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Effect of Repeated Presentation on Sweetness Intensity of Binary and Ternary Mixtures of Sweeteners

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Abstract

The purpose of the present study was to determine the effect of repeated presentation of the same sweet stimulus on sweetness intensity ratings. The sweet stimuli tested in this study were binary and ternary blends of 14 sweeteners that varied widely in chemical structure. A trained panel evaluated the sweetness intensity over four sips of a given mixture presented at 30 s intervals. The individual components in the binary sweetener combinations were intensity-anchored with 5% sucrose, while the individual sweeteners in the ternary mixtures were intensity-anchored with 3% sucrose (according to formulae developed previously). Each self-mixture was also evaluated (e.g. acesulfame-K—acesulfame-K). The main finding of this study was that mixtures consisting of two or three different sweeteners exhibited less reduction in sweetness intensity over four repeated sips than a single sweetener at an equivalent sweetness level. Furthermore, ternary combinations tended to be slightly more effective than binary combinations at lessening the effect of repeated exposure to a given sweet stimulus. These findings suggest that the decline in sweetness intensity experienced over repeated exposure to a sweet stimulus could be reduced by the blending of sweeteners.

Key words: bulk sweeteners, high potency, repeated presentation, sweeteners, synergism

Introduction

Prolonged uninterrupted application of a taste stimulus to the tongue reduces the responsivity to that stimulus (McBurney, 1969). This phenomenon is termed adaptation. Continuous prolonged exposure of the tongue to a variety of taste stimuli has been studied in humans (McBurney, 1966; DuBose *et al.*, 1977; Lawless and Skinner, 1979; DuBose and Meiselman, 1979; Meiselman and Buffington, 1980; Bujas *et al.*, 1991) and in animals (Smith *et al.*, 1975, 1978; Schiffman *et al.*, 1992) using a gravity flow system that delivers a constant stream of a taste solution over the dorsal tongue surface. In real-life eating situations, however, tastes of a single substance are seldom prolonged but rather are interrupted by short time intervals between bites or sips. The effect of repeated exposure to a single stimulant (with short interruptions) on taste intensity ratings differs from that of prolonged continuous flow in that it may involve some degree of taste system recovery from adaptation. For example, the sweetness of sucrose decreased less over time when consumed intermittently in water (Schiffman *et al.*, 1994) or in sweetened yogurt (Theunissen *et al.*, 2000b) than with uninterrupted application to the tongue by gravitational flow (DuBose and Meiselman, 1979) or by filter paper (Gent and McBurney, 1978; Theunissen *et al.*, 2000a,b). This smaller reduction in sweetness with repeated tasting is presumably the result of two processes: adaptation and recovery.

The biochemical mechanisms by which adaptation occurs at the receptor level in the taste system are not known but desensitization of the sweet taste receptor (Schiffman *et al.*, 1994), intracellular cascades that involve protein kinase A (Varkevisser and Kinnamon, 2000), or inositol phospholipid hydrolysis, intracellular Ca^{2+} mobilization and protein kinase C-mediated phosphorylation (Ozaki and Amakawa, 1992) may play a role. The rate or degree of adaptation can also be modified by a number of factors, such as extracellular citrate ions (Ogawa and Caprio, 1999), extracellular tannic acid (Schiffman *et al.*, 1994) and other components in a mixture with sweet taste compounds (Schiffman *et al.*, 1995, 2000). Mouth movements that move solution away from receptors have also been reported to interfere with the adaptation process (Theunissen and Kroeze, 1996; Theunissen *et al.*, 2000a).

The sweetness intensity of a number of sweeteners has been shown to decrease after repeated tasting over a 2 min interval (Schiffman *et al.*, 1994). High-potency sweeteners (such as aspartame, saccharin or acesulfame-K) tended to exhibit more reduction in sweetness over time than bulk sweeteners (such as sucrose) (Schiffman *et al.*, 1994; Froloff *et al.*, 1998). The absolute reduction in sweetness intensity over time, however, tends to vary depending upon the concentration and type of sweetener. One potential method that may blunt the reduction in responsivity with repeated tasting, especially for high potency sweeteners, is combining lower concentrations of two or more sweeteners in mixtures. It was hypothesized in this study that mixtures of sweeteners would show less reduction in sweetness over time than a single sweetener in part because each component of the mixture would be used at lower concentrations and hence produce less adaptation. Use of sweetener mixtures may also utilize different biochemical mechanisms that could potentially reduce the impact of adaptation.

In this study, repeated tastes of binary and ternary blends of sweeteners were presented to trained taste panelists to determine the effect on sweetness intensity ratings. The binary and ternary blends of stimuli tested in this study represented a broad spectrum of molecular types, from common carbohydrate sweeteners such as sucrose and fructose to several high-potency sweeteners such as aspartame and sucralose. These data on sweetener blends were compared with a previous research paper by Schiffman *et al.* (Schiffman *et al.*, 1994) in which a single sweetener was studied using the same protocol.

Materials and methods

Subjects

A trained panel of 17 subjects, 10 males and 7 females, participated in the study [for details of panel training see DuBois *et al.* (DuBois *et al.*, 1991)]. The minimum number of subjects who participated in any given session was 10, and the maximum number of subjects was 16. The mean age of the subjects was 48 ± 12 years. All subjects were from the Duke University or Durham, NC community. Subjects were paid for their participation. The study was approved by the Duke University Medical Center Institutional Review Board for Human Subjects.

Stimuli

Binary blends

Binary combinations of the following 14 food grade sweeteners were tested: three sugars (fructose, glucose, sucrose), three terpenoid glycosides [Highstevia RA (a commercial stevia blend), monoammonium glycyrrhizinate, rebaudioside-A], two *N*-sulfonylamides (acesulfame-K, sodium saccharin), two polyhydric alcohols (mannitol, sorbitol), one chlorodeoxysugar (sucralose), one dipeptide derivative (aspartame), one protein (thaumatin) and one sulfamate (sodium cyclamate). The sugars and polyhydric alcohols are defined as bulk sweeteners, and the glycosides, sulfonylamides, chlorodeoxysugar, dipeptide, protein and sulfamate are high-potency sweeteners. The sweeteners were dissolved in deionized water. The two sweeteners in any given mixture were anchored in sweetness intensity to sucrose, i.e. tested at levels expected to be equisweet with a given concentration of sucrose (Schiffman *et al.*, 1994). Each sweetener was tested at a concentration expected to be equivalent in intensity with 5% sucrose [according to formulae developed by DuBois *et al.* (DuBois *et al.*, 1991)]. Table 1 lists the concentrations of sweeteners tested in the study of binary blends. All possible combinations of these 14 sweeteners were tested in binary mixtures, including self-mixtures (e.g. 5% sucrose + 5% sucrose). The abbreviations used for each sweetener throughout the paper are also given in Table 1.

Ternary blends

The following eight food grade sweeteners were tested: two sugars (fructose, sucrose), two *N*-sulfonylamides (acesulfame-K, sodium saccharin), one chlorodeoxysugar (sucralose), one dipeptide derivative (aspartame), one protein (thaumatin) and one terpenoid glycoside (Highstevia RA). The sweeteners were dissolved in deionized water. Each sweetener was tested at a concentration expected to be equivalent in intensity with 3% sucrose [according to formulae developed by DuBois *et al.* (DuBois *et al.*, 1991)]. Table 1 lists the concentrations of sweeteners tested in this study, as well as the abbreviations used for each sweetener in this study. All possible combinations of the eight sweeteners were tested in ternary mixtures, including self-mixtures (e.g. 3% sucrose + 3% sucrose + 3% sucrose).

Procedure

The procedure used for repeated tasting in this study is similar to the one used in a previous study by this laboratory (Schiffman *et al.*, 1994). It is designed to quantify any changes in sweetness intensity that may occur with repeated tasting in a controlled laboratory setting using psychophysical procedures with a trained panel. The order of presentation of the blends was randomized with two sweetener blends tested at each taste panel session. Each mixture was presented four consecutive times with 30 s between each sample. A given sweetener was not tested in more than one of the two mixtures in a session. Panelists were not told that each set of four samples was actually the same sample repeated four times. The presentation order of the samples was the same for all panelists. Panelists were asked to give sweetness, bitterness and sourness intensity ratings for each sample. The sample size was ~10–15 ml of solution served in 30 ml plastic medicine cups. Each of the four samples of a given mixture was coded with a different random three-digit number.

Prior to evaluating the samples, each trained panelist tasted basic taste references, according to the method described by DuBois *et al.* (DuBois *et al.*, 1991). These

Table 1 Concentrations of sweeteners tested for binary mixtures (expected to be equivalent in sweetness intensity to 5% sucrose) and ternary mixtures (expected to be equivalent in sweetness intensity to 3% sucrose)

Sweetener (abbreviation)	Binary blend concentrations, p.p.m. (M)	Ternary blend concentrations, p.p.m. (M)
Acesulfame-K (ace)	356.06 (1.77×10^{-3})	163.95 (8.15×10^{-4})
Aspartame (apm)	254.55 (8.65×10^{-4})	129 (4.39×10^{-4})
Fructose (fru)	39 060 (0.217)	23 300 (0.129)
Glucose (glu)	83 670 (0.464)	N/A
Highstevia (ste)	150 (1.86×10^{-4})	50 (6.21×10^{-5})
Monoammonium Glycyrrhizinate (MAG)	456.52 (5.44×10^{-4})	N/A
Mannitol (man)	78 710 (0.4320)	N/A
Na Cyclamate (cyc)	1583.1 (7.87×10^{-3})	N/A
Na Saccharin (sac)	112.59 (5.49×10^{-4})	58.51 (2.85×10^{-4})
Rebaudioside-A (reb)	200.0 (2.07×10^{-4})	N/A
Sorbitol (sor)	86 108 (0.4726)	N/A
Sucralose (sucr)	78.631 (1.98×10^{-4})	46.55 (1.17×10^{-4})
Sucrose (suc)	50 000 (0.146)	30 000 (0.088)
Thaumatin (tha)	3.529 (1.60×10^{-7})	1.52 (6.91×10^{-8})

included six sweet references: 2 sweet (2% sucrose), 5 sweet (5% sucrose), 7.5 sweet (7.5% sucrose), 10 sweet (10% sucrose), 12 sweet (12% sucrose) and 15 sweet (16% sucrose). These sucrose standards have been used in previous studies (Schiffman *et al.*, 1994, 1995; Portmann and Kilcast, 1996; Hutteau *et al.*, 1998). Panelists also tasted two bitter references, labeled 2 bitter (0.02% caffeine) and 4 bitter (0.03% caffeine), and one sour reference, labeled 2 sour (0.01% citric acid). These concentrations cover the range of bitter and sour components typically found in the sweeteners we have tested. Concentrations used for the bitter and sour references were based upon previous evaluations by a portion of the current panelists, as well as other trained panelists.

Panelists refrained from eating or drinking anything other than water for 30 min prior to the tasting session. During each taste session, the panelists swirled a given sample around in their mouths for 5 s and then expectorated. Immediately following expectoration of a sample, the experimenter began timing 30 s. During this 30 s, the panelists completed the three ratings scales (sweet, bitter and sour intensity) for the sample on Toshiba laptop computer units using CSA software (Computerized Sensory Analysis, Version 4.3, Compusense Inc., 1994). The intensity of an attribute was noted by making a mark with a light pen on a 15-point line scale, which was anchored at 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5 and 15. After the 30 s expired, the panelists sipped the second sample and swirled it for 5 s. This procedure was repeated for the third and fourth samples. The experimenter instructed the panelists when to sip, expectorate and rate each sample using a stopwatch to time the intervals. The panelists did not rinse with water, eat crackers or re-taste their references during the 'repeated sips' procedure for a given mixture. After the fourth sample of a mixture was evaluated, the experimenter set the timer for 5 min. During this time delay, subjects rinsed their mouths thoroughly with deionized water and, if necessary, ate unsalted-top crackers to rid their mouths of lingering attributes of previous samples. This procedure was repeated with a second mixture in four consecutive samples.

Results

Analysis of variance—binary mixtures

General linear models (GLMs) were estimated (using SAS statistical software for Windows Version 8) to examine whether binary mixtures of sweeteners exhibit more, the same or less decline in sweetness intensity with repeated sips than their constituent self-mixtures. The GLM procedure uses the method of least squares to fit general linear models including analysis of variance when group means are not equal. For each binary mixture comparison, the binary mixture was compared with the average of its two self-mixtures. Any subject who did not participate in all three conditions (evaluations of the binary mixture and both self-mixtures) was excluded from analysis, so that the data could be analyzed using a within-subjects design. This was done to prevent possible unequal weighting of values in the self-mixture and mixture conditions. GLMs were estimated examining the effect of stimulus (mixture versus average of the constituent self-mixtures), sip (sip 1 through sip 4) and stimulus × sip effects.

There was an effect of stimulus for 53 out of 91 of binary mixtures. For all significant stimulus effects, the binary mixtures exhibited higher overall sweetness intensity ratings across four sips than the average of self-mixtures. There was a significant effect of sip for virtually all of the binary mixtures as well as self-mixtures in which the mean sweetness intensity decreased over the four repeated sips. The only binary mixtures that did not have significant sip effects (i.e. did not decrease over the four sips) were glu–man, glu–sor and glu–tha.

There was a stimulus × sip interaction for the following binary mixtures: ace–MAG, apm–cyc, cyc–fru, cyc–glu, cyc–MAG, cyc–man, cyc–sac, cyc–sor, cyc–sucr, cyc–tha, fru–MAG, fru–reb, fru–sac, fru–sucr, MAG–reb, reb–sucr and sac–tha. Figure 1 depicts the sweetness intensity ratings (based on least squares means) for sip 1 through sip 4 for those binary mixtures exhibiting a significant stimulus × sip interaction, as well as the least squares means for the ratings of their respective constituent self-mixtures. Least squares means are the most unbiased estimates of means when using GLM.

For binary mixtures in which there was a stimulus × sip interaction, the following comparisons were made: binary mixture sip 1 versus self-mixture sip 1, binary mixture sip 1 versus binary mixture sip 4 and self-mixture sip 1 versus self-mixture sip 4. Sweetness intensity ratings of mixtures for sip 1 were higher than those for sip 1 of the average of the constituent self-mixtures for the following binary blends with significant stimulus × sip interactions: apm–cyc, cyc–fru, cyc–glu, cyc–sac, cyc–sor, cyc–sucr, fru–sac, fru–sucr and reb–sucr (for cyc–tha, the opposite was true). Significant reduction in sweetness intensity ratings from sip 1 to sip 4 occurred in all binary mixtures (with a significant stimulus × sip interaction), as well as their constituent self-mixtures. For all significant stimulus × sip effects, the binary mixtures exhibited less decline in sweetness ratings over four sips than the average of their constituent self-mixtures.

Analysis of variance—ternary mixtures

General linear models were also estimated to examine whether ternary mixtures of sweeteners exhibit more, the same or less reduction in sweetness intensity with repeated sips than their constituent self-mixtures. For each ternary mixture comparison (e.g. ace–suc–tha), the sweetness intensity of the mixture was compared with the average sweetness intensity of its three constituent self-mixtures (e.g. ace–ace–ace, suc–suc–suc, tha–tha–tha). Only those panelists who participated in all three conditions (evaluating the ternary mixture and all three constituent self-mixtures) were included in the analysis. General linear models (GLM) were estimated examining the effect of stimulus (mixture versus average of the constituent self-mixtures), sip (sip 1 through sip 4) and stimulus × sip interactions.

There was an effect of stimulus for 39 out of 56 ternary mixtures. For all significant stimulus effects, the ternary mixtures exhibited higher overall sweetness intensity ratings than the average of the three self-mixtures. There was an effect of sip for almost all of the ternary mixtures evaluated in which mean sweetness intensity declined over four repeated sips. The only ternary blends that did not have a significant effect of sip were ace–suc–tha, apm–sac–ste, apm–ste–tha, apm–suc–tha, sac–ste–tha and suc–sucr–tha.

There was a stimulus × sip interaction for the following ternary mixtures: ace–apm–fru, ace–apm–sac, ace–apm–tha, ace–fru–ste, ace–sucr–tha, apm–fru–ste, apm–fru–sucr, apm–fru–tha, apm–sac–ste, fru–sac–ste, fru–sac–suc, fru–sac–sucr, fru–sac–tha, fru–ste–tha, fru–suc–sucr, fru–suc–tha, fru–sucr–tha, sac–ste–suc, sac–ste–tha, sac–suc–sucr and sac–sucr–tha. Figure 2 shows the mean sweetness intensity ratings for sip 1 through sip 4 of each ternary mixture (based on least squares means) with a significant stimulus × sip interaction and the mean of its constituent self-mixtures.

For ternary mixtures in which there was a stimulus × sip interaction, the following comparisons were made: ternary mixture sip 1 versus self-mixture sip 1, ternary mixture sip 1 versus ternary mixture sip 4 and self-mixture sip 1 versus self-mixture sip 4. Sweetness intensity ratings of mixtures for sip 1 were higher than those for sip 1 of the average of the constituent self-mixtures for the following ternary mixtures with a significant stimulus × sip interaction: ace–apm–fru, ace–apm–sac, ace–apm–tha, ace–fru–ste, ace–sucr–tha, apm–fru–ste, apm–fru–tha, apm–sac–ste, fru–sac–ste, fru–sac–tha and sac–sucr–tha (for fru–ste–tha, the opposite was true). Significant reduction in sweetness intensity from sip 1 to sip 4 occurred in 13 out of 21 ternary mixtures, with significant stimulus × sip interactions (ace–apm–fru, ace–apm–sac, apm–fru–ste, apm–fru–sucr, apm–fru–tha, fru–sac–ste, fru–sac–suc, fru–sac–tha, fru–suc–sucr, fru–suc–tha, fru–sucr–tha, sac–suc–sucr, sac–sucr–tha). All of the constituent self-mixtures demonstrated a significant decline in sweetness intensity ratings from sip 1 to sip 4. For all significant stimulus × sip interactions, the ternary mixtures exhibited less decline in sweetness ratings over four sips than the average of their constituent self-mixtures.

Percentage decreases in sweetness for binary and ternary mixtures

Percentage decreases in sweetness intensities from sip 1 to sip 4 were calculated in both the binary and ternary mixture studies. Figure 3 compares the mean percent decline of each binary blend versus the mean percent decline for the corresponding self-mixtures for the following sweeteners: acesulfame-k, aspartame, fructose, glucose, MAG, mannitol, rebaudioside-A, Na saccharin, sorbitol, Highstevia, sucrose, sucralose and thaumatin, respectively. The range in percentage decrease of all binary blends was 6.5% (man–sor) to 52.6% (ace–sac). For 87% of binary mixtures, the mean percent decrease in sweetness intensity was numerically lower than the percent decrease of their respective self-mixtures. Some binary blends differed greatly in the percentage they declined in sweetness across sips relative to the mean of their constituent self-mixtures (e.g. the cyc–tha blend differed by 27.7 percentage points). For a small

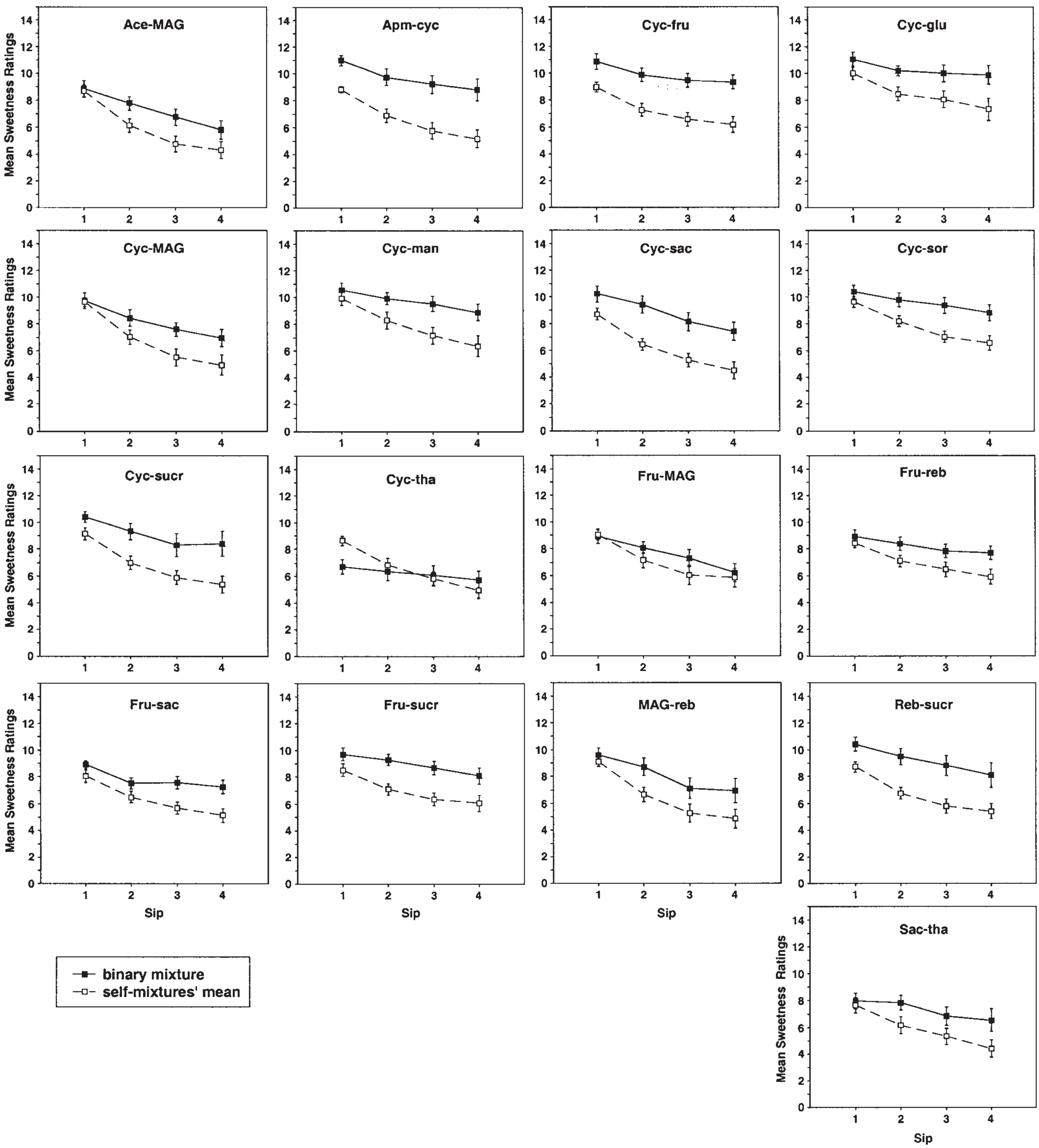

Figure 1 Sweetness intensity ratings (based on least squares means) for sip 1 through sip 4 of each binary mixture with a significant stimulus × sip interaction, respectively, as well as the least squares means for the ratings of its constituent self-mixtures.

proportion (11% of the binary mixtures), the percent decrease from sip 1 to sip 4 in comparison to that of the mean of the constituent self-mixtures was minimal (within 1%). The sweeteners that exhibited the greatest mean difference between decline in sweetness of blends and corresponding self-mixtures were Na cyclamate (19.5%), thaumatin (11.4%) and acesulfame-K (10.6%).

Figure 4 compares mean percent decline of each ternary

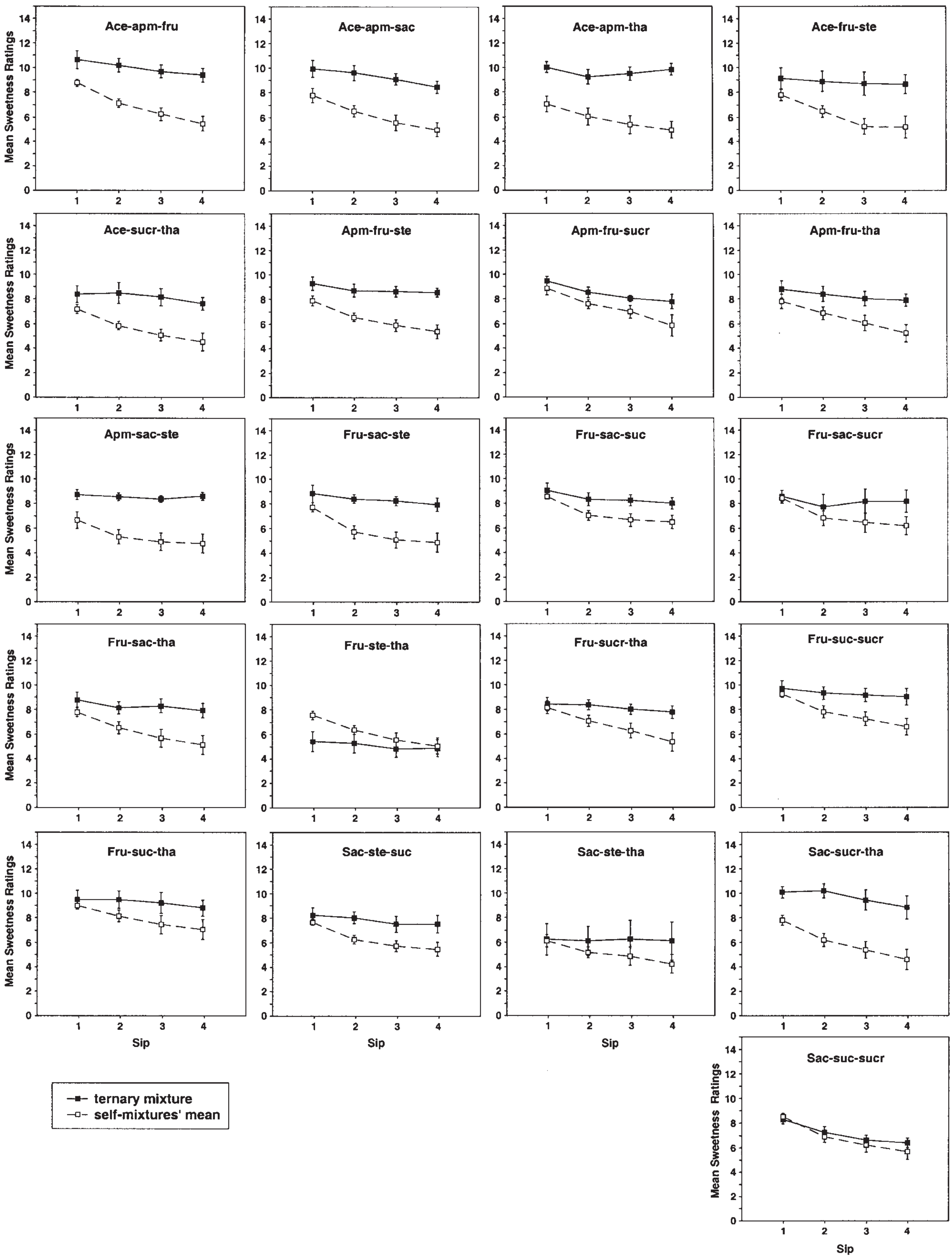

Figure 2 Sweetness intensity ratings (based on least squares means) for sip 1 through sip 4 of each ternary mixture with a significant stimulus × sip interaction, respectively, in addition to the least squares means for the ratings of its constituent self-mixtures.

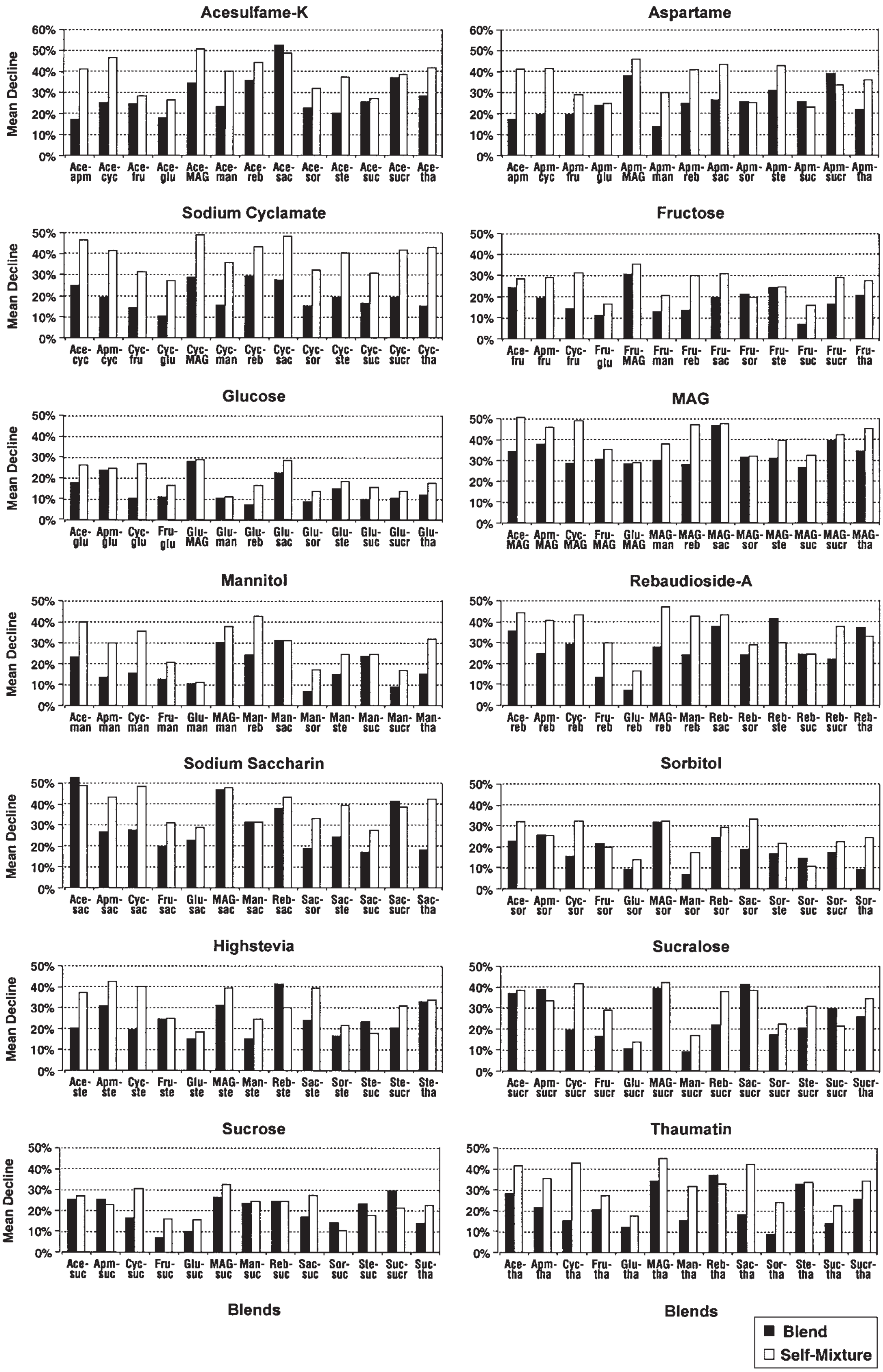

Figure 3 Mean percent decline of each binary blend with its mean constituent self-mixture for each combination of acesulfame-K, aspartame, fructose, glucose, Highstevia, MAG, mannitol, rebaudioside-A, Na cyclamate, Na saccharin, sorbitol, sucralose, sucrose and thaumatin, respectively.

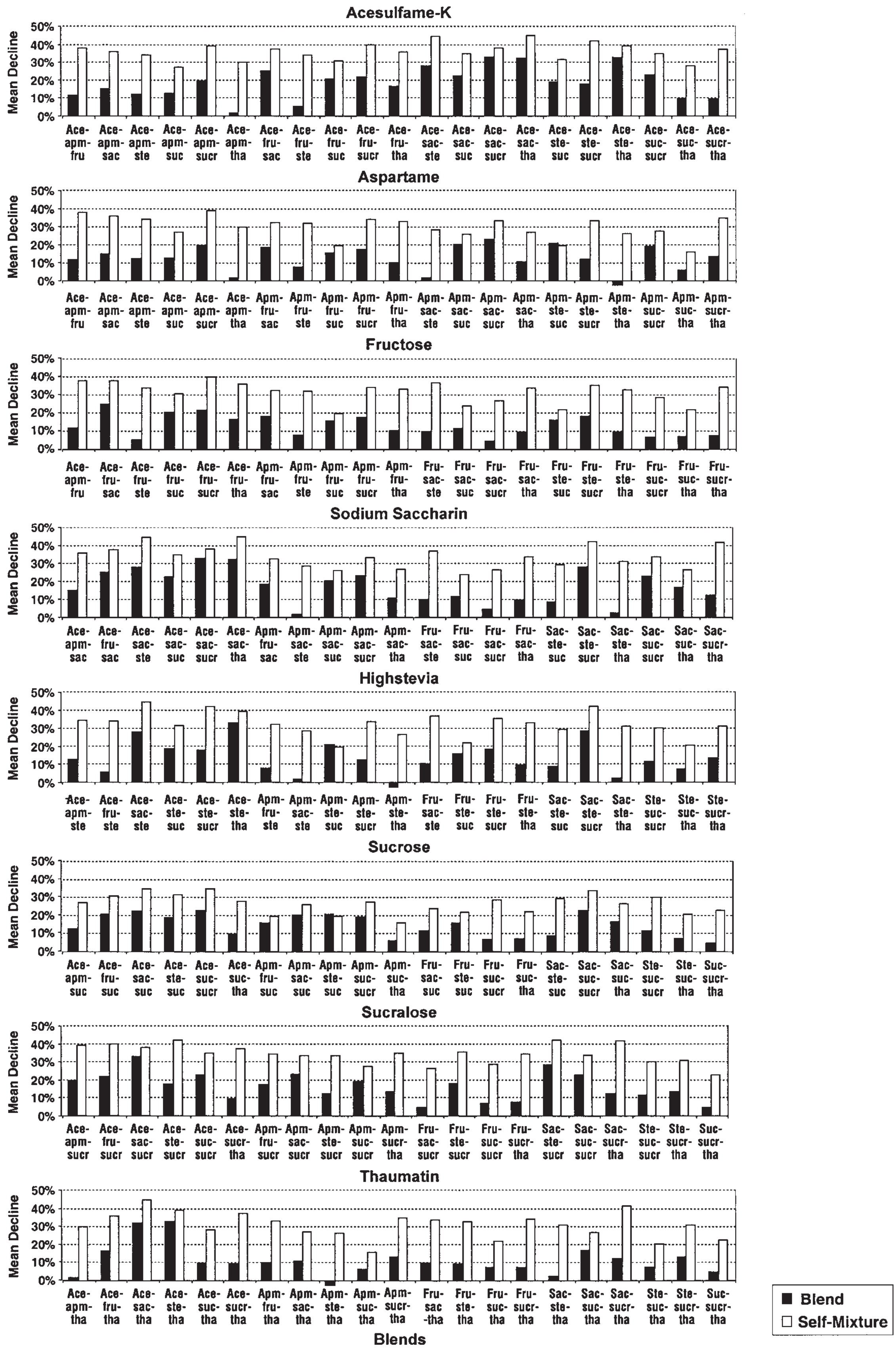

Figure 4 Mean percent decline of each ternary blend with its mean constituent self-mixture for each combination of acesulfame-K, aspartame, fructose, Highstevia, Na saccharin, sucralose, sucrose and thaumatin, respectively.

blend with the mean of its constituent self-mixtures for acesulfame-K, aspartame, fructose, Na saccharin, High-stevia, sucrose, sucralose and thaumatin, respectively. All but one of the ternary mixtures (apm–ste–tha) decreased in intensity from sip 1 to sip 4. The ternary mixture with the greatest percent decline from sip 1 to sip 4 was ace–sac–sucr at 32.9%. For ternary blends, 98% of mixtures had a numerically lower percent decline in sweetness ratings from sip 1 to sip 4 compared with their corresponding self-mixtures. Furthermore, the decrease in sweetness for 61% of ternary sweetener blends differed 15 or more percentage points from their self-mixtures. All of the sweeteners tested in ternary blends, with the exception of sucrose blends, differed to a large degree from the self-mixtures in the amount of decline in sweetness from sip 1 to sip 4.

Discussion

The purpose of the present study was to determine if the blending of sweeteners in either binary or ternary combinations would influence sweetness intensity ratings over the course of repeated sips. The findings indicate that the mixing of sweeteners in both binary and ternary combinations results in a smaller decrement in sweetness intensity with repeated sips relative to use of a single sweetener (Schiffman *et al.*, 1994). When sweetness intensity ratings of mixtures are compared with the corresponding mean intensity ratings of constituent self-mixtures (e.g. ace–apm versus the mean of ace–ace and apm–apm for binary mixtures), the percent decline from sip 1 to sip 4 was reduced for the vast majority of both binary and ternary blends. For stimulus mixtures in which a stimulus × sip interaction occurred, the rate of decline in sweetness intensity was lower for blends of different sweeteners than self-mixtures (see Figures 1 and 2). Nineteen percent (19%) of binary mixture blends and 37.5% of the ternary mixture blends had a significant stimulus × sip interaction. These significant interactions indicate that combining lower concentrations of two or more sweeteners in blends can blunt the reduction in sweetness that occurs with repeated sips.

For blends for which there was a reduction in percentage decrease in sweetness intensity rating but no stimulus × sip interaction, the reduction could be an artifact of the shape of the intensity functions for each sweetener or actual synergy among the individual components. The shape of the psychophysical functions for unmixed components has been shown to play a role in the sweetness intensity of mixtures for a variety of sweet compounds (DeGraaf and Frijters, 1987, 1988; DeGraaf *et al.*, 1987; Frijters and DeGraaf, 1987; Frijters and Oude Ophuis, 1983; McBride, 1988; Schifferstein, 1995, 1996). Synergism has been shown to occur for some of the sweetener combinations tested here (Schiffman *et al.*, 1995, 2000). Synergism occurs when the intensity of the mixture is greater than the theoretical sum of the intensities of the individual components (Frank *et al.*, 1989; Ayya and Lawless, 1992; Verdi and Hood, 1993; Birch, 1996). The analysis of variance indicated that there was an effect of stimulus (blends versus self-mixtures) for the majority of binary and ternary mixtures. For all significant stimulus effects, the binary and ternary mixtures exhibited higher overall sweetness intensity ratings than the average of the self-mixtures. Thus, the binary and ternary blends had higher sweetness intensity over the four sips compared with the self-mixtures. For example, if a binary sweetener blend started at an intensity of 12 at sip 1 (rather than the expected 10, i.e. 5 sweetness plus 5 sweetness) and the average of its corresponding self-mixtures started at an intensity of 10, and both decreased with the same slope to intensities of 9 and 7 respectively after four sips, the percentage decrease in sweetness for the blend [i.e. (12–9)/12 or 25%] would be less than for the average of the self-mixtures [i.e. (10—7)/10 or 30%]. Thus, smaller percentage changes in responsivity to mixtures over four sips (when no stimulus × sip interaction was found) may be a numerical consequence of the higher initial sweetness intensities resulting from either the psychophysical functions or the synergy of the individual components.

Ternary combinations tended to be even more effective than binary combinations in blunting the reduction in sweetness with repeated sips. A comparison of binary and ternary mixtures involving only the sweeteners that were tested in both studies (acesulfame-K, aspartame, fructose, Na saccharin, stevioside, sucralose, sucrose and thaumatin) yielded the following results. The average decrease in sweetness after four sips for the ternary mixtures involving these eight sweeteners (excluding self-mixtures) was 14.5%, whereas the average decrease for binary mixtures for the eight sweeteners (excluding self-mixtures) was 23.0%. (The average decrease in sweetness after four sips for both binary and ternary self-mixtures involving these eight sweeteners was 31%.) For example, binary blends containing fructose experienced 7.9% less decline in sweetness on average than its self-mixtures while ternary blends containing fructose experienced 18.7% less than its corresponding self-mixtures. Binary blends containing thaumatin experienced on average 11.4% less decline in sweetness than its self-mixtures while ternary blends containing thaumatin experienced 19.6% less than its corresponding self-mixtures. Binary blends containing acesulfame-K experienced on average 10.6% less decline in sweetness than its self-mixtures while ternary blends containing acesulfame-K experienced 17.5% less than its corresponding self-mixtures. Reduced blunting of sweetness with repeated sips for ternary blends relative to binary blends may occur because they are accessing a greater variety of receptor types at lower concentrations for each component sweetener in the mixture.

Sucrose was the sweetener that demonstrated the smallest sweetness reduction from the first to the fourth sip in binary, ternary and self-mixture blends. Sucrose (and other bulk sweeteners) are thought to activate a wide variety and number of sweet receptor types (Faurion *et al.*, 1980; Schiffman *et al.*, 1981, 1986; Tonosaki and Funakoshi, 1989), while high-potency sweeteners are thought to activate a more limited number of receptor types. However, it should be noted here that biochemical investigations of sweetener receptors are still in their infancy. To date only one human sweetener receptor type has been isolated (Nelson *et al.*, 2001). This receptor is a G-protein-coupled receptor that is a hetero-dimer T_1R_2/T_1R_3.

The binary mixture with the greatest percentage decrease in sweetness (ace–sac) was present in several ternary mixtures, which also displayed significant sweetness reduction (ace–fru–sac, ace–sac–ste, ace–sac–sucr and ace–sac–tha). Previous studies have hypothesized that acesulfame-K and Na saccharin may activate the same receptor type because they cross-adapt (Schiffman *et al.*, 1981) and are not synergistic with one another (Schiffman *et al.*, 1995).

Overall, this study shows that the reduction in sweetness intensity with repeated sips could be reduced if multiple sweeteners are included in the mixture. However, our understanding of the mechanisms involved in this blunting of taste awaits further discoveries in biochemistry, molecular biology and genetics. Recent molecular cloning studies have led to the identification of a number of taste receptor cell-specific G-protein-coupled receptors (GPCRs) (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Kitagawa *et al.*, 2001; Li *et al.*, 2001; Montmayeur *et al.*, 2001; Max *et al.*, 2001; Nelson *et al.*, 2001). These studies are a major step toward understanding the variety of receptors that are involved in the complicated molecular mechanisms of taste transduction.

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