DISSERTATION

# SENSORY AND FUNCTIONAL PROPERTIES OF MONOSODIUM GLUTAMATE

Submitted by

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In partial fulfillment of the requirements

For the degree of Doctor of Philosophy

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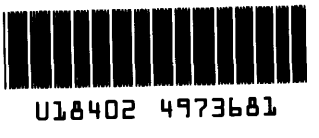

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MARIA ELIZABETH GIOVANNI ENTITLED SENSORY AND FUNCTIONAL PROPERTIES OF MONOSODIUM GLUTAMATE BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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## ABSTRACT OF DISSERTATION

### Sensory and Functional Properties of Flavor Potentiators

Flavor potentiators have been used for centuries to improve food flavor. However, neither the taste transduction mechanisms nor the behavior of flavor potentiators in food are fully understood. The objectives of this research were: 1. To determine the relationship between salivary glutamate and perception of MSG and NaCl; 2. To characterize the time-intensity profiles (TI) of flavor potentiators; and, 3. To determine the effects of heat treatment and pH on levels of L-glutamic acid in simple food systems.

The first study consisted of collecting whole mouth saliva and determining thresholds to and perceived intensities of MSG and NaCl. A preliminary experiment indicated that perception of MSG may be influenced by salivary glutamate, gender, and ethnicity. The principal study with 60 subjects found no effect of ethnicity or gender on salivary glutamate or sodium levels. Female Asians had higher salivary sodium and rated the lower concentrations of NaCl as more intense. Psychophysical measures of MSG and NaCl were independent of salivary levels.

Twenty subjects, trained in TI methods, evaluated 20 samples of MSG, IMP, and GMP, singly and in combination. The TI profiles generated were atypical of other taste modalities. Time to maximum intensity was brief, followed by a plateau phase at maximum intensity with a long aftertaste. Sample intensities varied significantly, with mixtures of 10 and 5 mM MSG and 2.5 mM IMP and GMP having highest intensity and duration. These results indicated that flavor potentiators may increase total flavor in the mouth. Synergism among flavor potentiators was demonstrated.

To determine the effect of pH and heat on L-glutamic acid, 0.1% MSG was added to eleven simple food systems. Percent recovery was highest for tomatoes and lowest for beef broth. Fish broth and tomatoes had higher recoveries at pH 6 than pH 3; thus, pH altered L-glutamic acid levels. No effect of heat on L-glutamic acid levels was found.

The sensory and functional behavior of MSG is governed, in part, by the individuality of the subject, its temporal response, and the food system in which it is used.

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The friendship and support of my classmates was vital, as was the love from my husband Ted and my sons, Vincent and Paul.

## DEDICATION

This manuscript and the effort put into it is dedicated to Professor Rose Marie Pangborn, one of the pioneers of sensory evaluation. She was committed to the evolution of sensory evaluation as a scientific discipline and to the growth of her students,of which I was fortunate to be. May her commitment and her sense of humor continue through those of us who have been influenced by her.

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# CHAPTER 1

## Introduction

Food flavor historically has been improved by the addition of ingredients such as mushrooms, soy sauce, cheese, and other foods high in naturally-occurring flavor potentiators. Monosodium glutamate (MSG) was isolated in 1908 from seaweed by Ikeda, who described the flavor MSG imparts to foods as "umami". Umami originates from the Japanese word for deliciousness, while Americans describe this sensation as savory or meaty (Maga, 1983). MSG can intensify the flavor of foods to which it is added, and also can provide its own taste, including a sensation of fullness in the mouth. Disodium inosinate (IMP) and disodium guanylate (GMP) were later isolated and have becomes important flavor potentiators in commercial applications. These compounds act synergistically with MSG to enhance food flavor.

Neither the taste mechanism nor the interaction of flavor potentiators in the food matrix is well understood. Both NaCl and MSG have sodium as a cation, which is critical to the salty taste of NaCl and to the flavor-enhancing properties of MSG (other glutamate salts do not have the same

flavor potentiating properties). Salivary and dietary sodium are involved in the taste perception of NaCl, although the specific relationships are unclear. The role of salivary or dietary glutamate in taste perception has had little research and more exploration of the effects of saliva for gustation is needed (Christensen, 1986). Another consideration in understanding the mechanism of flavor potentiators is to study the impact of experience and taste sensitivity by different ethnic groups on the perception of MSG.

Sensory responses to flavor potentiators generally have been made at a single point in time. However, flavor is a dynamic event that changes as the food is ingested, chewed, and swallowed. Profiling taste changes over time can demonstrate masking, synergy, aftertaste, and other qualities of a stimulus. Time-intensity (TI) methods have been applied to a variety of taste modalities. TI can be used to describe chemoreception and to model sensory adaptation. Birch (1987) has suggested that the understanding of MSG taste could be improved by the use of TI data, such as reaction time and persistence data. No researchers have characterized the TI profiles for any of the flavor potentiators to date.

The effectiveness of specific flavor potentiators is dependent upon the food system. The effectiveness of MSG in protein systems such as fish, meat, and vegetable foods is well-established. However, why MSG is

effective in some food systems and not in others in not well understood. The fate of MSG could follow several paths, depending upon the other ingredients in the food system and the type of processing and storage conditions used. Glutamate may bind with protein, carbohydrate or fat, could be converted to another chemical, or may remain in the sodium form as free glutamate; however, these theories have not been tested. The first step is to examine the amount of glutamate liberated or bound by various treatments. An expert panel on MSG convened by the Life Sciences Research Office (1995) called for more studies to understand the effect of food composition and food processing on MSG stability.

Thus, the specific objectives of this research were:

1. To determine the relationship between salivary composition, namely L-glutamic acid and sodium, and taste perception of flavor monosodium glutamate (MSG) and sodium chloride using threshold and intensity measurements and secondarily, to examine the role, if any of gender (male or female) or ethnicity (Asian or non-Asian) in the taste perception of MSG and NaCl;

2. To characterize the time-intensity profiles of flavor potentiators; and,

3. To determine the influence of heat treatment and pH on levels of free glutamate in simple food systems of protein, carbohydrate (including vegetable), and fat.

To accomplish these objectives, three studies were conducted, as detailed in Chapters 3, 4, and 5.

# CHAPTER 2

## Literature Review

I. Chemistry of Flavor Potentiators

A. History of Flavor Potentiators

The choice to consume a particular food is generally based on its sensory properties, such as appearance, odor, taste, and texture, rather than any nutritional consequence that may result from eating the food (Baker, 1982). Food from Asian cultures has long been recognized as fulfilling the requirements for both good health and good taste. Much of the flavor in Asian food can be attributed to the use of broth from different plants and animals. Kikunae Ikeda of the University of Tokyo is considered the first to isolate monosodium glutamate (MSG) from the dried seaweed kombu or sea tangle (*Laminaria japonica*), and identify it as the compound responsible for enhancing or intensifying the flavor of foods in which it was used (Sjöstrom, 1972: Yamaguchi, 1991). Previous to Ikeda's discovery, glutamic acid had been isolated from wheat gluten Fitthausen; however, the flavor enhancing properties of the compound were not discovered at this time (Sjöstrom, 1972).

Ikeda described the taste MSG imparts to food as "umami", derived from the Japanese word for deliciousness (Yamaguchi, 1979; Maga, 1995). Americans generally describe this sensation as savory, meaty, or brothy. A process for extracting MSG from wheat and other flours was then developed. In the 1940's, large-scale production of MSG began in the United States using by-products from sugar production. By the following decade, this process did not meet the demand for MSG and in 1959, a group of bacteria was discovered which produce large amounts of MSG (Margalith, 1981). Soon after the isolation of MSG, nucleotides were reported to have flavor potentiating properties by Kodama in 1913 (Margalith, 1981). These compounds were isolated from two other broths commonly used for flavor in Japanese cooking: katsuobushi, made from dried flakes of the bonito fish, and shiitake, made from shiitake or black mushroom (*Lentinus edodus*) (O'Mahony and Ishii, 1985). The active ingredient in katsuboshi is the histidine salt of inosinic acid, now produced as disodium 5'-inosinate (or inosine monophosphate, IMP) and in shiitake, the active component is disodium 5'-guanylate (guanosine monophosphate, GMP).

B. Definition

MSG is generally considered to be a flavor enhancer or flavor potentiator, terms that are often used interchangeably. A flavor enhancer is "a substance added to supplement, enhance or modify the original taste and/or aroma of a food without imparting a characteristic taste or aroma of its own" (U.S. Code of Federal Regulations 170.3 [0][11]). This definition is considered to encompass both flavor enhancers and flavor potentiators. "Flavoring agents and adjuncts" are defined as "substances added to impart or help impart a taste or aroma in food." (21 CFR 170.3 [0][12]). However, Komata (1990) argued that glutamate and 5'-ribonucleotides are not flavor enhancers because they contribute the umami taste to food.

Practically, the terms used to label these compounds are sometimes used interchangeably and have various definitions. Sjöstrom (1972) noted that the term potentiator, borrowed from pharmacology, describes "an action wherein the agent by itself, in small quantities, has no effect on a biological system, but exaggerates the effect(s) of other agents in that system". Maga (1983) defined flavor potentiators as compounds that do not have their own sensory character but modify the sensory characteristics of other compounds via intensification or some form of masking. Yamaguchi (1991) noted that although no academic definition of flavor enhancer exists, they are generally

considered to be substances that do not have a flavor of their own. However, MSG can be perceived at suprathreshold concentrations; thus, the term flavor enhancer applies to MSG when used at subthreshold concentrations, at which levels it enhances flavor due to its synergistic interaction with the 5'-ribonucleotides.

Several researchers have experimentally defined flavor potentiation. Van der Heijden *et al.* (1983) stated that enhancement is used primarily for research describing flavor compounding effects, while potentiation is used in psychophysics when a substance increases the intensity of a stimulus with a different taste. In a patent for a food ingredient that contributes flavor and fiber, Cox (1991) defined flavor potentiators as compounds that increase the sensitivity of the taste buds. Cox considered flavor enhancers, including MSG, as compounds that act as solvents or detergents, freeing more flavors from foods, making them more available to the taste buds. In an experiment to determine the impact of MSG on flavorings, Kemp and Beauchamp (1994) defined flavor potentiators as compounds that increased the perceived intensity of the flavor of another compound, while a flavor enhancer was characterized as a substance that increased the perceived pleasantness of another substance. They suggested the term flavor modulator be used for MSG as they found differential suppression rather than true potentiation, a

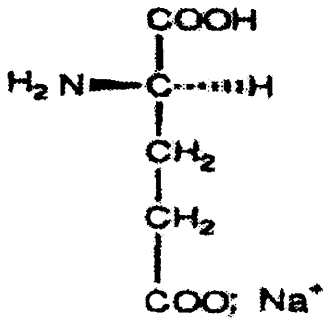
result most likely due to their choice of flavor systems in which to test MSG. A flavor integer is an absolute chemical such as vanillin, which by itself exerts its flavor in a food.

C. Production and Consumption of Flavor Potentiators

With the increasing demand for convenience and "instant" foods, which are usually produced using high heat processes that decrease flavor, food manufacturers have relied on flavor potentiators to provide the flavor impact that consumers demand at a reasonable cost. A variety of flavor potentiators are available for use, including monosodium glutamate, 5'-ribonucleotides, hydrolyzed vegetable protein, and yeast. Several comprehensive reviews have been published on flavor potentiators, including Kuninaka (1981), Maga (1983, 1995), Sjöstrom (1972), and Sugita (1990).

1. Monosodium Glutamate

As the sodium salt of one of the most abundant amino acids in nature, MSG is found endogenously in many foods and is added to others to potentiate their flavor (Figure 1).




L-GLUTAMATE, $Na^+$
(MSG)

Figure 1. Structure of monosodium glutamate

The primary use of MSG as a food additive is to potentiate flavor in meat, fish, poultry, vegetables, soups, and sauces. The recommended usage level is 0.02 to 0.8% by weight. The flavor potentiating mechanism of MSG is not understood, including the explanation why MSG is effective in some food systems such as meat, seafood, and vegetables, but not in others, for example, milk and cereals (Maga, 1987). As with sour and salty tastes, the taste of MSG is self-limiting, where a maximum hedonic score is reached at a low concentration of MSG and the taste of subsequently increased levels of compound in solution rapidly becomes unpleasant. MSG is water-soluble and mixes easily into foods before, during or after cooking, and so can be easily added by the consumer. Two low sodium glutamates, monoammonium glutamate and monopotassium glutamate, are also

commercially available (Anonymous, 1998).

Some researchers have demonstrated that MSG may mask or suppress off-flavors, such as bitterness, especially when combined with peptides (Arai, 1980), or sulfur notes (Margalith, 1981). MSG enhances several specific flavor characteristics such as continuity, complexity, mouthfulness, impact, and mildness, thus improving food palatability (Konosu *et al.*, 1987). At low concentrations, MSG does not impart its own taste to foods. In water, MSG has a unique taste sensation the Japanese call umami (Yamaguchi, 1991), described as a persistent sweet and salty taste with some tactile sensation (Margalith, 1981).

Daily consumption of MSG in the U.s. has been estimated to be between 0.4 to 0.55 grams per person using marketplace disappearance data (Life Sciences Research Office, 1995). Using production data, the Federal Register (1996) estimated that 28,000 tons of MSG were used in the United States in 1995, resulting in a per capita consumption of 0.5 to 1 gram of free glutamate per day. Rhodes *et al.* (1991) conducted an extensive survey of the MSG content of 228 foods commonly consumed in the United Kingdom that may contain added MSG. Using consumption data from the National Food Survey report, the per capita weekly intake of MSG was estimated to be 4.1 grams, or 0.6 grams per day, similar to the U.S. estimate.

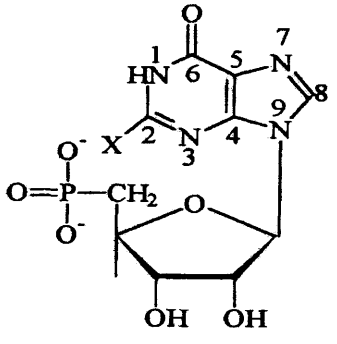
Industrial production of MSG began in 1909, using a process that extracted wheat gluten by washing the gluten from the starch (Anonymous, 1987). Crude gluten, containing up to 25% L-glutamic acid, was hydrolyzed, concentrated and crystallized. In 1965, developments in biotechnology led to the discovery that *Corynebacterium glutamicum* can accumulate large amounts of L-glutamate when grown on a media containing carbohydrate sources, nitrogen salts, and carefully controlled levels of biotin (Margalith, 1981). Under optimum conditions, *C. glutamicum* can produce 30 to 50 g per liter of MSG with a defective TCA (tri-carboxylic acid) cycle that preferentially converts α-ketoglutarate to glutamic acid rather than succinate. Fermentation using carbohydrate sources such as cane sugar, beets, or tapioca is still the commercial method used (Margalith, 1981; Anonymous, 1987; Kawakita, 1992).

2. 5'-ribonucleotides

5'-ribonucleotides are derived from nucleic acids and, as such, are naturally occurring in many animal and vegetable foods. Inosine 5'-monosphosphate (IMP) and guanosine 5'-monphosphate (GMP) are most often used in commercial applications. These compounds are also referred to as disodium inosinate and disodium guanylate, respectively. The synergism of 5'-ribonucleotides with MSG and with one another is well-

documented. In addition to imparting a umami taste to food, 5'-ribonucleotides also give the impression of increased thickness or mouthfeel (Yamaguchi and Kimizuka, 1979). They have also been found to suppress undesirable off-flavors (Woskow, 1964), including sulfur and bitter tastes (Schiffman and Gill, 1987). The chemistry, production and use of ribonucleotides have been reviewed by Kuninaka *et al.* (1964) and Shimazono (1964).

The chemical structure of 5'ribonucleotides consists of a nucleic acid base and the 5-carbon hemiacetal ribofuranose (Figure 2).




X: H, 5'-IMP

X: NH2, 5'-GMP

Figure 2. Chemical structure of 5' -ribonucleotide

For taste potentiating effects, the ribonucleotides require a purine base, hydroxylated at the 6 position, and a phosphate ester in the 5' position. Both the primary and secondary hydroxy groups must be dissociated for the savory taste; if esterified, the ribonucleotide loses this taste. GMP has a

more intense flavor potentiating effect that IMP (Kuninaka *et al.*, 1964). The flavor activity of GMP is 2.1 to 5.5 time greater than IMP, depending on concentration and the other constituents in the food (Shimazono, 1964).

IMP is predominantly found in animal foods, especially marine animals (Komata, 1990), and GMP occurs most often in vegetable foods, particularly mushrooms. These two 5'-ribonucleotides are most often used commercially. Early uses of ribonucleotides included 5'-xanthylate but it has relatively weak flavor potentiating activity (Trivedi, 1986). The two key parts of the molecule that contribute to its flavor potentiating activity are the purine base, hydroxylated in the 6 position, and the phosphate ester, which yields most potentiation in the 5" position (Margalith, 1981). Yamaguchi *et al.* (1971) tested the relative intensities of synthetic nucleotides and developed a universal mathematical relationship between flavor amino acids and 5'-nucleotides, demonstrating synergism.

In processed foods, IMP has the advantages of being more soluble in acidic and aqueous systems than GMP. IMP and GMP have their own taste quality, but also act synergistically with one another and with MSG to provide a greater flavor potentiating effect. IMP at 0.01% in a beef-noodle soup increased flavor intensity, vegetable flavor, and apparent viscosity, differences that were noticeable to consumers (Caul and Raymond, 1964).

Direct consumption levels of IMP and GMP have not been measured. Indirect estimates of 2500 mg/day of IMP among Americans have been made by Maga (1995). IMP is formed during the post-mortem enzymatic degradation of ATP and thus is primarily found in animal foods (Komata, 1990). Autolytic enzymes degrade ATP to AMP, which is deaminated to form IMP. However, further storage leads to the degradation of IMP to hypoxanthine, which has a bitter taste (Margalith, 1981).

The stability of IMP and GMP varies with the pH and temperature of the food system. They are easily split by phosphomonoesterases (Shimazono, 1964). High temperatures and low pH, such as found in canning, cause the hydrolysis of the glycosidic and phosphate bonds, rendering the ribonucleotide ineffective as a flavor potentiator. In addition, high processing temperatures can destabilize these compounds, as they are hydrolyzed to nucleosidic bases under acidic conditions and to their corresponding base under alkaline conditions. In general, heating a food product that contains 5'-ribonucleotides to temperatures up to 120°C has little effect on the resulting flavor (Shimazano, 1964; Maga, 1983; Sugita, 1990). Only one study was found using subjective evaluations to measure these changes (Kuhiba-Manabe *et al.,* 1991a). In this study, both IMP and GMP were found to be less thermally stable than MSG due to weak

glycosidic bonds and ester linkages. Heating IMP decreased concentrations to one-half of the difference threshold, an imperceptible level. Inosine, a bitter tasting compound, was formed at one-tenth its detection threshold. Thus, heating IMP did not affect its contribution to the umami taste or create off-flavors. No comparable studies for MSG were found. Analytical studies have shown that the cleavage of the phosphoric ester bond in IMP and GMP when these substances are heated can be depressed by $CaCl_2$, $MgCl_2$, or $MnCl_2$, but not by NaCl or KCl (Kuchiba *et al.*, 1990). The sensory impact of these salts was not determined. Other researchers found that 5'-ribonucleic acids are heat stable but are destroyed under acidic conditions and by phosphomonoesterase, which splits the phosphomonester linkage (Maga, 1983; Sugita, 1990). Phosphomonoesterase is found in many plant and animal products and should be inactivated via processing before the addition of IMP or GMP. GMP and IMP from yeast are hydrolyzed during heating and drying to guanosine and inosine, with longer heating times increasing hydrolysis (Fish, 1991).

Ribonucleotides are produced by direct fermentation, the breakdown of RNA, and chemical synthesis; however, the last method is not commercially used by the food industry (Trivedi, 1986). Direct fermentation uses a pure culture that produces an abundance of nucleotides. Cultures that

are most useful include *Bacillus subtilis, Brevibacterium ammoniagenes* and *C. glutamicum* (Margarlith, 1981). High production of nucleotides is induced through the use of mutant strains, for example, altering the sensitivity of IMP dehydrogenase in the bacteria so that it can tolerate higher levels of GMP (Margalith, 1981). Subjecting the bacteria to physiological stress also induces nucleotide products. RNA from yeast or bacteria can be degraded by 5'-phosphodiesterase to produce 5'-ribonucleotides. End products of this enzymatic hydrolysis are 5'-AMP, 5'-GMP, 5'-CMP, and 5'-UMP. AMP can be enyzmatically or chemically deaminated to 5'-IMP.

IMP and GMP have also been found to have antioxidant properties above $A_w$ 0.25 with the strongest antioxidant effect at $A_w$ 0.50, possibly through a chelating action; however, they are not as effective as BHT or EDTA (Kuchiba *et al.*, 1989).

3. Other Flavor Potentiating Compounds

In the 1970's, concerns about the safety of MSG received media attention, resulting in food manufacturers finding substitute compounds for MSG. One such suitable compound is hydrolyzed vegetable protein (HVP). HVP contributes approximately 25 mg of free glutamate per 100 g of HVP, and consumption of free glutamic acid from HVP is approximately 0.022 g/day per person (Federal Register, 1991). The glutamic acid content of

HVP from soy grits is 8.85%, as compared to 2.42% in beef extract (Dzanic *et al.,* 1985). HVP is also used as raw material for amino acid isolation and as an antioxidant in the meat, confectionery, and baking industries. Sources of proteins are primarily wheat gluten and defatted soy grits. Alternative sources include algae, alfalfa (Dzanic *et al.,* 1985), and other cereals. The best sources of HVP produce a light colored, low flavored product. Other sources may be less expensive and can be used in dark-colored and intensely flavored foods such as sausages.

Protein hydrolysates are nitrogenous compounds that improve the flavor of baked goods, soups, gravies, sauces, seasonings, and salt substitutes. Industry usage of protein hydrolysates estimates that average intake is 0.60% of Americans' total dietary intake. Hydrolysis of proteins by acids or enzymes yields many peptides and amino acids that have flavor potentiating properties. The resulting amino acids may be in the salt form, including glutamate. Glutamic and aspartic acids are sour when dissociated, yet yield a umami response when in the sodium salt form. Free amino acids play an important role in vegetable flavor. Two amino acids isolated from mushroom species, tricholomic acid and ibotenic acid, have fairly low thresholds and work synergistically with MSG but are not utilized commercially (Margalith, 1981). The source of protein can be any protein-

containing material such as soy meal, wheat and corn gluten, rice flour and animal proteins (Federal Register, 1991). Lieske and Konrad (1994) reviewed protein hydrolysis with an emphasis on meat flavoring systems.

In the 1960s, yeast was explored as an inexpensive, high-protein, vitamin-rich preparation for use in emergency food situations (Margalith, 1981). Although the high levels of nucleic acids and use of hydrocarbons for a substrate limit the use of yeast as a primary food source, their ability to improve flavor was explored further. Viable yeast can be enzymatically hydrolyzed into peptides and amino acids that have flavor potentiating properties. Patents using yeast to provide a meat-like flavor and to improve the palatability of low calorie foods have been issued (Margalith, 1981). Recent advances in biotechnology have yielded yeast extracts which are inexpensive to produce and improved in quality (Nagodawithana, 1992).

The taste of peptides can be bitter, sweet, sour, and salty, and peptides can also potentiate other flavors (Cagan 1987; Monjon and Solms, 1987). Most hydrophobic L-amino acids have a bitter taste, which indicates the bitterness may be due to the hydrophobicity of the amino acid side chain. Kato *et al.* (1989) have reviewed the taste of amino acids and peptides. Some peptides, including aspartame, thaumatin and monellin, have an intensely sweet taste, with the latter two 1600 and 3000 times sweeter than

sucrose, respectively. Different peptide fractions may be responsible for the umami taste, particularly those fractions that are at the N-terminus of dipeptides. In contrast, van den Oord and van Wassenaar (1997) did not find umami taste in 12 dipeptides and 4 tripeptides all containing glutamate. They concluded that the taste of glutamate is lost in the peptide. The structures of amino acids and peptides have been explored as stereochemical models for bitter and sweet taste transduction mechanisms. These theories are further discussed in the taste transduction mechanism section in the Literature Review.

Meat flavor is complex, with many flavor compounds forming as a result of heating the meat (Lieske and Konrad, 1994). Examples of reactions that occur with heating include proteolysis, denaturation, the Maillard reaction in conjunction with a reducing sugar, and synthesis and modification of new peptides. Low molecular weight peptides are important contributors to meat flavor (Lieske and Konrad, 1994). During cooking, these peptides are generated by proteolysis and peptide modifications of other compounds resulting from the Maillard reaction (amino acids and reducing sugars). Half of the low molecular weight peptides formed during cooking are hydrophilic and are usually sweet tasting, and the rest are hydrophobic, which are usually described as bitter or sour. When meat was

cooked, stored and re-cooked, the proportion of hydrophobic peptides increased (Spanier and Edwards, 1987).

Some amino acid combinations have a meaty or savory flavor, which can be potentiated with ribonucleotides (Fuke and Konosu, 1991; Pennisi, 1992; Zhang and Ho, 1991). Isolation of the "beefy meaty peptide" (BMP) from beef was reported in 1992 (Spanier *et al.*, 1996). BMP, with an amino acid sequence of Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala, is reportedly more effective than MSG in enhancing savory flavor, particularly meat flavor. The sensory properties of this peptide had earlier been discribed as umami and salty due to a basic amino acid, lysine, at the N-terminal and two acidic amino acids, aspartate and glutamate, in the middle (Tamura *et al.*, 1989). Spanier *et al.* (1996) later synthesized BMP and proposed the acronym STEP, Savory Taste-Enhancing Peptide, for this compound. They also suggested a molecular mechanism for taste perception using a structure-function model. The taste quality and intensity of BMP is dependent on pH, with the most intense umami taste found at pH 6.5 (Wang *et al.*, 1996). This study also determined that the addition of BMP to diluted beef extract increased the meaty or savory flavor of the extract. MSG and NaCl were found to interact synergistically with BMP. Although most research has focused on meat flavor, Komata (1990) reviewed the compounds responsible

for seafood flavor, noting that free amino acids and peptides are significant compounds in the umami taste of seafood. Glutamate and ribonucleotides were found to be key components of crab flavor by Konosu *et al.* (1987). They extracted the flavor components from fresh crab and reconstructed the crab flavor using analytical methods.

Sodium chloride (NaCl) is commonly used to add its own taste and also to enhance the flavor of many foods. NaCl works in a wide variety of systems and is inexpensive; however, consumers believe overuse of sodium to be linked to health problems (Lynch, 1987). In sweet foods, such as watermelon, the use of salt to intensify the sweet flavor is common. Salt appears to differentially intensify desirable flavors and suppress off-flavors (Breslin and Beauchamp, 1995).

A wide variety of herbs, spices, juices, and other condiments, such as basil, lemon juice, chicken broth, catsup, and parmesan cheese are used to enhance flavor. Many of these condiments, particularly those from animal, vegetable, and fermented sources, contain high levels of naturally occurring glutamate and ribonucleotides.

D. Safety of Flavor Potentiators

The safety of flavor potentiators, most specifically MSG, has repeatedly been questioned in the past 20 years. MSG, IMP, GMP, and protein

hydrolysates are on the FDA's Generally Recognized as Safe (GRAS) list of food ingredients (Maga, 1995). These ingredients also have wide international acceptance. However, since Kwok's report (1986) of symptoms after consuming MSG, the metabolism, safety, and regulatory status of MSG have been extensively reviewed.

1. Glutamate Metabolism

Glutamate, a nonessential dicarboxylic amino acid, serves as an intermediary in gluconeogenesis, protein synthesis, neurotransmission, and amino acid metabolism. Despite large daily intakes of glutamate in the human diet (Giacometti, 1979), total plasma levels are relatively low, which indicates strict regulation of glutamate levels in various body systems (Munro, 1979). Free glutamate is present throughout the body, with the highest concentrations found in the muscles and the brain (Giacometti, 1979). The blood-brain barrier controls the rate of amino acid transport (Pardridge, 1979) and is virtually impermeable to glutamate except when doses exceeding 2 mg/kg body weight of MSG are given to infant mice or rats (Airoldi *et al.*, 1979).

Glutamate is a key amino acid in both anabolic and catabolic intracellular reactions. L-glutamic acid is split into α-ketoglutarate and ammonia by glutamate dehydrogenase. In the reverse reaction, L-glutamic

acid is synthesized from α-ketoglutarate, an intermediate in the tricarboxylic acid cycle of glycolysis. Glutamate links the metabolism of carbon and nitrogen, serving as an energy source and a reservoir for ammonia, and is an important component in the synthesis of peptides, proteins, and other small molecules. After ingestion, free glutamate is absorbed from the intestine by an active transport system, converted to alanine, then transaminated to pyruvate and α-ketoglutarate under normal load conditions in the small intestine (Meister, 1979). Free glutamate then passes through the liver and may be metabolized to glucose, lactate, glutamine, and other amino acids before entering the peripheral circulation. In the liver, it is metabolized to glucose, lactate, glutamine, and other amino acids. In muscle, glutamate is readily converted to alanine and glutamine. In the TCA cycle, it is oxidatively deaminated to 2-oxoglutarate, and can serve as an amino donor for many transamination reactions, several of which are dependent on Vitamin $B_6$. Glutamine, glutathione, and other nonessential amino acids are synthesized from glutamate, and it also serves as a precursor for proline. Glutamate can be an ammonium ion donor for urea in the liver, an ammonium ion acceptor in the brain, and a transporter of reducing equivalents. In the CNS, it is a major excitatory neurotransmitter; conversely, it can be decarboxylated to GABA (gamma-aminobutyric acid),

the major inhibitory neurotransmitter via a Vitamin $B_6$ dependent reaction. The metabolism of glutamate has been thoroughly reviewed by the Life Sciences Research Office (1995).

The absorption and metabolism of glutamate varies in normal adults (Stegink *et al.*, 1979). Ingestion of MSG in water at 1 g protein/kg body weight resulted in significant elevation of plasma glutamate levels, a result not found when the same dose of protein was ingested as part of a meal. Large dose feeding trials in animals have yielded conflicting results. During the 1970's, studies were reported that observed development of brain necrosis, lesions, and adverse behavior in rodents fed large doses of enteral and oral MSG up to 0.5 to 0.7 g MSG/kg body weight (Olney *et al.,* 1972). However, these conclusions are inconsistent with studies conducted with non-human primates, and their relevance to the human response to MSG as consumed is not apparent. Inconsistencies in methodology and data presentation do not allow drawing comparisons among studies and establishing conclusions. Chronic feeding studies of a variety of animals, including dogs and monkeys, have not resulted in any neurological lesions or toxicity in these species (Heywood and Worden, 1979; Kenney, 1987). The $LD_{50}$ for L-MG has been established in mice as 19.9 g/kg body weight, which equals more than three pounds in a 70 kg human (Anonymous, 1987).

When administered with food, MSG has been found to increase food intake (Sticker-Krongrad *et al.*, 1992) and enhance metabolism, including increases in diet-induced thermogenesis and respiratory quotient (Viarouge *et al.*, 1992).

2. MSG Symptom Complex

Apparent sensitivity to MSG was first reported by Dr. Robert Ho Man Kwok, a Chinese immigrant (Kwok, 1968). He claimed to experience numbness of the neck, arms and upper back, general weakness, and heart palpitations within 15 to 20 minutes after eating food served in Chinese restaurants in the U.S. He suggested cooking wine, monosodium glutamate, and sodium as possible "obscure" sources of his symptoms. However, his letter caused an influx of responses from others regarding strange reactions experienced after eating Chinese food. Chinese Restaurant Syndrome, as the collection of symptoms later became called, encompasses a variety of acute reactions, including burning or numbness on the back of the neck, arms, or chest, facial pressure or tightness, headaches, and nausea. Less frequently reported symptoms are bronchospasm in asthmatics, psychiatric reactions, behavioral changes, peripheral neuropathy, and cardiac arrhythmias (Kenney, 1987). In 1995, an expert panel was convened by FASEB to review the safety of MSG (Life Sciences Research Office). They developed

the term "MSG-symptom complex" to describe the syndrome as more accurately representing possible exposure and reaction to MSG.

Baby food manufacturers voluntarily stopped adding MSG to baby food in 1969, after brain lesions were reported in infant monkeys administered high levels of glutamate directly into the brain (Olney, 1979). The use of MSG in all processed foods began to decline due to antidotal reports, and protein hydrolysates replaced MSG in many foods. In 1973, FDA requested FASEB to convene an expert panel to review the safety of MSG. In 1976, the panel concluded that studies implicating MSG in producing brain lesions or other symptoms were not reproducible and deemed MSG as safe for the general population (Life Sciences Research Office, 1995). Between 1980 and 1995, FDA's Center for Food Safety and Applied Nutrition received 661 reports of complaints of adverse reactions to MSG (Federal Register, 1996).

The variety of reported symptoms have led to many theories of the causes of the disorder. Most theories are based on the potential excitotoxicity of glutamate at either central or peripheral receptors. Stimulation of central nervous system (CNS) and peripheral glutamate receptors activate body systems such as the endocrine, gastrointestinal, and cardiovascular (Life Science Research Office, 1995). However, none of

these mechanisms have been confirmed through clinical studies. Others have proposed that the syndrome may be due to reactions to normal digestive processes (Morselli and Garattini, 1970; Kenney, 1987); chemoreceptor stimulation acting via a secondary agent such as GABA, serotonin or histamine (Kenney and Tidball, 1972); an in-born error of metabolism (Reif-Lehrer, 1976); Vitamin $B_6$ deficiency (Folkers *et al.*, 1984); and, power of suggestion (Kerr *et al.*, 1979). Contaminants in food have also been proposed as the cause of MSG symptom complex. Suggested compounds include histamine and other biogenic amines from fermentation or incompletely hydrolyzed vegetable proteins (Best, 1992) or compounds in the 1% of impurities in MSG, which have not been identified (Life Sciences Research Office, 1995). People with the syndrome are more likely to experience discomfort after a meal and men report symptoms less frequently than women, who are also more likely to be deficient in Vitamin $B_6$.

Reports of adverse reactions are antidotal and have not been documented in scientifically controlled studies. Thus, the symptoms experienced could be due to other ingested items, either food or toxins from another source, or other unaccounted factors. Accurate estimation of the incidence of sensitivity in the general population is difficult. Reports of MSG symptom complex are generally limited to North American (United

States and Canada), Western Europe, and Australia (Life Science Research Office, 1995). In the U.S., voluntary complaints are registered through the U.S. Food and Drug Administration's Adverse Reaction/reporting Monitoring System (ARMS) and vary in reporting of patient history, nutritional status, and condition of exposure. Nonetheless, this system has found that eight percent of these reports contained complaints that were potentially life-threatening, such as seizures and dysrhythmia. Interestingly, this syndrome has overwhelmingly been associated with Chinese food. Adverse reactions have also been attributed to Japanese, Italian and French restaurants, but no data were supplied to support this statement (Reif-Lehrer, 1976). Several researchers have used food symptomatology surveys to determine adverse reactions to MSG in the general population (Meiselman, 1987). Many respondents reported associating adverse reactions with food and women complained more frequently than men. Most respondents could not list symptoms associated with "Chinese Restaurant Syndrome" although 8% to 56% of respondents said they had heard of "Chinese Restaurant Syndrome". Prospective epidemiological data, i.e., the observation of a test population consuming a potentially harmful compound over a long period of time with appropriate controls, are not available for MSG. No studies have been conducted that relate an oral challenge of MSG to appetite, growth, or

human development. A thorough review of adverse reactions and clinical studies was completed for the Center for Food Safety and Applied Nutrition of the FDA by the Life Sciences Research Office of FASEB (Life Sciences Research Office, 1995).

In general, dose-response assessments of MSG with human subjects have found that single doses of at least 3 grams of MSG in water only rather than as part of a meal, resulted in varying responses among sub-groups of sensitive people (Stegink *et al.*, 1979). However, it is unlikely that any person would consume 3 gram doses of MSG in a normal eating episode, or any amount close to the amounts used in testing. When MSG is administered in protein-containing foods, plasma glutamate levels are similar to levels found after consumption of a high protein meal (Anonymous, 1987). The composition of the meal and the condition of the subject, i.e., fed vs. fasted, impacts the systemic effect of a glutamate challenge. Objective physical measures, including arterial blood pressure, heart rate, and respiratory frequency, were not changed after ingesting MSG (Morselli and Garattini, 1970). Kenney and Tidball (1972) had subjects ingest an oral dose of 1 to 3 mg MSG in 150 ml of liquid (tomato juice or water) and reported a concurrent increase in plasma glutamate, which was not affected by the subjects' breakfast consumption. Kenney (1979) found

that human subjects varied in their responses to MSG. At a dose of 0.75%, some type of response was noted in some subjects. These responses were attributed to ingesting a high carbohydrate and sodium intake or due to irritation of the esophagus. Conversely, Gore and Salmon (1980) did not find a dose-related effect. Tanphaicnitr *et al.* (1983) found no adverse reactions among 50 Thai adults to meals prepared with or without 3 g of added MSG.

Some asthmatics appear to be sensitive to MSG. Oral administration resulted in delayed onset of bronchospasms, 6 to 12 hours post-challenge (Life Science Research Office, 1995). These results are impacted by the design of the experiments, including the dose and type of asthma medication, if used during the trial. Discontinuation of medication during the trial may have contributed to increased susceptibility, as controlled studies were able to reproduce the MSG effects in asthmatic patients who were not on medication.

From the mixed results of human and animal studies, members of the Life Science Research Office Expert Panel concluded that sufficient evidence existed to suggest but not establish causality by MSG in this syndrome (Life Science Research Office, 1995). The members of the Expert Panel recommended that women should avoid exposure to large amounts of

MSG, particularly on an empty stomach or in the absence of a meal, because they more frequently report sensitivity to MSG, have varying neuroendrocrine responses, and have a higher incidence of Vitamin $B_6$ deficiency. Asthmatics should also avoid large doses due to apparently increased sensitivity. The panel also concluded that MSG symptom complex reactions are related to ingestion of L-glutamate, regardless of the source, i.e., whether endogenous or added to the food.

Members from a wide variety of organizations have reviewed MSG studies, concluding that MSG is safe at least for most people. Some of these organizations include The Joint Expert Committee of Food Additives of the United Nations (in 1987), The Food and Drug Administration, the European Communities Scientific Committee for Food (in 1991), The Federation of American Societies for Experimental Biology (independent reviews in 1978 and 1980), the Council on Scientific Affairs of the American Medical Association (in 1992), the American College of Allergy and Immunology, and newsletters such as the *Mayo Clinic Newsletter*, and the University of California's *Berkeley Wellness Letter*. However, in a review of the safety of MSG for the FDA, the Life Science Research Office Expert Panel (1995) noted that most of these reviews are lacking documentation of scientific literature.

The safety of the 5'-ribonucleotides has been demonstrated through extensive toxicity studies using various animal species, although studies with humans have not been reported (Kojima, 1974; Maga, 1995). The use of HVP or ribonucleotides in foods has not resulted in any reports of adverse reactions to these compounds.

3. Regulatory Status and Labeling Requirements

MSG is listed in the Code of Federal Regulations (21 CFR 182.1) as an example of a safe ingredient, when used as intended (Federal Register, 1991). Based on currently available information about usage and reports of adverse reactions, the Select Committee on GRAS Substances of FASEB has given both MSG and protein hydrolysates GRAS status. However, they recommended that additional information would be required if consumption of these substances increased. Flavor enhancers are not flavorings; therefore, they are not exempt from specific listing in the ingredient declaration requirements and must be listed by their common or usual name. In 1940, the FDA stated that MSG was an artificial flavoring when added to food, but later determined that MSG should be declared on the label by its common or usual name because it naturally occurs in food (Hac *et al.*, 1949).

Current FDA regulations (21 CFR 101.22(h)(5)) and USDA rulemaking require that any product containing added MSG in any amount must include the term "monosodium glutamate" in the list of ingredients on the label. When free glutamate occurs naturally in an ingredient that is added to a food product, such as hydrolyzed vegetable protein or Parmesan cheese, only the ingredient by its standard or usual name is declared on the label. Based on the recommendations of the 1995 FASEB panel, FDA is developing proposals for the most effective labeling method that would protect consumers from "inadvertently ingesting levels of MSG or other forms of free glutamate that could cause an adverse reaction." (Federal Register, 1996). Internationally, no labeling restrictions exist for MSG. The Expert Panel of the United Nation's World Health and Food and Agriculture Organizations have given MSG and Acceptable Daily Intake of "not specified", or no quantitative limit, the panel's most favorable classification for food additives.

As with MSG, GMP and IMP are considered GRAS by the FDA. These ingredients are permitted in foods due to their natural source (Maga, 1995). An informal letter of opinion covers the labeling of 5'-ribonucleotides, including GMP and IMP. A regulation clarifying the label requirements for these ingredients has not been necessary because these

compounds have always been considered flavor enhancers and they must be listed by their common or usual name (Federal Register, 1991).

Protein hydrolysates are generally added for both flavoring and flavor enhancing functions; therefore, the protein source of hydrolysates must be identified by their common or usual name on the ingredient list, including the protein source, e.g., soy protein hydrolysate (Falci *et al.*, 2001). This labeling requirement is in response to consumer concern about food allergies as some of the proteins used to make HVP are known to be allergenic. Additionally, FDA recommends that processors declare allergenic ingredients in a spice, flavor, or color. Processors may also voluntarily place precautionary labeling statements on food packaging, such as "may contain (ingredient)" if the food does not contain that ingredient, but is processed in a plant where it could be contaminated by the ingredient.

E. Chemistry of Monosodium Glutamate

1. Properties

Monosodium glutamate is the sodium salt of the amino acid glutamic acid, which is crystalline in a dry form. Table 1 details molecular information about MSG. Commercial MSG is 99% pure; the remaining 1% has not been identified (Life Science Research Office, 1995). Glutamic acid is one of the most prevalent amino acids found in nature, with the L-

enantiomer being predominate. Only the L form has taste enhancing activity; the D-form is tasteless. Structurally it is related to the amino acids aspartic acid, asparagine, and glutamine; however, these compounds do not possess the flavor potentiating properties of glutamate.

Table 1. Specifications for monosodium glutamate

| |
|---|
| Molecular Weight of MSG: 187.3 |
| Composition (Percent by weight)<br>Glutamate: 78.2%<br>Sodium: 12.2%<br>Water: 9.6% |
| 1 g MSG contains 12.2 mg sodium |
| Specific rotation<br>$[\alpha]_D^{20°}$ +24.8° to +25.3° |

The anion component of flavor potentiators is believed to be the part of the compound that creates the umami taste (Yamaguchi, 1991). The taste of glutamic acid is not the same as MSG. Kirimura *et al.* (1969) described the taste of L-glutamic acid as sour with a slight umami taste. When sodium is added, the umami taste becomes apparent.

As an ampholyte, MSG can react as an acid or a base depending upon the pH of the food (Figure 3). Its flavor potentiating power is limited to the pH range of 5.5 to 8.0. In acidic conditions, MSG dissociates to glutamic acid hydrochloride. As pH is increased, glutamic acid is formed. In alkaline conditions, disodium glutamate is formed. Komata (1990) has explained the

decrease in umami taste intensity at extremely high or low pH levels. These varied pHs cause the α -carboxyl radical under acidic conditions to become a carboxyl group and under alkaline conditions the α -amino radical changes to an amino group. Both of these chemical changes prevent the binding of the α-amino radical with the α -carboxyl radical due to low static electrical strength binding.

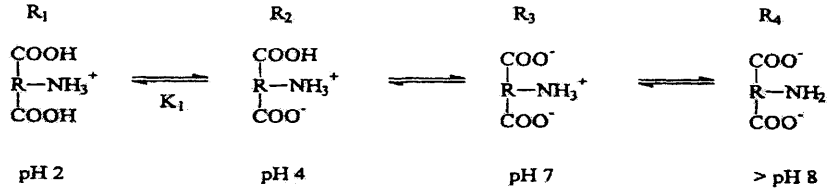



Figure 3. Major ionic forms of glutamic acid

The weak ionic bond between sodium and glutamate frees glutamate to participate in a variety of reactions depending upon pH, heat, and enzymes present. In the presence of reducing sugars, the Maillard reaction can occur. Birch (1987) has linked MSG's chemistry with its perceived taste. He noted that the solution properties of MSG, including its apparent molar volume, relate to the fit of the molecule with the structure of water, making it more accessible to sites on the taste cell receptor.

In an effort to better understand flavor potentiation, several researchers have studied both naturally occurring and synthetic compounds similar to MSG and the 5"-ribonucleotides. Yamaguchi *et al.* (1971) found

that the erytho form of L-tricholomic acid and L-ibotenic acid had a higher flavor potentiating ability than MSG. Two synthesized compounds, monosodium DL-threo-α -hydroxy glutamate and monosodium DL-homocystate had some flavor potentiating ability, compared to other synthesized compounds; however, none were greater than MSG. Kuninaka (1981) reviewed research that indicated a stable five-membered ring with electrostatic force between the negatively charged carboxyl group and the positively charged amino group had flavor activity, possibly because it could be in close contact with the receptor. Peptides containing glutamate have also been studied (Maga, 1983). In general, peptides that had a umami taste were more acidic, polar and hydrophilic; bitter tasting compounds were hydrophobic. Komata (1990) found that either adding or deleting a carbon from MSG produces a weaker umami taste than MSG. Other chemical derivatives of MSG do not have a umami taste, including the acetylation of the α amino radical, esterification of the α -carboxyl radical, and methylation of the α -hydrogen. Replacing the α -hydrogen with a hydroxy radical produces a umami taste in its threo form but not in the erythro form (Maga, 1983). Replacing the α -carboxyl radical of MSG with a sulfonium radical results in monosodium L-homocystate, which has a stronger umami taste than MSG.

2. Analytical Methods

A variety of methods have been used to determine glutamate levels in foods. These are microbiological assays (Hac *et al.*, 1949), which are no longer used; paper, gas, and liquid chromatography (Sporns, 1982), volumetric and flurometric methods (Coppola *et al.*, 1975); refractive index; and methods utilizing glutamate derivatization (Nguyen and Sporns, 1984). The most commonly used methods will be discussed.

Chromatographic methods are generally used for detection and quantification of MSG and 5'-ribonucleotides. Before the development of high performance liquid chromatography, paper and column methods were used (Fenandez-Flores *et al.*, 1969). The AOAC Official Method of Analysis for MSG is a column extraction (Anonymous, 1990). In this method, glutamate is extracted from food and concentrated with water or with an acetone-water mix if starch is present. Serine, threonine, and aspartic acid are pre-eluted from a 25 ml sample aliquot with 120 ml of 0.8 N HCl, using a flow rate of approximate 0.5 ml/min. The flow is adjusted and the glutamic acid is eluted with HCL. The elutant is neutralized with 50% NaOH and the amount of glutamic acid present is determined by the formal potentiometric titration procedure. Gas chromatography can be used to quantitate MSG by forming a derivative from MSG and using an

appropriate detector. GC methods have been described by Conacher *et al.* (1979) and Gal and Schilling (1972), including a procedure to counter the effects of interfering substances (Nakanishi, 1983).

High performance liquid chromatography, or HPLC, has been used by several researchers to measure levels of flavor potentiators. Nguyen and Sporns (1984) described a rapid HPLC technique that identifies and quantitates MSG, IMP, GMP, chloride, aspartate, and pyroglutamic acid. This method yielded excellent recoveries with no derivatization or gradient elution. Two detectors were used: UV absorbance for the low amounts of IMP and GMP occurring in food, while refractive index was used for glutamate and the other compounds. Daniels et al. (1995) did an in-depth analysis of the glutamic acid content in foods with HPLC. Extraction of glutamate was done with 0.02 M potassium phosphate and other food components were subsequently precipitated with acetone. Free glutamic acid was derivatized and separated by HPLC with detection at 254 nm. HPCL was performed using 2 pumps and a variable wavelength UV detector. The method produced a clean derivative of glutamic acid, with an average mean recovery of 95 $\pm$ 17%. HPLC has also been used for quantification of 5'-ribonucleotides. Yokoi *et al.* (1987) simultaneously analyzed 5'-mononucleotides and nucleosides by HPLC with a sodium

phosphate buffer. The method is useful for quantitative analysis in the commercial production of 5'-ribonucleotides. Fish (1991) used ion-pairing reversed phase HPLC to quantify 5'-mononucleotides in yeast. This method, which is not affected by the ionic composition of the samples, can be used to monitor nucleotide levels during processing.

Enzymatic analyses are sensitive and relatively easy to perform (Skurray and Pucar, 1988; Hirosue *et al.*, 1986). Enzymatic analysis begins with diluting the sample to yield glutamate concentrations within the test limits and removing any cloudy or other interfering substances. L-glutamic acid is oxidatively deaminated by nicotinamide-adenine dinucleotide (NAD) to 2-oxoglutarate in the presence of glutamate dehydrogenase. The NADH converts idiotro-tetrazolium chloride to a formazan, which can be measured spectrophotometrically at 492 nm. Eleven different European laboratories collaboratively confirmed the replicability of this analysis (Hattula and Wallin, 1991). A different analytical technique using a glutamate oxidase biosensor was reported by Dehart *et al.* (1993). Using samples with little preparation, the glutamate diffuses through an enzyme membrane and is oxidized to α-ketoglutarate, ammonia, and hydrogen peroxide. The hydrogen peroxide is oxidized at a platinum anode, producing a current

proportional to glutamate in the sample, in the range of 20 to 1870 mg/liter. Subsequent reports of the use of this method have not been found.

3. Levels in Food

As an amino acid, glutamate is found in many foods, in free and bound forms. In the free form and with the presence of sodium, monosodium glutamate can be formed. Thus, glutamate in food can be from three sources: 1) naturally occurring in the food; 2) formed in the food from protein hydrolysis during the food preparation and cooking process, which releases bound glutamate; or, 3) added to the food as MSG or as another food ingredient that contains glutamate, such as mushrooms or Parmesan cheese added to a food for flavor. When MSG is added to a food, the resulting glutamic acid becomes indistinguishable from the naturally occurring free glutamic acid in the food. Consequently, any analytical technique used to determine free MSG measures total free glutamic acid from all sources (Daniels *et al.*, 1995).

Several publications have compiled lists of glutamate levels found in foods (Kuninaka, 1981; Giacometti, 1979; Maga, 1983, 1995; Skurray and Pucar, 1988; Sugita, 1990; Rhodes *et al.*, 1991; Daniels *et al.*, 1995). Some examples of foods that are high in glutamate are milk from humans and cows and several vegetables, including mushrooms, tomatoes, and peas. The

post harvest treatment of vegetables also impacts the levels of glutamate. Sjöstrom and Crocker (1948) reported that MSG improved the flavor of vegetables, whether they were raw, cooked, canned, or frozen, by decreasing sharpness, bitterness, and metallic flavors while increasing the appropriate flavor notes. Fresh young peas and sweet corn contained higher levels of glutamate than more mature vegetables from the same field (Hac *et al.*, 1949). During storage, raw vegetables lost 25-35% glutamic acid content but cooked vegetables had little loss. Skurray and Pucar (1988) found that glutamate content in tomatoes increased from 50.3 to 292 mg/100 gram with ripening.

Reported levels of glutamic acid in food vary (see Chapter 5, Table 12 for a summary of the literature). A variety of factors may account for this variation. These reports originate from different geographical areas, including Asia, Europe, and North America, and the food samples most likely came from local sources. Differences in growing and feeding conditions for plants and animals may impact these results. The analytical method used to determine glutamic acid content differed among studies, including the method of extraction of glutamic acid from the food. Factors such as inhomogeneity, the presence of fat, and interference from fermentation products were cited as reasons for variation in L-glutamic acid

levels in Parmesan cheese (Daniels *et al.*, 1995). Processing and storage conditions, including the temperature and pH, affect the levels of glutamate in food. Glutamic acid levels can be increased if conditions favorable to protein hydrolysis are present, or can be decreased through participation in chemical reactions, such as the Maillard reaction (Yoong *et al.*, 1994). Thus, to be meaningful, glutamic acid must be measured in the final form of the food as consumed.

In addition to food ingredients used to add flavor to food, many food additives contain glutamic acid. When vegetable broth is commercially hydrolyzed into its component amino acids, the glutamate content is between 5.6 to 14.2% (Dzanic *et al.*, 1985). HVP is typically used at levels of 0.06%, which result in low levels of glutamate in the food. Autolyzed yeast extracts average 5.2% free glutamic acid. The high level of glutamate found in cheese and soy sauce are a natural by-product of the fermentation process used to produce these foods. In a review of the umami taste of seafood, Komata (1990) notes that shellfish and crustaceans contain more glutamate than fish, and at least twice as much glutamate than meat. Marine animals also contain high levels of IMP, relative to other foods. The 5'-nucleotides often naturally occur in foods with glutamate.

4. Chemical Interactions

The interaction of flavoring compounds with the protein, carbohydrate, and lipid components of food is an active area of research (Leland, 1997; McGorrin and Leland, 1996), especially due to the interest in developing low- and fat-free foods that taste like their full-fat counterparts. Most of this research has focused on volatile flavors, rather than water-soluble compounds such as amino acids, peptides, and ribonucleotides. Similar interactions may also occur with non-volatile compounds. The lipophillic nature of most flavoring compounds determines their functionality in a specific food matrix. Volatile compounds interact with non-flavor components in the food matrix through binding onto non-volatile substrates, partitioning between oil, water, and gas phases, and the release of the flavor compound from the food into the gas phase (McGorrin, 1996). The vapor pressure of the flavor component determines its behavior in the gas, water, and lipid phases of the food matrix.

The chemical properties of the flavorants and their relative concentrations determine the type of interactions that will occur. Binding between a flavor compound and a food component can be physical or chemical, resulting in a decreased flavor impact (Overbosch *et al.*, 1991). The amount of flavor available after binding is determined by the amount of flavor compound bound relative to its total concentration, the degree of

binding reversibility during mastication, and the solution characteristics of the product, such as the oil:water ratio, and the use of emulsifiers. Other factors affecting the interaction between flavors and food components include pH, moisture content, and the heterogeneous surface of most food, which influences the number of active sites for adsorption.

The flavor of protein compounds, including peptides and amino acids, is easily perceived in the mouth because they are already water-soluble. Proteins are often flavor precursors, interacting with other components of the food system upon heating. Proteins also carry flavors and release flavor-active peptides and amino acids, including glutamate, upon denaturation. During food processing and storage, flavor compounds can be generated, destroyed, altered, or bound to other food components, or a mix of these may occur. The hydrophobic regions of proteins and amino acids can also react with flavoring compounds, depending upon polarity, to form new compounds (Fischer and Widder, 1997).

Due to their complex nature, proteins can undergo a variety of chemical interactions with flavor compounds. The binding of flavor molecules to protein is dependent upon the degree of denaturation, the temperature, and the pH (McGorrin, 1996), all affecting the binding and/or adsorption of flavor compounds by the protein (O'Neill, 1996).

Denaturation of the protein increases the absorption of flavor compounds by exposing more hydrophobic regions of the protein, which absorb the flavors (Leland, 1997), making them unavailable for perception as they must be in a volatile state to be perceived. Two primary kinds of interaction occur between protein molecules and flavor compounds: 1) reversible physical adsorption via van der Waals interaction, and 2) chemical reactions via covalent or electrostatic linkages, which form chemical bonds such as salt, amides, ester formation, and condensation of aldehydes with amine and sulfur groups (Fischer and Widder, 1997).

Franzen and Kinsella (1974) used gas chromatography to study the interaction of carbonyl flavor compounds with food proteins to determine which reaction conditions minimized flavor binding. Soy concentrate bound the most flavor due to its high carbohydrate content, whereas protein decreased the concentration of headspace volatiles in aqueous systems. They found that flavor-protein interactions depend on the amount, type, and composition of the protein, the flavor compound, the presence of solvents, such as water, and the type and quantity of other components in the food such as lipids. Further research by Damodaran and Kinsella (1980) demonstrated that ketones undergo hydrophobic binding with bovine serum

albumin. Conformational changes in the protein increased its binding capacity.

Several factors determine the interaction between proteins and food flavors. The molecular mass of a protein is proportional to its ability to bind flavor compounds (Overbosch *et al.,* 1991). They also reported that as pH decreased from 6.89 to 4.55, binding of heptanal to whey protein decreased and the binding of 2-nonanone slightly increased. A pH-dependant reaction was also found for arginine, which is structurally related to glutamic acid, as foods high in arginine specifically and irreversibly bound diacetyl. Temperature affects the interaction between proteins and flavors. Heat treatment affects the structure and functional properties of proteins, causing conformational changes, protein aggregation, or both (O'Neill, 1996). These changes modify the nature and the extent of the interaction between flavors and proteins, with the protein binding more or less of the flavor compound, depending upon the amount of thermal treatment. For example, whey proteins will unfold with heating, thereby binding a greater amount of flavor compounds so that they are not available for flavoring the food, resulting in less flavor intensity (Hansen and Booker, 1996). This phenomenon is particularly important when formulating products with protein-based fat replacers. Chemical modification of proteins by compounds such as

solvents also changes the binding behavior by changing the conformation and decreasing the number of binding sites.

Simple carbohydrates, such as sucrose or fructose, increase the intensity of many flavors. Reducing sugars react with amino acids to form non-enzymatic browning compounds in the Maillard reaction, which is affected by concentration, pH, temperature, salt, and water activity, among other factors (Godshall, 1997). For example, at 180°C, glucose will react with glutamic acid to form furans and pyrans that give burnt sugar and chicken aromas. Carbohydrates also influence flavor by altering the viscosity of food. Overbosch *et al.* (1991) noted that polysaccharides such as pectin and methyl-cellulose decrease volatility more than simple sugars. Complex carbohydrates interact with flavor compounds in several ways: adsorption, entrapment, complexation, salting out, diffusion, and encapsulation (Godshall, 1997). Generally, carbohydrates decrease the volatility of flavor molecules due to nonspecific molecular interactions. However, mono- and disaccharides can exhibit a salting out effect that increases the volatility of these compounds relative to water.

Among starches, those with a low amylose content have a weak binding capacity while starches such as corn and wheat, with a high amylose content, have a greater binding capacity due to the hydrophobic regions

inside its helical structure that retain lipophilic flavors (McGorrin, 1996). Simple sugars can serve as carriers, although they weakly bind flavors. Starches also affect the flavor of food by altering the viscosity. Starches serve as inclusion complexes to encapsulate flavors, making them more stable during heat treatment and other processing treatments.

Macromolecules such as carboxymethylcellulose affect flavor release by altering the texture of the food, which may be more important than phase partitioning due to the influence of texture on the transport of the flavor in the food matrix as thickening agents inhibit flavor transport and release, reducing flavor strength (de Roos, 1997). Large molecules in the mixture also affect diffusion rates. Lipids also influence the mass transport of flavor compounds, determining the rate at which flavor compounds partition throughout the different parts of the food matrix. Food lipids have the largest impact on food flavor because they are solvents for the lipophilic flavor molecules and they reduce the rate of release of flavors into the air and aqueous phase during consumption, due to their influence on the vapor pressure. They also are involved in flavor release by stabilizing flavors, altering viscosity, and generating flavors, as lipids are often precursors of flavor compounds (de Roos, 1997). Lipids solublize many volatile flavor substances, which are generally lipophilic. Solid fats have a lower biding

capacity than oils, and the binding capacity is also dependant on chain length and degree of unsaturation, with short chain unsaturated fats binding more flavors than long chain saturated fats (McGorrin, 1996). The structure, temperature, and type of lipid determine the distribution of flavoring substances between the lipid and water phases. When fat is decreased or eliminated in a food, the flavor release is affected because flavors have a lower vapor pressure in lipids, thereby a higher odor threshold, than in water. Decreasing fat in a food thus allows for a rapid release of flavor compounds, resulting in a short-lived flavor impression.

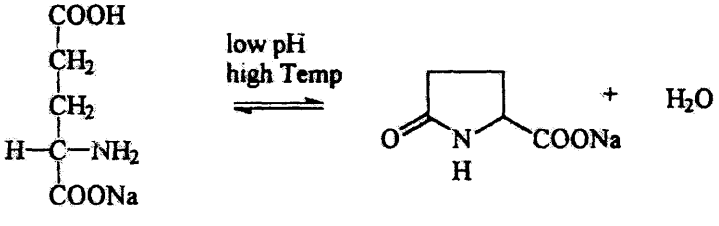
Few studies have been done in complex food systems, which generally consist of protein, lipid, carbohydrate, and water existing in various phases, with three or four phases occurring together, stabilized by emulsions and foams (Land, 1996). Flavor release is also dependent on the length of time the food is in the mouth, temperature of the food, and the degree of mastication (Overbosch *et al.*, 1991; Land, 1996). For a review of the influence of food composition on flavor release in the mouth, see Overbosch *et al.* (1991)

5. Stability of Flavor Potentiators

MSG is indefinitely stable during storage at room temperature, due to its non-volatile, non-hygroscopic nature (Maga, 1995). When mixed in a

food system, its chemical form is dependent upon the pH of the system due to its ampholytic nature, as previously discussed. Storage and cooking times have been found to decrease the amount of glutamate in fresh peas and corn (Hac *et al.*, 1949). At high temperatures, glutamate can react with available reducing sugars via the Maillard reaction, yielding non-enzymatic browning compounds that contribute color and flavor to foods. Temperatures above 100°C and low pH levels will result in a partial dehydration of MSG to its lactam, pyrrolidone carboxylic acid (Figure 4). The contribution of pyrrolidone carboxylic acid (PCA) to off-flavors in processed foods has been studied since the 1950s. The content of glutamine, glutamic acid, and PCA was measured by partition chromatography in a variety of fruit and vegetables before and after heat processing and throughout a two year storage period (Mahdi *et al.*, 1959). The glutamic acid levels remained constant throughout processing and storage but glutamine levels decreased as PCA levels increased with processing and during storage. However, the concentration of PCA in most of the foods tested was not found to be a major factor in flavor deterioration (Mahdi *et al.*, 1961). When PCA was added to food, the samples with lower levels of PCA were preferred. The taste of PCA was described as bitter, medicinal, chemical, and sour. Later, Lin *et al.* (1970, 1971) studied the formation of PCA in thermally processed

spinach puree before and after storage. The largest increase in PCA was found at 240°F, with a decrease in the amount generated as processing temperature was increased to 300°F. PCA levels also increased during storage, with more PCA generated under higher storage temperatures. PCA was also found in fresh spinach. This study showed that PCA decreased the quality of foods not only by contribution of bitter taste but also by decreasing the pH of the food, which impacted its color. They did not study the precursor of the PCA, but speculated that it was glutamine based on earlier research. Acree and Lee (1975) demonstrated the kinetics of the conversion of glutamine to PCA to be pseudo-first order in a model system. This reaction was catalyzed by acetic acid.




5-pyrrolidone-2-carboxylate

Figure 4. Formation of pyrrolidone carboxylic acid

A variety of studies have examined the stability of MSG and secondary reactants under heat processing conditions. Nguyen and Sporns (1984) studied the decomposition of flavor potentiators, including MSG, in a concocted "soup". After retorting the soup for 30 minutes at 124°C, 94% of

MSG was recovered. When glucose was omitted from the soup, 93% was recovered. The recoveries were 96% and 100% with the omission of starch and casein, respectively. These recoveries indicate that MSG was stable, relative to IMP and GMP, which were also tested. A small amount of glutamate may have reacted with glucose in the Malliard reaction or been converted to PCA. Additionally, some of the glutamate recovered could have been generated from protein degradation of the casein that was an ingredient in the soup. Gayte-Sorbier *et al.* (1985) studied the effect of pH, temperature, time, and oxygen level on the stability of glutamic acid and MSG. No changes in either compound were found in samples stored at room temperature for 24 hours. After three days of storage at pH <7, glutamic acid was converted to PCA. Upon further study (Airaudo *et al.*, 1987), pyroglutamic acid was found to be stable in the pH range 2.5 to 11.0. Outside of this range, it was converted to glutamine in a reversible reaction. Raising the temperature increased the rate of this reaction. Samples had higher loses of glutamic acid under acidic pH, when stored with oxygen as compared to nitrogen gas, and when stored at room temperature compared to samples in cold (4°C) storage.

Ribonucleotides are more thermally liable than MSG. Under canning conditions (124°C), Nguyen and Sporns (1984) determined that up to 50% of

IMP and GMP were hydrolyzed to their corresponding bases, with IMP having less hydrolysis than GMP. The path of decomposition was determined to be phosphate hydrolysis to the nucleoside (inosine or guanosine), followed by base hydrolysis to hypoxanthine and guanine, which are flavorless. Longer heating times and lower pH increased the extent of hydrolysis. At pH 9, no hydrolysis was found but at pH 1.5,the nucleotides were completely hydrolyzed to their bases. Adding casein, glucose, and starch to the mix did not affect the hydrolysis of the nucleotides.

The stability of these nucleotides at intermediate pH levels was studied by Shaoul and Sporns (1987). At room temperature, they were very stable but at canning temperatures (121°C), extensive hydrolysis occurred. At pH 5, the hydrolysis half-lives of IMP, GMP, and AMP were determined to be 63, 41, and 51 minutes respectively. These rates were increased at pH 3 because the phosphate bond and the glycosidic bond were hydrolyzed simultaneously. When the temperature was slightly decreased to 100°C, Matoba *et al.* (1988) determined that IMP and GMP had longer half lives (at pH 4.0, 8.7 hr and 6.4 hr, respectively), with a one-third reduction in half-life with a 10°C increase in temperature. Both compounds followed first order kinetic degradation, with IMP degrading more slowly than GMP and

with the phosphate bond hydrolyzed more readily than the glycosidic bond. The chloride salts of calcium, magnesium, and maganese were found to depress this degradation, but sodium and potassium did not affect the rate of hydrolysis (Kuchiba *et al.*, 1990). This depression was due to the formation of a ring structure between the divalent metals and the nucleotide.

The degradation of IMP to hypoxanthine in fish and poultry was found to be an indicator of deterioration and contributed bitter taste to the food (Jones, 1969). Kuchiba-Manabe *et al.* (1991a) found that 5'-ribonucleotides reacted with oxidized oils, providing an antioxidant effect. Heating IMP at 95°C for 15 hours in water resulted in an increase in intensity over the control for the three lowest concentrations tasted. The main degradation product was inosine, which had a bitter taste; however, the amount of inosine formed under these conditions was only one-tenth of its detection threshold. Thus, under these conditions thermal degradation of IMP would probably not affect flavor, but this conclusion must be tested in a food system. The peroxidation of methyl linoleate was found to degrade IMP and GMP during storage, with higher water activity resulting in a faster degradation rate (Kuchiba-Manabe *et al.*, 1991b). During the first week of this study, hydroperoxides hydrolyzed the nucleotides and during the second

week carbonyl compounds contributed to the degradation, with different reaction mechanisms for IMP and GMP.

## II. Sensory Properties of Flavor Potentiators

### A. Flavor Perception

#### 1. Umami: A Fifth Primary Taste?

The notion of the four "basic" or "primary" tastes of sweet, sour, salty, and bitter has been accepted for over 100 years (Komata, 1990). However, the criteria for the qualities of a basic taste have not been established (O'Mahony and Ishii, 1985) and the concept of only four tastes has been challenged both by neurophysiologists and psychologists (O'Mahony and Ishii, 1987). In a review of the umami taste of seafood, Komata (1990) proposed five conditions for a taste to be considered "basic": 1. A unique receptor for the compound exists on the taste cells; 2. The stimulus is transducted by a single neural fiber; 3. The quality is different from other tastes; 4. The taste cannot be created by a combination of other tastes; and 5. The taste is perceived in common foods. All five of these conditions have been met by umami.

The first two criteria have been demonstrated for MSG in a variety of neurobiological studies. In an electrophysiological study of rat taste responses to various amino acids, Yoshii *et al.* (1986) found that MSG did

not potentiate responses to NaCl, HCl, quinine, and sucrose. Single fiber analyses in animal models were used to demonstrate that umami substances are independent and do not enhance neural activity of other tastes (Kurihara, 1997). MSG does not have the same neural response as that generated by sweet, sour, salt, and bitter (Scott and Plata-Salaman, 1991). In 1996, Chaudhari *et al.* cloned a metabotropic glutamate receptor found only in the taste buds. The specificity of this receptor for glutamate was confirmed using LAP-4, the specific ligand that produces the same neural response as glutamate. A more complete discussion of the receptor mechanism for glutamate is presented in the taste transduction mechanism section of the Literature Review.

The third criterion, the unique taste quality of MSG, has been demonstrated in a variety of sensory studies, most conclusively by multidimensional scaling (MDS). This multivariate statistical technique allows characterization of a taste stimulus in a three-dimensional space, using behavioral, electrophysiological, or psychophysical data. Schiffman *et al.* (1980) had subjects subject the similarity and intensity of 13 sodium salts to each other and to NaCl. Subjects also evaluated the taste of the salts using semantic differential scales. The taste of MSG was different from the other salts as well as sucrose, citric acid, and quinine, occupying a unique

area in the multidimensional arrangement. Subjects varied in their description of MSG as good, salty, bitter, sour, sweet, sharp, alkaline, or aftertaste. Yamaguchi (1987) also used MDS to evaluate the flavor similarity of meat, fish, and vegetable stocks. On the graph, the response to MSG was outside the spaces characterizing the responses to other tastes, including quinine, tartaric acid, and various vegetables. Responses to meat stock were most closely related to umami, particularly when elicited by the 5'-ribonucleotides. Faurion (1987) used factor analysis of thresholds to ten different stimuli to demonstrate that MSG is a distinctive taste quality. The independent taste of MSG was corroborated with electrophysiological measures in hamster taste bud pores, demonstrating a unit response profile independent of NaCl, HCl, sucrose, quinine, and PTC. Behavioral and electrophysiological studies with mice have also demonstrated that the taste of MSG is located in a taste space (via MDS) separate from other taste compounds (Ninomiya and Funakoshi, 1987). Although the taste space occupied by MSG is closer to NaCl than sucrose, HCl or quinine on the MDS space, the use of amiloride has a minimal effect on the neural response to MSG. Thus, the taste quality of MSG is different from saltiness (Scott and Plata-Salaman, 1991).

Other sensory methods have found that MSG has different taste qualities than other stimuli. Yamaguchi and Kimizuka (1979) used untrained subjects to describe the taste of foods with MSG. Words used to describe beef broth with MSG included meaty, vegetable-like and mouthfeel terms. In a study by Halpern (1987), MSG was called sour, sweet, and salty more often than any other samples except tartaric acid, sucrose, and NaCl, respectively, and two words were often used to describe MSG. However, these responses may have been biased by the words suggested in the instructions (O'Mahony and Ishii, 1987). Halpern (1987) found that the taste quality of MSG changes with stimulus duration. When given freedom to choose any word to describe the taste of MSG, subjects used both food and non-food words as often as the four traditional taste qualities. MSG was described with different quality descriptors than other stimuli, including "brothy" and "fishy" (Kelling and Halpern, 1988). Zwillinger and Halpern (1991) used MSG, distilled water, sodium saccharin, and a citric acid-saccharin mix to determine successive changes in taste quality. MSG yielded multiple quality responses on 25.5% of the trials, far more than given for the citric acid-saccharin mixture (9%) or saccharin (1%). Subjects described the taste of MSG more frequently with non-traditional taste descriptors, using bouillon, soapy, tomato, milk, and vegetable. Hettinger *et al.* (1996) found

that MSG had a higher frequency of responses in the "other" category than D-MSG, NaCl, and CaCl. Responses were limited to the four traditional taste qualities of sweet, sour, salty, and bitter, plus soapy, sulfurous, metallic, other, and none, which may have limited the results.

Regarding the fourth criterion, combining other taste compounds does not reproduce the umami taste. For example, some peptides have a umami taste, but others do not. Additionally, glutamate does not increase the perceived intensity of compounds that represent the four "basic" tastes of sucrose, citric acid, quinine, and NaCl. Using intensity measures and threshold testing, Yamaguchi and Kimizuka (1979) found that neither MSG nor IMP increased the intensity of the four tastes or altered thresholds to sucrose, NaCl, and quinine sulfate. One exception was a decreased threshold to tartaric acid due to the decrease in pH. Evidence for a lack of potentiation of these compounds has also been demonstrated in rats with electrophysiologic experiments (Yoshii *et al.*, 1986). In contrast, Kemp and Beauchamp (1994) found that threshold concentrations of MSG increased sucrose and quinine sulfate thresholds. Suprathreshold concentrations of MSG suppressed sweetness and bitterness intensities, increased the perceived saltiness of NaCl, and did not affect the perceived sourness of citric acid. The impact of ribonucleotides on the tastes of sweet, sour, salt,

and bitter is unclear. Woskow (1964) tested a 50:50 mixture of IMP and GMP at five concentrations in model systems. Suppression of bitter and sour was found at all concentrations tested. Enhanced saltiness at the highest concentration and enhanced sweetness at the two highest concentrations also were reported.

The fifth criterion is that the taste is perceived in common foods. The umami taste is found in many food systems such as meat, vegetables, and seafood. Mushrooms, soy sauce, and seaweed are examples of foods that have umami as a predominant taste. These foods, which are high in umami compounds have historically been used to add and enrich food flavor (Kuninaka *et al.*, 1964; Maga, 1983).

2. Threshold Measurements

Sensory threshold testing is used to determine the taste sensitivity of a subject, often to evaluate the impact of age, disease, or some type of treatment on taste sensitivity (Meilgaard *et al.*, 1991). Two different types of sensory thresholds can be measured. Detection thresholds determine the concentration of a specific compound at which a subject is able to perceive the stimuli as different from the control water sample. Recognition thresholds are the lowest concentration at which the subject can correctly identify the quality of the specified compound. Generally, recognition

thresholds are two to three times higher than detection thresholds due to subject expectations. Identification of the taste quality can result in an artificially low threshold (Marks and Marshall, 1999). Because sensory thresholds do not predict a subject's ability to perceive suprathreshold tastes, they cannot be extrapolated to results from difference, intensity, or hedonic tests, all of which use suprathreshold concentrations. Interestingly, Schiffman *et al.* (1994) reported that the optimally preferred concentration of MSG in food systems tended to be lower than either the detection or recognition threshold of MSG in the food, but higher than the detection threshold for MSG in water.

Various methods can be used to determine thresholds. The most commonly used is the method of ascending limits, in which the stimulus is directly compared to distilled water. Each subsequent sample increases slightly in stimulus concentration (Jellinek, 1985). Other methods are discussed by Bartoshuk (1978) and Beauchamp *et al.* (1990). The American Society for Testing and Materials (ASTM) Committee E-18 on Sensory Evaluation recommended a variation on the method of ascending limits that uses a 3-AFC (Alternative Forced Choice) to determine threshold (ASTM, 1991). These methods were compared by Gonázlez-Viñas *et al.* (1998), with the ASTM method recommended due to its simplicity, both for the

experimenter and the subject. Delwiche and O'Mahony (1996) described a threshold test using signal detection theory, which accounted for changes in the oral milieu that occur whenever a stimulus is introduced into the mouth. Recently, a computerized method using an adaptive maximum-likelihood staircase procedure has been developed and found to yield reliable, accurate threshold with fewer samples (Linschoten *et al.*, 2001).

Each of these methods can yield different threshold measurements and are subject to different biases (Bartoshuk, 1978). Some methodological reasons for differing results include different test methods, method of stimulus delivery, area of the tongue stimulated (threshold decreases with area of the tongue stimulate, Morino and Langford, 1978), rinsing method used between samples (O'Mahony, 1972a), orientation of the subject to the task, changes in adaptation (McBurney and Pfaffmann, 1963), carry-over or masking effects created by previous stimuli (Delwiche and O'Mahony, 1996; Smith and van der Klaauw, 1995), criterion shift, fatigue, and water taste phenomenon, which is the detection of a taste in water due to adaptation to stimulus concentration and is influenced by stimulus quality (Bartoshuk, 1978).

Reported detection thresholds for MSG range between 0.016 mM to 0.625 mM (Table 1). Large inter-subject variation has been found in MSG

Table 1. Reported detection thresholds to MSG.

| Source | MSG | Method |
|---|---|---|
| Yamaguchi and Kimizuka, 1979 | 0.625 mM | Ascending Method of Limits |
| Faurion, 1987 | 0.34±0.20 mM | N.R. |
| Schiffman *et al.*, 1991 [a] | 0.902 mM | Forced Choice Staircase |
| Yamaguchi, 1991 | 0.00064 mM | Descending Triangle tests |
| Ajinomoto, ~1992 | 0.016 mM | N.R. |
| Gonázlez-Viñas *et al.*, 1998[b] | 1.01 mM | Ascending Method of Limits |

[a] Elderly subjects not included
[b] Mineral water used as carrier
N.R.: Not Reported

thresholds, as for other compounds. The threshold for MSG was found to be lower than the threshold for sucrose, approximately equal to NaCl, and higher than the thresholds for quinine and tartaric acid (Yamaguchi and Kimizuka, 1979). The threshold of MSG can be decreased 100-fold if 5'-nucleotides are added due to synergism between the compounds (Yamaguchi, 1991). To determine the effect of cations on umami, Yamaguchi (1991) conducted threshold experiments of organic and inorganic salts and flavor potentiators having sodium and potassium as cations, using a series of triangle tests. Results found that the threshold of MSG is due to the anionic component, whereas the threshold of IMP was found to be due to sodium, a cation. The umami taste of IMP has been found

to be unrecognizable until MSG is added at a level four times the detection threshold of IMP (Yamaguchi, 1991).

Sensory thresholds to flavor potentiators increase with age, indicating a decline in sensitivity. Schiffman *et al.* (1991) reported that the detection thresholds for five glutamate salts were five times higher for elderly subjects (mean age 87 years) as compared to young subjects (mean age 26 years). The recognition thresholds were almost four times as high for the elderly subjects. Addition of IMP at 0.1 mM lowered the thresholds for the younger subjects, while 1 mM of IMP was required to achieve the same affect for the elderly subjects. In addition, suprathreshold concentrations of the glutamate salts were perceived to be less intense by the elderly group when compared to the young. In a later study, it was reported that the threshold to MSG in a food system was not changed by adding 0.5 M IMP (Schiffman *et al.,* 1994). Similar results of the relationship between age and threshold have been reported by other researchers for NaCl (Stevens *et al.*, 1991).

3. Quality and Intensity of Flavor Potentiators in Water and Food Systems

The measurement of suprathreshold concentrations of substances more accurately represents a subject's taste experience than threshold tests (Bartoshuk, 1978). Flavor potentiators change both the flavor quality and intensity of foods in which they are used. In a series of early studies, MSG

was found to enhance the flavor of meats and vegetables. Sjöstrom and Crocker (1948) reported that adding approximately 0.2% MSG to vegetables prepared in various ways (raw, cooked, canned, and frozen) yielded a smoother flavor than the control vegetables without added MSG. The flavor intensity of carrots and cauliflower was increased with added MSG, and MSG decreased the off-flavors of sharp, bitter, and metallic in the vegetables. According to Sjöstrom *et al.* (1955), MSG increased the saltiness and sweetness of foods, and decreased sour and bitter notes.

The taste qualities of flavor potentiators are unlike any other compounds. Multi-dimensional scaling has been used to demonstrate that flavor potentiators occupy a "taste space" that is different from other tastes (Schiffman *et al.*, 1980). This space is shared by foods such as meats and vegetables (Yamaguchi, 1991). Flavor potentiators have another unique property. Depending upon the food system and the concentration used, they can either increase the natural flavor of a food or add the umami taste.

The intensity of a stimulus can be modeled by a power function. The slope of this function indicates changes in perceived intensity of a stimulus with changes in stimulus concentration and has been used in psychophysical studies to characterize stimuli (Moskowitz, 1971, 1977; Giovanni, 1981). The slope of the power function for MSG is less steep than the slopes

typically found for sweet, sour, salty, and bitter, indicating that the umami taste intensity is not as dependant on concentration as the primary tastes (Yamaguchi, 1991). The taste intensity of IMP increases even less than MSG with increase in concentration (Sugita, 1990). Thus, the mechanism by which these compounds potentiate flavor is not simply dependent on the concentration of the compound. In addition to intensifying flavor, flavor potentiators also have tactile sensations such as continuity and fullness (Yamaguchi, 1991).

Flavor potentiators impact the flavor of different foods to varying degrees. To study this phenomenon, Maga (1987) had fifty trained panelists evaluate the taste intensity of proteins from eight sources: red meat, poultry, fish, vegetable, cereal, legume, egg and milk. These extracts were evaluated with four different treatments: no umami compounds, 0.015% MSG, 0.010% IMP, and 0.004% GMP. These samples were also evaluated under four conditions: the protein extract alone, with the flavor potentiators, with 1% vegetable oil, or with 1% starch. All samples were adjusted to pH 5.5. MSG enhanced the flavor of meat proteins most, followed by fish, vegetables, cereals, legumes, egg, and milk. For MSG, the addition of oil resulted in increased enhancement of the intensity of the vegetable proteins and starch increased the intensity of the cereal proteins. IMP and GMP

increased the intensity of fish and vegetable proteins the most, meat and cereal moderately, and legumes, milk, and egg the least. The addition of oil and/or starch did not change the flavor intensity of any protein source with IMP or GMP. Although panel consistency was monitored, no statistical treatment of these data was reported. The functionality and taste mechanism of MSG, IMP, and GMP are dependent on protein source in the food and the presence or absence of non-protein compounds. Halpern (2000) reported that the hedonic responses to MSG, NaCl, and ribonucleotides in fried rice, boiled rice, Chinese noodles, or potatoes varied, depending upon the food system and combination of flavor potentiators. Each food required a different blend of flavor potentiators and other ingredients, such as soy sauce, to be liked.

Although the majority of research on flavor potentiators has focused on taste, impact of MSG on aroma is unclear. Sjöstrom *et al.* (1955) found that MSG reduced the intensity of aroma off-notes in vegetables. Wagner *et al.* (1963) found that added IMP to dehydrated beef noodle soup increased the aroma. Maga and Lorenz (1972) used gas chromatography to measure differences in the volatile composition of beef broth headspace due to the use of 0.05% MSG, IMP, and GMP singly and in combination. The results demonstrated that, in all cases, the addition of enhancers altered peak areas.

The flavor enhancers did not contribute additional peaks and no peaks found in the control broth disappeared. Headspace analysis of water and potentiators in solutions yielded no measurable peaks. Thus, the impact of the flavor enhancers was to increase the intensity of existing volatile compounds. Peak area increased using MSG alone, indicated by the ratio of control peak to test peak, ranged from 1.39 to 1.89 times for various peaks. When the ribonucleotides were added to MSG in the beef broth, the area ratios ranged from 2.02 to 3.52. No sensory evaluations of the beef broth were made. The combination of MSG, IMP and GMP provided the most increase in peak area, indicating a synergist effect, as has been demonstrated with taste. They proposed that the enhancing effect was due to a chemical reaction or bonding that occurred with the addition of the enhancers rather than an alteration of the vapor pressure of the volatile compounds, as the concentration of the flavor enhancers were too low to impact the vapor pressure of the broth. In a sensory study, Yamaguchi and Kimizuka (1979) found that the addition of MSG at 0.05% and at 0.2% did not increase the aroma intensity of beef consommé. Faurion (1987) had subjects assess MSG with and without retronasal airflow, and found no differences between the delivery system in the evaluation of MSG at suprathreshold levels, concluding that MSG is specifically a taste. However, the model system

used did not allow MSG to interact with other components of a food system, which may have altered the results.

A sensory study by Voirol and Daget (1989) found that MSG had no effect on the subjective odor character of beef flavor mixed in water. Flavor by mouth (retronasal perception) was determined to increase more rapidly with concentration of beef flavoring than odor by nasal evaluation. NaCl was found to increase the total flavor intensity of the beef broth, and also increase the salt intensity. MSG had only a slight effect on overall flavor intensity but no impact on the individual flavor notes that were evaluated in the study. When odor only was evaluated, the beef broth with MSG had slightly more intense odor overall and in meaty and spicy character. Tartar (1989) studied MSG, IMP, and NaCl singly and in combination in water and in broths using nasal inhalation, oral evaluation with the nose closed, and retronasal evaluation. The perception of the overall perceived aroma intensity of the carriers was not affected by the addition of the flavor potentiators, even the distilled water control. The broths, chicken and beef, were not significantly different in aroma intensity. Both broths were commercially prepared, so they most likely contained significant amounts of flavor portentiators. Hettinger *et al.* (1996) determined that the unique taste of MSG is not eliminated by the use of nose clamps, but their data did

demonstrate some differences in quality between nose-open and nose-closed conditions. Rolls (2000) found that MSG in a food system with an olfactory stimulus such as garlic results in a stronger response, suggesting that MSG interacts with volatile compounds to enhance flavor.

The synergism between MSG and ribonucleotides has been well-documented (Yamaguchi, 1967; Maga, 1983; Komata, 1990). Synergism results when the behavioral or physiologic response to a mixture of compounds is greater than the sum of the responses to the individual compounds. The synergism between umami compounds has been demonstrated electrophysiologically in rats (Cagan *et al.*, 1979; Yoshii *et al.*, 1986) and is not found with other taste compounds (Yamaguchi, 1979). Kuninaka *et al.* (1964) reported that thresholds of IMP and GMP were decreased more than 100-fold by the addition of 0.1% MSG.

This synergism can be used to a food processor's advantage by adding varying levels of these compounds to appropriate food systems, thereby intensifying their flavor. They can be used in place of more expensive ingredients to provide flavor while achieving a cost reduction. Naturally occurring compounds within foods to enhance flavor, specifically the umami taste (Yamaguchi, 1987). The potentiating effect of MSG may be due to synergism between added MSG and naturally occurring ribonucleotides in

food. The umami taste is especially enhanced in animal and fish stocks, while the flavor of vegetable broth also includes sweet and sour flavor notes. When IMP is added, the flavor notes of the vegetable broth include a more intense umami taste (Yamaguchi and Kimizuka, 1979). No controlled studies of this phenomenon have been found, whereby a concentration series of MSG is added to foods with high levels of naturally occurring ribonucleotides. Fuke and Konosu (1991) studied a variety of seafood, including abalone and snow crab, to demonstrate that for some foods, the synergism between glutamic acid and the 5'-ribonucleotides is a requirement for production of a characteristic flavor. The role of individual compounds in producing the umami taste is of interest. Komata (1990) described the method of omission as a way to determine the contribution of compounds to this taste. First, the chemical composition of a specific food is determined. Mixtures of the components of this food are synthesized. Test samples are then prepared that omit individual components of the mixture to determine qualitatively and quantitatively the unique contribution of each component to the umami taste. The relationship between amino acids and nucleotides is also of interest physiologically because interactions between proteins and nucleic acids are intermediate steps in important biochemical reactions.

Various combinations of IMP, GMP, MSG, and NaCl have been studied extensively. The synergistic increase in intensity is dependent upon the concentrations used. Yamaguchi (1967) used probit analysis to develop a mathematical model of the synergistic relationship between MSG and IMP. This model was based on several experiments with 100 subjects. The taste of MSG alone was found to follow the Weber-Fechner equation:

$$S = c \log R$$

where S is the perceived intensity, R in the concentration of the stimulus, and c is a constant, unique for each compound (Yamaguchi, 1967). When IMP was tested alone, this relationship did not apply; the subjective intensity changed very little with an increase in stimulus concentration, and c was very close to zero. When the compounds were mixed, the relationship was determined via sensory testing to be:

$$Y = u + \gamma uv$$

where y is perceived intensity, u is the concentration of MSG in g/dl, v is the concentration of IMP in g/dl and $\gamma$ is a constant (Figure 5).

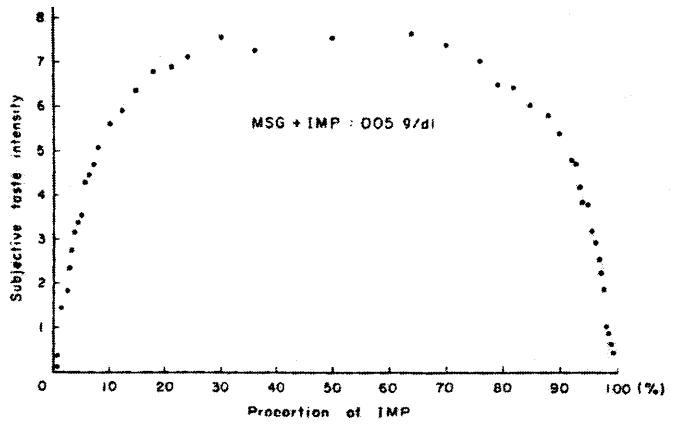



Figure 5. Graph of relationship between MSG and IMP (Yamaguchi, 1967)

This equation was found to express the umami intensity of the mixture of various amino acids and nucleotides, with u and v representing the concentrations of amino acids and nucleotides equivalent to the concentration of MSG and IMP in the mixture (Yamaguchi *et al.*, 1971). When a 50:50 mixture of MSG and IMP was used, the relative taste intensity increased sixteen-fold over MSG alone (Yamaguchi, 1979). Addition of compounds representing the four basic tastes of sweet, sour, salt, and bitter did not alter the synergism of IMP and GMP, while addition of histidine and arginine suppressed this synergism due to the buffering capacity of these amino acids (Yamaguchi, 1979).

4. Hedonic Evaluations and Impact on Consumption

Because flavor potentiators enhance the natural flavor intensity of foods such as meat, fish and vegetables, they also can improve palatability. However, the pleasantness of MSG is dependent upon its use in a food system. Low concentrations of MSG and NaCl in water, in single and binary mixtures, were given neutral hedonic scores, which decreased as stimuli concentration increased (Yamaguchi and Takahashi, 1984a). When added to a variety of food systems, including clear soup, miso soup, eggs, and fried rice, pleasantness scores followed a normal hedonic function, with maximum pleasantness at 0.3% or 0.9%, depending upon the food system. Thus, the umami taste of MSG appears to be self-limiting, as are the tastes of sour, salty, and bitter; only the sweet taste is not self-limiting (Moskowitz, 1977; Yamaguchi, 1979). Even infants have demonstrated an aversion to MSG in water but a preference for MSG in soup (Beauchamp and Pearson, 1991; Beauchamp *et al.*, 1998).

The Semantic Differential Technique was used by Yamaguchi (1979) to determine that the addition of 0.05% and 0.2% MSG in beef consommé significant increased palatability, compared to beef consommé with no added MSG. This difference was not found when 1.2% or 0.8% NaCl was used. Yamaguchi and Takahashi (1984b) used response surface

methodology (RSM) to determine the optimum levels of NaCl and MSG in Japanese clear soup. As MSG level was increased, less NaCl was required to maintain the maximum palatability score. Thus, synergism between NaCl and MSG may allow for a reduction in dietary sodium, thereby making a sodium-restricted diet more palatable. A subsequent study using an actual meal in a dining situation confirmed that NaCl could be reduced by 30% by adding 0.6 g/person of Ajinomoto seasoning (97.5% MSG, 2.5% 5'-ribonucleotides), resulting in significantly higher palatability and diner satisfaction than using NaCl alone (Yamaguchi, 1987). Turoila *et al.* (1990) found that reducing NaCl in beef broth could not be compensated for by the addition of MSG or other flavors (onion, allspice, and marjoram). Chi and Chen (1992) used RSM to demonstrate that NaCl and MSG must be added to chicken broth in inversely proportional amounts to maintain the maximum hedonic score. The different results from these studies may be due to the differences in concentration of MSG used (the Japanese study used higher levels), differences in experimental design, and cultural variations. In a study that evaluated the acceptance of low salt soups over a 5-week period, the pleasantness ratings of soups with 0.2% MSG and 0.05% 5'-ribonucleotides were unchanged, compared to soups with 0.3% and 0.5% NaCl, which decreased in pleasantness over the course of the study

(Roininen *et al.*, 1996). Sodium and glutamate independently improved the flavor of chicken broth and subjects preferred chicken broth with 0.01 M MSG compared to chicken broth with 0.01 M NaCl (Okiyama and Beauchamp, 1998).

Differences between cultures in taste concepts and experience with food likely influence the different umami preferences found. O'Mahony and Ishii (1987) found that Japanese and Americans used different terms to describe the taste quality of MSG. In addition to "salty", Americans used many other non-primary words, such as bouillon and fishy, while Japanese subjects used descriptors related to umami. In a taste sorting experiment, O'Mahony (1991) concluded that Japanese and Americans agreed in their concept of umami, except for GMP, which was not included in the umami concept as often by the Americans as it was by the Japanese. Prescott *et al.* (1992) examined differences in liking of umami between Japanese and Australians. Both groups used a nine-point hedonic scale to evaluate degree of liking for MSG, IMP, and GMP in still mineral water. For MSG, the Japanese subjects gave higher liking scores to higher MSG and GMP concentrations, but not IMP, than their Australian counterparts. The differences are most likely due to increased familiarity with umami among the Japanese subjects. For all three umami compounds, degree of liking

decreased significantly over concentrations, most likely due to using water as the carrier. Bell and Song (1999) found that the perception of the umami taste was a common determinant of food preferences among Japanese consumers, but not for Australian, Singaporean, or Indonesian consumers. Thus, differences among ethnic groups are apparently due to different experiences with foods in each culture.

The role of flavor potentiators in food intake, nutritional status, and food preferences has been studied in humans of various ages and animals. Vazquez *et al.* (1982) studied taste and flavor preferences, measured by intake, in malnourished and well-nourished Mexican infants (2 to 24 months). All infants preferred soup with MSG to plain soup. The malnourished infants had a higher intake of the soup with added casein hydrolysate soup compared to the control soup; in contrast, well-nourished infants preferred the control soup. Sugita (1990) has found that infants responded to MSG and sucrose in a similar fashion.

Murphy (1987) reported the results of two experiments that examined the relationship between age and preference for MSG and casein hydrolysate in soup. The subjects were either elderly (>65 years of age) with a low nutritional status as determined by biochemical indicators or young subjects (18 – 26 years of age) in good health. The elderly subjects who were of

lower nutritional status preferred higher concentrations of MSG than the young subjects and subjects of higher nutritional status. Thus, perception of flavor potentiators appears to be mediated by nutritional status as well as changes in chemosensory function. Schiffman and Warwick (1988) suggested that adding various flavor enhancers alleviated anorexia in the elderly due to diminished chemosensory functioning. Rogers and Blundell (1990) used beef consommé to examine the effect of MSG, hunger and food intake among undergraduate volunteers. Consumption of the soup with or without MSG initially decreased appetite, indicating that the sensory stimulus alone does not reduce appetite. Intake of food at a subsequent meal was not affected by consumption of MSG. However, consuming soup with MSG led to a faster recovery of appetite as compared to consuming the control soup.

Bellisle *et al.* (1991) reported that 0.6% MSG added to foods increased consumption rate and intake of foods upon first exposure and also over time among 3 healthy young subjects. In a second study, elderly people in institutional care significantly increased food consumption, thereby increasing calories, calcium and magnesium when MSG was added to vegetable soup and mashed potatoes, although individuals varied greatly in their intake. Individual variation and preferences may confound any long-

term impact of MSG to improve nutritional status through intake. Therefore, MSG should be added only to foods in which it increases palatability and provides necessary nutrients. Young and elderly subjects were also studied by Schiffman *et al.* (1994) to determine thresholds to MSG singly and with IMP. The average detection threshold of the elderly subject group was 2.8 times higher than the average threshold of the young subjects. For both groups, the combination of MSG and IMP in chicken broth, corn, onion soup, steak and tomato soup was preferred over using only MSG in these foods.

The impact of MSG on preference and consumption has also been demonstrated in animals. Torii *et al.* (1997) concluded that taste preference for MSG is observed when rats have adequate balanced protein in their diet, but when protein status is compromised, the rats preferred solutions with NaCl. Racotta and Hernandez-Garcia (1989) measured differences in food intake between male and female rats with subcutaneous administration of MSG. At a level of 6 g/kg, female rats significantly decreased food intake while increasing water intake, while male rats significantly increased both food and water intake at this level of MSG. A demonstrated preference for solutions of MSG over water by rats and dogs has been reported by Naim *et al.* (1991). In dogs, MSG and sucrose stimulated pancreatic secretions,

while bitter, sour, and salty tastants did not. Rats fed no protein or a poor quality protein had a reduced preference for umami substances; thus, preference for MSG is found only when dietary protein is balanced. These preferences appear to be due to sensory response to the compounds rather tan post-ingestion effects.

Intracerebroventricular injections of MSG had an immediate impact upon increasing food intake in rats, an effect that lasted for 24 hours (Stricker-Krograd *et al.,* 1992). Venous injection of MSG had a greater impact on intake than systemic injection. In these studies, the mechanisms for intake were hypothesized to be mediated by neuromodulators as demonstrated in rats. Brain cells in the hypothalamus of lysine-deficient rats responded preferentially to lysine over MSG, demonstrating neuronal plasticity (Torii *et al.*, 1997). When amino acid status was normal, the cells responded to MSG. Peripheral glutamate sensors appear to play an essential role in amino acid homeostasis and dietary protein intake in rats (Torii *et al.*, 1998). Neural responses to MSG deceased after feeding macaques to behavioral satiety, demonstrating a satiety mechanism (Rolls, 1997).

5. Time-Intensity Studies

Flavor is a dynamic, not static, event. However, most sensory measures are made at a single point in time, which can fail to demonstrate

flavor changes over time such as masking, synergy, or aftertaste. The perception of the quality and intensity of a given attribute changes as the product is taken into the mouth, chewed or moved in the mouth, and then swallowed. Time-intensity methods (TI) allow subjects to indicate changes in quality and intensity over time. Reviews of TI methodologies and uses of TI data have been published (Lee and Pangborn, 1986; Halpern, 1991).

First attempts to record TI responses used a variety of methods subject to human error, including auditory signals to prompt the subject to record a response (McNulty and Moskowitz, 1974) or having subjects watch a stopwatch to determine when to record numerical intensities (Guinard *et al.*, 1985). In 1978, Larson-Powers and Pangborn (1978) had subjects move a pen across a strip-chart recorder to indicate intensity changes. Computers were initially used to digitize TI curves, and later judges drew the curves with a joystick interfaced to a microcomputer (Guinard *et al.,* 1985) and more recently with a mouse (Yoshida, 1986). Computerized methods improve accuracy, decrease data entry and analysis time, provide a more interesting task for subjects to complete, and prevent subjects from seeing their responses, which can influence subsequent responses (Schmitt *et al.*, 1984; Guinard *et al.*, 1985). The Dynamic Flavor Profile integrates time intensity and descriptive analysis methods to create a series of "wire"

diagrams that represent both qualitative and quantitative changes over time (DeRovira, 1996).

TI measurements have been conducted with a variety of tastants in different modalities. TI has been used to profile the sweetness of simple and artificial sweeteners (Larson-Powers and Pangborn, 1978), the saltiness of tomato juice (Pecore, 1979), sweetness of sucrose (Lawless and Skinner, 1979), determine relative sweetness among sugars (Harrison and Bernhard, 1984), quantify bitterness in beer with different ethanol and iso-alpha acid levels (Pangborn *et al.*, 1983), measure the effect of repeated ingestion on temporal bitterness in beer (Guinard *et al.*, 1986a) and temporal astringency in wine (Guinard *et al.*, 1986b), compare the bitterness of caffeine and quinine and quantify perception of bitterness by PTC (1-phenyl-2-thiourea) tasters and non-tasters (Leach and Noble, 1986), determine the relationship between sweetness hedonic and intensity assessments with repeated stimulus presentation (Lee *et al.*, 1992), characterize meat tenderness and chewing activity (Duizer *et al.*, 1994), measure flavor release in different gel systems (Guinard and Marty, 1995), examine differences in chemoreception of sweet and bitter compounds (Guinard *et al.*, 1995), and determine the relationship between salivary flow and flavor release (Guinard *et al.*, 1997).

Variation among subjects is even more pronounced in TI evaluations than in other sensory test methods. Individual subjects are fairly consistent in their responses, often tracing an idiosyncratic pattern that characterizes their responses to stimuli within a set (Larson-Powers and Pangborn, 1978; Schmitt *et al.*, 1984; Leach and Noble, 1986). Whether or not this pattern continues across modalities has not been studied. The accuracy of estimating the intensity of MSG stimuli and the uniformity of the TI curves among subjects was increased by calibrating subjects to a NaCl series (O'Mahony and Wong, 1989).

Differences in the test procedures account for some of the variability among subjects. These include quantity of the sample used, number of samples, whether the samples were swallowed or expectorated and the timing of the action, the rinsing procedure between samples, subject training, type of TI equipment, use of a standard, and experience, or lack thereof, with a stimulus. Moving the tongue, cheeks, and other muscles in the oral cavity disrupts adaptation to the stimulus and can lead to an increase in the perceived intensity of a stimuli (O'Mahony and Wong, 1989), as can repeated ingestion of samples (Guinard *et al.*, 1986a).

Stimulus duration and mode of stimuli presentation differ in TI experiments, depending upon the objective of the test, yielding results that

are not comparable between studies (Kelling and Halpern, 1988). Yoshida (1986) did not find differences in sweetener TI profiles between expectorating and swallowing the samples. Differences among the subjects in reaction time were likely due to the amount of time taken by a subject to manually move the mouse in responding to the stimulus. Halpern (1987) has noted that verbal reports are faster than manual movements. Most TI studies use whole mouth sipping procedures, but Halpern (1991) used a closed flow delivery apparatus that delivered a controlled volume of stimuli only to the anterodorsal region of the tongue tip to study reaction time and qualitative changes in the taste of various stimuli.

There is no consensus on the appropriate treatment of TI data. Usually, individual curves or specific measures from the curves are arithmetically averaged into a panel mean for both intensity and time (Overbosch *et al.*, 1991). Various parameters are measured from the curve and can be statistically analyzed. The most common parameters measured are illustrated in Figure 6.

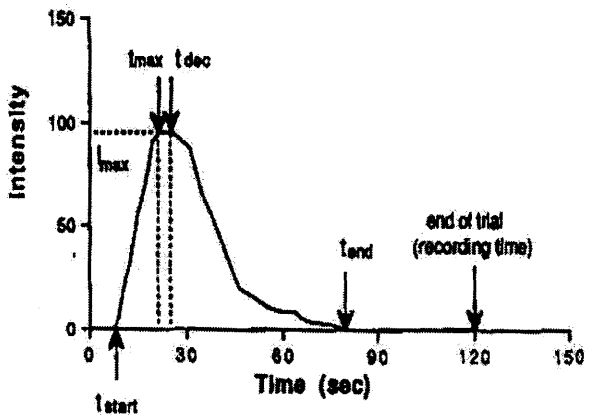



Figure 6. Typical time intensity curve with characteristics. From Liu and MacFie (1990).

Data analysis and results are affected by the number of intensity readings per second (Halpern, 1991). Problems in data handling include a long reaction phase, a fluctuating plateau, a non-monotonic decrease in intensity, and lack of an endpoint or return to zero (Liu and MacFie, 1990).

DuBois and Lee (1983) proposed an abbreviated analysis for screening compounds that focuses on appearance time and extinction time. Schmitt *et al.* (1984) used regression analysis to quantify the rates of increase and decrease in beer bitterness, using a linear rate constant, K, to study differences between beers. To develop a model that would be useful in understanding changes in perception with changes in stimulus strength over time, the power law was applied to TI data (Overbosch, 1986). A differential equation was derived to relate the threshold of perception to the

time course of the stimulus, considering adaptation. This model reconciled the different shapes of individual subject curves by dividing the curves into ascending and descending portions (Overbosch *et al.,* 1986). The intensity of each curve was normalized using the geometric mean of each subject's maximum intensity. However, this method does not allow for a maximum intensity plateau, multiple peaks, or non-zero points on the curve. To compensate for these problems, a more flexible method was proposed that normalizes across time and intensity, using geometric means (Liu and MacFie, 1990). In contrast to these procedures, Duzier *et al.* (1994) found that removing subject individuality by normalizing the data lost too much information, including significance due to treatment.

Principal Component Analysis (PCA) has been used to examine the shape of the principal curves and to examine subject variability. Van Buuren (1992) applied PCA to TI curves in a study of subject variability, noting that the TI curve may be more influenced by characteristics of the subject rather than the product. Principal curves accounted for more variance than averaging the curves, giving summary curves that more accurately described the products. In another use of PCA to decrease the effect of individual differences, Dijksterhuis (1993) had five subjects evaluate the bitterness of drinks with different levels of caffeine and sugar.

Because the average curve may not be a good representation of the characteristic curve, he applied PCA across samples. Although the analysis separated the average curves by percent variability into a principal component, the difference between samples was obscured by the analysis. Therefore, non-centered PCA was used, which reflects the position of the mean for each sample. Although the first curve, which accounted for most of the variation, indicated differences among samples, the other curves were difficult to interpret. In a second paper, Dijksterhuis *et al.* (1994) applied Principle Curve Analysis, a variant of PCA, to TI data from sweet and bitter samples. Non-centered PCA was again found to model most aspects of the individual TI curves.

Factor loadings of PCA can be used to classify subjects and interpret the principal curves by showing relationships among stimuli. Dijksterhuis and van den Broek (1995) used PCA to focus on shape differences in the TI curves, which allowed segmentation by subject and identification of outliers. They also discussed isotropic scaling, in which the TI curves can be modified differently in the time and intensity directions by determining weighting factors for each dimension and comparing these factors among stimuli and subjects. PCA and Cluster Analysis were applied to TI data to examine the differences among individuals in the temporal perception of

sweet and bitter compounds (Guinard *et al.,* 1995). These techniques used inter-individual subject differences to group the 23 stimuli together on the profile parameters of maximum intensity, time to maximum intensity, duration, and area under the curve.

Few TI studies have been conducted with flavor potentiators. An unpublished study by Chung (1981) was conducted to determine the influence of L-glutamic acid and NaCl in water and broths on human salivary flow and simultaneous temporal taste responses. A key point in this study is that L-glutamic acid, rather than MSG, was used. These compounds have two different tastes; yet, L-glutamic acid was labeled MSG in the report. Results indicated that NaCl was a better sialogogue, or salivary stimulator, than L-glutamic acid. TI responses varied with concentration of L-glutamic acid and NaCl. Generally, the two compounds had similar TI profile parameters, except maximum intensity (greater for NaCl) and duration of the taste sensation (longer for L-glutamic acid). Sodium, potassium, and calcium output correlated positively with salivary flow. Tarter (1989) studied the effects of MSG, NaCl, and IMP, singly and in combinations. Adding NaCl to MSG yielded the largest increase in beef broth flavor intensity, whereas adding IMP to MSG was more effective in water. Subjects more easily discriminated between MSG, IMP, and NaCl

when the stimuli were dissolved in beef broth rather than water. For MSG, beef broth also yielded a longer time to maximum intensity and duration than using water as the medium.

O'Mahony and Wong (1989) first calibrated subjects to a NaCl concentration series and then had them evaluate NaCl and MSG taste intensity changes over time using this internalized scale. The purpose of the study was to examine sensory adaptation, not to characterize the TI curve for MSG. Sensory adaptation had a greater effect on taste intensity loss than salivary dilution. NaCl tasted stronger than equimolar MSG, except for three minutes after expectoration when MSG was more intense. To determine the relation between salivary flow and gustatory response, Guinard *et al.* (1998) used MSG in chicken broth as one of seven food systems. They found that maximum intensity and total duration of MSG in chicken broth increased with stimulus strength, which also increased mean salivary flow. This study did not characterize the TI curve for MSG.

Various researchers have related TI events to the chemoreception of gustation and olfaction. To model sweetness perception, Birch (1981) proposed the "orderly queue hypothesis", which considers the timing and direction of the approach, alignment, and localized concentration of stimulus molecules. However, DuBois and Lee (1983) considered this explanation to

be "unlikely", proposing that temporal differences in sweetness are due to the rapid diffusion of receptor-specific molecules. They noted that non-receptor sites also bind the stimulus molecules, blocking their perception. TI methods can model sensory adaptation (Overbosch *et al.*, 1991) and sensory interactions among food ingredients, such as flavor release, masking, additivity, enhancement, synergism, and other dynamic sensory properties (Larson-Powers and Pangborn, 1978; Harrison and Bernhard, 1984). In sweetness models, Birch (1981) noted that factors such as diffusion, localized concentration of stimuli near receptors, reaction time, molecular stereochemistry and alignment, and competitive processes could be studied from TI curves. TI has been used to calculate the apparent binding affinity of a molecule to a receptor, $K_m$ (Shamil *et al.*, 1988). $K_m$ represents the concentration of the substrate at which half the receptor sites are filled and is determined from a Lineweaver-Birk-type plot of the magnitude estimation rates (maximum intensity divided by the time taken to reach maximum intensity) and the concentration of the molecule. The $K_m$ is analogous to the $K_m$ used in enzyme kinetics. The greater the $K_m$ value, the lower the degree of affinity of the molecule for the substrate. Overbosch *et al.* (1991) proposed that flavor release is related to mass flow, not stimulus concentration, and TI methods are better at studying this phenomenon than

other sensory methods. Birch (1987) believes that TI measures are more effective when correlating sensory and physiochemical measures.

In using TI to model olfaction, de Wijk (1989) concluded that temporal integration of odorants was directly related to the molecular diffusion rate through the olfactory mucous layer and adaptation processes were affected by stimulus duration. Birch (1987) called for characterization of the TI curve for MSG, noting that it would be useful for understanding taste properties of MSG, such as persistence, reaction time, and differentiation of MSG from other sapid molecules, which would broaden understanding of MSG chemoreception and the dynamic concept of receptor mechanisms.

Multivariate analyses applied to TI data of bitter and sweet stimuli indicate taste space dimensions that might represent receptor mechanisms, different binding sites, and different binding characteristics among stimuli. Guinard *et al.* (1995) applied Principal Component Analysis and Cluster Analyses techniques to TI data from 23 sweet or bitter stimuli. They proposed that inter-individual differences in sensitivity corresponded to the number of receptor mechanisms involved in chemoreception. The segregation between data from the sweet and bitter stimuli suggested that sweet and bitter do not appear to share common receptor mechanisms. For

sweetness, one receptor mechanism appears to be responsible for sugars and small molecules, while another system is responsible for larger sweet molecules. Two receptor mechanisms were also proposed for bitterness: one for PROP/PTC taste and the other for compounds that use a low-specificity membrane receptor.

B. Taste Transduction Mechanisms

Unlike other sensory receptor systems, the transduction of taste is mediated by a variety of mechanisms. The following discussion summarizes the anatomy, receptor mechanisms, and chemical properties of compounds involved in taste transduction mechanisms, with an emphasis on mammalian systems and glutamate. Lindemann (2001) has published a comprehensive review of this subject.

1. Anatomy

Taste is perceived via the taste buds, onion-shaped bulbs embedded in the taste papilla (Figure 7). Three types of taste papillae are present on the

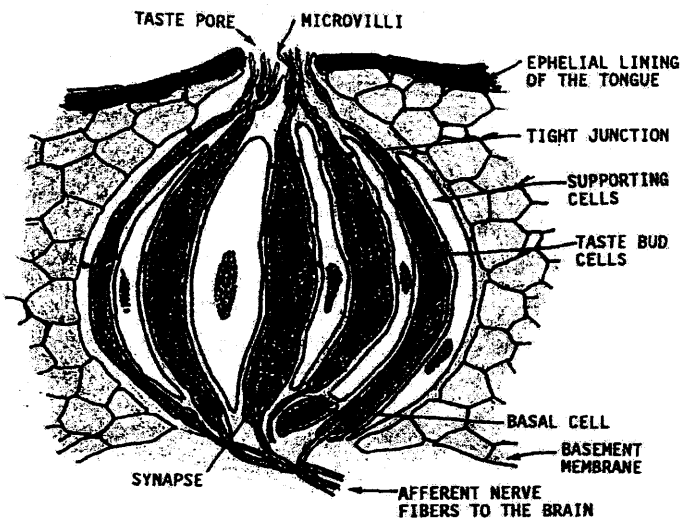



Figure 7. Diagram of a taste bud and associated structures in vertical section.

tongue: fungiform, on the anterior two-thirds of the tongue; foliate, in the posterior region; and circumvallate, on the posterior edge of the tongue in a V-shaped row. The theory that different tastes are perceived on different regions of the human tongue is now known to be in error, although different areas of the tongue apparently are more sensitive to different stimuli (Kinnamon, 2000). Stimulation of a single papilla is enough to identify the quality of a stimulus. The chorda tympani nerve (VII cranial) innervates the

fungiform papillae and the other two types are innervated by the glossopharyngeal nerve (IX cranial). Denervation of the bilateral chorda tympani and glossopharyngeal nerves of mice suggested that the glossopharyngeal nerve is important to the discrimination between umami and salty components of MSG while the CT nerve is important for perception of the sodium component of MSG (Ninomiya and Funakoshi, 1989). Using electrophysiological and behavioral experiments with rats, Sako *et al.* (2000) demonstrated that the CT and greater superficial petrosal (GSP) nerves were more responsive to MSG and IMP that the GL, and that the CT nerve plays a more important role than the GSP nerve in mediating the taste of umami substances. Taste buds on the soft palate are innervated by the GSP nerve and are particularly sensitive to sweet and umami compounds, rather than bitter and sour. The oral cavity is also innervated by the trigeminal or V cranial nerve, which is involved in tactile sensations such as hot, e.g., capsaicin, and cool, e.g., menthol (Restrepo, 1997).

Sapid compounds are carried in saliva, diffusing through the mucous covering the surface of the tongue into the taste pore (Spielman, 1990). Transduction is initiated when the compound comes in contact with the membranes of the taste receptor cells, small bipolar cells extending from the base of the taste bud to the taste pore on the surface of the tongue. The

interaction between the compound and the taste cell leads to a change in membrane conductance, depolarization, and transmitter release onto afferent neurons for transmission to the central nervous system.

Although each taste bud contains 50 to 150 taste receptor cells, less than one-third of the taste cells within a bud are innervated, possibly due to the turnover of taste cells every few days (Kinnamon and Cummings, 1992). In addition to the taste receptor cell, a variety of basal cells are found, depending on the species. In mammals, basal cells are not innervated. In fish and amphibians, synapses from the basal cells to the primary afferent neurons are found, and the taste receptor cells also synapse with other receptor cells, indicating that the taste bud may be capable of processing information in these species. In some species, the processing of taste may be peripheral, at least in part, whereas in other species it appears to be central.

2. Receptor Mechanisms

The relationship between stimulus molecules, taste cells, and taste sensation is complex. Many compounds have multiple taste qualities and a single taste receptor cell can respond to more than one compound. A specific taste may be the result of several different transduction mechanisms. Taste transduction mechanisms can be categorized into two groups: 1. Ionotropic channels, using apical ion channels; and, 2. Metabotropic

receptors, or receptor-mediated systems. A variety of pathways can be used by a single taste stimulus (Lindemann, 2001). Afferent nerve fibers appear to have a hierarchy of sensitivities, responding preferentially to one compound over another via the population of afferent nerve fibers activated. Taste coding may also be due to unique patterns of action potentials initiated by specific stimuli, with intensity coded by the total number of action potentials occurring at a given time (Scott and Plata-Salaman, 1991). Recent evidence supports the idea that nerve cell activity patterns are critical to coding for taste (Smith and Margolskee, 2001). In mammals, many taste nerve fibers and receptor cells respond to a variety of taste qualities (Lindemann, 2001). Additionally, taste stimuli of one quality often differ in chemical properties (e.g., molecular size and shape). Thus, central processing is apparently critical for taste identification. Recent developments in molecular biology have initiated the identification of specific receptor-protein mechanisms (Chaudhari *et al.*, 2000; Kinnamon, 2000).

In ionotropic channels, salt and sour compounds interact directly with the ion channels. Sodium influx causes cell depolarization, which activates the voltage-gated calcium channels, resulting in calcium influx. Potassium, calcium, and sodium channels are found on taste cell membranes; thus, the

concentration of these ions in saliva may be important in cell depolarization and repolarization (Spielman, 1990). Sour taste is initiated by protons diffusing through apical sodium channels (Kinnamon and Margolskee, 1996) or through $H^+$-gated channels (Lindemann, 2001). The tight junctions of taste buds are permeable to $H^+$ ions, allowing them to enter the paracellular pathway and interact with basolateral ion channels in taste cells. In response to the decrease in extracellular pH, intracellular pH also decreases, indicating that $H^+$ ions probably enter the taste cells through ion channels in the taste cell membrane. Receptor mechanisms may also play a role in sour taste as acid-sensing ion channels and other types of channels may be important (Kinnamon, 2000).

Metabotropic receptor-mediated events governing transduction of sweet, bitter, and umami tastes require compounds initiating these tastes to bind to a membrane receptor, initiating a signaling cascade of second messengers. These tastes are created by a diverse group of chemicals, including carbohydrates, proteins, and peptides. Taste receptors are coupled to guanine nucleotide-binding regulatory proteins (G protein), called GPCRs (G-protein coupled receptors). The binding of a tastant to a GPCR activates a G protein subunit within the taste receptor cell, which initiates a second-messenger cascade resulting in TRC depolarization and calcium release.

Recently, a group of gustducin-linked GPCRs were cloned; this research has been reviewed by Kinnamon (2000). Several of these G protein subunits have been identified in taste receptor cells, such as □-gustducin, which is expressed selectively in TRCs. Gustducin was so named because of its similarity in structure and activity to transducin, which is a key G protein subunit in rod vision. The subunit then activates an effector enzyme such as adenylyl cyclase, phosphodiesterase, or phospholipase. These enzymes control the second messenger cascade, including cyclic nucleotides and inosine triphosphate, which regulate the activity of the basolateral ion channels, leading to the entry of calcium into the TRC and thus, depolarization. The activation of specific protein subunits determines the messengers in the cascade, which result in the perception of a specific taste.

Sweet taste is created by many compounds, including carbohydrates, proteins, peptides, halogenated sugars, aminoacyl sugars, and N-sulfonyl amides (Brand and Bryant, 1994). Receptors responding to sweet taste are of the T1R family of receptors, which are expressed primarily in palate as well as fungiform taste buds. One key receptor, T1R3, has been most identified and is highly expressed selectively in a subset of TRCs (Margolskee, 2001). Cyclic AMP and $IP_3$ appear to be the second messengers involved in the sweet taste transduction mechanism. Taste cells

appear to respond differently to nutritive and non-nutritive sweeteners, with the sugars elevating intercellular calcium via influx, but the latter group using inositol triphosphate ($IP_3$)-mediated calcium released from intracellular stores. Both of these mechanisms can take place in the same taste cell, causing cell depolarization. Researchers also have suggested an amiloride-sensitive pathway in humans and other mammals. Several sweet receptors have recently been cloned, including a heterodimer, T1R2/T1R3 (Lindemann, 2001). Sako and Yamamoto (1999) have presented evidence of a link between binding of umami and sweetener substances.

A wide variety of chemical structures have a bitter taste, including quaternary amines, acetylated sugars, alkaloids, some amino acids, and some inorganic salts. Compounds producing bitter tastes are primarily lipophilic, whereas most taste stimuli are hydrophilic. The taste threshold to bitter compounds is lower than those of sweet, sour, and salt compounds and is only slightly lower than that of umami. Bitter taste is determined by the T2R/TRB receptors, which are coupled to gustducin. The T2Rs share 30-70% sequence identity, with differences between the receptors occurring in the extracellular loops. These receptors are found primarily on the circumvallate papillae and can accommodate the structural variation of bitter-tasting compounds. Bitter compounds bind to the T2R family of taste

receptors, resulting in activation phosphodiestaerase as the effector enzyme, leading to a decrease in cNMP levels.

The T2R receptors are co-expressed with the $\alpha$ -subunit of gustducin (Lindemann, 2001). The transduction mechanisms for the bitter taste of both denatonium and PROP have been elucidated (Kinnamon, 2000). The stimulus binds to the receptor subunit nT2R$\beta$, which activates $\alpha$-gustducin. This G protein stimulates PDE, resulting in a decrease of intracellular cAMP, but the function of this decrease is not known. Concurrently, the G-protein $\beta$3/$\delta$13 partners stimulate phosphodipase C-$\beta$2, causing $IP_3$ and diacylglycerol to be produced, thereby releasing $Ca^{2+}$ from intracellular stores. Due to the structural variety and number of bitter-tasting compounds, other receptors must also be involved in bitter taste transduction. Interestingly, some bitter and sweet compounds are structurally similar, with many non-nutritive sweeteners having a bitter aftertaste. Possibly, some T2Rs may also respond to sweet stimuli.

The taste of the various amino acids are generally described as sweet, sour, salty, or umami (Solms, 1969). Several theories have been proposed to explain this varying tastes, including the position of ammonium and carboxylate groups, and the chirality and molecular volume, shape, and length of the side chain (Belitz *et al.*, 1979). Glutamate is a major excitatory

neurotransmitter in the mammalian central nervous system. In 1996, Chaudhari *et al.* cloned a metabotropic glutamate receptor from rodent taste buds. This protein, mGluR4, was found only in the taste buds, and not in the lingual epithelium. Behavioral studies with rats have confirmed that L-AP4, the specific ligand for mGluR4, produces the behavioral same response as MSG. MSG alters membrane conductance in a reversible, concentration dependent manner (Teeter *et al.*, 1997; Lin and Kinnamon, 1999).

Chaudhari and Roper (1997) suggested that glutamate might lead to closure of cation channels, producing hyperpolarizing receptor potentials. Also, stimulating circumvallate and foliate taste epithelia with MSG decreased levels of cAMP. Larger decreases in cAMP were found when a combination of MSG and IMP was used as a stimulus. Although other glutamate receptors are found in the taste buds, such as NMDA (Oh *et al.* 1999), they do not appear to be taste receptors. Chaudhari *et al.* (2000) reported that a truncated form of brain mGluR4 is a taste receptor for glutamate and therefore, less sensitive than the receptor found in the brain. The taste form of mGluR4 is truncated in the N-terminal regions, which reduces the binding region for glutamate. The G protein subunit has not been identified but is likely α-gustducin or α -transducin. After binding to the receptor, the G protein subunit activates PDE, which decreases

intracellular cAMP. Glutamate elicits both depolarizing and hyperpolarizing responses (Kinnamon, 2000). Evidence of calcium-permeable glutamate receptors in taste cells has also been reported. These receptors may be presynaptic autoreceptors or post-synaptic receptors (Kim *et al.*, 1999; Caicedo *et al.*, 2000). Rolls (2000) reported neurophysiological research in rats suggesting that part of the umami flavor may result from stimulation of both olfactory and gustatory glutamate receptors. Indications of a separate taste area in the orbitofrontal cortex for glutamate have also been reported (Rolls, 2000).

Electrophysiologic studies in rats have demonstrated the synergism of 5'-ribonucleotides with various amino acids. Yoshii *et al.* (1986) proposed that receptor membranes have multiple receptor sites with different dissociation constants for each amino acid. These researchers also suggested that 5'-ribonucleotides have specific binding sites in the receptor membranes closely associated with the amino acid receptors, thereby increasing the affinity of amino acids to their respective receptors. Neurons that responded to IMP were more related to responses to MSG than any of the other "basic" tastes (Rolls, 2000). Using L-$AP_4$, an agonist of the mGluR4 receptor, taste synergy with MSG and IMP was found, with evidence that similar receptors may be involved with the synergistic response (Delay *et al.,* 2000).

Alternatively, a convergence of signaling pathways from separate receptors or separate sensory cells onto common afferent fibers may create this synergistic response (Chaudhari *et al.*, 2000).

3. Structure-Function Theories

Several theories have been considered concerning the structure of a compound and its perceived taste. Using molecular information about the structure and shape of molecules, specific stereochemistry is related to the configuration of taste receptors. Birch (1981) proposed that sweetness perception was due to a combination of factors, including molecular stereospecificity with a receptor, the diffusion properties and localized concentration of stimuli near the receptor, the length of time the stimuli has to approach and align with the receptor, and the speed at which the stimuli pass through the receptor membrane. The cloning of sweet receptors of the T1R family indicate that receptor mechanisms may be primarily responsible for sweet taste.

Structure-function theories have also been postulated to explain the taste potentiating effect of MSG with 5'-ribonucleotides. Saint-Hilaire and Solms (1973) suggested that nucleotides may stabilize the helical structure of the proteins at the receptor surface, thus increasing the receptor surface available for tastant and receptor interaction. An increase in receptor sites

for glutamate when mixed with 5'-ribonucleotides has been demonstrated by Torii and Cagan (1980) and a second binding site for a taste modifying protein adjacent to the active bonding site may alter the glutamate receptor affinity of the information provided to the receptor (Kinnamon and Cummings, 1992; Kumazawa *et al.*, 1991). The finding that only the L-isomer of MSG has taste, but the D-isomer is tasteless, suggests that the receptor for L-glutamate is chiral or selective for one optical isomer (Hettinger *et al.,* 1996). Rolls (1997) found that stimulating neurons in the primary taste cortex of macaques with IMP correlated better with responses to MSG than to any prototypical tastant. Similar results have been found for GMP and glutamate in rat fungiform taste buds (Kinnamon, 1997). Cells that are sensitive to glutamate may or may not respond to GMP, suggesting different receptors for these compounds. Others have found evidence that GMP uncovers "hidden" glutamate receptors via allosteric interactions (Cagan, 1987). Alternatively, Yoshii *et al.* (1986) described a mechanism whereby GMP causes an increase in the binding affinity of glutamate to the receptors.

Birch (1987) noted that temporal factors might be important to describe chemoreception. He has detailed the stereochemistry of MSG, suggesting that its structure could elicit the taste of sweetness at the amino-

carboxy end, sourness by active protons, saltiness from the sodium attached to the carboxy group, and bitterness by the interaction of hydrogen-bonding polar, steric and hydrophobic factors (Birch, 1987). This paper also details the hydration and molar volume of MSG, discussing how this may impact the strength of the receptor bond via a "lock and key" principle, with MSG reaching a deeper region of the receptor.

C. Saliva

1. Function and Composition

Saliva is a viscous colorless oral secretion that is usually alkaline and is secreted into the mouth by the salivary glands (Ellison, 1978). Approximately 500 to 750 ml of saliva is secreted daily, most being reabsorbed into the body by swallowing. Three glands produce most of the saliva: the parotid, submaxillary or submandibular, and the sublingual, with salivary composition varying somewhat from each gland (Bradley, 1991). Saliva coats the oral cavity, inhibiting bacterial growth and transporting components from blood plasma into the mouth. The von Ebner glands are contained in the tongue and supply saliva to the circumvalliate and foliate papillae. The saliva from these glands appears to be involved in taste transduction mechanisms (Spielman, 1990; Bradley, 1991). Saliva is the solvent for water-soluble compounds, many of which provide flavor.

Enzymes are also present in saliva. Amylase begins the breakdown of starch and glycogen, proteases are present to begin cleaving proteins, and lipases may be important in fat digestion (Bradley, 1991). The enzymatic composition of saliva is dependent upon the secretion gland and the taste stimuli. A strong stimulus, such as salt, activates the submandibular and sublingual glands more than the parotid gland, which increases the proportion of lysozyme to α-amylase (Noble, 2000). In addition to saliva's role in digestion and oral health, it has other important functions. These include formation of the bolus to be transported down the esophagus, lubrication for swallowing, and cleansing and antimicrobial effects, protecting both the teeth and taste buds from bacteria.

Saliva consists of 99% water and small amounts of various organic compounds, including mucin, glycoproteins, protein, salts, and enzymes. Many other taste active compounds, including sodium, potassium, chloride, calcium and bicarbonate, are present in saliva, usually at levels below their detection threshold. These compounds may play a role in the changes in the ionic environment at the apical surface of the taste cell (Spielman, 1990). This review will focus on protein compounds in saliva. For complete reviews on salivary composition, see Bradley (1991), Ellison (1978), and Shannon *et al.* (1974).

Protein accounts for a relatively high percentage of organic components in saliva, but free amino acids are generally present in very low levels. Large peptides and proteins can be hydrolyzed extracellularly by proteases into 2 to 5 amino acid residues that can be transported directly into bacteria, which then decarboxylate amino acids to maintain a pH of 5.82 (Kleinberg *et al.*, 1978). Peptide synthesis and glycosylation occur in the rough endoplasmic reticulum and Golgi cisternae of the acinar cells (Bradley, 1991). These proteins are released by exocytosis. However, when the parasympathetic nervous system is stimulated, the proteins appear to be released by a different mechanism.

Nucleotides, particularly cylic AMP, have been found in salivary glands (Shannon *et al.,* 1974). Proline-rich proteins are also present in saliva. They appear to be important in protecting the surface of teeth and they may function as carrier proteins in gustation (Spielman, 1990; Bradley, 1991). Other proteins are synthesized and secreted by ductal cells, including growth factors and digestive enzymes. Glutamate is one of the primary amino acids in glycoproteins that coat the entire oral mucosa, protecting the soft tissues from environmental hazards and auto-digestion by enzymes (Ellison, 1978). When glutamate is released from protein, it could bind with sodium, one of the most prevalent salivary cations, precipitating the umami

response. Other important compounds, such as vasoactive and regulatory peptides, have also been identified in saliva, with their release governed by alpha-adrenergic or hormonal control. Secretion of these compounds into saliva followed by swallowing and reabsorption results in an insignificant increase in the plasma levels of these compounds (Bradley, 1991).

Salivary flow rate is controlled by the autonomic nervous system, and is affected a parasympathic response. When food is smelled, salivary flow is increased via a parasympathetic response from the chemoreceptors. This response is also responsible for flow increases when a person just thinks about food. When food is introduced into the mouth, flow rate increases due to mechanoreceptors. The composition and flow rate of saliva varies depending upon a several factors, including time of day, the gland from which saliva is collected, the agent used to stimulate salivation, and the health of the subject, including diet and medication. Differences in these experimental factors among studies can lead to incongruous results (Kapur *et al.*, 1966). Flow rate also impacts salivary composition, but different salivary collection methods do not alter the measurement of whole mouth salivary flow rate (Navazesh and Christensen, 1982). When flow rate increases for any reason, sodium concentration increases and potassium concentration decreases (Prader *et al.*, 1955).

Salivary flow rate and composition vary with time of day; with the highest flow rates generally occurring during the early afternoon (Christensen, 1986). Sodium content was found to be highest before breakfast, decreasing throughout the day (Grad, 1954). These high sodium levels in the morning occur concurrently with low potassium levels, which increase throughout the day (Prader *et al.*, 1955). Grad (1954) also found that gender and age influenced salivary sodium. Men who were forty years of age or older had higher levels than women and also higher levels than men under forty years. Salivary sodium is affected by renin and aldosterone levels, which increase with a decrease in dietary sodium (Wotman *et al.*, 1973). Salivary protein increases with an increase in flow rate, and stimulation with NaCl may also increase salivary protein and calcium (Spielman, 1990). Guinard *et al.* (1998) found salivary total protein, sodium, and potassium concentrations were dependant on salivary flow rate; however, these salivary components did not impact the temporal perception of taste attributes in actual food systems, including the umami taste of broth with added MSG.

The contribution to flow rate by major salivary glands also may account for differences among individual subjects. Christensen *et al.* (1986) found that salivary sodium and flow rate were not correlated for group data

but individual data were correlated, perhaps because of differences in flow rate from major salivary glands. Higher flow rates can dilute a stimulus faster and to a greater extent than lower flow rates. A high flow rate also allows stimuli to be cleared from the mouth in a shorter period of time, which affects the subject's adaptation to the stimuli.

Some research has suggested that lack of salivary flow does not decrease taste sensitivity, as might seem logical (van Pelt, 1988; Christensen *et al.*, 1983). However, Bradley (1991) reports research supporting decreased taste acuity with reduced salivation. Research by Temmel *et al.* (2000) also found decreased taste sensitivity with xerostoma, a condition in which salivary flow is severely reduce, with no improvement in taste senstivity with a saliva replacement. Patients with xerostoma must increase the amount of effort and time required to eat, thereby decreasing the amount eaten, which may have adverse nutritional consequences (Kapur *et al.*, 1966).

Laboratory experiments have found that salivary composition varies with stimuli and stimuli delivery method. Introducing the stimulus changes the composition of the saliva in the oral cavity, which can alter taste sensitivity. Most experiments that quantify salivary composition do not stimulate salivary flow with a chemical stimulant, but use a taste-neutral

object such as a rubber band or unflavored chewing gum. In one study, parotid saliva collected under sour stimulation had the highest sodium concentration as compared to sweet, salt, and bitter stimuli. The stimulus alters salivary composition by affecting flow rate, stimulus dilution, and post-absorptive events. For example, an acid stimulus increases flow rate, which increases bicarbonate levels. Depending upon the quantity of the stimulus used, saliva may account for ten percent of the total volume of the stimuli-saliva mixture in the mouth, effectively increasing the concentration of very dilute stimuli (Christensen, 1986). Pre-test condition of subjects also must be considered, such as the composition and timing of any consumption by the subject prior to testing.

The nature of the stimulus can also affect salivary flow rate. Pangborn and Chung (1981) found that NaCl in water induced a salivary flow rate twice as high as that induced by L-glutamic acid. In commercial beef broth, L-glutamic acid was more effective at increasing salivary flow than in commercial chicken broth or water. However, the initial L-glutamic acid content of these carriers was not measured. When L-glutamic acid and NaCl were tested in combination, a type of mixture suppression of salivary flow resulted, possibly due to interactions between the compounds. This

study did not demonstrate fatigue in the salivary glands with continuous stimulation by L-glutamic acid and NaCl.

Different methods of stimulus delivery include placing filter paper circles on the tongue, flowing solutions over a fixed area of the tongue, and drinking of the stimulus by the subject. These methods vary the quantity and rate of delivery. If the results of the experiment are to be extrapolated to an eating experience, the test design needs to mimic actual consumption behavior as closely as possible. Instructions given to subject to swallow or expectorate and to move the mouth or hold it still influence results. Chewing movements have been found to increase salivary sodium concentrations (Delwiche and O'Mahony, 1996), so mouth movements must be controlled. The type and duration of the interstimulus rinse is also very important. In conducting threshold tests for the salt taste, O'Mahony and Heintz (1981) found that rinsing between samples significantly lowered the NaCl concentration in the mouth, a practice not usually done in threshold tests. Using signal detection measures, Delwiche and O'Mahony (1996) found that elevated salivary sodium levels, brought about by chewing, resulted in a decreased sensitivity to NaCl. They proposed that this result occurs because chewing increased secreted sodium, which mixes with the water used for rinsing, increasing the "taste zero" or taste baseline, making

subsequent samples more difficult to detect. Therefore, taste sensitivity depends upon receptor sensitivity and the setting of the taste zero or baseline by the surrounding medium. For a more thorough discussion of experimental factors, the reader is referred to Delwiche and O'Mahony (1996), Bradley (1991), Christensen (1986), Bartoshuk (1974, 1980) and Dawes (1967).

The health of the subject influences salivary sodium levels (Dewiche and O'Mahony, 1996). Salivary sodium is elevated above levels found in healthy subjects among patients with cystic fibrosis, adrenal insufficienty (Addison's Disease), and Sjögren's syndrome, whereas hypertensive patients have lower salivary sodium levels. None of these conditions have been found to alter sodium taste sensitivity. Various pharmacological agents have been found to change salivary composition, thereby affecting taste (Christensen, 1986). The impact of restricting dietary sodium on salivary sodium levels in not clear and is discussed in the next section.

2. Role of Saliva in Taste Perception

Saliva contains taste active compounds, including salts, amino acids, and glucose. Although present at very low concentrations, these compounds could effectively increase the concentration of these compounds in very dilute stimuli. Saliva dilutes tastants and moves them through the oral cavity

at different rates, contributing to subject variability because of individual differences in salivary composition and flow rate. Salivary buffers can alter the pH of stimuli, and especially influence the perception of acid stimuli (Christensen, 1986). Additionally, the pH in the oral cavity, due in part to salivary pH, and of the stimuli could affect the perception of MSG because it is an ampholyte, although this effect of pH has never been experimentally measured. These differences are magnified when only a small amount of a stimulus is used, for example, with delivery systems such as filter paper circles. Using a small stimulus volume magnifies salivary effects; therefore, a larger stimulus quantity will decrease individual subject variation, thereby increasing subject sensitivity (Christensen, 1986).

Sensitivity to a particular tastant depends on the interaction between the tastant and the receptor and on the oral and microenvironment around the taste pore. Although not well understood, the microenvironment may change during gustation, controlling access and removal of stimuli, or it may serve merely to carry the stimuli to the receptors. Determining the composition of saliva, either whole mouth or from a specific gland, may not indicate the composition of the micropore environment.

Adaptation is a common phenomenon in sensory tests of taste and odor. Also known as fatigue, adaptation is the change in sensitivity of a

sensory system caused by a stimulus, usually resulting in the decrease or disappearance of a sensation. Adaptation modifies the perceived intensity of subsequent stimuli. If the prior stimulus is strong, the subsequent stimulus is perceived as tasteless. Expectorating a stimulus and then adding fresh adapting solution every minute during a five minute period increased perceived intensities when compared with evaluations made by subjects under continuous adaptation (Meiselman, 1968). Saliva dilutes the stimulus and carries it away from receptors, but does not impact taste intensity loss as significantly as sensory adaptation. These factors, stimuli and saliva, work together to set the "taste zero" or baseline against which a subject makes an evaluation. The taste zero for any compound is based on the concentration of that compound in the mouth, by readjusting to the adapting solution (Delwiche and O'Mahony, 1996). If the taste zero decreases, then sensitivity increases and suprathreshold concentrations are perceived as being more intense. Mouth movements disrupt the oral environment to the extent that adaptation is not complete (Meiselman, 1968; O'Mahony and Wong, 1989). Adaptation can also modify the perceived quality of stimuli. Adaptation to NaCl eliminated the perceived saltiness of fifteen organic and inorganic salts, including MSG, but increased the perceived sour and bitter intensity of these salts (Smith and van der Klaauw, 1995). Only the anterior

tongue surface was stimulated so as to eliminate the influence of saliva. Although the NaCl adapting solution decreased the perceived saltiness of MSG, the total perceived intensity was not decreased because the perceived intensities of bitter and especially sour were increased.

The role of salivary components in taste perception has been explored primarily with sodium (Bartoshuk, 1978; Delwiche and O'Mahony, 1996). The saltiness of NaCl was first believed to be due to the chloride anion because salts with a common cation but different anions had similar thresholds (Bartoshuk, 1980). However, Beidler (1966) used neural responses with salts to determine that the cation was responsible for stimulating the salt chemoreceptors and the anions were, in fact, inhibitory.

Subjects with low levels of salivary sodium would be expected to be more sensitive to sodium stimuli (Bartoshuk, 1978; Bradley, 1991). NaCl should taste salty only when it is present in the mouth in concentrations that are higher than the concentration of salivary sodium. Thus, the detection threshold to NaCl should be greater than the level of salivary sodium. However, this theory is not always confirmed by empirical testing. Detection thresholds have been found to increase (indicating lower sensitivity) with increasing salivary sodium (Bartoshuk, 1974; 1978) and recognition thresholds were found to correlate positively with salivary

sodium (Morino and Langford, 1978). Taste sensitivity to NaCl, as determined by signal detection measurement, was reduced when salivary sodium levels were increased by chewing a flavorless gum (Delwiche and O'Mahony, 1996). In contrast, Pecore (1979) and Christensen *et al.* (1983) did not find a relationship between salivary sodium and NaCl detection thresholds, with subjects having normal ranges of salivary sodium. Christensen has found that subjects with very high levels of salivary sodium have lower than average detection thresholds to NaCl, perhaps because high levels of salivary sodium supercedes the effect of adaptation (Christensen *et al.*, 1983; Christensen, 1986). It should be noted that taste sensitivity is generally determined via threshold measurements, which generally do not indicate sensitivity to suprathreshold concentrations. In a review of the role of NaCl in the salt taste, Lynch (1987) noted that temporarily restricting dietary sodium results in subjects rating salty foods as tasting more pleasant. However, longer restriction of sodium causes salty foods to taste less pleasant (Beauchamp, 1981).

The role of dietary sodium in sodium sensitivity and salivary levels is also not clear. Dietary intake can impact the composition of saliva by pre-absorptive routes through oral concentration and flow linked changes and by post-absorptive endocrine mechanisms, specifically changing levels of

aldosterone, the primary sodium-retaining hormone (Christensen *et al.*, 1986; Wotman *et al.*, 1973). In general, a decrease in dietary sodium appears to result in decreased salivary sodium but the converse may not be true. Severe or moderate restriction of dietary sodium has been found to result in lower salivary sodium (Christensen *et al.*, 1986). The converse result, higher salivary sodium with increased salt intake, has not always been found. High levels of salt intake have been found to increase salivary sodium levels by Damodaran and Kinsella (1980), and Bradley (1991) but not by Pradar *et al.* (1955) and Wotman *et al.* (1973). The method of determining salt intake varied among these studies, thus influencing the results. Dietary restriction of sodium is very difficult in a free-living group of subjects, so diet intake is often self-reported via frequency questionnaires. The data from these reports can be inaccurate and may be influenced by the design of the questionnaire and subjects' beliefs of what they think they "should" be eating or what the experimenter anticipates (Kerr *et al.*, 1979; Meiselman, 1987).

Affective evaluations are influenced by changes in sodium consumption. Pecore (1979) found that sodium intake was directly related to the amount of NaCl added to unsalted tomato juice in an *ad libitum* procedure. However, no significant relationships between sodium intake

(measured by a self-administered questionnaire) and discrimination, intensity, and hedonic sensory ratings of salt taste were found. Salt depletion led to a preference for higher sodium levels in soup and crackers compared to subjects intake during normal consumption, possibly due to subjects' experience eating low salt foods, rather than a physiological response (Bertino *et al.*, 1983). Beauchamp *et al.* (1990) found that individual subjects' threshold to NaCl decreased during sodium depletion, which was experimentally controlled with diuretics, in six of ten subjects. Supra-threshold intensity judgements were not affected by sodium state, but preference judgements of salt in foods indicated that subjects preferred higher concentrations of salt during the sodium depleted time periods. They also reported that the subjects had an increased desire for salty foods, as determined by a self-reported questionnaire. Creating a true sodium depletion state in humans is difficult. The impact of post-ingestive factors in the salt appetite for salt appears to be weak compared to the role of sensory factors (Rowland, 1990).

Many factors affect the results from these experiments and can account for the differences found in these studies (Bartoshuk, 1980; Delwiche and O'Mahony, 1996). Individual differences among subjects in salivary composition and flow rate due to age, gender, diet, health, and other

factors is most likely the primary reason for incongruent results. Different experimental methods also lead to differing results. Saliva may be collected under different conditions than the conditions present during sensitivity testing. The adaptation conditions change during sensitivity testing, depending upon the rinsing procedure used, if re-sampling of the stimulus is permitted, the volume of the stimului, the size and region of the tongue that is stimulated (Morino and Langford, 1978; Bagla *et al.*, 1997), and the method of saliva collection, which could artificially increase salivary sodium due to stimulation of salivary flow rate. The analytical method used to measure salivary sodium varies among experiments, including controlling for interfering substances. Bartoshuk (1978) states that confusion among subjects between the taste of the stimuli and the water taste can account for the unexpected result of a lower detection threshold with higher salivary sodium levels.

Only one study of salivary composition and taste perception of MSG was found. Yamaguchi *et al.* (1987) reported average salivary glutamate levels as $1.8 \times 10^{-5}$ M. They concluded that the level of glutamate in the saliva was sufficient to decrease both the detection and recognition threshold of IMP. As MSG was added to IMP, umami intensity increased logarithmically, demonstrating a synergistic effect. They postulated that

salivary glutamate could act synergistically with the nucleotides in a food to produce the umami taste. The need for more research in exploring the relationship among salivary composition, taste perception, and diet has been presented by Christensen *et al.* (1986) and Smith and van der Klaauw (1995), who emphasized the importance of relating human sensation and perception to transduction events.

# CHAPTER 3

## The Relationship Between Salivary Composition and Taste Perception of Flavor Potentiators

The primary objective of these experiments was to determine the relationship between salivary composition, namely L-glutamic acid and sodium, and taste perception of monosodium glutamate (MSG) and sodium chloride (NaCl) using threshold and intensity measurements. A secondary objective was to examine the roles, if any, of gender (male or female) and ethnicity (Asian or non-Asian) in the taste perception of MSG and NaCl.

### I. Study 1. Preliminary Experiment

#### A. Materials and Methods

##### 1. Subjects

Twenty-two subjects, students and staff at Colorado State University, Fort Collins, CO participated in the entire study, with three additional subjects completing some of the tests. The group consisted of five Asian men, six Asian women, three non-Asian men and eleven non-Asian women. Subjects ranged in age from 21 to 52 years of age. Because adverse physical reactions to MSG have been reported by some people (Life Sciences

Research Organization, 1995), potential subjects were told that they would taste MSG as part of the study and were asked if they had ever experienced or thought they may have experienced a reaction after consuming MSG or foods that may have contained MSG. If the response to this question was negative, they were recruited for the study. All subjects gave their informed consent (Appendix I) and were compensated with refreshments after each test.

2. Procedure

Subjects completed the testing over a three-week period. All testing was conducted between 9 a.m. and 12 p.m. In the first week, each subject expectorated at least 2 ml of whole mouth saliva into a 5 ml vial on three different days. Subjects refrained from eating or drinking anything but water one-half hour prior to expectoration and refrained from drinking water ten minutes prior to the test to prevent salivary dilution of L-glutamic acid. Salivary stimuli were stored immediately after collection at 4°C for L-glutamic acid determination later that day. Bench-top testing with five subjects determined that storing salivary stimuli in the refrigerator or freezer for up to 3 months did not alter the levels of L-glutamic acid in the saliva. Subjects completed a food frequency questionnaire to estimate their intake of MSG (Appendix II).

In week two, subjects tasted stimuli with five concentrations of MSG (Ajinomoto Interamericana Ind E Com LTD, Brazil) at five levels in distilled water, unsalted canned tomato juice (Sacramento brand, Sacramento, CA; pH=4.2; naturally occurring L-glutamic acid content = 70 mg/l), and beef broth (Table 1). The beef broth was prepared fresh each day by simmering 2 pounds of beef soup bones in 6 quarts of tap water for 60 minutes over low heat. The bones and pieces of meat were removed from the broth, which was then refrigerated overnight. Two hours before the test, the broth was removed from the refrigerator, pieces of fat were skimmed from the broth, which was then poured through a cheesecloth to removed additional solids. The naturally occurring L-glutamic acid content of the beef broth was measured as 25.7 mg/l and pH was determined to be 6.5. MSG levels of the stimuli were chosen to be comparable to L-glutamic acid concentrations in foods, both naturally-occurring and added. Stimuli were prepared on a weight/volume percent basis by adding the appropriate number of grams to 500 ml of each food system.

Table 1. MSG concentrations (percent wt/vol) in three food systems.

| Modality | Percent MSG (w/v) | | | | |
|---|---|---|---|---|---|
| Water | 0 | 0.025 | 0.05 | 0.075 | 0.1 |
| Tomato Juice | 0 | 0.025 | 0.05 | 0.1 | 0.2 |
| Beef Broth | 0 | 1 | 2 | 4 | 6 |

Approximately 2 oz (60 ml) of each stimulus was served in a randomized order at room temperature in 3-ounce white plastic cups, coded with three digit random numbers. Tasting was done by subjects in individual portable booths. Prior to testing, subjects were familiarized with the umami taste by tasting the four stimuli of MSG in distilled water, in order from least to most intense. Before intensity evaluations were made, subjects' preferences for MSG in tomato juice and in beef broth were determined by having the subjects rank the five MSG stimuli in order from most well-liked (ranked first) to least well-liked (ranked last) to determine which stimulus was most preferred.

On each of three separate days during a one week period, subjects tasted the concentration series of MSG in distilled water first, followed by tomato juice, and lastly beef broth, so that each test was replicated three times. The overall intensity, umami intensity, and salt intensity of each stimulus was evaluated using an unstructured 146 mm line scale, anchored with the words "None" and "Extreme" at the left and right scale ends, respectively (Appendix III). For tomato juice and beef broth the subjects also rated the intensity of tomato and beef flavor, respectively.

In the third week, detection and recognition thresholds were determined using the Ascending Concentration Series Method of Limits

(Jellinek, 1985). Sensitivities to sucrose, citric acid, NaCl, caffeine, and MSG were determined, and the test with MSG was replicated.

3. Chemical Analysis

The concentration of L-glutamic acid in the canned tomato juice, the prepared beef broth, and the subjects' saliva was determined using an enzymatic test kit (Boehringer Mannheim BMGH, Germany). In this analysis, L-glutamic acid is oxidatively deaminated by nicotinamide-adenine dinucleotide (NAD) to 2-oxoglutarate with L-glutamate dehydrogenase. The NADH formed in this reaction converts iodonitro tetrazolium chloride to a formazan, which is red. The intensity of the red color is proportional to the amount of L-glutamic acid that was in the stimulus. The red color of the formazan is measured spectrophotometrically at 492 nm. A Spectronic 21 (Bausch and Lomb, Rochester, NY) spectrophotometer was used for all glutamic acid analyses.

The concentration of L-glutamic acid was determined using the general equation for calculating the concentration relative to a standard:

$$C\ (g/l) = (\Delta A_{stimulus} / \Delta A_{standard}) \times C_{standard}$$

where $\Delta A_{stimulus}$ = change in absorbance of stimulus; $\Delta A_{standard}$ = change in absorbance of standard; and $C_{standard}$ = concentration of the standard, as specified in the test kit.

4. Statistical Analyses

Data were analyzed using SPSS 8.0 (SPSS, Chicago, IL). Analyses of variance (General Linear Model) and Pearson's Product Moment Correlation tests were conducted. Differences between stimuli were determined using the LSD (Least Squares Difference) test.

B. Results

Table 2 details the means and standard deviations for the four experimental variables. Average salivary L-glutamic acid for 25 subjects was 9.50±11.0 mg/l, or 0.0646 mM, ranging from 0.740 to 48.1 mg/l (0.0503 to 0.327 mM). Significant differences were found among subjects in salivary L-glutamic acid levels ($p \leq 0.001$; Table 3). Salivary L-glutamic acid did not vary among the three replications, demonstrating that for each subject, salivary L-glutamic acid did not vary from day to day (Table 3). No differences in salivary L-glutamic acid due to ethnicity or gender were found. Ethnicity influenced preference for MSG in tomato juice and beef broth (Tables 2 and 3). Asians preferred 0.15% MSG as compared to non-Asians, who preferred 0.11% ($p=0.045$). Dietary intake of MSG, as derived from the food frequency questionnaire, differed between Asians and non-Asians ($p=0.007$; Table 3). Asians consumed relatively more MSG than non-Asians.

The five concentrations of MSG in water and beef broth differed significantly in intensity for all attributes evaluated ($p \leq 0.001$ for both; Table 4). However, in unsalted tomato juice, no significant differences were found among the concentrations for any of the attributes. The inability of the subjects to differentiate the samples indicates that the addition of MSG to unsalted tomato juice did not increase its overall flavor, umami flavor, tomato flavor, or salt intensity, perhaps due to its high level of endogenous L-glutamic acid or it's low pH. Graphs of perceived intensity across stimulus concentrations by ethnicity and gender for all attributes in each modality are in Appendix IV.

For overall intensity in water, neither the two lowest concentrations of MSG nor the two highest concentrations were significantly different (Table 5). The umami taste of all stimuli differed, with the exception of the highest two. The salt intensities of the 0 and 1% concentrations were less than the others and the highest concentration had more salt intensity than the middle stimulus ($p \leq 0.001$). For overall flavor and salt intensity of beef broth, all stimuli except 1% and 2% MSG were perceived as different (Table 5; $p \leq 0.001$). The umami intensity of 0% MSG was less than the other stimuli, while that of the highest concentration was more intense. Beef flavor was

most intense in the two highest concentrations, followed by the 1% and 2% concentrations, which were more intense in beef flavor than 0% MSG.

Differences in perceived intensities due to ethnicity or gender were found for all attributes of tomato juice and beef broth (except salt intensity in beef broth) and in water, only salt intensity was different (Table 4). For each attribute, mean intensity scores with standard deviations for the five stimuli in each modality are presented in Table 5. Intensity scores were averaged across concentrations for each attribute in each food systems and factored by subjects' ethnicity and gender (Table 6). Although the intensity of the tomato juice stimuli did not differ across subjects, differences were found when intensity scores were factored by ethnicity and gender. For overall intensity in tomato juice, males and Asians gave higher scores than females and non-Asians ($p=0.003$ and 0.002, respectively; Table 4). The interaction between these factors was highly significant ($p\leq0.001$). Asian males rated the stimuli as more intense than the other three groups, and non-Asian females rated the stimuli as more intense than non-Asian males and Asian females, which did not differ in their ratings. In beef broth, overall intensity was rated higher by Asians than non-Asians ($p\leq0.001$); no differences were found between males and females.

For umami intensity in tomato juice, males gave higher scores to the stimuli than females ($p=0.001$). The ethnicity by gender interaction was also significant ($p=0.002$) with the same trend as overall intensity: Asian males gave the highest intensity scores to the stimuli. In beef broth, Asians rated the umami taste intensity of MSG in beef broth as more intense than non-Asians ($p\leq0.001$); no significant differences due to gender or interaction between ethnicity and gender were found. Differences were also found in tomato flavor intensity in tomato juice between groups. Asians gave significantly higher ratings to the stimuli than non-Asians ($p\leq0.001$). This latter direction in difference was also found for beef flavor intensity ($p\leq0.001$). Interaction between ethnicity and gender was highly significant for tomato flavor ($p\leq0.001$), with Asian males giving higher scores to the stimuli than the other groups. This interaction was not significant for beef broth.

Non-Asians and males gave the water stimuli higher salt intensity scores than females and Asians ($p=0.002$ and 0.024, respectively). The ethnicity by gender interaction was not significant. The saltiness of the tomato juice stimuli was rated as more intense by males than females ($p\leq0.001$), with Asian males giving significantly higher intensity scores than the other three groups ($p=0.002$). No differences due to ethnicity or gender

of subjects were found for salt intensity in beef broth. Both genders and ethnicities were consistent in their evaluation of the stimuli, as no interaction of gender by stimuli or ethnicity by stimuli was found (Table 4). Subjects differed in their evaluation of the stimuli for all attributes across all modalities ($p \leq 0.001$; Table 7). Only in water did replications differ ($p=0.023$ and 0.039 for overall and salt intensity, respectively). For overall intensity, average scores for replication 1 were greater than those for replications 2 and 3, and for salt intensity, the scores given to the first two replications were greater than those for replication 3.

Mean detection and recognition thresholds to MSG, sucrose, citric acid, NaCl, and caffeine are detailed in Tables 8a and 8b, respectively. No significant difference was found between replications for either the detection or the recognition threshold, so the data were averaged. The mean detection threshold for MSG, averaged across the subjects' gender and ethnicity, was $0.00895 \pm 0.00038$ g/100 ml (0.0478 mM). Males had a higher detection threshold for MSG than females ($p=0.050$; Tables 8a and 8b). No other differences in detection thresholds due to ethnicity or gender were found for MSG.

The detection threshold for NaCl was $0.0219 \pm 0.00016$ g/100 ml (3.78mM). Non-Asians had a higher threshold for NaCl than Asians and

thresholds for males were higher than those for females ($p=0.004$; Tables 8a and 8b). The ethnicity by gender interaction was also significant, with non-Asian males having higher thresholds than the other groups ($p=0.004$). None of the detection thresholds for the other compounds tested differed by ethnicity or gender. The recognition thresholds to citric acid and NaCl differed between gender and ethnicity (Tables 9a and 9b). For citric acid, non-Asians had a higher recognition threshold than Asians ($p=0.042$), with non-Asian males having the highest thresholds ($p=0.048$). Asian females had higher recognition thresholds for NaCl than non-Asian females ($p=0.027$). No other differences in recognition thresholds were found between the groups.

Correlations between several experimental variables were significant ($p\leq0.05$; Table 10). Dietary MSG intake was negatively correlated with salt detection threshold ($r= -0.490$, $p=0.024$). The detection threshold for MSG was weakly negatively correlated with salivary glutamate ($r= -0.422$, $p=0.072$). Detection and recognition thresholds were positively correlated for citric acid and caffeine ($r=0.725$, $p\leq0.001$; $r=1.00$, $p\leq0.001$). The detection threshold for sour was also positively correlated with the recognition threshold for sweet ($r=0.564$, $p=0.018$). The interpretation of these correlations is not apparent, especially considering the low number of

subjects and the biased threshold measurement method used (see the Literature Review discussion onThreshold Measurements).

This preliminary experiment demonstrated that salivary L-glutamic acid and sensitivity to MSG varied considerably among subjects; thus, a group mean did not represent individual experience. Subjects varied approximately 65-fold in salivary L-glutamic acid levels. Asian males gave higher intensity scores to MSG in water and two food systems than females and non-Asian males. Non-Asian males had the highest mean threshold to NaCl and perceived MSG in water as saltier than the other groups. Because the small sample size limited the interpretation of the results, Study 2 was conducted with more subjects, balanced for ethnicity and gender, and included fewer test variables. In addition, a more precise method of measuring thresholds was used.

## II. STUDY 2. PRINCIPAL EXPERIMENT

### A. Materials and Methods

#### 1. Subjects

Sixty non-smoking subjects were recruited at the University of Colorado Health Sciences Center by posting signs around campus and via personal contact. Subjects were screened for availability, cigarette smoking, medication, and sensitivity to MSG. Subjects were balanced for gender and

ethnicity: 15 Asian women, 15 non-Asian women, 15 Asian men, and 15 non-Asian men. Mean subject age was 34.8 years, and ranged from 23 to 58 years. Subjects gave their informed consent (Appendix V) and were compensated monetarily upon completion of the one-hour study.

2. Procedure

Subjects completed the testing in one session, which lasted approximately 45 minutes and was conducted between 9 a.m. and 3 p.m. over a three week period. Subjects were told to refrain from eating or drinking anything, except water, for one hour prior to the test, and to refrain from drinking water 15 minutes prior to the test. Subjects completed a questionnaire designed to estimate their intake of foods high in MSG and NaCl (Appendix VI).

The three-phase test was completed as follows:

1. A 2 ml stimulus of whole mouth saliva was expectorated into a 15 ml plastic centrifuge tube with a screw top. Stimuli were frozen immediately for sodium and L-glutamic acid analysis at a later time.
2. The threshold of each subject to NaCl first and then MSG was determined using the Maximum-Likelihood Staircase Procedure (Linschoten *et al.*, 2001). This procedure used a two-alternative

forced-choice procedure in which a stimulus is paired with a blank. Both the stimulus and the blank were presented at the same time, and subjects were required to pick the stimulus with a taste, or, if they are unable to detect a taste, to make their best guess. Subsequent stimuli of higher or lower concentrations were presented based on the correctness of the subject's response. The subject chose to swallow or expectorate the stimulus, but was asked to swallow a small amount. Subjects rinsed between stimulus pairs with distilled water until the taste of the stimuli had been removed.

3. Subjects used a modified magnitude estimation method to rate the overall taste intensities of five concentrations of NaCl and of MSG. In this method, subjects estimated the strength of each stimulus by moving a metal tab along a non-graduated linear scale of 30 cm, labeled "None" on the left end and "Extreme" on the right end of the scale. Subjects were asked to rate overall intensity of the stimuli rather than a particular taste to prevent confusion about the meaning of a specific attribute and to avoid the need for references or training of subjects to a specific taste. Subjects were instructed to make ratio judgments by moving a tab on a metal

plate a distance to the right corresponding to their perception of the intensity of the stimulus, relative to the previous one. On the back of this device, unseen by the subject, the experimenter read and recorded the intensity estimates, defined as mm distance from the left starting point. Subjects tasted the NaCl stimuli series first, followed by the MSG stimulus series. For each tastant, the five concentrations were replicated three times, with stimuli completely randomized across stimuli and replications. Subjects took approximately ten minutes to evaluate the fifteen stimuli, with a five-minute break between the tastants. Subjects had the option of swallowing or expectorating the stimulus, but were asked to swallow a small amount of stimulus to allow the stimulus to reach all areas of the mouth. Subjects rinsed after each stimulus with distilled water until the taste of the stimulus had been removed.

Data from the threshold and intensity evaluations were recorded on the data collection sheet (Appendix VII).

3. Stimuli

The stimuli for the two tests were as follows:

Threshold Determinations: Stimuli were selected according to the Adaptive Maximum-Likelihood Staircase Procedure (Linschoten, *et*

*al.,* 2001). Twenty concentrations of each compound in 0.25 log steps were prepared from stock solutions, using double distilled water for all stimuli preparations. Stimulus concentrations ranged from 0.00003 M to 1.8 M for NaCl and 0.00001 M to 0.64 M for MSG. For MSG, two of the first ten subjects were able to discriminate between water and 0.00001 M MSG stimulus, thus, an additional stimulus of MSG, 0.000006 M, was added, resulting in 21 stimuli. The specific concentrations for each stimulus are detailed in Appendix VIII. The test was administered using a whole mouth sip-and-spit method. The stimuli and the blanks were presented in 30 ml plastic medicine cups containing 5 ml of liquid. Subjects were requested to take the first sample in their mouth, swish it around and spit it out, then rinse with distilled water. Subjects repeated this procedure with the second sample. They chose the sample which had a taste different from water.

Intensity Ratings: The concentrations for NaCl were 0.01, 0.032, 0.1, 0.32 and 1.0 M; and, for MSG were 0.63, 1.25, 2.5, 5.0, and 10.0 mM. These stimuli were prepared from a stock solution and diluted for each concentration with double-distilled water. Approximately five ml of each stimulus were served in one oz (30 ml) clear plastic

medicine cups at room temperature. NaCl was served first because the taste of MSG can remain in a subject's mouth for longer than 10 minutes after expectoration and rinsing.

4. Test Conditions

All testing was done at the Rocky Mountain Taste and Smell Center at the University of Colorado Health Sciences Center, Denver, CO. Testing was conducted individually with each subject in a quiet room with incandescent lighting at ambient temperature. Although comments were not solicited, any voluntary comments made by the subjects were recorded.

5. Chemical Analyses

Each subjects' salivary stimulus was analyzed for L-glutamic acid and sodium as follows:

L-glutamic acid: An enzymatic test kit was used to determine the quantity of L-glutamic acid in each stimulus, as described for Study 1.

Sodium: Atomic absorption spectrophotometry was used to quantify salivary sodium. All standards and stimuli were prepared using deionized water and plastic containers to prevent adsorption of sodium onto the container surface. The partial ionization of sodium in an air-acetylene flame was suppressed by adding potassium chloride to yield a final concentration of 2000μg/ml potassium in the standards and stimuli. A series of five

standard solutions were prepared from a stock of 1 ppm: 0.001, 0.002, 0.005. 0.010, and 0.020 ppm. Saliva samples were diluted 1:50 (0.2 ml saliva plus 1 ml KCl solution, brought to 10 ml total volume) for analysis. An AA/AE Spectrophotometer (Instrumentation Laboratory, Wilmington, MA) was used, with an air-acetylene flame through a single slot burner head, with a 0.5 nm bandpass. The light source was a hollow sodium cathode, the wavelength was set at 589.0 nm, and the current was 8 mAMPs.

6. Statistical Analyses

Mean thresholds for MSG and NaCl were determined using the Maximum-Likelihood Staircase Procedure program, which also yielded confidence intervals for each threshold. Intensity data were normalized to decrease the differences in scale usage by untrained subjects and to remove outliers. Normalization began by converting all 0 values to 0.1. Then the log mean of the three replications was calculated for each subject. A weighting factor for each subject was determined by dividing the group mean of all values by the individual subject's arithmetic average across the five concentrations. Each log mean value was then multiplied by this weighting factor. Analysis of variance of these values was conducted using Stat ANOVA (Berkeley, CA), which also determined means, standard deviations, and differences among intensity responses to MSG and NaCl.

Salivary concentrations of sodium and L-glutamic acid and dietary estimates of NaCl and MSG intake were correlated with the threshold and intensity determinations using Pearson's Product Moment Correlation (SPSS 8.0, Chicago, IL). The food frequency questionnaire was scored by assigning numbers to categories proportional to the frequency of consumption of that item (Appendix VI).

B. Results

Means and standard deviations for the eight experimental variables measured in this study are summarized in Table 11. Mean salivary L-glutamic acid was 9.13 $\pm$ 6.05 mg/l, or 0.049 mM, very close to the mean level found in Study 1 (9.50 mg/l). The range for these subjects was approximately 50-fold (0.448 to 22.6 mg/l), and sujbects did not differ in salivary glutamate levels. No significant differences due to ethnicity or gender were found ($p \geq 0.05$; Table 12). Mean salivary sodium was 5.44 $\pm$ 2.44 ppm (0.237 $\pm$ 0.106 mM), ranging from 1.54 to 22.4 ppm (0.0670 to 0.974 mM) and subjects differed in salivary sodium levels ($p=0.0058$; Table 12). Although no differences due to ethnicity and gender were found, the interaction between these two factors was significant: Asian females had significantly more salivary sodium than Asian males and non-Asian females ($p=0.004$).

The mean threshold for MSG was 1.07 ± 0.927 mM (0.0200 g/100 ml ± 0.0173 g/100 ml). Subjects were significantly different in their MSG threshold ($p=0.049$), ranging from 0.0016 to 3.31 mM (0.0000299 to 0.0619 g/100ml; Table 11). Mean NaCl threshold was 2.78±1.91 mM (0.0162 g/100 ml ± 0.0112 g/100 ml) and ranged from 0.0912 to 2.59 mM (0.000533 g/100 ml to 0.0151 g/100 ml). Subjects also differed in their NaCl thresholds ($p=0.0106$). No differences due to ethnicity or gender were found in thresholds.

The five concentrations of MSG and of NaCl were differentiated by the subjects ($p\leq0.001$; Table 13). Mean intensity scores for MSG and NaCl by ethnicity and gender are presented graphically in Figures 1 and 2, and numerically in Tables 14 and 15, which detail the grand means for each group, i.e., the intensity scores averaged across the five stimuli for each group. Subjects differed in their evaluation of the intensity of the MSG stimuli ($p\leq0.001$; Table 13), but not NaCl. This lack of difference between subjects for NaCl may be due to the 0.32 and 1.0 M stimuli, which were rated as very intense, creating a "ceiling effect" whereby the subjects ran out of space on the "Extreme" end of the scale to indicate intensity (Figure 2). For NaCl, replications and the replications by stimulus interaction were significant ($p\leq0.001$ and $p=0.005$, respectively; Tables 13 and 16a),

indicating that once the subjects had tasted several of the NaCl stimuli, they changed their scaling to accommodate the higher concentrations. Generally, they rated the three middle stimuli as less intense on the third compared to the first replication. Although the subjects rinsed thoroughly and waited 5 minutes between the threshold and intensity tests, tasting the NaCl intensity series after the threshold series (which was very low in concentration) influenced the subjects' perception of the intensity stimuli due to carry over effects. To decrease the effect of this carry-over bias, after the 21$^{st}$ subject, the 0.032 M NaCl stimulus was verbally labeled as "slightly intense" and given to the subject to taste before evaluating the stimulus series. This reference oriented the remaining subjects to the more concentrated stimuli in the intensity series.

When the MSG data were factored by ethnicity and gender, results showed that the MSG stimuli were rated as more intense by females than males ($p=0.028$; Tables 13 and 14). The ethnicity by gender interaction was significant for MSG: Asian males rated the stimuli as less intense than the other three groups ($p\leq0.001$). For NaCl, Asians rated the stimuli as less intense than non-Asians ($p\leq0.001$; Table 15). A ethnicity by concentration interaction was also found: the Asians rated the 0.10 M and 0.32 M stimuli as less intense than the non-Asian subjects ($p=0.055$; Table 16b). For NaCl,

the Asians also had a higher slope of the power function than non-Asians ($p=0.049$; Tables 11 and 12). This finding indicates that Asians perceived the stimuli as increasing more in intensity with concentration than non-Asians. When interpreting the results from NaCl, it must be noted that the log-linear function for NaCl was not straight, due to the "ceiling effect" caused by the extreme intensity scores given to the two most concentrated stimuli (Figure 2).

Mean dietary intake of MSG was higher for Asian subjects than non-Asians ($p\leq0.001$; Tables 11 and 12). No gender differences in MSG intake were found. Dietary intakes of NaCl were also higher for Asians than non-Asians ($p=0.039$). Correlation coefficients among the eight experimental variables were determined (Table 17). Dietary MSG intake was positively correlated with salivary sodium ($r=0.268$, $p=0.038$) and NaCl intake($r=0.802$, $p\leq0.001$). However, neither salivary sodium nor salivary L-glutamic acid were correlated with any other variables, including thresholds or the slopes of the psychophysical functions. Voluntary comments made by subjects indicated that the aftertaste of MSG was difficult to rinse away, and one subject noted that the taste intensity of MSG increased after the stimulus was expectorated. Two other subjects said that MSG was more detectable in the back of the mouth. Three Asian subjects comments that the taste of

MSG was familiar, and one non-Asian subject said it tasted like seaweed. Two non-Asian subjects said that MSG was more difficult to evaluate than NaCl because the taste of MSG was unfamiliar.

III. Discussion

The results of these studies did not lead to any straightforward conclusions about the relationship of taste perception to salivary composition, diet, gender, and ethnicity. Neither Study 1 nor Study 2 found significant correlation between salivary L-glutamic acid or sodium levels and thresholds to MSG or NaCl, or to the perceived intensity of MSG or NaCl. However, other significant relationships were found in the data, particularly the effect of ethnicity and gender on salivary L-glutamic acid and sodium, thresholds to MSG and NaCl, and perceived intensity of MSG and NaCl.

A. Salivary Composition

Mean salivary L-glutamic acid was 9.50 mg/l (0.065 mM) and 9.13 mg/l (0.062 mM), for Study 1 and Study 2, respectively. These means are the same order of magnitude as 0.018 mM, the average level of salivary L-glutamic acid reported by Yamaguchi et al. (1987), which was the only report of salivary glutamate found in the literature. Salivary L-glutamic acid varied significantly among subjects in Study 1, but not in Study 2. In Study

1, average salivary glutamate ranged from 0.740 to 48.1 mg/l, and in Study 2, the range was 0.448 to 22.6 mg/l. Results from Study 1 indicate that salivary glutamate levels were consistent for each subject over the three different days that saliva was collected. Salivary glutamate was not significantly related to any of the variables measured in either study, including ethnicity, gender, or dietary intake. However, a weak negative correlation between MSG threshold and salivary glutamate was found ($p=0.072$).

Mean salivary sodium, measured only in Study 2, was $5.44 \pm 2.44$ ppm ($0.237 \pm 0.106$ mM) and differed significantly among subjects, ranging from 1.54 to 22.4 ppm (0.0.670 to 0.531 mM). Several Asian females had very high salivary sodium levels that could have inflated the mean, so the median was determined to be 4.60 ppm (0.200 mM). The average salivary sodium level determined in this experiment is approximately one-tenth less than values reported in the literature, which ranged from 1.5 mM to 27.24 mM (Table 18). The results in the present study may be more accurate than the studies reported in Table 18 because most of these studies used flame photometry, which is less sensitive than atomic absorption spectrophotometry. In addition, the present study corrected for interfering substances, which has not been reported in other studies. A second reason

for the differences between this study and others was the method of collecting saliva. Many of the studies inserted a device into the mouth to collect saliva and any object or stimulus inserted into the mouth increases salivary flow, resulting in an increase in salivary sodium (Guinard *et al.*, 1998). Errors in pipeting and diluting the viscous saliva may account for differences among studies. Also, most reports in the literature used a relatively small number of subjects; thus, the means reported may not be representative of a larger population.

The determination of salivary composition is impacted by a variety of factors, some inherent to the subject and others due to differences in experimental procedures. Subjects' salivary flow rate, gender, diet, age, medications, and health have all been found to contribute to variability in salivary composition (Bradley, 1991). Experimental procedures vary in the time of day saliva is collected (salivary sodium was found to be highest in the morning and decrease throughout the day (Grad, 1954)), the method used and the glands from which saliva is collected, and the analytical method used to measure the concentration of salivary components. In the following discussion, the effect of ethnicity and gender, diet, method of collecting saliva, and analytical procedures impacting salivary sodium are discussed; comparable studies of these factors on salivary glutamate have not been

done. For a more complete discussion of the many variables that effect the determination of the concentration of salivary components, see the Literature Review discussion about Saliva, Function and Composition.

Ethnicity and gender accounted for differences in salivary sodium levels in Study 2. Asian females had more salivary sodium than Asian males and non-Asian females. Conversely, Chung (1981) found that Asian subjects and males had significantly higher salivary sodium than non-Asians and females. The gender by ethnicity interaction was not determined for these twenty subjects.

Diet likely accounted for some of the variation among subjects in salivary composition. In Study 1, no relationships were found between salivary composition and intake of MSG, as measured by a dietary frequency questionnaire. In Study 2, salivary sodium was positively correlated with MSG intake; MSG intake and NaCl intake were also positively correlated. Other researchers have suggested that diet is related to salivary composition. Chung (1981) found salivary sodium positively correlated with dietary sodium, using a food frequency questionnaire. Although increased sodium intake might be expected to increase salivary sodium, this result has not always been found. A decrease in dietary sodium resulted in decreased salivary when subjects decreased sodium intake by

50% (Christensen *et al.*, 1986). However, the converse conclusion, higher salivary sodium with increased salt intake, has not always been found. High levels of salt intake have been found to increase salivary sodium levels by Damodaran and Kinsella (1980), and Bradley (1991) but not by Pradar *et al.* (1955) and Wotman *et al.* (1973). Many researchers have used a survey or questionnaire to measure intake, subsequently grouping subjects into low, medium and/or high intake groups. Grouping subjects this way often results in very small sample sizes and reduces the power of statistical tests. Thus, differences that are small but nonetheless real are not discerned by the analysis. Additionally, questionnaires have significant biases, making the results highly dependent upon the method used and the accuracy of subjects' reports (Kerr, *et al.,* 1979). The influence of dietary intake on salivary composition might be more accurately studied by controlling the subjects' diet but no experiments of this type were found with the exception of Christensen *et al.* (1986).

The directly proportional relationship between salivary flow rate and salivary sodium has been well documented (Prader *et al.*, 1955; Guinard *et al.,* 1998). The effect of salivary flow rate on glutamate concentrations has not been documented. Salivary calcium and protein levels increased with flow rate and with stimulation of parotid saliva by NaCl (Pangborn and

Chung, 1981; Spielman, 1990). Thus, salivary stimulation with NaCl could increase levels of L-glutamic acid from increased protein. Any stimulation of the salivary glands increases salivary flow (Spielman, 1990) and some stimuli are more effective than others. Pangborn and Chung (1981) reported that solutions of NaCl generally produced more saliva and a higher flow rate than solutions of L-glutamic acid. When included in a food system, NaCl in beef broth produced higher flow rates than NaCl in chicken broth or distilled water. Salivary flow rate increased as a function of NaCl and L-glutamic acid concentration, with large variability among subjects but not between replications, indicating that the flow rates of individuals were consistent. Guinard *et al.* (1998) found that acidic stimuli elicited the highest flow rate and the highest salivary sodium among seven different stimuli in differing food systems. Semi-solid and solid foods resulted in higher parotid salivary protein content than liquid stimuli, which yielded saliva with highest sodium concentrations. MSG in soup was found to be a relatively strong sialgogue, compared to the other six food systems studied.

Incongruity in results could be due to different procedures used to collect saliva. Among methods of collecting saliva, collecting whole mouth saliva caused the least discomfort to the subject and was considered to be functionally more relevant (Kapur *et al.,* 1966). In measuring whole mouth

flow rate, Navazesh and Christensen (1982) found no significant differences in salivary flow rate among four different methods of collecting saliva: draining, spitting, suction, and swab. They suggested that whole mouth flow is functionally more relevant than measuring salivary flow from a single gland. Salivary composition also depends upon the contribution of different autonomic fibers to the salivary glands (Christensen, 1986).

Researchers have also used different analytical procedures used to measure salivary sodium. Morino and Langford (1978) used sterox to dilute saliva, but most studies do not specify the dilutant. Flame photometry has been most often used to measure salivary sodium, but more recent experiments have used atomic absorption spectrophotometry (AAS). AAS is a better analytical method for the quantification of sodium because it is more sensitive and efficient (Pomeranz and Meloan, 1994). Interference or self-absorption between similar elements (i.e., wavelengths emitted from one element absorbed by another) is a problem in flame photometry. Although interference can also be a problem with AAS, two experimental controls were used with the analysis in the present study that were deemed critical to obtaining accurate sodium readings: 1. the use of plastic, not glass, containers throughout the analysis as sodium adsorbs onto glass; and, 2. the use of potassium chloride to suppress the partial ionization of sodium in the

air-acetylene flame. These two controls have not been reported in any published literature and may be a reason for the higher salivary sodium levels found by other researchers.

B. Thresholds

The detection threshold for MSG was 0.00895 ± 0.00038 g/100 ml (0.478 ± 0.020 mM) for Study 1, and 1.07 ± 0.927 mM for Study 2, ranging from 0.0016 to 3.31 mM. Both of these values are within the ranges of 0.34 to 1.01 reported in the literature for MSG (Table 19). Study 1 used the Ascending Concentration Series Method of Limits, in which the subject receives samples of increasing intensity, so the threshold from this experiment may be artificially low due to subjects' expectations of the samples. Study 2 used the more accurate Maximum Likelihood Staircase Method (Linschoten *et al.,* 2001); thus, this mean is probably closer to the true mean. The threshold to NaCl in Study 1, determined with the Method of Ascending Limits, was 0.0219 g/100 ml ± 0.00016 (3.75 ± 0.0273 mM). Using the Maximum Likelihood Staircase Method, the mean detection threshold for NaCl was 2.78±1.91 mM, ranging from 0.0912 to 2.59 mM. The mean NaCl threshold determined in Study 2 is somewhat lower than the thresholds determined by other experimenters, which ranged from 3.42 mM to 7.7 mM (Table 19). The threshold for MSG was found to be lower than

the threshold for sucrose, approximately equal to NaCl, and higher than the thresholds for quinine or tartaric acid (Yamaguchi and Kimizuka, 1979). In Study 1, citric acid had the lowest threshold (0.0056 g/100 ml), followed by MSG (0.00895 g/100 ml), caffeine (0.0109 g/100 ml), NaCl (0.0219 g/100 ml) and sucrose, which had the highest threshold (0.108 g/100 ml). The glutamate cation may contribute to the threshold of MSG at least as much as the sodium anion (Yamaguchi, 1991).

The relationship between MSG and the tastes of sweet, sour, salty, and bitter is not clear. Early researchers theorized that MSG increased the sensitivity of taste receptors for the basic tastes of sweet, salty, sour and bitter compounds (Sjöström *et al.*, 1955). However, MSG has not been found to have a consistent effect on the perception of these compounds in model systems (Maga, 1983). Pilgrim *et al.* (1955) found that MSG increased the intensity of salt and bitter tastes in model systems but not sour and sweet. However, the same study found that the thresholds for salt and bitter were unaffected by MSG while the thresholds for sweet and sour were increased. The taste intensities of sweet, salty, sour and bitter compounds in model systems were not changed by the addition of MSG, and the converse was also true (Yamaguchi and Kimizuka, 1979) and detection thresholds of compounds commonly used to elicit basic tastes were not affected

(Meiselman, 1987; Yamaguchi, 1987). The threshold of MSG was not largely affected by other taste substances except IMP, which lowered the threshold of MSG (Yamaguchi *et al.*, 1996). The same study found that MSG did not affect the thresholds of sucrose, NaCl, and quinine sulfate but the threshold to tartaric acid was increased by MSG due to the change in pH.

Significant variation among individual subjects' thresholds was found in both studies. Large inter-subject variability in thresholds has been reported by other experimenters (Faurion, 1987). Pecore (1979) noted individual variability might have confounded any relationship between sensory response and the measured independent variables of sodium intake and salivary sodium. Although thresholds vary among individuals, they are stable within an individual, changing only under specific conditions, such as aging (Schiffman, *et al.,* 1991) or disease (Bartoshuk, 1978). In Study 1, the threshold test for MSG was replicated on a different day with no difference between the two days.

Gender and ethnicity accounted for some of the variability among subjects in Study 1. Males had higher thresholds than females for NaCl and MSG and non-Asian males had significantly higher thresholds for NaCl than the other three groups. However, these differences were not found in Study 2. Other experimenters have not found differences between ethnic groups or

gender. Druz and Baldwin (1982) did not find any differences among Americans, Nigerians, and Korean in their study of thresholds to sucrose, citric acid, caffeine, and NaCl. Yamaguchi (1991) found no differences in sensitivity to umami between Japanese and Americans of European descent. In contrast, Ishii *et al.* (1992) found that Japanese subjects were better able to discriminate MSG and sucrose than their American counterparts, while no difference between the groups was found for NaCl. In this study, the experimenters tested both sets of subjects in their own countries using the appropriate language. Men and women do vary in their sensitivity of 6-n-propylthiouracil (PROP), a bitter tasting compound (Duffy and Bartoshuk, 2000). PROP sensitivity has been linked to differences in fungiform papillae density and may also affect food preferences.

The relationship between diet and threshold is not clear. For Study 1, dietary glutamate was negatively correlated with NaCl threshold; thus, people who ate more glutamate may have been more sensitive to salt. No significant correlation between dietary sodium intake and threshold for NaCl were found. In Study 1, a weak positive correlation was found between sodium intake and discrimination of NaCl in water but not in tomato juice, suggesting that threshold response in distilled water cannot be extrapolated to food systems. Most other studies also relied on subjects' self-reported

intake to determine dietary status of a specific compound. Taste perception of NaCl in tomato juice, determined via threshold, preference, and intensity measures was not related to dietary intake (Pecore, 1979). Morino and Langford (1978) determined that the recognition threshold to salt was positively correlated to salivary sodium. In this study, pre- and post-test levels of salivary sodium were averaged; thus, the results are biased by residual sodium left in the mouth from the threshold stimuli. Putting subjects on a very low sodium diet did result in lower thresholds for six of ten subjects (Beauchamp *et al.,* 1990) but this relationship was not significant for the group. The subjects also had an increased preference for salty foods, measured conceptually, but did not differ in their suprathreshold perception of NaCl when compared to their normal sodium state. An effect of intake on threshold has been determined for caffeine by Tanimura (1994), who found that caffeine users were less sensitive to its bitter taste than non-users.

Methods of determining thresholds vary among experimenters, many of whom use only a small number of subjects. Limiting the number of subjects compromises the results because of the large variation among individual subjects' thresholds, as previously discussed. The Ascending Concentration Series Method of Limits (Jellinek, 1985; Meilgaard *et al.,*

1991) is biased by carryover effects and by subject expectation, as the subject knows that the stimuli are increasing in concentration. Recognition thresholds are also biased by subject expectations (Delwiche and O'Mahony, 1996) and are generally two to three times higher than detection thresholds (Schiffman *et al.*, 1980). Yamaguchi (1991) recommended use of a warm-up sample (a recognizable concentration of stimulus) to clarify the flavor of the compound of interest. However, this practice skews the results towards an artificially low threshold because knowing the taste quality lowers the threshold (Marks and Marshall, 1999). Gonázlez-Viñas *et al.* (1998) used mineral water as a carrier, which contributed sodium and other ions to the stimuli.

The method of stimulus delivery can also affect the threshold (Morino and Langford 1978). In this study of salivary sodium and recognition threshold, dropping a NaCl solution on the tongue resulted in higher recognition thresholds than a cuplick method, whereby subjects inserted their tongue into the solution, probably because a larger area of the tongue was stimulated with the latter method. The volume of the stimuli put into the mouth and whether it is swallowed or expectorated can also affect the results (Christensen, 1986). The use of a rinse alters the concentration of compounds in the mouth, and probably not in a uniform manner throughout

the oral cavity. Differences in threshold testing procedures are discussed in the Literature Review, Threshold Measurement.

Whether or not to use a rinse during threshold testing merits discussion because a rinse alters sensory adaptation and thus, the results of threshold testing (McBurney and Pfaffman, 1963; O'Mahony, 1972a; Bartoshuk, 1978). Any residual stimulus left in the mouth can affect the perception of subsequent stimuli and change sensitivity. Thus, threshold methods that do not use a rinse may not be accurate because residuals from previous stimuli are present. However, using a rinse yields a lower threshold by removing stimuli and other salivary components and by diluting subsequent stimuli. Rinsing also introduces uncontrolled variables such as volume of rinse, time the rinse is held in the mouth, the thoroughness of rinsing, the amount of mixing between the rinse and saliva, and the affect of the rinse solution on salivary flow (McBurney and Pfaffman, 1963). Delwiche and O'Mahony (1996) found that subjects had to expectorate saliva three times to clear the mouth of any prior residue. In these studies, the procedure used between stimuli, *ad libitum* rinsing of approximately 30 to 60 seconds, together with whole mouth stimulation, did not allow the environment in the oral cavity to return to normal.

Adaptation, the decrease in a response due to exposure to a stimulus, can alter the perception of subsequent stimuli. Meiselman (1968) found that complete adaptation to sucrose, NaCl and quinine did not occur. The inability of a subject to adapt to very dilute concentrations of MSG has also been reported (Ishii, 1997). Three Asian women in Study 2 were able to detect the lowest concentration of MSG. This extreme sensitivity may be due to an afterimage effect of MSG, which is a sensation that continues after the stimulus is removed. This effect is similar to dark adaptation in vision, whereby a subject continues to perceive light after a more intense light stimulus has been removed (e.g., the lasting sensation of light when a flash photograph is taken). The sensitivity to MSG may also be linked to high salivary sodium levels as Asian females in Study 2 had significantly higher salivary sodium. Perhaps high levels of salivary sodium enhance the perception of MSG, similar to the additive effect proposed for the detection threshold to NaCl by Christensen (1986). Because this additive effect may only be apparent at high levels of salivary sodium, it was not enough to effect a significant relationship between salivary sodium and perception of MSG in these studies. After adaptation to NaCl, subjects perceived MSG as less salty and more sour than NaCl (Smith and van der Klaauw, 1995). Adaptation to any tastant is difficult because any movement of the tongue or

mouth causes disadaptation by exposing previously unexposed receptors to the residual stimulus.

In contrast to these results for MSG, Bartoshuk (1980) has stated that a tastant can only be perceived at concentrations above that of the adapting solution, which is most often saliva. If true, then an individual subject's threshold to a compound must be greater than their salivary levels of that compound. This result has been confirmed for sodium by some experimenters using thresholds (McBurney and Pfaffman, 1963; Bartoshuk, 1974; Spielman, 1990) and using signal detection measures (Delwiche and O'Mahony, 1996). However, other studies have not found this relationship. In Study 1, a weak negative correlation was found between salivary glutamate and threshold to MSG (high levels of salivary glutamate are related to increased sensitivity to MSG). Christensen (1986) reported that subjects with unusually high salivary sodium (600 μg/ml stimulated) were more sensitive to NaCl, with lower than average detection thresholds. One reason suggested for this finding is that salivary sodium levels may require a large decline before the threshold is measurably changed. Another postulate is that the salivary sodium mixes with the sodium in the stimulus, yielding a lower threshold, which is particularly significant at low salivary sodium concentrations (the additive effect). Bartoshuk (1980) attributes these

seemingly anomalous results to the use of a rinse, which alters adaptation to salivary components, and the confusion between the tastes of NaCl and water (Bartoshuk, 1974). The differences between the results of these studies may also be due to the time of day saliva was collected, including the last time the subject ate or drank anything, and whether or not the method of collection stimulated the salivary glands, which would cause sodium levels to increase.

One key consideration when determining the relationship between salivary composition and taste perception is that the introduction of a stimulus will stimulate salivary flow, thereby changing the concentration of salivary components and affecting taste perception (Delwiche and O'Mahony, 1996). Thus, obtaining an accurate understanding of this relationship is difficult if not impossible because the stimulus changes the variable of interest. Neither obtaining saliva prior to tasting, which indicates individual status, nor during tasting, which represents the environment in the mouth, are accurate assessments. Additionally, the environment around the micropore of the taste bud may not be represented by a saliva sample.

The role of salivary composition in flavor potentiation has not been systematically studied. Salivary components that are know to act as flavor potentiators in food, such as glutamate and nueclotides, could originate from

several sources. Nucleotides, particularly cyclic AMP, have been found in salivary glands (Shannon *et al.*, 1974) and could blend with salivary glutamate and sodium to contribute to flavor potentiation. Although glutamate is present in the glycoprotein coating the mouth, the levels of free amino acids in saliva are very low (Ellison, 1978) and may not appear to be have a measurable role in taste perception. Yamaguchi *et al.* (1987) suggested that salivary glutamate may act synergistically with ribonucleotides in food to produce the umami taste. Saliva from the von Ebner's gland may contain proteins that bind tastants (Bradley, 1991).

Another possible interaction could be due to the weak ionic bond between sodium and L-glutamic acid in MSG, allowing salivary sodium to combine readily with dietary glutamate, or the opposite could occur. The buffering effect of saliva may also contribute to flavor potentiation. Saliva has a pH of 5.82 and has a buffering action on acids produced by oral bacteria, which can damage the teeth (Bradley, 1991). The same buffering capacity may affect the perception of MSG, which is an ampholyte and exists in one of four different ionization states depending upon the pH of the surrounding system (Maga, 1983). When introduced into the mouth, any MSG in food could release its sodium ion upon contact with the more alkaline saliva. Additionally, the pH of the food may impact the flavor

potentiation of MSG. More acid foods would have less stable MSG. Thus, as the pH increases, glutamic acid becomes more ionized and would form a stronger bond with sodium. Salivary proteases may initiate protein hydrolysis, releasing free amino acids including glutamate. Other sources of salivary glutamate are from plasma and from residual glutamate in the oral cavity after eating. Among adults, plasma glutamate concentration increases with increasing doses of MSG in water (Life Sciences Research Office, 1995), which may result in increases in salivary glutamate. No studies of the effect of the quality, quantity, or timing of food intake on salivary glutamate levels have been conducted.

C. Intensity Evaluations

Thresholds determine sensitivity to compounds at very low concentrations, which is useful for examining differences among subjects, such as the impact of aging on taste sensitivity (Schiffman *et al.*, 1991) or to determine a subject's sensitivity for a particular task (Meilgaard *et al.*, 1991). However, thresholds do not indicate the ability of an individual to perceive a compound at suprathreshold levels. Therefore, the intensities of MSG and NaCl at suprathreshold levels were evaluated. In Study 1, MSG was tasted in four different carriers: distilled water, unsalted tomato juice, chicken broth, and beef broth. To eliminate the confounding effect of carrier

flavor, MSG and NaCl in distilled water were used in Study 2. Perception of NaCl was included to determine its relationship to perception of MSG and because salivary sodium was measured.

1. Differences Among Stimuli

Subjects were able to differentiate among the concentrations of MSG and NaCl with a few exceptions. In Study 1, no differences were found among the five levels of MSG in unsalted tomato juice for all attributes, perhaps due to the high level of endogenous L-glutamic acid in tomato juice or its low pH. Yamaguchi and Kimizuka (1979) determined that adding MSG to cream of tomato soup did not alter its flavor profile, but MSG did change the profiles of other meat and vegetable food systems tested. In Study 1, the lowest two and highest two concentrations of MSG in water were not significantly different in intensity for the three attributes tested. In beef broth, the middle samples were not differentiated for the four attributes tested. Thus, the carrier used affected the perceived intensity of MSG.

Intensity differences in these attributes should be interpreted with caution because subjects were not trained with references for each attribute, which ensures that all subjects are using the same concept for the attribute in question. O'Mahony (1991) has found that without a reference stimulus, subjects may use different concepts for a given attribute, increasing data

variability. In Study 1, subjects were given the MSG stimuli in water before recording their perceived intensities to familiarize themselves with the umami taste. Differences between males and females or Asians and non-Asians may be due to similarities within these groups in defining the meaning of these attributes rather than perceptual differences. Therefore, in Study 2, subjects were asked to evaluate only the overall intensity of the stimuli to decrease this source of variation.

The slope of the taste, or psychophysical, function indicates the perceived change in taste intensity vs. the physical change of the stimuli concentration and can be defined by a log-linear function for the tastes of sweet, sour, salt, and bitter (Moskowitz, 1971). This relationship, the Weber-Fechner law, can be used to characterize taste stimuli (Stevens, 1975). MSG follows this function but the slope is less steep than the slopes for sweet, sour, bitter, and salty (Yamaguchi, 1979). Thus, MSG increases less in intensity per unit change in stimulus concentration than the other four tastes, indicating the unique taste quality of MSG (Yamaguchi and Kimizuka, 1979). Possibly this less steep slope indicates that L-glutamic acid is released more slowly from the taste receptor than sodium. Study 2 also demonstrated that the slope of the taste function for MSG was less steep than that of NaCl (0.959 vs. 0.989) although the slopes were not

significantly different, which may be due to the extreme perceived intensity of the two highest NaCl samples. In Study 2, subjects did not discriminate among the three highest levels of NaCl due to adaptation to the high levels, increasing the slope of the taste function by decreasing the perceived intensity of the lower concentrations, which is called recruitment (Bartoshuk, 1980).

The context effect of the stimuli set on both hedonic and intensity judgments has been well-documented (Bertino *et al.*, 1983; Meilgaard *et al.*, 1991). Bartoshuk (1980) found that adaptation to the stimuli increased the slope of the psychophysical function by decreasing the perceived intensity of the lower concentrations. Saltiness and pleasantness of soups with high and low concentrations of NaCl were affected by the frequency of stimuli of high and low concentrations and the order of presentation of these stimuli (Riskey, 1982). Context effect also impacts the evaluations of subsequent replications. The difference found between replications in both studies is most likely due to subjects' experience with the stimuli and use of the scale throughout the testing.

2. Differences Among Subjects

As usually found in sensory tests with minimally trained subjects, subjects were a significant source of variation in the intensity data from both

studies. Grouping the subjects by ethnicity and gender accounted for some of the variability between subjects, but the results of the two studies were opposing. In Study 1, Asians, particularly males, gave higher scores to the stimuli than the other subjects. Males also gave higher scores than females. In contrast, Asian male subjects in Study 2 generally gave lower scores to the stimuli and females gave higher scores than males. For NaCl, non-Asians rated the samples as more intense than the Asians. The slope of the taste function for NaCl was steeper for Asians, indicating that they perceived lower concentrations of NaCl as less intense than non-Asians and that perceived intensity increased more per unit of NaCl for Asians than non-Asians. The test procedures differed between the two studies to the extent that the results of one cannot be directly compared to the other. Bertino *et al.* (1983) also found that Asians rated lower concentrations of NaCl as more salty and higher concentrations as less salty than Americans of European descent.

The differences found between Asians and non-Asians in these studies may be due to a number of factors, and are most likely cultural rather than genetic. Asians and non-Asians have different diets, taste preferences, experience with the umami taste, and attach different meanings to taste descriptors (Ishii and O'Mahony, 1987). Japanese and Americans sort tastes

in the same way, indicating that taste is conceptualized in a comparable manner if the concept is defined (Ishii and O'Mahony, 1987). However, these concepts were not defined in the studies reported here. Americans do not have a convenient word to describe umami and tend to use "salty" as a descriptor for MSG (O'Mahony and Ishii, 1985). Thus, lack of context definition may account for the differences between Asians and non-Asians rather than a perceptual difference between the ethnicitys. Many Japanese described the taste of MSG as "ajinomoto", the company name of the primary manufacturer of MSG in the world, while the Chinese called the taste "wei jing". Most of the Asian subjects were foreign students in the U.S., whose place of birth and primary residence was in East Asia (80% in Study 1 and 87% in Study 2) and most of them continued their traditional eating habits while in the United States. Bertino *et al.* (1983) suggested that differences in hedonic and intensity scores given to salty and sweet samples by Asians, specifically Taiwanese, and Americans of European descent may be related to the ability to taste phenylthiocarbamide (PTC) or its functional analogue, 6-n-propylthiouracil (PROP). The ability to taste these bitter compounds is related to number of taste buds and sensitivity to tastes other than bitter. A higher percentage of people who can taste these compounds are found among Asians than Caucasians (Bertino *et al.*, 1983).

Dietary intake of both MSG and NaCl was higher among Asians than non-Asians, as measured in Study 2. However, no significant correlations between dietary intake of MSG or NaCl and any of the sensory measures were found in either study. No studies of the relationship between consumption and sensory response to MSG in humans were found in the literature. In a study with rats, Kimura *et al.* (1987) found that rats fed a high protein diet preferred umami substances, specifically MSG over IMP. Consumption of a low protein diet elicited an increase in NaCl intake with a corresponding decrease in MSG intake. These changes were attributed to different taste sensitivities created by the different diets, as the rats had an increased taste threshold to MSG on a low protein diet. They also had increased plasma levels of glutamic acid, which may be related to taste preferences. The role of sodium intake in human supra-threshold perception of NaCl is not clear. Pecore (1979) found that sodium intake was directly related to the amount of NaCl added to unsalted tomato juice in an *ad libitum* procedure, with the high sodium intake group adding more salt than the low and medium intake groups. However, no significant relationships between sodium intake, as measured by a questionnaire, and sensory responses of discrimination, intensity, and liking of salt taste were found. Chung (1981) found that subjects with a lower sodium intake as determined

by a food frequency questionnaire gave lower intensity scores to stimuli of NaCl and MSG, singly and in combination. These results may be due to the inherent variability found when dietary intake is self-reported (Kerr *et al.*, 1979). The only method to accurately determine dietary intake is to control the subjects' diets, which was done by Beauchamp *et al.* (1990) in a study of sodium depletion and salt taste with ten subjects. During the depletion period, preference judgments for salt tended to be higher and saltier foods were imagined to be more pleasant than during the pre- and post-depletion periods. However the slope of the power function for saltiness or sweetness did not change during the depletion period.

D. The Relationship Between MSG and NaCl

MSG is often classified as a salty taste (Halpern, 1987), possibly due to the presence of the sodium ion. The flavor of L-glutamic acid is different from that of MSG (Yamaguchi, 1991) and is described by this experimenter as sour with a slight brothy taste. L-glutamic acid, whether endogenous or added to a food, contributes a umami taste and in many cases, a taste also described as salty. In Study 1, subjects found significant differences in the salt taste intensity of MSG in water and beef broth, but not in tomato juice. Whether or not MSG contributes a salt taste is dependent upon the concentration and food system used, and also the sensory methodology.

According to Maga (1983), high levels of MSG have a salty taste and moderate levels tasted sweet. In a study of NaCl substitutes in oatmeal, MSG was not considered salty at any of the 5 levels evaluated (0.0% to 0.4%). Maximum saltiness (4 on a 15 point intensity scale) was achieved with 0.1% MSG (Lynch, 1987).

MSG has been suggested as a replacement for NaCl in salt restricted diets due to its lower sodium content (13% vs 33%, respectively). Bartoshuk *et al.* (1974) expressed concern about this recommendation. They found that subjects added approximately 67% more sodium to tomato juice in an *ad libitum* procedure when they used MSG rather than NaCl to make the juice as palatable as possible. The authors noted that the use of MSG could actually increase sodium intake due to MSG having a less intense salt taste than NaCl. Their study was criticized by Ebert (1975), who noted that tomato juice was not an appropriate medium due to its high endogenous MSG content, that the levels of MSG used by the subjects were much higher than recommended, and that a brief exposure to a compound does not relate to dietary habits. He indicated that MSG, when used at recommended levels, could increase the palatability of low-sodium foods. In their reply, Bartoshuk *et al.* (1974) noted that synergism between NaCl and MSG had not been demonstrated in other studies. However, later studies have found

that as MSG is added to a food, less NaCl is needed for maximum palatability; the converse has also been found to be true. Yamaguchi and Takahashi (1984a) used Response Surface Methodology to determine this inverse relationship in a soup. They recommended that an optimal level of MSG can be used to decrease sodium consumption while the palatability is maintained. Because the endogenous levels of neither MSG nor NaCl were measured, the recommended levels of MSG and NaCl should not be extrapolated to other food systems. A study using rats also found this inverse relationship between MSG and NaCl (Kimura *et al.,* 1987). Rats eating a moderate of high protein diet increased consumption of MSG and decreased NaCl intake, possibly due to a relationship between plasma glutamate and taste sensitivity. Rats on a low protein diet had higher plasma glutamic acid levels and were less sensitive to MSG than the other two groups.

In 1990, Tuorila *et al.* used an *ad libitum* mixing procedure to discover that flavoring beef broth with combinations of allspice, marjoram, onion, and MSG did not decrease the amount of salted beef broth added to these mixtures. To confirm these results, a different group of subjects evaluated the pleasantness of these mixtures. Again, the addition of flavors did not alter the preferred level of NaCl in the broth. Thus, they did not find

the compensatory effect of MSG on preferred levels of NaCl. The authors concluded that salt preference was independent from other tastes, but they also noted that other flavors might yield different conclusions. The saltiness of MSG at suprathreshold levels was estimated as 30% of NaCl in molar sodium comparison and less than 10% of NaCl by weight (Yamaguchi, 1991). Adding 0.01% IMP to 0.07% MSG yielded a solution with no salty taste, but with a stronger umami taste than MSG alone, and led to the conclusion that the salty taste of MSG is not the same as that of NaCl or other salts. Chi and Chen (1992) used Response Surface Methodology to examine the relationship between MSG and NaCl in chicken broth. Using a small number of trained panelists (in the U.S.A. as compared to Yamaguchi and Takahashi's study in Japan), Chi and Chen found maximum hedonic scores were obtained with levels of MSG and NaCl similar to those of Yamaguchi and Takahashi (1984a; 0.33% MSG and 0.83% NaCl vs. 09.38% and 0.81%, respectively). These levels were slightly higher in a spiced chicken broth, which also yielded higher hedonic scores. They concluded that a high hedonic score for low sodium chicken broth could be maintained by increasing MSG levels. Riha *et al.* (1997) had subjects rate the saltiness and other taste attributes of solutions with NaCl and L-arginine, L-lysine, or L-asparagine. Although arginine alone was not salty, stimuli with NaCl in

combination with low and moderate concentrations of arginine (50, 75, or 100 mM) had increased saltiness. Lysine had less effect on saltiness and asparagine had no effect on saltiness of NaCl. Future studies of the relationship between NaCl and MSG must use references when possible and define the attributes tested; specify the type of subjects used; detail dietary intake, and measure the levels of MSG and NaCl naturally-occurring and added to stimuli.

E. Preferences for MSG

A umami substance alone in water does not taste pleasant, but adding these compounds to food increases their hedonic scores (Yamaguchi, 1991). The addition of MSG to a food increases both preference and intake of the food as compared to the same food without MSG (Yamaguchi, 1979; Murphy, 1986; Rogers and Blundell, 1990; Bellisle *et al.*, 1991). Preferences for the stimuli were determined in Study 1, with a particular interest in differences between Asians and non-Asians. Higher concentrations of MSG in tomato juice were preferred by Asians but they indicated a preference for lower levels of MSG in beef broth. Thus, preference for MSG is dependent upon the food system, perhaps due to differences between cultures or the high levels of endogenous MSG in tomato juice. Yamaguchi and Takahashi (1984b) had a semi-trained panel,

assumedly Japanese, respond to sucrose, NaCl, tartaric acid, caffeine, and MSG singly and in binary combinations in water and food systems appropriate to the compounds of interest. MSG in water did not receive pleasantness ratings above zero, but adding 0.37% w/v NaCl raised the score slightly. The use of low levels (0.3% w/v) MSG in a clear soup, miso soup, fried rice, and egg custard yielded higher scores, which dropped sharply as MSG concentration increased. As Meiselman (1987) noted, the evaluation of any stimuli in water or a simple food system cannot be extrapolated to hedonic evaluations in complex food systems, which is particularly relevant with MSG because the resultant flavor is highly dependent upon the food system. Study 2 did not include an affective sensory measurement because water was used as the medium.

The influence of culture on sensory responses is an important consideration when interpreting test results and comparing studies using different groups of subjects (Tuorila *et al.*, 1990). Druz and Baldwin (1982) determined that Americans consumed more sweet food than either Nigerians or Koreans, with the latter group eating more salty foods. These dietary patterns were related to hedonic responses to sweet, sour, salty, and bitter stimuli in tomato juice and sweet and salty tastes in applesauce. Koreans and Nigerians gave higher hedonic scores to 0.2% and 0.4% NaCl in tomato

juice than Americans. Asians gave higher hedonic scores than Americans to salty solutions in water, possibly because Asians are more familiar with the taste of salty solutions in their diet, such as soy sauce (Bertino *et al.*, 1983). An interlaboratory study examined the hedonic response of Japanese and Australians to sucrose, NaCl, caffeine, citric acid, MSG, IMP, and GMP (Prescott *et al.*, 1992). The researchers concluded that the groups were more similar than different in their responses. At 40 mM MSG, Australians gave the stimuli higher hedonic scores than the Japanese, who had less dislike for the higher concentrations of both MSG and GMP. No differences were found between the groups for NaCl. These results were attributed to the fact that the Japanese subjects were more familiar with the umami taste. However, the application of these results is limited because water, not food, was used as the medium. Taste preferences of compounds within food systems are expected to be much more subject to individual variation (Prescott *et al.*, 1992). Recently, Bell and Song (1999) compared hedonic evaluations from Japanese, Australian, Singaporean, and Indonesian consumers to determine the sensory factors most import to each culture in liking of foods. The Japanese were the only group for which umami was a significant factor. The authors concluded that differences among groups are

due to the experiences within each culture and should be considered when developing food products.

F. MSG Interactions in Food Systems

The sensory response to MSG has often been studied in model and simple food systems rather than in a complex food system. However, as previously discussed, the perception of both the quality and the intensity of MSG is dependant upon the system in which it is tested. Studies also vary in the type of food system, the kind and amount of umami compound, interactions with other ingredients, and the sensory methodology used. Thus, it is imperative that the food system in which MSG is tested be well-defined, including the determination of pH and endogenous levels of sodium and L-glutamic acid. A subjective comparison of the intensity means of all stimuli tested in Study 1 demonstrated that the overall intensity and umami and salt intensities were lower when MSG was tasted in water as compared to tomato juice and beef broth. Statistical differences among the systems were also found. Subjects found significant differences in the intensity of the stimuli for water and beef broth but not for tomato juice, possibly due to its high levels of endogenous MSG or its low pH. Thus, the perception of MSG is highly dependent upon the food system in which it is tested; however, why these perceptions vary is not well understood.

Early studies determined that MSG improved the quality of meat, poultry, and vegetables by increasing desirable flavors, suppressing off-flavors, and improving mouthfeel and aroma (Cairncross and Sjöström, 1948; Sjöström and Crocker, 1948; Sjöström *et al.*, 1955). The mechanisms by which MSG works are not understood. One early theory proposed that MSG increased the sensitivity of the taste receptors, but as previously discussed, MSG does not appear to interact with the "basic" tastes of sweet, salt, and bitter; some evidence has been found that it decreases sensitivity to sour (Yamaguchi *et al.*, 1996). The taste, flavor, and somatosensory effects of five levels of MSG and NaCl were studied using pure tastes (sucrose, citric acid, NaCl, and quinine) and seven flavors (extracts of celery, lemon, and meat, and flavors of butter, pistachio, citral, and menthol) by Kemp and Beauchamp (1994). Sub-threshold concentrations of MSG and NaCl did not effect the perception of the stimuli, whereas levels above threshold gave mixed results. At threshold concentrations, sweet stimuli with NaCl and sweet and bitter stimuli with MSG were most intense, possibly due to the added sweet and bitter tastes that have been reported for MSG and NaCl at threshold levels, rather than a potentiation effect. The only effects that were found at higher concentrations were due to decreases in intensity of lemon and mint flavors, most likely caused by suppression or masking. MSG did

increase salt perception with higher concentrations due to its salty component. The authors noted that the potentiating effects of NaCl and MSG not found in this study of model solutions may be found using real food systems.

From this research, Kemp and Beauchamp suggested that NaCl and MSG altered the taste and retronasal attributes of foods by differential effects, that is, contribution of specific flavors while suppressing other flavors. However, the effect of MSG on aroma is unclear. Using gas chromatography, Maga and Lorenz (1972) found that MSG, IMP, and GMP increased the total peak area of all peaks observed; when combined, the flavor potentiators demonstrated synergism in a peak with the highest increase. The headspace of water and potentiator combinations did not yield any peaks. These results may have been due to chemical reactions or bonds between the potentiators and the volatile components, or less likely, the potentiators may have increased the volatility of some compounds by changing the vapor pressure. However, using a sensory panel, Voirol and Daget (1989) found that MSG did not increase the aroma intensity of beef broth.

Neither of these studies determined endogenous concentrations of NaCl or MSG in their respective beef broths. In fact, none of the studies

reviewed for this project reported endogenous levels of NaCl or MSG. Because small amounts of MSG and L-glutamic acid can significantly affect the flavor of a food, differences in the results of the studies reported herein may be due to differences in the endogenous levels of MSG, L-glutamic acid, and/or NaCl of the systems in which these compounds were tested. As previously noted, tomato juice is naturally high in endogenous L-glutamic acid (0.35 g/l) while beef broth, prepared from raw materials rather than a commercially prepared broth, was low in L-glutamic acid (0.04 g/l). Other researchers have found beef to be low in L-glutamic acid, relative to the amounts in pork and chicken (Kato *et al.*, 1989), resulting in a weaker umami taste in beef, which was increased by adding L-glutamic acid or IMP to beef broth (Nishimura and Kato, 1988; Fuke and Konosu, 1991).

The effectiveness of flavor potentiators is dependant upon the food system in which they are used, particularly the types of proteins, amino acids, and other compounds in the food, the pH of the food, and heat treatment of the food. Maga (1987) added MSG, IMP, and GMP to 17 difference protein sources. The protein was purified from a wide variety of sources including red meat, poultry, fish, vegetables, grains, eggs, milk, and legumes, mixed with demineralized water, and adjusted to pH 5.5. Two additives were added to each protein system, corn oil and corn starch, to

determine how a fat and a carbohydrate affect the functionality of the flavor potentiators. Fifty experienced judges assessed the intensity of the stimuli using a 100-point scale, with data averaged and compared. MSG was most effective in meat systems and enhanced the intensity of beef and chicken more than lamb, pork, or turkey proteins. To a lesser extent, MSG intensified the flavors of fish, vegetable, cereal, legume, egg, and milk proteins. These differences in intensity may be due to the composition or structure of the proteins tested. GMP and IMP were most effective in fish proteins, followed by meat, cereal, legume, milk, and egg protein systems. The effectiveness of the flavor potentiators varied within each protein system. In meat, they were most effective with chicken and least with lamb; in cereal, the effect was greatest for rice and least for corn; and with vegetables, green bean flavor was most intensified. With MSG, the addition of corn oil resulted in an increased intensity of the vegetable proteins, whereas the starch increased the intensity of the cereal proteins. Adding the oil and/or starch to the protein systems with IMP and GMP did not significantly change the flavor intensity of any of the samples. Protein systems from peanuts and soybeans did not have an increase in taste intensity with any of the umami compounds, possibly due to the globular-shaped proteins in these foods. Thus, the effect of flavor potentiators on a

food system appears to be dependent upon the flavor potentiator, the proteins system, and other compounds in the food that may interact with the flavor potentiators.

The presence of other compounds that are available to react with glutamic acid can influence the resulting flavor of a food. MSG synergistically potentiates flavor when combined with 5'-ribonucleotides (Yamaguchi, 1979, 1991) and with the beefy meaty peptide (Wang et al., 1996). The contribution of glutamic acid to the characteristic tastes of peptides has been widely studied (Spanier et al.,1996; Lieske and Konrad, 1994; Fuke and Konosu, 1991; Tamura et al., 1989; Kato et al., 1989; and Nishimura and Kato, 1988). These reports note that when glutamic acid or any of the other amino acids in a flavoring peptide change sequence or are removed, the flavor of the resultant compound is dramatically altered. Additionally, the pH of the system may effect the perception of MSG, which is most effective as a flavor potentiator between pH 5.5 and 8.0 (Maga, 1983). Because MSG is an ampholyte, it can exist in different ionic forms depending upon the pH of the solution (see the Literature Review section on Chemistry of MSG). Between pH 5.5 and 8.0, the most predominant form of glutamic acid contains two negatively charged carboxyl groups and one positively charged amino group. Thus, the lack of significant differences

among the MSG stimuli in tomato juice may be due to the acidity of the juice, which had a pH of 4.35, while the beef broth was more neutral at pH 6.60. In an early study, Cairncross and Sjöström (1948) found that changing the pH by $\pm$0.5 units yielded a chicken broth with flavor changes greater than those produced solely by glutamate. Alterations in chemoreception with changes in pH were thought to be due to altering the charge distribution on the stimulus molecules, with maximum stimulation near the isoelectric point; however, Tierney and Atema (1988) have suggested that this phenomenon is due to ionization changes in the protein receptor. Their enzymatic model in the trout gustatory system accounted for small changes in pH and the differential response of receptors.

The ability of flavor potentiators to impact flavor intensity and quality appears to be dependent upon the types of proteins and other compounds in a food, including any endogenous L-glutamic acid and NaCl, and the pH of the food. The fact that these compounds do not function equally in all foods is not well understood. Additionally, flavor potentiators in food undergo chemical changes with processing, heating, and storage, which affect the quality of foods. These changes must be studied using both sensory and instrumental techniques to determine the most effective use of flavor potentiators in food systems.

Table 2. Study 1: Means ±standard deviations for four experimental variables, grouped by ethnicity and gender.

| Group (N) | Salivary L-glutamic acid (mg/l) | % MSG Preferred in Tomato Juice | % MSG Preferred in Beef Broth | MSG Intake |
|---|---|---|---|---|
| Total (25) | 9.50±11.0 | 0.13±0.0068 | 3.57±2.54 | 32±9.8 |
| Range | 0.740-48.1 | 0.025-0.200 | 0 – 6 | 10-55 |
| Asian (11) | 5.61±4.23 | 0.15±0.0027[b] | 2.40±2.41 | 38±9.3[b] |
| Non-Asian (14) | 12.8±13.8 | 0.11±0.0040[a] | 4.46±2.33 | 28±8.0[a] |
| Male (8) | 4.55±3.05 | 0.13±0.0050 | 3.63±2.13 | 30±11 |
| Female (17) | 12.0±12.7 | 0.13±0.0055 | 3.53±2.80 | 34±9.3 |
| Asian, Male (5) | 3.48±3.34 | 0.16±0.0048 | 3.00±2.45 | 33±7.7 |
| Asian, Female (6) | 7.48±4.31 | 0.14±0.0048 | 1.80±2.49 | 42±8.9 |
| Non-Asian, Male (3) | 6.35±1.59 | 0.038±0.0077 | 4.67±1.15 | 24±15 |
| Non-Asian, Female (11) | 14.7±15.3 | 0.13±0.0020 | 4.40±2.63 | 29±5.6 |

Means within a column between darkened lines that have different superscripts are significantly different ($p \leq 0.05$).

Table 3. Study 1: F-ratios and probability values (p value) from analysis of variance factored by ethnicity (E), gender (G), subjects, and replications (Reps) for four experimental variables (N=22).

| Factor | Salivary L-glutamic acid | | % MSG Preferred in Tomato Juice | | % MSG Preferred in Beef Broth | | MSG Intake | |
|---|---|---|---|---|---|---|---|---|
| | F-ratio | p value | F-ratio | p value | F-ratio | p value | F-ratio | p value |
| Ethnicity | 1.16 | 0.295 | 4.61 | 0.045 | 3.65 | 0.071 | 8.87 | 0.007 |
| Gender | 1.67 | 0.211 | 1.23 | 0.282 | 0.43 | 0.520 | 3.73 | 0.067 |
| E x G | 0.22 | 0.640 | 3.04 | 0.098 | 0.17 | 0.681 | 0.42 | 0.525 |
| Subjects | 5.17 | <0.001 | 0.66 | 0.603 | 0.25 | 0.804 | 0.25 | 0.801 |
| Reps | 0.72 | 0.493 | | | | | | |

Table 4. Study 1: F-ratios and probability values (p value) from analysis of variance factored by ethnicity (E), gender (G) and concentration (Conc., C) for MSG in water, tomato juice, and beef broth (N=22).

| Factor | Overall Intensity | | Umami | | Tomato/Beef Flavor | | Saltiness | |
|---|---|---|---|---|---|---|---|---|
| | F-ratio | p value | F-ratio | p value | F-ratio | p value | F-ratio | p value |
| Water | | | | | | | | |
| Ethnicity | 0.02 | 0.900 | 0.57 | 0.454 | Not Applicable | | 10.20 | 0.002 |
| Gender | 1.13 | 0.291 | 0.73 | 0.396 | | | 5.28 | 0.024 |
| Conc. | 25.11 | <0.001 | 19.38 | <0.001 | | | 6.65 | <0.001 |
| E x G | 0.36 | 0.549 | 0.15 | 0.699 | | | 0.01 | 0.945 |
| G x C | 0.59 | 0.674 | 0.33 | 0.859 | | | 0.62 | 0.649 |
| E x C | 1.98 | 0.104 | 1.16 | 0.338 | | | 0.79 | 0.557 |
| Tomato Juice | | | | | | | | |
| Ethnicity | 10.22 | 0.002 | 3.69 | 0.058 | 15.87 | <0.001 | 2.16 | 0.146 |
| Gender | 9.48 | 0.003 | 12.61 | 0.001 | 3.53 | 0.064 | 18.95 | <0.001 |
| Conc. | 1.10 | 0.365 | 0.23 | 0.922 | 1.30 | 0.275 | 0.39 | 0.813 |
| E x G | 38.54 | <0.001 | 10.11 | 0.002 | 14.04 | <0.001 | 10.66 | 0.002 |
| G x C | 0.79 | 0.535 | 0.27 | 0.895 | 0.37 | 0.830 | 0.33 | 0.855 |
| E x C | 0.31 | 0.873 | 0.32 | 0.863 | 0.41 | 0.805 | 0.14 | 0.968 |
| Beef Broth | | | | | | | | |
| Ethnicity | 43.12 | <0.001 | 28.99 | <0.001 | 19.49 | <0.001 | 0.40 | 0.530 |
| Gender | 0.22 | 0.612 | 2.62 | 0.109 | 0.23 | 0.631 | 0.14 | 0.710 |
| Conc. | 17.81 | <0.001 | 12.46 | <0.001 | 5.30 | 0.001 | 23.35 | <0.001 |
| E x G | 3.24 | 0.075 | 1.58 | 0.212 | 0.17 | 0.680 | 1.46 | 0.230 |
| G x C | 0.19 | 0.940 | 0.20 | 0.940 | 0.14 | 0.964 | 0.99 | 0.415 |
| E x C | 1.31 | 0.270 | 1.18 | 0.328 | 0.62 | 0.652 | 0.31 | 0.870 |

Table 5. Study 1: Mean intensity scores* with standard deviations (s.d.) for five concentrations of MSG in water, tomato juice and beef broth for four attributes (N=22).

| Modality | Overall Intensity | Umami | Tomato/Beef Flavor | Saltiness |
|---|---|---|---|---|
| (%w/v) | Mean±s.d. | Mean±s.d. | Mean±s.d. | Mean±s.d. |
| Water | | | | |
| 0 | 24.4±25.8[a] | 24.3±26.8[a] | Not applicable | 13.3±17.6[a] |
| 0.025 | 34.5±32.9[a] | 37.3±36.4[b] | | 20.7±25.6[a] |
| 0.05 | 65.5±37.8[b] | 64.4±39.0[c] | | 31.2±28.8[b] |
| 0.075 | 83.8±36.2[c] | 85.0±41.1[d] | | 39.6±36.7[bc] |
| 0.1 | 87.5±33.2[c] | 86.7±34.3[d] | | 42.7±36.5[c] |
| Tomato Juice | | | | |
| 0 | 76.1±36.5 | 47.2±36.2 | 76.7±34.6 | 36.9±32.8 |
| 0.025 | 79.8±32.6 | 51.8±37.5 | 77.5±30.8 | 39.3±32.1 |
| 0.05 | 82.7±29.8 | 47.7±34.1 | 85.0±30.5 | 41.0±32.9 |
| 0.1 | 79.5±31.9 | 55.3±36.1 | 84.6±31.5 | 43.1±35.9 |
| 0.2 | 84.4±34.0 | 52.9±39.3 | 87.3±29.8 | 44.3±36.7 |
| Beef Broth | | | | |
| 0 | 48.1±33.9[a] | 43.1±36.0[a] | 46.3±39.2[a] | 16.6±19.1[a] |
| 1 | 76.2±35.6[b] | 74.6±37.7[b] | 60.9±35.6[b] | 32.7±28.5[b] |
| 2 | 78.1±36.0[b] | 77.6±36.3[b] | 65.3±38.5[b] | 41.3±34.9[b] |
| 4 | 93.0±27.3[c] | 82.8±36.4[b] | 79.6±34.0[c] | 65.3±40.1[c] |
| 6 | 108.9±25.5[d] | 101.1±30.3[c] | 88.5±32.4[c] | 85.8±32.9[d] |

For each attribute, means with different superscripts within a column between darkened lines are significantly different ($p \leq 0.05$). Means between modalities were not compared.

*Scale: 0 mm = None; 146 mm = Extreme.

Table 6. Study 1: Mean intensity scores* with standard deviations (s.d.) averaged across five concentrations of MSG in three modalities grouped by ethnicity and gender for four attributes (N=22).

| Modality | Overall Intensity Mean±s.d. | Umami Mean±s.d. | Tomato/Beef Flavor Mean±s.d. | Saltiness Mean±s.d. |
|---|---|---|---|---|
| Water | | | | |
| Total | 59.1±34.6 | 59.6±35.7 | Not applicable | 29.5±24.0 |
| Asian (A) | 59.3±38.7 | 55.7±38.8 | | 21.4±23.9[a] |
| Nonasian(N) | 29.0±32.4 | 61.8±33.8 | | 34.1±23.0[b] |
| Male(M) | 62.8±33.4 | 55.1±34.6 | | 35.2±25.2[b] |
| Female(F) | 57.7±35.2 | 61.2±36.1 | | 27.4±23.4[a] |
| A, M | 64.7±41.9 | 54.0±40.3 | | 27.7±24.9 |
| N, M | 60.9±23.2 | 56.1±29.1 | | 42.6±24.0 |
| A, F | 56.0±37.1 | 56.7±38.7 | | 17.5±22.9 |
| N, F | 58.5±34.6 | 63.3±35.1 | | 31.8±22.4 |
| Tomato Juice | | | | |
| Total | 80.5±25.5 | 51.0±30.7 | 82.2±24.8 | 40.9±27.0 |
| Asian | 83.5±32.1[b] | 56.2±41.7 | 91.1±28.3[b] | 44.2±33.3 |
| Nonasian | 78.6±20.5[a] | 47.8±21.0 | 76.8±20.8[a] | 38.9±22.4 |
| Male | 93.9±34.0[b] | 71.1±41.2[b] | 93.4±32.8 | 61.2±25.5[b] |
| Female | 76.3±20.8[a] | 44.7±23.5[a] | 78.7±20.8 | 34.6±24.3[a] |
| A, M | 113±29.0[b] | 85.3±47.9[b] | 110.0±29.4[b] | 72.5±21.5[b] |
| N, M | 65.1±15.4[a] | 50.0±11.4[a] | 68.2±18.5[a] | 44.4±21.9[a] |
| A, F | 65.7±17.6[a] | 38.7±25.3[a] | 79.6±20.8[a] | 27.2±26.9[a] |
| N, F | 81.1±20.5[a] | 47.4±22.3[a] | 78.3±20.9[a] | 37.9±22.5[a] |
| Beef Broth | | | | |
| Total | 80.9±31.9 | 75.8±33.4 | 68.1±31.6 | 48.3±31.9 |
| Asian | 98.1±31.0[b] | 93.3±35.2[b] | 83.5±33.6[b] | 48.9±30.8 |
| Nonasian | 70.3±27.2[a] | 65.1±27.5[a] | 58.7±26.3[a] | 48.0±32.8 |
| Male | 85.1±35.9 | 84.9±37.8 | 68.9±33.5 | 49.2±26.3 |
| Female | 79.2±30.2 | 72.2±31.0 | 67.8±30.9 | 48.0±24.3 |
| A, M | 105.0±35.3 | 103.0±39.7 | 83.2±37.9 | 53.5±28.0 |
| N, M | 65.5±24.5 | 66.6±26.1 | 54.6±21.3 | 45.0±24.8 |
| A, F | 94.1±28.1 | 87.3±31.5 | 83.7±31.5 | 46.2±32.6 |
| N, F | 71.7±28.7 | 64.7±28.2 | 59.9±27.7 | 48.9±35.0 |

For each attribute, means with different superscripts within a column between darkened lines are significantly different ($p \leq 0.05$). Means among the three modalities were not compared.

*Scale: 0 mm = None; 146 mm = Extreme

Table 7. Study 1: F-ratios and probability values (p values) from analysis of variance factored by subjects and replications for MSG in water, tomato juice and beef broth (N=22).

| Factor | Overall Intensity | | Umami | | Tomato/Beef Flavor | | Saltiness | |
|---|---|---|---|---|---|---|---|---|
| | F | p value | F | p value | F | p value | F | p value |
| Water | | | | | | | | |
| Subject | 2.56 | <0.001 | 3.18 | <0.001 | Not applicable | | 7.40 | <0.001 |
| Rep | 3.80 | 0.023 | 2.61 | 0.075 | | | 3.29 | 0.039 |
| Tomato Juice | | | | | | | | |
| Subject | 14.64 | <0.001 | 28.64 | <0.001 | 14.81 | .000 | 23.52 | <0.001 |
| Rep | 0.44 | 0.647 | 0.37 | 0.689 | 0.65 | .521 | 1.88 | 0.154 |
| Beef Broth | | | | | | | | |
| Subject | 5.18 | <0.001 | 6.14 | <0.001 | 7.70 | .000 | 2.41 | 0.001 |
| Rep | 0.22 | 0.805 | 0.42 | 0.661 | 0.81 | .838 | 2.88 | 0.058 |

Table 8a. Study 1: Mean detection thresholds (g/100 ml) with standard deviations (s.d.) for MSG, sucrose, citric acid, NaCl and caffeine grouped by ethnicity and gender.

| Factor | MSG (N=20) Mean±s.d. | Sucrose (N=22) Mean±s.d. | Citric Acid (N=22) Mean±s.d. | NaCl (N=21) Mean±s.d. | Caffeine (N=16) Mean±s.d. |
|---|---|---|---|---|---|
| Total | 0.00895±0.00038 | 0.108±0.0055 | 0.00536±0.00071 | 0.0219±0.00016 | 0.0109±0.00040 |
| Asian | 0.0103±0.00072 | 0.113±0.0061 | 0.00500±0 | 0.0200±0[a] | 0.0142±0.000017 |
| Nonasian | 0.00823±0.00032 | 0.105±0.0052 | 0.00557±0.00014 | 0.0227±0.00026[b] | 0.00946±0.00027 |
| Male | 0.0118±0.00041[b] | 0.117±0.0083 | 0.00633±0.00027 | 0.0267±0.00033[b] | 0.0140±0.00093 |
| Female | 0.0080±0.00047[a] | 0.104±0.0029 | 0.00500±0 | 0.0200±0[a] | 0.00992±0.00019 |
| A, M | 0.0135±0 | 0.117±0.0064 | 0.00500±0 | 0.0200±0[a] | 0.0200±0 |
| A, F | 0.00788±0.00012 | 0.110±0.108 | 0.00500±0 | 0.0200±0[a] | 0.0103±0.000041 |
| N, M | 0.00925±0.0015 | 0.117±0.0064 | 0.00767±0.0062 | 0.0333±0.0015[b] | 0.00800±0 |
| N, F | 0.00805±0.00037 | 0.101±0.00070 | 0.00500±0 | 0.0200±0[a] | 0.00978±0.00096 |

Means within a column with different superscripts within a column between darkened lines are significantly different ($p \leq 0.05$). Means among tastants were not compared.

Table 8b. Study 1: F-ratios and probability values from analyses of variance in detection thresholds for MSG, sucrose, citric acid, NaCl and caffeine factored by ethnicity (R) and gender (G).

| Factor | MSG (N=20) | | Sucrose (N=22) | | Citric Acid (N=22) | | NaCl (N=19) | | Caffeine (N=16) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | F-ratio | p value | F-ratio | p value | F-ratio | p value | F-ratio | p value | F-ratio | p value |
| Ethnicity | 1.60 | 0.225 | 0.059 | 0.810 | 3.13 | 0.094 | 11.2 | 0.004 | 3.12 | 0.103 |
| Gender | 4.49 | 0.050 | 0.009 | 0.925 | 3.13 | 0.094 | 11.2 | 0.004 | 1.23 | 0.289 |
| E x G | 1.88 | 0.189 | 0.009 | 0.925 | 3.13 | 0.094 | 11.2 | 0.004 | 2.59 | 0.134 |

Table 9a. Study 1: Mean recognition thresholds (g/100 ml) ± standard deviations (s.d.) for MSG, sucrose, citric acid, NaCl, and caffeine grouped by ethnicity and gender.

| Factor | MSG (N=9) Mean±s.d. | Sucrose (N=17) Mean±s.d. | Citric Acid (N=21) Mean±s.d. | NaCl(N=19) Mean±s.d. | Caffeine (N=16) Mean±s.d. |
|---|---|---|---|---|---|
| Total | 0.0166±0.00099 | 0.432±0.226 | 0.00876±0.0067 | 0.0605±0.0020 | 0.0109±0.00040 |
| Asian(A) | 0.0161±0.00079 | 0.429±0.263 | 0.00663±0.0031[a] | 0.0833±0.0083 | 0.0142±0.000017 |
| Nonasian(N) | 0.0169±0.00055 | 0.435±0.211 | 0.0101±0.0080[b] | 0.0500±0.0079 | 0.00946±0.00027 |
| Male (M) | 0.0195±0.00028 | 0.500±0.355 | 0.0113±0.011 | 0.0720±0.0028 | 0.0140±0.00093 |
| Female (F) | 0.0151±0.00065 | 0.412±0.185 | 0.00773±0.0046 | 0.0564±0.0048 | 0.00992±0.00019 |
| A, M | 0.0180±0.00083 | 0.500±0.436 | 0.0050±0[a] | 0.0600±0[ab] | 0.0200±0 |
| A, F | 0.0143±0.00060 | 0.375±0 | 0.0076±0.0037[a] | 0.1070±0.0004[b] | 0.0103±0.000042 |
| N, M | 0.0225±0 | 0.500±0 | 0.0177±0.0130[b] | 0.0900±0.0041[ab] | 0.0080±0 |
| N, F | 0.0155±0.00081 | 0.423±0.222 | 0.00780±0.0051[a] | 0.0427±0.0064[a] | 0.0098±0.00096 |

Means with different superscripts within a column between darkened lines are significantly different ($p \leq 0.05$).

Table 9b. Study 1: Table 11. F-ratios and probability values (p values) from analysis of variance in recognition thresholds to MSG, sucrose, citric acid, NaCl and caffeine factored by ethnicity and gender.

| Factor | MSG (N=9) | | Sucrose (N=17) | | Citric Acid (N=21) | | NaCl (N=19) | | Caffeine (N=16) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | F-ratio | p value | F-ratio | p value | F-ratio | p value | F-ratio | p value | F-ratio | p value |
| Ethnicity(E) | 1.01 | 0.361 | 0.027 | 0.871 | 4.82 | 0.042 | 0.786 | 0.389 | 3.12 | 0.103 |
| Gender(G) | 3.54 | 0.119 | 0.381 | 0.548 | 1.54 | 0.232 | 0.000 | 0.988 | 1.23 | 0.289 |
| E x G | 0.323 | 0.594 | 0.027 | 0.871 | 4.53 | 0.048 | 6.02 | 0.027 | 2.59 | 0.134 |

Table 10. Study 1. Matrix of correlation coefficients (r) for 14 experimental variables.

| | Salivary Glutamate | % MSG Pref. In Juice | % MSG Pref. In Beef | MSG Intake | Citric Acid DT | Citric Acid RT | NaCl DT | NaCl RT | Caffeine DT | Caffeine RT | MSG DT | MSG RT | Sucrose DT | Sucrose RT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Salivary Glutamate** | 1.000[a] | | | | | | | | | | | | | |
| | . | | | | | | | | | | | | | |
| | 24[c] | | | | | | | | | | | | | |
| **% MSG Pref. In Juice** | -0.348 | 1.000 | | | | | | | | | | | | |
| | 0.112[b] | . | | | | | | | | | | | | |
| | 22 | 23 | | | | | | | | | | | | |
| **% MSG Pref. In Beef** | 0.048 | 0.339 | 1.000 | | | | | | | | | | | |
| | 0.831 | 0.123 | . | | | | | | | | | | | |
| | 22 | 22 | 23 | | | | | | | | | | | |
| **MSG Intake** | 0.049 | -0.071 | -0.307 | 1.000 | | | | | | | | | | |
| | 0.819 | 0.748 | 0.154 | . | | | | | | | | | | |
| | 24 | 23 | 23 | 25 | | | | | | | | | | |
| **Citric Acid DT** | -0.079 | -0.261 | 0.026 | 0.171 | 1.000 | | | | | | | | | |
| | 0.734 | 0.253 | 0.911 | 0.446 | . | | | | | | | | | |
| | 21 | 21 | 21 | 22 | 22 | | | | | | | | | |
| **Citric Acid RT** | 0.030 | -0.265 | 0.053 | -0.030 | 0.725** | 1.000 | | | | | | | | |
| | 0.901 | 0.259 | 0.824 | 0.898 | <0.001 | . | | | | | | | | |
| | 20 | 20 | 20 | 21 | 21 | 21 | | | | | | | | |
| **NaCl DT** | -0.097 | -0.344 | 0.160 | -0.490* | -0.073 | 0.128 | 1.000 | | | | | | | |
| | 0.684 | 0.138 | 0.501 | 0.024 | 0.755 | 0.591 | . | | | | | | | |
| | 20 | 20 | 20 | 21 | 21 | 20 | 21 | | | | | | | |
| **NaCl RT** | -0.393 | -0.255 | -0.294 | 0.301 | 0.117 | 0.116 | 0.238 | 1.000 | | | | | | |
| | 0.106 | 0.355 | 0.236 | 0.210 | 0.632 | 0.648 | 0.327 | . | | | | | | |
| | 18 | 19 | 18 | 19 | 19 | 18 | 19 | 19 | | | | | | |
| **Caffeine DT** | -0.032 | 0.053 | 0.278 | -0.026 | -0.122 | -0.006 | -0.122 | -0.182 | 1.000 | | | | | |
| | 0.906 | 0.846 | 0.316 | 0.925 | 0.652 | 0.983 | 0.652 | 0.517 | . | | | | | |
| | 16 | 16 | 15 | 16 | 16 | 15 | 16 | 15 | 16 | | | | | |
| **Caffeine RT** | -0.032 | 0.053 | 0.278 | -0.026 | -0.122 | -0.006 | -0.122 | -0.182 | 1.000** | 1.000 | | | | |
| | 0.906 | 0.846 | 0.316 | 0.925 | 0.652 | 0.983 | 0.652 | 0.517 | <0.001 | . | | | | |
| | 16 | 16 | 15 | 16 | 16 | 15 | 16 | 15 | 16 | 16 | | | | |
| **MSG DT** | -0.422 | -0.066 | 0.020 | -0.005 | -0.101 | -0.073 | 0.135 | 0.176 | 0.427 | 0.427 | 1.000 | | | |
| | 0.072 | 0.781 | 0.935 | 0.984 | 0.672 | 0.767 | 0.582 | 0.470 | 0.113 | 0.113 | . | | | |
| | 19 | 20 | 19 | 20 | 20 | 19 | 19 | 19 | 15 | 15 | 20 | | | |
| **MSG RT** | -0.604 | -0.137 | 0.184 | -0.151 | 0.559 | 0.565 | -0.289 | 0.409 | 0.310 | 0.310 | 0.583 | 1.000 | | |
| | 0.085 | 0.725 | 0.662 | 0.699 | 0.118 | 0.113 | 0.488 | 0.314 | 0.499 | 0.499 | 0.099 | . | | |
| | 9 | 9 | 8 | 9 | 9 | 9 | 8 | 8 | 7 | 7 | 9 | 9 | | |
| **Sucrose DT** | -0.238 | 0.035 | 0.098 | 0.163 | 0.242 | -0.039 | -.0125 | -0.050 | -.0184 | -.0184 | 0.241 | 0.392 | 1.000 | |
| | 0.300 | 0.880 | 0.673 | 0.469 | 0.279 | 0.867 | 0.590 | 0.840 | 0.495 | 0.495 | 0.306 | 0.296 | . | |
| | 21 | 21 | 21 | 22 | 22 | 21 | 21 | 19 | 16 | 16 | 20 | 9 | 22 | |
| **Sucrose RT** | -0.481 | 0.045 | 0.341 | -0.112 | 0.564* | 0.166 | 0.069 | 0.504 | 0.036 | 0.036 | 0.501 | 0.459 | 0.110 | 1.000 |
| | 0.059 | 0.867 | 0.181 | 0.668 | 0.018 | 0.539 | 0.800 | 0.066 | 0.912 | 0.912 | 0.057 | 0.300 | 0.675 | . |
| | 16 | 16 | 16 | 17 | 17 | 16 | 16 | 14 | 12 | 12 | 15 | 7 | 17 | 17 |

[a] Stimulus correlation coefficient, r.; [b] p-value; [c] Number of subjects (N)

Table 11. Study 2: Means with standard deviations (s.d.) for eight experimental variables, grouped by ethnicity and gender.

| Group (N) | Salivary L-glutamic acid (mg/l) Mean±s.d. | Salivary Sodium (ppm)* Mean±s.d. | Threshold MSG (mM) Mean±s.d. | Threshold NaCl (mM) Mean±s.d. | Power Function Slope-MSG Mean±s.d. | Power Function Slope-NaCl Mean±s.d. | MSG Intake Mean±s.d. | NaCl Intake Mean±s.d. |
|---|---|---|---|---|---|---|---|---|
| Total (60) | 9.13±6.05 | 5.44±2.44 | 1.07±0.927 | 2.78±1.91 | 0.959±0.467 | 0.990±0.224 | 31.3±10.0 | 17.8±5.20 |
| Range | 0.448 - 22.6 | 1.54-22.4 | 0.0016 - 3.31 | 0.0912 - 7.59 | 0.118 - 2.09 | 0.505 - 1.38 | 10 – 61 | 3 – 32 |
| Asian (A) | 9.48±5.87 | 5.73±2.46 | 1.09±0.990 | 1.76±1.48 | 0.975±0.440 | 1.04±0.252[b] | 35.6±10.1[b] | 19.1±5.1[b] |
| NonAsian(N) | 8.78±6.29 | 5.53±2.87 | 1.06±0.880 | 1.07±2.12 | 0.944±0.499 | .929±0.245[a] | 27.0±7.9[a] | 16.4±4.9[a] |
| Male (M) | 10.5±5.60 | 5.53±3.61 | 1.06±0.85 | 2.06±1.78 | 0.939±0.441 | 1.03±0.252 | 29.4±8.7 | 19.0±5.3 |
| Female (F) | 7.79±6.30 | 5.73±2.81 | 1.09±1.01 | 1.40±1.81 | 0.980±0.497 | 0.937±0.249 | 33.2±10.9 | 16.5±4.8 |
| A, M (15) | 10.0±6.63 | 4.55±1.40[a] | 1.22±0.920 | 1.81±1.76 | 0.936±0.395 | 1.07±0.254 | 37.3±11.2 | 20.3±5.5 |
| A, F (15) | 8.93±5.19 | 6.83±2.75[b] | 0.950±1.06 | 1.71±1.19 | 1.01±0.492 | 1.01±0.255 | 33.9±9.0 | 17.9±4.6 |
| N, M (15) | 10.9±4.53 | 5.72±2.34[ab] | 0.890±0.780 | 2.32±1.83 | 0.942±0.497 | 0.995±0.253 | 29.1±9.2 | 19.6±4.8 |
| N, F (15) | 6.65±7.19 | 4.55±2.44[a] | 1.22±0.970 | 1.09±2.27 | 0.946±0.518 | 0.862±0.226 | 24.9±5.9 | 15.2±4.8 |

Means with different superscripts within a column between darkened lines are significantly different ($p \leq 0.05$).
*Number of subjects was 57 due to loss of sample.

Table 12. Study 2: Analysis of variance in eight experimental measures factored by ethnicity and gender (N=60).

| Group | Salivary L-glutamic acid | | Salivary Sodium | | Threshold MSG | | Threshold NaCl | |
|---|---|---|---|---|---|---|---|---|
| | F | p | F | p | F | p | F | p |
| Subject | 0.931 | 0.339 | 8.21 | 0.006 | 4.05 | 0.049 | 6.97 | 0.0106 |
| Ethnicity (E) | 0.207 | 0.651 | 2.56 | 0.199 | 0.014 | 0.907 | 0.014 | 0.907 |
| Gender (G) | 3.02 | 0.088 | 2.50 | 0.195 | 0.015 | 0.902 | 2.04 | 0.159 |
| E x G | 1.05 | 0.310 | 8.98 | 0.004 | 1.48 | 0.228 | 1.47 | 0.230 |

| Group | Power Function Slope-MSG | | Power Function Slope-NaCl | | MSG Intake | | NaCl Intake | |
|---|---|---|---|---|---|---|---|---|
| | F | P | F | p | F | p | F | p |
| Subject | 0.498 | 0.308 | 0.503 | 0.309 | 4.29 | 0.043 | 4.06 | 0.048 |
| Ethnicity (E) | 0.066 | 0.799 | 4.07 | 0.049 | 13.9 | <0.001 | 4.47 | 0.039 |
| Gender (G) | 0.114 | 0.737 | 2.98 | 0.090 | 2.71 | 0.105 | 3.63 | 0.062 |
| E x G | 0.088 | 0.768 | 0.227 | 0.636 | 0.168 | 0.683 | 0.001 | 0.979 |

Table 13. Study 2: F-ratios and probability values (p) from analysis of variance of five concentrations of MSG and NaCl factored by subject, concentration, replication, gender, and ethnicity (N=60).

| Factor | MSG | | NaCl | |
|---|---|---|---|---|
| | F | p | F | p |
| Subject (S) | 6.41 | <0.001 | 0.922 | 0.642 |
| Rep | 0.971 | 0.397 | 14.7 | <0.001 |
| Concentration (C) | 97.1 | <0.001 | 873 | <0.001 |
| S x Rep | 0.680 | 0.999 | 0.127 | 1.000 |
| C x Rep | 0.240 | 0.984 | 2.80 | 0.005 |
| Ethnicity (E) | 0.448 | 0.503 | 26.6 | <0.001 |
| Gender (G) | 4.83 | 0.028 | 2.16 | 0.142 |
| E x G | 21.8 | <0.001 | 0.104 | 0.742 |
| G x C | 0.256 | 0.906 | 0.212 | 0.932 |
| E x C | 0.693 | 0.597 | 2.32 | 0.055 |

Table 14. Study 2: Mean intensity scores[a] with standard deviations (s.d.) for five concentrations of MSG grouped by ethnicity and gender (N=60; 3 reps).

| MSG | 0.63 mM Mean±s.d. | 1.25 mM Mean±s.d. | 2.5 mM Mean±s.d. | 5.0 mM Mean±s.d. | 10.0 Mm Mean±s.d. | Grand Mean |
|---|---|---|---|---|---|---|
| Total | 14.22±14.7 | 17.67±16.5 | 40.16±40.5 | 75.36±54.5 | 124.6±73.9 | 54.40 |
| Asian (A) | 12.74±14.5 | 19.03±16.8 | 39.27±33.2 | 74.44±52.3 | 115.5±60.3 | 52.20 |
| NonAsian(N) | 15.72±15.1 | 16.30±16.4 | 41.05±47.2 | 76.28±57.5 | 133.6±85.4 | 56.59 |
| Male (M) | 12.83±10.4 | 15.65±11.3 | 37.65±32.3 | 65.73±68.7 | 114.6±57.0 | 49.29[a] |
| Female (F) | 15.63±17.9 | 20.17±20.2 | 42.65±47.0 | 85.02±66.0 | 134.6±85.7 | 59.61[b] |
| A, M | 9.340±7.03 | 16.79±12.8 | 30.93±25.1 | 55.95±98.0 | 100.7±51.4 | 42.74 |
| N, M | 16.32±13.7 | 14.54±11.8 | 44.40±39.4 | 75.50±39.4 | 128.5±63.0 | 55.85 |
| A, F | 16.13±19.0 | 22.27±20.0 | 47.61±38.8 | 92.93±59.1 | 130.4±66.4 | 61.87 |
| N, F | 15.12±16.8 | 18.06±20.3 | 37.70±55.1 | 77.10±72.9 | 138.8±105 | 57.36 |

Means with different superscripts within a column between darkened lines of a column are significantly different ($p \leq 0.05$). All stimuli intensity scores, based on the total mean (first row) are significantly different ($p \leq 0.001$).
[a]Scale: Modified magnitude estimation scale; see Statistical Analysis section for normalization procedure.

Table 15. Study 2: Mean intensity scores[a] with standard deviations (s.d.) for five concentrations of NaCl grouped by ethnicity and gender (N=60; 3 reps).

| NaCl | 0.010 M Mean±s.d. | 0.032 M Mean±s.d. | 0.10 M Mean±s.d. | 0.32 M Mean±s.d. | 1.0 M Mean±s.d. | Grand Mean |
|---|---|---|---|---|---|---|
| Total | 6.54±7.92 | 26.91±33.5 | 115.9±77.7 | 228.8±67.5 | 300.9±78.8 | 135.8 |
| Asian (A) | 4.66±6.54 | 19.62±16.6 | 90.94±52.9 | 214.8±54.7 | 301.8±78.5 | 126.4[a] |
| NonAsian(N) | 8.42±8.81 | 34.20±43.6 | 140.8±90.6 | 242.9±76.7 | 300.1±80.4 | 145.3[b] |
| Male (M) | 4.46±4.22 | 22.00±23.3 | 106.0±55.5 | 225.9±44.8 | 300.1±72.0 | 131.7 |
| Female (F) | 8.63±9.65 | 31.84±34.8 | 125.7±69.6 | 231.7±82.5 | 301.9±87.5 | 140.0 |
| A, M | 2.78±2.33 | 19.78±18.1 | 89.88±50.8 | 209.0±44.0 | 293.9±69.2 | 123.1[a] |
| N, M | 6.13±6.10 | 24.22±28.4 | 122.2±60.1 | 242.7±45.5 | 306.2±75.4 | 140.3[b] |
| A, F | 6.54±8.69 | 19.50±15.6 | 92.00±26.6 | 220.5±64.7 | 309.6±88.6 | 129.6[a] |
| N, F | 10.7±10.6 | 44.18±54.1 | 159.3±113 | 242.9±100 | 294.1±87.3 | 150.2[b] |

Means with different superscripts within a column between darkened lines of a column are significantly different ($p \leq 0.05$). All stimuli intensity scores, based on the total mean (first row) are significantly different ($p \leq 0.001$).
[a]Scale: Modified magnitude estimation scale; see Statistical Analysis section for normalization procedure.

Table 16a. Mean intensity scores for five concentrations of NaCl for three replications (N=60).

| Replication | 0.010 M | 0.032 M | 0.10 M | 0.32 M | 1.0 M |
|---|---|---|---|---|---|
| 1 | 1.70 | 4.87[b] | 15.62[c] | 23.37[b] | 28.54 |
| 2 | 0.847 | 3.70[b] | 11.61[b] | 22.46[b] | 28.21 |
| 3 | 0.582 | 2.54[a] | 9.80[a] | 20.98[a] | 28.62 |

Means with different superscripts within a column are significantly different ($p \leq 0.05$).

Table 16b. Mean intensity scores for five concentrations of NaCl for Asians and Non-Asians (N=60).

| Ethnicity | 0.010 M | 0.032 M | 0.10 M | 0.32 M | 1.0 M |
|---|---|---|---|---|---|
| Asians | 0.647 | 3.09 | 10.4[a] | 21.1[a] | 27.8 |
| Non-Asians | 1.41 | 4.28 | 14.2[b] | 23.4[b] | 29.1 |

Means with different superscripts within a column are significantly different ($p \leq 0.05$).

Table 17. Study 2. Matrix of correlation coefficients (r) for ten experimental variables (N=60).

| | Salivary Glutamate | Salivary Sodium | MSG Threshold | NaCl Threshold | Slope MSG Power Function | Slope NaCl Power Function | MSG Intake | NaCl Intake | Age |
|---|---|---|---|---|---|---|---|---|---|
| Salivary Glutamate | 1.000 | | | | | | | | |
| | . | | | | | | | | |
| Salivary Sodium | -0.054[a] | 1.000 | | | | | | | |
| | 0.682[b] | . | | | | | | | |
| MSG Threshold | -0.053 | -0.112 | 1.000 | | | | | | |
| | 0.690 | 0.392 | . | | | | | | |
| NaCl Threshold | -0.137 | -0.096 | 0.106 | 1.000 | | | | | |
| | 0.298 | 0.465 | 0.421 | . | | | | | |
| Slope MSG Power Function | -0.056 | -0.100 | 0.195 | 0.127 | 1.000 | | | | |
| | 0.671 | 0.448 | 0.134 | 0.335 | . | | | | |
| Slope NaCl Power Function | -0.045 | -0.030 | -0.009 | 0.151 | 0.176 | 1.000 | | | |
| | 0.671 | 0.819 | 0.945 | 0.248 | 0.180 | . | | | |
| MSG Intake | 0.146 | 0.268* | 0.048 | 0.022 | 0.025 | 0.157 | 1.000 | | |
| | 0.265 | 0.038 | 0.715 | 0.865 | 0.851 | 0.232 | . | | |
| NaCl Intake | 0.095 | 0.206 | 0.068 | 0.032 | -0.136 | 0.102 | 0.802** | 1.000 | |
| | 0.469 | 0.115 | 0.605 | 0.809 | 0.300 | 0.437 | <0.001 | . | |
| Age | -0.146 | 0.033 | 0.077 | -0.040 | -0.148 | 0.095 | -0.086 | 0.044 | 1.000 |
| | 0.265 | 0.802 | 0.561 | 0.764 | 0.258 | 0.471 | 0.515 | 0.739 | |

[a] Stimulus correlation coefficient, r.
[b] p-value

Table 18. Reported salivary sodium levels.

| Source | Subjects | Mean | Std. Dev. | Range | Method of Collection | Analytical Method |
|---|---|---|---|---|---|---|
| Giovanni, this study | 57 | 0.237 mM | 0.106 | 0.0670 – 1.05 | Whole mouth expectoration | AAS with correction |
| Grad, 1954 | 101 | 11.38 mEq/l | 1.3 | N.R. | N.R. | Flame photometry |
| Prader *et al.*, 1955 | N.R. | N.R. | N.R. | 12-36 mEq/l | N.R. | Flame photometry |
| McBurney and Pfaffman, 1963 | 5 | 4.3 mM | N.R. | 2.2-12.5 | N.R. | N.R. |
| Dawes, 1969* | N.R. | 1.5mM | N.R. | N.R. | N.R. | N.R. |
| Wotman *et al.*, 1973 | 6 | 11.9 mEq/l | 3.8 | 8.1-15.7 | Stimulated by chewing | N.R. |
| Shannon *et al.*, 1974 | N.R. | 13.5 mEq/l | N.R. | N.R. | N.R. | N.R. |
| Morino and Langford, 1978 | 27 | N.R. | N.R. | 0.75-1.4 mM (est. from graph) | "resting sodium" | Flame phtometry |
| Bartoshuk, 1980 | 2 | 16.5 mM | N.R. | 8-19 mM | N.R. | Flame photometry |
| Chung, 1981 | 20 | 2.66 mEq/l | 0.69 | N.R. | Cap on inner check | A.A.S. |
| Christensen *et al.*, 1986 | N.R. | 3.7 mM | N.R. | N.R. | N.R. | N.R. |
| Bradley, 1991 | N.R. | 2.7 mEq/l | N.R. | N.R. | N.R. | N.R. |
| Delwiche and O'Mahony, 1996 | 4 | 7.8 mM | N.R. | 4.9-12.2 | Whole mouth expectoration | A.A.S. |
| Guinard *et al.*, 1998 | N.R. | 27.24 mM | N.R. | N.R. | N.R. | N.R. |

N.R.: Not Reported

Table 19. Reported Thresholds to MSG and NaCl.

| Source | MSG | NaCl | Method |
|---|---|---|---|
| Giovanni, Study 1 | 0.478±0.020 mM | 3.75±0.0273 mM | Ascending Method of Limits |
| Giovanni, Study 2 | 1.07±0.927 mM | 2.78±1.91 mM | Maximum Likelihood |
| McBurney and Pfaffman, 1963[a] | N.R. | 4.3±0.14 mM | Ascending Method of Limits |
| Bartoshuk, 1974[b] | N.R. | 6.2 mM | Forced Choice Staircase |
| Yamaguchi and Kimizuka, 1979 | 0.625 mM | N.R. | Ascending Method of Limits |
| Druz and Baldwin, 1982 | N.R. | 0.007% w/v | Forced Choice |
| Faurion, 1987 | 0.34±0.20 mM | N.R. | N.R. |
| Beauchamp *et al.*, 1990[a] | N.R. | 7.7 mM | Forced Choice |
| Schiffman *et al.*, 1991[c] | 0.902 mM | N.R. | Forced Choice Staircase |
| Ajinomoto, ~1992 | 0.016 mM | N.R. | N.R. |
| Gonazlez-Viñas *et al.*, 1998[d] | 1.01 mM | 3.42 mM | Ascending Method of Limits |

[a] Threshold interpolated from graph.
[b] Water rinse before each trial.
[c] Elderly subjects not included
[d] Mineral water used as carrier
N.R.: Not Reported

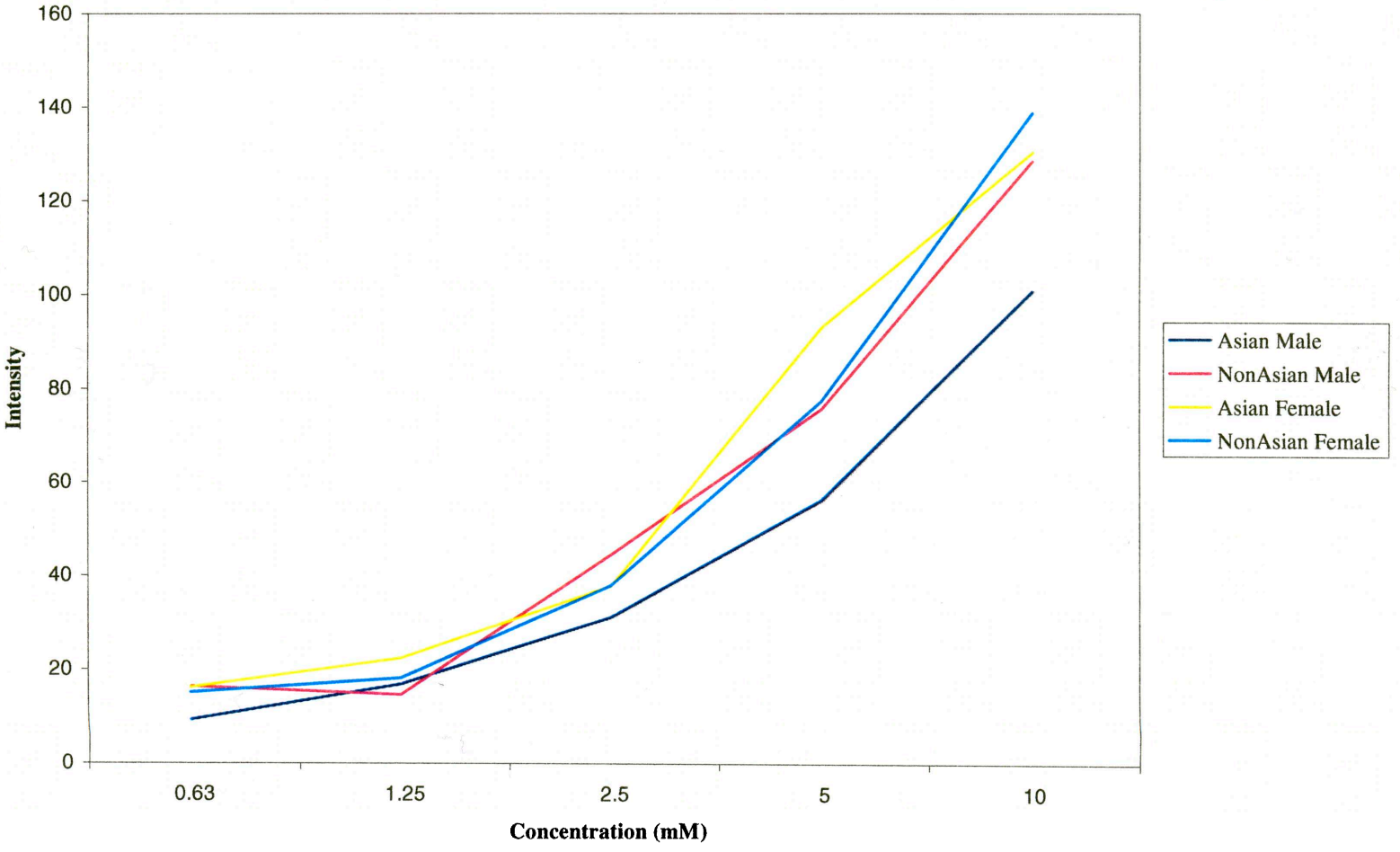



Figure 1. Mean intensity responses to increasing concentrations of MSG in water for four groups of subjects (Study 2, N=60).

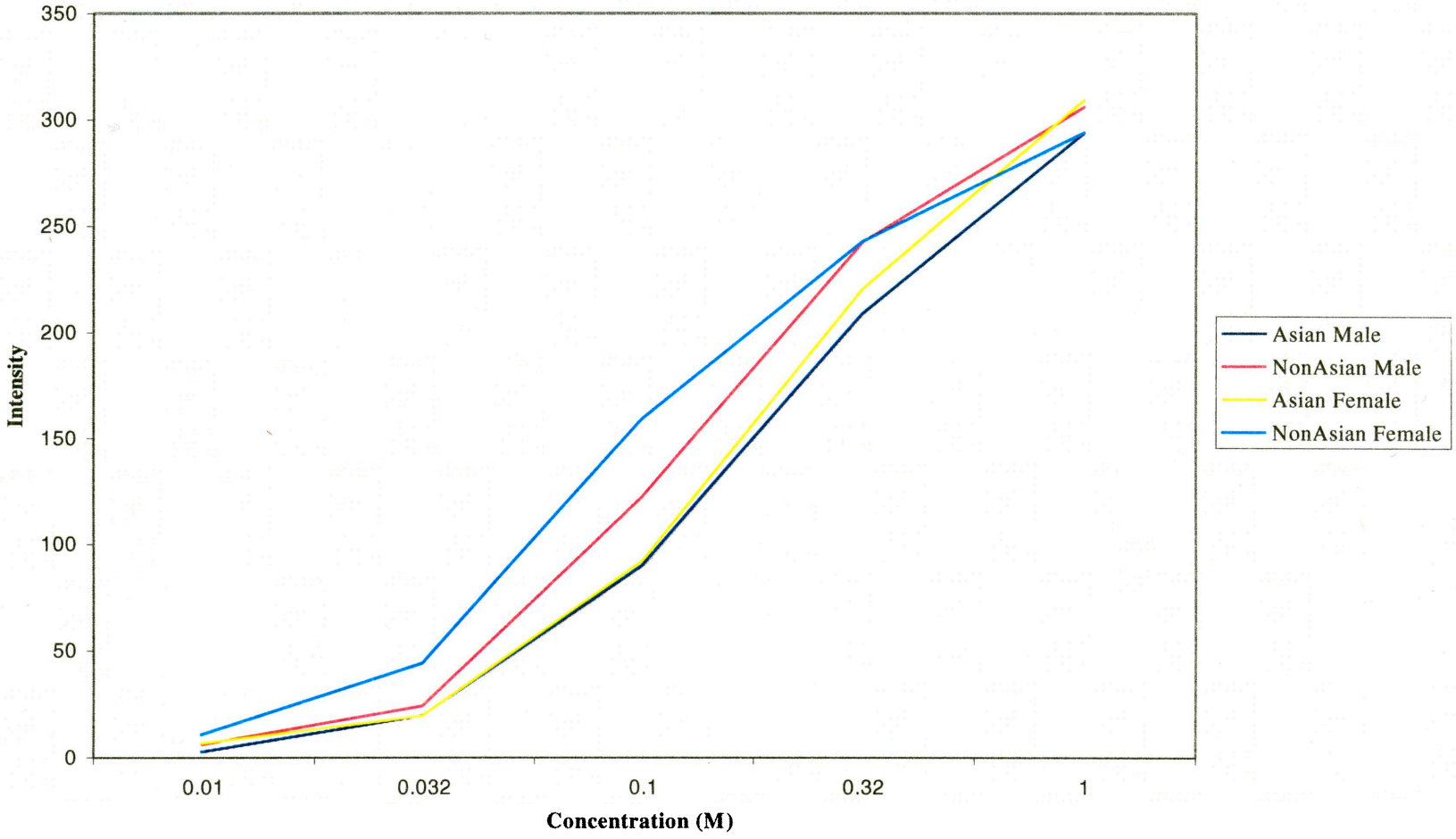



Figure 2. Mean intensity responses to increasing concentrations of NaCl in water for four groups of subjects (Study 2, N=60).

# CHAPTER 4

## Time-Intensity Profiles of Flavor Potentiators

The primary objective of this study was to characterize the time-intensity (TI) profiles for selected flavor potentiators; and, secondly, to determine any differences in the curve parameters due to gender, ethnicity (Asian and non-Asian), salivary glutamate levels, and dietary consumption.

### I. Materials and Methods

#### A. Subjects

Twenty healthy non-smoking subjects, from 21 to 48 years of age, were recruited based on availability and interest in the study. The panel was comprised of seven Asian females, four Asian males, three non-Asian females and six non-Asian males. Subjects were self-reported as not having MSG symptom complex (Life Sciences Research Institute, 1995) and gave their informed consent (Appendix I). They were compensated monetarily upon completion of the study.

#### B. Stimuli

The design consisted of 20 stimuli at various concentrations. Seven stimuli were single solutions of each flavor potentiator in purified water

(Milli-RO 4/5 Filtration and Reverse Osmosis system in series with a Milli-Q system: ion exchange and activated charcoal, Millipore Corp., Bedford, MA):

Monosodium Glutamate (MSG): 2.5 mM, 5 mM, 10 mM
Disodium 5'-Guanylate (GMP): 0.63 mM, 2.5 mM
Disodium 5'-Inosinate (IMP): 0.63 mM, 2.5 mM

Twelve stimuli were mixtures of each concentration of MSG with an equal volume of each of the 5'-ribonucleotide stimuli, and one stimulus was an equal-volume mix of 2.5 mM MSG, 2.5 mM GMP, and 2.5 mM IMP. These concentrations were chosen to cover the ranges of these compounds used in food and used in previous sensory tests. All chemicals were food grade and provided courtesy of Ajinomoto, Inc. (Tokyo, Japan). Ten ml stimuli were served at room temperature (22°C) in one ounce plastic opaque cups coded with three-digit random numbers. Stimuli were evaluated in duplicate. Five stimuli were served per session according to a randomized complete block design, for a total of eight sessions.

C. Procedure

Subjects completed two training sessions. The first session consisted of a discussion about flavor potentiators and the use of time-intensity equpment. Subjects tasted a 70 mM NaCl standard, 2.5 and 10 mM MSG, and 2.5 mM GMP. They completed a questionnaire designed to estimate

their intake of foods high in naturally-occurring and added glutamic acid (Appendix VI). In the second session, subjects practiced the time-intensity procedure with 2.5 and 10 mM MSG, 2.5 mM GMP and IMP, 5 mM MSG:0.63 mM IMP, and 2.5 mM MSG:0.63 mM GMP. Subjects were trained to evaluate the overall taste intensity of each stimulus, rather than focusing on a specific taste attribute. Subjects also selected 70 mM NaCl as a standard to use during evaluations, corresponding to a value of 75 on the scale from None (0) to Extreme (100).

Subjects did not eat or drink one hour prior to testing, with the exception of water, and refrained from drinking water 15 minutes prior to testing. Upon entering the taste booth, each subject provided approximately two ml of whole mouth saliva in a 5 ml glass vial with a screw-top lid. The saliva samples were immediately frozen for L-glutamic acid analysis. Next, the subjects evaluated the overall taste intensity of each stimulus using a computer-interfaced apparatus described by Guinard *et al.* (1985, 1995). This system allowed the subject to move a variable resistor sliding rod ("joystick") up and down a slot that served as an intensity scale, indicating increases and decreases in taste intensity. The NaCl standard was tasted before each session and was available for re-tasting as needed. To begin the evaluation, subjects took in the 10 ml stimulus, gently moving it to all areas

of their mouth. After 20 seconds, the stimulus was expectorated. Subjects were instructed to be consistent in their mouth movements. Stimulus evaluation continued until either the subject no longer perceived any taste or until the taste did not change in intensity. The latter criterion was determined during practice evaluations, because some subjects said the taste continued without a decrease in intensity for a very long time after expectoration. A four minute rest period was provided between stimuli during which subjects rinsed at least four times with distilled water, which was provided *ad libitum*. Appendix IX details the written instructions given to the subjects.

D. Test Conditions

Sessions were held in individual booths, at room temperature (22°C) with white incandescent lights (200 W) in each booth and fluorescent room lighting. Each booth was equipped with a sink for stimulus expectoration.

E. Glutamic Acid Determinations

Saliva samples were analyzed for L-glutamic acid content within 48 hours of collection using an enzymatic test kit (Boehringer Mannheim GmBh, Germany) as detailed in Chapter 3, Chemical Analyses.

F. Statistical Analyses

Four profile parameters were derived from each subjects' time-intensity curves: time to maximum intensity (Tmax), maximum intensity

(Imax), total duration of the evaluation (DUR), and area under the curve (AREA). Subjects were divided into three salivary glutamate groups based on their average salivary glutamate levels (high: 0.04165 – 0.1012 g/l; medium: 0.02120 – 0.03881 g/l; and, low: 0.00521-0.01586 g/l) and three intake groups based on their score on the food frequency questionnaire (high: 41-50; medium: 29-38; and, low: 13-25). One- and two-way analyses of variance using "Stat ANOVA" software (Abacus Software, Berkeley, CA) were performed to determine significant differences among stimuli, subjects, salivary glutamate, and dietary glutamate intake groups, and between gender, ethnicity, and replications.

II. Results

A. Time-Intensity (TI) Profiles of MSG, IMP, and GMP

TI profiles were arithmetically averaged across replications and subjects for each stimulus. In general, the profiles were atypical of those reported for other taste modalities (Figures 1-4). Tmax was short, followed by a plateau phase at Imax. The average profiles indicated a slight decrease in intensity before expectoration, after which the intensity increased again, sometimes higher than the initial intensity. This second intensity peak was relatively short and was followed by a gradual decrease in intensity. The average slope of the profiles as they decreased in intensity from Imax to 10

on the intensity scale was –0.814/sec (±0.140), and did not differ among stimuli. For most samples, DUR was very long, although aftertaste was difficult to quantify as subjects were instructed to continue the evaluation until the taste no longer changed in intensity, making the endpoint of the evaluation subjective. For a more accurate measure of aftertaste, subjects should have continued with the evaluation until all taste was gone from the mouth, but some subjects reported this would have taken at least ten minutes. The stimuli were different on all profile parameters except Tmax ($p \leq 0.001$; Table 1). Subjects also differed in their responses to these parameters ($p \leq 0.001$), as did replications, except for Imax ($p \leq 0.05$). Differences among subjects due to gender, ethnicity, salivary glutamate, and glutamate intake were also found and will be discussed.

Means for the TI parameters of all stimuli are summarized in Table 2. Tmax was very short for all flavor potentiators, between 14.3 and 20.6 seconds, and did not differ among the stimuli. Imax, DUR and AREA were generally highest for the binary mixtures of MSG with 2.5 mM ribonucleotides. For all parameters, singular stimuli had the smallest averages. Some numerical values for parameters extracted from the curves (Table 2) do not directly correspond to points on the graphs in Figures 1 – 4. This incongruity is due to differences in deriving the data for the profiles and

for the parameters. To construct the mean profiles, each subjects' intensity value at each second was averaged. To obtain the values in Table 2, the four parameters were measured from each subjects' graph, irrespective of the time this parameter was reached, and then analysis of variance was applied to the data. Liu and MacFie (1990) noted that the Imax of a mean curve may not be the same as the mean value of the Imax for the subjects' curves due to the averaging method used.

The taste potentiating effects of the nucleotides with MSG are demonstrated in the TI profiles for single and binary stimuli of 10 mM MSG (Figure 1). Addition of the nucleotides yielded a higher Imax and resulted in longer DUR and larger AREA for all stimuli except 10 mM MSG:0.63 mM IMP ($p \leq 0.05$). The binary stimuli were not significantly different from each other for Imax or AREA, but the 10 mM MSG:0.63 mM IMP stimulus was significantly shorter in duration than the 10 mM MSG with either 2.5 mM IMP or GMP (Table 2). Stimuli decreased slightly in intensity prior to expectoration and increased to approximately the same intensity after expectoration, leading to a relatively flat peak at Imax. The profiles for 5 mM MSG single and binary stimuli are very similar to those for 10 mM MSG (Figure 2). Imax and AREA were smaller for 5 mM MSG alone than for all the binary mixtures. All binary stimuli except 5 mM MSG:0.63 mM

IMP had greater DUR than all of the single stimuli. Similar results were found for 2.5 mM MSG (Figure 3), which had smaller Imax, DUR and AREA than all of the binary mixtures ($p \leq 0.05$).

Figure 4 profiles the curves for the 5'-ribonucleotides singly and the tertiary mix of 2.5 mM MSG:IMP: GMP. The mixture had greater Imax than the single stimuli of MSG and ribonucleotides. This stimulus had a longer duration than the stimuli of 5 and 2.5 mM MSG and 0.63 mM IMP and GMP, and a larger area than all single stimuli but 2.5 mM GMP (Table 2; $p \leq 0.05$). When comparing the single stimuli with each other, the 2.5 mM ribonucleotides and the 10 mM MSG were significantly greater in Imax than the other single stimuli. 2.5 mM MSG and 0.63 IMP had significantly shorter DUR and smaller AREA than 2.5 mM GMP. With a few exceptions, the tertiary mixture did not differ in Imax, DUR or AREA from any of the binary stimuli.

Additional differences were found among the binary combinations (Table 2). Imax of 10 mM MSG:2.5 mM IMP was significantly higher than 5 mM MSG: 0.63 mM IMP and all binary stimuli of 2.5 mM MSG except with 2.5 mM GMP. The Imax of 2.5 mM MSG:0.63 mM GMP was also significantly less than all 5 and 10 mM MSG binary stimuli except 5 mM MSG:0.63 mM IMP, and 2.5 mM MSG:2.5 mM GMP. The duration of 10

mM MSG with 2.5 mM IMP or GMP was longer than 10 mM MSG:0.63 mM IMP and 2.5 mM MSG with 0.63 mM IMP or GMP. The 10 mM MSG:2.5 mM GMP was also longer in DUR than 5 mM MSG:0.63 mM IMP. The AREA of 2.5 mM MSG:2.5 mM IMP was greater than each concentration of MSG with 0.63 mM IMP and 2.5 mM MSG:0.63 mM GMP.

After tasting, some subjects voluntarily commented that the stimuli, particularly those with MSG, had a tactile sensation in the mouth. They described this sensation as prickly, astringent, or slightly drying, like the sensation resulting after eating very salty food.

B. Shapes of Individual Profiles

Subjects varied in their responses to the flavor potentiators ($p \leq 0.001$, Table 1), with visual observation of the profiles revealing differences among subjects in profile shape. Each subject used a consistent pattern that generally followed one of four shapes, which were subjectively grouped: (Figure 5): 1. Typical, which is similar to curves found in other taste modalities (3 subjects, see Liu and MacFie, 1990); 2. Staircase, with the subject perceiving constant intensities for short durations that decreased suddenly, rather than gradually (4 subjects); 3. Mesa, appearing similar to a flat-topped mountain, indicating a short time to maximum intensity, a long

plateau of constant intensity, and either a sudden decrease in the intensity or, more likely, an end to the evaluation due to no perceived change in intensity (9 subjects); and, 4. Bimodal, having two separate peaks with a "valley" between them, indicating that intensity increased, decreased and then increased again (4 subjects). The staircase shape was used by almost half of the subjects, which may be due to use of the joystick and to recording frequency, which was once per second; a more frequent recording may have produced a smoother curve. No consistencies among subjects sharing a profile shape were revealed by qualitatively comparing the groups.

C. Effect of Subjects' Characteristics on Perception of Flavor Potentiators

Table 3 details the mean TI parameters for subjects grouped by gender, ethnicity, salivary glutamate, and glutamate intake. Males rated the stimuli as less intense ($p \leq 0.001$) but had a longer DUR ($p \leq 0.001$) and a larger AREA ($p \leq 0.05$), indicating that they may not perceive flavor potentiators as intense overall but might experience a longer aftertaste than females. As a group, Asians had a longer Tmax ($p \leq 0.01$), DUR ($p \leq 0.001$), and a larger AREA ($p \leq 0.001$) than non-Asians, but no differences in Imax were found. These results could be due to experiential, dietary, or genetic factors. Interactions between gender and ethnicity were not significant for the four parameters measured.

Mean salivary glutamate was 0.177 mM ± 0.109 mM (0.026 g/l± 0.016 g/l). Salivary glutamate varied among subjects (p=0.003) ranging from 0.034 mM to 0.686 mM (0.005 to 0.101 g/l). No significant difference was found in salivary glutamate between males and females. However, Asians had lower levels of salivary glutamate than non-Asians, 0.122 mM and 0.287 mM, respectively (p=0.03). The gender by ethnicity interaction was not significant. Subjects with lower salivary glutamate had profiles with lower Imax and longer DUR than those with high and medium salivary glutamate levels ($p \leq 0.001$); those with the highest salivary glutamate levels had the shortest DUR. For the other measures, the medium salivary glutamate group had the shortest Tmax, the highest Imax, and the largest AREA ($p \leq 0.001$, 0.001, and 0.01, respectively).

Asians consumed more glutamate in their diet, with a mean intake score of 34.4 (±9.7) from the food frequency questionnaire as compared to a score 24.9 (±9.1) for the non-Asian subjects (p=0.04). Gender did not influence glutamate consumption. Subjects with high glutamate intake rated the stimuli as less intense than the other two groups, as indicated by faster Tmax, smaller Imax, and shorter DUR ($p \leq 0.05$). Subjects with medium intake had the slowest Tmax, highest Imax, and largest AREA. Salivary and dietary glutamate measures were not significantly correlated (Table 4).

None of the profile parameters, Tmax, Imax, DUR, or AREA were correlated, with the exception of a positive correlation between DUR and AREA ($p \leq 0.001$), or with salivary glutamate or dietary glutamate intake.

III. Discussion

In 1987, Birch called for characterization of the TI curve for MSG, noting that it would be useful for understanding taste properties and chemoreception of MSG, such as persistence, reaction time, and differentiation of MSG from other sapid molecules. In this study, the TI profiles were atypical of profiles generated for sweet, sour, salt, and bitter tastes (Larson-Powers and Pangborn, 1978; Pangborn *et al.*, 1983; Schmitt *et al.*, 1984; Guinard, *et al.*, 1995). The profiles had a brief time to maximum intensity (Tmax), followed by a maximum intensity (Imax) of a relatively long plateau that included an intensity decrease before and increase after expectoration. The increase in intensity after expectoration is due to changes in the oral milieu caused by the mouth movements required for expectoration (O'Mahony and Wong, 1989). The aftertaste (DUR) was very long, particularly for some subjects. Similar profiles were found by Tartar (1989) for MSG, IMP, and NaCl singly and in combination in water and commercially prepared beef broth. Profiles for L-glutamic acid in water and beef broth (referred to as MSG in the manuscript) did not follow the same

pattern as the profiles in the present study (Chung, 1981). Instead, L-glutamic acid had lower Imax than NaCl, but the L-glutamic acid stimuli had a longer DUR than NaCl, especially when tasted in commercial beef broth, as compared to commercial chicken broth or water. The higher fat content of the chicken broth was hypothesized to suppress the flavor impact of L-glutamic acid. Characterization of the TI profile of flavor potentiators in a food system would be more applicable to normal food consumption, but water was used in this study as the medium to establish the profiles without the confounding effects of other components, including flavor compounds.

The profile parameters are affected by the frequency of recording intensity measurements. In this study, recordings were made every second. Halpern (1991) recommended that recordings be made at least ten times per second for accurate data and good resolution of the TI curve. The short time to Tmax found for all stimuli may indicate that the binding time, signal transmission, or both, are brief for flavor potentiators. Dubois and Lee (1983) suggested that a short Tmax for nutritive sweeteners may be explained by rapid and specific binding. Different methods of measuring reaction times have yielded different results. In studies of reaction time, MSG had a longer reaction time than other stimuli tested (Kelling and Halpern, 1987, 1988; Zwillinger and Halpern, 1991), indicating a longer

binding time, and once bound, a quick maximum response by the receptor. However, reaction time measures the initial response of the subject to the stimulus in milli-seconds, not the time to reach the maximum taste intensity.

As expected for the single stimuli, Imax increased as flavor potentiator concentration increased, but a concentration-dependent response was not found for DUR. An increase in DUR with stimulus strength was found for MSG in commercially produced chicken soup by Guinard *et al.* (1998). The increase in perceived intensity of IMP with concentration is less per unit than for MSG (Yamaguchi, 1991). Synergism of IMP and GMP with MSG was demonstrated in this study. GMP was more effective than IMP at the lower concentration (0.63 mM); at 2.5 mM, GMP and IMP were equally effective in increasing stimulus taste intensity. The synergism of ribonucleotides with MSG has been documented (Yamaguchi *et al.*, 1971) and mathematically modeled (Yamaguchi and Kimizuka, 1979; Yamaguchi, 1991). Subjects in this study antedoctally reported that the flavor potentiators, most notably MSG, had a tactile sensation that was drying. This feeling has been described as mouthfulness (Yamaguchi, 1987) and as a feeling of satisfaction that may continue for half an hour (Birch, 1987).

The large AREA indicates that flavor potentiators may increase total flavor in the mouth, as compared to other stimuli. The plateau region seen

for the stimuli demonstrates the duration of maximum intensity is longer for flavor potentiators than for other reported stimuli. In one report, TI measures of bitterness demonstrated broad peak height (Leach and Noble, 1986), which may contain valuable information about the duration of the sensation (Schmitt *et al.,* 1984), such as binding affinity, ligand interaction, and the receptor type (ionotropic or metabotropic). Bitter compounds studied by Guinard *et al.* (1995) also demonstrated plateaus, whereas artificial sweeteners had more than one peak.

The aftertaste of the stimuli in this experiment was considerably longer than the aftertaste for sweet, sour, salt, and bitter compounds. Kuninaka *et al.* (1964) and Tartar (1989) have reported a long or strong aftertaste for MSG and the 5'-ribonucleotides. In a hedonic study of model systems of MSG and NaCl, Pangborn (1980) reported that MSG had a "distracting carry-over" effect that increased subject variability, despite thorough rinsing between stimuli. This aftertaste, which lasted longer than the taste after expectoration of NaCl, may be due to a slower adaptation rate for MSG than for NaCl (O'Mahony and Wong, 1989). The measurement of aftertaste in this study was not precise because subjects varied in their determination of the end of the evaluation. In a study of bitterness, the evaluation was ended after the taste intensity had been constant for at least

one minute (Leach and Noble, 1986). Kelling and Halpern (1987) noted that the decrease in intensity takes longer than the onset or peak output periods, and can be sustained for a long time. Perhaps the subjects in this study who experienced a very long aftertaste were affected by the excitatory nature of glutamate on the peripheral receptors.

Adaptation, stimulus dilution, and removal of stimuli by saliva primarily influence aftertaste. A persistent response may represent a localized binding or high concentration of stimuli molecules, or both, with slow release of the flavor potentiator from the receptor (Birch, 1987). Strong binding between the receptor and molecule, perhaps due to the chirality of the molecules or their solution characteristics, also may impact the persistence of the response (DuBois and Lee, 1983). Birch (1987) has proposed that the efficacy of the bond between a receptor and a tastant is controlled by a "lock and key" principle. Based on the apparent molar volume of MSG, he theorized that MSG accesses a deeper region of the taste receptor than bitter compounds. MSG's long aftertaste could be analogous to the first few minutes of dark adaptation in vision, with the threshold remaining high even after the stimulus is removed, or in the case of taste, extremely decreased due to expectoration or swallowing. The gradual decrease in taste intensity after Imax (average slope of –0.814/sec) is less

steep than slopes reported for bitterness, -1.47 for caffeine and –1.95 for quinine (Leach and Noble, 1986). They postulated that the rate of intensity development may be controlled by adsorption of the species to a receptor site. If it is strongly bound, it will be adsorbed faster than desorbed, leading to a longer duration of aftertaste. Conversely, a slow desorption of the molecule from the receptor can also prolong aftertaste. In the present study, the decreasing slopes were not significantly different among stimuli. However, Tartar (1989) found that the taste of a mixture of MSG and IMP declined at a slower rater than MSG alone.

Gender differences in TI results have been reported. In this study, males rated the samples as less intense, but had a longer DUR and larger AREA than females. In contrast, Guinard *et al.* (1998) found that males gave higher intensity responses than females to MSG in soup, whereas females perceived the other six stimuli as more intense. Males had higher parotid salivary flow, but this did not appear to be related to perception. Chung (1981) found that males gave higher maximum intensity ratings to the stimuli than females, and along with Asian subjects, had significantly higher parotid flow, higher sodium and lower potassium and calcium outputs. Because Asians are more familiar with the taste of MSG, they may have a different taste response to umami. In this study, Asians had shorter

Tmax, longer DUR, greater AREA, and lower salivary glutamate than non-Asians. This result may be due to Asians' familiarity with umami or to physiological differences. Asians and Caucasians do not differ in their thresholds to MSG (Yamaguchi, 1991), but Japanese more readily identify umami taste than Americans (O'Mahony and Ishii, 1987).

Differences in profile parameters among high, medium, and low salivary glutamate and dietary glutamate intake groups were not consistent. Subjects with low salivary glutamate had the lowest Imax and longest DUR. The group of subjects with medium salivary glutamate had the highest Imax, the largest AREA, and the shortest Tmax. Perhaps a moderate level of salivary glutamate enhanced the response. However, conclusions should be made only carefully due to the small sample size. Low salivary glutamate, found among Asians, may mitigate their intensity response to flavor potentiators, due to dietary, experiential, or genetic factors. People who have low salivary glutamate levels may not saturate all receptors with low stimulus concentrations and those with high salivary glutamate may have reached maximum binding for the available glutamate receptor sites.

Subjects with high glutamate intake, again Asians, perceived the stimuli as less intense. Those subjects with medium glutamate intake had the most intense response to the samples. Tanimura (1994) used TI to study

differences in perceived bitterness between caffeine users and non-users. Caffeine users had lower peak height, indicating a less intense bitter response. When drawing conclusions from studies of dietary intake, several factors must be considered. Generally, dietary intake is self-reported by the subject and may not be accurate due to forgetfulness or unwillingness to report actual consumption. Current behavior is considered to be indicative of past consumption (Hankin *et al.*, 1978). Although sensory data are often related to dietary consumption, sensory tests seldom reflect normal eating situations.

Neither salivary nor dietary glutamate were correlated with any of the four TI profile parameters. Guinard *et al.* (1998) did not find any relationship between salivary sodium and total protein and TI parameters. Conversely, Chung (1981) reported that dietary sodium was sometimes positively correlated with maximum intensity, but this result was dependent upon the tastant and the media used. No relationships between salivary sodium and profile parameters were found, and no differences between Asians and non-Asians were found in dietary sodium intake.

Variation in individual subject profiles is often found in TI experiments. This variability is complicated by a lack of consensus regarding treatment of TI data (Liu and MacFie, 1990). Perceptual

differences in threshold and suprathreshold sensitivity, adaptation, salivary composition, or consumption differences may also account for some of the variation among subjects (Birch, 1981; Leach and Noble, 1986; Guinard *et al.*, 1995). After expectoration, some subjects perceived a gradual decrease in taste and others perceived different rates of decrease, but a small group perceived an increase in intensity, which is not surprising due to mouth movements (O'Mahony and Wong, 1989). Moving the tongue, cheeks, and other muscles controlling the oral cavity disrupts adaptation to the stimulus and changes the oral milieu, leading to an increase in the perceived intensity of a stimuli. The impact of this effect has been observed to be greater for compounds that have a long aftertaste, such as umami and bitter (Cubero-Castillo and Noble, 1998). Variations among subjects in the rinsing method and time interval used between stimuli affect adaptation, thus influencing results. Subjects also vary in the number of mouth rinses required to clear the mouth. O'Mahony (1972b) found that five mouth rinses effectively reduced stimuli residuals within 5 mM of pre-experimental levels. Ad-lib rinsing procedures were both uncontrolled and ineffective. The quantity, composition, and salivary flow rate also affect perception of some stimuli (Guinard *et al.*, 1998). Chung (1981) found that parotid salivary flow was positively correlated to DUR, and inversely correlated to salivary potassium

and calcium levels. Salivary flow rate has been demonstrated to be directly proportional to maximum perceived intensity of sweetness and cherry flavor in chewing gum (Guinard, *et al.,* 1997). Guinard *et al.* (1998) found parotid saliva flow was proportional to MSG concentration in chicken broth, but salivary flow did not affect the gustatory TI parameters.

One method of examining variation among individual subjects is to group the subjects by their characteristic profile shapes. The subjects within these groups could be studied for shared characteristics to determine any relationship between profile shape and subject characteristics. In this study, four shapes were used to subjectively group the profiles: typical, staircase, mesa, and bimodal. However, an examination of the subjects in each of these groups did not reveal any apparent commonalties, as determined by attributes included in this study (i.e., none of the profile shapes included subjects of only one ethnicity or gender). Other investigators have grouped subjects by profile shapes. Guinard *et al.* (1995) grouped subjects into binomial, staircase, and long tail profiles. Cubero-Castillo and Noble (1998) grouped subjects as typical, curve not back to baseline, and maximum intensity reached after expectoration with a long plateau. The subjects within these groupings could be examined for other commonalties that they share to determine the significance, if any, of the profile shape.

Standards are sometimes used in TI to increase consistency among subjects and to provide a conditioning stimulus (Guinard *et al.*, 1995). Use of a standard can cause stimuli residuals to build up in the mouth, resulting in an underestimation of intensity values (O'Mahony, 1972b). O'Mahony and Wong (1989) trained subjects to estimate MSG intensity directly in mM concentration of NaCl by calibrating subjects with NaCl standards. This training did make the TI shapes more uniform among the subjects and increased the accuracy of stimuli intensity estimation. A standard can provide a "built in" validity check and can also be used to indicate when a subject is at a specified level of accuracy. The 70 mM NaCl standard used in this experiment was not consistently used by all subjects, which may have contributed to variability among subjects. The standard should have been matched to a MSG intensity series, as NaCl tastes stronger than equimolar MSG stimuli (O'Mahony and Wong, 1989). Guinard *et al.* (1998) used the highest of three concentrations of a stimulus series as a standard in TI evaluations, corresponding to an intensity value of 75 on a 100-point TI scale. Results corroborated that the standard was used appropriately, as the highest intensity scores average 75.

Table 1. Analysis of variance of the time-intensity profile parameters (degrees of freedom (df) and F-ratios are shown with significance levels).

| | Df | Time to Max. Intensity (sec) | Maximum Intensity | Total Duration (sec) | Area Under the Curve |
|---|---|---|---|---|---|
| **Stimuli** | 19 | 1.18 | 26.46*** | 4.03*** | 7.31*** |
| **Subjects** | 19 | 24.95*** | 16.87*** | 57.58*** | 16.00*** |
| **Replications** | 1 | 3.94* | 0.31 | 5.76* | 6.02* |
| **Gender** | 1 | 2.33 | 40.65*** | 102.18*** | 8.79* |
| **Ethnicity** | 1 | 8.73** | 2.17 | 26.75*** | 14.69*** |
| **Salivary Glutamate** | 2 | 22.14*** | 31.55*** | 21.99*** | 6.35** |
| **Glutamate Intake** | 2 | 96.07*** | 41.75*** | 10.06*** | 14.00*** |

*,**,*** Significant at $p<0.05$, 0.01, and 0.001, respectively.

Table 2. Means of time-intensity profile parameters for the stimuli and significant differences among stimuli according to Fisher's LSD ($p \leq 0.05$; n = 20, 2 replications).

| Stimuli | Time to Max. Intensity (sec) Tmax | Maximum Intensity Imax | Total Duration (sec) DUR | Area Under the Curve AREA |
|---|---|---|---|---|
| 10 mM MSG | 18.2 | $49.8^{bc}$ | $68.6^{abcde}$ | $2458.2^{abcd}$ |
| 5 mM MSG | 15.1 | $47.5^{b}$ | $61.0^{abc}$ | $1933.9^{ab}$ |
| 2.5 mM MSG | 14.3 | $38.6^{a}$ | $51.5^{a}$ | $1315.3^{a}$ |
| 2.5 mM IMP | 16.3 | $50.2^{bc}$ | $63.9^{abcd}$ | $2235.3^{abc}$ |
| 0.63 mM IMP | 15.5 | $38.6^{a}$ | $54.4^{ab}$ | $1431.5^{a}$ |
| 2.5 mM GMP | 19.7 | $56.5^{cd}$ | $74.3^{cdef}$ | $2999.1^{bcde}$ |
| 0.63 mM GMP | 19.2 | $43.2^{ab}$ | $58.9^{abc}$ | $1820.9^{ab}$ |
| 10mM MSG:2.5mM IMP | 19.0 | $80.4^{h}$ | $95.7^{gh}$ | $4591.2^{ghij}$ |
| 10mM MSG:0.63mM IMP | 19.6 | $75.1^{fgh}$ | $72.6^{bcdef}$ | $3539.7^{defgh}$ |
| 10mM MSG:2.5mM GMP | 16.7 | $75.5^{fgh}$ | $96.4^{h}$ | $4707.6^{hij}$ |
| 10mM MSG:0.63 GMP | 19.3 | $77.6^{gh}$ | $89.3^{fgh}$ | $4558.8^{ghij}$ |
| 5mM MSG:2.5mM IMP | 17.6 | $75.8^{fgh}$ | $83.6^{efgh}$ | $3956.3^{efghij}$ |
| 5mM MSG:0.63mM IMP | 16.2 | $70.4^{efg}$ | $76.7^{cdefg}$ | $3441.5^{cdefg}$ |
| 5mM MSG:2.5mM GMP | 20.6 | $78.3^{gh}$ | $95.3^{gh}$ | $4784.5^{ij}$ |
| 5mM MSG:0.63 GMP | 20.1 | $74.1^{fgh}$ | $85.6^{efgh}$ | $4067.1^{efghij}$ |
| 2.5mM MSG:2.5mM IMP | 20.5 | $71.7^{efg}$ | $84.2^{efgh}$ | $5053.8^{j}$ |
| 2.5mM MSG:0.63mM IMP | 17.6 | $69.3^{ef}$ | $72.9^{bcdef}$ | $3292.1^{cdef}$ |
| 2.5mM MSG:2.5mM GMP | 17.5 | $72.5^{fgh}$ | $95.8^{gh}$ | $4421.4^{fghij}$ |
| 2.5mM MSG:0.63mM GMP | 19.6 | $64.3^{de}$ | $74.1^{cdef}$ | $2991.1^{bcde}$ |
| 2.5mM MSG:2.5mM IMP:2.5mM GMP | 19.9 | $70.7^{efg}$ | $82.9^{defgh}$ | $3771.7^{efghi}$ |

MSG: Monosodium Glutamate
IMP: Disodium 5'-inosinate
GMP: Disodium 5'-guanylate
Means sharing superscripts are not significantly different ($p<0.05$).

Table 3. Means with standard deviations of time-intensity parameters for subject groups (gender, ethnicity, salivary glutamate, glutamate intake) and significant differences according to Fisher's LSD ($p \leq 0.05$; n = 20, 2 replications).

| Subject Group | Time to Max. Intensity (sec) | Maximum Intensity | Total Duration (sec) | Area Under the Curve |
|---|---|---|---|---|
| Gender | | | | |
| Males (n=10) | 18.4± 9.3 | 60.8±21.4[a] | 90.5±55.7[b] | 3549±2840[b] |
| Females (n=10) | 19.3±11.7 | 67.3±23.5[b] | 63.3±28.6[a] | 3191±3136[a] |
| Ethnicity | | | | |
| Asian (n=11) | 19.1±11.5[b] | 63.8±24.4[a] | 79.9±53.2[b] | 3638±3564[b] |
| Non-Asian (n=9) | 17.0±10.4[a] | 64.3±20.6[a] | 73.2±35.8[a] | 3038±2059[a] |
| Salivary Glutamate | | | | |
| High (0.042 – 0.101 g/l; n=6) | 19.5±10.3[b] | 64.9±18.8[b] | 64.1±35.6[a] | 2700±1977[a] |
| Medium (0.021 – 0.039 g/l;n=5) | 14.2±10.5[a] | 72.2±23.6[c] | 75.5±29.4[b] | 4086±3940[c] |
| Low (0.005 – 0.016 g/l;n=9) | 19.7±11.3[b] | 58.9±23.3[a] | 86.2±57.0[c] | 3416±2860[b] |
| Glutamate Intake | | | | |
| High (41-50; n=4) | 12.6±7.3[a] | 56.2±19.8[a] | 66.5±41.3[a] | 2826±4129[a] |
| Medium (29-38; n=8) | 23.8±12.2[c] | 68.8±23.8[c] | 76.8±42.0[b] | 3750±2805[b] |
| Low (13-25; n=8) | 15.2±8.7[b] | 63.2±21.8[b] | 82.1±21.7[b] | 3259±2409[a] |

Means for each parameter sharing superscripts within darkened lines are not significantly different (see appropriate significance level in Table 2).

Table 4. Correlation matrix of time-intensity profile parameters and glutamate measures.

| | Tmax | Imax | DUR | AREA | Salivary Glutamate | Glutamate Intake |
|---|---|---|---|---|---|---|
| Tmax | 1.000 | | | | | |
| Imax | -0.04316 | 1.000 | | | | |
| DUR | -0.1910 | 0.08823 | 1.000 | | | |
| AREA | -0.1535 | 0.4170 | 0.8879*** | 1.000 | | |
| Salivary Glutamate | -0.2254 | -0.1160 | -0.2404 | -0.3167 | 1.000 | |
| Glutamate Intake | 0.2580 | -0.3105 | -0.1515 | -0.1544 | -0.3260 | 1.000 |

***Significant at $p \leq 0.001$.

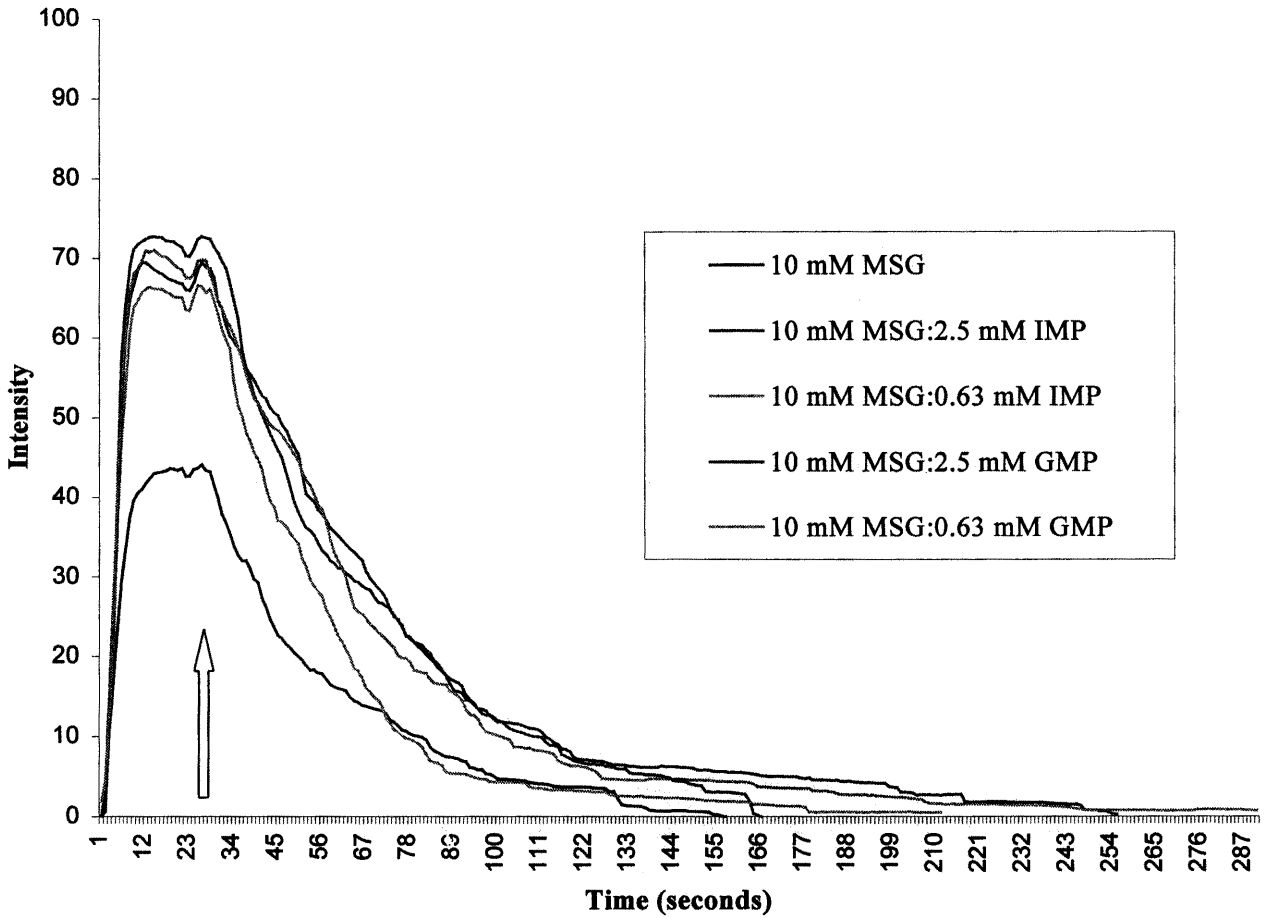



Figure 1. Average Time-Intensity Profiles for 10 mM MSG, and with Two Concentrations of IMP and GMP (n=20, 2 reps).

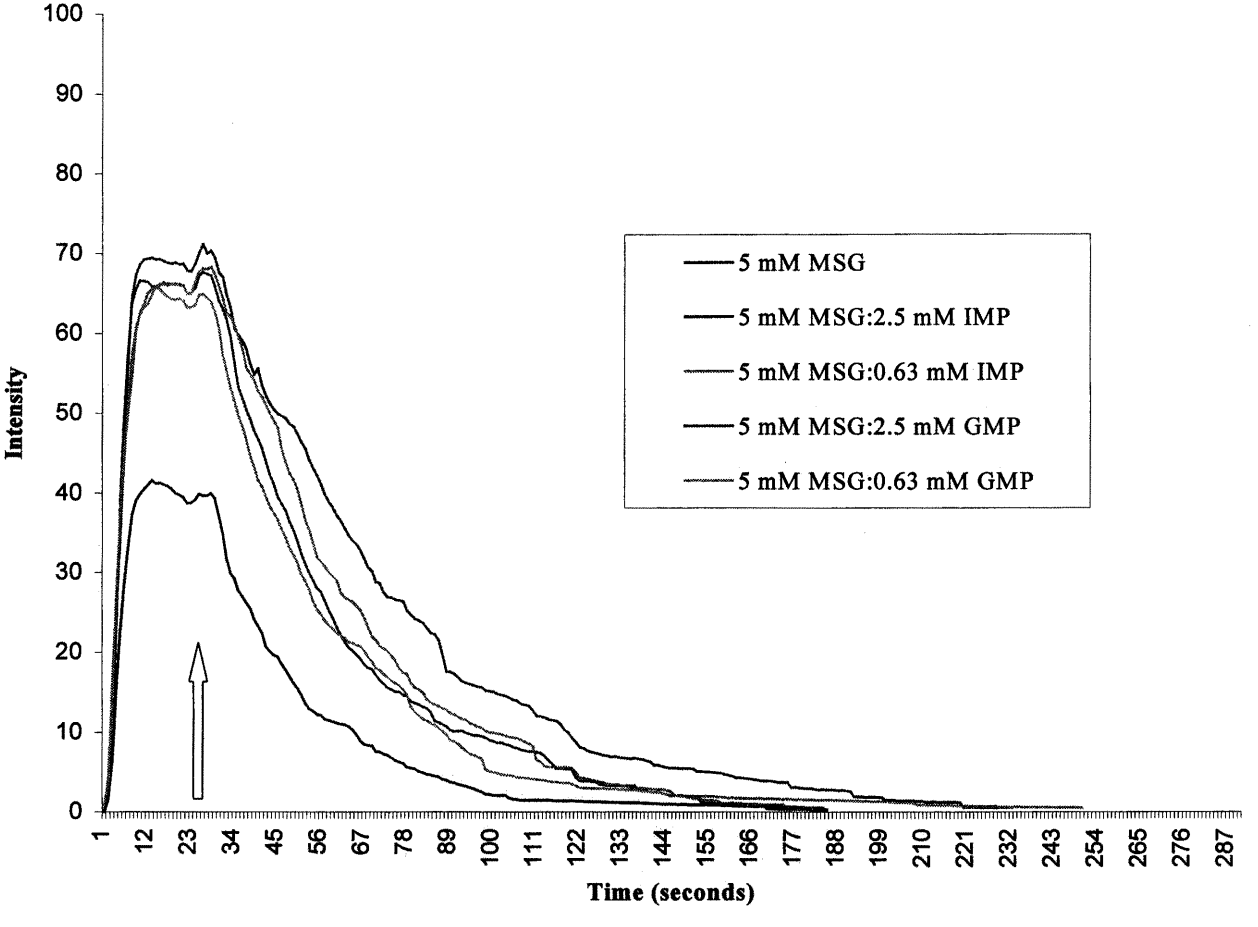



Figure 2. Average Time-Intensity Profiles for 5 mM MSG, and with Two Concentrations of IMP and GMP (n=20, 2 reps).

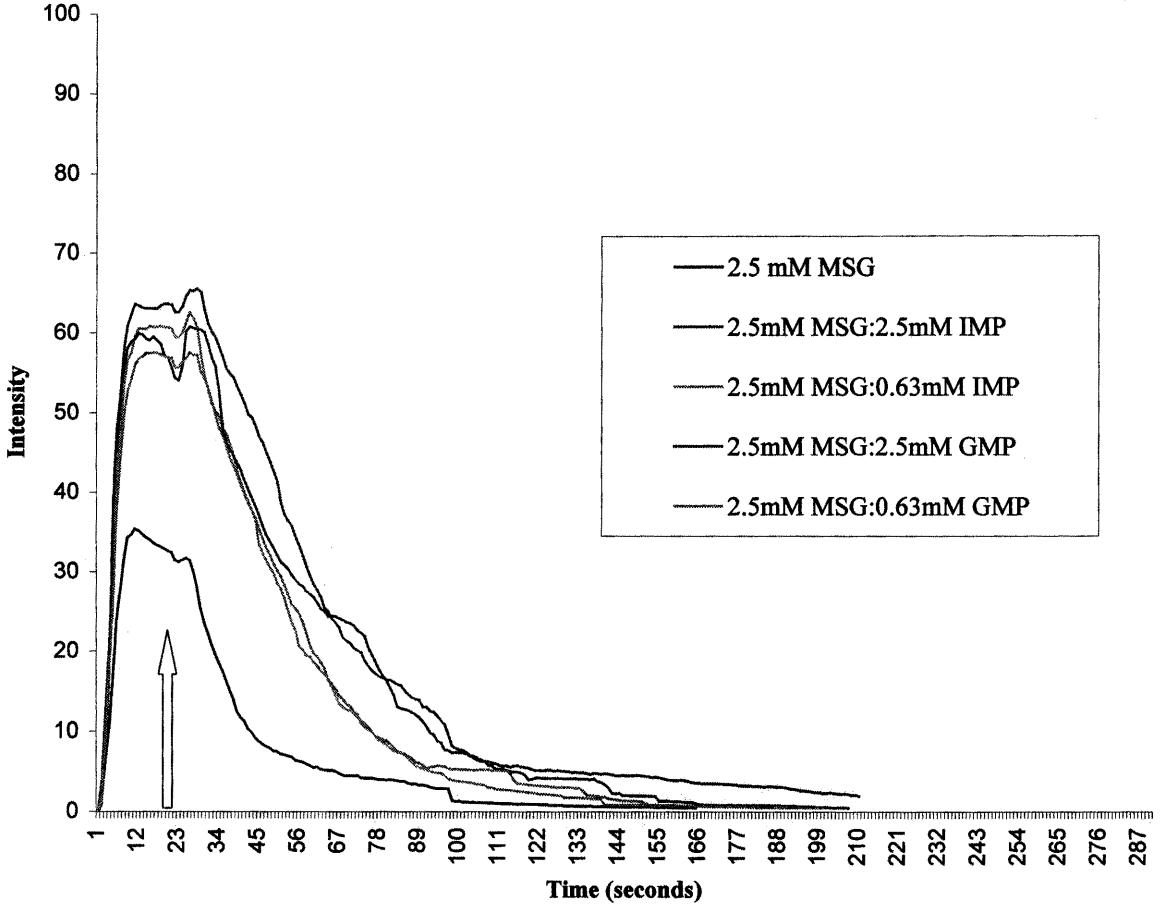



Figure 3. Average Time-Intensity Profiles for 2.5 mM MSG, and with Two Concentrations of IMP and GMP (n=20, 2 reps).

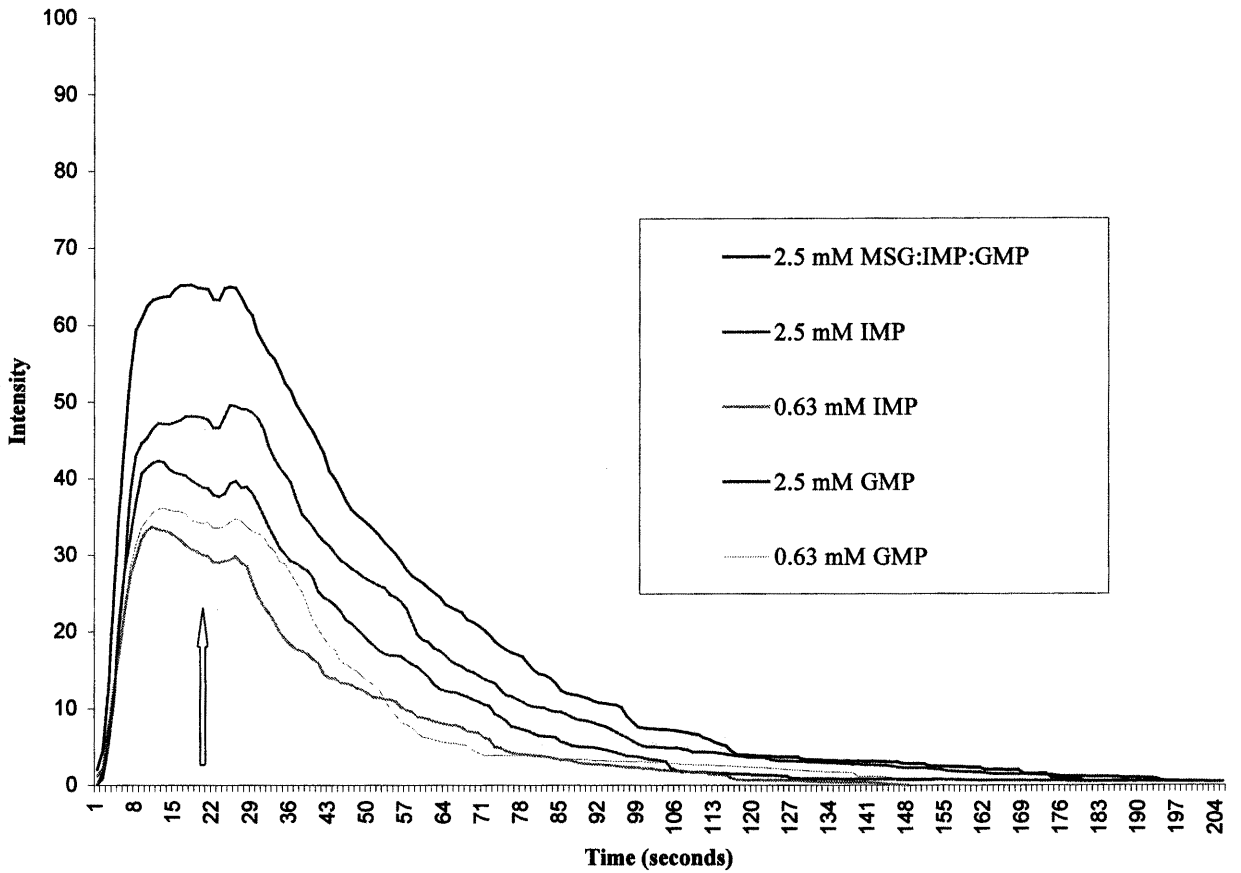



Figure 4. Average Time-Intensity Profiles for Two Concentrations of IMP and GMP, and 2.5 mM MSG:IMP:GMP (n=20, 2 reps).

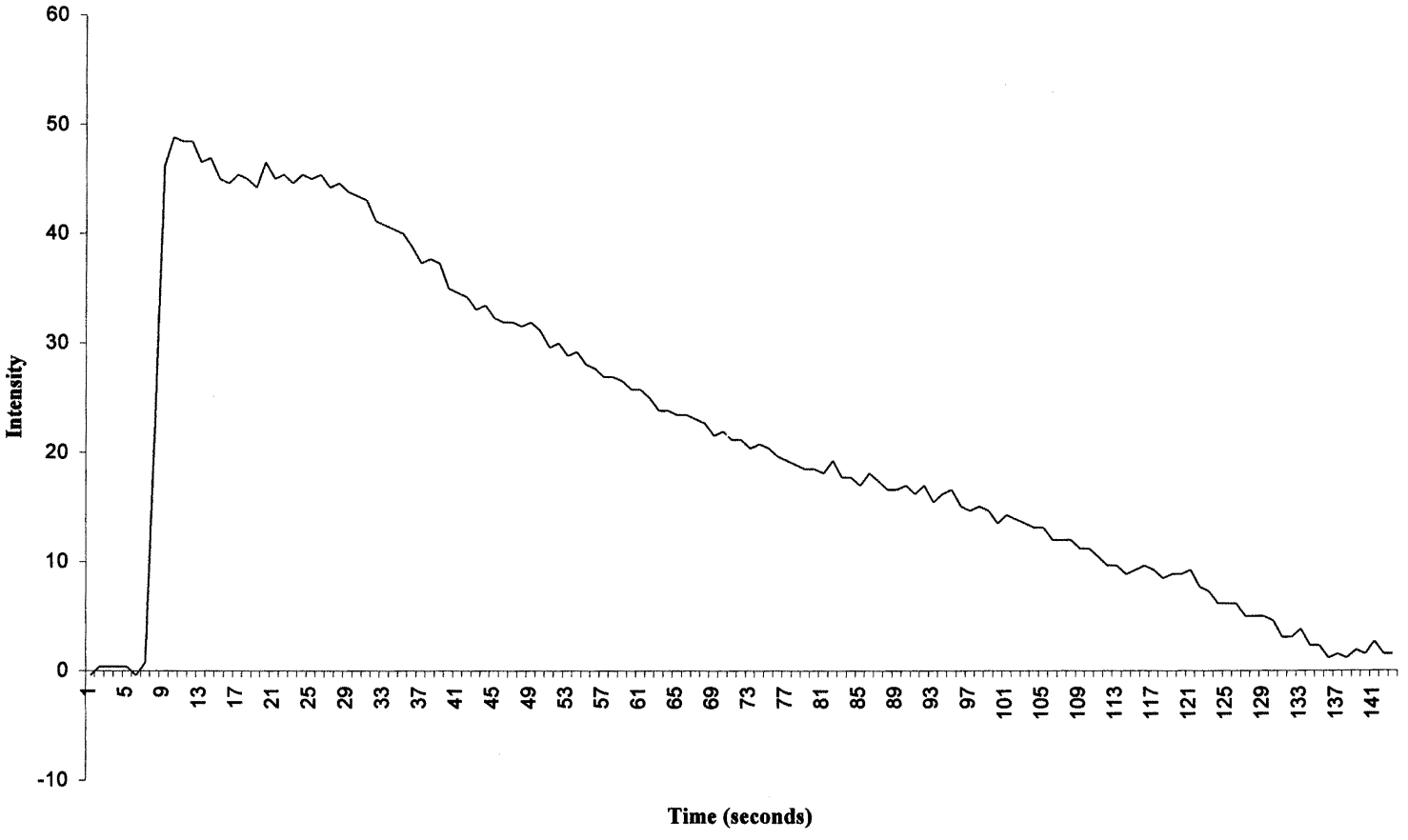



Figure 5A. Time-Intensity Curve: Typical Shape

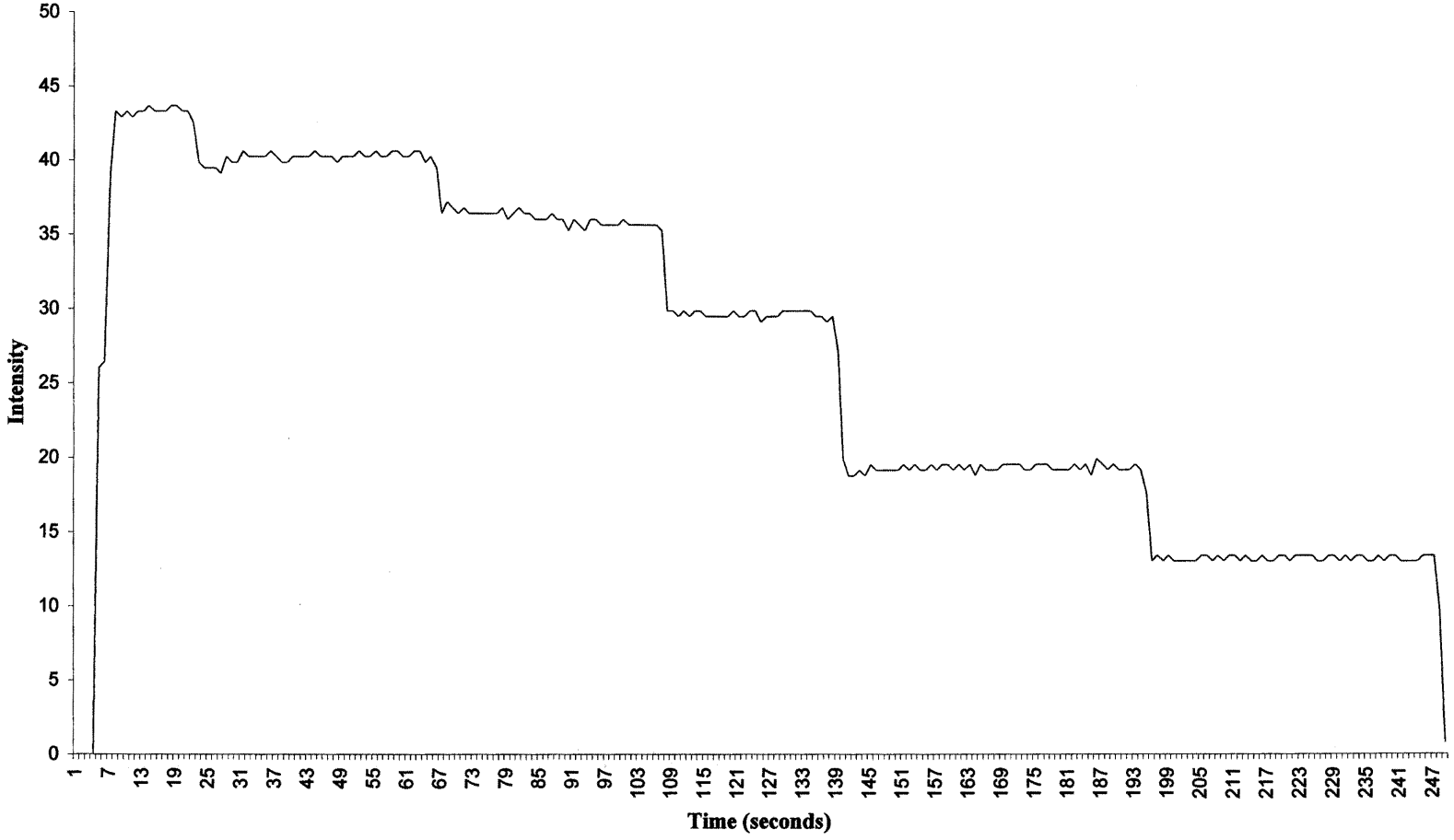



Figure 5B: Time-Intensity Curve: Staircase Shape

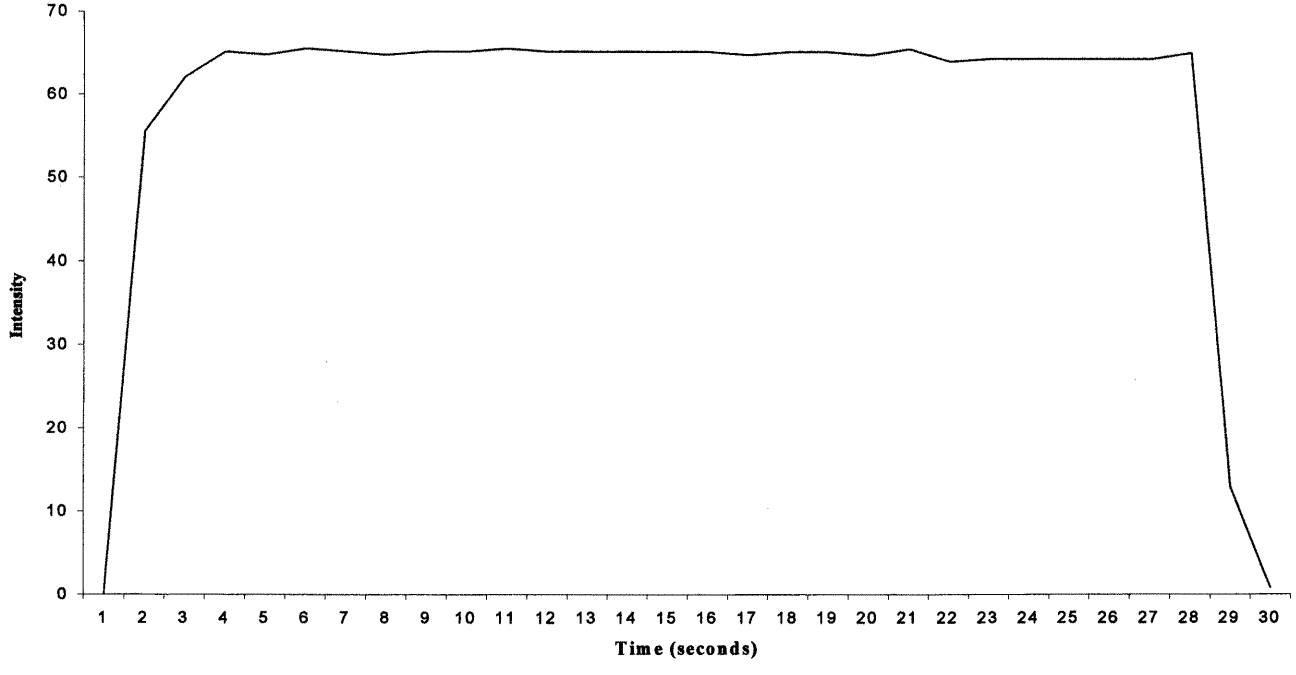



Figure 5C. Time –Intensity Curve: Mesa Shape

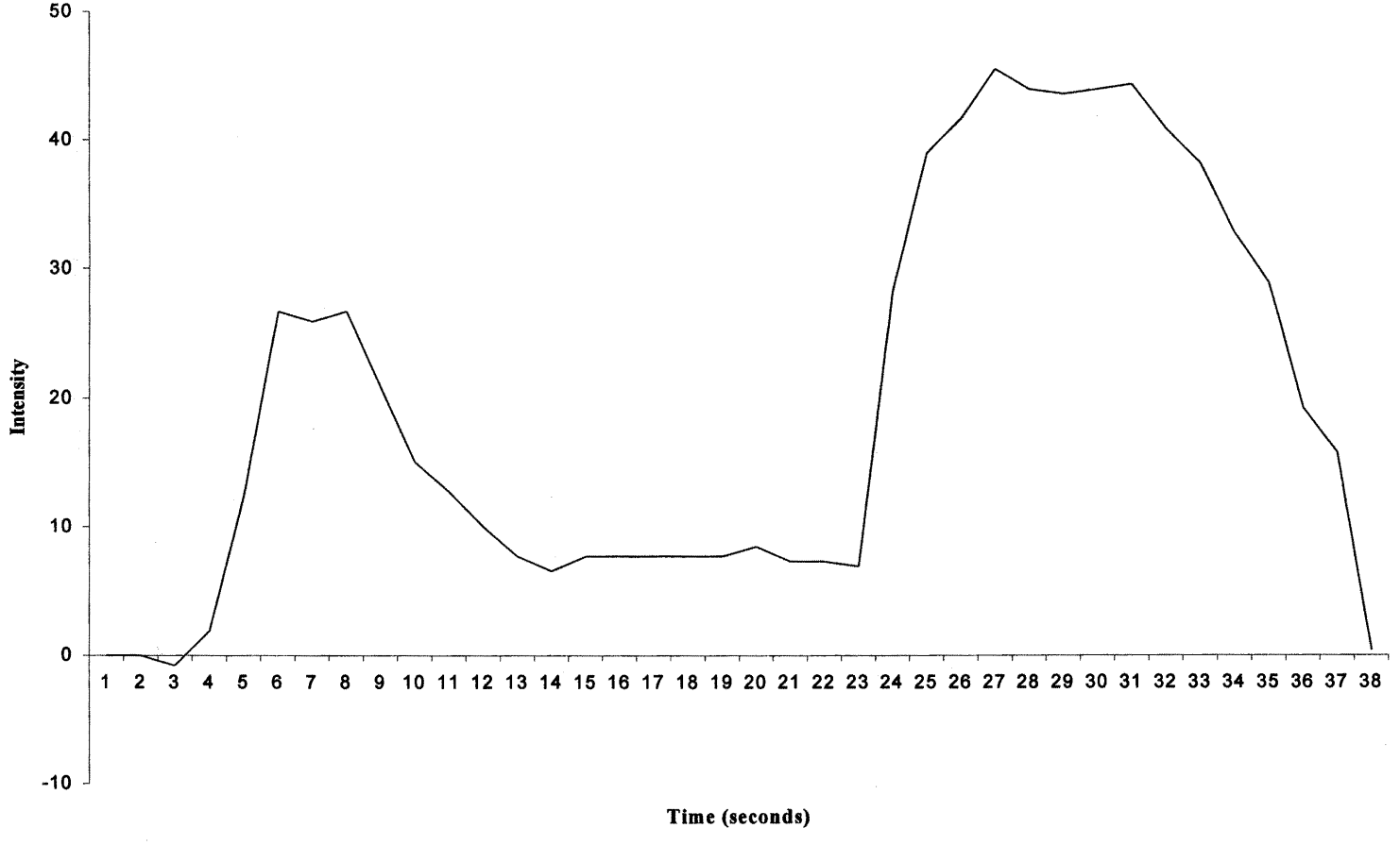



Figure 5D. Time-Intensity Curve: Bimodal Shape

# CHAPTER 5

## Effect of Heat Treatment and pH on Levels of Free L-Glutamic Acid in Simple Food Systems

The objective of this study was to determine the influence of heat treatment and pH on the levels of free L-glutamic acid in simple food systems of protein, carbohydrate (including vegetable), and fat.

### I. Materials and Methods

#### A. Food Systems

Eleven different food systems were tested to determine L-glutamic acid concentration. Four food categories were tested: protein, carbohydrate, vegetable, and fat. All samples were tested immediately after preparation.

##### 1. Protein Systems

Beef broth: 2.5 pounds of beef soup bones were added to three quarts (80 oz) of tap water and simmered over low heat for one hour in a covered six-quart stock pot. The broth was refrigerated

overnight. The bones were removed and fat was skimmed from the top with a stainless steel spoon before use.

Chicken broth: One stewing chicken (approximately 3 pounds) was added to 2 quarts (64 oz) of tap water and simmered over low heat for 1 hour in a covered six-quart stock pot. The broth was refrigerated overnight. Bones and skin were removed and fat was skimmed from the top with a stainless steel spoon before use.

Fish broth: Four frozen haddock filets, approximately 1.25 pounds total weight, were added to 2 quarts (64 oz) of tap water and simmered over low heat for 20 minutes in a covered six quart stock pot. The broth was refrigerated overnight. The fish was removed the following morning.

Parmesan Cheese (100%, Kraft Foods, Inc., Glenview, IL): 30 grams of parmesan cheese was brought to volume in a 100 ml volumetric flask with distilled water (30% w/v).

Soy Flour (100% soybeans , Wild Oats Markets, Boulder, CO): 10 grams of soy flour was brought to volume in a 100 ml volumetric flask with distilled water (10% w/v). Nutrient content of 1 cup (100 g): 20 g protein, 11 g fat; 18 g carbohydrate; 1 mg sodium; 8.1 g fiber

2. Carbohydrate Systems

Sucrose (Topco Associates, Skokie, IL): 15 grams of sucrose was brought to volume in a 50 ml volumetric flask with distilled water (30% w/v).

Cornstarch (Argo® 100% Cornstarch, CPC International Inc., Englewood Cliffs, NJ): 10 grams of cornstarch was brought to volume in a 100 ml volumetric flask with distilled water (10% w/v).

3. Vegetable systems

Mushrooms: Fresh button mushrooms were rinsed and quartered. 200 grams of mushrooms were pureed with 200 ml water in a blender. 20 ml of the puree were brought to volume in a 100 ml volumetric flask with distilled water (10% w/v).

Tomatoes: Fresh Roma tomatoes were blanched for 30 seconds, plunged into cold water and peels removed. 2 cups of tomatoes were pureed with 2 cups distilled water. 20 ml of the puree were brought to volume in a 100 ml volumetric flask with distilled water (10% w/v).

4. Fat Systems

Butter: One cube of sweet cream unsalted butter (pasteurized cream, annato for color; King Soopers, Denver, CO) was melted over low heat. 15 ml of melted butter was brought to volume in a 50 ml volumetric flask with distilled water (30% w/v).

Corn oil (Topco Associates, Skokie, IL): 15 ml of corn oil was brought to volume in a 50 ml volumetric flask with distilled water (30% w/v).

B. Treatments

Three treatments were used:

1. Three temperature levels: 1. Room, with samples left at room temperature (24°C); 2. Mild (63°C), heated in a water bath set at constant temperature; and 3. Severe (100 °C), heated in a household pressure cooker with rack. Samples were heated for 30 minutes.
2. Two pH levels: 3 and 6. Samples were adjusted with NaOH or HCl to pH 3 or 6 before heating. The pH of each sample was measured again after the heat treatment, when all

samples had cooled to room temperature. pH was measured with a Corning 320 Digital meter.

3. Added MSG: 0% (control) and 0.05% (4.325 g/l glutamic acid, Treatment).

For each food system, 13 samples were prepared, including a "base" or untreated sample. This sample had no added MSG, was not pH adjusted, and was left at room temperature. Of the 12 remaining samples, six were control samples with no added MSG and six were treatment samples with 0.05% MSG (Ajinomoto, Ajinomoto Interamericana Ind E Com LTD, Brazil), added on a weight/volume basis. According to the Life Sciences Research Office (1995), 1.27 g of MSG yields 1.00 g of L-glutamic acid (i.e., MSG is 78.74% L-glutamic acid), resulting in a final concentration of 0.03937 g L-glutamic acid in each 100 ml sample. The test was replicated once. For each food system, 100 ml of sample prepared as described above was poured into an 8 ounce Mason jar and closed with band and lid. Jars were shaken before pH was adjusted. The jars were shaken again after the heat treatment, before pH was measured.

C. L-Glutamic Acid Analysis

L-glutamic acid content in each sample was determined using an enzymatic test kit (Boehringer Mannheim GmbH, Germany). In this reaction, L-glutamic acid was oxidatively deaminated by nicotinamide-adenine dinucleotide (NAD) to 2-oxoglutarate in the presence of glutamate dehydrogenase (GIDH). Using diaphorase as a catalyst, the NADH formed converted iodonitro tetrazolium chloride (INT) to a formazan, which was measured spectrophotometrically in the visible range at 492 nm. An aliquot of sample was removed from each sample of each food system that would result in a reading between 0.07 and 0.7 g/l of L-glutamate, within the sensitivity range of the enzymatic test. The formula used to calculate the concentration of L-glutamate was adjusted to account for the different dilution schemes for the different food systems and for the addition of MSG.

The calculation of free L-glutamic acid was based on the use of a standard, provided with the test kit, according to the following equation:

$$C_{sample} = (\Delta A_{sample} / \Delta A_{standard}) \times C_{standard} * \text{Dilution Factor}$$

C = concentration (g L-glutamic acid/l sample solution)

$\Delta A$ = change in absorbance due to the addition of GIDH (glutamate dehydrogenase)

D. Statistical Analyses

Analysis of variance was used to determine significant differences among the samples, with added MSG, heat treatment, and pH level as factors. Main effects only were evaluated. One way analysis of variance was used to determine if the replications differed. Recovery of added glutamic acid was calculated for each sample by subtracting the amount of L-glutamic acid in the control sample from the amount in the treatment sample. This difference was divided by 0.03937, the amount in grams of L-glutamic acid added to each sample, and multiplied by 100 to convert the recovery to percent.

II. Results

The L-glutamic acid content of the eleven food systems, without heat treatment or pH adjustment, ranged from 0 g/l for the carbohydrate and fat systems to 1.13 g/l for tomatoes ("Base", Table 1). Parmesan cheese had the second highest level, 0.444 g/l, and mushrooms were third highest, 0.325 g/l. Among the animal protein systems, chicken broth contained 0.134 g/l, beef broth 0.0300 g/l, and

fish broth 0.0145 g/l. Soy flour had 0.0692 g/l, which was more L-glutamic acid than measured in either the beef or fish broth. Table 1 also details the L-glutamic acid content of the control and treatment samples of the food systems without added MSG (C) and with 0.05% MSG (T), at the two pH levels and the three heat treatments used in this study, averaged across two replications. The relationships among these data are best understood by examining the results from analysis of variance, detailed in Tables 2 through 5.

For all food systems, the addition of MSG increased the amount of L-glutamic acid ($p \leq 0.001$) when the values were averaged across heat treatment and pH levels (Table 2). Differences between the C and T samples in L-glutamic acid levels were determined for each food system. The differences ranged from a low of 0.2437 g/l for beef broth to a high of 0.5678 g/l for tomatoes, with an average of 0.3993 g/l. This average level is very close to the amount of L-glutamic acid added to the systems, which was 0.3937 g/l. Low levels of L-glutamic acid were unexpectedly found in the control samples for sucrose, cornstarch, corn oil, and butter (Table 2). Possibly these foods contained very low levels of L-glutamic acid (from corn or milk

proteins, respectively) or these values may be an indication of measurement error. A sample of MSG in water would have accounted for such error. Experimental error (e.g., inaccuracy of the spectrophotometer especially at high absorbency, or the affect of light on the samples) was controlled for by using a L-glutamic acid standard, provided with the test kit.

The amount of L-glutamic acid recovered from each sample varied widely (Table 3). The percent recovery was 172% for tomatoes, which was significantly greater than all the other food systems ($p \leq 0.001$). This recovery is more than 100%, indicating that the tomatoes may have liberated some L-glutamic acid. Corn oil, sucrose, butter, and fish broth were similar in recovery, near 100%. Fish broth was also similar to chicken broth, Parmesan cheese, cornstarch, and mushrooms. Parmesan cheese was not significantly different from starch, mushrooms, and soy flour, but was different from beef broth, which had the lowest recovery (61.4%). The recovery of L-glutamic acid from two of the food systems was affected by pH (Table 3). For fish broth and tomatoes, the L-glutamic acid levels of the samples adjusted to pH 6 were significantly higher

than the samples at pH 3 ($p=0.0003$). This may be due to more L-glutamic acid liberated by these food systems in the less acidic environment. The recovery of L-glutamic acid from the food systems was not affected by heat treatment, nor interaction between heat treatment and pH.

Heat treatment of the samples only affected the L-glutamic acid content of one food system: cornstarch ($p=0.0119$; Table 4). The sample held at room temperature had significantly less L-glutamic acid than the samples heated to 63°C and at 100°C. This result is likely due to large variability in the data for cornstarch, as the cornstarch gelled upon heating, making measurements of glutamic acid and pH difficult. The glutamic acid content of the food systems was not altered by pH (Table 5). For both butter and corn oil, L-glutamate from replication 1 was higher than L-glutamate from replication 2 (Table 6). The variation between replications may be due to the lipophobic nature of MSG.

Mean pH of the untreated food systems ranged from 4.49 for tomatoes to 6.66 for fish broth ("Base", Table 7). The addition of MSG to corn oil resulted in a significantly lower pH ($p=0.0026$; Table

8). This difference may be due to the difficulty of adjusting the pH of the fat food systems. No other food systems differed in pH as a result of MSG addition. Heat treatment resulted different pH levels only in fish broth: the fish broth heated to 100°C was significantly higher than the pH of fish broth at room and moderate temperatures ($p=0.0005$; Table 9). Not surprisingly, adjusting the pH to 3 or 6 resulted in significant differences in the pH of all the food systems ($p\leq0.0001$; Table 10). The two treatment pH levels remained different even after heat treatment and the addition of MSG. The high standard deviations for the fat systems (corn oil and butter) show the large variability in the pH of these systems due to measurement problems. Differences in pH between replications were found for beef broth ($p=0.0002$), Parmesan cheese ($p\leq0.0001$), sucrose ($p=0.0063$), and corn oil ($p=0.0141$; Table 11). The differences were mixed as to whether replication 1 or replication 2 was at a higher or lower pH than the corresponding replication. The difference between replications is not surprising because of the difficulty in adjusting the pH of the fat systems. To compensate for this inconsistency, an electrode designed for high-fat samples should have been used.

## III. Discussion

The levels of L-glutamic acid measured in this experiment were generally less than reported ranges (Table 12; note that units from this experiment changed to mg/100g to match values reported in the literature). The L-glutamic acid content of the Parmesan cheese measured in this study was less than the range of reported values (44.4 mg/100 g as compared to 2170, 1200, and 516 mg/100 g in Table 12). The level of L-glutamic acid in mushrooms was also less than reported ranges (32.5 mg/100 g vs. 2400, 192, and 180 mg/100 g). The levels in beef broth were more consistent with published values, perhaps because beef has a relatively low concentration of L-glutamic acid when compared to other protein foods (Komata, 1990).

Reported levels of L-glutamic acid in most foods vary widely and depend upon whether the food is fresh or processed (e.g., canned, frozen, soup), among other factors. This variability may be due to the use of different analytical methods, including purification and extraction methods. In the present study, soy flour and cornstarch formed a suspension, and cornstarch also gelled upon heating. These samples should have been filtered prior to analysis. Large variation in

the L-glutamic acid content of foods has been reported by other researchers (Skurray and Pucar, 1988; Rhodes *et al.*, 1991; Daniels *et al.*, 1995). Daniels *et al.* (1995) suggested that large variability in the L-glutamate levels of Parmesan cheese may be due to lack of homogeneity in the sample, the presence of fat, and interference by fermentation products.

Variability in the L-glutamic acid content of foods may be due to factors unrelated to the experimental procedure. Skurray and Pucar (1988) found that the glutamic acid content of fresh tomatoes increased with ripening: from 50.3 mg/100 g in green tomatoes to 292 mg/100 g in red tomatoes. In contrast, Hac *et al.* (1949) found that for corn and peas, the L-glutamic acid content decreased with maturity, perhaps due to the changes in the starch matrix of these vegetables as they mature, compared to tomatoes. Heat processing, exposure of a food to oxygen, and storage time can affect free L-glutamic acid levels in processed foods (Gayte-Sorbier *et al.*, 1985; Airaudo *et al.*, 1987). Skurray and Pucar (1988) have compared L-glutamic acid in fresh tomatoes, canned tomatoes, and canned tomato juice. The levels decreased with processing (292, 202, and 109

mg/100 g, respectively). They found that canned mushrooms also had less L-glutamic acid than fresh: 34 mg/100 g (without added MSG) and 192 mg/100 g respectively. In contrast, Komata (1990) found that the L-glutamic acid of tomatoes increased with heat processing from 140 mg/100 g in fresh tomatoes to 260 mg/100 in canned tomato juice. Komata did not indicate whether or not MSG was added to the juice. Most reports of L-glutamic acid levels do not usually indicate whether food tested was fresh or processed, and whether or not MSG was added to the food.

The percent recovery of L-glutamic acid from each system was calculated as an indication of the degradation of added MSG or the generation of L-glutamic acid. An increase in percent recovery could mean L-glutamic acid was released from the food due to heat or pH, while a percent recovery less than 100% could mean that L-glutamic acid had been degraded, reacted with another compound, or bound into another molecule. Tomatoes had the highest percent recovery of 172%, indicating that glutamic acid was released. Daniels *et al.* (1995) recovered 197% of L-glutamic acid from fresh tomatoes, suggesting that the recovery was high because the spiking level was

low compared with the level of L-glutamic acid naturally occurring in the tomato. With the exception of cornstarch, the non-protein foods (corn oil, sucrose, and butter) had percent recoveries slightly higher than 100%. Parmesan cheese, cornstarch, mushrooms, and soy flour had percent recoveries between 90% and 69%, indicating that added L-glutamic acid may have reacted or complexed with other components in the food system. Beef broth had the lowest recovery, possibly due to its low endogenous L-glutamic acid content or because most of the L-glutamic acid combined with other available amino acids to form polypeptides. Although beef broth has low levels of free L-glutamic acid, glutamic acid is a component of many of the peptides responsible for beef flavor (Spanier *et al.*, 1996).

Fish broth and tomatoes had a higher percent recovery at pH 6 than pH 3, indicating that pH affected the amount of free L-glutamic acid. The pH of the system dictates the flavor potentiation of MSG, which is most effective between pH 5.5 to 8.0. This pH range corresponds to complete ionization of the MSG molecule (Nagodawithana, 1994). It had been hypothesized that proteins may hydrolyze in the acid environment, thereby increasing the levels of

glutamic acid, but this was not found. Possibly, more L-glutamic acid was liberated from protein molecules in a neutral environment, making it available to bind with sodium. Heating the protein systems may have liberated all L-glutamate that was available.

The L-glutamic acid content was affected by heat in only one food system, cornstarch, which had a significant decrease in L-glutamic acid content with heat treatment. This finding could be due to the starch binding L-glutamic acid or in some way facilitating its degradation to another compound, or it could be the result of experimental variation. The cornstarch formed a cloudy suspension that may have artificially increased the absorbency values on the spectrophotometer. MSG appears to be very stable under most heat processing conditions. The primary degradation product of glutamic acid is pyrrolidone carboxylic acid (PCA) (Gayte-Sorbier *et al.*, 1985), which has a bitter taste (Solms, 1969). In an aqueous medium, L-glutamic acid and PCA are in equilibrium (Pintauro, 1976). Early research found 0.1% glutamic acid to be stable with no PCA formation at pH 5.5 and 6.0 at 240°F for 40 minutes (Mahdi *et al.*, 1959). In a study with a "soup" concocted with glucose, casein, or

starch, MSG was found to be stable, with PCA undetectable in samples (Ngyuen and Sporns, 1984).

A series of experiments in France optimized conditions favorable to the formation of PCA from glutamic acid (Gayte-Sorbier *et al.*, 1985; Airaudo *et al.*, 1987). Both glutamic acid and MSG were stable when stored at room temperature for 24 hours. At acidic pH levels, PCA formation was found, while glutamic acid levels decreased. Samples stored in the presence of oxygen lost glutamic acid faster than those with nitrogen. Room temperature samples also lost more glutamic acid than samples stored at 4°C. Perhaps the lack of change in L-glutamic acid content found in these experiments was due to the relatively low temperatures or short heating times used.

Table 1. Mean L-glutamic acid content (g/l) of 11 simple food systems, subjected to 3 heat treatments and 2 pH levels (2 replications).

| Temperature | | Room (24°C) | | | | Moderate (63°C) | | | | Severe (100°C) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | | 3 | | 6 | | 3 | | 6 | | 3 | | 6 | |
| Food System | Base | C | T | C | T | C | T | C | T | C | T | C | T |
| Chick.Broth | 0.13 | 0.13 | 0.46 | 0.16 | 0.62 | 0.14 | 0.50 | 0.15 | 0.51 | 0.14 | 0.53 | 0.15 | 0.55 |
| Fish Broth | 0.02 | 0.02 | 0.41 | 0.02 | 0.44 | 0.01 | 0.45 | 0.01 | 0.52 | 0.01 | 0.38 | 0.01 | 0.44 |
| Beef Broth | 0.03 | 0.03 | 0.27 | 0.03 | 0.25 | 0.03 | 0.27 | 0.03 | 0.29 | 0.01 | 0.27 | 0.03 | 0.29 |
| Parmesan | 0.44 | 0.52 | 0.86 | 0.53 | 0.90 | 0.53 | 0.92 | 0.51 | 0.86 | 0.51 | 0.88 | 0.55 | 0.92 |
| Soy Flour | 0.07 | 0.07 | 0.42 | 0.05 | 0.23 | 0.05 | 0.37 | 0.07 | 0.32 | 0.10 | 0.37 | 0.06 | 0.37 |
| Tomatoes | 1.13 | 1.22 | 1.76 | 1.12 | 1.74 | 1.11 | 1.81 | 1.04 | 1.90 | 1.03 | 1.37 | 1.05 | 2.12 |
| Mushrooms | 0.33 | 0.20 | 0.66 | 0.39 | 0.69 | 0.23 | 0.62 | 0.31 | 0.48 | 0.27 | 0.57 | 0.28 | 0.73 |
| Corn Starch | 0 | 0 | 0.39 | 0 | 0.39 | 0 | 0.39 | <0.01 | 0.35 | <0.01 | 0.25 | <0.01 | 0.28 |
| Sucrose | 0 | 0 | 0.44 | <0.01 | 0.50 | 0 | 0.50 | 0 | 0.49 | <0.01 | 0.46 | 0 | 0.49 |
| Butter | 0 | 0 | 0.48 | <0.01 | 0.44 | 0 | 0.50 | 0 | 0.51 | 0 | 0.50 | 0 | 0.42 |
| Corn Oil | 0 | 0 | 0.51 | 0 | 0.47 | 0 | 0.50 | 0 | 0.53 | 0 | 0.48 | 0 | 0.47 |

Base: No added MSG; not heat treated or pH adjusted.
C: Control, No added MSG.
T: Treatment, 0.05% w/v MSG

Table 2. Effect of added MSG on free L-glutamic acid content: Means (g/l) averaged across heat treatments, pH levels, and replications, standard deviations, F-ratios, and probability levels from analysis of variance for 11 food systems (2 reps, 12 d.f.)

| Food System | No Added MSG Mean±Std. Dev. | 0.1% Added MSG Mean±Std. Dev. | F-ratio | p-value | Difference (Test-Control) |
|---|---|---|---|---|---|
| Chicken Broth | 0.1431±0.0112 | 0.5280±0.0698 | 356.72 | ≤0.001 | 0.3849 |
| Fish Broth | 0.0138±0.0029 | 0.4387±0.0536 | 930.94 | ≤0.001 | 0.4249 |
| Beef Broth | 0.0276±0.0162 | 0.2713±0.0187 | 1045.45 | ≤0.001 | 0.2437 |
| Parmesan Cheese | 0.5228±0.0381 | 0.8870±0.0583 | 313.30 | ≤0.001 | 0.3645 |
| Soy Flour | 0.0666±0.0262 | 0.3448±0.0825 | 156.15 | ≤0.001 | 0.2782 |
| Tomatoes | 1.0930±0.1439 | 1.781±0.2876 | 39.30 | ≤0.001 | 0.5678 |
| Mushroom | 0.2790±0.0753 | 0.6275±0.1126 | 47.04 | ≤0.001 | 0.3366 |
| Corn Starch | 0.0001±0.0025 | 0.3444±0.0672 | 455.36 | ≤0.001 | 0.3443 |
| Sucrose | 0.0006±0.0011 | 0.4793±0.0307 | 3124.6 | ≤0.001 | 0.4787 |
| Butter | 0.0001±0.0003 | 0.4748±0.0941 | 372.10 | ≤0.001 | 0.4747 |
| Corn Oil | 0.0001±0.0003 | 0.4942±0.0991 | 463.44 | ≤0.001 | 0.4942 |

Table 3. Mean percent recovery of L-glutamic acid from 11 simple food systems, averaged across pH and averaged by pH treatment (3 heat treatments, 2 replications; 10 d.f.).

| Food System | Across pH | By pH | |
|---|---|---|---|
| | Mean±Std. Dev. | pH 3 | pH 6 |
| | | Mean±Std.Dev. | Mean±Std. Dev. |
| Chicken Broth | 95.7±15.3$^{c}$ | 89.9±10.4 | 102.2±18.4 |
| Fish Broth | 105.0±11.9$^{bc}$ | 99.2±9.9$^{b}$ | 112.1±11.4$^{a}$ |
| Beef Broth | 61.4±7.9$^{e}$ | 60.3± 7.8 | 62.5±8.5 |
| Parmesan Cheese | 90.4±16.7$^{cd}$ | 90.9±22.0 | 89.9±11.2 |
| Soy Flour | 69.1±18.6$^{de}$ | 76.7±18.2 | 61.5±17.7 |
| Tomatoes | 172.1±68.0$^{a}$ | 134.1±46.1$^{b}$ | 210.0$^{a}$±60.6$^{a}$ |
| Mushroom | 84.4±30.5$^{cd}$ | 96.0±24.9 | 72.7±33.9 |
| Corn Starch | 86.1±16.2$^{cd}$ | 87.5±19.0 | 84.7±14.6 |
| Sucrose | 119.0±7.7$^{b}$ | 114.0±8.3 | 123.1± 5.2 |
| Butter | 119.0±21.2$^{b}$ | 122.2±14.0 | 114.0±27.6 |
| Corn Oil | 123.1±22.8$^{b}$ | 125.2±24.0 | 121.0±23.6 |

Significance: Across pH: Means within the column that do not share a superscript are significantly different at $p \leq 0.001$.

By pH: Means within a row that have differing superscripts are significantly different at $p=0.0003$.

Table 4. Effect of heat treatment on free L-glutamic acid content: Means (g/l) averaged across added glutamate, pH levels, and replications, standard deviations, F-ratios, and probability values from analysis of variance for 11 food systems. (2 reps, 8 d.f.)

| | Room (24°C) | Moderate (63°C) | Severe (100°C) | F-ratio | p-value |
|---|---|---|---|---|---|
| Food System | Mean±Std. Dev. | Mean±Std. Dev. | Mean±Std. Dev. | | |
| Chicken Broth | 0.3414±0.2276 | 0.3254±0.1941 | 0.3399±0.2126 | 0.2507 | 0.7810 |
| Fish Broth | 0.2208±0.2209 | 0.2483±0.2519 | 0.2096±0.2147 | 2.7268 | 0.0924 |
| Beef Broth | 0.1451±0.1221 | 0.1528±0.1324 | 0.1505±0.1391 | 0.3698 | 0.6960 |
| Parmesan Cheese | 0.7004±0.2031 | 0.7023±0.2013 | 0.7121±0.1986 | 0.1255 | 0.8828 |
| Soy Flour | 0.1937±0.1600 | 0.1991±0.1558 | 0.2243±0.1662 | 0.7157 | 0.5023 |
| Tomatoes | 1.4565±0.3484 | 1.4642±0.4371 | 1.3910±0.5017 | 0.1494 | 0.8623 |
| Mushroom | 0.4835±0.2224 | 0.4273±0.1912 | 0.4650±0.2277 | 0.4548 | 0.6417 |
| Corn Starch | 0.1963±0.2109[a] | 0.1878±0.2002[b] | 0.1348±0.1444[b] | 5.7308 | 0.0119 |
| Sucrose | 0.2349±0.2516 | 0.2483±0.2656 | 0.2366±0.2528 | 0.9610 | 0.4013 |
| Butter | 0.2310±0.2635 | 0.2510±0.2732 | 0.2303±0.2503 | 0.3050 | 0.7408 |
| Corn Oil | 0.2483±0.2782 | 0.2568±0.2822 | 0.2364±0.2596 | 0.2651 | 0.7701 |

Table 5. Effect of pH on free L-glutamic acid content: Means (g/l) averaged across heat treatments, added MSG, and replications, standard deviations, F-ratios, and probability values from analysis of variance for 11 food systems (2 reps, 12 d.f.).

| | pH = 3 | pH = 6 | | |
|---|---|---|---|---|
| Food System | Mean±Std. Dev. | Mean±Std. Dev. | F-ratio | p-value |
| Chicken Broth | 0.3168±0.1919 | 0.3543±0.2196 | 3.3710 | 0.0829 |
| Fish Broth | 0.2127±0.2108 | 0.2398±0.2378 | 3.7920 | 0.0673 |
| Beef Broth | 0.1467±0.1279 | 0.1522±0.1289 | 0.5441 | 0.4702 |
| Parmesan Cheese | 0.7002±0.2001 | 0.7097±0.1926 | 0.2132 | 0.6498 |
| Soy Flour | 0.2287±0.1678 | 0.1827±0.1428 | 4.2820 | 0.0532 |
| Tomatoes | 1.3822±0.3542 | 1.4915±0.4788 | 1.0730 | 0.3139 |
| Mushroom | 0.4265±0.2101 | 0.4907±0.2063 | 1.7090 | 0.2076 |
| Corn Starch | 0.1738±0.1875 | 0.1721±0.1831 | 0.0108 | 0.9186 |
| Sucrose | 0.2327±0.2432 | 0.2472±0.2582 | 2.8670 | 0.1076 |
| Butter | 0.2458±0.2601 | 0.2290±0.2529 | 0.4680 | 0.5026 |
| Corn Oil | 0.2512±0.2716 | 0.2431±0.2630 | 0.1240 | 0.7288 |

Table 6. Effect of replications on free L-glutamic content: Means (g/l) averaged across added lutamate, heat treatments, and pH levels, standard deviations, F-ratios, and probability values from analysis of variance for 11 food systems (2 reps, 12 d.f.).

| | Replicate 1 | Replicate 2 | F-ratio | p-value |
|---|---|---|---|---|
| Food System | Mean±Std. Dev. | Mean±Std. Dev. | | |
| Chicken Broth | 0.331±0.213 | 0.340±0.201 | 0.164 | 0.690 |
| Fish Broth | 0.226±0.225 | 0.226±0.225 | 0.001 | 0.988 |
| Beef Broth | 0.152±0.121 | 0.147±0.136 | 0.469 | 0.502 |
| Parmesan Cheese | 0.689±0.181 | 0.721±0.209 | 2.521 | 0.130 |
| Soy Flour | 0.228±0.169 | 0.183±0.142 | 4.002 | 0.061 |
| Tomatoes | 1.542±0.363 | 1.330±0.453 | 0.541 | 0.474 |
| Mushroom | 0.458±0.215 | 0.459±0.206 | 0.001 | 0.976 |
| Corn Starch | 0.160±0 | 0.186±0 | 2.853 | 0.125 |
| Sucrose | 0.236±0.248 | 0.244±0.254 | 0.733 | 0.403 |
| Butter | 0.272±0.287 | 0.203±0.216 | 7.749 | 0.012 |
| Corn Oil | 0.292±0.306 | 0.202±0.212 | 15.520 | 0.001 |

Table 7. Mean pH levels of 11 simple food systems, with 3 heat treatments at 2 pH levels (2 replications).

| Temperature | | Room (24°C) | | | | Moderate (63°C) | | | | Severe (100°C) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | | 3 | | 6 | | 3 | | 6 | | 3 | | 6 | |
| Food System | Base | C | T | C | T | C | T | C | T | C | T | C | T |
| Chick. Broth | 6.34 | 2.39 | 2.93 | 6.25 | 5.94 | 2.83 | 2.98 | 6.09 | 5.91 | 3.12 | 3.10 | 6.05 | 5.92 |
| Fish Broth | 6.66 | 3.07 | 2.99 | 6.01 | 6.01 | 2.98 | 2.97 | 5.99 | 5.98 | 3.29 | 3.21 | 6.00 | 6.10 |
| Beef Broth | 6.61 | 2.96 | 2.79 | 5.99 | 6.30 | 2.79 | 2.94 | 5.94 | 5.99 | 3.01 | 3.12 | 5.97 | 6.07 |
| Parmesan | 5.06 | 3.53 | 3.40 | 5.48 | 5.52 | 3.63 | 3.59 | 5.36 | 5.55 | 3.71 | 3.64 | 5.31 | 5.39 |
| Soy Flour | 6.16 | 3.23 | 3.18 | 6.05 | 6.15 | 3.31 | 3.32 | 5.99 | 6.01 | 3.41 | 3.36 | 5.96 | 6.01 |
| Tomatoes | 4.49 | 3.13 | 3.06 | 5.66 | 5.74 | 3.20 | 3.14 | 5.54 | 5.66 | 3.19 | 3.17 | 5.58 | 5.69 |
| Mushroom | 6.27 | 3.13 | 3.04 | 6.11 | 6.02 | 3.17 | 3.14 | 6.08 | 6.08 | 3.20 | 3.14 | 5.97 | 6.08 |
| Corn Starch | 5.27 | 2.91 | 2.86 | 5.68 | 5.83 | 3.00 | 2.84 | 5.99 | 5.81 | 2.97 | 2.82 | 6.05 | 5.82 |
| Sucrose | 6.50 | 3.02 | 3.06 | 5.85 | 5.92 | 3.04 | 3.05 | 6.03 | 5.98 | 3.07 | 3.05 | 6.38 | 5.91 |
| Butter | 5.28 | 3.95 | 3.20 | 5.87 | 5.58 | 4.28 | 3.42 | 5.82 | 5.94 | 4.04 | 3.61 | 5.57 | 6.31 |
| Corn Oil | 5.60 | 6.55 | 3.07 | 7.90 | 8.03 | 4.39 | 4.44 | 8.58 | 6.23 | 5.76 | 3.14 | 7.68 | 6.81 |

Base: No added MSG; not heat treated or pH adjusted.
C: Control, No added MSG.
T: Treatment, 0.05% w/v MSG

Table 8. Effect of added MSG on pH: Means (g/l) averaged across heat treatments and pH levels, standard deviations, F-ratios, and probability values from analysis of variance for 11 food systems (2 reps, 12 d.f.).

| | No Added MSG | 0.1% MSG | F-ratio | p-value |
|---|---|---|---|---|
| Food System | Mean±Std. Dev. | Mean±Std. Dev. | | |
| Chicken Broth | 4.44±1.65 | 4.47±1.65 | 0.126 | 0.727 |
| Fish Broth | 4.56±1.51 | 4.54±1.56 | 0.203 | 0.658 |
| Beef Broth | 4.44±1.62 | 4.53±1.69 | 0.953 | 0.342 |
| Parmesan Cheese | 4.50±0.95 | 4.51±1.04 | 0.032 | 0.860 |
| Soy Flour | 4.66±1.40 | 4.67±1.45 | 0.126 | 0.727 |
| Tomatoes | 4.38±1.27 | 4.41±1.34 | 0.497 | 0.490 |
| Mushroom | 4.61±1.51 | 4.58±1.54 | 0.237 | 0.632 |
| Corn Starch | 4.43±1.55 | 4.33±1.56 | 3.572 | 0.075 |
| Sucrose | 4.56±1.60 | 4.49±1.51 | 1.210 | 0.286 |
| Butter | 4.92±1.14 | 4.68±1.41 | 0.790 | 0.386 |
| Corn Oil | 6.69±1.91 | 5.11±2.05 | 12.161 | 0.003 |

Table 9. Effect of heat treatment on pH: Means averaged across added glutamate, pH and replications, standard deviations, F-ratios, and probability values from analysis of variance for 11 food systems (2 reps, 8 d.f.).

| | Room (24°C) | Moderate (63°C) | Severe (100°C) | F-ratio | p-value |
|---|---|---|---|---|---|
| Food System | Mean±Std. Dev. | Mean±Std. Dev. | Mean±Std. Dev. | | |
| Chicken Broth | 4.38±1.85 | 4.45±1.66 | 4.55±1.54 | 1.198 | .325 |
| Fish Broth | 4.52±1.59[a] | 4.48±1.61[a] | 4.65±1.50[b] | 12.086 | .001 |
| Beef Broth | 4.51±1.79 | 4.41±1.69 | 4.54±1.59 | 0.695 | .512 |
| Parmesan Cheese | 4.48±1.11 | 4.53±1.02 | 4.51±0.92 | 0.374 | .694 |
| Soy Flour | 4.65±1.55 | 4.65±1.44 | 4.68±1.39 | 0.310 | .738 |
| Tomatoes | 4.39±1.39 | 4.38±1.30 | 4.41±1.32 | 0.105 | .901 |
| Mushroom | 4.57±1.60 | 4.62±1.57 | 4.60±1.53 | 0.263 | .772 |
| Corn Starch | 4.32±1.54 | 4.41±1.60 | 4.41±1.63 | 1.248 | .312 |
| Sucrose | 4.46±1.53 | 4.52±1.59 | 4.60±1.66 | 1.665 | .217 |
| Butter | 4.65±1.33 | 4.86±1.32 | 4.88±1.30 | 0.292 | .751 |
| Corn Oil | 6.38±2.32 | 5.91±2.22 | 5.41±1.90 | 1.541 | .241 |

[ab] Means within a row that share a superscript are not significantly different at stated p-value.

Table 10. Effect of adjusted pH on pH: Means averaged across added MSG, heat treatments, and replications, standard deviation, F-ratios, and probability values from analysis of variance for 11 food systems (2 reps, 12 d.f.).

| | pH = 3 | pH = 6 | F-ratio | p-value |
|---|---|---|---|---|
| Food System | Mean±Std. Dev. | Mean±Std. Dev. | | |
| Chicken Broth | 2.89±0.26 | 6.03±0.14 | 1235.26 | ≤0.001 |
| Fish Broth | 3.08±0.14 | 6.01±0.05 | 9792.38 | ≤0.001 |
| Beef Broth | 2.93±0.22 | 6.04±0.39 | 1095.34 | ≤0.001 |
| Parmesan Cheese | 3.58±0.24 | 5.43±0.22 | 1580.45 | ≤0.001 |
| Soy Flour | 3.30±0.08 | 6.02±0.09 | 5271.48 | ≤0.001 |
| Tomatoes | 3.15±0.07 | 5.64±0.11 | 4359.41 | ≤0.001 |
| Mushroom | 3.14±0.10 | 6.05±0.13 | 3462.15 | ≤0.001 |
| Corn Starch | 2.90±0.12 | 5.86±0.18 | 2803.63 | ≤0.001 |
| Sucrose | 3.05±0.05 | 6.01±0.27 | 2115.54 | ≤0.001 |
| Butter | 3.75±0.53 | 5.85±0.80 | 58.30 | ≤0.001 |
| Corn Oil | 4.44±1.71 | 4.36±1.28 | 41.78 | ≤0.001 |

Table 11. Effect of replication on pH: Means (g/l) averaged across added glutamate, heat treatments and pH level, standard deviations, F-ratios, and probability values of analysis of variance for 11 food systems (2 reps, 12 d.f.).

| | Replicate 1 | Replicate 2 | | |
|---|---|---|---|---|
| Food System | Mean±Std. Dev. | Mean±Std. Dev. | F-ratio | p-value |
| Chicken Broth | 4.45±1.77 | 4.46±1.53 | 0.0087 | 0.9266 |
| Fish Broth | 4.53±1.54 | 4.57±1.53 | 2.14 | 0.1603 |
| Beef Broth | 4.27±1.53 | 4.70±1.74 | 21.45 | 0.0002 |
| Parmesan Cheese | 4.71±0.96 | 4.31±0.99 | 72.27 | 0.0001 |
| Soy Flour | 4.69±1.46 | 4.63±1.39 | 2.15 | 0.1601 |
| Tomatoes | 4.37±1.27 | 4.42±1.34 | 2.38 | 0.1403 |
| Mushroom | 4.62±1.58 | 4.57±1.47 | 1.27 | 0.2752 |
| Corn Starch | 4.33±1.57 | 4.43±1.54 | 3.35 | 0.0839 |
| Sucrose | 4.63±1.63 | 4.43±1.48 | 9.56 | 0.0063 |
| Butter | 5.04±1.56 | 4.55±0.87 | 3.15 | 0.0929 |
| Corn Oil | 5.29±2.20 | 6.52±1.88 | 7.38 | 0.0141 |

Table 12. L-glutamic acid content (mg/100 g) of selected foods from published studies.

| Food | This Study | Hac *et al.*, 1949 | Orr and Watt, 1957 | Anon., 1987 | Skurray and Pucar, 1988 | Komata, 1990 | Rhodes *et al.*, 1991 | IGTC, 1990 |
|---|---|---|---|---|---|---|---|---|
| Beef | 3.0[A] | 50 | | | 60.7[D] | 11 | | 33 |
| Chicken | 13.4 | 30 | | | 205[C] | 40 | | 44 |
| Fish | 1.45 | 200[B] | | | 36.3 | 26 | | 20 |
| Parmesan Cheese | 44.4 | | | | 516 | | | 1200 |
| Soybean/ soy flour | 6.92 | | | | 63.2 | 66 | 2680 | |
| Mushrooms | 32.5 | 2400 | | | 192 | | 180 | |
| Tomatoes | 113 | | 231 | 246 | 292 | 140 | | |

[A]Freshly prepared broth
[B]Canned
[C]Soup
[D]Processed hamburger meat

# Chapter 6

## Conclusions

An understanding of the mechanism of flavor potentiators must include comprehension of both the human experience and the food system. Although the role of salivary composition in the taste perception of flavor potentiators is unclear, it should be considered as a factor in further research into these mechanisms. Additionally, gender, ethnicity, and diet are important considerations. The conclusions of these experiments demonstrate the unique behavior of flavor potentitors and lend support for future research.

1. The Relationship Between Salivary Composition and Taste Perception of Flavor Potentiators

Although thresholds to NaCl and MSG were positively correlated, no relationships between salivary L-glutamic acid or sodium and the two sensory measures of detection thresholds and perceived intensity were found. Ethnicity and gender were significant factors in the results. Asian females had higher salivary sodium than the other groups. Additionally, Asians perceived the lower concentrations of NaCl as less intense than non-

Asians, and had a steeper power function slope for perceived intensity (larger increases in perceived intensity per unit of NaCl) than non-Asians. Research by other experimenters finds that these differences are most likely due to different experiences with food, rather than a genetic trait. In these experiments, Asians had a higher dietary glutamate and NaCl intake than non-Asians.

These studies could be continued in a number of ways. They should be replicated using simple food systems, including in the design the ability to determine possible interactions with NaCl. Similar experiments to determine sensitivity to other flavor potentiators, specifically IMP and GMP, should be conducted to test the idea that salivary glutamate acts synergistically with IMP for the umami taste (Yamaguchi et al., 1987). Factors that affect salivary glutamate should be studied, as they have been for NaCl, including dietary intake, salivary flow rates, and diurnal variation. The theory that salivary glutamate levels in people with MSG-symptom complex may be different that non-sufferers could be tested. Future experiments must use precise techniques to sample saliva, including the methods of collecting, pipetting, and diluting of saliva, and appropriate analytical methods. Another research possibility is to determine the functionality of L-glutamic acid. Sensory properties of foods high in

endogenous L-glutamic acid could be determined, both with and without the addition of other flavor potentiating compounds, including 5'-ribonucleotides and NaCl.

2. Time Intensity Profiles of Flavor Potentiators

The time-intensity (TI) profiles for the flavor potentiators were atypical of curves generated from sweet, sour, salt, and bitter stimuli, and lend support to umami as a separate taste. The profiles quickly reached maximum intensity, which was followed by a relatively long plateau phase. The large area of the curve indicates that flavor potentiators may increase total flavor in the mouth. These parameters may yield clues to the taste mechanism of flavor potentiators (Birch, 1987), e.g., the long aftertaste may indicate that the flavor potentiator molecule is tightly bound to the receptor. Alternatively, the long aftertaste may be due to a slower adaptation rate for MSG, a phenomenon similar to dark adaptation in vision.

Differences in profile parameterize were found for the different flavor potentiators when tasted at different concentrations. Lower concentrations and single solution samples had were less intense and had shorter duration and smaller area. Binary samples with 0.63 mM IMP had the least intensity, and those with 2.5 mM GMP were generally more intense. Because lower concentrations of MSG were rated higher when paired with a high

ribonucleotide concentration, the ribonucleotide apparently has more of an impact on taste intensity than MSG. A 2.5 mM equi-mixture of the three flavor potentiators did not increase the intensity. Synergism was demonstrated between MSG and the 5'-ribonucleotides. Differences in curve parameters were found for men vs women, Asians vs. non-Asians, and salivary glutamate groups.

These experiments must be repeated using simple food systems so that the TI profiles can be used to examine the temporal properties of flavor potentiators in food systems and to further understand their perception. The profiles would have broader application for both food and chemoreception research if more detailed statistical analyses for the TI curve were to be employed in future research.

3. Effect of Heat Treatment and pH on Levels of Free L-Glutamic Acid in Simple Food Systems

The amount of L-glutamic acid recovered was dependent upon the food system to which it was added. Regardless of heat or pH treatment, tomatoes had a recovery of 167%, indicating that L-glutamic acid was liberated. Beef broth had a 61% recovery, with glutamate either being bound or destroyed in the beef broth. For fish broth and tomatoes, the L-glutamic acid levels of the pH 6 samples were higher than the pH 3 samples;

due to more L-glutamic acid liberated in the less acidic environment. With one exception, cornstarch, heat treatment did not effect the resulting levels of L-glutamic acid in simple food systems to which MSG had been added.

The amount of free L-glutamic acid available for flavor potentiation is dependent upon the food system, but why MSG is effective in some food systems and not in others is still unknown. From these experiments, pH appears to play a critical role. Other evidence also points to pH, for example, the pH levels of most fruit species are too low for L-glutamic acid to be effective. Future studies into the effect of temperature and pH on MSG in food should include a sensory component that evaluates aroma, taste, and mouthfeel. Other processing conditions and methods to test could include irradiation, freezing, drying, and oxidation. Any studies of this type must account for the levels of endogenous L-glutamic acid in the food systems. Also of interest would be testing other flavor potentiators under these conditions.

Future research into the mechanism by which MSG potentiates flavor must combine both analytical and sensory methods and should include foods with a wide range of endogenous L-glutamic acid, which must be measured and factored into the results of these experiments. This information would be useful for different purposes. A better understanding of interactions

among food components and flavor potentiators, including MSG, 5'-ribonucleotides, NaCl, and yeast and vegetable proteins, and the role of endogenous L-glutamic acid would allow for the development of improved food flavors. Additionally, improved foods could be developed, including more flavorful low fat and non-fat food and foods that require flavor masking, such as savory foods made with soy. Although an increased dietary intake of vegetables has been encouraged by many groups to decrease risk of cancer and other diseases, consumption of vegetables remains below recommended levels. Flavor potentiators could be used to develop more flavorful vegetable products, including vegetable-based snack foods that could increase consumption. Lastly, the use of flavor potentiators could improve food intake and thereby the quality of life for people with diminished taste and smell and others who are nutritionally compromised.

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## LIST OF APPENDICES

APPENDIX I. Threshold and Intensity Tests, Study 1 and Time-Intensity Test. Consent Form

COLORADO STATE UNIVERSITY

INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

FOR PEOPLE WHO ARE NOT SENSITIVE TO MONOSODIUM GLUTAMATE

TITLE OF PROJECT: The Effect of Salivary Glutamate and Glutamine Levels on Perception of Umami Taste

NAME OF PRINCIPAL INVESTIGATOR: Joseph A. Maga, PhD

NAME OF CO-INVESTIGATOR: Maria Buscarello

CONTACT NAME AND PHONE NUMBER FOR QUESTIONS/PROBLEMS: Maria Buscarello, (303) 491-6763 or (303) 237-1162

SPONSOR OF PROJECT: Non-sponsored.

PURPOSE OF THE RESEARCH: The purpose of this research project is to determine if levels of the amino acids glutamate and glutamine, which naturally occur in saliva, affect perception of the uamim taste. Umami is a Japanese word that describes a taste that is savory or meaty and the perception of enhanced flavor in foods that contact flavor potentiators. This research will: 1. Measure the levels of the amino acids glutamate and glutamine in human saliva; and, 2. Determine the effect of salivary glutamate and glutamine levels on the ability to perceive umami taste, both as its own savory taste and as a flavor potentiator.

You will participate in one to three sessions per week for twenty weeks or less, with each session lasting a maximum of one-half hour. Well established sensory evaluation procedures will be used in this research project.

PROCEDURES/METHODS TO BE USED: You will follow one or more of these procedures depending upon the test:

1. Saliva will be collected by non-induced salivation into a container for analysis of glutamate and glutamine levels.
2. Determination of taste thresholds to sweet, salt, sour, bitter and umami will be determined by standard sensory evaluation methods. Compounds used will be sucrose, sodium chloride, citric acid, caffeine and monosodium glutamate (MSG).
3. Distilled water, unsalted tomato juice and beef broth with varying amounts of MSG will be tasted and evaluated for degree of liking and intensity. MSG levels will be equivalent to the amount found in the current food supply. Cuspidors will be provided for expectoration, in the event you would rather not swallow the samples.

RISKS INHERENT IN THE PROCEDURES: No risks are inherent in these procedures, except possible discomfort in tasting samples that you do not like. Cuspidors will be provided to expectorate samples, if desired. The only risk is a reaction to MSG, to which some individuals are sensitive. IF YOU HAVE A REACTION TO MSG OR YOU ARE PREGNANT OR NURSING A BABY, YOU SHOULD NOT PARTICIPATE IN THIS STUDY IF:

- YOU HAVE A REACTION TO MONOSODIUM GLUTAMATE (MSG), OR
- YOU ARE PREGNANT OR NURSING A BABY, OR
- YOU HAVE ALLERGIES TO TOMATO JUICE OR BEEF BROTH.

 Subjects initials_____ Date_____

I understand that it is not possible to identify all potential risks in an experimental procedure, but I believe that reasonable safeguards have been taken to minimize both the known and the potential, but unknown, risks.

BENEFITS: You will realize no immediate benefits, except a better understanding of your ability to perceive taste. In the longer term, this research will lead to a better understanding of the function of taste potentiators.

CONFIDENTIALITY: Information from this study will be coded so that it cannot be identified with you personally, and will remain confidential to the full extent of the law. Data will be grouped for statistical analysis and published anonymously.

LIMITATION OF LIABILITY: Because Colorado State University is a publicly-funded state institution, it may have only limited legal responsibility for injuries incurred as a result of participation in this study under a Colorado law known as the Colorado Governmental Immunity Act (Colorado Revised Statutes, Section 24-10-101, et seq.) In addition, under Colorado law, you must file any claim against the Univeristy within 180 days after the date of the injury.

In light of these laws, you are encouraged to evaluate your own health and disability insurance to determine whether you are covered for any injuries you might sustain by participating in this research, since it may be necessary for you to rely on your individual coverage for any such injuries. If you sustain injuries which you believe were caused by Colorado State University or its employees, we advise you to consult an attorney. Questions concerning treatment of subjects' rights may be directed to Celia Walker at (970) 491-1563.

PARTICIPATION: Participation is voluntary and all participants will read the following statement:

I understand that my participation in this research is voluntary. if I decide to participate in the study, I may withdraw my consent and stop participating at a time without penalty or loss of benefits to which I am otherwise entitled.

I have read and understand the information stated and willingly sign this consent form. My signature also acknowledges that I have received, on the date signed, a copy of this document of 2 pages.

_____
Subject name (printed)

_____ _____
Subject signature Date

_____ _____
Co-investigator signature Date

 Subjects initials_____ Date_____

APPENDIX II. Threshold and Intensity Tests, Study 1. Food Frequency Questionnaire

Please tell us a little about yourself:

Name:_____

Date of Birth:_____ Gender: _____ Male _____ Female

Race: _____White, not Hispanic _____African American _____Hispanic

_____Asian (country:_____) _____Native American

Please list any medications which you have taken during the past four weeks, including prescription and over-the-counter drugs:

---

How often do you eat the following foods (Mark the appropriate space)?

| Food | >1x/day | 3-6x/week | 1-2x/week | 1-3x/month | <1x/month | Never |
|---|---|---|---|---|---|---|
| Hard Cheese (Parmesan, Romano) | 5* | 4 | 3 | 2 | 1 | 0 |
| Soy Sauce | | | | | | |
| Light Soy Sauce | | | | | | |
| Other Asian Cooking Sauces | | | | | | |
| Please list:_____ | | | | | | |
| Tomatoes (Fresh or canned, Not sauce) | | | | | | |
| Tomato Sauces (For pasta, Pizza, etc) | | | | | | |
| Ketchup | | | | | | |
| Salsa | | | | | | |
| Hot Sauce | | | | | | |
| Mushrooms | | | | | | |
| Table Salt | | | | | | |
| Salt Substitutes | | | | | | |
| MSG | | | | | | |
| Grapes | | | | | | |
| Nectarines or peaches | | | | | | |
| Broccoli | | | | | | |
| Grape Juice | | | | | | |
| Tomato Juice | | | | | | |
| Instant Soup | | | | | | |
| Instant "Lunch", e.g., Cup of Noodles | | | | | | |

*Numerical values used to score food intake. These scores were not revealed to the subject.

APPENDIX III. Threshold and Intensity Tests, Study 1. Instructions and Scoresheets for Subjects

PARTICIPANT INSTRUCTIONS – FLAVOR INTENSITY

The purpose of today's session will be for you to evaluate three sets of samples for several taste qualities. One of these taste qualities is umami. Umami is a Japanese word meaning "deliciousness" and refers to the savory, meaty taste associated with MSG. Use the set of reference samples labeled "NONE" and "EXTREME" as reference points for all evaluations of umami made this week. Take a small sip of each reference before you begin the test. Salt refers to the taste associated with table salt.

Taste the samples from left to right. Each sample is coded with a random number to identify its scale on the scoresheet. For each taste quality, indicate the intensity of each sample by drawing a vertical line ( ) through the scale at the point best representing your perception. Your answer cannot be right or wrong; indicate which point on the scale best represents your perception of the flavor intensity.

Rinse well between each sample, using water and cracker, if you find it useful. If you prefer, you can use the spit cup, rather than swallow the sample; however, if you use the spit cup, be sure all taste buds on your tongue have experienced the sample.

When you have finished the set of water samples, please dispose of the cups in the trash can, set the tray on the table, and help yourself to any set of tomato juice samples. Follow this same procedure for the beef broth.

When you have completed the three sets, please help yourself to a reward on the back table, by the door! See you on Monday, November 27.

*Have a Nice and Tasteful Thanksgiving Holiday and*

*Thank You for Your Help!*

Scoresheet: Water

Name_____ Date_____ Set No._____

Taste the samples from left to right. Mark a vertical line through the horizontal scale at the point that best represents the intensity of each characteristic. Rinse well between samples. Use space under the scale or the back of the scoresheet to make comments, if desired.

OVERALL FLAVOR INTENSITY

Sample

_____ None Extreme

_____ None Extreme

_____ None Extreme

_____ None Extreme

_____ None Extreme

_____ None Extreme

INTENSITY OF UMAMI FLAVOR

_____ None Extreme

_____ None Extreme

_____ None Extreme

_____ None Extreme

_____ None Extreme

INTENSITY OF SALT FLAVOR

_____ None Extreme

_____ None Extreme

_____ None Extreme

_____ None Extreme

_____ None Extreme

Scoresheet: Tomato Juice

Name_____Date_____Set No._____

Taste the samples from left to right. Mark a vertical line through the horizontal scale at the point that best represents the intensity of each characteristic. Rinse well between samples. Use space under the scale or the back of the scoresheet to make comments, if desired.

OVERALL FLAVOR INTENSITY

Sample

| | | |
|---|---|---|
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |

INTENSITY OF UMAMI FLAVOR

| | | |
|---|---|---|
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |

INTENSITY OF TOMATO FLAVOR

| | | |
|---|---|---|
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |

INTENSITY OF SALT FLAVOR

| | | |
|---|---|---|
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |

Scoresheet: Beef Broth

Name_____Date_____Set No._____

Taste the samples from left to right. Mark a vertical line through the horizontal scale at the point that best represents the intensity of each characteristic. Rinse well between samples. Use space under the scale or the back of the scoresheet to make comments, if desired.

OVERALL FLAVOR INTENSITY

Sample

| | | |
|---|---|---|
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |

INTENSITY OF UMAMI FLAVOR

| | | |
|---|---|---|
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |

INTENSITY OF BEEF FLAVOR

| | | |
|---|---|---|
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |

INTENSITY OF SALT FLAVOR

| | | |
|---|---|---|
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |
| _____ | None | Extreme |

Preference Scoresheet

Name_____Date_____Set_____

PREFERENCE: BEEF BROTH

Taste the samples from left to right. Rank the samples according to your preference, or liking, by writing the rank in the space next to the code number, using the following numbers.

1 Like most
2
3
4
5 Like least

You must use all five numbers; there can be no ties. Rinse weel between samples. Use the space at the bottom to write any comments, if desired.

| Sample No. | Rank |
|---|---|
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |
| _____ | _____ |

Threshold Scoresheet

SET NO:_____

THRESHOLD TEST

Name:

You will receive a concentration series with samples of one taste quality (sweet, sour, salty, or bitter). The samples are arranged in order of increasing concentration. First rinse your mouth with the control water in order to become familiar with its taste. For most accurate results, swallow only a small amount of the sample; expectorate the remainder.

Start with the first sample and continue with the second, third, fourth, etc. RETASTING OF SAMPLES IS NOT ALLOWED. Taste each sample once and rate its taste; do not retaste it or previous samples.

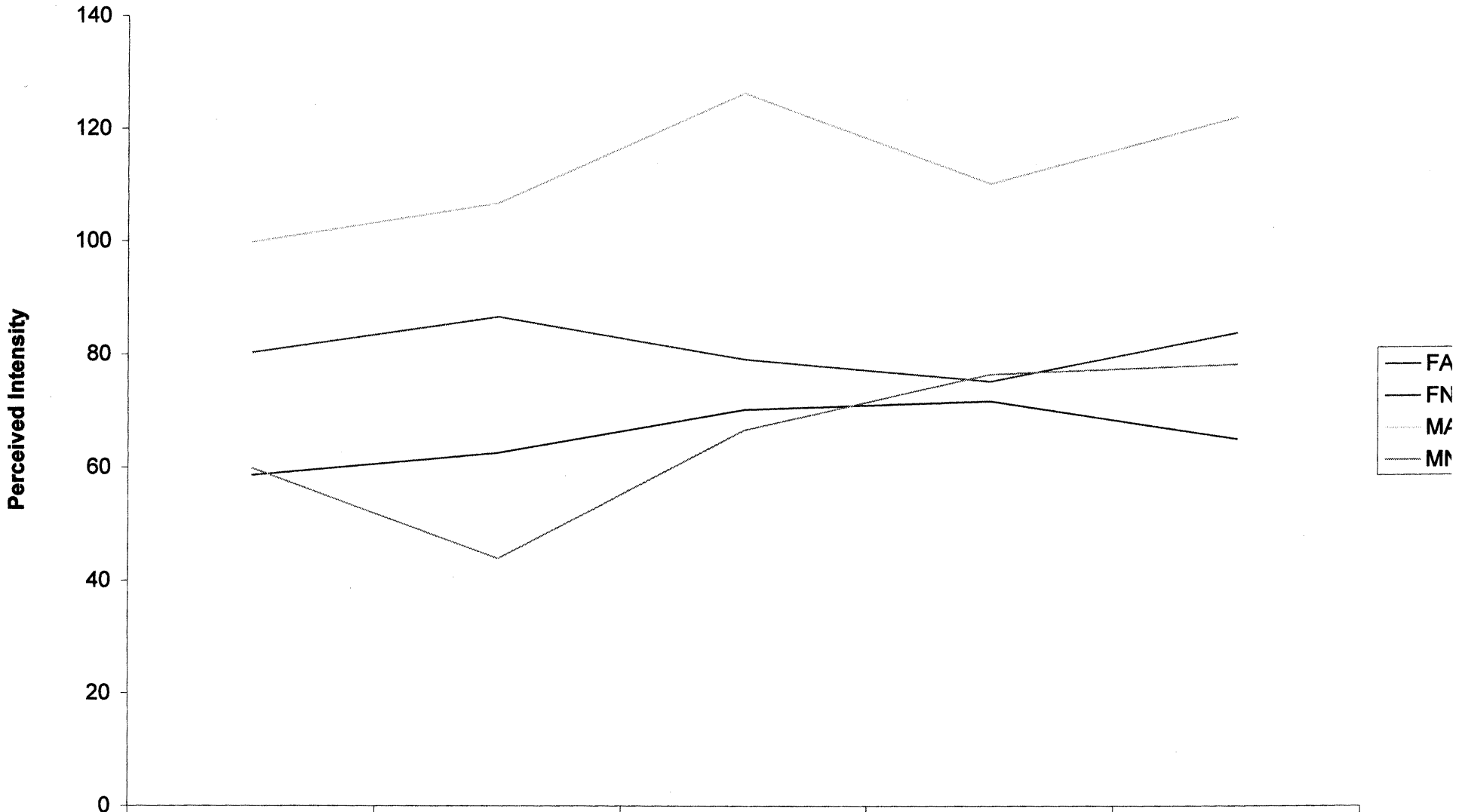
Describe the taste of each sample, along with any mouthfeel. Determine also the taste intensity of each sample by using the following scale:

0 = No taste/tastes like control water
? = Different from control water but taste cannot be identified
1 = Threshold; very weak; can identify a taste
2 = Weak
3 = Pronounced/distinct
4 = Strong
5 = Very strong

| Sample No. | Taste quality/mouthfeel | Intensity |
|---|---|---|
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |

# APPENDIX IV. GRAPHS OF MEAN PERCEIVED INTENSITY RESPONSES TO MSG CONCENTRATIONS IN THREE FOOD SYSTEMS, BY ETHNCITY AND GENDER

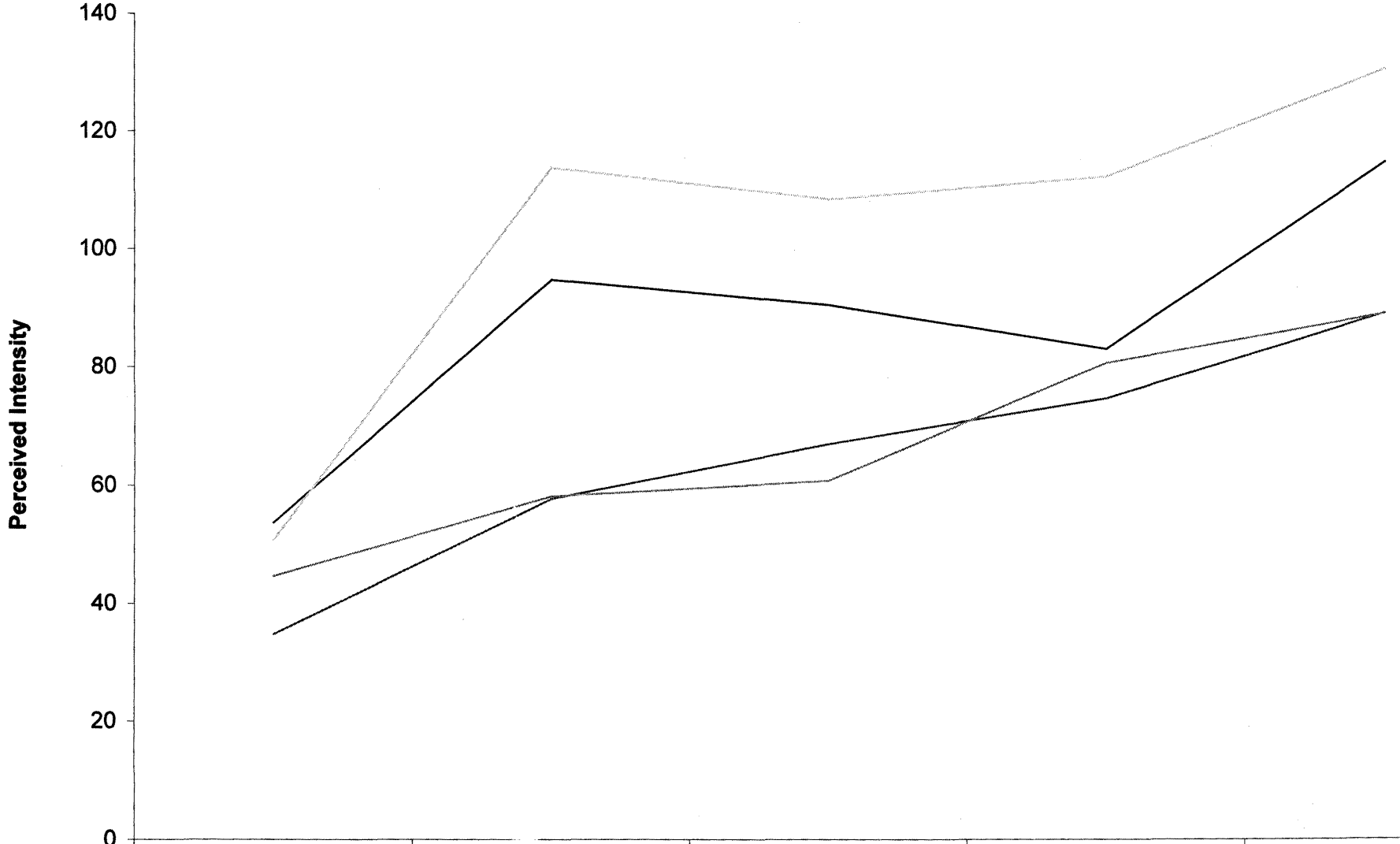
## MSG in Beef Broth: Overall Intensity




| | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|
| FA | 58.7 | 62.7 | 70.3 | 71.7 | 65.1 |
| FN | 80.4 | 86.7 | 79.2 | 75.2 | 83.9 |
| MA | 99.9 | 106.8 | 126.4 | 110.3 | 122.3 |
| MN | 60 | 44 | 66.7 | 76.5 | 78.3 |

Concentration (% MSG w/v)

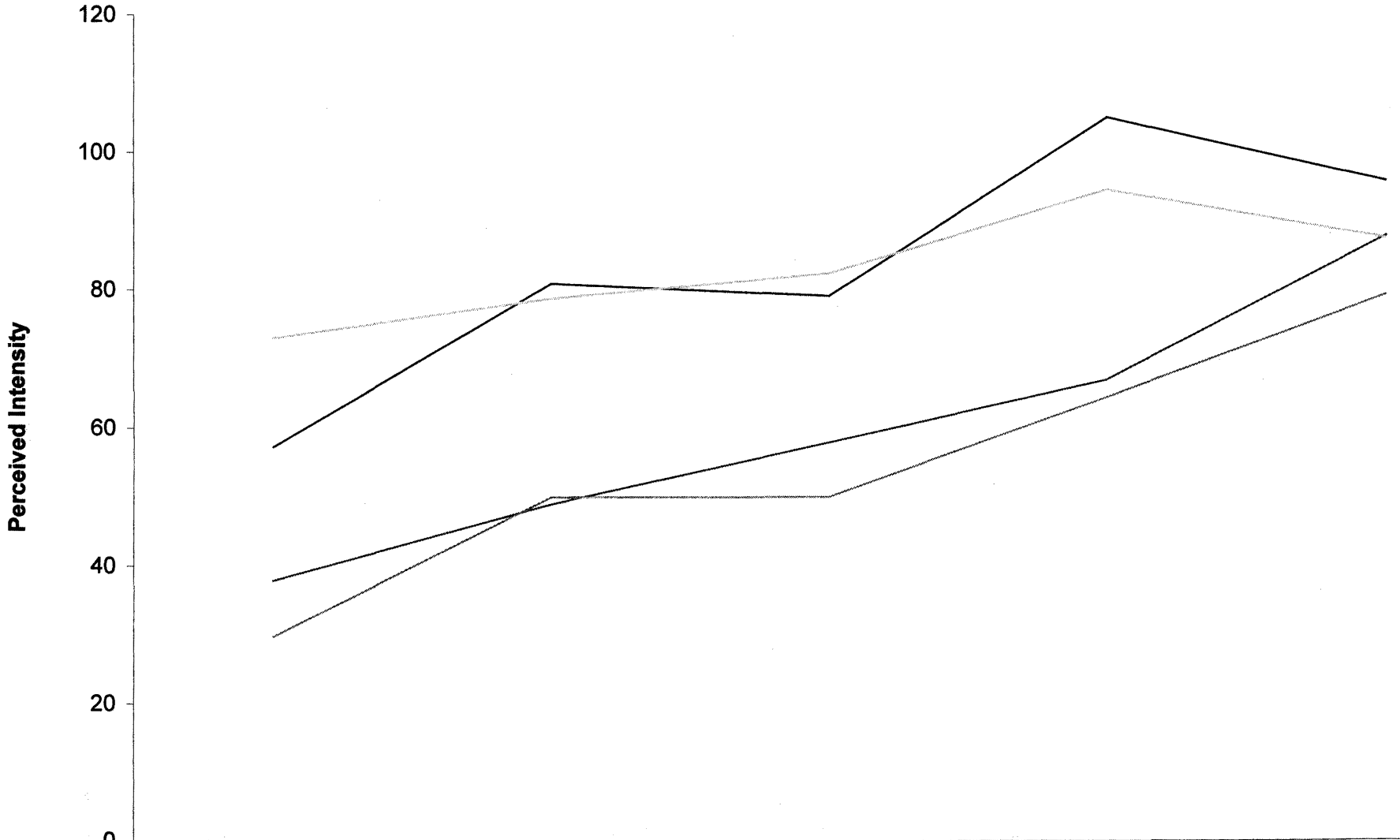
## MSG in Beef Broth: Umami Intensity




| | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|
| FA | 53.8 | 94.7 | 90.4 | 83 | 114.7 |
| FN | 34.9 | 57.8 | 67 | 74.6 | 89.1 |
| MA | 50.8 | 113.7 | 108.4 | 112.3 | 130.6 |
| MN | 44.7 | 58.2 | 60.8 | 80.6 | 89 |

Concentration (% MSG w/v)

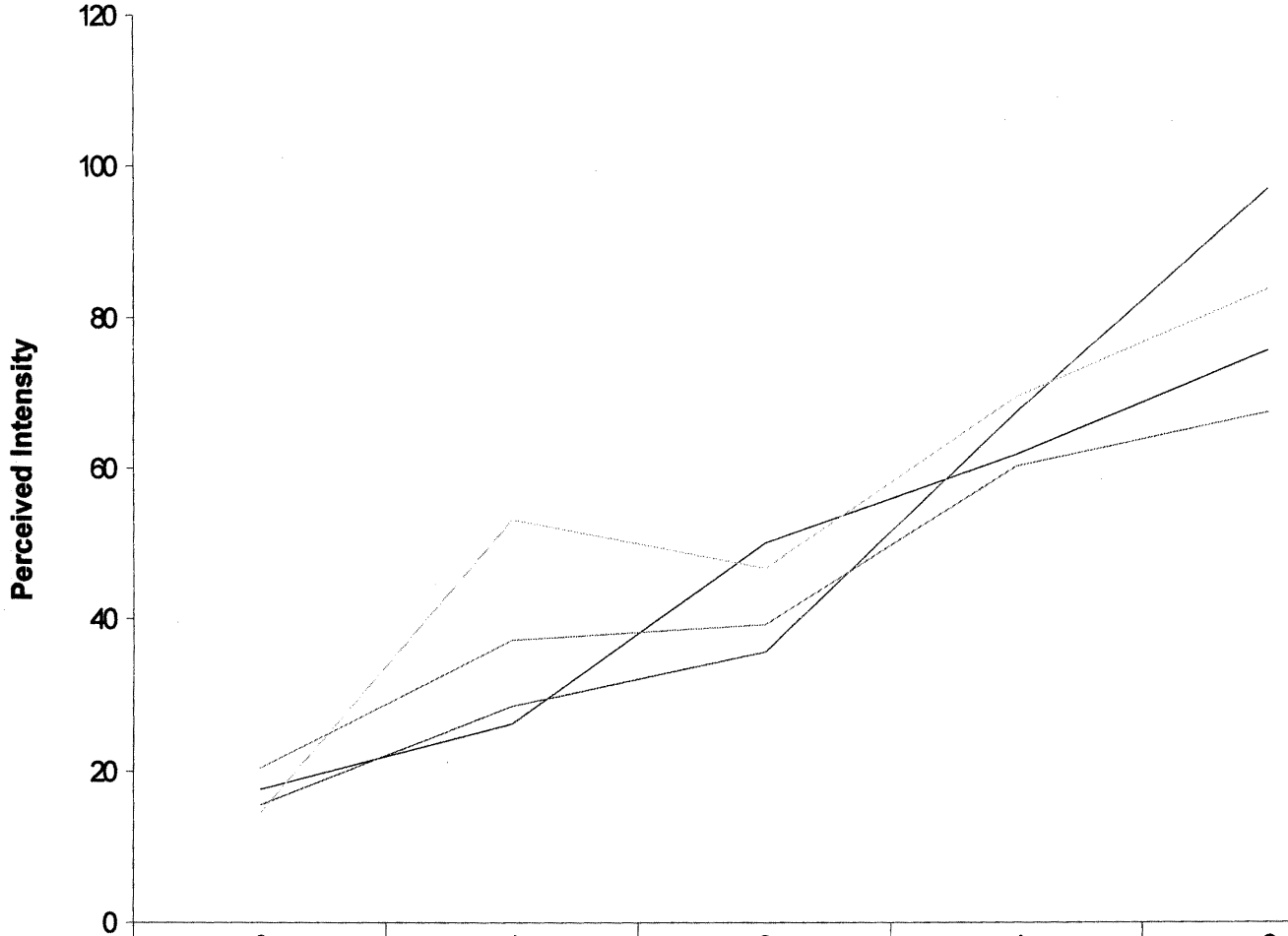
## MSG in Beef Broth: Beef Flavor Intensity




| | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|
| FA | 57.3 | 80.8 | 79.1 | 105.1 | 95.9 |
| FN | 37.9 | 49 | 57.9 | 66.9 | 87.9 |
| MA | 73 | 78.7 | 82.4 | 94.6 | 87.6 |
| MN | 29.8 | 50 | 50 | 64.4 | 79.3 |

Concentration (% MSG w/v)

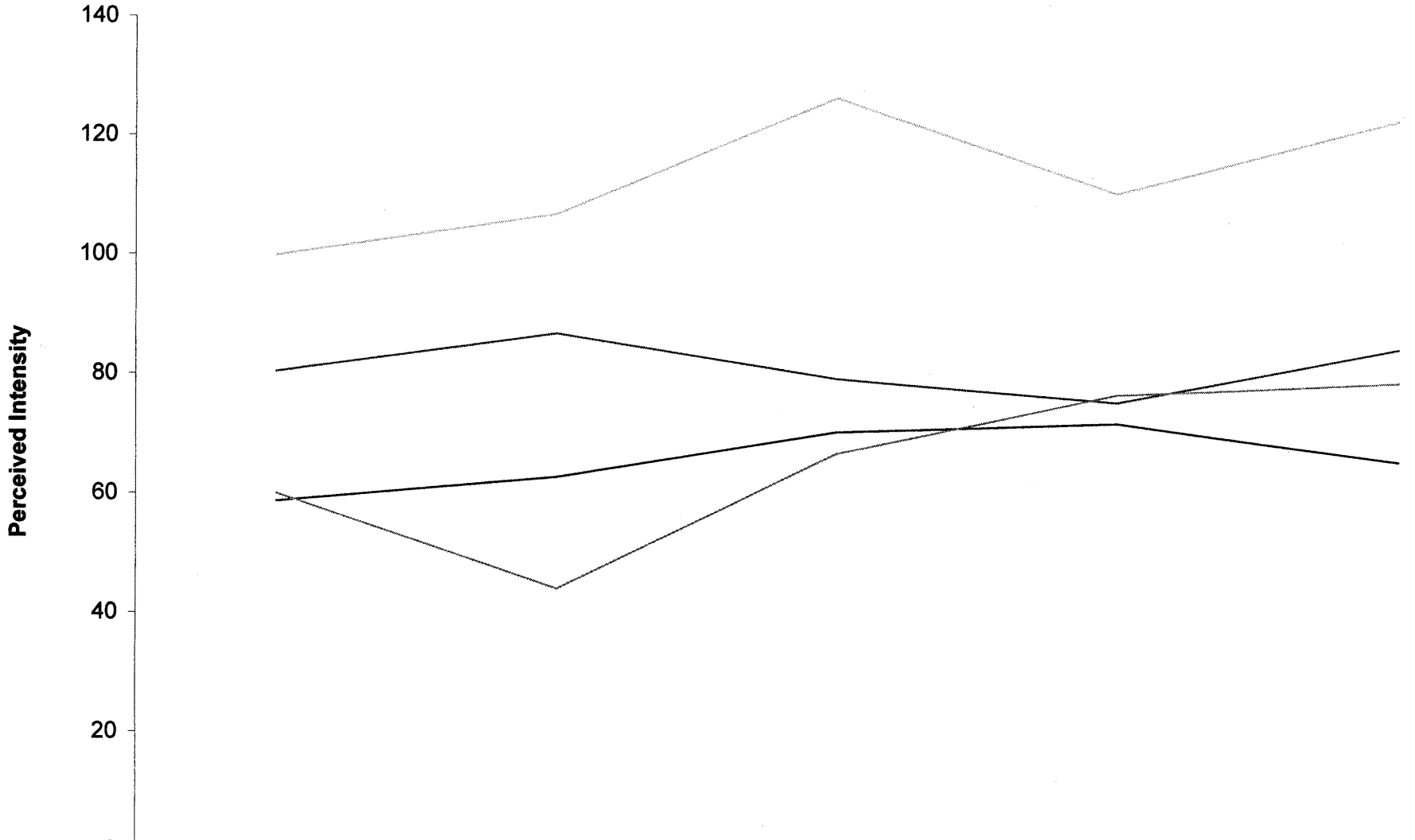
## MSG in Beef Broth: Salt Intensity




| | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|
| FA | 17.5 | 26.2 | 50 | 61.7 | 75.5 |
| FN | 15.6 | 28.5 | 35.8 | 67.3 | 97.1 |
| MA | 14.6 | 53.1 | 46.7 | 69.4 | 83.7 |
| MN | 20.4 | 37.4 | 39.4 | 60.2 | 67.4 |

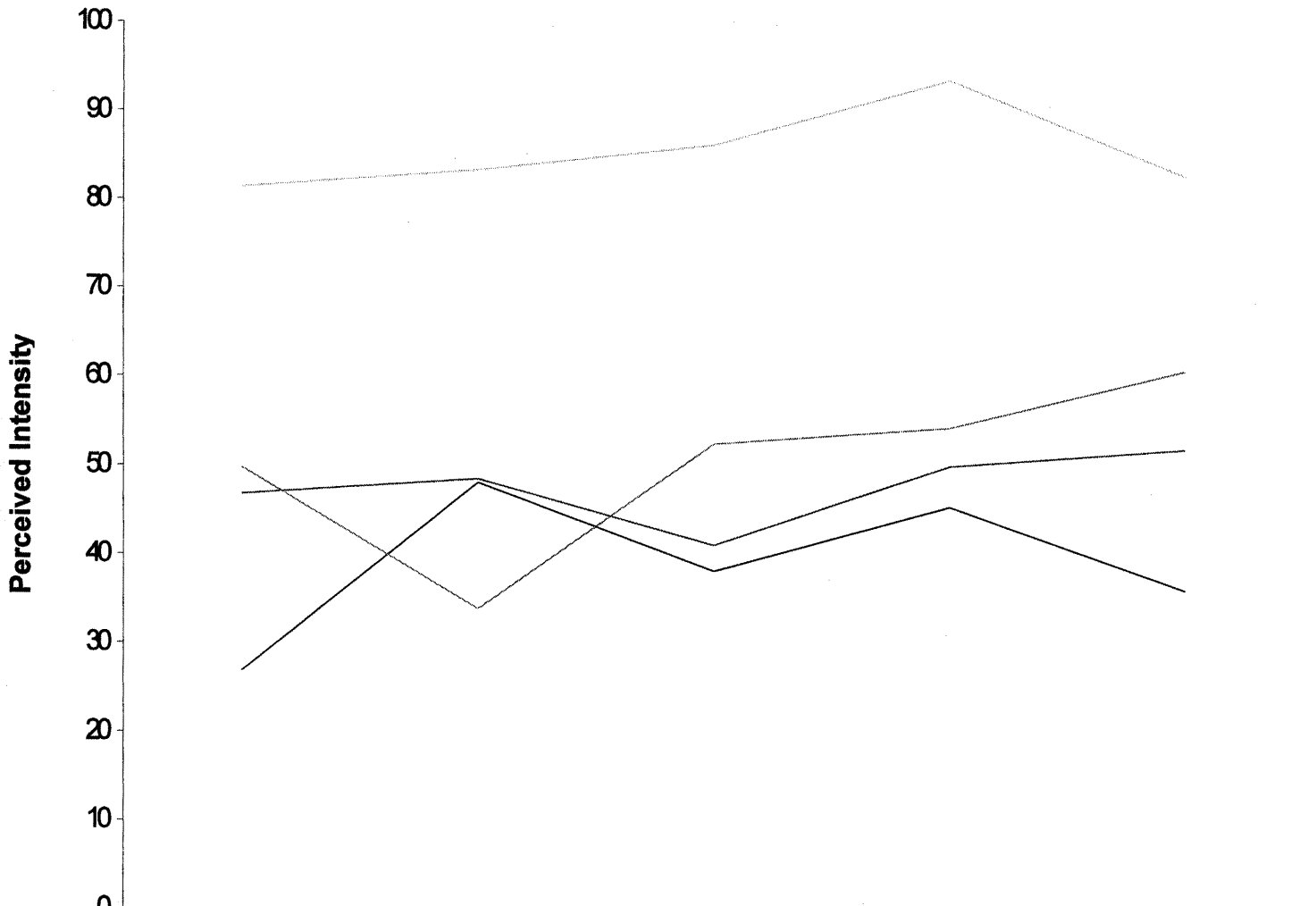
Concentration (% MSG w/v)

## MSG in Tomato Juice: Overall Intensity




| | 0 | 0.03 | 0.05 | 0.1 | 0.2 |
|---|---|---|---|---|---|
| FA | 58.7 | 62.7 | 70.3 | 71.7 | 65.1 |
| FN | 80.4 | 86.7 | 79.2 | 75.2 | 83.9 |
| MA | 99.9 | 106.8 | 126.4 | 110.3 | 122.3 |
| MN | 60 | 44 | 66.7 | 76.5 | 78.3 |

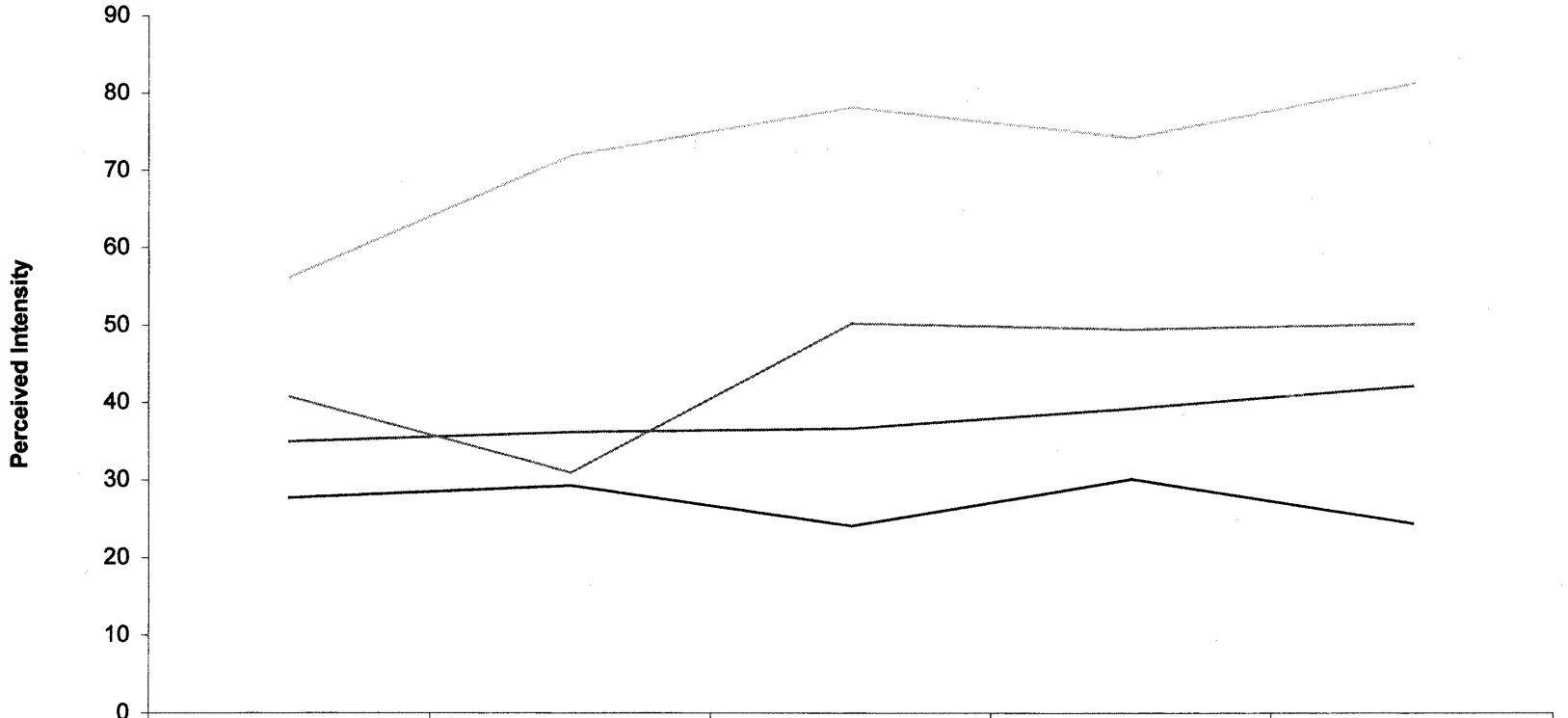
**Concentration (% MSG w/v)**




| | 0 | 0.03 | 0.05 | 0.1 | 0.2 |
|---|---|---|---|---|---|
| FA | 26.8 | 47.9 | 37.9 | 45.1 | 35.6 |
| FN | 46.7 | 48.3 | 40.8 | 49.7 | 51.5 |
| MA | 81.4 | 83.2 | 86 | 93.3 | 82.3 |
| MN | 49.7 | 33.7 | 52.2 | 54 | 60.3 |

Concentration (%MSG w/v)

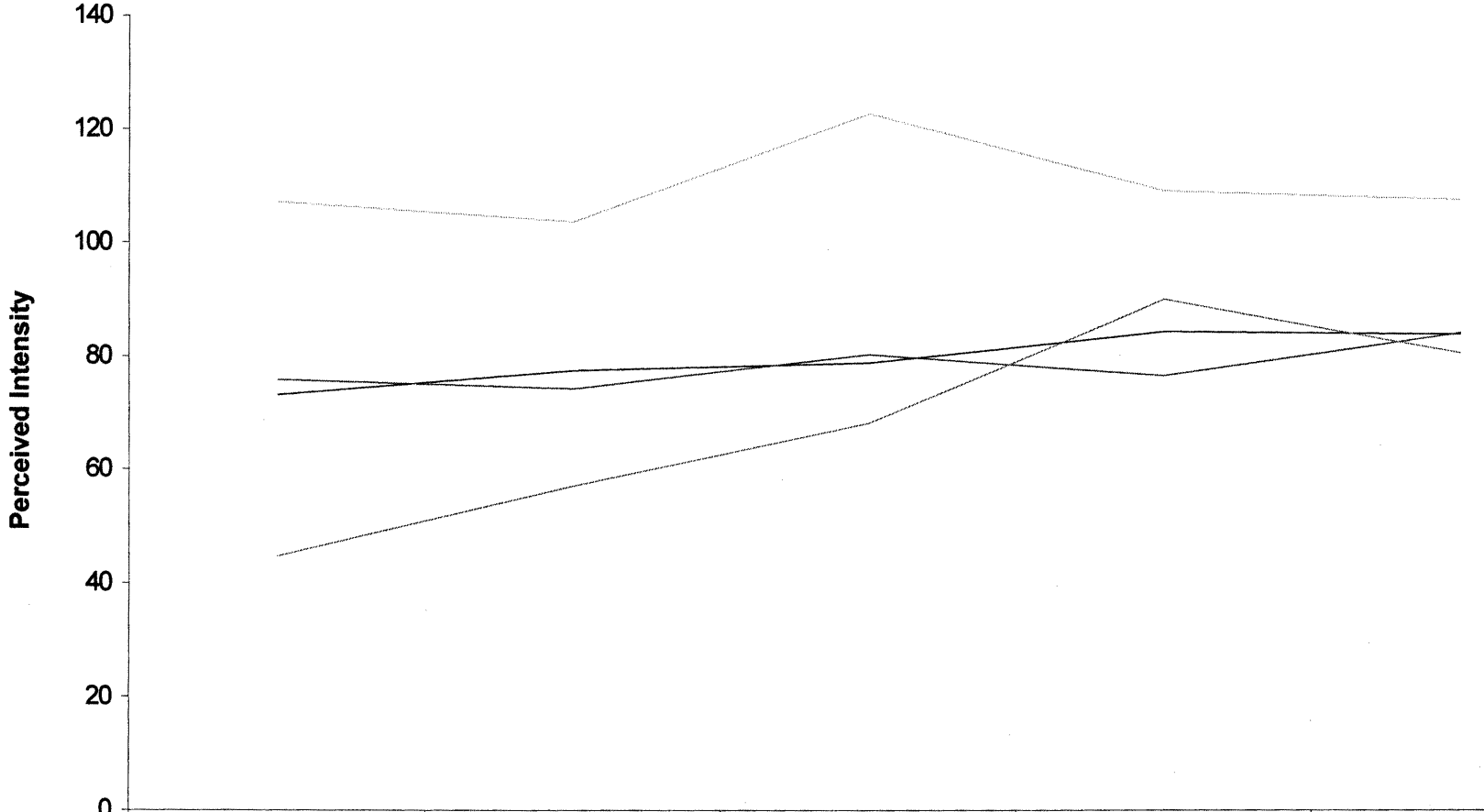
## MSG in Tomato Juice: Salt Intensity




| | 0 | 0.03 | 0.05 | 0.1 | 0.2 |
|---|---|---|---|---|---|
| FA | 27.8 | 29.4 | 24.2 | 30.2 | 24.5 |
| FN | 35 | 36.3 | 36.7 | 39.3 | 42.2 |
| MA | 56.3 | 72.1 | 78.3 | 74.3 | 81.3 |
| MN | 40.8 | 31 | 50.3 | 49.5 | 50.2 |

Concentration (% MSG w/v)

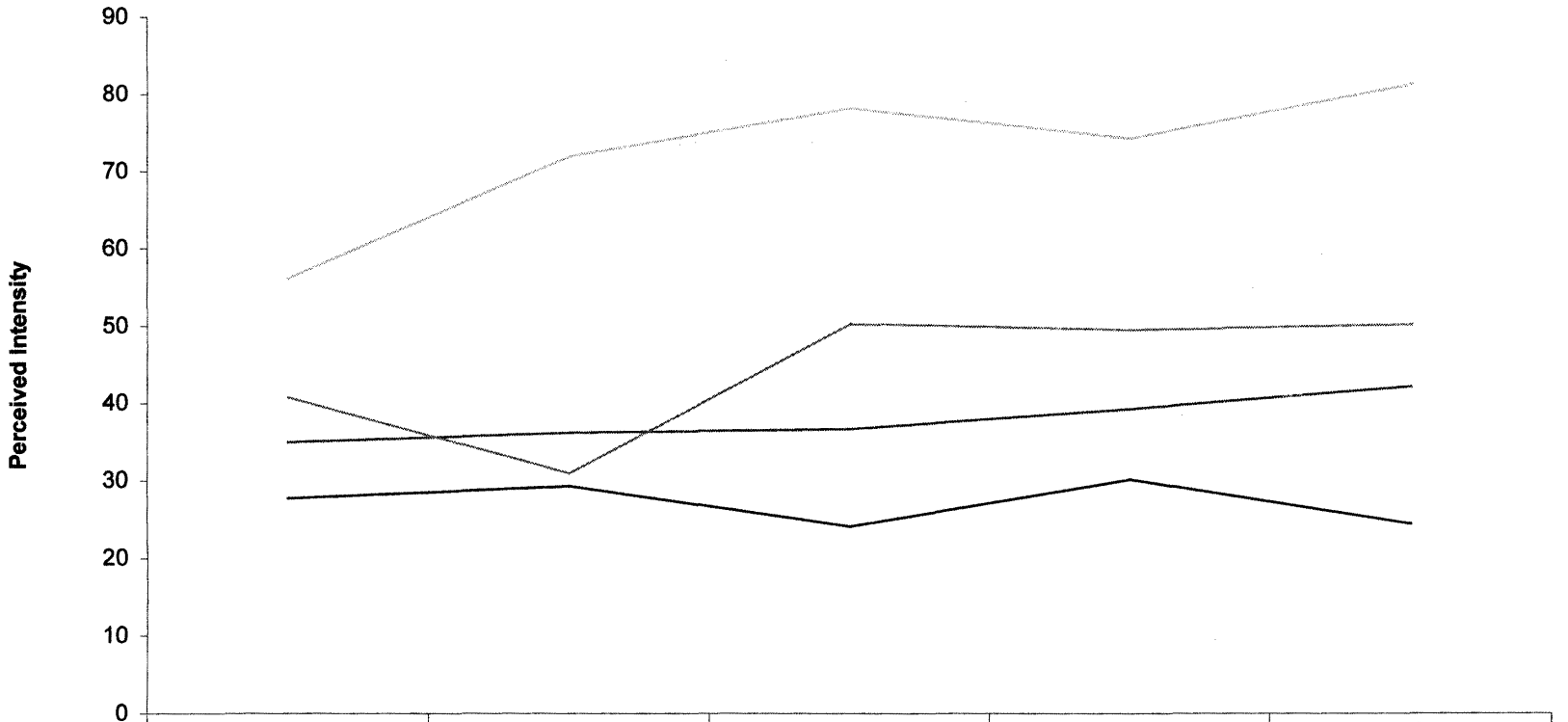
## MSG in Tomato Juice: Tomato Flavor Intensity




| | 0 | 0.03 | 0.05 | 0.1 | 0.2 |
|---|---|---|---|---|---|
| FA | 73.1 | 77.4 | 78.9 | 84.6 | 84.1 |
| FN | 75.8 | 74.2 | 80.4 | 76.8 | 84.4 |
| MA | 107.2 | 103.7 | 122.9 | 109.4 | 107.8 |
| MN | 44.7 | 57 | 68.2 | 90.2 | 80.8 |

Perceived Intensity (% MSG w/v)

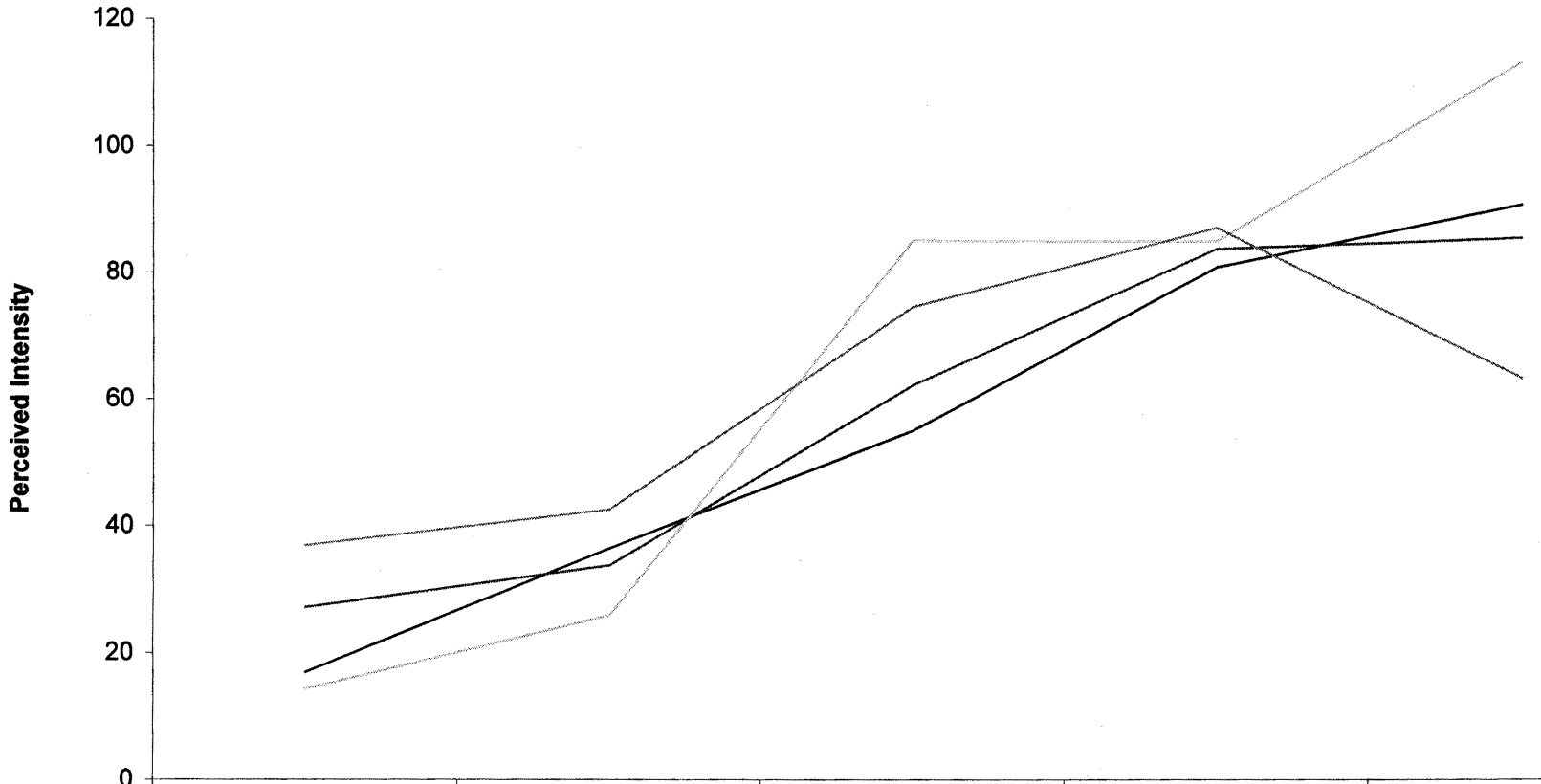
## MSG in Tomato Juice: Salt Intensity




| | 0 | 0.03 | 0.05 | 0.1 | 0.2 |
|---|---|---|---|---|---|
| FA | 27.8 | 29.4 | 24.2 | 30.2 | 24.5 |
| FN | 35 | 36.3 | 36.7 | 39.3 | 42.2 |
| MA | 56.3 | 72.1 | 78.3 | 74.3 | 81.3 |
| MN | 40.8 | 31 | 50.3 | 49.5 | 50.2 |

Concentration (% MSG w/v)

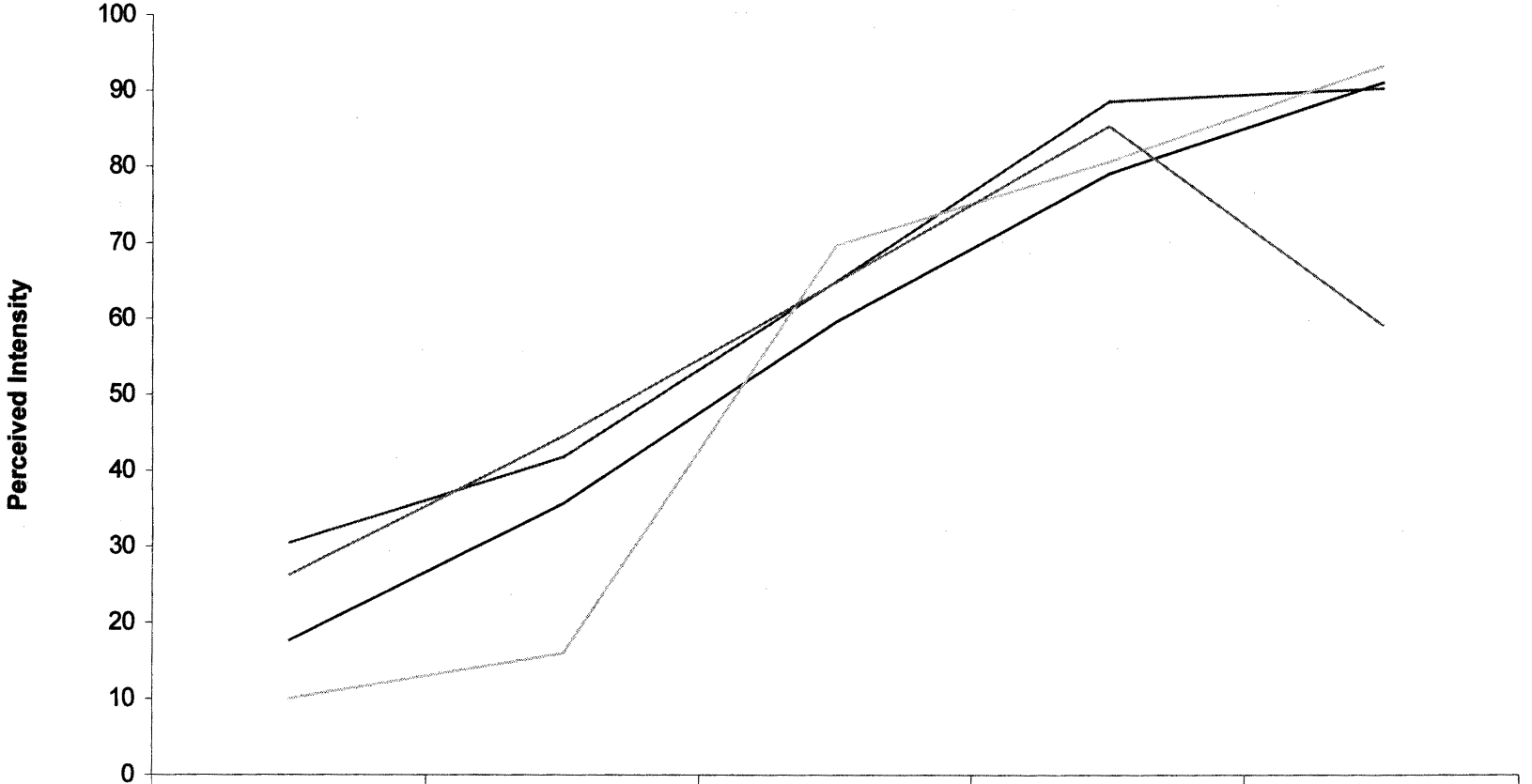
## MSG in Water: Overall Intensity




| | 0 | 0.03 | 0.05 | 0.08 | 0.01 |
|---|---|---|---|---|---|
| FA | 16.9 | 36.5 | 55.1 | 80.9 | 90.7 |
| FN | 27.1 | 33.8 | 62.3 | 83.8 | 85.5 |
| MA | 14.3 | 25.9 | 85.1 | 85 | 113.2 |
| MN | 36.9 | 42.6 | 74.6 | 87.1 | 63.4 |

**Concentration (% MSG w/v)**

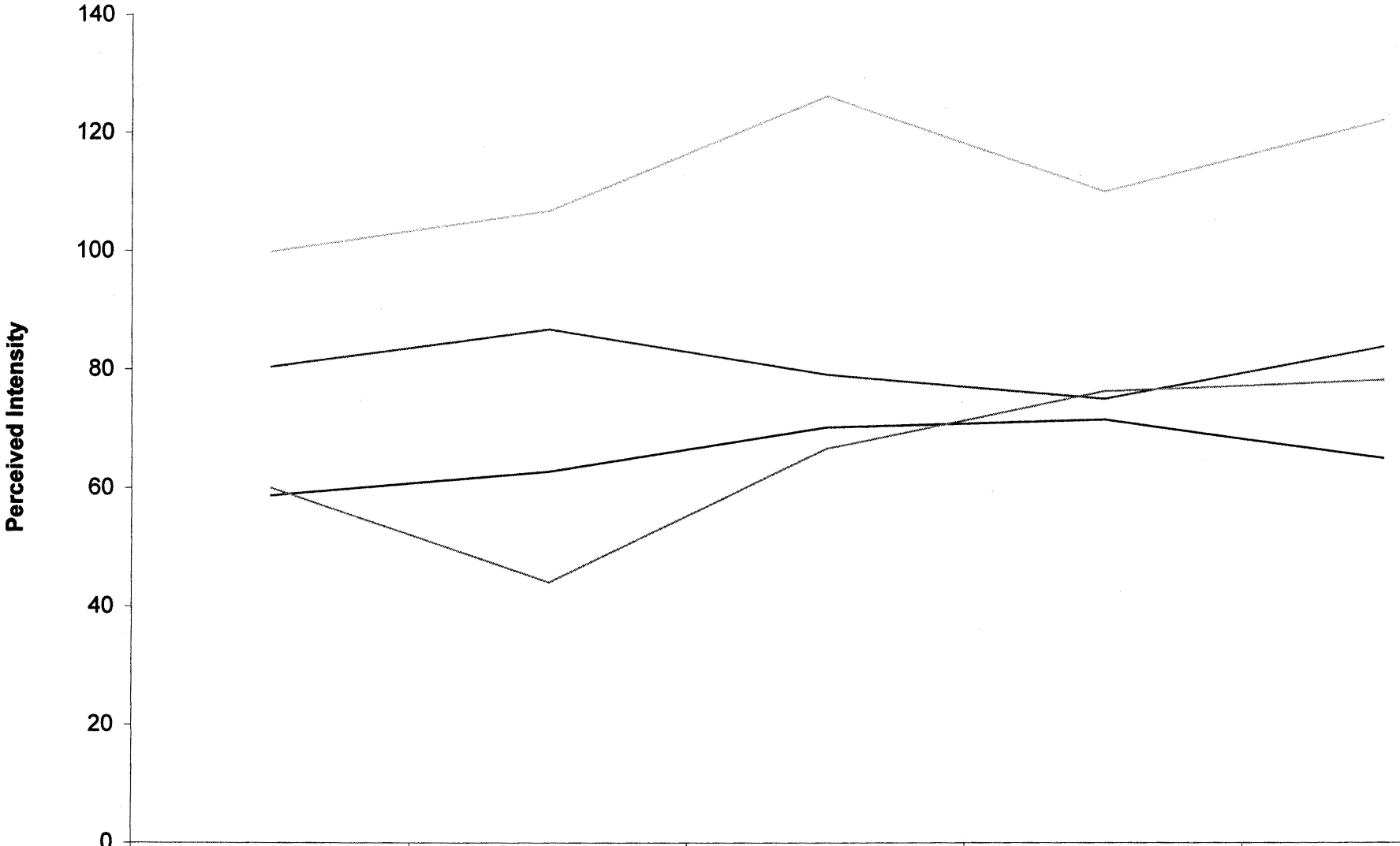
## MSG in Water: Umami Intensity




| | 0 | 0.03 | 0.05 | 0.08 | 0.01 |
|---|---|---|---|---|---|
| FA | 17.7 | 35.8 | 59.7 | 79.1 | 91.2 |
| FN | 30.6 | 41.9 | 65 | 88.7 | 90.4 |
| MA | 10.1 | 16 | 69.8 | 80.7 | 93.4 |
| MN | 26.3 | 44.7 | 64.9 | 85.4 | 59.2 |

Concentration (% MSG w/v)

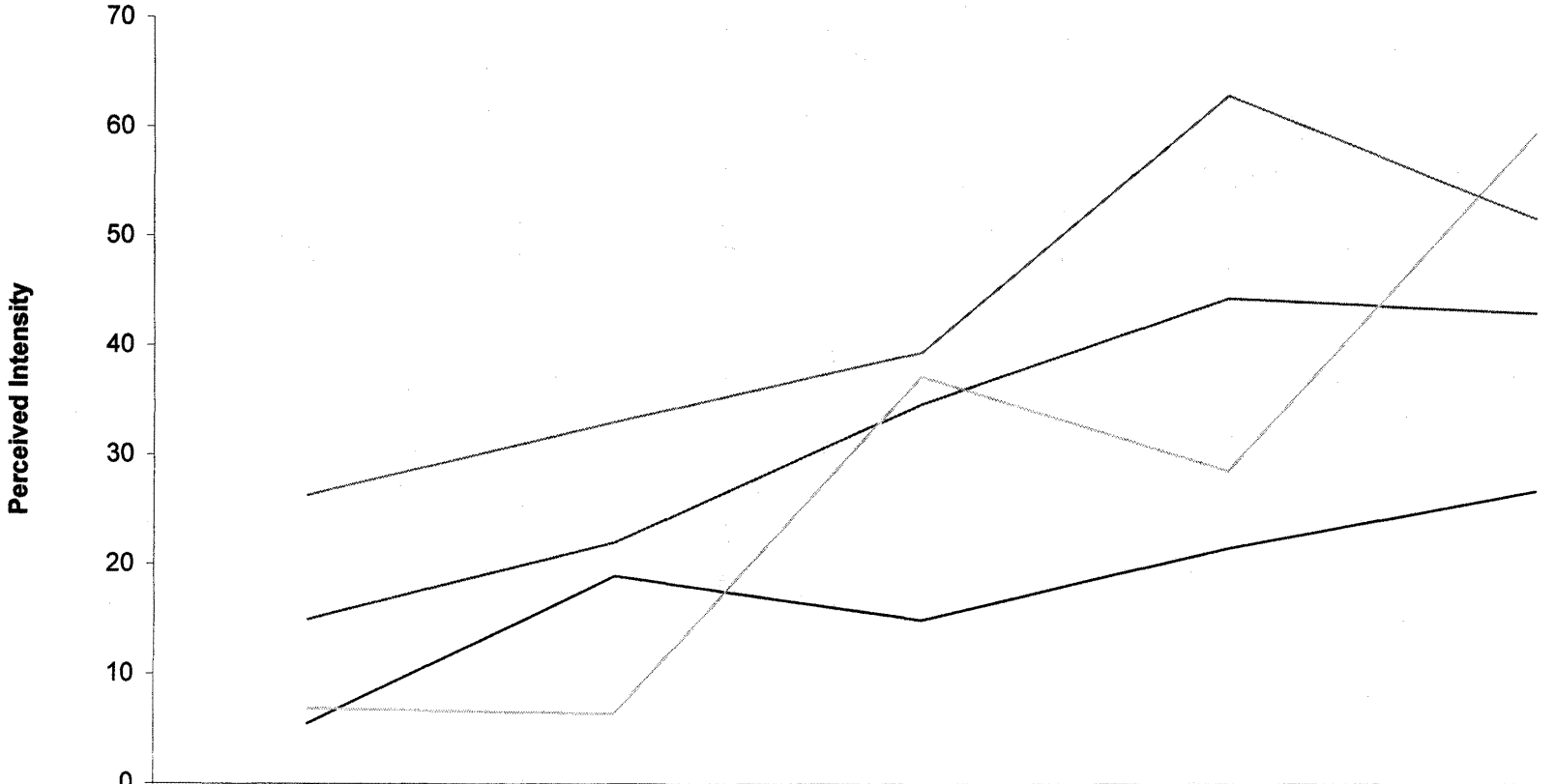
## MSG in Tomato Juice: Overall Intensity




| | 0 | 0.03 | 0.05 | 0.1 | 0.2 |
|---|---|---|---|---|---|
| FA | 58.7 | 62.7 | 70.3 | 71.7 | 65.1 |
| FN | 80.4 | 86.7 | 79.2 | 75.2 | 83.9 |
| MA | 99.9 | 106.8 | 126.4 | 110.3 | 122.3 |
| MN | 60 | 44 | 66.7 | 76.5 | 78.3 |

**Concentration (% MSG w/v)**

## MSG in Water: Salt Intensity




| | 0 | 0.03 | 0.05 | 0.08 | 0.01 |
|---|---|---|---|---|---|
| FA | 5.5 | 18.9 | 15 | 21.6 | 26.8 |
| FN | 15 | 22 | 34.7 | 44.4 | 43 |
| MA | 6.9 | 6.4 | 37.2 | 28.7 | 59.4 |
| MN | 26.3 | 33 | 39.4 | 62.9 | 51.6 |

**Concentration (% MSG w/v)**

# APPENDIX V. Threshold and Intensity Tests, Study 2. Consent Form

**COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD**

**SUBJECT CONSENT**
September 1, 1997 (Version 1)

**ROCKY MOUNTAIN TASTE AND SMELL CENTER:**
**TASTE PERCEPTION STUDY**

## PROJECT DESCRIPTION

You are being asked to take part in a study to determine your ability to taste certain compounds. You will be asked to complete several tests of your sense of taste and to provide a sample of your saliva. You are being asked to be in this study because you have indicated that you are healthy and have no obvious problems with either your taste or your smell.

## PROCEDURES INVOLVED

If you agree to take part in this study, you will complete the following procedures, which will last approximately one hour:

1. You will provide approximately 1 teaspoon of saliva, collected by having your spit into a small bottle. Your saliva will be analyzed for naturally occurring substances. Your ability to taste different substances will be related to levels of these substances.

2. You will be asked to complete several tasks involving your sense of taste. You will be asked to taste small amounts of common substances that are used in food and to report your experiences. These tests are non-invasive.

## DISCOMFORTS AND RISKS

There are no risks involved in these procedures. If you have had any health problems related to eating salt (sodium chloride) or MSG (monosodium glutamate), you should not participate in this study. If you are pregnant or nursing a baby, you should not participate in this study.

## BENEFITS

There are no direct advantages of this procedure to you. Monetary compensation is being offered for participation in this study, and you will be paid $15 for one hour, which is the time it will take for you to complete the study.

**INJURY AND COMPENSATION**

Risk of injury in this study is minimal. If you are hurt by this research, we will provide medical care if you want it, but you will have to pay for the care that is needed. You will not be paid for any other loss as a result of the injury, such as loss of wages, pain and suffering. Further information can be obtained by calling Maria Buscarello at 237-1162.

**STUDY WITHDRAWAL**

You may stop being in the study without affecting the loss of benefits to which you are entitled. If the investigator believes that you are experiencing any reaction to the test, you will be asked to withdraw from the study.

**INVITATION FOR QUESTIONS**

Please ask questions about any aspect of this research or this consent either now or in the future. You can direct your questions to Maria Buscarello at 237-1162 or Dr. Miriam Linschoten at 315-6600. If you have questions regarding your rights as a research subject, please call Vicky Starbuck, Secretary of the COMIRB, at 315-8081.

**CONFIDENTIALITY**

Your investigator will treat your identity with professional standards of confidentiality. Since some questions you are being asked may provide information that you do not want others to know, the questionnaires will have a code number, but not you name. All records will be kept under lock and key. Data will be grouped for statistical analyses and may be published in scientific journals, but your identity will not be revealed. Federal regulations limit the release of information obtained during research studies and this information can only be release to others under subpoena from a court.

**AUTHORIZATION**

I HAVE READ THIS PAPER ABOUT THE STUDY OR IT WAS READ TO ME. I KNOW WHAT WILL HAPPEN, BOTH THE POSSIBLE GOOD AND BAD. I CHOOSE TO BE IN THIS STUDY. I KNOW I CAN STOP BEING IN THE STUDY WITHOUT PENALTY. I WILL GET A COPY OF THIS CONSENT FORM.

_____

Subject name (printed)

_____ _____

Subject signature Date

_____ _____

Investigator signature Date

APPENDIX VI. Threshold and Intensity Tests, Study 2 and Time-Intensity Test: Food Frequency Questionnaire

Name:_____Gender: F M Date of Birth:_____
(Month/Year)

Race: _____African American _____Hispanic _____Native American _____White (not Hispanic)

_____Asian (Country:_____) Country of birth: _____

What medications have you taken during the past two weeks (including over-the-counter):

_____

How often do you eat the following foods (X the appropriate space):

| Food | > 1x/day | 3-6x/week | 1-2x/week | 1-3x/month | <1x/month | Never |
|---|---|---|---|---|---|---|
| **Parmesan** | 5* | 4 | 3 | 2 | 2 | 0 |
| **Soy Sauce** | | | | | | |
| **Lite Soy Sauce** | | | | | | |
| **Other Asian Sauces** | | | | | | |
| **Can, Fresh Tomatoes** | | | | | | |
| **Tomato Sauces** | | | | | | |
| **Ketchup** | | | | | | |
| **Salsa** | | | | | | |
| **Hot Sauce** | | | | | | |
| **Mushrooms** | | | | | | |
| **Table Salt** | | | | | | |
| **MSG** | | | | | | |
| **Grapes** | | | | | | |
| **Peaches & Nectarines** | | | | | | |
| **Broccoli** | | | | | | |
| **Tomato & Vege Juice** | | | | | | |
| **Instant Soups, Ramen** | | | | | | |
| **Instant "Lunch"** | | | | | | |
| **Salted snacks** | | | | | | |
| **Seasoned Snacks** | | | | | | |
| **Sausage, lunch meat** | | | | | | |
| **Canned, frozen meals** | | | | | | |

*Numerical values used to score food intake. These scores were not revealed to the subject.

## APPENDIX VII. Threshold and Intensity Tests, Study 2: Data Collection Sheet

NAME: TELEPHONE: #

DATE: AGE: DOB: SEX:

THRESHOLDS

| | NaCl | MSG |
|---|---|---|
| # | | |
| log □ | | |
| □ | | |
| ci | | |
| %/p | | |

MAGNITUDE ESTIMATION

NaCl

| | replication 1 | Replication 2 | replication 3 |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |

MSG

| | replication 1 | replication 2 | replication 3 |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |

## APPENDIX VIII. Threshold and Intensity Tests, Study 2: Threshold Concentration Series for MSG and NaCl (Maximum Likelihood Staircase Procedure)

**NaCl**

-2.00000 estimated alpha
3.50000 fixed beta

| Step | Log | Moles/liter | |
|---|---|---|---|
| 1 | -4.50 | 0.00003 | [take 10 ml step 3, add 90 ml H20] |
| 2 | -4.25 | 0.00006 | [take 10 ml step 4, add 90 ml H20] |
| 3 | -4.00 | 0.00010 | [take 10 ml step 5, add 90 ml H20] |
| 4 | -3.75 | 0.0018 | [take 10 ml step 6, add 90 ml H20] |
| 5 | -3.50 | 0.00032 | [take 10 ml step 7, add 90 ml H20] |
| 6 | -3.25 | 0.00056 | [take 10 ml step 8, add 90 ml H20] |
| 7 | -3.00 | 0.00100 | [take 10 ml step 9, add 90 ml H20] |
| 8 | -2.75 | 0.00178 | [take 10 ml step 10, add 90 ml H20] |
| 9 | -2.50 | 0.00316 | [take 10 ml step 11,add 90 ml H20] |
| 10 | -2.25 | 0.00562 | [take 10 ml step 12, add 90 ml H20] |
| 11 | -2.00 | 0.01000 | [take 10 ml step 13, add 90 ml H20] |
| 12 | -1.75 | 0.01778 | [take 10 ml step 14, add 90 ml H20] |
| 13 | -1.50 | 0.03162 | [take 10 ml step 15, add 90 ml H20] |
| 14 | -1.25 | 0.05623 | [take 10 ml step 16, add 90 ml H20] |
| 15 | -1.00 | 0.1000 | [take 10 ml step 17, add 90 ml H20] |
| 16 | -0.75 | 0.17783 | [1.04 gr NaCl/100/ml] |
| 17 | -0.50 | 0.31623 | [1.85 gr NaCl/100/ml] |
| 18 | -0.25 | 0.56234 | [3.29 gr NaCl/100/ml] |
| 19 | 0.00 | 1.00000 | [5.84 gr NaCl/100/ml] |
| 20 | 0.25 | 1.7783 | |

**MSG**

-2.75000 estimated alpha
3.50000 fixed beta

| Step | Log | Moles/liter | |
|---|---|---|---|
| 1 | -5.25 | 0.000006 | [take 10 ml step 5, add 90 ml H20] |
| 2 | -5.00 | 0.00001 | [take 10 ml step 6, add 90 ml H20] |
| 3 | -4.75 | 0.00002 | [take 10 ml step 7, add 90 ml H20] |
| 4 | -4.50 | 0.00003 | [take 10 ml step 8, add 90 ml H20] |
| 5 | -4.25 | 0.00006 | [take 10 ml step 9, add 90 ml H20] |
| 6 | -4.00 | 0.00010 | [take 10 ml step 10, add 90 ml H20] |
| 7 | -3.75 | 0.0018 | [take 10 ml step 11, add 90 ml H20] |
| 8 | -3.50 | 0.00032 | [take 10 ml step 12, add 90 ml H20] |
| 9 | -3.25 | 0.00056 | [take 10 ml step 13, add 90 ml H20] |
| 10 | -3.00 | 0.00100 | [take 10 ml step 14, add 90 ml H20] |
| 11 | -2.75 | 0.00178 | [take 10 ml step 15, add 90 ml H20] |
| 12 | -2.50 | 0.00316 | [take 10 ml step 16,add 90 ml H20] |
| 13 | -2.25 | 0.00562 | [take 10 ml step 17, add 90 ml H20] |
| 14 | -2.00 | 0.01000 | [take 10 ml step 18, add 90 ml H20] |
| 15 | -1.75 | 0.01778 | [take 10 ml step 19, add 90 ml H20] |
| 16 | -1.50 | 0.03162 | [take 10 ml step 20, add 90 ml H20] |
| 17 | -1.25 | 0.05623 | [take 10 ml step 21, add 90 ml H20] |
| 18 | -1.00 | 0.1000 | [1.47 gr MSG/100/ml] |
| 19 | -0.75 | 0.17783 | [2.62 gr MSG/100/ml] |
| 20 | -0.50 | 0.31623 | [4.65 gr MSG/100/ml] |
| 21 | -0.25 | 0.56234 | [8.27 gr MSG/100/ml] |

Appendix IX. Time-Intensity Tests. Instructions

## TIME INTENSITY INSTRUCTIONS

1. Write your initials (the first initial of your first and last name) on the tape that is on the bottle. Salivate, or spit, saliva into the bottle until the amount of saliva in the bottle reaches the bottom of the tape.

2. Place the bottle with the saliva by the small door and flip the light switch to signal that you are ready for the sample set.

3. Rinse two times, spitting the water out into the disposal.

4. Taste a small amount of the "Salt Standard". Rinse twice after tasting the standard. Feel free to taste the salt standard as often as needed throughout the test to refresh your memory.

5. When the green light comes on, press the red button. A yellow light will come on. Immediately put the entire contents of the first samples into your mouth. Gently swirl the sample around your mouth until it has covered your oral cavity. Rate the **overall flavor intensity** of the sample by moving the "joystick" up as the sample becomes more intense and down as it becomes less intense. Do not move the joystick if the sample intensity does not change.

6. When the red light comes on (after 20 seconds), spit out the entire sample. Continue to use the joystick to indicate how much the flavor intensity increases of decreases. Do not move your mouth until the taste sensation is gone.

7. When you no longer taste anything, push the joystick back to none until you feel and hear it "click" into place. The red light will come back on.

8. The rid light will stay on for 4 minutes. During this time, rinse your mouth at least 4 times. If you need more water, please flip the switch to "on" and place your cup by the small door.

9. When the green light comes on, begin again at step 5. Repeat steps 5 through 8 for the remaining samples.

THANK YOU VERY MUCH!