Subject: *Gorko 11 Report*

To: ███████████████

From: ███████████████

Date: *August 05, 2013*

srl
Excellence in Research

Contents

1   BACKGROUND

Consumers desire great tasting, mid- to low-calorie beverages that are naturally sweetened. TCCC is partnering with Pure Circle to gain sensory fundamentals on CC-00276 and CC-00293 that represent different purity grades (95% & 80% respectively) of a new natural sweetener steviol glycoside for mid- to low-calorie beverages. CC-00276 and CC-00293 sweeteners would expand our portfolio by allowing for the development of mid to low calorie beverages that fall under the following platforms: Natural; Wellness and Nutrition & Beyond.

The project was delivered in two modules:

> Module 1 to investigate the performances of CC-00276, CC-00293 and RebA (stand-alone and in blends), sucrose and aspartame (stand-alone) at 10%SE.

> Module 2 to investigate a direct comparison of CC-00276, CC-00293 and RebA, sucrose and aspartame at 8%SE.

2   OBJECTIVES

Business: To grow the still and sparkling beverage categories by providing mid to low calorie naturally sweetened beverage options.

Project: CC-00276 and CC-00293 DA panel work.

Study: Descriptive analysis and temporal profile of RX80, RX95 and other sweeteners.

3   ACTION STANDARD

TCCC

4 SIGNIFICANT FINDINGS

The steviol glycosides CC-00276 and CC-00293 were tested against three other sweeteners (Rebaudioside A, Sucrose and Aspartame) and blends (of each of the steviol glycosides with the other 3 sweeteners and also with erythritol acid, and of sucrose with Reb A) in three solution systems (phosphoric acid, citric acid and water) and two matrices (carbonated and non-carbonated).

The results show that:

- Aspartame provided the closest sensory experience to sucrose, and Reb A the furthest.
- The two CC steviosides gave similar sensory experiences, in between those of Reb A and Aspartame, with CC-293 slightly, but consistently, closer to Aspartame (except for bitterness).
- The differences between sensory experiences could be analysed with a high degree of orthogonality by the two first principal components (PC) of the 12 sensory descriptors, where
    - the first principal component that is dominated by the perception of bitterness and bitter aftertaste distinguishes very clearly between the different sweeteners and was only slightly affected by the different beverage systems. Other relevant descriptors for the scores of this PC were sweetness linger and liquorice aftertaste in the same direction, and smoothness and smoothness aftertaste in the reverse direction;
    - the second principal component which is dominated by the perceptions of sourness and sweetness and sour aftertaste distinguished very clearly the different systems (acids and carbonation) and was little influenced by the sweeteners.
- The different factors that distinguish the samples: sweetener, its concentration (between 8 and 10%), the base solution and carbonation, have mostly independent effects, with very few statistically significant interactions. This implies that the effect of changing a sweetener was on average about the same, whether there was more or less of it (between 8 and 10%), whether the liquid was water, a phosphoric or a citric acid solution, and whether it was carbonated or not.
- Thus, the most salient feature of the sensory experience to these sweeteners was that aspartame gave a more bitter taste and aftertaste, sweetness linger and liquorice taste and aftertaste than sucrose, CC-293 and CC-276 even higher, and Reb A the highest of these tastes/aftertastes.
- The solution system and the carbonation both influence the sensory perception, with citric acid giving a more sour and less sweet perception than phosphoric acid, and water the most sweet / less sour. On average, carbonation increased appearance time, bitter taste and aftertaste, sour taste and aftertaste and sweet taste and sweetness linger, while decreasing liquorice taste and aftertaste and smoothness and smoothness aftertaste. However, carbonation caused a clear shift in the principal component plot with an increase of the score of the second principal component, associated with sourness/sweetness, towards greater sourness, while lowering the score of the first principal component associated more strongly with bitterness, towards lower bitterness (and also lower sweetness linger and liquorice aftertaste, and higher smoothness and smoothness aftertaste, all of which contribute to this PC).

- The sensory features that distinguished the sweeteners the most were appearance time, bitter taste and aftertaste, liquorice taste and aftertaste and sweetness linger. In all cases a similar sequence was found, from lower to higher average score: sucrose -> aspartame -> CC 293 -> CC276 -> Reb A, except for bitter taste and aftertaste where the order of the two steviosides was reversed (CC 293 having a slightly higher score than CC 276). Reb A provided a lower sweet taste than the other sweeteners, which all had similar average sweetness scores, and with the two steviosides slightly above all others.
- In particular, the most important factors that influenced the sensory experience were the following (see table 1 for interactive effects):
    - **appearance time**: the differences caused by the different sweeteners were the most important feature, with sucrose having the lowest score, followed by aspartame, then CC-293, then CC-276 and finally RebA, all with statistically significant differences. Carbonating and increasing concentration from 8 to 10% both caused a slight fall in the appearance time of all sweeteners. Appearance time was slightly higher in citric acid and slightly lower in water.
    - **smoothness and smoothness aftertaste**: the sweetener, the solution and carbonation all contributed significantly to smoothness and its aftertaste. Both were higher with sucrose and lower with Reb A, with no statistically significant differences between the two CC steviosides and aspartame, although the smoothness of aspartame was slightly higher. Water provided a greater smoothness than the 2 acids, and carbonation lowered smoothness, which is likely due to the higher acid content due to carbonic acid.
    - **carbonation**: this sensation was simply explained by the carbonation itself, with no significant differences in perception caused by any other factor.
    - **bitter taste and aftertaste**: the sweeteners were the main cause of bitter taste and its aftertaste, with sucrose giving the lowest score, followed by aspartame, then CC-276, then CC-293 and Reb A being the most bitter. The difference between the two CC steviosides was however not statistically significant, whereas the others were. Bitterness was more discernible with lower concentration and in water than the two acids (except that there were no differences for aftertaste). Carbonation increased bitterness.
    - **sour taste and aftertaste**: sourness was defined by the solution itself, with the sweetener having a very little influence, although still within statistical significance (specially for the aftertaste). There was no sourness for any sweetener in water, only with acids, with citric acid giving higher scores than phosphoric acid. Reb A, CC-276 and CC-293 slightly enhanced sourness, especially when compared to sucrose, although the two steviosides gave a slightly, but statistically significant, lower sour taste score than Reb A, and CC-276 a slightly higher score than CC-293 for aftertaste. Increasing the sweetener concentration lowered the sourness perception slightly, while carbonating increased it, possibly due to the carbonic acid

- o **sweet taste and sweetness linger**: the concentration of sweetener influenced the sweet taste the most, followed by the solution, with the sweetener itself and carbonation having a much milder influence. In the former case the only difference was a slightly lower sweet taste of Reb A compared to the other four. The sweetness perception was higher in water and lower in citric acid. However, its lingering perception was affected quite substantially by the sweetener, with sucrose having the lowest lingering score, followed by aspartame, then the two CC steviosides and Reb A having the longest lingering effect. Lingering was enhanced slightly by carbonation and was higher in water and lower in citric acid.
  - o **liquorice taste and aftertaste**: this sensation was only detected in Reb A and the CC steviosides, being stronger in water and lower in the two acids. Increasing the sweetener concentration and carbonating both caused a loss of this taste. These influences were similar for the aftertaste, but even more pronounced.
- Mixing two different sweeteners provided scores gradually moving from those of one of the sweeteners to the other in an expectable gradual manner. However, some synergistic effects were found in some cases, where the mix provided a slight, but significantly different, experience to that of either the sweeteners on their own (see table 2). Erythritol had a small effect, the most noticeable being a greater decrease in the liquorice taste and aftertaste than even sucrose mixes had.
- The panel performance and consistency was very good, giving a low error of measurement of the sensory experience with the trained panel.

Summary tables of main findings (note also that the error due to replicate measurements and to differences between panel members is far smaller than all other potential sources of error and variability):

Table 1. Which factors influence the sensory experience, and by how much

a) Appearance and mouthfeel

		Appearance Time	Carbonation	Smoothness	Smoothness Aftertaste
System factors					
Sweetener	statistical significance	**YES**	no	**YES**	**YES**
	% influence on score	**88.0%**	0.00%	**31.1%**	**34.5%**
	average effect	max. 2.64 increase (from sucrose to Reb A)	--	max. 0.62 decrease (from sucrose to Reb A)	max. 0.57 increase (from sucrose to Reb A)
Concentration (increase from 8 to 10%)	statistical significance	**YES**	**YES**	no	no
	% influence on score	**1.6%**	**0.001%**	0.00%	0.00%
	average effect	lowers score by 0.24	--	--	--
Solution	statistical significance	**YES**	no	**YES**	**YES**
	% influence on score	**2.0%**	0.00%	**18.1%**	**18.3%**
	average effect	max. 0.32 increase (from water to citric acid)	--	max. 0.61 decrease (from water to citric acid)	max. 0.58 increase (from water to citric acid)
Carbonation	statistical significance	**YES**	**YES**	**YES**	**YES**
	% influence on score	**2.5%**	**99.70%**	**31.4%**	**16.1%**
	average effect	increases score by 0.29	increases score by 5.12	decreases score by 0.78	decreases score by 0.46
Relevant interactions	% influence on score	--	--	1.56%	1.30%
	main features	--	--	no difference between phosphoric and citric acid with aspartame. score decresed with concentration with citric acid, and increased in the other 2	decreased caused by carbonation higher in sucrose
Noise factors					
different panelists	% influence	0.20%	0.001%	0.10%	0.1%
panellist consistency	% influence	0.00%	0.003%	0.02%	1.5%
other 2-way interactions	% influence	1.30%	0.297%	3.12%	9.5%
all other sources of error	% influence	4.40%	0.301%	14.50%	20.0%

Table 1. **Which factors influence the sensory experience, and by how much**

b) Bitterness and sourness

		Bitter Taste	Bitter Aftertaste	Sour Taste	Sour Aftertaste
System factors					
Sweetener	statistical significance	**YES**	**YES**	**YES**	**YES**
	% influence on score	**69.1%**	**55.6%**	**0.4%**	**1.5%**
	average effect	max. 1.42 increase (from sucrose to Reb A)	max. 1.04 increase (from sucrose to Reb A)	max. 0.23 increase (from sucrose to Reb A)	max. 1.42 increase (from sucrose to Reb A)
Concentration (increase from 8 to 10%)	statistical significance	**YES**	**YES**	**YES**	**YES**
	% influence on score	**5.2%**	**1.7%**	**0.3%**	**0.4%**
	average effect	lowers score by 0.23	lowers score by 0.11	lowers score by 0.09	lowers score by 0.23
Solution	statistical significance	**YES**	no	**YES**	**YES**
	% influence on score	**0.3%**	0.10%	**96.0%**	**85.2%**
	average effect	max. 0.07 increase (from water to citric acid)	--	none in water, to 1.46 in citric acid	max. 0.07 increase (from water to citric acid)
Carbonation	statistical significance	**YES**	**YES**	**YES**	**YES**
	% influence on score	**3.7%**	**2.6%**	**0.2%**	**0.9%**
	average effect	increases score by 0.19	increases score by 0.14	increases score by 0.13	increases score by 0.19
Relevant interactions	% influence on score	2.30%	5.30%	0.30%	1.89%
	main features	increase caused by carbonation higher in sucrose and minimal in RebA. Higher bitterness in water han in the acids for all sweetneers except sucrose where it is the opposite	increase caused by carbona ion higher in sucrose and aspartame. One panellist more sensitive than o hers to lingering bitterness with Reb A and aspartame.	samples with he 2 steviosides had similar sourness to those with aspartame in uncarbonated, but had similar sourness to Reb A, and above aspartame, for uncarbonated	samples with the 2 steviosides had similar sourness to hose with aspartame in uncarbonated, but had similar sourness to Reb A, and above aspartame, for uncarbonated. Higher sour aftertaste in citric versus phosphoric acid more pronounced without carbonation
Noise factors					
different panelists	% influence	0.40%	3.20%	0.10%	1.6%
panellist consistency	% influence	0.10%	0.30%	0.00%	0.0%
other 2-way interactions	% influence	2.60%	6.90%	0.80%	2.3%
all other sources of error	% influence	16.20%	24.3%	2.0%	6.2%

Table 1. Which factors influence the sensory experience, and by how much

c) Sweetness and liquorice

		Sweet Taste	Sweetness Linger	Liquorice	Liquorice Aftertaste
System factors					
Sweetener	statistical significance	**YES**	**YES**	**YES**	**YES**
	% influence on score	**3.5%**	**0.4%**	**31.2%**	**33.2%**
	average effect	max. 0.13 increase (from RebA to CC293)	max. 1.31 decrease (from sucrose to Reb A)	max. 0.41 increase (from sucrose to Reb A)	max. 0.46 increase (from sucrose to Reb A)
Concentration (increase from 8 to 10%)	statistical significance	**YES**	**YES**	**YES**	**YES**
	% influence on score	**62.7%**	**0.3%**	**0.9%**	**1.8%**
	average effect	increases score by 1.14	lowers score by 0.07	lowers score by 0.04	lowers score by 0.07
Solution	statistical significance	**YES**	**YES**	**YES**	**YES**
	% influence on score	**17.4%**	**96.0%**	**6.3%**	**10.9%**
	average effect	max. 0.73 decrease (from water to citric acid)	max. 0.30 decrease (from water to citric acid)	max. 0.11 decrease (from water to phosp.acid)	max. 0.17 decrease (from water to phosp.acid)
Carbonation	statistical significance	**YES**	**YES**	**YES**	**YES**
	% influence on score	**2.6%**	**0.2%**	**2.6%**	**1.3%**
	average effect	increases score by 0.23	increases score by 0.16	decreases score by 0.07	decreases score by 0.06
Relevant interactions	% influence on score	1.4%	1.6%	16.1%	17.90%
	main features	different sweetness perceptions in different solutions for all sweeteners except aspartame. In carbonated samples all sweeteners except Reb A had similar sweetness levels (while all were different in uncarbonated samples)	greater difference in sweetness lingering between panel members for Reb A	liquorice taste of CC-293 was higher in citric acid. 3 membersof the panel less sensitive han the others,and in agreement between themselves	liquorice aftertaste of CC-293 was higher in citric acid. 3 members of the panel less sensitive than the others,and in agreement between hemselves
Noise factors					
different panelists	% influence	0.6%	1.6%	8.40%	7.0%
panellist consistency	% influence	0.1%	0.1%	0.00%	0.1%
other 2-way interactions	% influence	2.1%	2.2%	5.10%	5.9%
all other sources of error	% influence	9.5%	20.0%	29.40%	21.8%

Table 2. Which mixes show synergistic effects

Mixes are referred to by x/y with x being % of the first sweetener mentioned for the pair

Synergies	Appearance Time	Liquorice	Sweetness linger	Bitterness	Sour aftertaste
CC-276 and Reb A	Mixes of 2.5/7.5 and 5/5 have lower appearance time than either pure sweetener	Mixes of 5/5 have lower liquorice taste and aftertaste than either pure sweetener	Mixes of 5/5 have lower sweetness linger than either pure sweetener	--	--
CC-276 and Sucrose	--	--	--	bitter taste and aftertaste in carbonated samples remains the same for all mixes with CC-293, whereas with uncarbonated samples it falls gradually as CC-293 is replaced by sucrose	sour aftertaste remains the same for all mixes with CC-293
CC-293 and Reb A	Mixes of 2.5/7.5 and 5/5 have lower appearance time than either pure sweetener	Mixes of 5/5 have lower liquorice taste and aftertaste than either pure sweetener	Mixes of 5/5 have lower sweetness linger than either pure sweetener	--	--
CC-293 and Sucrose	--	--	--	bitter taste and aftertaste in carbonated samples remains the same for all mixes with CC-276, whereas with uncarbonated samples it falls gradually as CC-276 is replaced by sucrose	sour aftertaste remains the same for all mixes with CC-276
Sucrose and Reb A	--	Liquorice taste and aftertaste higher with 7.5/2.5 than for pure Reb A	--	--	--
CC-276 and Erythritol	--	--	--	--	--
CC-293 and Erythritol	--	--	--	--	--

5   RECOMMENDATION

[To be filled in by TCCC]

6   PRODUCT DESCRIPTION

Tables 3 and 4 indicate the variants tested via Descriptive Analysis and Temporal profile. Variants of module I include pure sweeteners and blends such that the total concentration of sweetener was 10%. Those in module II tested each sweetener on its own in the 3 solutions systems and 2 matrices and with the total concentration of sweetener of 8%.

Table 3. Variants for Descriptive Analysis and Temporal Profiles of module 1

SWEETENER(S)	SYSTEM					
	Phosphoric Acid		Citric Acid		Water	
	No Carbonation	With Carbonation	No Carbonation	With Carbonation	No Carbonation	With Carbonation
CC-00293	MI Variant 1	MI Variant 61	MI Variant 21	MI Variant 81	MI Variant 41	MI Variant 101
CC-00293 + RebA (7.5% + 2.5%)	MI Variant 2	MI Variant 62	MI Variant 22	MI Variant 82	MI Variant 42	MI Variant 102
CC-00293 + RebA (5% + 5%)	MI Variant 3	MI Variant 63	MI Variant 23	MI Variant 83	MI Variant 43	MI Variant 103
CC-00293 + RebA (2.5% +7. 5%)	MI Variant 4	MI Variant 64	MI Variant 24	MI Variant 84	MI Variant 44	MI Variant 104
CC-00293 + Sucrose (2.5% + 7.5%)	MI Variant 5	MI Variant 65	MI Variant 25	MI Variant 85	MI Variant 45	MI Variant 105
CC-00293 + Sucrose (7.5% + 2.5%)	MI Variant 6	MI Variant 66	MI Variant 26	MI Variant 86	MI Variant 46	MI Variant 106
CC-00293 + 1% erythritol	MI Variant 7	MI Variant 67	MI Variant 27	MI Variant 87	MI Variant 47	MI Variant 107
CC-00293 + 2% erythritol	MI Variant 8	MI Variant 68	MI Variant 28	MI Variant 88	MI Variant 48	MI Variant 108
CC-00276	MI Variant 9	MI Variant 69	MI Variant 29	MI Variant 89	MI Variant 49	MI Variant 109
CC-00276 + RebA (7.5% + 2.5%)	MI Variant 10	MI Variant 70	MI Variant 30	MI Variant 90	MI Variant 50	MI Variant 110
CC-00276 + RebA (5% + 5%)	MI Variant 11	MI Variant 71	MI Variant 31	MI Variant 91	MI Variant 51	MI Variant 111
CC-00276 + RebA (2.5% +7. 5%)	MI Variant 12	MI Variant 72	MI Variant 32	MI Variant 92	MI Variant 52	MI Variant 112
CC-00276 + Sucrose (2.5% + 7.5%)	MI Variant 13	MI Variant 73	MI Variant 33	MI Variant 93	MI Variant 53	MI Variant 113
CC-00276 + Sucrose (7.5% + 2.5%)	MI Variant 14	MI Variant 74	MI Variant 34	MI Variant 94	MI Variant 54	MI Variant 114
CC-00276 + 1% erythritol	MI Variant 15	MI Variant 75	MI Variant 35	MI Variant 95	MI Variant 55	MI Variant 115
CC-00276 + 2% erythritol	MI Variant 16	MI Variant 76	MI Variant 36	MI Variant 96	MI Variant 56	MI Variant 116
RebA + Sucrose (2.5% + 7.5%)	MI Variant 17	MI Variant 77	MI Variant 37	MI Variant 97	MI Variant 57	MI Variant 117
RebA + sucrose (7.5% + 2.5%)	MI Variant 18	MI Variant 78	MI Variant 38	MI Variant 98	MI Variant 58	MI Variant 118
Sucrose	MI Variant 19	MI Variant 79	MI Variant 39	MI Variant 99	MI Variant 59	MI Variant 119
Aspartame	MI Variant 20	MI Variant 80	MI Variant 40	MI Variant 100	MI Variant 60	MI Variant 120

Table2. Variants Needed for Descriptive Analysis and Temporal Profiles of Module 2

SWEETENER(S)	SYSTEM					
	Phosphoric Acid		Citric Acid		Water	
	No Carbonation	With Carbonation	No Carbonation	With Carbonation	No Carbonation	With Carbonation
CC-00276	MII Variant 1	MII Variant 16	MII Variant 6	MII Variant 21	MII Variant 11	MII Variant 26
CC-00293	MII Variant 2	MII Variant 17	MII Variant 7	MII Variant 22	MII Variant 12	MII Variant 27
RebA	MII Variant 3	MII Variant 18	MII Variant 8	MII Variant 23	MII Variant 13	MII Variant 28
Sucrose	MII Variant 4	MII Variant 19	MII Variant 9	MII Variant 24	MII Variant 14	MII Variant 29
Aspartame	MII Variant 5	MII Variant 20	MII Variant 10	MII Variant 25	MII Variant 15	MII Variant 30

7 METHODOLOGY

7.1 Data collection

Samples were analysed by SRL's sweetener panel who previously completed the Gorko 1 research , each providing their score of intensity of 12 descriptors of the sensory experience in a scale of 1 to 10. The sensory descriptors characterized the appearance time taste/flavor mouthfeel, and aftertaste.

Study Design

- Full descriptive profile and temporal profile.

- Complete Block Design (CBD); all products randomized and balanced within solution system (phosphoric acid, citric acid, water) carbonated and non-carbonated beverage systems.
- Each assessor evaluated the samples sequentially. Each product was evaluated three times by each assessor.
- Rest period and palate cleansing were in accordance to Gorko1 research.
- 15 ml of sample was served chilled (4 ˚C +/ 1˚C) in odor free cups.

Test Protocol

- The assessors were instructed to take a sip of the sample, rate the appearance time and the sweetness experience, and then ingest. They rated the mouth feel and taste/flavor of the samples and three minutes after ingestion then rated the intensity of the sweetness linger and aftertaste.

Panel Calibration:

- The panel was calibrated at the beginning of each test session. In addition the panel also received anchor references representing early (sucrose), middle (stevioside) and late (thaumatin) appearance times. In addition, blind samples were inserted during each test session to monitor panelist/panel performance.

Palate cleanser procedure:

Palate cleanser procedure:

1. Take a sip of 5% sucrose solution, and expectorate.
2. Take a sip of warm water, and expectorate.
3. Take a sip of 0.75% salt solution, and expectorate.
4. Take a sip of warm water, and expectorate.
5. Rinse with miner grade water.
6. Clean lips with a paper tissue.

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A rest period of 10 minutes was allowed between sample evaluations.

7.2 Lexicon

INITIAL EVALUATION	
Attribute	**Definition**
Appearance Time	The time until you experience the maximum sweetness
MOUTHFEEL:	

Attribute	Definition
Smoothness	The velvety, silky sensation of the sample, ranging from harsh to smooth.
Carbonation	The amount of tingling, burning sensation in the mouth and throat.

TASTE/FLAVOUR:	
Attribute	**Definition**
Sweet Taste	The taste stimulated by sucrose other sugars and artificial sweeteners.
Bitter Taste	Taste stimulated by certain substances such as quinine, caffeine, sucrose octa-acetate.
Sour Taste	The sour taste associated with citric acid, phosphoric or malic acid.
Licorice Flavour	Fruity flavour associated with liquorice or anise.

AFTERTASTE:	
Attribute	**Definition**
Sweetness Linger	The intensity of the sweet taste, 3 minutes after ingestion.
Smoothness	The velvety, silky sensation of the sample, ranging from harsh to smooth.
Bitter Aftertaste	The bitter aftertaste, stimulated by certain substances such as quinine, caffeine, sucrose octa-acetate.
Sour Aftertaste	The sour taste associated with citric acid, phosphoric or malic acid.
Licorice Aftertaste	Fruity aftertaste associated with liquorice or anise.

7.3 Data analysis

7.3.1 Outline of the methodology

The data was analysed in two subsets:

a) All data obtained with a single sweetener only, pooling together the results of module II with those of module I containing only one sweetener.

This subset of data contains almost a full factorial design of all possible combinations of 5 sweeteners (sucrose, aspartame, Reb A, CC-276 and CC-293), 3 solutions (water, phosphoric acid and citric acid), 2 concentrations (8 and 10%) and 2 carbonations (uncarbonated and carbonated). The only element missing for a full factorial design is that there were no data for Reb A at 10% concentration. This

implies that the interactive effect between sweetener and concentration could not be calculated fully, but all other two-way interactive effects could be determined.

The corresponding experimental design is shown in table 5. This set of data was handled with an Analysis of Variance of each of the 12 sensory descriptors and a Principal Component Analysis. The panel performance was analyzed jointly by treating the member of the panel and the number of repeat as factors, thus determining the portion of the variance (and sum of squares) of the data that was explained by variability in the repeatability of the assessments of each panelist and the variability between different panelists. This provided an additional validation of the accuracy of the panel. These were thus treated as fixed noise factors in a first ANoVA. All interactions and these two noise factors were ultimately pooled with the error, except for significant two-way interactions observed in the first ANoVA run. Panel variability proved to be a very small component of the overall error of the ANoVA against which the influence of the factors was assessed.

Table 4. Subset of data for the analysis of single sweetener samples

Sweetener	Concentration	Solution	Carbonation	Variant No.
CC-00293	10%	phosphoric acid	uncarbonated	MI V 1
CC-00276	10%	phosphoric acid	uncarbonated	MI V 9
Sucrose	10%	phosphoric acid	uncarbonated	MI V 19
Aspartame	10%	phosphoric acid	uncarbonated	MI V 20
CC-00293	10%	citric acid	uncarbonated	MI V 21
CC-00276	10%	citric acid	uncarbonated	MI V 29
Sucrose	10%	citric acid	uncarbonated	MI V 39
Aspartame	10%	citric acid	uncarbonated	MI V 40
CC-00293	10%	water	uncarbonated	MI V 41
CC-00276	10%	water	uncarbonated	MI V 49
Sucrose	10%	water	uncarbonated	MI V 59
Aspartame	10%	water	uncarbonated	MI V 60
CC-00293	10%	phosphoric acid	carbonated	MI V 61
CC-00276	10%	phosphoric acid	carbonated	MI V 69
Sucrose	10%	phosphoric acid	carbonated	MI V 79
Aspartame	10%	phosphoric acid	carbonated	MI V 80
CC-00293	10%	citric acid	carbonated	MI V 81
CC-00276	10%	citric acid	carbonated	MI V 89
Sucrose	10%	citric acid	carbonated	MI V 99
Aspartame	10%	citric acid	carbonated	MI V 100
CC-00293	10%	water	carbonated	MI V 101
CC-00276	10%	water	carbonated	MI V 109
Sucrose	10%	water	carbonated	MI V 119
Aspartame	10%	water	carbonated	MI V 120
CC-00276	8%	phosphoric acid	uncarbonated	MII V 1
CC-00293	8%	phosphoric acid	uncarbonated	MII V 2
Reb A	8%	phosphoric acid	uncarbonated	MII V 3
Sucrose	8%	phosphoric acid	uncarbonated	MII V 4
Aspartame	8%	phosphoric acid	uncarbonated	MII V 5
CC-00276	8%	citric acid	uncarbonated	MII V 6
CC-00293	8%	citric acid	uncarbonated	MII V 7
Reb A	8%	citric acid	uncarbonated	MII V 8
Sucrose	8%	citric acid	uncarbonated	MII V 9
Aspartame	8%	citric acid	uncarbonated	MII V 10
CC-00276	8%	water	uncarbonated	MII V 11
CC-00293	8%	water	uncarbonated	MII V 12
Reb A	8%	water	uncarbonated	MII V 13
Sucrose	8%	water	uncarbonated	MII V 14
Aspartame	8%	water	uncarbonated	MII V 15
CC-00276	8%	phosphoric acid	carbonated	MII V 16
CC-00293	8%	phosphoric acid	carbonated	MII V 17
Reb A	8%	phosphoric acid	carbonated	MII V 18
Sucrose	8%	phosphoric acid	carbonated	MII V 19

Sweetener	Concentration	Solution	Carbonation	Variant No.
Aspartame	8%	phosphoric acid	carbonated	MII V 20
CC-00276	8%	citric acid	carbonated	MII V 21
CC-00293	8%	citric acid	carbonated	MII V 22
Reb A	8%	citric acid	carbonated	MII V 23
Sucrose	8%	citric acid	carbonated	MII V 24
Aspartame	8%	citric acid	carbonated	MII V 25
CC-00276	8%	water	carbonated	MII V 26
CC-00293	8%	water	carbonated	MII V 27
Reb A	8%	water	carbonated	MII V 28
Sucrose	8%	water	carbonated	MII V 29
Aspartame	8%	water	carbonated	MII V 30

b) The various subgroups composed of every pair of sweetener mixes, with all data of module I thus further subdivided into 4 subgroups: all mixes with CC-276, all mixes with CC-293, all mixes with sucrose, all mixes with Reb A.

In each of the subgroup, each pair was analyzed independently for the 6 systems (3 solutions and 2 carbonations), seeing how the change in concentration from one pure sweetener to the other influenced each of the sensory descriptors. A Principal Component Analysis of the entire set of data was also performed to enable all samples to be plotted in the same chart, thereby differentiating the various samples and the effect of the sweeteners and the beverage system.

These subgroups are listed in table 6. In the case of the PC graphs, the position of a solution containing just 10% of Reb A was also added by estimating the score from marginal means addition out of the score of the 8% solution in module II, as the analysis of the results showed that a simple linear marginal means addition was a good estimate of scores for all other cases, as interactive effects were mostly negligible. Table 6 is organized with the CC-steviosides first, and then Sucrose, without repeating the same subgroups.

Table 5. Subset of data for the analysis of sweetener mixes

Subgroup	Sweetener 1	Sweetener 2	Solution	Carbonation	Variant No.
CC-293 / Reb A	CC-293 10%		phosphoric acid	uncarbonated	MI V 1
	CC-293 7.5%	Reb A 2.5%	phosphoric acid	uncarbonated	MI V 2
	CC-293 5%	Reb A 5%	phosphoric acid	uncarbonated	MI V 3
	CC-293 2.5%	Reb A 7.5%	phosphoric acid	uncarbonated	MI V 4
	CC-293 10%		citric acid	uncarbonated	MI V 21
	CC-293 7.5%	Reb A 2.5%	citric acid	uncarbonated	MI V 22
	CC-293 5%	Reb A 5%	citric acid	uncarbonated	MI V 23
	CC-293 2.5%	Reb A 7.5%	citric acid	uncarbonated	MI V 24
	CC-293 10%		water	uncarbonated	MI V 41
	CC-293 7.5%	Reb A 2.5%	water	uncarbonated	MI V 42
	CC-293 5%	Reb A 5%	water	uncarbonated	MI V 43
	CC-293 2.5%	Reb A 7.5%	water	uncarbonated	MI V 44
	CC-293 10%		phosphoric acid	carbonated	MI V 61
	CC-293 7.5%	Reb A 2.5%	phosphoric acid	carbonated	MI V 62
	CC-293 5%	Reb A 5%	phosphoric acid	carbonated	MI V 63
	CC-293 2.5%	Reb A 7.5%	phosphoric acid	carbonated	MI V 64
	CC-293 10%		citric acid	carbonated	MI V 81
	CC-293 7.5%	Reb A 2.5%	citric acid	carbonated	MI V 82
	CC-293 5%	Reb A 5%	citric acid	carbonated	MI V 83
	CC-293 2.5%	Reb A 7.5%	citric acid	carbonated	MI V 84
	CC-293 10%		water	carbonated	MI V 101
	CC-293 7.5%	Reb A 2.5%	water	carbonated	MI V 102
	CC-293 5%	Reb A 5%	water	carbonated	MI V 103
	CC-293 2.5%	Reb A 7.5%	water	carbonated	MI V 104
CC-293 / Sucrose	CC-293 10%		phosphoric acid	uncarbonated	MI V 1
	CC-293 7.5%	Sucrose 2.5%	phosphoric acid	uncarbonated	MI V 6
	CC-293 2.5%	Sucrose 7.5%	phosphoric acid	uncarbonated	MI V 5
		Sucrose 10%	phosphoric acid	uncarbonated	MI V 19
	CC-293 10%		citric acid	uncarbonated	MI V 21
	CC-293 7.5%	Sucrose 2.5%	citric acid	uncarbonated	MI V 26
	CC-293 2.5%	Sucrose 7.5%	citric acid	uncarbonated	MI V 25
		Sucrose 10%	citric acid	uncarbonated	MI V 39
	CC-293 10%		water	uncarbonated	MI V 41
	CC-293 7.5%	Sucrose 2.5%	water	uncarbonated	MI V 46
	CC-293 2.5%	Sucrose 7.5%	water	uncarbonated	MI V 45
		Sucrose 10%	water	uncarbonated	MI V 59
	CC-293 10%		phosphoric acid	carbonated	MI V 61
	CC-293 7.5%	Sucrose 2.5%	phosphoric acid	carbonated	MI V 66
	CC-293 2.5%	Sucrose 7.5%	phosphoric acid	carbonated	MI V 65
		Sucrose 10%	phosphoric acid	carbonated	MI V 79
	CC-293 10%		citric acid	carbonated	MI V 81

Subgroup	Sweetener 1	Sweetener 2	Solution	Carbonation	Variant No.
	CC-293 7.5%	Sucrose 2.5%	citric acid	carbonated	MI V 86
	CC-293 2.5%	Sucrose 7.5%	citric acid	carbonated	MI V 85
		Sucrose 10%	citric acid	carbonated	MI V 99
	CC-293 10%		water	carbonated	MI V 101
	CC-293 7.5%	Sucrose 2.5%	water	carbonated	MI V 106
	CC-293 2.5%	Sucrose 7.5%	water	carbonated	MI V 105
		Sucrose 10%	water	carbonated	MI V 119
CC-293 / Erythritol	CC-293 10%		phosphoric acid	uncarbonated	MI V 1
	CC-293 9%	Erit. 1%	phosphoric acid	uncarbonated	MI V 7
	CC-293 8%	Erit. 2%	phosphoric acid	uncarbonated	MI V 8
	CC-293 10%		citric acid	uncarbonated	MI V 21
	CC-293 9%	Erit. 1%	citric acid	uncarbonated	MI V 27
	CC-293 8%	Erit. 2%	citric acid	uncarbonated	MI V 28
	CC-293 10%		water	uncarbonated	MI V 41
	CC-293 9%	Erit. 1%	water	uncarbonated	MI V 47
	CC-293 8%	Erit. 2%	water	uncarbonated	MI V 48
	CC-293 10%		phosphoric acid	carbonated	MI V 61
	CC-293 9%	Erit. 1%	phosphoric acid	carbonated	MI V 67
	CC-293 8%	Erit. 2%	phosphoric acid	carbonated	MI V 68
	CC-293 10%		citric acid	carbonated	MI V 81
	CC-293 9%	Erit. 1%	citric acid	carbonated	MI V 87
	CC-293 8%	Erit. 2%	citric acid	carbonated	MI V 88
	CC-293 10%		water	carbonated	MI V 101
	CC-293 9%	Erit. 1%	water	carbonated	MI V 107
	CC-293 8%	Erit. 2%	water	carbonated	MI V 108
CC-276 / Reb A	CC-276 10%		phosphoric acid	uncarbonated	MI V 9
	CC-276 7.5%	Reb A 2.5%	phosphoric acid	uncarbonated	MI V 10
	CC-276 5%	Reb A 5%	phosphoric acid	uncarbonated	MI V 11
	CC-276 2.5%	Reb A 7.5%	phosphoric acid	uncarbonated	MI V 12
	CC-276 10%		citric acid	uncarbonated	MI V 29
	CC-276 7.5%	Reb A 2.5%	citric acid	uncarbonated	MI V 30
	CC-276 5%	Reb A 5%	citric acid	uncarbonated	MI V 31
	CC-276 2.5%	Reb A 7.5%	citric acid	uncarbonated	MI V 32
	CC-276 10%		water	uncarbonated	MI V 49
	CC-276 7.5%	Reb A 2.5%	water	uncarbonated	MI V 50
	CC-276 5%	Reb A 5%	water	uncarbonated	MI V 51
	CC-276 2.5%	Reb A 7.5%	water	uncarbonated	MI V 52
	CC-276 10%		phosphoric acid	carbonated	MI V 69
	CC-276 7.5%	Reb A 2.5%	phosphoric acid	carbonated	MI V 70
	CC-276 5%	Reb A 5%	phosphoric acid	carbonated	MI V 71
	CC-276 2.5%	Reb A 7.5%	phosphoric acid	carbonated	MI V 72
	CC-276 10%		citric acid	carbonated	MI V 89

Subgroup	Sweetener 1	Sweetener 2	Solution	Carbonation	Variant No.
	CC-276 7.5%	Reb A 2.5%	citric acid	carbonated	MI V 90
	CC-276 5%	Reb A 5%	citric acid	carbonated	MI V 91
	CC-276 2.5%	Reb A 7.5%	citric acid	carbonated	MI V 92
	CC-276 10%		water	carbonated	MI V 109
	CC-276 7.5%	Reb A 2.5%	water	carbonated	MI V 110
	CC-276 5%	Reb A 5%	water	carbonated	MI V 111
	CC-276 2.5%	Reb A 7.5%	water	carbonated	MI V 112
CC-276 / Sucrose	CC-276 10%		phosphoric acid	uncarbonated	MI V 9
	CC-276 7.5%	Sucrose 2.5%	phosphoric acid	uncarbonated	MI V 14
	CC-276 2.5%	Sucrose 7.5%	phosphoric acid	uncarbonated	MI V 13
		Sucrose 10%	phosphoric acid	uncarbonated	MI V 19
	CC-276 10%		citric acid	uncarbonated	MI V 29
	CC-276 7.5%	Sucrose 2.5%	citric acid	uncarbonated	MI V 34
	CC-276 2.5%	Sucrose 7.5%	citric acid	uncarbonated	MI V 33
		Sucrose 10%	citric acid	uncarbonated	MI V 39
	CC-276 10%		water	uncarbonated	MI V 49
	CC-276 7.5%	Sucrose 2.5%	water	uncarbonated	MI V 54
	CC-276 2.5%	Sucrose 7.5%	water	uncarbonated	MI V 53
		Sucrose 10%	water	uncarbonated	MI V 59
	CC-276 10%		phosphoric acid	carbonated	MI V 69
	CC-276 7.5%	Sucrose 2.5%	phosphoric acid	carbonated	MI V 74
	CC-276 2.5%	Sucrose 7.5%	phosphoric acid	carbonated	MI V 73
		Sucrose 10%	phosphoric acid	carbonated	MI V 79
	CC-276 10%		citric acid	carbonated	MI V 89
	CC-276 7.5%	Sucrose 2.5%	citric acid	carbonated	MI V 94
	CC-276 2.5%	Sucrose 7.5%	citric acid	carbonated	MI V 93
		Sucrose 10%	citric acid	carbonated	MI V 99
	CC-276 10%		water	carbonated	MI V 109
	CC-276 7.5%	Sucrose 2.5%	water	carbonated	MI V 114
	CC-276 2.5%	Sucrose 7.5%	water	carbonated	MI V 113
		Sucrose 10%	water	carbonated	MI V 119
CC-276 / Erythritol	CC-276 10%		phosphoric acid	uncarbonated	MI V 9
	CC-276 9%	Erit. 1%	phosphoric acid	uncarbonated	MI V 15
	CC-276 8%	Erit. 2%	phosphoric acid	uncarbonated	MI V 16
	CC-276 10%		citric acid	uncarbonated	MI V 29
	CC-276 9%	Erit. 1%	citric acid	uncarbonated	MI V 35
	CC-276 8%	Erit. 2%	citric acid	uncarbonated	MI V 36
	CC-276 10%		water	uncarbonated	MI V 49
	CC-276 9%	Erit. 1%	water	uncarbonated	MI V 55
	CC-276 8%	Erit. 2%	water	uncarbonated	MI V 56
	CC-276 10%		phosphoric acid	carbonated	MI V 69
	CC-276 9%	Erit. 1%	phosphoric acid	carbonated	MI V 75

Subgroup	Sweetener 1	Sweetener 2	Solution	Carbonation	Variant No.
	CC-276 8%	Erit. 2%	phosphoric acid	carbonated	MI V 76
	CC-276 10%		citric acid	carbonated	MI V 89
	CC-276 9%	Erit. 1%	citric acid	carbonated	MI V 95
	CC-276 8%	Erit. 2%	citric acid	carbonated	MI V 96
	CC-276 10%		water	carbonated	MI V 109
	CC-276 9%	Erit. 1%	water	carbonated	MI V 115
	CC-276 8%	Erit. 2%	water	carbonated	MI V 116
Reb A / Sucrose	Reb A 7.5%	Sucrose 2.5%	phosphoric acid	uncarbonated	MI V 18
	Reb A 2.5%	Sucrose 7.5%	phosphoric acid	uncarbonated	MI V 17
		Sucrose 10%	phosphoric acid	uncarbonated	MI V 19
	Reb A 7.5%	Sucrose 2.5%	citric acid	uncarbonated	MI V 38
	Reb A 2.5%	Sucrose 7.5%	citric acid	uncarbonated	MI V 37
		Sucrose 10%	citric acid	uncarbonated	MI V 39
	Reb A 7.5%	Sucrose 2.5%	water	uncarbonated	MI V 58
	Reb A 2.5%	Sucrose 7.5%	water	uncarbonated	MI V 57
		Sucrose 10%	water	uncarbonated	MI V 59
	Reb A 7.5%	Sucrose 2.5%	phosphoric acid	carbonated	MI V 78
	Reb A 2.5%	Sucrose 7.5%	phosphoric acid	carbonated	MI V 77
		Sucrose 10%	phosphoric acid	carbonated	MI V 79
	Reb A 7.5%	Sucrose 2.5%	citric acid	carbonated	MI V 98
	Reb A 2.5%	Sucrose 7.5%	citric acid	carbonated	MI V 97
		Sucrose 10%	citric acid	carbonated	MI V 99
	Reb A 7.5%	Sucrose 2.5%	water	carbonated	MI V 118
	Reb A 2.5%	Sucrose 7.5%	water	carbonated	MI V 117
		Sucrose 10%	water	carbonated	MI V 119

7.3.2 Data analysis methods and interpretation of results

a) Analysis of variance

The first analysis groups the results of 54 different samples (2 carbonations X 3 solutions X 4 sweeteners = 24 for 10% concentration plus 2 carbonations X 3 solutions X 5 sweeteners = 30 for 8% concentration). Each was assessed 3 times by 8 panelists, therefore, each sensory descriptor provided 54 X 3 X 8 = 1296 data points. In all cases, this was not 1296 instances of a same number, there was a variability, wider in some descriptors than in others. The Analysis of Variance quantifies this variability and then assesses how much of it is explained because:

i.   the composition of the samples was different (the system factors)

ii. there is variability in the system, which could be divided in 3 elements, allowing to further validate that the error eventually induced by a panel with different individuals is sufficiently small. The sources of variability were thus that:

    a.   a panel is composed by different individuals;

    b.   each individual provided 3 different assessments of each sample blindly (i.e., not knowing they were repeats, and given randomly amongst all samples)

    c.   there may be many other sources of white noise (potential differences between different bottles, variation in the effective temperature of ingestion depending on how long a taster waits to process a particular sample, etc.)

The influence of the system factors was further subdivided in 3 types of effects:

i.1. the average effect. This quantifies the average impact of changing a factor on its own, regardless of the values that other factors happen to have. If the influence of a system factor is totally independent of all others, then this portion of variability quantifies all its impact on the variability of the data.

i.2. the two-way interactions. This quantifies the fact that the influence of a factor may depend on the value of another factor. For illustration: if the sweetness perception of the different sweeteners was different in water than it was in citric and phosphoric acids, then there would be a significant interactive effect Sweetener-by-Solution, and in this case these two factors would explain a portion of the variability of the data equal to the sum of their average effects plus the interactive effect.

i.3. higher-order interactions. This quantifies more complex interactive effects between factors. For illustration: if the Sweetener-by-Solution interactive effect was noticed in carbonated samples but did not exist in uncarbonated, there would be a 3-way effect Sweetener-by-Solution-by-Carbonation.

All higher order interactions were actually pooled with the white noise (as the total was found to be quite acceptable as an error in an ANoVA analysis). Therefore, only two explanations for the influence of the

system factors were retained for analysis: that a factor has an average effect independent of all other factors; that a factor actually has a different impact depending on one other factor, for all possible pairs.

Thus, a first ANoVA provided the following interpretations for the variability of the sensory scores:

i. the fact that samples were sweetened with sucrose, or aspartame, or Reb A, or CC-276, or CC-293 and changing from any sweetener to any other may have an average impact on the sensory score.

ii. the fact that samples were sweetened with 8% or with 10% concentration of the sweetener and there could be an average difference on sensory perception depending on the level of concentration.

iii. the fact that some samples were water, some phosphoric acid and some citric acid solutions, and changing the solution could have an average influence on the score

iv. the fact that some samples were carbonated and others were not, and carbonation could have an average impact on the score

v. the fact that repeats of the same sample by the same person may vary (if panel members were inconsistent, this error would be a significant part of the overall error, otherwise, the consistency of the panelists is validated).

vi. the fact that different members of the panel may have different perceptions (if different individuals had different opinions this portion of the error would be significant, otherwise, it is validated that the panel is consistent).

vii. the fact that the influence of each factor could be different depending on the value of one other factor:

    a. different results for the same sweetener depending on the solution (and vice-versa)

    b. different results for the same sweetener depending on carbonation

    c. different results for the same sweetener depending on the repeat sampling by each panelist (i.e. here we can check if the panel was more consistent with some sweeteners and less with others)

    d. different results for the same sweetener depending on the member of the panel (i.e., here we can check if there was panel disagreement on just one, or more. sweeteners)

    e. different results for the same solution depending on the concentration

    f. different results for the same solution depending on carbonation

    g. different results for the same solution depending on the repeat sampling by each panelist

    h. different results for the same solution depending on the member of the panel

    i. different results for the same solution depending on carbonation

    j. different results for the same solution depending on the repeat sampling by each panelist

    k. different results for the same solution depending on the member of the panel

    l. different repeatability of different members of the panel (i.e. here we can check if on average some panelists were significantly more consistent than others)

viii. all sources of error (pooled together):

a. neglecting the sweetener-by-concentration interactive effect, and all 3 and higher order effects

b. everything else that could contribute to variability and uncertainty (different bottles, different days, etc.)

The ANoVA quantifies these influences in two ways. First, the total variability is quantified by the total sum of squares of the data (which is the sum of all squared differences between each data point and the average of all data points, termed the grand average):

$$SSD = \sum_{i=1}^{n}(y_i - \bar{y})^2$$

where y_i are the various data points, n is the total number of points (1296) and $\bar{y}$ is the grand average, equal to:

$$\bar{y} = \frac{T}{n}$$

where T is the sum of all data points:

$$T = \sum_{i=1}^{n} y_i$$

The influence of the average effect of each factor explains a portion of this SSD, which is given by:

$$SS_{Factor\ j} = \left[\sum_{k=1}^{L_j} \frac{\left(\sum_{i=1}^{n} y_{i,k}\right)^2}{n_k}\right] - \frac{T^2}{n}$$

where j are the system factors (1 to 4), L_j is the number of levels of factor j (5 for sweetener, 3 for solution and 2 for concentration and carbonation), with k being the specific levels, and $y_{i,k}$ are only the data points obtained with factor j having level k.

The 2-way interactive effects explain additional portions of SSD, given by:

$$SS_{Factor\ j\ by\ Factor\ x} = \left[\sum_{k=1}^{L_j} \sum_{m=1}^{L_x} \frac{\left(\sum_{i=1}^{n} y_{i,k,m}\right)^2}{n_{k,m}}\right] - SS_{Factor\ j} - SS_{Factor\ x} - \frac{T^2}{n}$$

where k are the levels of factor j and m are the levels of factor x, $n_{k,m}$ is the number of data points obtained with factor j set at level k and factor x set at level m, and $y_{i,k,m}$ are only the data points obtained with these two factors set at levels k and m.

The error is the difference between the total SSD and all portions explained by the average and interactive effects:

$$SS_{error} = SSD - \sum_{j=1}^{6} SS_{Factor\ j} - \sum_{j=1}^{5} \sum_{m=j+1}^{6} SS_{Factor\ j\ by\ Factor\ x}$$

Once the ANoVA validates that the panel is consistent and the variability that it could create is negligible compared to other sources of error, the corresponding portions of the sums of squares were then pooled with the error (in practice, what we have is 24 repeat assessments of 54 samples).

Table 7 details the decomposition of the ANoVA elements in the sequence of analysis, and what "error" means and is composed of.

Table 6. ANoVA decomposition of causes of data variability

Element	Reason for variability	1st analysis	final interpretation of significance
system factors	average effect of changes in sweetener	quantified	quantified
	average effect of changes in concentration	quantified	quantified
	average effect of changes in solution	quantified	quantified
	average effect of changes in carbonation	quantified	quantified
noise factors	average effect of repeat	quantified	pooled w. error
	average effect of panelists	quantified	pooled w. error
interactions between system factors	interactive effect sweetener by concentration	pooled w. error	pooled w. error
	interactive effect sweetener by solution	quantified	quantified if 1st analysis revealed significance, otherwise pooled with error
	interactive effect sweetener by carbonation	quantified	quantified if 1st analysis revealed significance, otherwise pooled with error
	interactive effect concentration by solution	quantified	quantified if 1st analysis revealed significance, otherwise pooled with error
	interactive effect concentration by carbonation	quantified	quantified if 1st analysis revealed significance, otherwise pooled with error
	interactive effect solution by carbonation	quantified	quantified if 1st analysis revealed significance, otherwise pooled with error
interactions with noise factors	interactive effect sweetener by repeat	quantified	pooled w. error
	interactive effect sweetener by panelist	quantified	pooled w. error
	interactive effect concentration by repeat	quantified	pooled w. error
	interactive effect concentration by panelist	quantified	pooled w. error
	interactive effect solution by repeat	quantified	pooled w. error
	interactive effect solution by panelist	quantified	pooled w. error
	interactive effect carbonation by repeat	quantified	pooled w. error
	interactive effect carbonation by panelist	quantified	pooled w. error
higher order interactions	all 3rd and higher order interactive effects	pooled w. error	pooled w. error
error	all other (unexplained) sources of variability	pooled w. error	pooled w. error

The importance of each effect compared to the error can be determined by an F-test. The total variance and the variance due to each factor are determined by dividing the sums of squares by the respective degrees of freedom, and the F-value of each factor or interaction is equal to the variance due to it divided by the variance of the error. If this value is greater than the F-distribution value for the number of degrees of freedom of the error, the effect has statistical significance. Although this is an important test, the number of data points is so large than the number of degrees of freedom of the error is high. As the panel was trained

and all sources of error such as differences between bottles, etc., were carefully controlled, the portion of sum of squares due to the error is generally expected to be small, and thus the variance due to the error will likely be very small. The best visual perception of the ANoVA results is simply a pie chart of the sums of squares, which gives a very immediate view of which factors dominate the response, how consistent the panel is, and are any interactive effects relevant. The outcome of the ANoVAs performed is shown in this visual manner.

A second important set of graphs are the means plots. If all interactive effects of a given factor are negligible, then the means plots give the full picture of what the influence of that factor is. These are simple plots of the averages of all data for each of the levels of the factor. If interactive effects are negligible, whatever the values of other factors, the difference between having one level of that factor or one other is equal to the difference of the two means. The standard impact of choosing a given level of that factor is equal to the marginal mean. The marginal mean of the level of a factor is the difference between this average and the grand average. If all interactive effects were negligible, then the score of a given sample is predicted well by simply adding to the grand average the marginal means of the levels of each factor.

If an interactive effect was deemed significant, then the means plot should also be drawn for the various means of all combinations of levels of the two factors. This will reveal the nature of the interaction, which can be one of the following:

- the extent of the influence of factor j is much higher in one (or more) level of the factor x than at other(s) - in the limit, a factor actually had no effect in one or more levels of the other (for illustration, sour taste being detected only in citric, phosphoric or carbonated solution, and not in uncarbonated water)
- the influence of a factor is the complete opposite in different levels of the other factor - for instance, factor j increases the intensity of the sensory experience at one level of factor x, but actually decreases the intensity at another level of factor x.

7.3.3   Principal component analysis

There are 12 sensory descriptors. They can be analyzed one by one, or grouped together in spider-graphs, which can be a bit confusing, depending on the complexity of the data. Sometimes, plots of principal components provide an integrated overall view of similarity/distinguishability that while not capturing everything in detail, gives a simple overall picture based on the main sources of distinguishability of the data.

A principal component is a linear combination of the scores of all 12 sensory descriptors. It was found that although several principal components would have to be extracted if one were to use them to analyse the data in full, just the first two principal components provided very clear graphs distinguishing the main differences between the samples in a highly orthogonal manner.

The principal component analysis was applied to the totality of raw data (150 samples x 3 repeats x 8 panelists = 3600 data points). The principal components are given by:

$$PC_i = \sum_{k=1}^{12} a_{i,k} \times s_k$$

where k are the sensory descriptors, s_k the score of descriptor k and $a_{i,k}$ the coefficients for PC i. The coefficients were determined by maximizing the orthogonality between different PC's with the normalized Varimax method, using the software Statistica.

Orthogonality is a very useful feature in graphs to analyse data, it means that a sample may slide in a graph as one of the axis changes value with minimum change on the other axis (the opposite would be a high correlation, where change in one axis causes an equally proportional change in the other). Graphs of PC_i versus PC_j tend to be orthogonal because that is precisely how the PC coefficients are determined: to maximise orthogonality. Indeed, it was found that plots of the average PC_2 versus average PC_1 of the samples clustered the data and showed the main features of distinguishability of the samples. The graphs allow a comparison of the effect of changing a sweetener, in total or gradually, together with the differences between the 6 systems. How close data points are in a graph like this is a measure of how similar the samples are in their main distinguishable sensory features.

8 DETAILED ANALYSIS

8.1 Panel performance and accuracy of the sensory methods

Panel performance was assessed in two complementing ways:

- by testing specifically for reproducibility, discrimination and agreement based on the ability of the assessors to reproduce their ratings, discriminate between the different attributes and agree with the panel consensus as individuals and also on their ability to reproduce their results and discriminate between the different samples as a group.
- by using the results of the ANoVA of the data with single sweetener, considering that the different panel individual and the replicate number are noise factors, contrasting the variance explained by difference between individuals and between replicates of the same individual with that explained by the system factors. This provided an assessment of the error induced by variability of the sensory panel methodology as an analytical method.

8.1.1 Panel performance

Table 7. Reproducibility, discrimination and agreement of the panel

	Reproducibility	Discrimination	Agreement
	Number of attributes with reproducibility comparable to group performance (p>0.5)	Number of attributes significantly different at 90% CL individually (p<0.1)	Number of attributes that correlates well to the panel consensus (r>0.7)
GRK121301	83%	100%	100%
GRK121302	75%	100%	100%
GRK121303	83%	100%	100%
GRK121304	75%	100%	100%
GRK121305	83%	100%	100%
GRK121306	75%	100%	100%
GRK121307	75%	100%	100%
GRK121308	83%	100%	100%

Green (70-100%): Good performance.

Yellow (50-70%): Fair performance.

Red (less than 50%): Poor performance.

Overall, the performance of the individual panellists can be described as very good. All assessors were able to reproduce their ratings, discriminate between the different products and agree with the panel consensus.

8.1.2 Accuracy of the sensory methodology

The ANoVA of the subset of data grouping samples with one single sweetener provided a quantification of the variability induced by the panel itself, as a method of measurement. Figure 1 shows the portion of the variability of the data explained by the factors, and explained by the error, with the panel being individualised as a source of error. This allows to validate that the panel consistency is good, as it can be seen that the portions of sums of squares due to repeat, to panel member, and all interactions of these are much smaller than everything else (barely visible in fig. 1). It The sum of squares of carbonation is not shown in full in figure 1), as it simply relates to samples being carbonated or not and thus the total value is over 8000 and a graph with that scale would dwarf all other descriptors that actually are of much greater interest (if half the samples are not carbonated and everyone would rate them 0, and everyone would rate the other half at the mid-scale of 5, for instance, then the grand average would be 2.5 and the sum of squares would be 1296 times 2.5^2, which is 8,100; if the samples would have been strongly carbonated this value would be even bigger)

Figure 1 also shows clearly that interactive effects are equally very mild. One or two have been deemed significant in some cases, and removing these, all others can safely be pooled with the error.
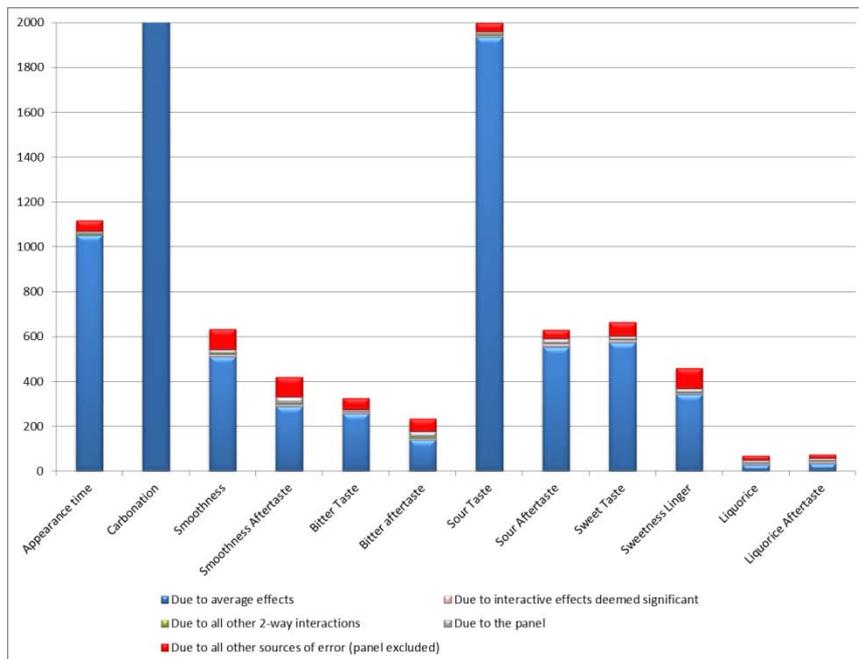
Figure 1. Sums of squares of the Analysis of Variance of the data for samples with single sweeteners The carbonation bar is truncated (max. value 8,508)

8.2 Difference between the sweeteners and relative importance of all factors

This section of the report discusses the first subset of data, composed by the results of module II and module I with single sweeteners. A broad overview is provided in figure 2, showing which factors dominate each of the sensory descriptors. Each will then be analyzed in detail. In this summary figure all sources of error and interactive effects neglected are pooled with the error. Again, carbonation is not shown in full, as it would simply show that carbonating half the samples caused a sum of squares of over 8000 in the scores of carbonation.
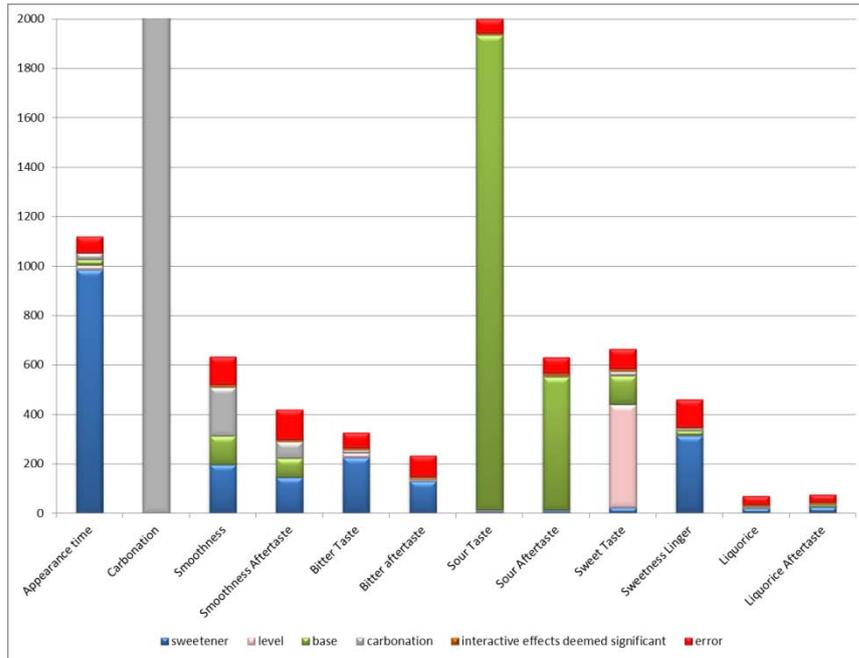
Figure 2. Sums of squares of the Analysis of Variance of the data for samples with single sweeteners showing the importance of the average affect of each system factor. The carbonation bar is truncated (max. value 8,508, of which 8,483 is the portion due to the carbonation factor).

Figure 2 shows some general features of the results:

- appearance time, bitter taste and aftertaste, sweetness linger and liquorice taste and aftertaste were dictated essentially by the sweetener used.
- sour taste and aftertaste were defined primarily by the solution.
- sweet taste was influenced mostly by the concentration, with the solution also having a noticeable effect;
- smoothness and smoothness aftertaste were influenced by the sweetener, the solution and the carbonation;
- carbonation w not affected by any factor other than the sample having been carbonated or not. None of the other factors changed the tingling perception of carbonation in any noticeable manner.

8.2.1 Overall distinguishability (with principal component analysis)

The principal component analysis was performed in all data, not just this subset. The method extracted 4 principal components with eigenvalues above 1.0, which would explain just 67.4% of the variance of the data. The scree plot is shown in figure 3. This is a plot of the eigenvalues which the principal component method calculates to determine the loading factors and coefficients of the principal components. The greater an eigenvalue, the greater the percentage of the variance of the data that the principal component describes. Differences between eigenvalues will decrease and if negligible the principal component is no longer needed. A plot like fig. 3 where all differences are still significant shows a system where one cannot properly replace the 12 descriptors by a smaller number of principal component without losing a significant part of the features of the data.
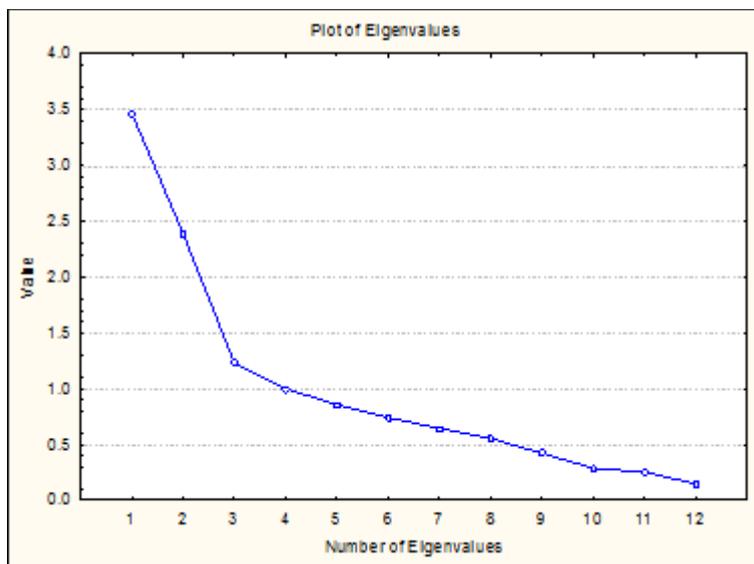
Figure 3. Scree plot of the principal component analysis

As fig. 3 shows, while after the 3rd PC each adds a small portion to the description of the data, there is no descriptor that could actually be removed, all are necessary for a full analysis. This is simply a mirror image of the fact that there is a low correlation between the 12 descriptors, each "tells its own story" and none is perfectly correlated to one other. This is a good feature of the results. However, the main principal components also reveal the most marked distinguishable features of the data and will provide a visual distribution of the samples in a graphic space that quantify differences and similarities.

Usually, eigenvalues above 1 are deemed significant when it is not possible to test for statistical significance, and so the first 4 PC's were deemed to be the most representative, as they have eigenvalues above 1. The loading factors and the coefficients of the first 4 principal components are shown in table 9. The dominant elements in each PC (those with loading factor above 0.7) are highlighted in bold and red. Loading factors are a normalized relative importance of the weight of the score of a descriptor on the value of the PC, varying from -1 to 1, with 0 being no weight and -1 or 1 maximum importance. The coefficients

are the $a_{i,k}$ values used to calculate a PC value as a linear combination of the scores of the descriptors. It is noted that when calculating the score of a PC using the coefficients, descriptors with loading factors below 0.7 may still be relevant, particularly because the variables are all in a scale of 0 to 10, but in practice, some variables occupy a small part of this scale only (for instance, liquorice taste and aftertaste is generally below 1.0, while sweetness is above 5).

Table 8. Results of the Principal Component Analysis

	Loading factors				Coefficients			
	PC 1	PC 2	PC 3	PC 4	PC 1	PC 2	PC 3	PC 4
Appearance Time	0.4426	-0.1313	0.0612	0.0078	0.1699	-0.0707	-0.0618	0.0247
Bitter Aftertaste	**0.8142**	-0.0005	0.0797	0.0198	**0.3096**	-0.0189	-0.0978	0.0540
Bitter Taste	**0.8556**	0.0197	0.1151	0.0034	**0.3181**	-0.0055	-0.0793	0.0394
Carbonation	0.0628	0.0919	**0.8282**	-0.0116	-0.1306	0.1693	**0.5363**	-0.0155
Liquorice	0.0099	-0.0044	0.0119	**-0.9936**	-0.0409	-0.0124	0.0145	**-0.9872**
Liqoorice Aftertaste	0.5591	0.2503	-0.4144	-0.1427	0.2821	0.0337	-0.3293	-0.1108
Smoothness	-0.4886	0.3745	-0.6950	0.0337	-0.0798	0.0926	-0.3010	0.0234
Smoothness Aftertaste	-0.5314	0.4000	-0.5527	0.0211	-0.1243	0.1282	-0.1974	0.0079
Sour Aftertaste	0.0239	**-0.8419**	0.2778	0.0034	-0.0089	**-0.3639**	0.0449	-0.0081
Sour Taste	-0.0133	**-0.7325**	0.1348	-0.0172	-0.0026	**-0.3320**	-0.0234	-0.0276
Sweet Taste	0.0571	**0.7096**	0.3114	-0.0249	-0.0625	**0.3875**	0.2997	-0.0168
Sweetness Linger	0.6919	0.3317	0.2395	-0.0309	0.2169	0.1699	0.0791	0.0008
% variance explained	24.26%	18.73%	15.99%	8.43%				
Cumulative explained	24.26%	42.99%	58.99%	67.42%				

The results, taken together with fig. 2, show clearly the main features of the results:

- Samples have a different bitterness (taste and aftertaste), which is largely due to the different sweeteners used. They also have different sweetness linger, smoothness and smoothness aftertaste and appearance time, which all contribute somewhat to the score of PC1. All these characteristics relate mostly to the sweetener;
- Samples have a different sourness (taste and aftertaste) and a different sweet taste, which is largely due to the different solutions used and to the concentration of sweetener (whatever the sweetener);
- Samples have different carbonations (this is a redundant result, as we already know that half were carbonated and half were not);
- Samples have a different liquorice taste, which is largely due to the sweetener used.

As PC1 is dominated by the sweetener and PC2 by the solution system, plots of PC2 versus PC1 should clearly differentiate the samples, clustering them in one axis in terms of differences between sweeteners

resulting specifically in different bitterness, and in the other axis in terms of differences due largely to the solution, which affects particularly sourness. This plot is shown in figure 4 for the data of the first subset. As there are many data points, to visualize better the plot was divided in a) for the samples with 8% concentration and b) for the samples with 10% of sweetener. As panel repeatability and consistency was very good and the error induced by the panel is perfectly well pooled in the overall error, each data point shown is the average of all 24 repeats of each sample (8 panelists x 3 repeat assessments).

(a)   (b)
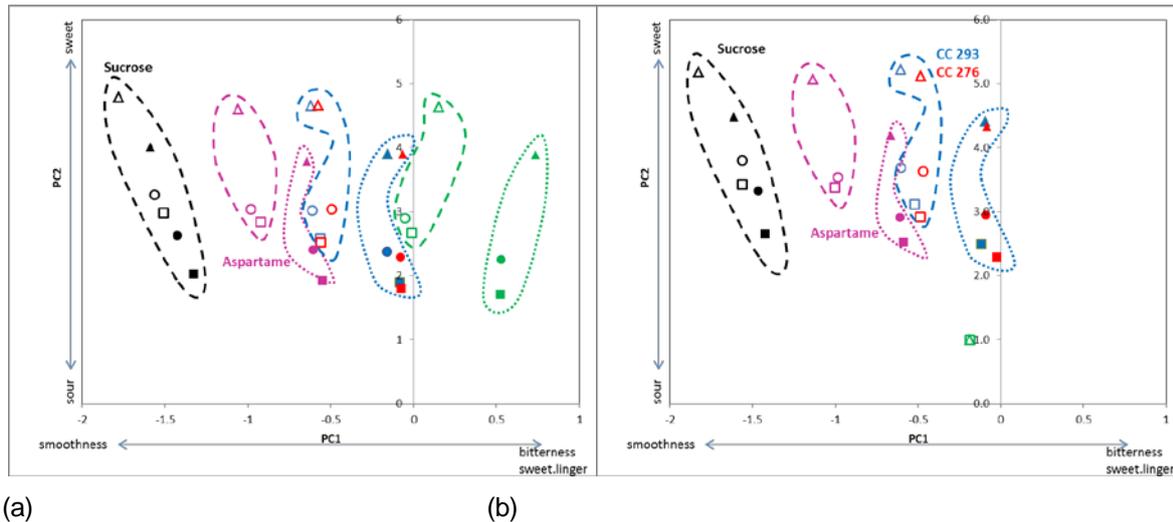
Figure 4. Average scores of principal components 1 and 2 of the samples with single sweeteners with a) 8% concentration and b) 10% concentration.

Sweeteners are surrounded by dotted lines and shown in:
black (sucrose), purple (aspartame), blue (CC-293), red (CC-276), and green (Reb A)
open symbols: carbonated samples
closed symbols: uncarbonated samples
circles: phosphoric acid
squares: citric acid
triangles: water

Sweeteners are clearly separated into groups with sucrose on the left, then aspartame, then the two CC steviosides and then Reb A well to the right. The two steviosides are very close together, although CC-293 is always to the left of the corresponding solution system of CC-276. That means that although other factors can be more important (solution, carbonation, concentration) then all other things being equal, CC-293 gives a slightly lower PC1 score than CC-276. The nature and consequence of this difference is better analyzed in the detail of the sensory descriptors and statistically significant differences. However, the positioning in the graph indicates that the difference is far less than that between either of them and aspartame, and aspartame and sucrose.

With regards to carbonation in PC1, it is apparent that carbonated samples are clearly to the left of the corresponding uncarbonated ones. It is important to note that this does not imply that the bitter perception of the sweeteners is more noticeable in a still than in a carbonated base, it will be shown later that the score for bitter taste and bitter aftertaste is actually higher in carbonated beverages, not the other way around. This position in PC1 results from the overall contribution of all relevant terms of the PC (sweetness linger,

smoothness and smoothness after taste, appearance time, and liquorice aftertaste). As sweetness and smoothness have average scores higher than bitter taste and aftertaste, the score of the PC is more influenced by effects in the descriptors that have higher scores just because of a scale effect, even if the coefficients are lower. The scores of carbonated CC's are similar to those of uncarbonated aspartame, and those of carbonated Reb A to those of uncarbonated CC's. The influence of carbonation is less pronounced in sucrose than in the other sweeteners. Carbonation can be seen in the PC2 axis to cause an increase in this score as well, which is strongly related to a greater sweetness / lower sourness that dominate this component.

Figure 5 shows the shifts in samples that occur with the change of levels in each factor. The arrows connect the points of one level with another, for samples where everywhere else is the same. The lengths and slopes of the lines are similar, suggesting that interactive effects are largely negligible, and therefore that the consequence of a change in a factor is largely independent of all others. It can be seen that increasing the concentration increases the PC2 score slightly with little influence on PC1 (increases sweetness/decreases sourness with little effect on bitterness); carbonation moves in both PC's as described before; the solution moves from water to phosphoric acid to citric acid with falling PC2 score (more sourness, less sweetness), and the sweetener moves PC1 right to left from Reb A to CC-276 then CC-293 then aspartame and then sucrose, with little change in PC2 (sourness/sweetness). An interactive effect is also apparent, in that changing from water to the acid solutions increases PC1 (bitterness/sweetness linger/smoothness) slightly with sucrose and aspartame, but actually *decreases* it for Reb A, and in the case of the two CC's, there is very little effect on PC1 at all.

(a)   (b)
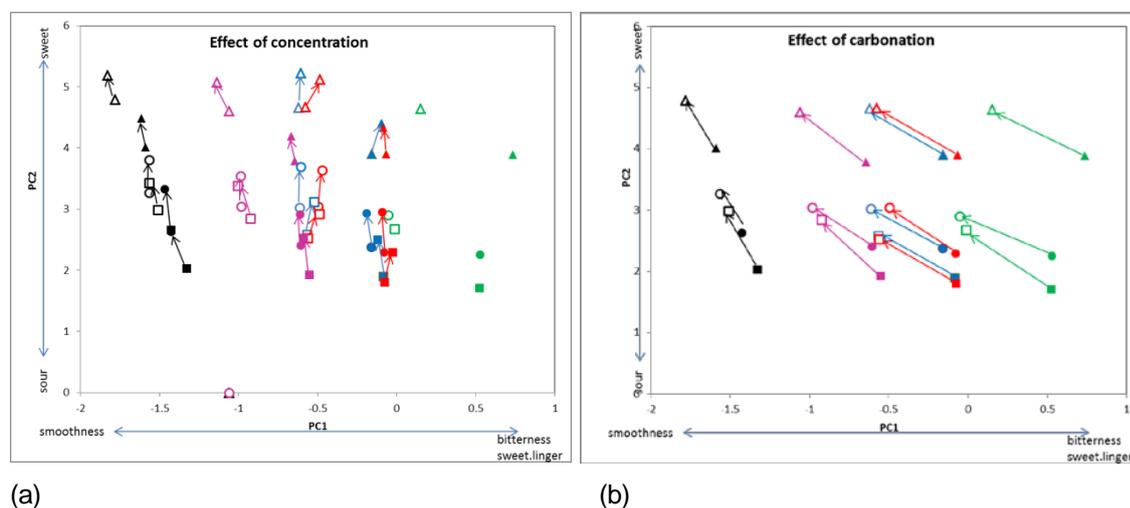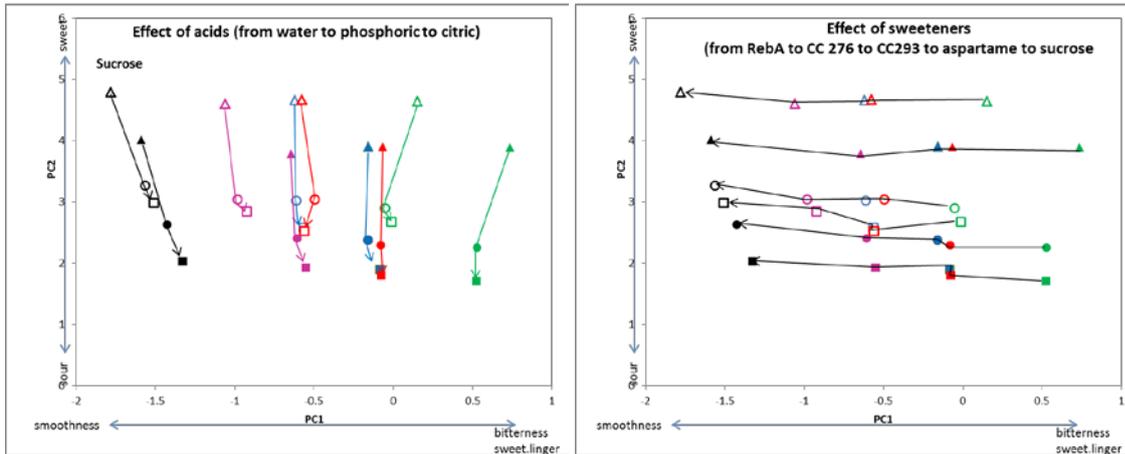
(c)   (d)

(c)   (d)

Figure 5. Changes in the average scores of principal components 1 and 2 of the samples with single sweeteners caused by changes in the system factors. a) concentration of sweetener, b) carbonation, c) solution, d) sweetener

Sweeteners are surrounded by dotted lines and shown in:
black (sucrose), purple (aspartame), blue (CC-293), red (CC-276), and green (Reb A)
open symbols: carbonated samples
closed symbols: uncarbonated samples
circles: phosphoric acid
squares: citric acid
triangles: water

8.2.2 One-by-one analysis of the sensory descriptors

For each sensory descriptor, the ANoVA results are shown first with a pie chart of the raw sums of squares and then the mean plots. If a factor has no statistical significance when all sources of error are pooled (including the panel), the bars are filled in light colour, so all means plots of dark shaded bars mean that changing the factor has a statistically significant effect in the score of the sensory descriptor. When interactive effects were considered to be significant, the means plots subdividing between the different combinations of levels of the interactive pair are then shown. In the means plots of the system factors the error bars show the 95% spread of the mean, which is causes not only by the error, but mostly by the variability due to the other factors, so these bars are not to be read as indicators of statistical significance.

a) Appearance time
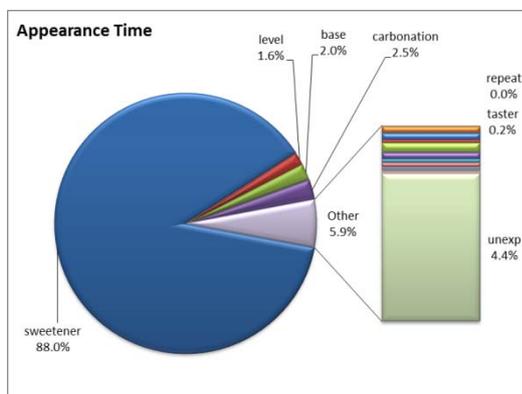
Figure 6. Percent of the total sum of squares explained by each system and noise factors, 2-way interactions, and all other sources of variability for the scores of appearance time in samples with a single sweetener;

Appearance time is essentially determined by the sweetener. There were no significant interactions.
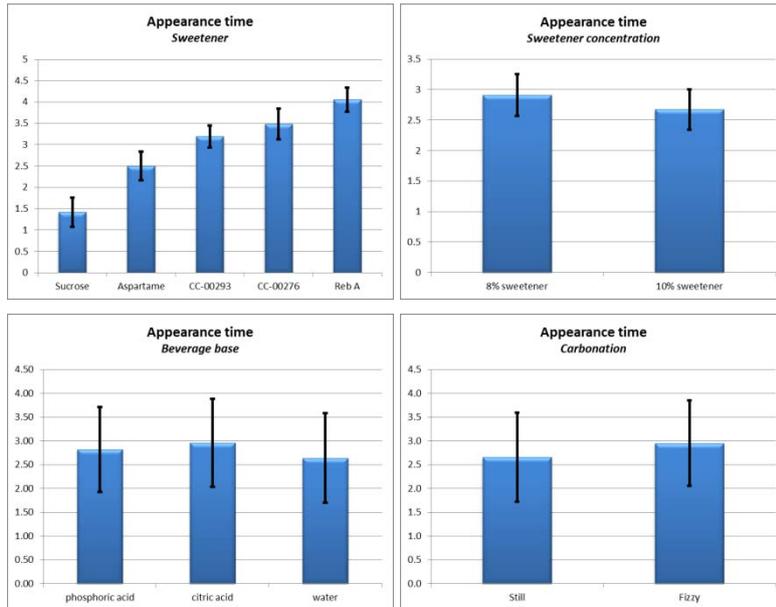
Figure 7. Means plots of the system factors for appearance time

Appearance time is lower in sucrose, then aspartame, then CC-293, then CC-276 and finally Reb A. It is slightly higher in lower concentration and in carbonated samples and it is slightly lower in water and higher in citric acid. Appearance time is estimated well by marginal means addition.

b) Carbonation
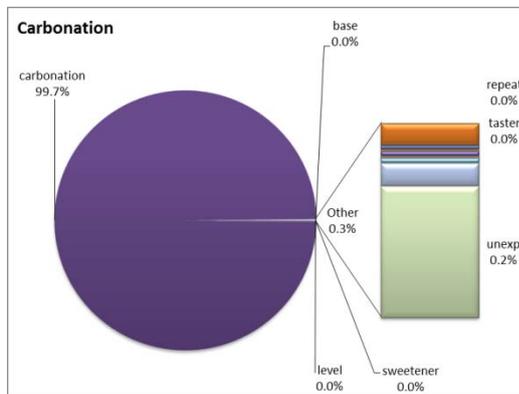
Figure 8. Percent of the total sum of squares explained by each system and noise factors, 2-way interactions, and all other sources of variability for the scores of carbonation in samples with a single sweetener;

The perception of carbonation is influenced just by the carbonation of the sample, no other factor is really relevant.
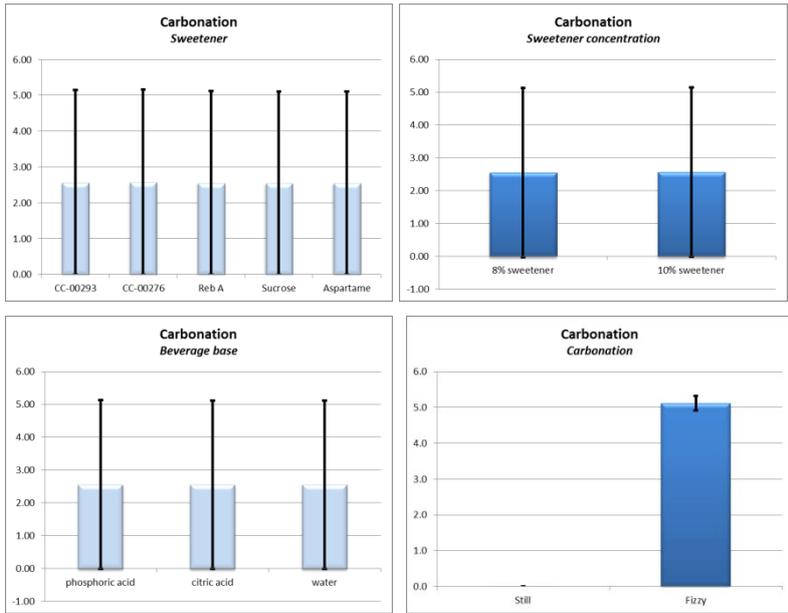
Figure 9. Means plots of the system factors for carbonation

c) Smoothness
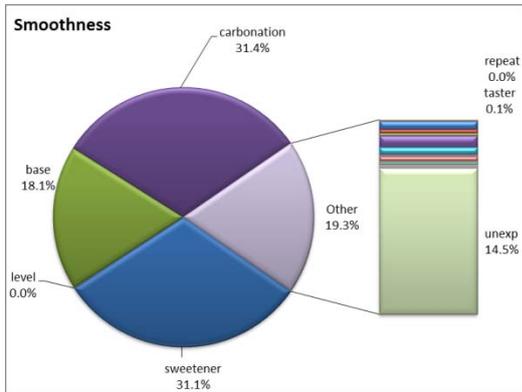
Figure 10. Percent of the total sum of squares explained by each system and noise factors, 2-way interactions, and all other sources of variability for the scores of smoothness in samples with a single sweetener

The sweetener, the solution and carbonation all contribute to the sensation of smoothness. The concentration of the sweetener had no statistical significance. There were two small interactive effects with some significance: between the concentration of the sweetener and the solution, and between the sweetener and the solution.
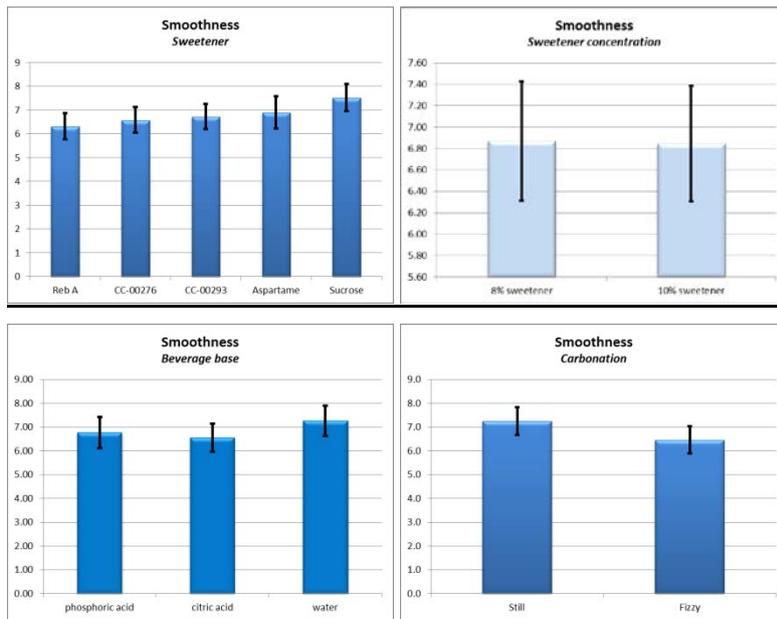
Figure 11. Means plots of the system factors for smoothness

Sucrose gave the smoothest sensation, followed by aspartame, then CC-293, then CC-276, then Reb A. Water increases smoothness and citric acid provided the lowest. Carbonation decreased smoothness.
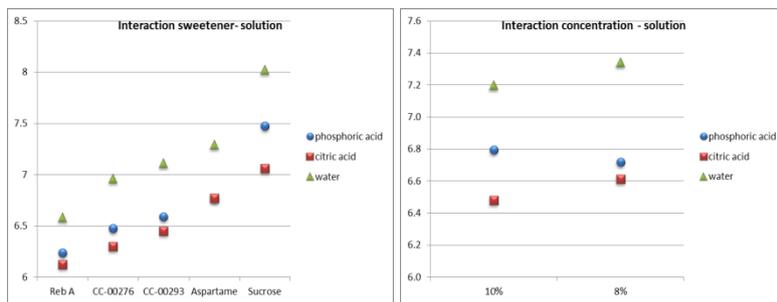
Figure 12. Means plots of relevant interactive effects for smoothness

With aspartame the slightly higher smoothness with phosphoric acid compared to citric was not observed. Smoothness increased with concentration for water and citric acid, but actually decreased with phosphoric acid. The average effect is therefore almost negligible because it averages these reverse effects, but it does nevertheless have a small influence.

d) Smoothness aftertaste
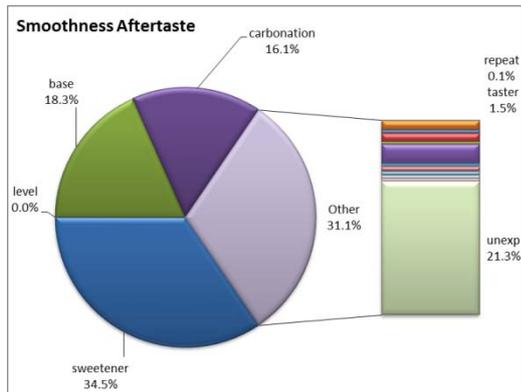
Figure 13. Percent of the total sum of squares explained by each system and noise factors, 2-way interactions, and all other sources of variability for the scores of smoothness aftertaste in samples with a single sweetener;

Very similar result to smoothness, with carbonation showing a small interactive effect with the sweetener. Concentration did not have a statistically significant effect, but in this case there was no relevant interaction either.
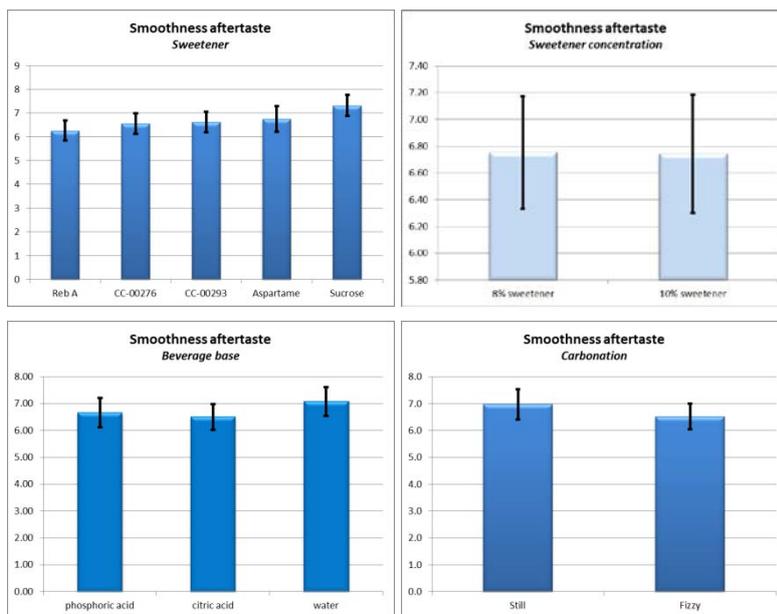
Figure 14. Means plots of the system factors for smoothness aftertaste
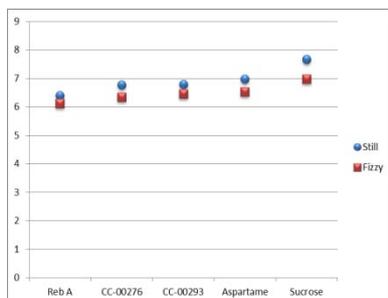
Figure 15. Means plots of relevant interactive effects for smoothness aftertaste

Carbonation has a stronger impact on reducing the smoothness aftertaste of sucrose.

e) Bitter taste
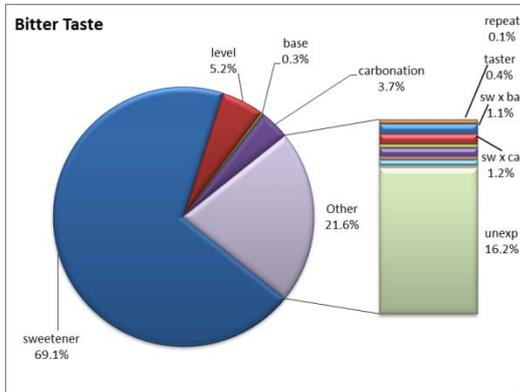
Figure 16. Percent of the total sum of squares explained by each system and noise factors, 2-way interactions, and all other sources of variability for the scores of bitter taste in samples with a single sweetener;

The sweetener is the main cause of bitterness, with its concentration being the second most important factor. Although the solution has a statistically significant effect, it is the most relevant, with a significant interactive effect with the sweetener. There is also a significant interaction between sweetener and carbonation. The higher proportion of unexplained random variation in the data compared to other descriptors is simply due to a scaling factor, as bitterness scores are low (generally below 1, with panelists scoring with differences up to 0.1).
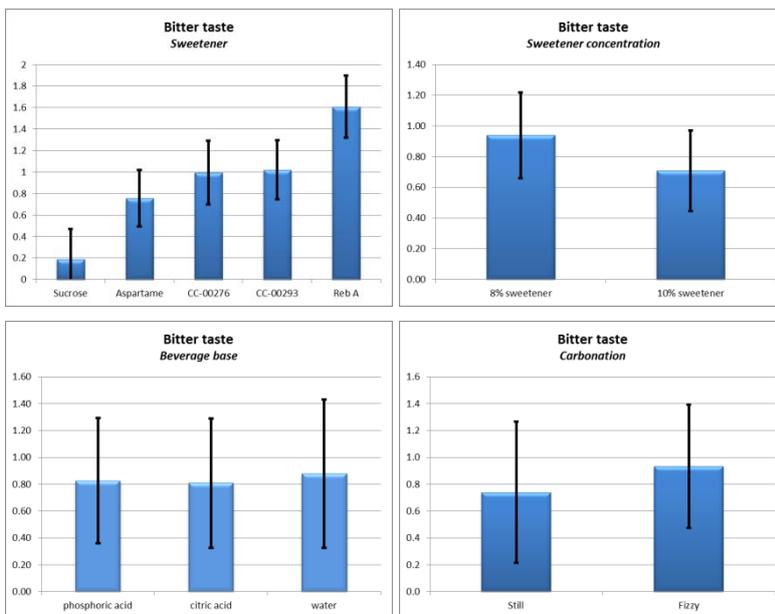
Figure 17. Means plots of the system factors for bitter taste

Bitterness was much lower in sucrose and much higher in Reb A - see interactions in fig. 18. It was slightly lower in aspartame than the 2 CC steviosides, which have very similar means. The bitterness perception was significantly lower with the higher concentration of sweetener and significantly higher with water, compared to the two acids which gave similar results. This may be due to greater sweetness lowering the perception of bitterness. As will be shown in the other sensory descriptors, the concentration of sweetener increases sweet taste significantly, and the two acids give sourness, which water lacks. The increased bitterness due to carbonation is therefore also likely to be due to the carbonic acid created by the carbonation. It has also been found that sour taste correlates to some extent with sweet taste (reversely), as they are pooled in PC2. Therefore, greater sweetness and less sourness (higher PC2 score) decreased bitterness perception slightly (note the scales of the graphs, the differences of the means for concentration, solution and carbonation are however all very small).
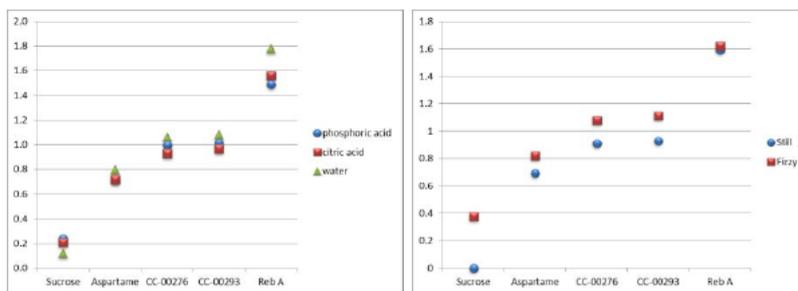
Figure 18. Means plots of relevant interactive effects for bitter taste

The interaction between sweetener and solution shows an interesting reversal. While with sucrose there was a higher bitterness with the acid solutions, with all other sweeteners it was the opposite, especially with the most bitter, i.e. Reb A. This is again probably due to a mild link between sourness and bitterness, as water generates no perception of sourness (see results for that descriptor later). The effect of carbonation was more significant with sucrose, and not significant with Reb A, which again is due to the same link, as carbonation increases sourness (see results for sourness later), which is probably due to the carbonic acid. Note that the average score for sucrose without carbonation is actually 0. It jumps to 0.4 with carbonation bringing the carbonic acid to the taste.

f) Bitter aftertaste
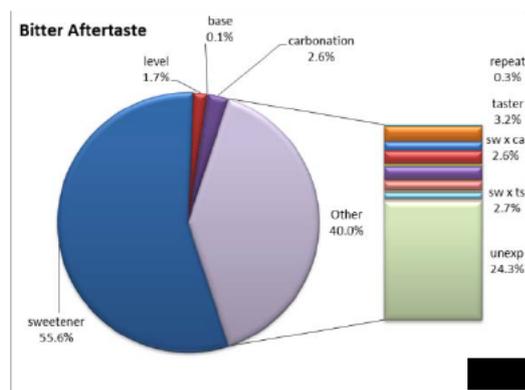
Figure 19. Percent of the total sum of squares explained by each system and noise factors, 2-way

interactions, and all other sources of variability for the scores of bitter aftertaste in samples with a single sweetener;

Results were similar to that of bitter taste itself, only that differences that were smaller were now so attenuated that they no longer had statistical significance. This was the case of the influence of the solution and of the interaction between sweetener and solution.
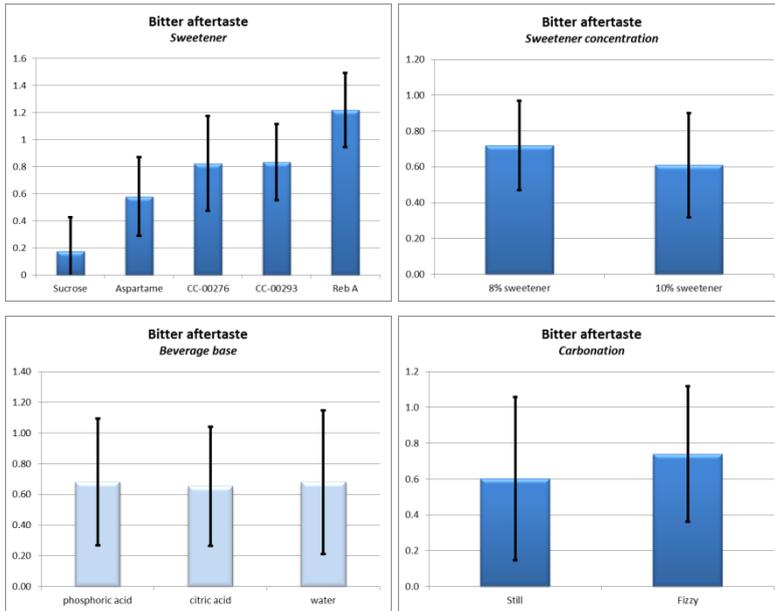
Figure 20. Means plots of the system factors for bitter aftertaste
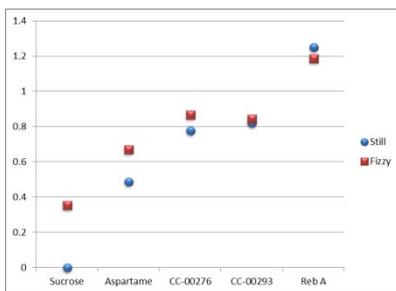
Figure 21. Means plots of relevant interactive effects for bitter aftertaste

g) Sour taste
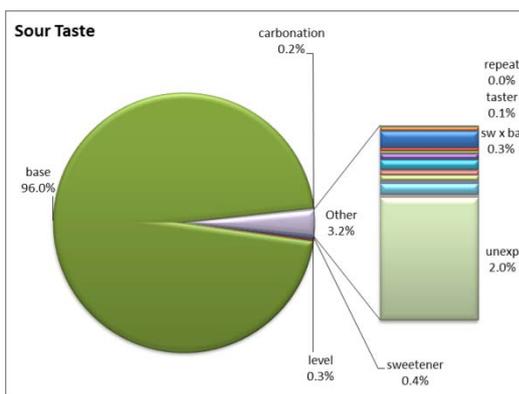
Figure 22. Percent of the total sum of squares explained by each system and noise factors, 2-way interactions, and all other sources of variability for the scores of sour taste in samples with a single sweetener;

Sour taste was essentially a consequence of the solution, although it showed a significant interactive effect with the sweetener.
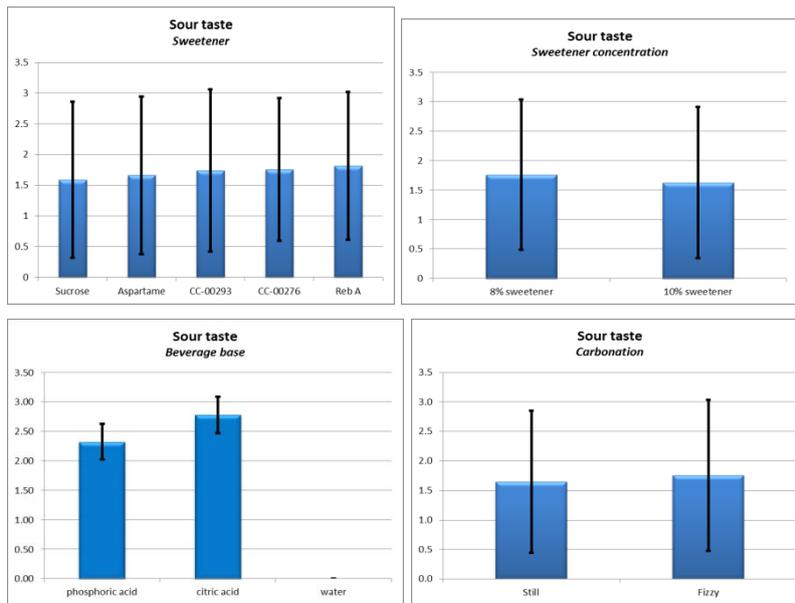
Figure 23. Means plots of the system factors for sour taste

Quite simply, there was no sourness with water, and citric acid gave more sourness than phosphoric acid. There was a very small effect of the sweetener with slightly higher sourness the higher the bitterness of the sweetener, but this should be analysed with the interactive means plot, as that effect was significant.
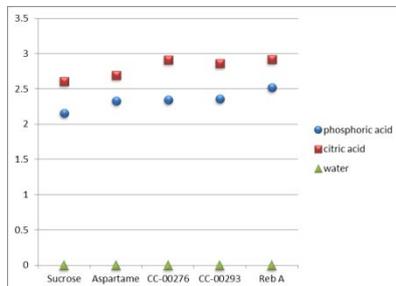
Figure 24. Means plots of relevant interactive effects for sour taste

The interactive effect between sweetener and solution was that while with phosphoric acid the samples of the two steviosides and aspartame had no statistically significant differences in sourness scores, with citric acid the two steviosides had no statistically significant differences in sourness scores with Reb A, and now significantly above aspartame. Note however that these are very small differences.

h) Sour aftertaste
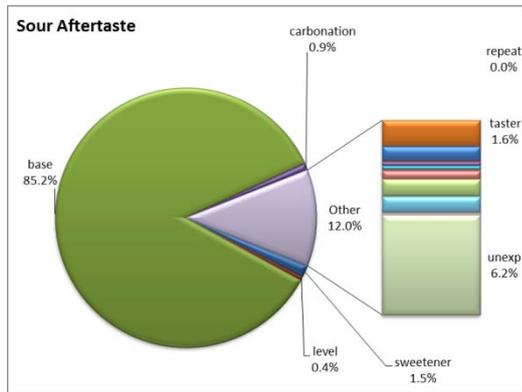
Figure 25. Percent of the total sum of squares explained by each system and noise factors, 2-way interactions, and all other sources of variability for the scores of sour aftertaste in samples with a single sweetener;

Results were similar to those of the sour taste itself, with a slightly higher importance of the other factors.
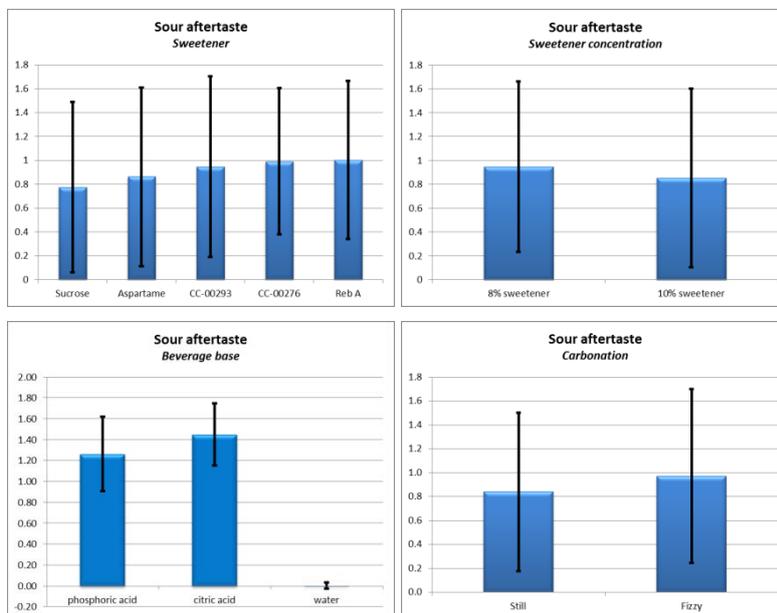
Figure 26. Means plots of the system factors for sour aftertaste
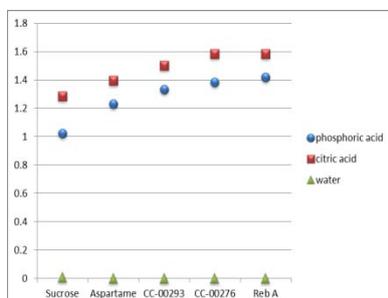
Figure 27. Means plots of relevant interactive effects for sour aftertaste

i) Sweet taste
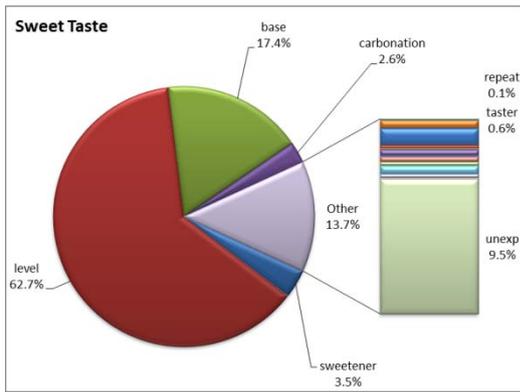
Figure 28. Percent of the total sum of squares explained by each system and noise factors, 2-way interactions, and all other sources of variability for the scores of sweet taste in samples with a single sweetener;

The concentration of sweetener was the dominant factor, and the solution was actually much more important than the sweetener. The solution and the sweetener had a small interactive effect and there was also an interesting interaction between sweetener and carbonation.
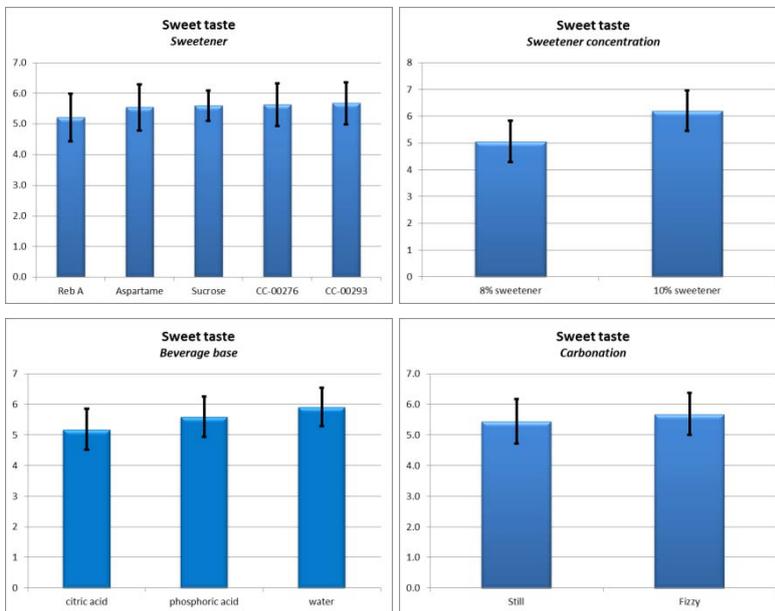
Figure 29. Means plots of the system factors for sweet taste

While the sweetness intensity increased significantly with an increase in the concentration, there was a small difference with citric acid giving lower sweetness than phosphoric acid and water the highest. This is likely to be due to a sweetness/sourness correlation found in the pooling of these two taste sensations in PC2; that is, the more sour the solutions, the lower the corresponding sweetness. However, while the carbonated samples were slightly more sour (see results for sourness), carbonating also increased the perception of sweetness slightly. All sweeteners showed similar sweetness except Reb A, which was slightly below. It is however important to view this in the context of the interaction with carbonation, which is shown below.
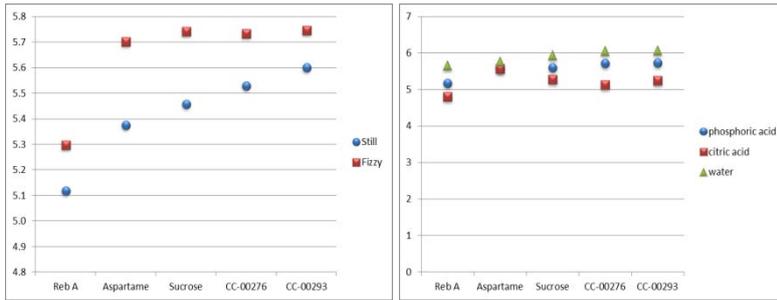
Figure 30. Means plots of relevant interactive effects for sweet taste

In carbonated samples there was no difference in sweet taste between the 4 sweeteners other than Reb A. In uncarbonated samples however there was a clear differentiation between the sweeteners, with aspartame slightly less sweet, followed by sucrose, then CC-276 and CC-293 being the sweetest. Reb A is clearly less sweet than the others.

The interaction between sweetener and solution was simply related to aspartame, where the sweetness perception was independent of the solution. With all other sweeteners, there was a higher sweet taste with water, followed by phosphoric acid and then citric acid. Within this sequence, greater sweetness correlates with lower sourness.

j) Sweetness linger
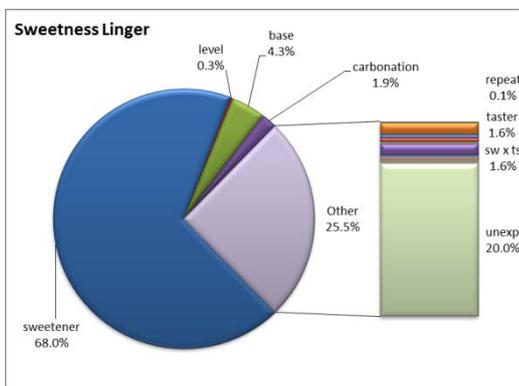
Figure 31. Percent of the total sum of squares explained by each system and noise factors, 2-way interactions, and all other sources of variability for the scores of sweetness linger in samples with a single sweetener;

Sweetness linger is the only aftertaste that has a completely different influence to the taste at time of ingestion. While, as seen before, the immediate sweetness perception is similar to all sweeteners and depends essentially on how much of it there is, the sweetness linger has little to do with quantity of the sweetener, and is dictated by the sweetener itself. The solution also plays an albeit small role in the lingering.
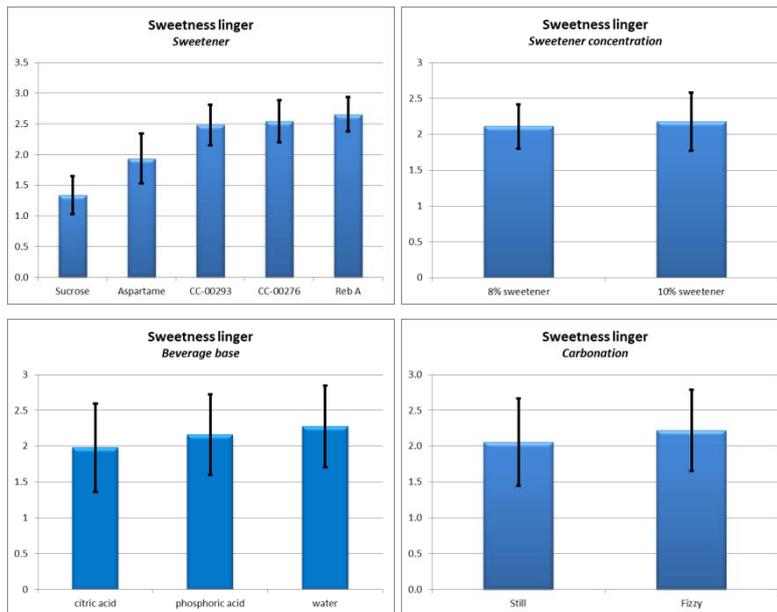
Figure 32. Means plots of the system factors for sweetness linger

Sucrose had the least lingering, followed by aspartame, and Reb A had the most lingering. The two steviosides, being very similar, were closer to Reb A than to aspartame. Lingering increased slightly as sourness diminished; this may be related to the sweet/sour connection, as sourness correlates with less sweetness, more sour solutions "destroy" the lingering effect more quickly. However, carbonation (which increases sourness) also increased lingering, the same opposite effect to sourness found for the sweet taste itself. It is therefore consistent with the possibility that carbonation enhances the sweet taste, and hence strengthens its lingering, in spite of the carbonic acid.
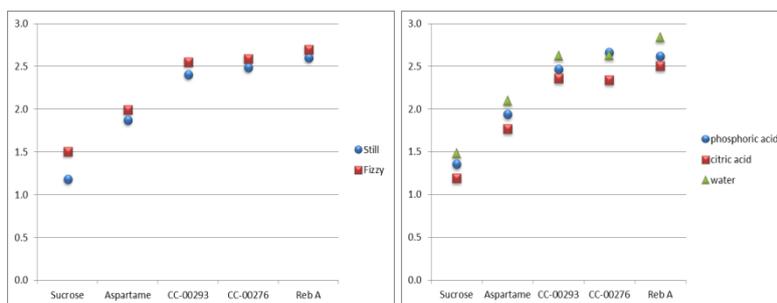
Figure 33. Means plots of relevant interactive effects for sweetness linger

While carbonation had masked the different sweetness of all but Reb A, it did nothing for sweetness linger, the results were similar whether the sample was carbonated or not. Sweetness linger with CC-276 had a slightly different pattern to the others, with phosphoric acid clearly lowering the lingering effect.

k) Liquorice taste
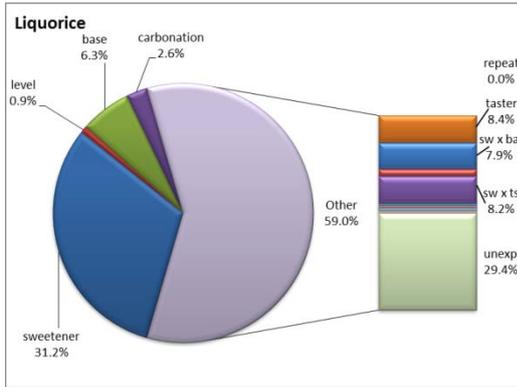
Figure 34. Percent of the total sum of squares explained by each system and noise factors, 2-way interactions, and all other sources of variability for the scores of liquorice taste in samples with a single sweetener;

Liquorice taste is mostly due to the sweetener, with an interactive effect with the solution. General sources of error and differences between tasters are more meaningful proportionally due to a scaling effect, as liquorice is a very faint taste (scores below 1, most of them below 0.4), as the means plots show.
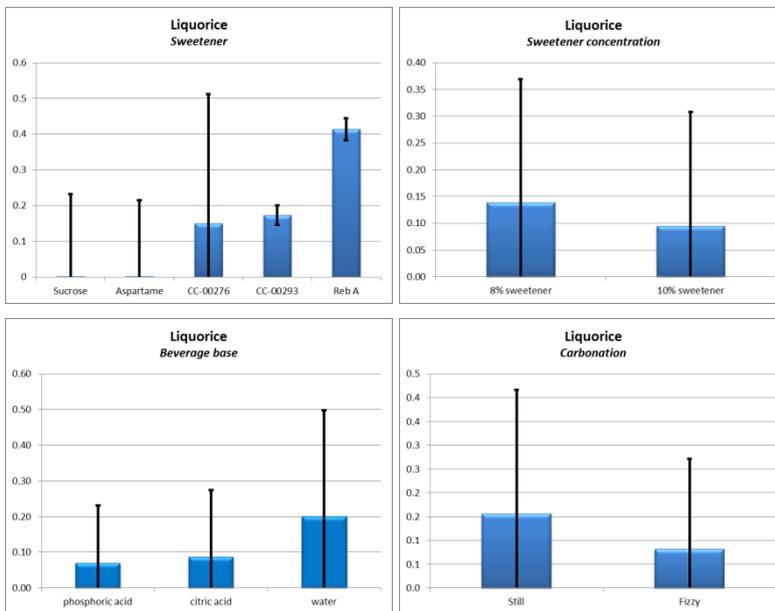
Figure 35. Means plots of the system factors for liquorice taste

There is no liquorice taste with sucrose nor aspartame. It is similar in the two steviosides, and much higher in Reb A. Note that however averages are well below 1 in a scale of 1 to 10, so this is a mild taste.
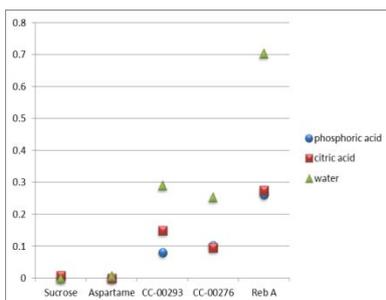
Figure 36. Means plots of relevant interactive effects for liquorice taste

The liquorice taste is much more discernible in water than in the two acids.

l) Liquorice aftertaste
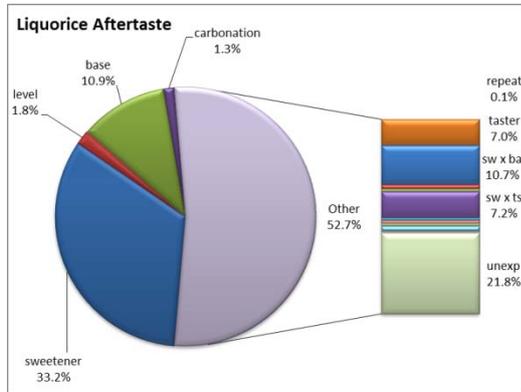
Figure 37. Percent of the total sum of squares explained by each system and noise factors, 2-way interactions, and all other sources of variability for the scores of liquorice aftertaste in samples with a single sweetener;

The results for the liquorice aftertaste were similar to those of the taste itself, but even fainter.
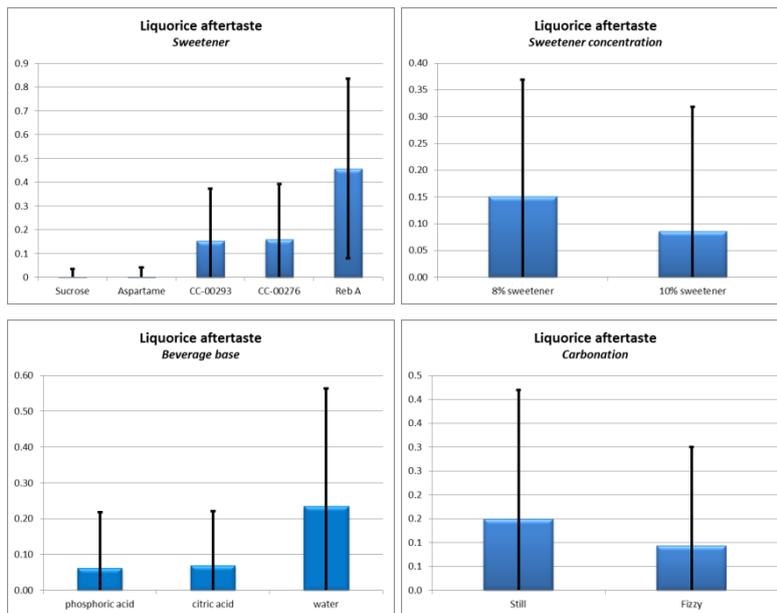
Figure 38. Means plots of the system factors for liquorice aftertaste
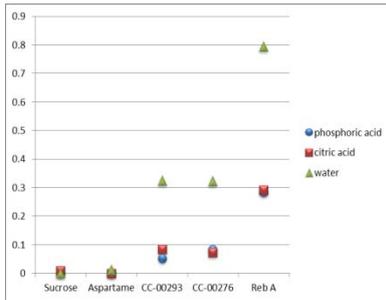
Figure 39. Means plots of relevant interactive effects for liquorice aftertaste

8.3 Performance of sweetener mixes

8.3.1 Overall distinguishability (with principal component analysis)

This analysis is performed with the data of module I only. However, the result of the first analysis suggested that a simple marginal means addition is a good estimate of the score of a sample, and therefore the data for Reb A at 10% concentration was estimated from that at 8%, in order to show the likely position of this point in the graphs. All the sample averages for PC1 and PC2 are shown in figure 40 below. From this figure it is evident that all mixes are located within a gradual progression from one pure sweetener to the other, but because variations in the PC2 scale are small when only the sweetener changes, this single graph does not allow for a very good visualization. The following set of charts (figure 41) therefore show the points divided into 6 graphs, one for each solution system.
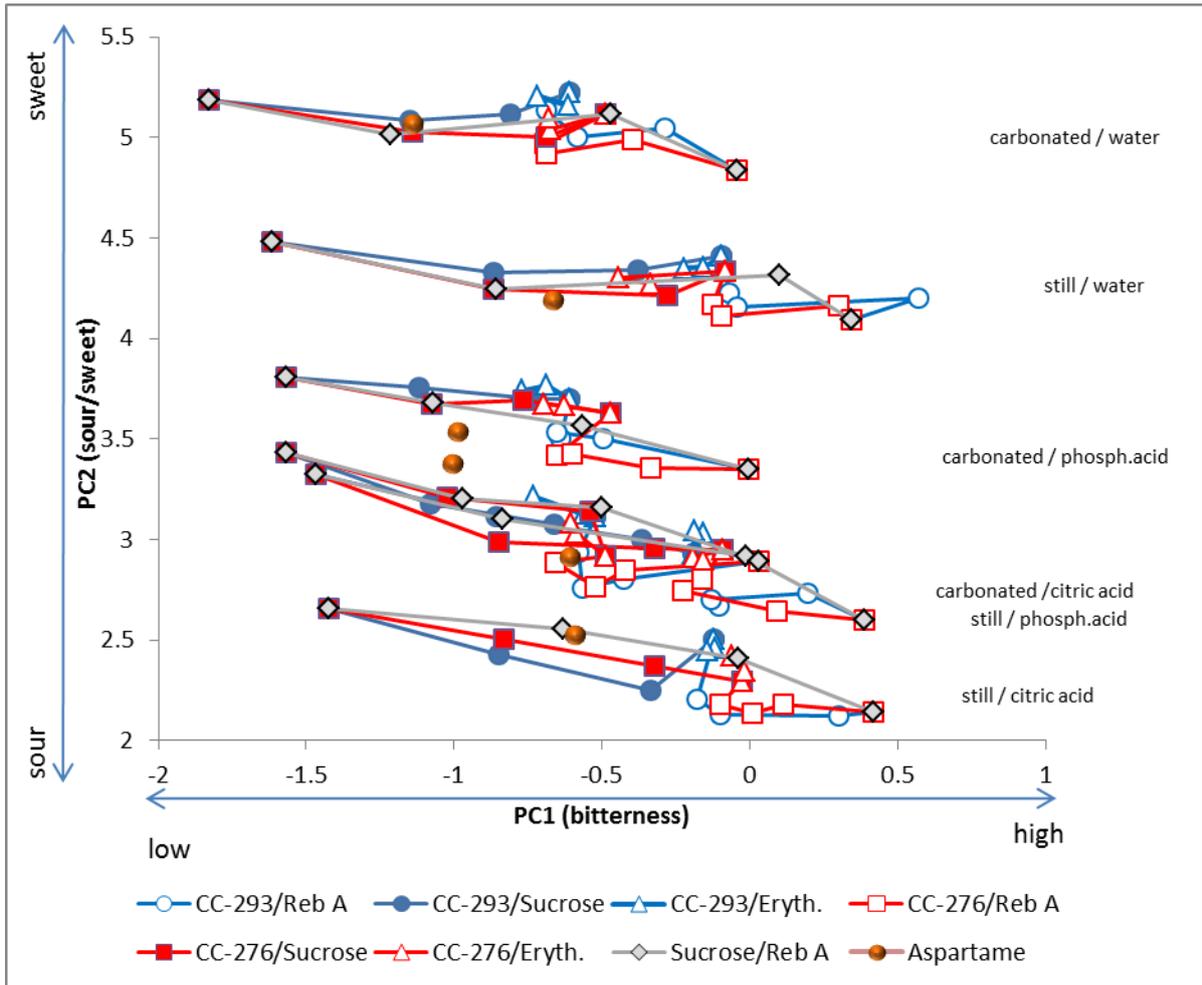
Figure 40. Average scores of principal components 1 and 2 of all samples of module 1
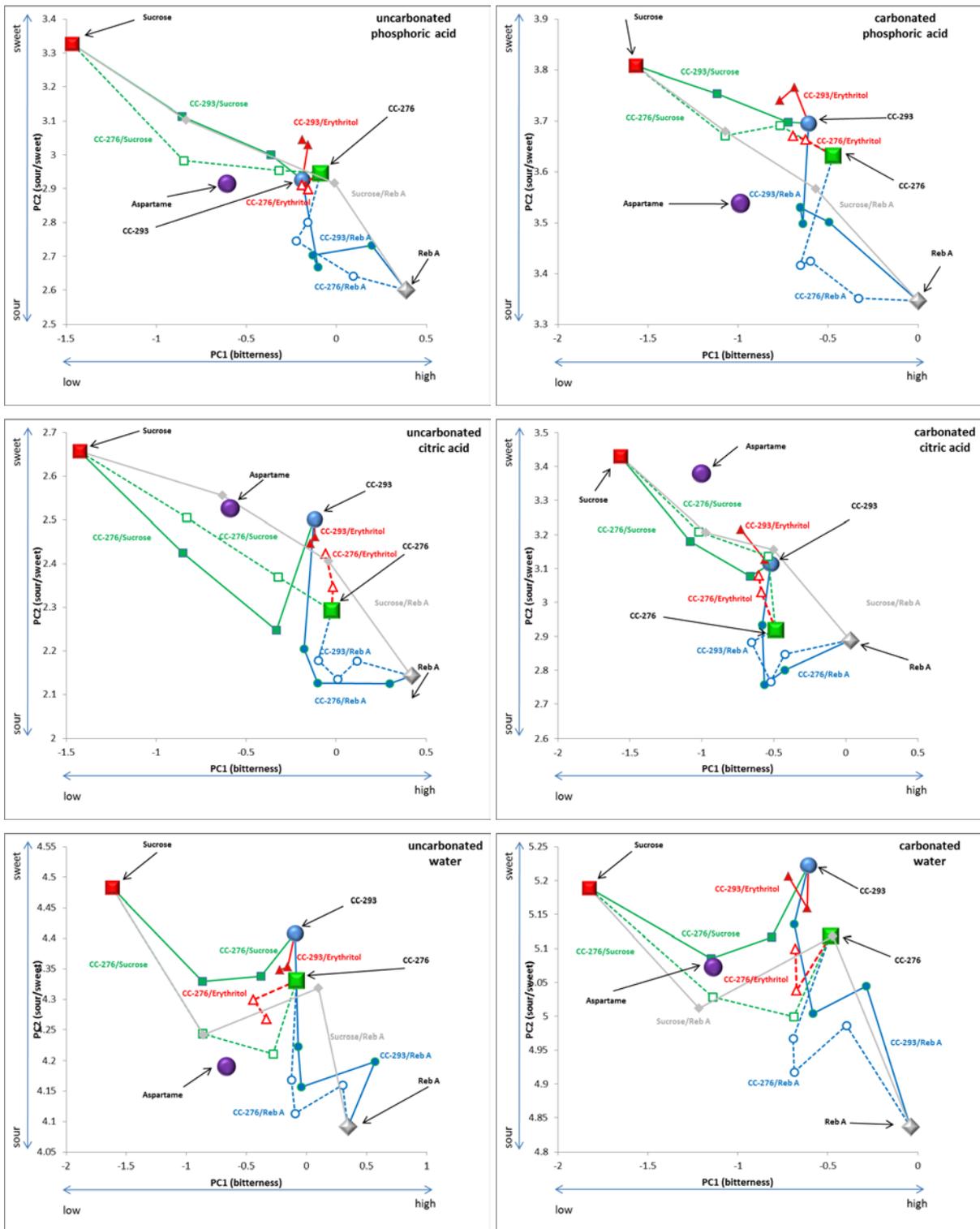
Figure 41. Average scores of principal components 1 and 2 of the samples of module 1 in a) phosphoric acid, b) carbonated phosphoric acid, c) uncarbonated citric acid, d) carbonated citric acid, e) uncarbonated water, f) carbonated water.

To facilitate identification between the two steviosides, mixes with CC-276 are linked with dotted lines (mixes sucrose/Reb A are a grey, thus fainter, solid line).

In general, the progression from Sucrose to Reb A is fairly linear, as is that of Sucrose to either of the steviosides, but only in the acid solutions. In contrast, those of sucrose to each of the steviosides in water, and particularly all of those = towards Reb A, are more curved due to the location of the 100% stevioside concentration pushing the PC1 scores slightly to the right and the PC2 slightly above, compared to the mixes. Samples sweetened with mixes of any of the steviosides with either sucrose or Reb A therefore tend to be less sweet/more sour although less bitter (less sweetness linger / more smoothness) than those with the stevioside alone (except mixes with sucrose in citric or phosphoric acid), compared to a simple proportional change corresponding to the proportion in the mix. This is investigated in greater detail below, by analyzing the impact of each sensory descriptor on the progressive replacements.

8.3.2   One-by-one analysis of the sensory descriptors

For each sensory descriptor, the variation of the score with the composition of the mix is shown for every pair. For all graphs:

- carbonated samples: open symbols and dashed lines
- uncarbonated samples: closed symbols and full lines
- samples with phosphoric acid: circles and blue colour
- samples with citric acid: squares and red colour
- water samples: triangles and green colour

As in most cases the changes are very similar in the 6 solutions systems, the average of all 6 was determined and plots of the average change of the sensory descriptors with the change in proportion of the mix are then also show for each of the steviosides to provide a global comparison for these two sweeteners (for comparison the score of aspartame is also shown as a dotted line).

a) Appearance time
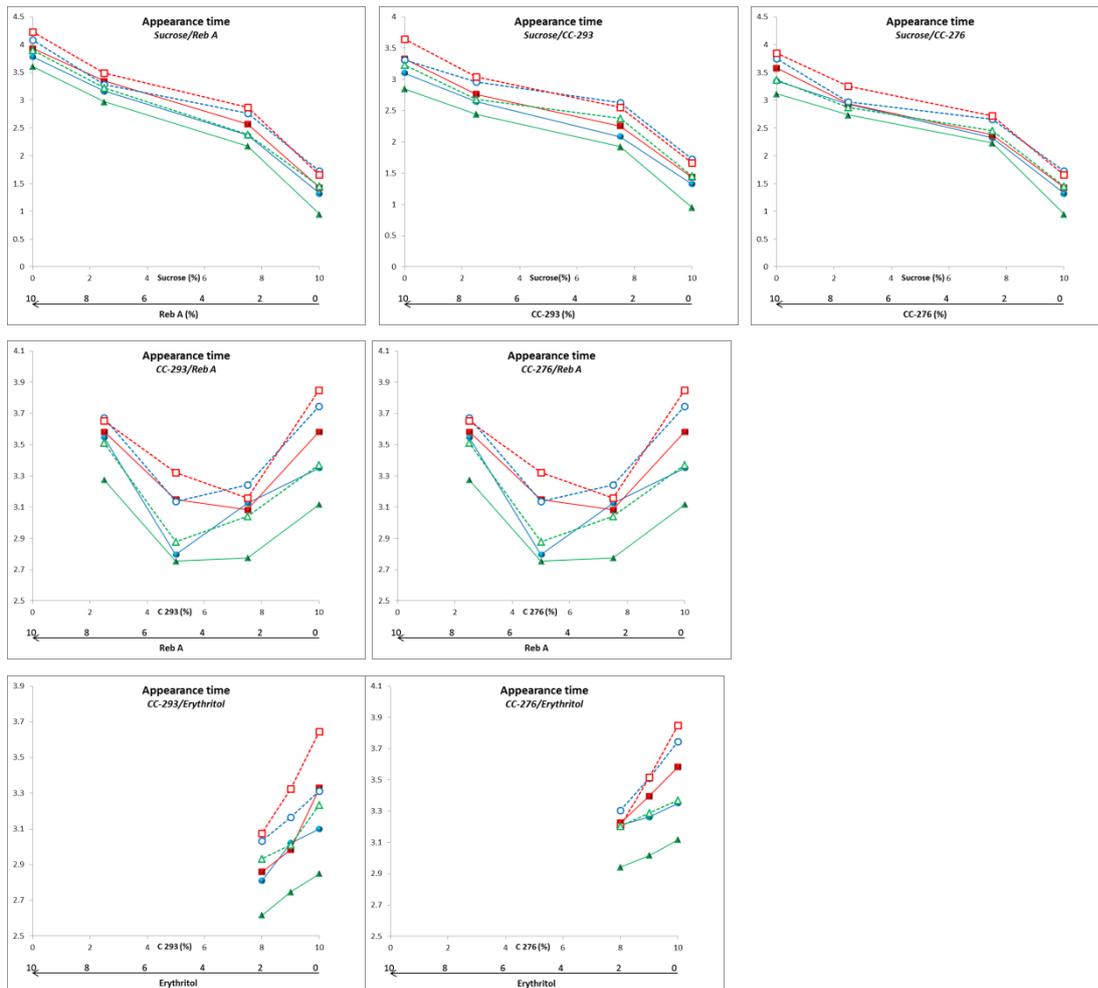
Figure 42. Change in the appearance time score for every sweetener pair tested
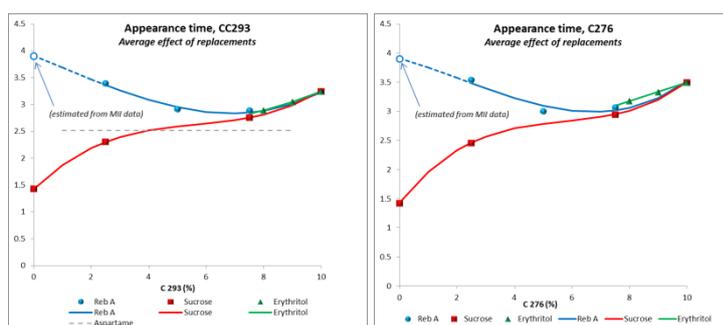
Figure 43. Average effect on appearance time of replacing the steviosides with other sweeteners

The appearance time changes from the one of sucrose to Reb A, CC-276 or CC-293 in a progressive manner explained just by the different proportions of sweeteners with difference appearance times, that is, no synergistic effect was observed. The same is true for erythritol, although in this case the difference is small (note the scale in the graphs), replacing either stevioside for erythritol up to 2% decreases the

appearance time. However, there was a synergistic effect in the Reb A mixes with either of the steviosides, with appearance time having a minimum between 25 and 50% replacement of a stevioside by Reb A. As Reb A replaced the stevioside the appearance time fell slightly, just as much as with the erythritol, in spite of the fact that Reb A has a higher appearance time than the steviosides do. In fact, a mix with 75% Reb A already has the same appearance time as the stevioside.

b) Carbonation

No results are shown for carbonation because they are redundant. All mixes show the same feature as those seen in the first subset of data: all uncarbonated samples have a score of 0 and all carbonated ones have a score of around 5.1.

c) Smoothness
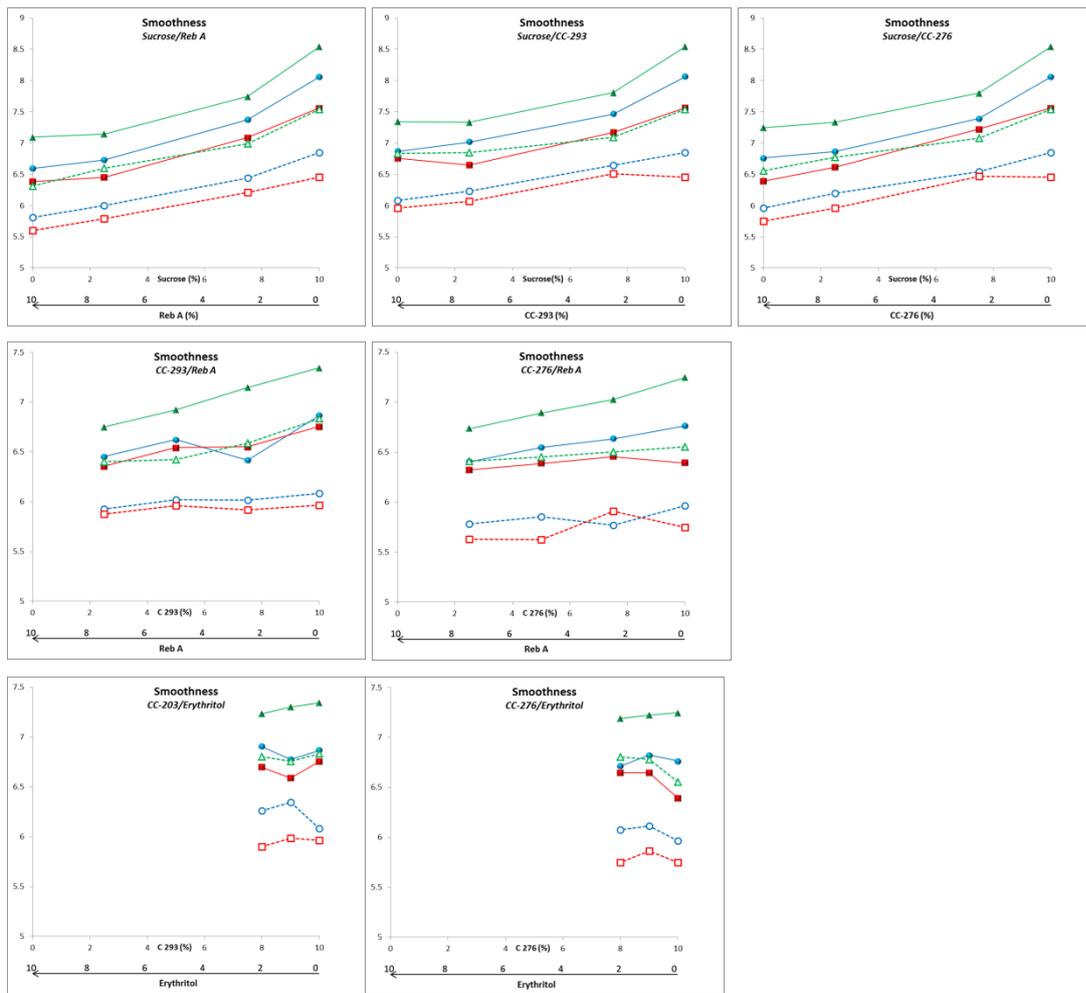
Figure 44. Change in the smoothness score for every sweetener pair tested
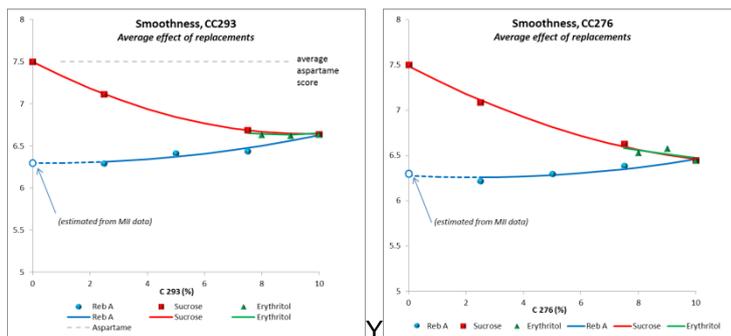
Figure 45. Average effect on smoothness of replacing the steviosides with other sweeteners

Smoothness simply changes gradually from that of one sweetener to the other. Erythritol has no effect on smoothness.

d) Smoothness aftertaste
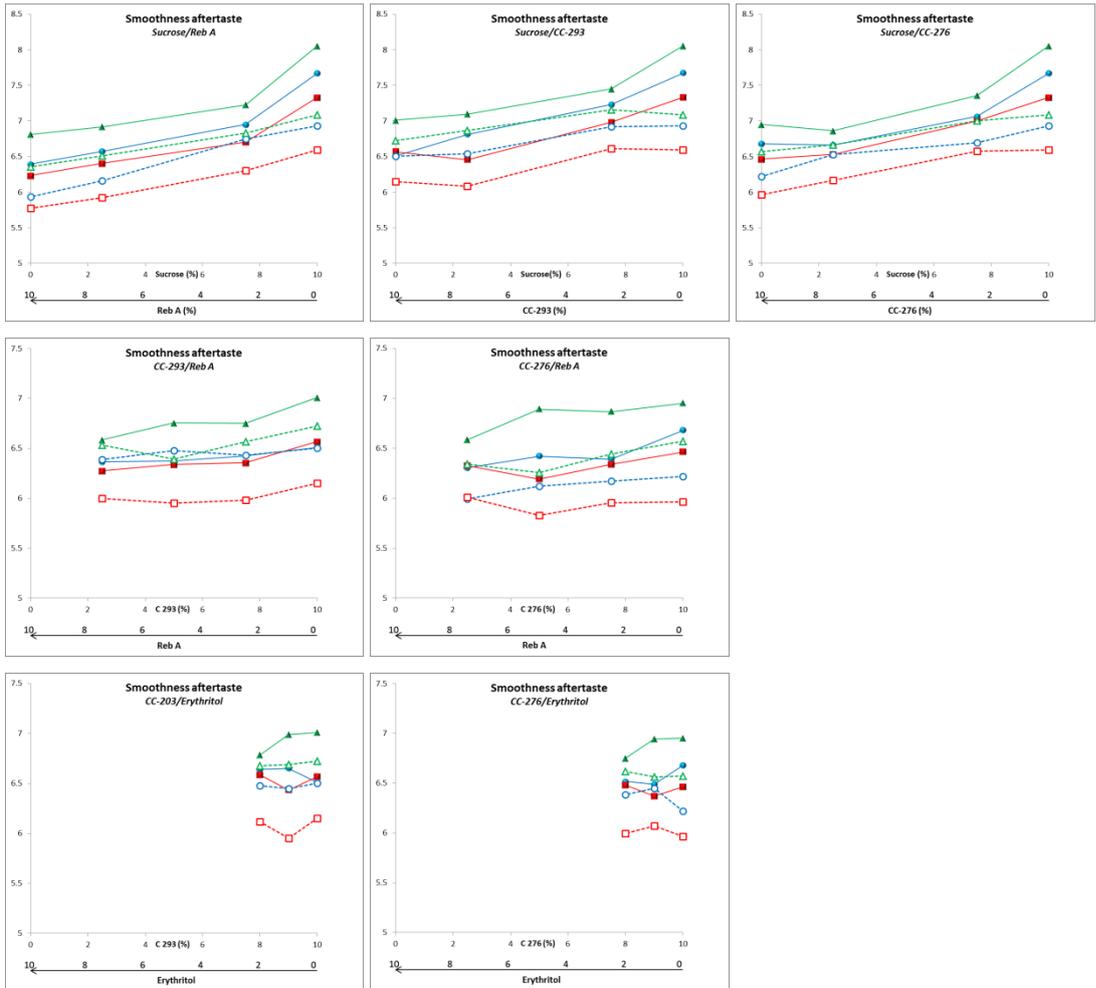
**Figure 46. Change in the smoothness aftertaste score for every sweetener pair tested**
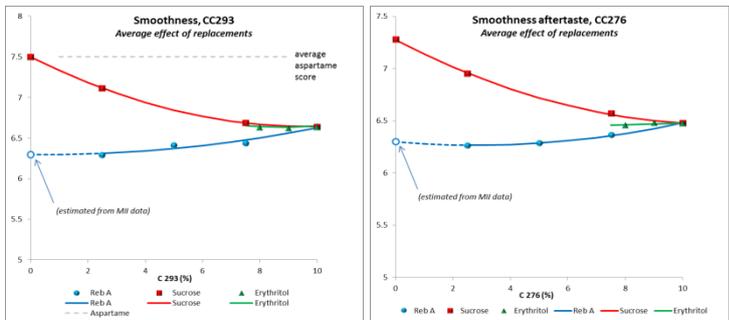
Figure 47. Average effect on smoothness aftertaste of replacing the steviosides with other sweeteners

Similar results as for smoothness.

e) Bitter taste
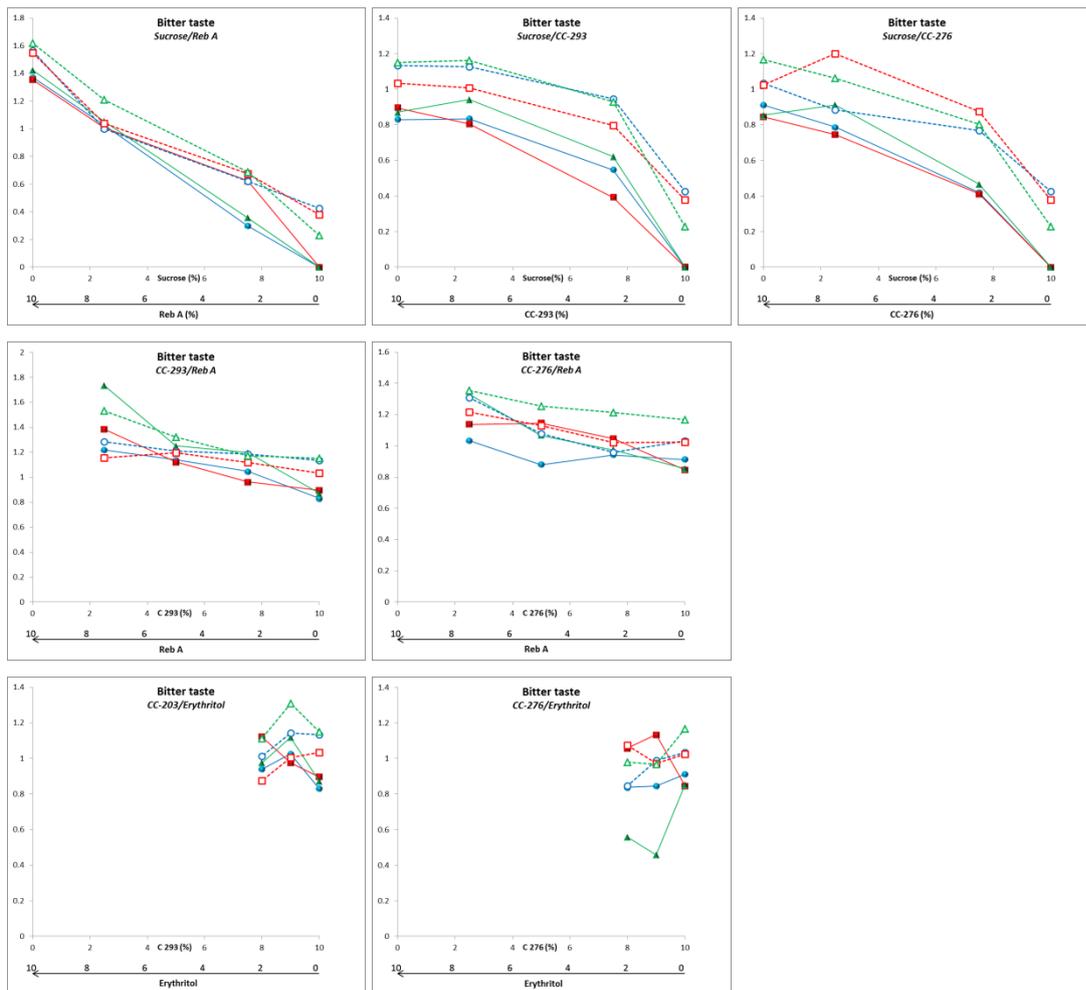
Figure 48. Change in the bitter taste score for every sweetener pair tested
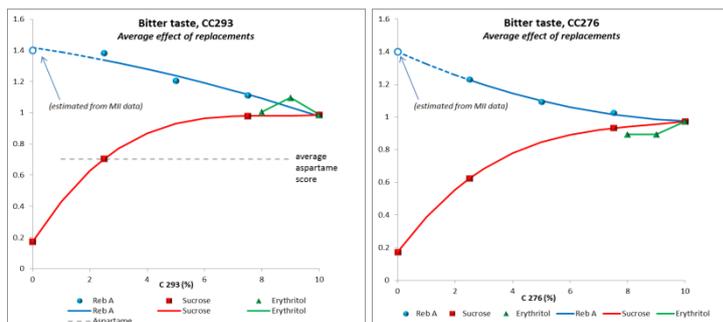
Figure 49. Average effect on bitter taste of replacing the steviosides with other sweeteners

All changes are gradual, but the replacement of either of the steviosides by sucrose shows a marked curvature, with the bitter taste falling very little initially (bitter scores of samples with 2.5% sucrose and 7.5% of either of the steviosides are about the same as those of with only the stevioside). Mixes with 2.5% of sucrose have about the same score of bitter taste as aspartame.

f) Bitter aftertaste
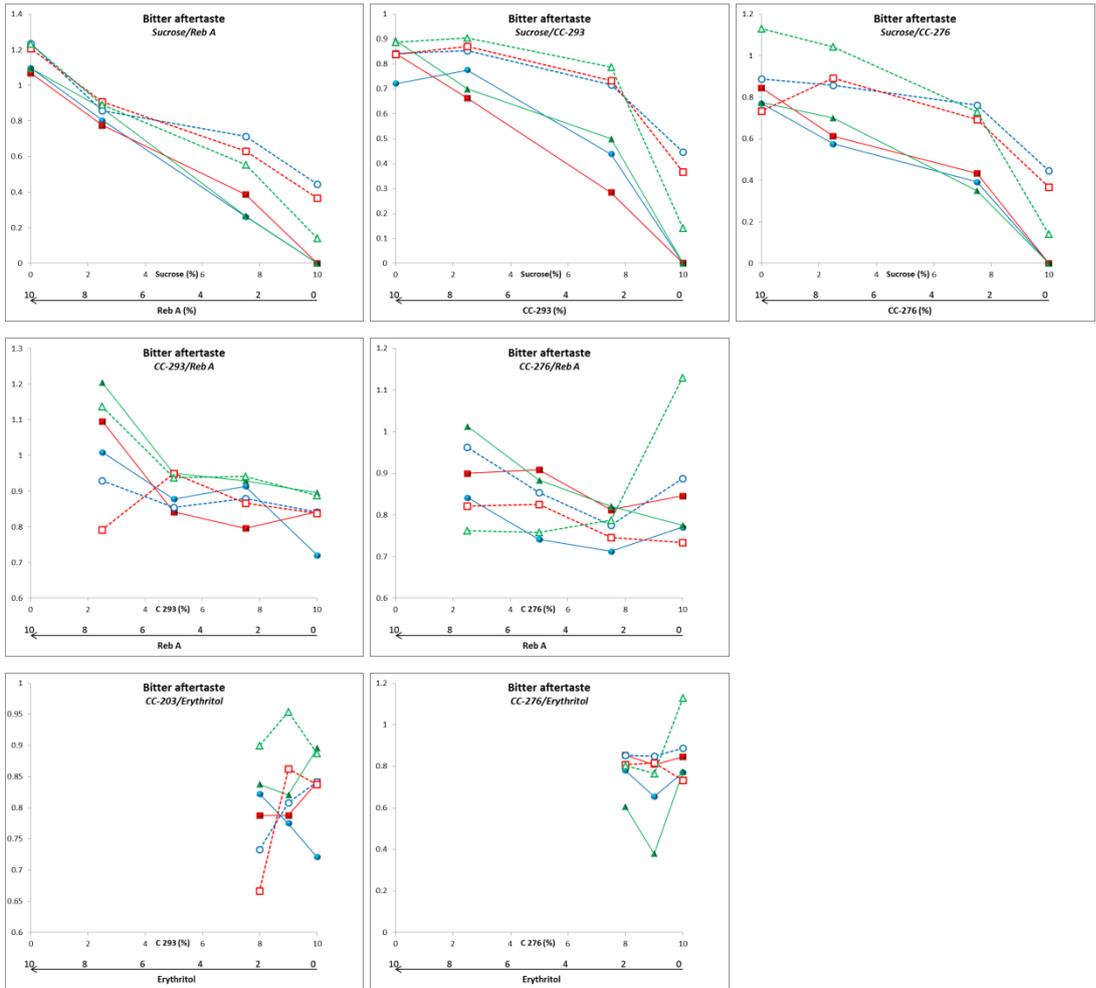
Figure 50. Change in the bitter aftertaste score for every sweetener pair tested
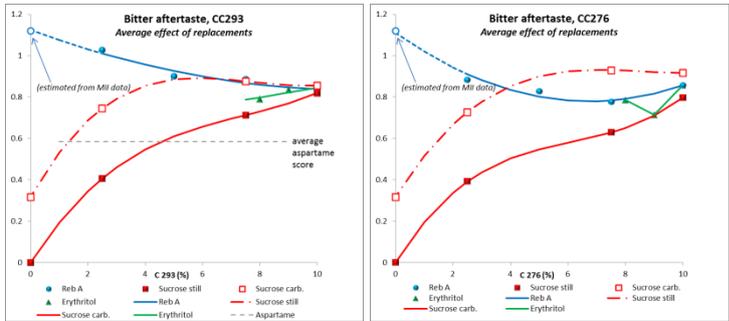
Figure 51. Average effect on bitter aftertaste of replacing the steviosides with other sweeteners

In carbonated samples the small fall of bitterness for mixes of either stevioside as they were replaced by sucrose was even smaller. With carbonation, even the 2.5% sucrose/7.5% stevioside had a similar bitter aftertaste to that of the stevioside on its own.

g) Sour taste
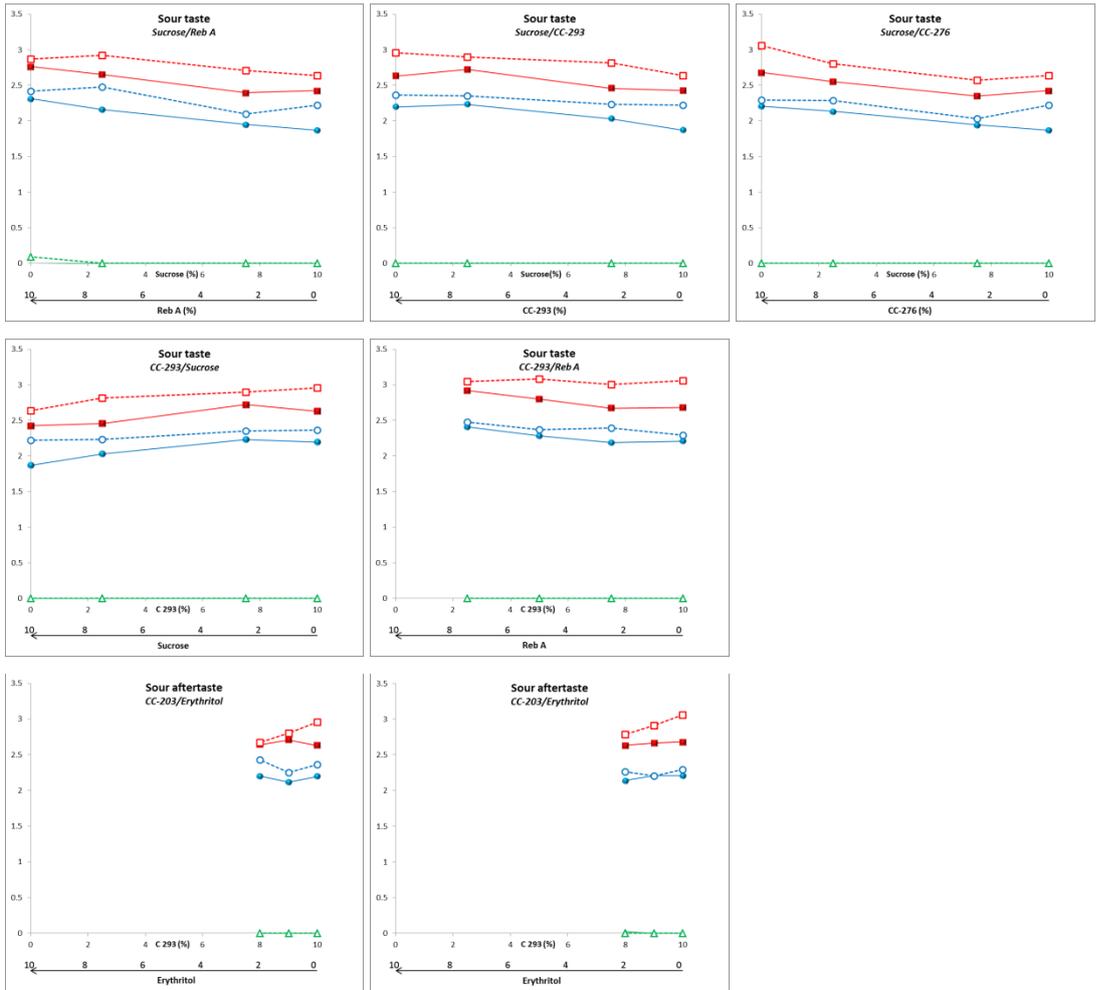
Figure 52. Change in the sour taste score for every sweetener pair tested
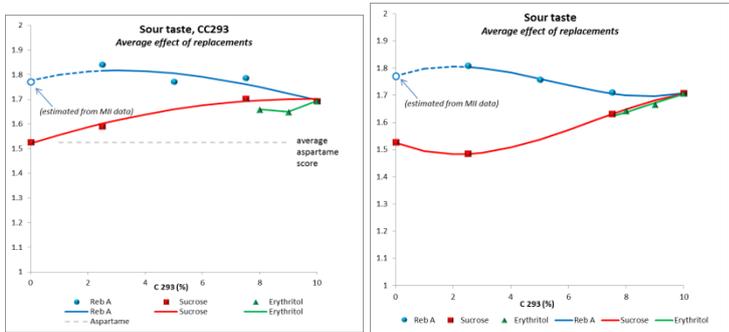
Figure 53. Average effect on sour taste of replacing the steviosides with other sweeteners

The sweeteners do not influence sourness very much, and therefore the mixes involve very small variations.

h) Sour aftertaste
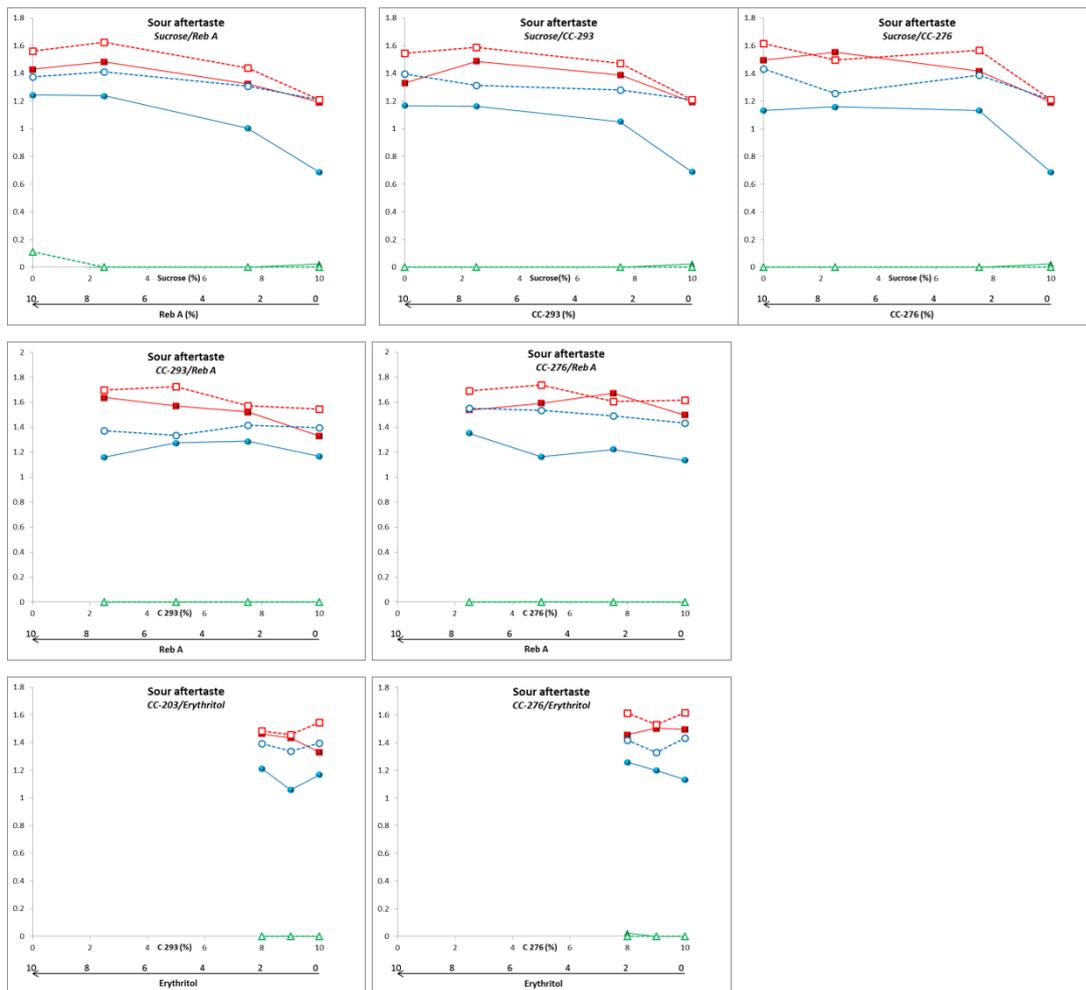
Figure 54. Change in the sour aftertaste score for every sweetener pair tested
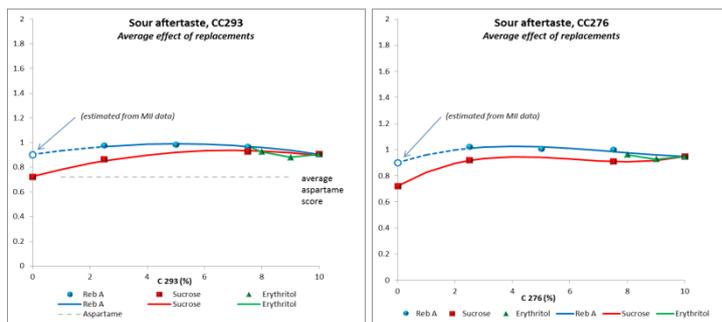
Figure 55. Average effect on sour aftertaste of replacing the steviosides with other sweeteners

Similar results to those of sour taste.

i) Sweet taste
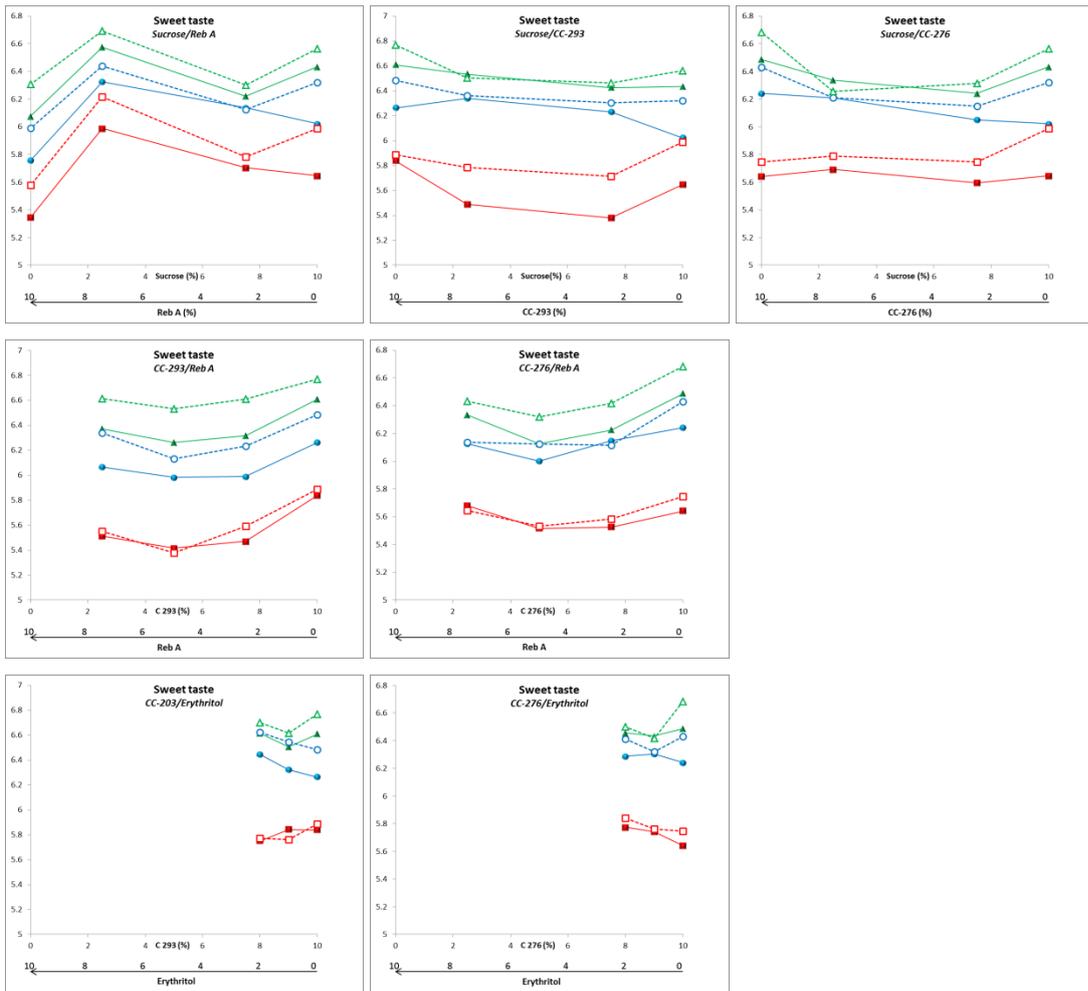
Figure 56. Change in the sweet taste score for every sweetener pair tested
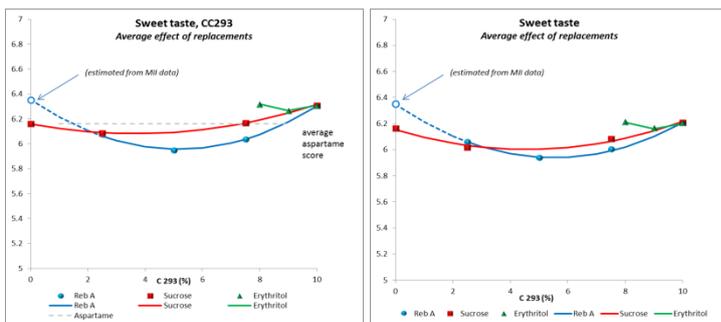
Figure 57. Average effect on sweet taste of replacing the steviosides with other sweeteners

The differences between samples involved only small variations, but there was a general tendency for a point of minimum, meaning that the sweet taste of most mixes 25/75 and 50/50 showed a slightly lower sweet taste than either pure sweetener. Erythritol had a small effect.

j) Sweetness linger
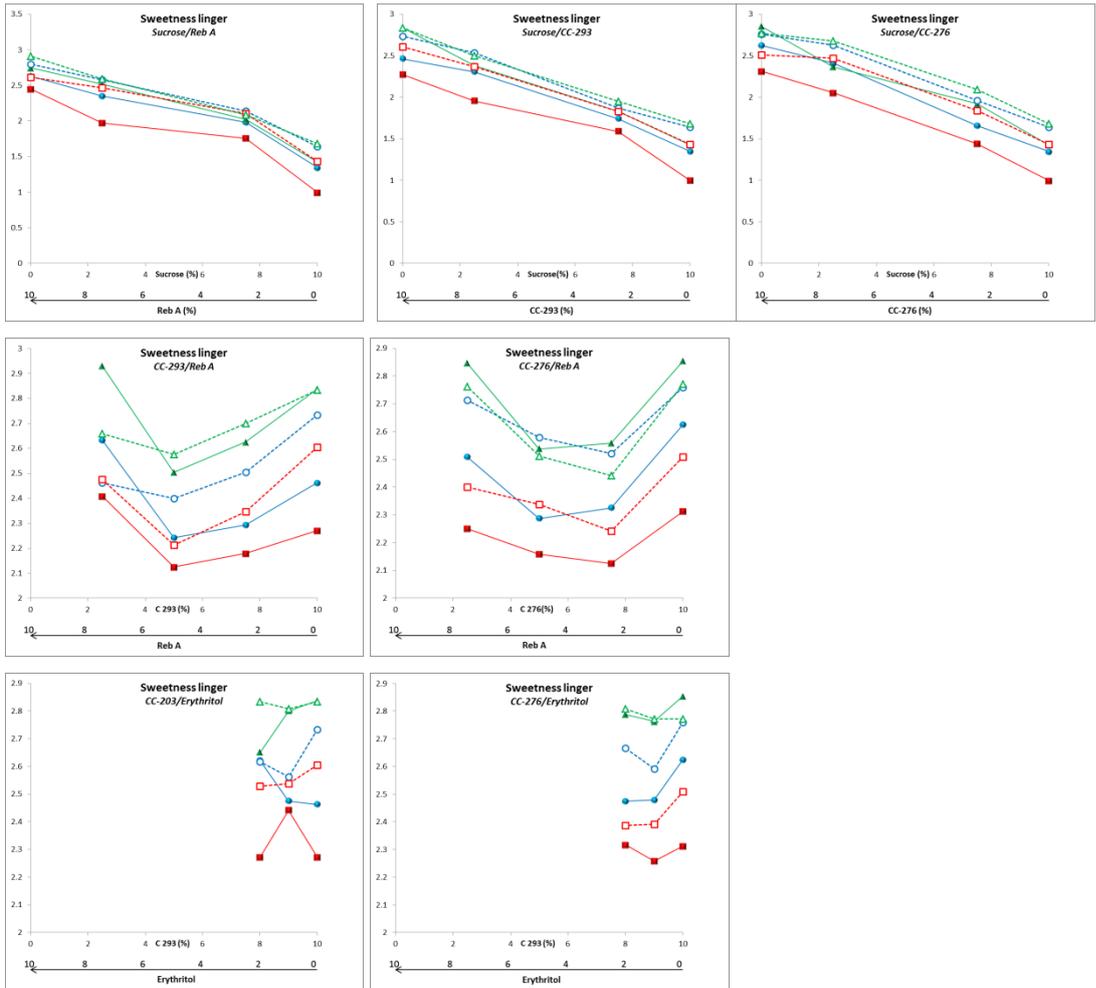
Figure 58. Change in the sweetness linger score for every sweetener pair tested
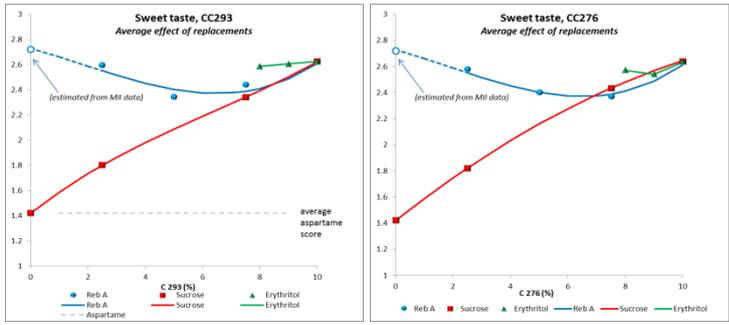
Figure 59. Average effect on sweetness linger of replacing the steviosides with other sweeteners

Although being a small effect, it is evident that there is a minimum in the sweetness linger score for mixes of Reb A with the steviosides, showing a small synergistic effect. Erythritol had a negligible effect on sweetness linger.

k) Liquorice
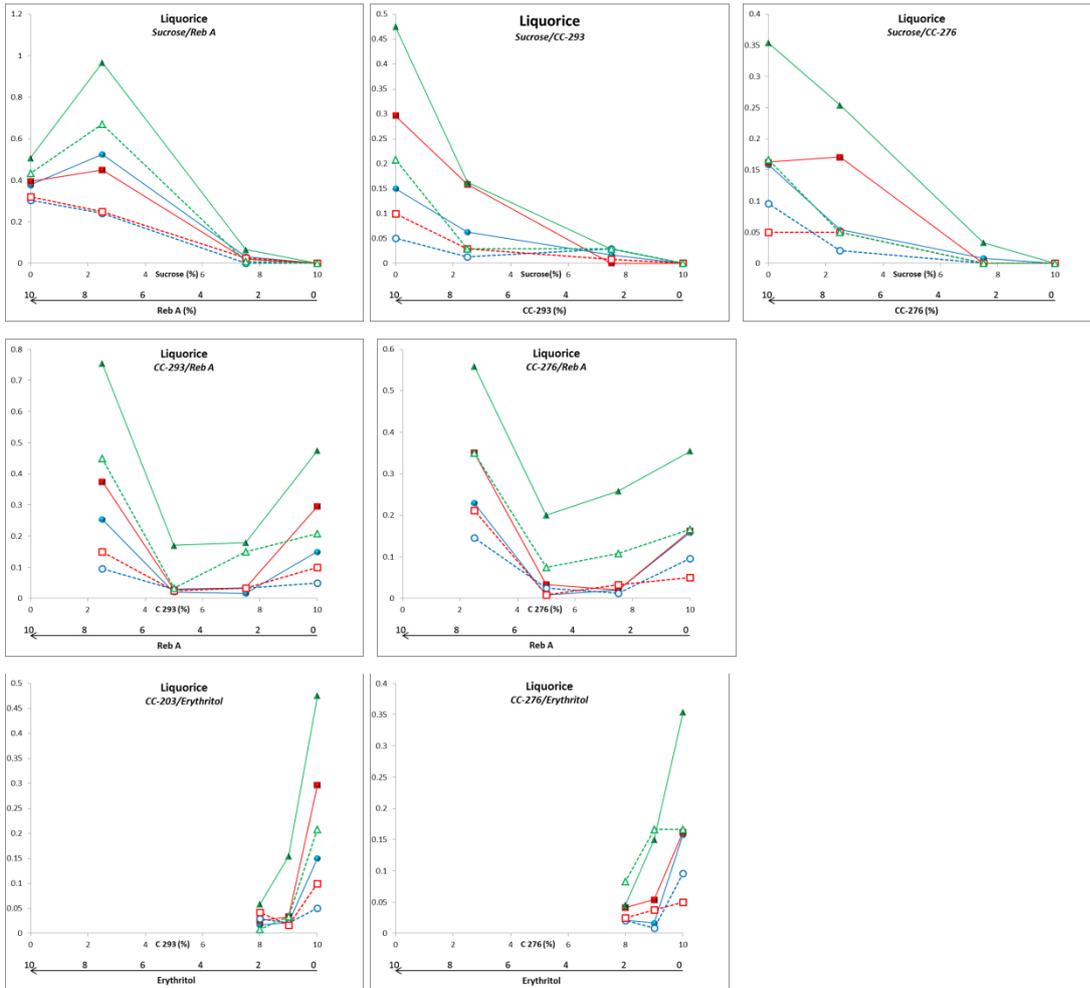
**Figure 60. Change in the liquorice taste score for every sweetener pair tested**
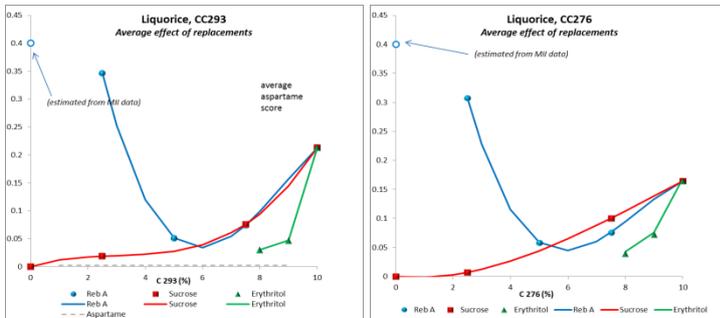
Figure 61. Average effect on liquorice taste of replacing the steviosides with other sweeteners

The results showed a clear minimum for the mixes between the steviosides and Reb A, so although a small difference, the mixes have a lower score than either on its own. Erythritol has an interesting impact on liquorice, bringing it down significantly, close to 0, already with 1% mix in 9% of the steviosides, and clearly lower than the simple replacement effect with sucrose or the synergistic effect with Reb A..

l) Liquorice aftertaste
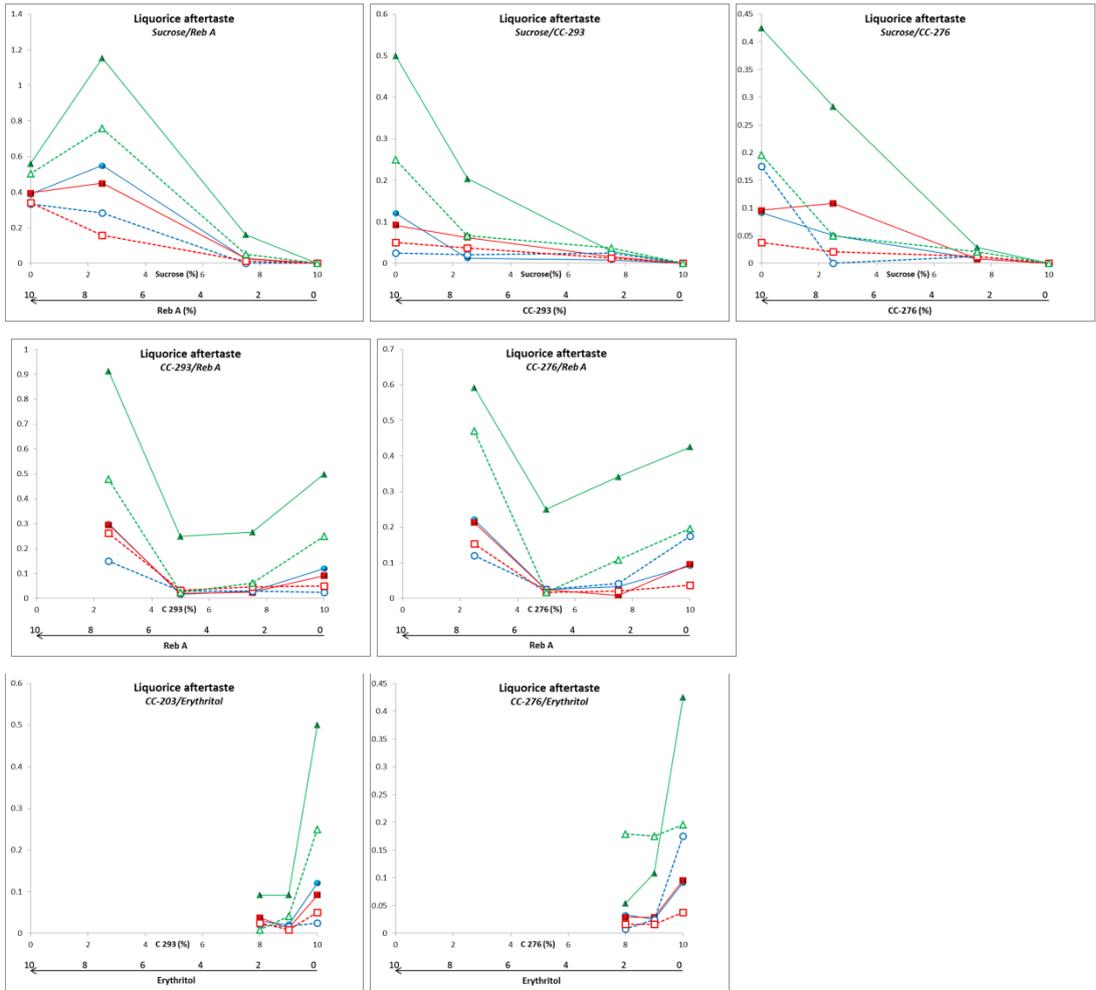
Figure 62. Change in the liquorice aftertaste score for every sweetener pair tested
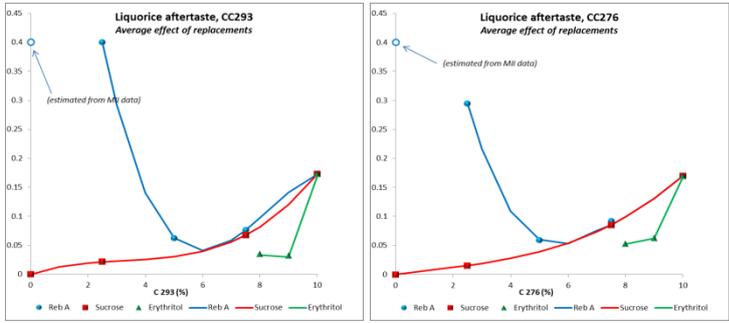

Figure 63. Average effect on liquorice aftertaste of replacing the steviosides with other sweeteners

The results for liquorice aftertaste were similar to those of the taste itself.