

APPENDIX C

Sensory Analysis of Major and Minor Steviol Glycosides

Technical Effect of Minor Steviol Glycosides

Summary: To evaluate the technical effect of minor steviol glycosides, a series of sensory tests were conducted with stevia leaf extracts containing varying amount of selected major steviol glycoside components (Reb A, B, C, D, M, Stevioside) and a group of large number of minor steviol glycosides. The sensory results clearly show that minor steviol glycosides contribute to sweetness intensity and the overall taste profile of stevia sweeteners used in beverage and food applications.

Products: The following stevia leaf extracts were used for studying the sensory effect of minor steviol glycoside components in food application.

Stevia Leaf Extract (SLE)	Major Steviol Glycosides		Minor Steviol Glycosides
Reb A 97	Reb A, Stevioside	>98%	<2%
PC Alpha	Reb A, B, C	>98%	<1%
SLE-Reb DM	Reb D, M, A	>96%	<2%
SLE-Minor SG	Reb A, D, M, C, Stevioside	<50%	>45%
SLE-SLE-A95	Reb D, M, A	~90%	<10%

Summary of Sensory Evaluation: The following sensory tests, detailed later in this document, were conducted in three food matrices to show the contribution of minor steviol glycosides on sensory attributes.

1. Reduced sugar Chocolate Milk with PureCircle Alpha and SLE-Minor (Test) was tested against the same formulation with PureCircle Alpha (Control). Test samples containing SLE-MINOR (minor steviol glycosides) had significantly higher chocolate flavor and directionally improved after taste, dairy note and overall liking.
2. Reduced sugar barbeque flavored peanut with Reb A 97 and SLE-Minor (Test) was compared against the peanut samples with Reb A 97 only (Control). Compared to the control sample, the test sample containing SLE-Minor showed significant increase in heat/spice intensity, smoke, chili flavor and directional improvement in saltiness and suppressed sweet intensity.
3. Acidified water samples made with 5% sugar (Control), 200 ppm SLE-A95 (Test 1) and 200 ppm SLE-Reb DM.(Test2) were compared. The Test 1 sample with SLE-A95 had significantly lower astringency, lower off-notes, and sweet aftertaste (at 95%, confidence) compared to Taste 2 sample with SLE-Reb DM. Test 1 sample had more sugar-like attributes.



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Chocolate Milk With SLE-Minor

Application:	Dairy Beverage
Panel Date:	08 January 2014
Client:	Internal/Design Team

SUMMARY

To evaluate the contribution of SLE-Minor, a stevia leaf extract containing minor steviol glycosides, to a dairy product, two 50% reduced sugar chocolate milk samples were prepared and tested by a consumer panel of 30 company employees. The consumer panel evaluated those two samples of chocolate milk for overall acceptance and attribute intensities (chocolate flavor, dairy notes, sweetness, bitterness and aftertaste). The two samples included: 1) a 50% sugar reduced control sample containing PureCircle Alpha (steviol glycoside sweetener) and 2) 50% sugar reduced test sample containing PureCircle Alpha and 80 ppm SLE-Minor. Test samples containing SLE-Minor (minor steviol glycosides) had significantly higher chocolate flavor and directionally improved after taste, dairy note and overall liking.

PROJECT OBJECTIVE

The project objective is to assess if the addition of stevia extract with minor steviol glycosides has an effect on key taste attributes in various beverage applications.

TEST OBJECTIVE

The test objective is to determine if the flavor profile and overall acceptance of a Control sugar reduced sample of chocolate dairy drink (containing Purecircle Alpha) differs from a reduced sugar Test sample of the same beverage containing SLE-Minor.

SAMPLE PREPARATION

1. Blend the stevia and emulsifiers/stabilizers with the other dry ingredients.
2. Add to the milk using good agitation. Do not over mix once the stevia is added.
3. Pasteurize, homogenize and package.
4. Serve chilled in 1 oz cup.

	50% Total Sugar Reduction with PureCircle Alpha	50% Total Sugar Reduction with PC Alpha & SLE-Minor
Dairy Formula		
2% Reduced fat Milk	96.5803	96.5753
Sugar	2.40	2.40
Cocoa Powder 10/12	0.80	0.80
Palsgaard 150 ChoMilk	0.20	0.20
PureCircle Alpha	0.0197	0.0197
SLE-MINOR		0.0080
Total	100	100

METHODOLOGY

• Nature of Participants:	Company employees
• Number of Sessions	1
• Number of Participants:	30
• Test Design:	Balanced, randomized within pair. Blind
• Sensory Test Method:	Intensity and acceptance ratings
• Environmental Condition	Standard booth lighting
• Attributes and Scales:	
	<ul style="list-style-type: none"> Overall Acceptance on a 10-pt hedonic scale where 10 = Extremely Like and 0 = Extremely Dislike Overall Liking, sweetness, bitterness, dairy notes, chocolate, and aftertaste. 10-pt continuous intensity scale where 0 = Imperceptible and 10 = Extremely Pronounced
• Statistical Analysis:	ANOVA (by Block) with Post Hoc Duncan's Test
• Sample Size	~1.5 oz. in a clear capped plastic cup
• Serving Temperature	Refrigerated temperature (~45°F)
• Serving/Panelists Instruction:	Samples served simultaneously. Panelists instructed to read ingredient statement, evaluate each sample.

RESULTS

Table 1 (below) summarizes the overall acceptance and mean attribute intensity results for each sample.

Summary of Mean-Scores, P-Values, and Significance Test Result Code - chocolate milk with 80 SLE-MINOR				
This test was performed on 30 panelists.				
Attribute	197 ppm of Alpha in chocolate milk (Control)	80 ppm of SLE-MINOR w/ alpha in chocolate milk	P-Value	Sig
Sweet Intensity	8.90 b	9.05 a	0.1557	*
Chocolate Flavor	6.89 b	7.53 a	0.0048	***
Dairy Note	4.12 b	4.44 a	0.1470	*
Bitterness	0.49	0.35	0.2473	NS
Bitter Aftertaste	0.71 a	0.55 b	0.1824	*
Sweet Aftertaste	2.66	2.82	0.5177	NS
Overall Liking	6.49 b	6.89 a	0.1908	*

* = 80% CI, ** = 90% CI, *** = 95%CI

- Both test samples containing SLE-MINOR had significantly higher chocolate flavor.

CONCLUSION

To evaluate the contribution of SLE-MINOR, a stevia extract containing minor steviol glycosides, to a dairy product, two 50% reduced sugar chocolate milk samples were prepared and tested by a consumer panel of 30 company employees. The consumer panel evaluated those two samples of chocolate milk for overall acceptance and attribute intensities (chocolate flavor, dairy notes, sweetness, bitterness and aftertaste) and found the Test samples containing SLE-MINOR (minor steviol glycosides) had significantly higher chocolate flavor and directionally improved after taste, dairy note and overall liking.



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Barbeque Flavored Peanut with SLE-Minor

Application:	Snack Food/Nut Product
Panel Date:	07 January 2014
Client:	Internal/Design Team

SUMMARY

Roasted Peanuts were used for testing the modification of taste profile by adding a stevia leaf extract (SLE-Minor) with high level of minor steviol glycosides. Thirty consumer panel members evaluated two samples of the peanuts for overall acceptance and attribute intensities (overall flavor, saltiness, sweetness, smoke flavor, spice/heat intensity, peanut flavor, chili powder flavor, bitterness and lingering sweet aftertaste intensity). The two samples included: 1) a 50% sugar reduced control sample containing Reb-A, 2) a 50% reduced sugar test sample containing Reb-A and 80 ppm SLE-Minor. The objective of the test was to determine if the addition of the stevia leaf extract containing minor glycosides, SLE-Minor, affects the taste profile of a savory snack food. The results indicated that the addition of SLE-Minor provided flavor and taste modification showing an increase in heat/spice intensity, smoke, and chili flavor compared to the control.

PROJECT OBJECTIVE

The project objective is to assess if the addition of stevia extract solids has an effect on key flavor attributes in various applications.

TEST OBJECTIVE

The test objective is to determine if the flavor profile and overall acceptance of a Control sample of barbeque flavored peanuts (containing only Reb A sweetener) differs from a Test sample of the same snack food (containing additional stevia leaf extract with minor steviol glycosides).

SAMPLE PREPARATION

- Mix peanuts with liquid ingredients, until peanuts are completely coated..
- Blend dry ingredients with Reb A and the test ingredient SLE-Minor.
- Add to the peanuts and mix well until they are fully coated.
- Serve at room temperature in 1 oz cups.

METHODOLOGY

Nature of Participants:	Company employees
Number of Sessions	1
Number of Participants:	30
Test Design:	Balanced, randomized within pair. Blind
Sensory Test Method:	Intensity and acceptance ratings
Environmental Condition	Standard booth lighting
Attributes and Scales:	
<ul style="list-style-type: none"> Overall Acceptance on a 9-pt hedonic scale where 9 = Like Extremely, 5 = Neither Like Nor Dislike, and 1 = Dislike Extremely 	
<ul style="list-style-type: none"> Overall Flavor, Saltiness, Sweetness, Smoke Intensity, Heat/spice intensity, peanut flavor, chili powder and Aftertaste Intensity (sweet and bitter) on a 10-pt continuous intensity scale where 0 = Imperceptible and 10 = Extremely Pronounced 	
<ul style="list-style-type: none"> Open Ended General Comments 	
<ul style="list-style-type: none"> Gender and Age 	
Statistical Analysis:	ANOVA (by Block) with Post Hoc Duncan's Test
Sample Size	~1.5 oz. in a clear capped plastic cup
Serving Temperature	Room temperature (~70°F)
Serving/Panelists Instruction:	Samples served simultaneously. Panelists evaluate each sample once.

SAMPLES

Samples with	Reb A (g)	Reb A + SLE-MINOR
Unsalted Peanuts	86.80	86.80
Vegetable oil	2.93	2.93
Sugar	5.88	5.88
Salt	2.93	2.93
Chilli powder	0.17	0.17
Cumin powder	0.29	0.29
Garlic powder	0.16	0.16
Cayenne pepper	0.16	0.16
Smoke liquid	0.73	0.73
Reb A	0.0243	0.0243
SLE-Minor		0.0080
Total wt. (g)	100	100

RESULTS

Table 1 (below) summarizes the overall acceptance and mean attribute intensity results for each sample.

Summary of Mean-Scores, P-Values, and Significance				
Test Result Code – Barbeque Flavored Peanuts - SLE-Minor				
This test was performed on 30 panelists.				
Attribute	243 ppm of Reb A (Control)	SLE-Minor with Reb A (Test)	P-Value	Sig
Sweet Intensity	2.10 a	1.72 b	0.0592	**
Saltiness	5.44 b	5.81 a	0.1182	*
Smoked Flavor	1.20 b	2.37 a	0.0041	***
Heat/Spice	2.26 b	2.86 a	0.0330	***
Peanut Flavor	8.83 a	8.26 b	0.0061	***
Chili Powder	3.52 b	3.95 a	0.0443	***
Bitterness	0.53	0.51	0.7419	NS
Bitter Aftertaste	0.41	0.42	0.7868	NS
Sweet Aftertaste	0.97 b	1.42 a	0.0133	***
Overall Liking	6.64 b	7.08 a	0.0483	***

* = 80% CI, ** = 90% CI, *** = 95%CI

- The results indicated that the addition of SLE-MINOR 80 ppm provided taste enhancement.
- Compared to the control sample, the test sample containing SLE-Minor showed significant increase in heat/spice intensity, smoke, chili flavor and directional improvement in saltiness and suppressed sweet intensity.

CONCLUSION

Roasted Peanuts were used for testing the modification of taste profile by adding stevia leaf extract containing minor steviol glycosides, SLE-Minor. Thirty consumer panel members evaluated two samples of the peanuts for overall acceptance and attribute intensities (overall flavor, saltiness, sweetness, smoke flavor, spice/heat intensity, peanut flavor, chili powder flavor, bitterness and lingering sweet aftertaste intensity). The two samples included: 1) a 50% sugar reduced control sample containing Reb-A, 2) a 50% reduced sugar test sample containing Reb-A and 80 ppm SLE-Minor. The objective of the test was to determine if the addition of the stevia leaf extract containing minor glycosides, SLE-Minor, affects the flavor and taste profile of a savory snack food. The results indicated that the addition of SLE-Minor at 80 ppm provided an increase in heat/spice intensity, smoke, and chili flavor compared to the control. Directionally SLE-Minor also enhance the saltiness in this snack sample



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Acidified Water with Sugar

Application:	Still Beverage
Panel Date:	5 October 2015
Client:	Internal/Design Team

SUMMARY

Fifteen company employees evaluated three samples of acidified water for overall acceptance and attribute intensities (sweetness, bitterness, astringency, acidity, off-notes and aftertaste). The three samples included: 1) 5% sugar, 2) SLE-A95 (test 1), and 3) SLE-Reb DM (test 2). The objective of the test was to determine if the taste profile of the acidified water with SLE-A95 is significantly different from sample with SLE-Reb DM. The results indicated:

- The Test sample 1 had significantly lower astringency, lower off-notes, and sweet aftertaste (at 95%, confidence). The Test 1 sample showed better sugar-like taste profile.

PROJECT OBJECTIVE

The project objective is to assess if the addition of minor stevia glycosides found in stevia extract (SLE-A95) has an effect on key flavor attributes.

TEST OBJECTIVE

The test objective is to determine if the flavor profile and overall acceptance of test sample 1 containing minor stevia glycosides differs from a product containing on only specific glycosides Reb D and Reb M.

SAMPLE PREPARATION

Weigh and blend all dry ingredients, then add to the water with good agitation. Agitate until dissolved. Samples were serve at room temperature in 2 oz cup.

SAMPLES

	Control	Test Sample 1	Test Sample 2
Water	95.00	98.9800	98.9800
Sucrose	5.00		
PureCircle SLE-A95		0.0200	
SLE-Reb DM			0.0200

METHODOLOGY

• Nature of Participants:	Company employees
• Number of Sessions	1
• Number of Participants:	15
• Test Design:	Balanced, randomized within pair. Blind
• Sensory Test Method:	Intensity and acceptance ratings
• Environmental Condition	Standard booth lighting
• Attributes and Scales:	
	<ul style="list-style-type: none"> Overall Liking on a 10-pt hedonic scale where 10 = Extremely Like and 0 = Extremely Dislike
	<ul style="list-style-type: none"> Sweetness, bitterness, peach flavor, tea flavor, acid intensity, astringency, and aftertaste. 10-pt continuous intensity scale where 0 = Imperceptible and 10 = Extremely Pronounced
• Statistical Analysis:	ANOVA (by Block) with Post Hoc Duncan's Test
• Sample Size	~2.0 oz. in a clear capped plastic cup
• Serving Temperature	Room Temperature
• Serving/Panelists Instruction:	Samples served simultaneously. Panelists instructed to read ingredient statement, evaluate each sample.

RESULTS

Table 1 (below) summarizes the overall acceptance and mean attribute intensity results for each sample.

Table 1: Mean Scores

Attribute	5% Sugar (Control)	SLE-A95 (Test 1)	SLE-Reb DM (Test 2)	P-Value	Sig
Sweetness	4.91	4.86	4.98	0.0997	
Bitterness	0.75b	0.98ab	1.23a	0.0083	***
Astringency	0.84b	1.02b	1.77a	0.0035	***
Acidity	0.99c	1.41b	1.99b	0.0012	***
Off-Note (Metallic/ Licorice)	0.52b	0.61b	1.13a	0.0081	***
Sweet Aftertaste	0.56c	0.99b	1.38a	0.0046	***
Bitter Aftertaste	0.33	0.37	0.53	0.5487	NS
Overall Liking	4.96a	4.53b	4.46b	0.1964	*

* = 80% CI, ** = 90% CI, *** = 95% CI

Fifteen company employees evaluated three samples of acidified water for overall acceptance and attribute intensities (sweetness, bitterness, astringency, acidity, off-notes and aftertaste). The three samples included: 1) 5% sugar, 2) SLE-A95 (test sample 1), and 3) SLE-Reb DM (test sample 2). The objective of the test was to determine if the flavor profile of the acidified water with SLE-A95 is significantly different from sample with SLE-Reb DM. The results indicated:

- The Test 1 sample with SLE-A95 had significantly lower astringency, lower off-notes, and sweet aftertaste (at 95%, confidence) compared to Taste 2 sample with SLE-Reb DM.
- The Taste 1 sample showed more sugar-like taste profile than the Taste sample 2

Chart 1: Acidified water 5 brix target – SLE-A95 vs SLE-Reb DM

