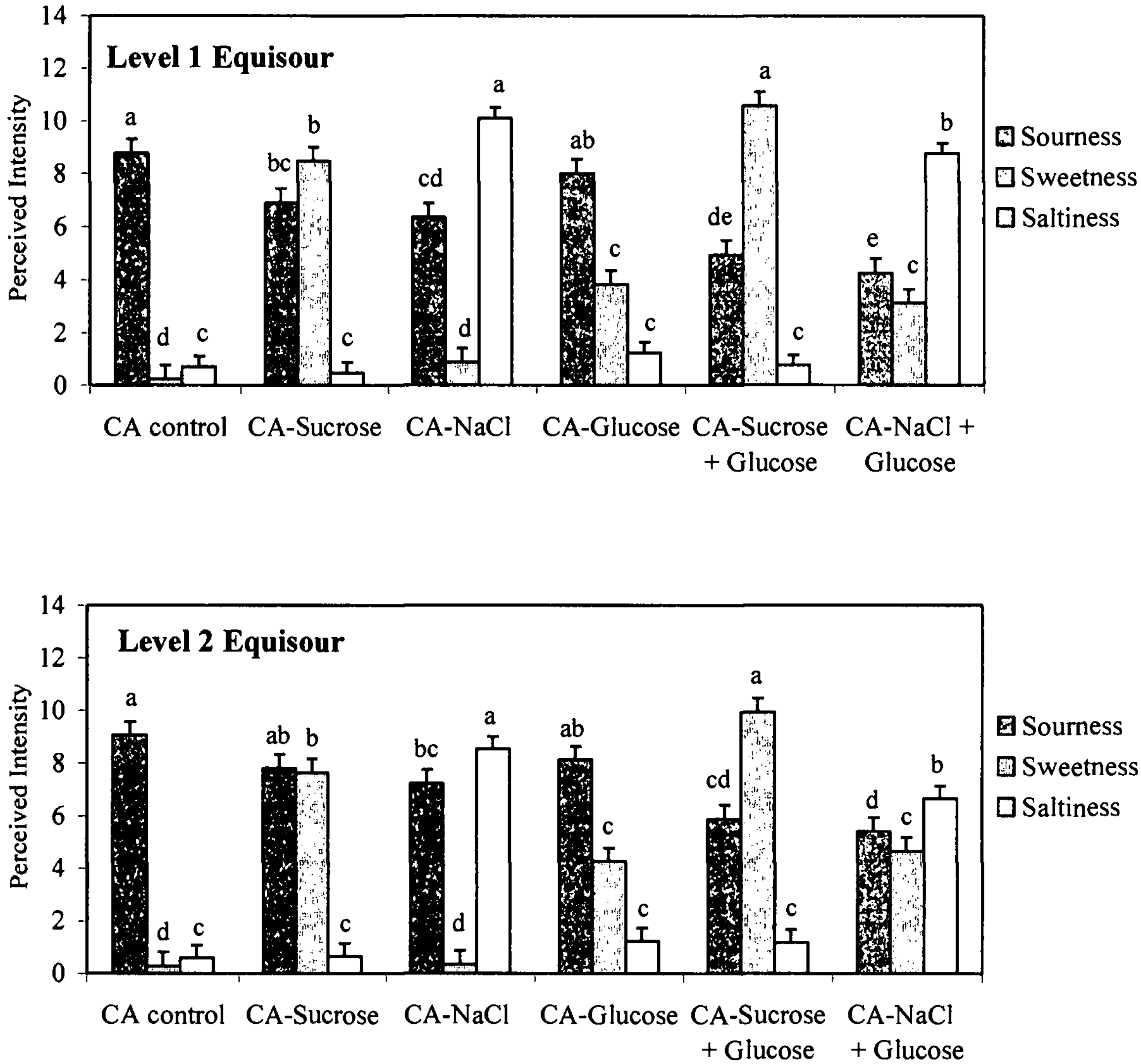


Figure 5.3 Effect of Addition of a Fixed Amount of Glucose to Equisour Mixtures of CA-Sucrose and CA-NaCl at Levels 1 and 2



Discussion

In the tertiary mixtures with fructose (Figures 5.2 a-b), the results indicated that the multiple masker made with NaCl and fructose was more effective at suppressing the sourness of CA as compared to that made from sucrose and fructose at equisour levels 1 (Figure 5.2 a) and 2 (Figure 5.2 b), since the sourness ratings corresponding to CA-NaCl + fructose were significantly lower than those for CA-sucrose + fructose at both the levels examined. The binary control ratings show the success of the sourness matching for CA-sucrose and CA-NaCl since the sourness ratings or the two mixtures were not significantly different at either sourness level. They also show that CA-sucrose and CA-fructose were not significantly different in sweetness, verifying the equisweet levels predicted for sucrose and fructose.

Thus from the results of the tertiary mixtures prepared with fructose it appears that taste quality does play a significant role in multiple masking, wherein a multiple masker comprising two maskers of two different taste qualities (e.g. one salty - NaCl and one sweet - fructose as used in this study) is found to be more effective in reduction of a target taste (in this case the sour taste of CA), as compared to another comprising two maskers belonging to the same taste quality (e.g. both sweet, i.e. sucrose and fructose as used in this study). However, earlier work on sourness suppression in binary mixtures of acids with sugars (Savant and McDaniel, 2001), and other psychophysical studies (McBride, 1989; Kennedy et al., 1997; Armstrong et al., 1998), have indicated that the sugar glucose utilized a different receptor site/mechanism from sucrose and fructose, while sucrose and fructose may utilize a common receptor/mechanism. An examination of the results in the context of these earlier findings suggests that a possible reason why the multiple masker made up of the sugars sucrose and fructose may not suppress the sourness of the target CA as much as the CA-NaCl multiple masker, may be on account of the fact that sucrose and fructose share a common receptor/mechanism, while NaCl and fructose by virtue of being a salt and a sugar that are variously transduced do not. If this were the case, then the underlying receptor/mechanism as opposed to the taste quality would better explain the resulting suppression in tertiary mixtures.

In order to confirm this hypothesis, the tertiary trials were repeated with the sugar glucose replacing fructose. The results from the tertiary experiments with glucose (Figures 5.3 a-b), indicated that the multiple masker made with NaCl and glucose was as effective at suppressing the sourness of CA compared to the sucrose – glucose multiple masker across equisour levels 1 (Figure 5.3 a) and 2 (Figure 5.3 b), since the sourness ratings corresponding to CA-NaCl + glucose were not significantly different from those for CA-sucrose + glucose at both the levels examined. This demonstrates that even though sucrose and glucose are grouped as imparting the same taste quality – one of sweetness, while NaCl and glucose have different taste qualities – salty versus sweet, there is no significant difference in the performance of multiple maskers made from either of the pairs with respect to suppression of the target sourness of CA.

Thus the findings of the present study indicate that a two-component multiple masker is more effective in suppressing a target taste if its two components stimulate different (versus similar) receptors/receptor mechanisms in the taste system, irrespective of whether they impart the same or different taste qualities. Thus taste quality is important only in that it is likely that substances evoking different taste qualities stimulate different receptors or receptor mechanisms. Furthermore, it can be seen form the sourness ratings of the binary controls, that glucose in mixture with CA did not significantly reduce CA sourness compared to the CA-only control (Figure 5.3 a-b), while sucrose and NaCl in mixture with CA significantly reduced CA sourness (Figures 5.2 a-b and 5.3 a-b) at both equisour levels. So while glucose was ineffective as a masker by itself, a multiple masker using glucose and sucrose or glucose and NaCl was more effective than glucose, sucrose or sodium chloride acting singly. This shows that the two-component masker is always more effective than either of its components acting independently, and that both components of a two-component masker do not necessarily have to be effective maskers individually in order for the two of them to function together as an effective multiple masker.

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VI. THESIS SUMMARY

Taste suppression has been closely studied in the context of bitterness. However, not much work has focused on the suppression of sourness, which is also considered an unpleasant and undesirable taste in various food and beverage formulations. Besides the dearth of literature in the area which makes its study valuable, sourness suppression is also important from the standpoint of increasing the usage of acids in their capacity as food preservatives. An understanding of the associated issues could ultimately help product developers and food processors utilize higher acid levels to ensure food sterility without simultaneously enhancing sourness.

Suppression or partial masking of sourness can be accomplished by the use of one or more maskers and by maskers of similar or different taste qualities. This research demonstrated that out of the sugars and salts examined, sucrose, fructose and sodium chloride (NaCl) constituted effective maskers of the sour taste of citric, lactic and malic acids in binary model mixture solutions while maintaining the solution pH below 4.4. In mixture with sugars it was found that suppression was not mediated by sugar molarity or weight, but was significantly influenced by its perceived intensity in most cases, suggesting that the underlying mechanisms are dominantly central. The data also supported the existence of a separate receptor site/mechanism for glucose and a peripheral component to suppression.

In mixture with salts it was found that while sodium acetate (NaAc) mixtures showed the most sourness reduction, they also showed the largest concurrent pH increase (above 4.4). Sodium gluconate (NaGluc) mixtures showed moderate suppression with citric and malic acids with pH increases remaining below 4.4, but showed little effect on lactic acid sourness. NaCl mixtures were the only ones that showed significant suppression without a pH increase and saltiness appeared to drive suppression in this case.

The tertiary trials indicated that a two-component multiple masker was always more effective than each component alone. Also the taste quality of the maskers by themselves did not appear to be a determinant of degree of suppression. Instead the receptors/receptor mechanisms stimulated by the components seemed to better relate to suppression i.e. a two-component masker was more effective when its components stimulated different as opposed to similar receptors/receptor mechanisms in the taste system, irrespective of taste quality. So a two-component masker made with a sugar and a salt could be either as effective or more effective than one made with two sugars, depending on whether the sugars stimulated different or similar receptors/receptor mechanisms. It was also observed that both components of a two-component masker did not have to be effective individually for them to function together as an effective multiple masker.

It remains to be determined whether the trends observed in model solutions will be maintained in more complex systems such as with added flavor components or in the form of emulsions, gels etc. More work of this nature could eventually lead us away from the current trial-and-error based approach to determining the ranges for mixture components in actual foods and beverages.

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