

Sensory Testing for Flavorings with Modifying Properties

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FEMA Science Committee develops Guidance for the Sensory Testing of Flavorings with Modifying Properties within the FEMA GRAS Program.

Flavorings with modifying properties (FMPs) are a type of flavouring widely used by the flavor industry to modify the flavor profile of a flavoring and the food to which it is added. In the last few years, the development of new FMPs has increased to help address consumer desire for healthy food alternatives, including reductions in sugar and salt, without compromising flavor. FMPs may not necessarily have or impart a specific characteristic flavor of their own but can modify the flavor profile by altering flavor attributes such as intensifying specific flavor characteristics (e.g., perceived fruitiness), reducing specific flavor characteristics, masking of off-notes or bitterness, or changing the time onset and duration of the perception of specific aspects of the flavor profile.

In the United States, the Expert Panel of the Flavor and Extract Manufacturers Association of the United States (FEMA) evaluates new flavor ingredients, including FMPs, to determine if they can be considered “generally recognized as safe” (GRAS) for their intended use as flavor ingredients under authority provided by the 1958 Food Additives Amendment to the Federal Food, Drug, and Cosmetic Act (Hallagan and Hall, 1995, 2009). The Expert Panel evaluates substances only for their use as flavor ingredients in human food; it does not evaluate substances for other uses in food (e.g., sweetening) or for uses in products other than human food (e.g., tobacco). Therefore, as part of their evaluation, to assure that the flavor ingredient is an appropriate candidate for consideration as FEMA GRAS, the Expert Panel a) considers if the new flavor ingredient is functioning to impart or modify flavor in the finished food product¹ under conditions of intended use and b) assesses the effect of the flavor ingredient in the finished food product under conditions of intended use.

To complete their evaluation, the FEMA Expert Panel requires sensory data to be submitted as part of the FEMA GRAS application process for FMPs. In a publication in *Food Technology* (Marnett et al., 2013) the FEMA Expert Panel requested that the flavor industry outline best practices for conducting sensory testing for FMPs to provide data for both items a) and b) above.

FEMA's Science Committee Sensory Data Task Force, composed of sensory scientists and regulatory experts from FEMA member companies, was formed to respond to the request and developed the document, “Guidance for the Sensory Testing of Flavorings with Modifying Properties within the FEMA GRAS Program,” which follows this article.

To provide guidance on whether the substance functions to impart or modify in the finished food product under conditions of intended use [item (a) above], the FEMA Sensory Data Task Force developed “Test 1.” Test 1 is used to demonstrate that the substance does not have inherent sweetness or saltiness under conditions of intended use as an FMP in the finished food product. This test is focused on sweetness and saltiness as the Codex definition¹ of flavoring precludes “exclusively sweet or salty taste” in the finished food product from the definition of flavor². Additionally, in the United States, if the candidate were exclusively sweet under conditions of its intended use in the finished food, it would not be performing the technical effect of flavor and would require separate regulatory authority to use for that technical effect³.

Test 1 recommends a two-alternative forced choice test (ASTM Designation E2164-08: Standard Test Method for Directional Difference Test) to show that the sweetness or saltiness of the FMP alone and at the maximum use level is less than that of the recognition threshold concentration of sucrose or sodium chloride in the sample matrix evaluated. The guidance provides a recognition threshold concentration of 1.5% for sucrose in a water base, and 0.25% for sodium chloride in a water base⁴. As these thresholds are only applicable in a water base, the option is provided for the FEMA GRAS applicant to develop a threshold in another food matrix (i.e., meat products).

To provide guidance on the assessment of the effect of the FMP on the relevant attributes in the finished food product under conditions of intended use [item b) above], the task force developed “Test 2.” Test 2 recommends a Two-Alternative Forced Choice (2-AFC, also known as Directional Difference Test, Paired Comparison Test), one test conducted for each attribute of interest or scaling methods, such as Descriptive Analysis (e.g., Quantitative Descriptive Analysis, Sensory Spectrum Method).

Test 1 and Test 2 provide methodologies to conclusively determine a substance is not “exclusively sweet or salty” under its conditions of intended use as a flavoring, and thus meets the definition of flavoring as established by Codex Alimentarius Guidelines for the Use of Flavourings (CAC/GL 66-2008)¹.

The Sensory Data Task Force evaluated standard food matrices that may be applicable to multiple food categories listed within the FEMA GRAS publications and in the U.S. Code of Federal Regulations (21 CFR 170.3(n)). This work is provided in Appendix A. Further guidance on assessing use levels of FMP's in chewing gum is provided in Appendix B.

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NOTES

¹ The Codex Alimentarius Guidelines for the Use of Flavourings (CAC/GL 66-2008) defines flavorings as “products that are added to food to impart, modify, or enhance the flavour of food with the exception of flavour enhancers considered as food additives under the Codex Class Names and the International Numbering System for Food Additives - CAC/GL 36-1989. Flavourings do not include substances that have an exclusively sweet, sour, or salty taste (e.g., sugar, vinegar, and table salt). Flavourings may consist of flavouring substances, natural flavouring complexes, thermal process flavourings, or smoke flavourings and mixtures of them and may contain non-flavouring food ingredients within defined conditions such as carriers, solvents, etc. Flavourings are not intended to be consumed as such.”

² Sour taste is also included but a recognition threshold for sour taste is not included in the Guidance.

³ Technical effect refers to the function of a food ingredient in food. Technical effect F05, flavors and flavor modifiers, refers to substances that impart, supplement, intensify, or modify the taste and/or aroma of a food. This category excludes [technical effect] of sweeteners (National Academy of Sciences, 1989).

⁴ These recognition thresholds were derived from a literature search of articles citing thresholds for taste sensations related to sweetness and saltiness. The FEMA Sensory Data Task Force filtered the literature by: 1) requiring articles citing “recognition thresholds,” not “detection thresholds,” with the reasoning that the sensation needs to be recognized as sweet or salty and 2) sample size of greater than or equal to 20 subjects/observations.

⁵ To be determined by FEMA GRAS applicant.

FEMA 2013 Science Committee Sensory Data Task Force

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References

Hallagan, J.B. and Hall, R.L. 1995. FEMA GRAS—A GRAS assessment program for flavor ingredients. *Regul. Toxicol. Pharmacol.* 21: 422.

Hallagan, J.B. and Hall, R.L. 2009. Under the conditions of intended use—new developments in the FEMA GRAS program and the safety assessment of flavor ingredients. *Food Chem. Toxicol.* 47: 267.

Marnett, L.J., Cohen, S.M., Fukushima, S., Gooderham, N.J., Hecht, S.S., Rietjens, I.M.C.M., Smith, R.L., Adams, T.B., Hallagan, J.B., Harman, C., McGowen, M.M., and Taylor, S.V. 2013. GRAS Flavoring Substances 26: The 26th publication by the Expert Panel of the Flavor and Extract Manufacturers Association provides an update on recent progress in the consideration of flavoring ingredients generally recognized as safe under the Food Additive Amendment. *Food Technol.* 67(8): 38-56.

NAS. 1989. 1987 Poundage and Technical Effects Update of Substances Added to Food. National Academy of Sciences, Washington, D.C.

Guidance for the Sensory Testing of Flavorings with Modifying Properties within the FEMA GRAS Program

Test 1

Inherent Sweetness or Saltiness of FMPs under Conditions of Intended Use

1.1 Objective

This test can be used to demonstrate that the FMP does *not* have inherent sweetness or saltiness under the conditions of intended use.

1.2 Test Description

Test 1: Is the sweetness or saltiness of the *FMP* alone (at maximum use level) less than that of the *recognition threshold concentration* of sucrose or sodium chloride (NaCl) (or other relevant substance) in the sample matrix evaluated?

- Where the *FMP* is intended to change specific attributes or the balance of attributes
- Where the *recognition threshold concentration* is 1.5% sucrose or 0.25% NaCl (or other relevant substance) in a water base, or the recognition threshold concentration sucrose, NaCl, or other relevant substance in an alternative sample matrix (see section 1.4.2 Recognition Threshold Concentration).

Note: The FEMA GRAS applicant can select an alternative relevant substance to sucrose or NaCl or an alternate sample matrix for recognition threshold concentrations; see section 1.4 Method Details, below.

This test may be appropriate if the FMP is intended to modify sweetness, sourness, saltiness, or bitterness; *or* if the FMP is inherently sweet or salty, regardless of whether the FMP is intended to modify sweetness or saltiness. For example, this test would be appropriate to show that an FMP which is intended to mask bitterness is not inherently sweet.

In this test, a Test Sample containing the FMP, which does not contain the ingredient or attribute which it modifies, is compared to a Control Sample which contains the recognition threshold concentration of sucrose or NaCl (or other substance), but which does not contain the FMP. The test(s) should demonstrate that the Test Sample has significantly less sweetness or saltiness than the Control Sample. For further details, see section 1.4 Method Details, below.

1.2 Recommended Method and Standard Methodology

The recommended method is:

- 2-Alternative Forced Choice (2-AFC, also known as Directional Difference Test, Paired Comparison Test)

Standard methodology recommendations include:

- *ASTM Designation E2164-08: Standard Test Method for Directional Difference Test*

1.4 Method Details

1.4.1 Sample Matrix

The simplest sample matrix is a water base. Additional or alternative relevant sample matrices (see Appendix A) are recommended if the anticipated maximum use level of the FMP in those categories *exceeds* that determined in water, or if a water base is not relevant.

For example:

- In a water base for an FMP displaying sweetness modification, a 2-AFC test compares the Test Sample of the FMP alone (i.e., without added sucrose) versus the Control Sample containing 1.5% sucrose.
- In a water base for an FMP displaying saltiness modification, a 2-AFC test compares the Test Sample of the FMP alone (i.e., without added NaCl) versus the Control Sample containing 0.25% NaCl.

If the FEMA GRAS applicant wishes to apply for a maximum use level higher than that determined in a water base, or use a sample matrix other than a water base, then the FEMA GRAS applicant must also determine the recognition threshold concentration of sucrose or NaCl in the chosen matrix, and use that determined threshold concentration for the Control Sample. In the case where the FEMA GRAS applicant chooses to use a sample matrix other than a water base, it is acceptable to use 1.5% sucrose or 0.25% NaCl as the threshold level in the chosen matrix as opposed to determining the

threshold concentration of sucrose or NaCl in the chosen matrix. Please see section 1.4.2 Recognition Threshold Concentration, below.

For example:

In a fat based matrix for an FMP displaying sweetness modification, a 2-AFC test compares the Test Sample of the FMP alone (i.e., without added sucrose) versus the Control Sample containing the recognition threshold of sucrose in a fat based matrix, as determined by the FEMA GRAS applicant.

Please see sections 1.4.3 Control Sample and 1.4.4 Test Sample, below, for further details.

1.4.2 Recognition Threshold Concentration

The recognition threshold concentrations of sucrose and NaCl in a water base have been determined by FEMA to be 1.5% sucrose and 0.25% sodium chloride, respectively.

Should the FEMA GRAS applicant wish to use alternative ingredient(s) to sucrose or NaCl in a water base, or to use an alternative matrix (e.g., simple matrix such as fat/oil based, alcohol based, or a more complex product matrix as listed in Appendix A), the FEMA GRAS applicant may need to make their own determination of the recognition threshold concentration of sucrose, NaCl, or other alternative ingredient(s) relevant to the FMP in question, for each desired alternative sample matrix.

For example:

- A FEMA GRAS applicant who wishes to evaluate an FMP in a water base versus a recognition threshold concentration of aspartame in a water base should determine the recognition threshold of aspartame in that water base.
- A FEMA GRAS applicant who wishes to evaluate an FMP in a fat based matrix should determine the recognition threshold of sucrose in that fat based matrix.

It is recommended to follow one of the suggested standard methodology documents for determining recognition thresholds. Note: FEMA GRAS applicant is recommended to use 3-AFC methodology to determine recognition threshold within the following standard methodologies:

- *ASTM Designation E679: Standard Practice for Determination of Odor and Taste Thresholds By a Forced-Choice Ascending Concentration Series Method of Limits*
- *ASTM Designation E1432: Standard Practice for Defining and Calculating Individual and Group Sensory Thresholds from Forced-Choice Data Sets of Intermediate Size*
- *INTERNATIONAL STANDARD ISO 13301: Sensory Analysis Methodology: General guidance for measuring odour, flavour and taste detection thresholds by a three-alternative forced-choice (3-AFC) procedure*

Important Note: The recognition threshold determined by the FEMA GRAS applicant may be adjusted by **adding one standard error** unit to the actual concentration determined. The FEMA GRAS applicant calculates standard error from their study, and uses the determined concentration plus one standard error unit as the concentration of sucrose, NaCl, or alternative ingredient in the Control Sample.

For example:

- A FEMA GRAS applicant determines the recognition threshold concentration of sucrose in a fat-based matrix to be 2.0%. The standard error in the experiment is calculated to be 0.25%. Thus the concentration of sucrose in the fat-based matrix should be $2.0\% + 0.25\% = 2.25\%$.

1.4.3 Control Sample

The Control Sample contains a recognition threshold concentration of sucrose, NaCl, or alternative ingredient(s) without the FMP added. In the cases of using sample matrices other than a water base, or the use of ingredient(s) other than sucrose or NaCl in a water base or other sample matrix, the FEMA GRAS applicant should conduct testing to determine the recognition threshold concentration. In the case where the FEMA GRAS applicant chooses to use a sample matrix other than a water base, it is acceptable to use 1.5% sucrose or 0.25% NaCl as the threshold level in the chosen matrix as opposed to determining the threshold concentration of sucrose or NaCl in the chosen matrix.

For example:

- 1.5% sucrose in a water base *without* the FMP added.
- 0.25% NaCl in a water base *without* the FMP added.
- A recognition threshold concentration of an alternative ingredient (plus one standard error unit) in a water base *without* the FMP added, as determined by the FEMA GRAS applicant.
- A recognition threshold concentration of sucrose (plus one standard error unit) in a sample matrix *without* the FMP added.
- A recognition threshold concentration of NaCl (plus one standard error unit) in a sample matrix *without* the FMP added.

1.4.4 Test Sample

The Test Sample contains the FMP alone, *without* the ingredient it is intended to modify. For example:

- For an FMP displaying sweetness modification, the test sample contains the FMP alone in a water base *without* added sweetener.
- For an FMP displaying saltiness modification, the test sample contains the FMP alone in a water base *without* added NaCl.
- For an FMP displaying fructose modification, the test sample contains the FMP alone in a sample matrix *without* added fructose.
- For an FMP displaying saltiness modification, the test sample is the FMP alone in a sample matrix *without* ingredients that could be modified by the FMP in question.
- For an FMP displaying bitterness modification, the test sample is the FMP alone in a water base *without* ingredients that could be modified by the FMP in question.

The concentration of the FMP in the Test Sample should support the conditions of intended use. Note that the use level determined from a sample evaluated in a water base can be applied to all product categories. Should the FEMA GRAS applicant wish to request a maximum use level *higher* than that determined in a water sample, or wish to test in an alternative sample matrix, they may do so by conducting their testing in alternative sample matrices. Please see section 1.4.1 Sample Matrix, above.

1.4.5 Attribute Tested

The attribute evaluated in the 2-AFC test should be directly related to the intended effect and/or inherent taste quality of the FMP.

- For FMP's displaying sweetness modification, the test sample should be compared to a sweet Control Sample and tested for sweetness.
- For FMP's displaying saltiness modification, the test sample should be compared to a salty Control Sample and tested for saltiness.
- For FMP's displaying sourness modification, the test sample should be compared to a sweet Control Sample and tested for sweetness.
- FMPs not displaying sweet or salt modification (i.e., bitterness maskers or sourness maskers), but which are inherently sweet or salty, should be compared to a sweet or salty Control Sample and tested for sweetness or saltiness (respectively).

Consider specifying maximum intensity over a specific period of time if the FMP changes temporal profile of sweetness or saltiness.

Consider the use of nose clips where aroma may interfere with the evaluation of sweetness or saltiness.

1.4.6 Subjects

It is recommended to complete testing with at least 30 responses. The minimum number of subjects is 10, each completing three replicates of the 2-AFC test.

The FEMA GRAS applicant is free to choose naïve, screened, or trained panelists.

Consider screening panelists for anosmia and ageusia.

1.4.7 Data Analysis

The FEMA GRAS applicant is required to demonstrate that the attribute intensity of the Test Sample is significantly less intense than that of the Control Sample.

It is recommended to use the binomial distribution to determine significance in the 2-AFC test with no replicates. Should the FEMA GRAS applicant complete testing with two or more replicates, the FEMA GRAS applicant must use an analysis, such as the beta-binomial, to account for replicates.

The alpha value will be set at 5%. The test should be a two-sided alternative.

1.4.8 Reporting

Reporting of results should include the number of panelists, replicates, frequency of responses, and either calculated p-value (two-sided alternative) demonstrating that $p < 0.05$, or the minimum number of selected responses required for significance at $\alpha = 0.05$ (two-sided alternative), demonstrating the number of responses selecting the Control Sample as more intense exceeds this minimum.

1.5 Sample Test and Results

1.5.1 Example 1

This example demonstrates a 2-AFC test for sweetness in water. *An FMP was evaluated in a 2-AFC test for sweetness.*

Control Sample: 1.5% sucrose in water

Test Sample: 10 ppm FMP in water

Thirty subjects completed a 2-AFC test for sweetness. Twenty-five responses indicated the Control Sample was sweeter. Five responses indicated the Test Sample was sweeter. Using a binomial distribution, the minimum number of responses required for significance at $\alpha = 0.05$ is 21 (two-sided alternative). Therefore, the Control Sample is significantly sweeter than the Test Sample ($p < 0.05$).

This result would suggest a 10 ppm maximum use level in water, which can be applied to any categories desired by the FEMA GRAS applicant.

1.5.2 Example 2

This example demonstrates a 2-AFC test for sucrose sweetness in an alternative sample matrix.

An FMP was evaluated in a 2-AFC test for sweetness.

Control Sample: FEMA GRAS applicant-determined recognition threshold of (in %) sucrose in 5% alcohol base

Test Sample: 25 ppm FMP in 5% alcohol base

Recognition Threshold Determination of sucrose in a 5% alcohol base:

The experiment followed the guidelines of ASTM Standard Method E679-04, for determining recognition threshold of sucrose in a 5% alcohol base. Ten different concentrations of sucrose in a 5% alcohol base were prepared. Each of these samples was presented with two samples of 5% alcohol base. The concentrations were increased by a factor of two per concentration step. Fifteen panelists completed the test, proceeding from the lower to higher concentrations. At each concentration level, panelists compared the three samples (two blanks and one sucrose sample) and indicated which sample was recognized as being sweet. Each panelist performed the test twice. The best-estimate recognition threshold for sucrose in a 5% alcohol base was found to be AA%¹ sucrose.

¹ To be determined by FEMA GRAS applicant.

In a subsequent test, 11 subjects completed three replicates of a 2-AFC test for sweetness. Twenty-four responses indicated the Control Sample was sweeter. Nine responses indicated the Test Sample was sweeter. Using a beta-binomial analysis, $p=0.016$ (two-sided alternative). Therefore, the Control Sample is significantly sweeter than the Test Sample ($p<0.05$).

This result would suggest a 25 ppm maximum use level in a 5% alcohol base.

Test 2 Effect of the FMP on Relevant Sensory Attributes

2.1 Objective

This test can be used to demonstrate the intended effect that the FMP has on relevant sensory attributes under the conditions of intended use.

2.2 Test Description

Test 2: Does addition of the FMP cause a significant difference (i.e., increase or decrease) in the sensory attributes being modified?

- Where the *FMP* is intended to increase or decrease specific attributes
- Where *attributes* are the specific attributes that are being modified by the FMP

In this test, a Test Sample containing the FMP is compared to a Control Sample that *does not contain* the FMP. The test(s) should demonstrate that the FMP significantly increases or decreases the relevant attributes. The attributes and the direction of the difference should support the intended use of the FMP.

2.3 Recommended Methods and Standard Methodology

The FEMA GRAS applicant can use one or more of a variety of methods to demonstrate significant changes in attributes. Each of the recommended methods has benefits and drawbacks, and the FEMA GRAS applicant is encouraged to employ the method that is best suited to their FMP in question.

The recommended methods are (but are not limited to):

- 2-Alternative Forced Choice (2-AFC; also known as Directional Difference Test, Paired Comparison Test); one test conducted for each attribute of interest; or
- Scaling methods, such as Descriptive Analysis (e.g., Quantitative Descriptive Analysis, Sensory Spectrum Method, Temporal Profiling)

Standard methodology recommendations include:

- *ASTM Designation E2164-08: Standard Test Method for Directional Difference Test*
- *Manual on Descriptive Analysis Testing, R.C. Hootman, Ed. 1992*

2.4 Method Details

2.4.1. Sample Matrix

The simplest sample matrix is a water base. Additional or alternative sample matrices are recommended to demonstrate efficacy in various product categories, or if a water base is not relevant (Appendix A). The sample matrix should contain the ingredient(s) and/or attribute(s) on which the FMP is effective. Please see section 2.4.2. Control Sample, below, for examples.

2.4.2. Control Sample

The Control Sample contains some level of the ingredient(s) or attribute(s) with which the proposed FMP is effective, but that does not contain the FMP. For example:

- A sample matrix containing some level of sucrose without the FMP added.
- A sample matrix containing some level of NaCl without the FMP added.
- In the case of a bitter masker or blocker: A sample matrix containing perceptible bitterness without the FMP added.
- In the case of a juiciness FMP: A sample matrix containing the ingredient(s) to be modified, but without the FMP added.

The FEMA GRAS applicant may include more than one Control Sample, if desired. For example, additional samples containing differing concentrations of relevant ingredients.

2.4.3. Test Sample

The Test Sample is the Control Sample to which the FMP has been added. The concentration of the FMP in the Test Sample should support the conditions of intended use.

- A sample matrix containing the same level of sucrose as the Control Sample, with the FMP added.
- A sample matrix containing the same level of NaCl as the Control Sample, with the FMP added.
- In the case of a bitter masker or blocker: A sample matrix with perceptible bitterness, containing the same ingredients as the Control Sample, with the FMP added.

The FEMA GRAS applicant may include more than one Test Sample, if desired. For example:

- Additional samples containing differing concentrations of FMP.

2.4.4 Attributes Tested

The attributes evaluated in the test(s) will be directly related to the intended effect of the FMP including temporal profiling as applicable. For example:

- For an FMP displaying saltiness modification, all tests should evaluate saltiness and other relevant attributes.
- For an FMP displaying sweetness modification, all tests should evaluate sweetness and other relevant attributes.
- For an FMP displaying temporal profile modification, all tests should evaluate that temporal profile attribute and other relevant attributes.

2.4.5 Subjects

2.4.5.1 2-AFC Testing

It is recommended to complete testing with at least 30 responses. The minimum number of subjects is 10, each completing three replicates of the test. The FEMA GRAS applicant is free to choose naïve, screened, or trained panelists.

2.4.5.2 Descriptive Analysis Testing

The FEMA GRAS applicant is referred to standard methodology for appropriate number of subjects and training procedures for panelists; see section 2.3 Recommended Methods and Standard Methodology, above. The FEMA GRAS applicant is free to choose naïve, screened, or trained panelists.

2.4.6 Data Analysis

The FEMA GRAS applicant is required to demonstrate that the intensity or temporal profile of the Test Sample is significantly different than that of the Control Sample for the attributes being modified.

The direction of the difference may depend on the type of flavor modification being sought.

The alpha value will be set at 5% for determining significant differences.

2.4.6.1 Analysis of 2-AFC Test Data

It is recommended to use the binomial distribution to determine significance in the 2-AFC test with no replicates. Should the FEMA GRAS applicant complete testing with two or more replicates, the FEMA GRAS applicant must use an analysis, such as the beta-binomial, to account for replicates.

2.4.6.2 Analysis of Descriptive Analysis Test Data

It is recommended to use a t-test for each attribute when evaluating a total of two samples. Analysis of Variance (ANOVA) is recommended for each attribute when evaluating more than two samples. Additional factors may be incorporated in ANOVA calculations (such as panelists, replicates, etc.).

If ANOVA is used for statistical calculations, a multiple comparison test should be employed to specify differences among three or more samples (such as Fisher's LSD, Tukey's HSD, etc.).

2.4.7 Reporting

2.4.7.1 2-AFC Test

Reporting of results should include the number of panelists, replicates, frequency of responses, and either calculated p-value (two-sided alternative) demonstrating that $p < 0.05$, or the minimum number of selected responses required for significance at $\alpha = 0.05$ (two-sided alternative), demonstrating the number of responses selecting the Control Sample as more intense exceeds this minimum.

2.4.7.2 Descriptive Analysis

Reporting of results should include the number of panelists, replicates, description of methods and attributes evaluated, and a table of mean responses including the lettering convention representing significant differences in attribute(s) ($p < 0.05$) using a multiple comparison test of the FEMA GRAS applicant's choice. Figure(s) such as histogram(s), spider plot etc. may be included with significant differences in attributes clearly identified.

2.5 Sample Test and Results

2.5.1 Example 1

This example demonstrates 2-AFC testing and binomial test results.

A FMP intended to modify astringency, bitterness, sweetness and lemon flavor intensity of a sucrose-sweetened lemon beverage was evaluated in four separate 2-AFC tests.

Control Sample: 5% sucrose in lemon-flavored water

Test Sample: 5% sucrose in lemon-flavored water containing 10 ppm sweet sucrose FMP

Ten subjects completed three replicates of a 2-AFC test for astringency, bitterness, sweetness and lemon flavor intensity. Twenty-two responses indicated the Test Sample was sweeter. Eight responses indicated the Control Sample was sweeter. Using a beta-binomial distribution, $p = 0.016$ (two-sided alternative). Therefore, the Test Sample is significantly sweeter than the Control Sample ($p < 0.05$).

Similarly, 23 responses indicated the Test Sample had a more intense lemon flavor. Seven responses indicated the Control Sample had the more intense lemon flavor. Using a beta-binomial distribution, $p = 0.005$ (two-sided alternative). Therefore, the Test Sample is significantly more intense in lemon flavor than the Control Sample ($p < 0.05$).

Twenty-one responses indicated the Test Sample was less bitter while five responses indicated the Control Sample was less bitter. Using a beta-binomial distribution, $p = 0.043$ (two-sided alternative). Therefore, the Test Sample is significantly less bitter than the Control Sample.

Twenty-four responses indicated the Test Sample was less astringent while six responses indicated the Control Sample was less astringent. Using a beta-binomial distribution $p = 0.001$ (two-sided alternative). Therefore, the Test Sample is significantly less astringent than the Control Sample.

2.5.2 Example 2

This example demonstrates Descriptive Analysis Testing and ANOVA results.

An FMP intended to modify sweetness of sucrose and other attributes is evaluated in a Descriptive Analysis test including sweetness and other attributes of interest.

Control Sample: 3% sucrose in a Lemon-Lime flavored carbonated soft drink (CSD)

Test Sample 1: 3% sucrose in a Lemon-Lime flavored carbonated soft drink (CSD) containing 10 ppm FMP

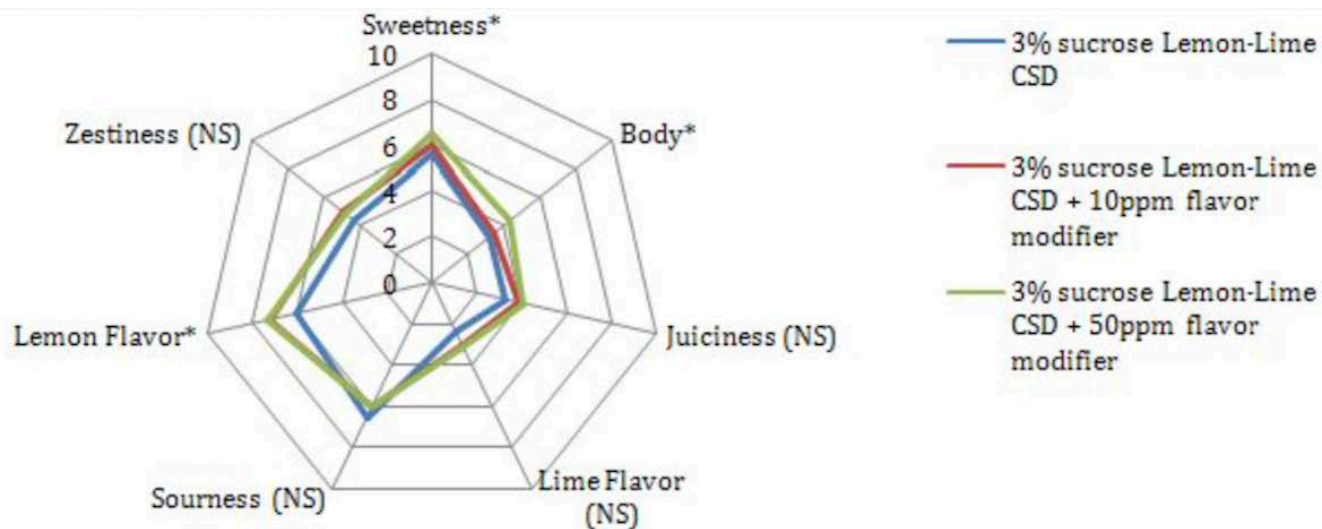
Test Sample 2: 3% sucrose in a Lemon-Lime flavored carbonated soft drink (CSD) containing 50 ppm FMP

The sensory characteristics of the samples were assessed by descriptive profiling. Descriptive vocabularies were created by three sensory experts. The panel (n=10) was trained to use the descriptive attributes. The trained sensory panel evaluated the samples in two replicate sessions by descriptive profiling by using a graphical 10 cm long intensity scale (0 = no attribute intensity, 10 = very intense) for seven attributes, including sweetness. The samples were served to the assessors coded with three-digit blinding codes and in random order in 2 oz. volumes. Water was provided to cleanse the palate between the samples. The data was collected by using computerized data collection software. Statistical analysis of the results was conducted using a three-factor ANOVA (factors were Sample, Panelists, and Replicates), and significant differences among samples were calculated using Tukey's HSD ($p < 0.05$).

Table 1. Mean scores for attributes of Lemon-Lime-flavored carbonated soft drinks. Differing letters within an attribute indicate significant differences using Tukey's HSD test ($p < 0.05$).

Sample	Sweetness	Body	Juiciness	Lime Flavor	Sourness	Lemon Flavor	Zestiness
3% sucrose Lemon-Lime CSD	5.6a	3.2a	3.3a	2.4a	6.5a	6.1a	4.4a
3% sucrose Lemon-Lime CSD + 10ppm FMP	6.2b	3.5a	3.9a	3.0a	6.0a	7.3b	5.0a
3% sucrose Lemon-Lime CSD + 50ppm FMP	6.5b	4.3b	4.1a	3.1a	6.0a	7.4b	4.9a

Figure 1. Spider plot of attributes of Lemon-Lime-flavored carbonated soft drinks. * = Statistically significant difference found among samples at $p < 0.05$. NS = No significant difference found among samples at ($p < 0.05$).



This test indicates that 50 ppm FMP significantly increases sweetness of a 3% sucrose Lemon-Lime-flavored carbonated soft drink ($p < 0.05$). In addition, 10 ppm FMP significantly increases sweetness and lemon flavor ($p < 0.05$), and 50 ppm FMP significantly increases both body and lemon flavor ($p < 0.05$).

3. Example Carried Through Both Tests

3.1 Example 1

This example demonstrates testing for a FMP which is intended to increase sweetness, mask bitterness, increase lime flavor, and increase lemon flavor.

Test 1:

An FMP was evaluated in a 2-AFC test for sweetness.

Control Sample: 1.5% sucrose in water

Test Sample: 10 ppm FMP in water

Fifteen subjects completed two replicates of a 2-AFC test for sweetness. Twenty-two responses indicated the Control Sample was sweeter. Eight responses indicated the Test Sample was sweeter. Using a beta-binomial analysis, $p=0.012$ (two-sided alternative). Therefore, the Control Sample is significantly sweeter than the Test Sample ($p<0.05$). This result would suggest a 10 ppm maximum use level in water, which can be applied to any categories desired by the FEMA GRAS applicant.

Test 2:

An FMP was evaluated in a series of 2-AFC tests for each relevant attribute.

Control Sample: Lemon lime CSD with 5% aspartame

Test Sample: Lemon lime CSD with 5% aspartame + 10 ppm FMP

Ten subjects completed three replicates of a 2-AFC test for each relevant attribute. Twenty-one responses indicated the Control Sample was more bitter. Nine responses out of 30 indicated the Test Sample was more bitter. Using a beta-binomial analysis, $p=0.043$ (two-sided alternative). Therefore, the Control Sample is significantly more bitter than the Test Sample ($p<0.05$). Twenty-three responses out of 30 indicated Test Sample was more sweet than the Control Sample ($p=0.005$; two-sided alternative), 22/30 ($p=0.016$ (two-sided alternative)) subjects indicated Test Sample was more lemon-flavored and 24/30 ($p=0.001$ (two-sided alternative)) more lime-flavored compared to Control Sample. Therefore, the Test Sample is significantly more sweet, significantly less bitter, and significantly more lemon- and lime-flavored than the Control Sample.

Recommendations¹ for Model Systems representing Food Categories Considered for FEMA GRAS**Table 1.** Model Systems representing food categories considered for FEMA GRAS.

FOOD CATEGORIES	MODEL SYSTEMS
Baked Goods	Crackers
Beverages Type I, Non-alcoholic	Soft drink, or Dairy drink
Beverages Type II, Alcoholic	Alcoholic drink
Breakfast cereals	Hot/cold cereal
Cheese	Topical Seasoning
Chewing gum	Chewing gum see Appendix B
Condiments & Relishes	Salad dressing
Confectionery & Frostings	Soft chewy candy
Egg Products	Frozen custard
Fats & Oils	Salad dressing
Fish Products	Broth or Meat patty
Frozen Dairy	Dairy drink
Fruit Ices	Soft drink
Gelatins & Puddings	Soft chewy candy
Granulated Sugar
Gravies	Gravy sauce
Hard Candy	Hard candy
Imitation Dairy Products	Dairy drink
Instant Coffee & Tea	Soft drink
Jams & Jellies
Meat Products	Broth or Meat patty
Milk Products	Dairy drink
Nut Products	Hot/cold cereal
Other grains	Hot/cold cereal
Poultry	Broth or Meat patty
Processed Fruits
Processed Vegetables	Broth or Gravy sauce
Reconstituted Vegetable Protein	Meat patty
Seasonings & flavors	Topical seasoning
Snack Foods	Topical seasoning
Soft Candy	Soft chewy candy
Soups	Broth
Sweet Sauce

¹ Recommendations for Model Systems and Processing Conditions contained herein are not requirements, rather they are suggested sample matrices for the purposes of conducting sensory testing as outlined in Section 1.4.1 and 2.4.1 in the “Guidance for the Sensory Testing of Flavorings with Modifying Properties within the FEMA GRAS Program”

Table 2. Suggested formulation and processing conditions of Model Systems for Test 1

Model System	Formulation	Processing/Notes
Crackers	Flour (50%), Shortening (8%), Salt (1%), NaHCO ₃ (0.5%), and water	Sheet dough (2-3mm); Convection oven 400°F/4 min; Conventional oven 350°F/10 min
Hot/cold cereal	Cereal (unflavored uncoated grain-based; e.g., oats), water, and salt	Use hot water to cook; evaluate either hot or cold
Frozen custard	Ice cream Mix (milk, cream, whey, nonfat milk, guar gum, mono- and diglycerides, polysorbate 80, xanthan, carrageenan) and egg yolks	Add egg yolks to 1.4% (w/w), mix and freeze
Hard candy	Citric acid, corn syrup solids/Isomalt, and water	Heat to 265°F, cool, and pour into molds
Soft chewy candy	Water, citric acid, and gelatin	Follow standard protocols
Chewing gum	Gum base	Follow standard protocols
Salad dressing	Oil (25-50%), vinegar (15-30%), water (15-30%), gum (0-1%), and salt (0.5-2%)	Hydrate the gum, add salt and acid in the end
Topical seasoning	Potato chips (unflavored plain), herbs, salt, maltodextrin, citric acid and cheese powder, oil and flow agent (silica)	Blend in the order of salt, maltodextrin, and acids with oil and cheese powder, herbs and silica. Heat chips to 200°F/3min, coat with 6-8% seasoning
Broth	Water, chicken/beef/vegetable/seafood solids (2-7%), salt (0-1%), fat/oils, corn starch (0-3%)	Pieces of meat / vegetables optional
Meat patty	Fresh ground meat/veg (80-100%), water (0-10%), starch/gum (1-5%), and salt (0-2%)	Ground to desired texture; cook consistently (oven 350°F/xmin)
Gravy sauce	Water, protein solids (0-2%), starch (0-3%), fat (0-5%), and salt (0.5-2%)	Dissolve starch followed by protein and rest of the ingredients, heat to 180°F and cool
Soft drink	Water, acidulant (0.01-0.4%), and preservatives (optional)	Use still or carbonated water; for without preservatives use hot fill
Alcoholic drink	Alcohol (15%), and acid (0-0.3%)	Alcohol 0.5-50%
Dairy drink	Protein (fresh milk or soy milk) 1-90%, and cream (0-30%)	Pasteurized /retorted per USDA guidelines (drink evaluation), or frozen (for frozen evaluation)

Table 3. Suggested formulation and processing conditions of Model Systems for Test 2

Model System	Formulation	Processing/Notes
Crackers	Flour (50%), Shortening (8%), Salt (1%), NaHCO ₃ (0.5%), and water	Sheet dough (2-3mm); Convection oven 400°F/4 min; Conventional oven 350°F/10 min
Hot/cold cereal	Cereal (unflavored uncoated grain-based; e.g., oats), water, sweetener, and salt	Use hot water to cook; evaluate either hot or cold
Frozen custard	Ice cream Mix (milk, cream, sugar, corn syrup, whey, nonfat milk, guar gum, mono- and diglycerides, polysorbate 80, xanthan, carrageenan), and egg yolks	Add egg yolks to 1.4% (w/w), mix and freeze
Hard candy	Sugar, citric acid, corn syrup solids/Isomalt, and water	Heat to 265°F, cool, and pour into molds
Soft chewy candy	Water, citric acid, gelatin, and sugar	Follow standard protocols
Chewing gum	Gum base, sorbitol, mannitol, and sweetener	Follow standard protocols
Fruit relish	Fruit, sugar, vinegar, salt, and herbs/flavors	Follow standard protocols
Salad dressing	Oil (25-50%), vinegar (15-30%), water (15-30%), gum (0-1%), sweetener (0-5%), and salt (0.5-2%)	Hydrate the gum, add sweetener and salt and acid in the end
Topical seasoning	Potato chips (unflavored plain), herbs, salt, maltodextrin, sweetener, citric acid and cheese powder, oil and flow agent (silica)	Blend in the order of salt, maltodextrin, and acids with oil and cheese powder, herbs and silica. Heat chips to 200°F/3min, coat with 6-8% seasoning
Broth	Water, chicken/beef/vegetable/seafood solids (2-7%), salt (0-1%), fat/oils, corn starch (0-3%)	Pieces of meat / vegetables optional
Meat patty	Fresh ground meat/veg (80-100%), water (0-10%), starch/gum (1-5%), and salt (0-2%)	Ground to desired texture; cook consistently (oven 350°F/xmin)
Gravy sauce	Water, protein solids (0-2%), starch (0-3%), fat (0-5%), and salt (0.5-2%)	Dissolve starch followed by protein and rest of the ingredients, heat to 180°F and cool
Soft drink	Water, acidulant (0.01-0.4%), sweetener (0-20% --- 10°Brix sugar equiv), and preservatives (optional)	Use still or carbonated water; for without preservatives use hot fill
Alcoholic drink	Alcohol (15%), sweetener (10°B), and acid (0-0.3%)	Ranges: alcohol 0.5-50%; sweetener 0-22%
Juice drink	Fruit juice (5-100%)	Sweetener, acidulant, preservative --- optional; Evaluate as liquid or frozen
Dairy drink	Protein (fresh milk or soy milk) 1-90%, sweetener (10°B total), and cream (0-30%)	Pasteurized /retorted per USDA guidelines (drink evaluation), or frozen (for frozen evaluation)

Appendix B

Assessing Usage Levels for Flavorings with Modifying Properties in Chewing Gum*

Evaluation of sweetness threshold for Flavorings with Modifying Properties (FMPs) in chewing gum can prove challenging and time-consuming. While the sweetness threshold in water can be useful for many applications, the release character of flavoring molecules in chewing gum can be much more complex than in an aqueous system. The partition coefficient of some molecules may mean that much of the added FMP may not be released from the gum matrix. Thus it is suggested that some FMPs could be used at a higher use level than that found in water, owing to the release properties of the FMP.

In an effort to improve usage levels of FMPs for use in a chewing gum base, the following guidelines are provided. If release of an FMP during chewing is less than 100%, the applicant may increase the use level of the FMP such that the effective release quantity is equivalent to that available in an aqueous system, as determined by the use level in water.

FMP Release Study:

The applicant is advised to evaluate the release of an FMP into the saliva during chewing. The release of the compound is then compared to the FMP sweetness threshold determined in water. If applicable, a multiplication factor is applied to the FMP usage level to ensure that its release permits the same quantity as that found in water.

Release can be measured in two ways: 1) direct quantification of compound release in saliva during chewing, or 2) indirect quantification of compound release, as measured by subtracting the amount measured in the gum bolus after chewing from the gum before chewing.

For direct quantification of FMP release in saliva, the gum base is first prepared with the FMP. The gum is then chewed by subjects for a prescribed period of time while saliva is constantly collected. The saliva is analyzed to quantify the amount of FMP released over the course of a typical chewing period versus the amount in the prepared gum base prior to chewing.

For indirect quantification of FMP as measured in the gum bolus after chewing, the gum base is first prepared with the FMP. Samples of gum are chewed by subjects for a prescribed period of time. The gum bolus is retained after chewing and FMP remaining in the bolus is quantified. The FMP release is quantified as the amount in the prepared gum base prior to chewing minus the amount of FMP in the chewed gum bolus.

In either case, amount of FMP released is used to calculate the increase in use level permitted for the chewing gum category.

Example: An FMP is found to have a use level of 10 ppm in water. When the same FMP is incorporated into chewing gum, it is demonstrated that only 25% (or $\frac{1}{4}$) of the quantity of FMP is released over the course of chewing the gum:
 $10 \text{ ppm} \times (100/25) = 40 \text{ ppm}$

Therefore, the applicant can request a use level of 40 ppm for the chewing gum use level table category.

Method in brief:

The applicant is referred to references such as Potineni and Peterson (2008) and Raithore and Peterson (2016) for brief descriptions of quantifying ingredients in chewing gum. The applicant is free to determine appropriate methods for quantification of the FMP in question, including solvents, high performance liquid chromatography (HPLC) or other quantification methods, and so on. The description below is further simplified from these references. The applicant is encouraged to review the details below when planning their evaluation.

Overview of direct quantification method:

An example of direct quantification method via saliva collection is described here. Subjects chew a standard piece of gum for a prescribed time period, and their saliva is collected over several time intervals. The amount of FMP is quantified and averaged across intervals to determine the total quantity released versus the amount in the prepared gum.

Subjects:

It is recommended that a minimum of three subjects complete the study. Subjects should refrain from eating or drinking anything other than plain water for at least 1 hour prior to the start of the test. The applicant should evaluate the subjects prior to testing to ensure they are familiar with the procedure, and that they release enough saliva to be able to measure the FMP in the expectorate. The applicant could consider standardizing chewing speed across panelists by using a metronome or similar method.

Alternatively, an artificial mouth can be used instead of subjects to complete the chewing experiment. An artificial mouth standardizes chewing speed and saliva volume, collecting a similar amount of saliva as produced by a human subject. Please see Krause et al., 2011.

Samples:

Chewing gum containing the FMP is prepared, and a standard size piece is evaluated by each subject. The applicant is free to determine the format and size of the gum pieces, such as a stick, tablet, or coated pellet.

Procedure:

Subjects rinse their mouths with water prior to starting the test. Subjects chew the piece of gum while simultaneously expectorating all saliva into a tared vessel, at various time intervals (for example, 5 minute intervals, such as 0-5 minutes, 5-10 minutes, and 10-15 minutes, for a total of 15 minutes of chewing.) The saliva at each of the three time intervals is sampled in triplicate, and used to quantify the total amount of FMP released in each of the time intervals, as well as the total quantity released during the entire 15 minutes. It is expected that the release of the FMP slows over the course of time, such that if one can demonstrate in the last interval (10-15 minutes) that very little additional release is seen, the chewing need not continue beyond 15 minutes. If the data suggest that a significant amount of FMP continues to be released during the last interval (10-15 minutes), additional intervals should be added to the test until such time that the FMP release slows significantly. However, it is expected that a 15 minute chewing time is sufficient to see significant tapering off of FMP release.

Specific details on how to analyze the saliva for quantifying the FMP will need to be determined by the applicant.

Data analysis:

The applicant should quantify the FMP released on a weight basis during each of the time intervals in order to a) confirm that release slows or is completed as time progresses; and b) sum the release from all time intervals to calculate the total weight of FMP released during the 15-minute chewing period. The total amount released during the entire chew is compared to the original amount added to the gum, expressed as a percentage released, which is to calculate the usage factor. For example, if 25% of the amount of FMP in the gum is released during the 15-minute chew, the usage level in water is multiplied by 100/25 to determine the use level in chewing gum.

Determining Sweetness Threshold of FMP in Chewing Gum:

It is recognized that the sweetness threshold of an FMP in gum, even at 100% release, may actually be different than that found in water using Test 1. Though complex, the applicant may alternatively determine the sweetness threshold of the FMP in a gum base through a saliva sampling method. First, the applicant must determine the threshold of sucrose in chewing gum, and then demonstrate the level of the FMP in chewing gum is significantly less than that of sucrose in order to determine the final usage level of the FMP.

References

Raithore, S., Peterson, D.G. 2016. Delivery of taste and aroma components in sugar-free chewing gum: mass balance analysis. *Chemosensory Perception*, 9: 182-192.

Potineni, R.V., Peterson, D.G. 2008. Mechanisms of flavor release in chewing gum: cinnamaldehyde. *Journal of Agricultural Food Chemistry*, 56(9): 3260-7.

Krause, A.J., Henson, L.S., Reinecciusa, G.A. 2011. Use of a chewing device to perform a mass balance on chewing gum components. *Flavour and Fragrance Journal*, 26: 47-54.

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