Sucrose-replacement by rebaudioside a in a model beverage

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Abstract Rebaudioside A (RA), a component of *Stevia rebaudiana*, is a non-caloric sweetener of natural origin, suitable to meet consumers' demand for sweet taste, but undesirable flavors were reported at high concentrations.

Aim of this study was to create a model beverage (ice-tea) in which sucrose was replaced increasingly by RA to identify optimal sensory profile for consumer acceptance.

Samples with 20 % and 40 % sucrose replacement by RA, respectively, showed very similar sensory profiles but were significantly higher in some flavor attributes, such as artificial sweetness, licorice-like and metallic, as well as in sweet and bitter aftertaste ($p < 0.05$) compared to the reference ice-tea.

In both hedonic tests, preference and acceptance samples with RA have been judged as comparable to the reference despite perception of some undesirable notes.

In view of the results of our study it can be stated that a replacement of 20 % or 40 % sucrose by RA in an ice-tea is achievable.

Keywords Rebaudioside A · Sucrose replacement · Ice-tea · Sensory evaluation

Introduction

According to new estimates by the World Health Organization in 2008, worldwide more than 1.4 billion adults (20 and older) were overweight. Of these about 500 million were obese (WHO 2013). The regulation of hunger and satiety plays an important role in maintaining energy balance and thus normal body weight. Studies on the satiating effect of liquid and solid

foods have shown that liquids have a low satiating effect because of their high speed of consumption and low oro-sensory residence time. Thus, they can easily lead to an overconsumption of energy and consequently to a higher body weight (De Graf 2012). Therefore the consumption of sugar-sweetened beverages can be considered as a possible dietary cause of obesity which is associated with a number of diseases including e.g. Type 2 diabetes, heart disease, and cancer (Anton et al. 2010; Cardello et al. 1999). Energy reduced foods are used to mitigate overconsumption of energy, in particular by individuals who are increasingly concerned about their health and appearance. This lead to an increasing demand for products with reduced energy especially those that use sweeteners as substitutes for sucrose (Trevizam Moraes and Bolini 2010). Substituting sugar with low calorie sweeteners may be an efficacious weight management strategy (Anderson et al. 2012).

The range of low-calorie sweeteners available to the food industry is expanding. It is essential for the food manufacturer, however, to have exact knowledge of both the relative sweetness and other sensory properties of various sweeteners in relation to different sucrose concentrations and in relation to the food matrix, since replacement of sucrose to produce energy reduced foods should not cause significant changes in the sensory characteristic of the product (Cardello et al. 1999).

Rebaudioside A (RA), one of the major sweet components of the plant *Stevia rebaudiana*, is a non-caloric sweetener of natural origin, 100–300 times more potent than sucrose. The safety of steviol glycosides (stevioside and rebaudioside A) has been extensively reviewed by national and international food safety bodies. Both the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) have reviewed the safety of steviol glycosides and have established an acceptable daily intake (ADI) of 4 mg per kg body weight per day (based on steviol equivalents) (EFSA 2010; EU 2011; EU 2012).

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Rebaudioside A is suitable to meet consumers' demand for sweet taste, however undesirable flavor notes (bitter, metallic, artificial sweetness, licorice like) were reported at high concentrations (Cardello et al. 1999; Cardoso and Bolini 2007; Cardoso and Bolini 2008; Palazzo et al. 2011; Prakash et al. 2008; Sass 2010).

Therefore the aim of this study was on the one hand to create and evaluate a model beverage (ice-tea) with sucrose being partially replaced by RA without off-flavor using quantitative descriptive analysis as a method providing a complete description of important sensory properties and on the other hand to assess the beverages on consumers' preference and acceptance.

Materials and methods

Samples

All ice-tea varieties used in the present study were prepared from commercially available Twinings Finest Ceylon-Tea. For the preparation, 1 tea-bag (2 g Ceylon tea) was infused with 200 ml boiling tap water for 2 minutes. The teas were then cooled down to 8 °C and stored in a refrigerator until consumption. For the sensory evaluation, the ice teas were adjusted to 10 °C. To investigate the effect of different degrees of sucrose reduction with rebaudioside A, four varieties of ice-tea were created, reference ice-tea A with sucrose only, sample B with 20 %, sample C with 40 % and sample D with 60 % sucrose replaced with RA, respectively.

The ingredients Twinings Finest Ceylon-Tea, lemon juice (100 % lemon juice from lemon concentrate), and sucrose (Wiener Zucker) were purchased from retail supermarkets, while rebaudioside A 97 % purity was kindly provided by Instantina Nahrungsmittel Entwicklungs- und Produktions Ges. m.b.H.

Methods

Just about right (JAR)

The optimum levels of sucrose and lemon concentrate in the created ice-tea, and the sweetness equivalent of rebaudioside A (RA) were determined by using a just-about-right (JAR) scale (Lawless and Heymann 1999). The evaluation of eight different samples for the optimal ratio between sucrose and lemon concentrate in ice-tea was conducted by 38 consumers (untrained individuals), while 14 trained panelists were determining sweetness equivalence in five samples.

For the evaluation of the optimum levels of sucrose and lemon concentrate in the ice-tea varieties the subjects were served 20 ml of each sample, with different sucrose and lemon concentrate ratios, and were asked to place the entire contents

in their mouth and evaluate sweetness and sourness by placing a mark on a five point unstructured line scale anchored “not intense enough” at the left, “just right” at the center, and “much too intense” at the right.

For the evaluation of the sucrose equivalent of rebaudioside A the “just right” point in the middle of the scale corresponded the sweetness of sucrose, i.e. the subjects should place a mark at this point if the sweetness of the sample with rebaudioside A was equal to the reference samples with sucrose only. The left of the scale was labeled “not sweet enough”, the right “much too sweet”.

Quantitative descriptive analysis

For Quantitative Descriptive Analysis (QDA) according to Stone et al. (1974), a panel of 17 trained assessors (ISO 1993) rated the intensity of selected taste and flavor attributes listed in Table 1, using a numeral ordinal 10-unit scale with the end values labeled as “imperceptible” and “very strong” of four created ice-tea beverages. The selection of the descriptors were done in accordance with the paper by Prakash et al. (2008) and Sass (2010). The samples were served in a

Table 1 Sensory descriptors and definitions for quantitative descriptive analysis

Descriptors	Definition
Taste	
Sweet	The basic taste of sucrose solution
Sour	The basic taste of citric acid solution
Bitter	The basic taste of caffeine solution
Flavor	
Metallic	Flavor caused by stainless cup or can
Artificial sweetness	Flavor associated with artificial sweeteners
Licorice like	Flavor associated with licorice
Lemon like	Flavor associated with lemon
Black tea	Flavor associated with black tea
Caramel	Flavor associated with caramels
Mouth feel	
Viscosity	Sticky characteristics to palate or mucosal surface in the oral cavity
Refreshment	Feelings associated with cooling caused by something such as mints
Astringent	Feelings associated with dry sensation associated with immature persimmons or green tea
Aftertaste	
Sweet	Long-lasting sweet taste 1 minute after swallowing
Sour	Long-lasting sour taste 1 minute after swallowing
Bitter	Long-lasting bitter taste 1 minute after swallowing
Metallic	Long-lasting metallic impression 1 minute after swallowing

randomized order and evaluated in duplicate sessions, in the morning and in the afternoon.

Training of the panelists for QDA Panel members were students of the Department of Nutritional Sciences aged 24–35 years and all had two years of experience developing descriptive languages. The training was run in four steps:

1. Introduction of the method to the panelists.
2. Familiarization of the panelists with the computer system (FIZZ Network System, Biosystemes, France).
3. Training the panelists using basic-taste solutions and the water solution of rebaudioside A. About 15 ml of basic tastes relevant for the evaluation (sweet, sour, bitter), higher than the threshold concentration, and Rebaudioside A in water solution in sucrose equivalent concentrations were presented to each assessor.
4. Training the panelists using the real product (ice-tea). The descriptors for ice-tea were discussed and agreed upon the definitions by the panelists as well as the using of the scale was trained during five sessions lasting each about 90 minutes, as suggested by Stone and Sidel (2004).

Hedonic evaluation

Additionally, the beverages were tested for preference using a ranking test (ISO 1988a) and for consumer acceptance using a hedonic 9-point scale (Lawless and Heymann 1999). In total 120 subjects participated in the hedonic evaluation. Due to various reasons (e.g. not completed test protocols), the results of 12 participants could not be used for statistical analysis for the preference test.

In the ranking test, the order of degree of preference of three samples A, B and C was evaluated. Due to the strong expression of the negative attributes (bitterness, metallic and licorice taste, artificial sweetness) identified during the QDA, sample D was excluded from the hedonic tests.

The best liked sample was ranked on first place, the least liked on last (three). The other sample was ranked in between. The rank sum for each sample was obtained by adding the single sums of each participants. The lowest rank sum indicates the best liked sample, the highest rank sum the least liked sample.

Due to the reason mentioned above, the acceptance test was conducted with the three samples: reference sample A and samples B and C with 20 % and 40 % replacement of sucrose by rebaudioside A, respectively.

All sensory evaluations were conducted in individual booths at the sensory laboratory of the Department of Nutritional Sciences of the University of Vienna, designed and equipped according to the International Organisation for Standardization (ISO 1988b).

Statistical analysis

For descriptive statistics and determination of statistical significance the software PASWStatistics 15.0 (Predictive Analysis SoftWare, SPSS Inc., Chicago Ill., USA) was used. Primarily the K-S-Test (Kolomogorov-Smirnov-Test) was used to test for normal distribution of the data. If data were normally distributed, they were further evaluated by ANOVA (Analysis of Variance), otherwise the non-parametric Mann–Whitney *U*-test was used. Differences were regarded as statistically significant at $p < 0.05$. The correlations between the attributes were calculated by Spearman test.

Results

Determinations by JAR scale

After the evaluation of eight samples with different concentrations of sucrose and lemon concentrate by 38 individuals, the optimal relation between the sweet and the sour taste in the created ice-tea was identified at levels of 7 % sucrose and 4 % lemon concentrate. The other samples were classified much too sour (samples with 6 % or 7 % sucrose and 6 % or 8 % lemon concentrate) or not sweet enough (samples with 6 % or 7 % sucrose and 2 % lemon concentrate) (Fig. 1).

The sweetness equivalent of RA for 7 % sucrose in ice-tea was 0.035 %. This value was defined by 12 of 14 assessors (86 % of respondents). Two individuals (14 % of respondents) decided to select the concentration of 0.04 % RA as the equivalent value for 7 % sucrose in the ice-tea sample. All other samples were either sweeter (RA concentration 0.045 %) or less sweet (RA concentration 0.03 %) than ice-tea with 7 % sucrose (Fig. 2).

Profile analysis (QDA)

Four ice-teas with different concentrations of sucrose and rebaudioside A were created for the QDA following the results of the optimal relation between sweet and sour taste estimated by the just about right (JAR) method and by taking into consideration the calculated equivalent of RA for sucrose: reference ice-tea (sample A) containing 7 % sucrose, ice-tea (sample B) with 20 % (5.6 % sucrose and 0.007 % RA), ice-tea (sample C) with 40 % (4.2 % sucrose and 0.014 % RA) and ice-tea (sample D) with 60 % (2.8 % sucrose and 0.021 % RA) sucrose replaced with RA, respectively.

Samples B and C, with 20 % and 40 % sucrose replacement by RA, respectively, showed very similar sensory profiles but were significantly higher in some flavor attributes, such as artificial sweetness, licorice-like and metallic as well as in sweet and bitter aftertaste ($p < 0.05$) compared to the reference

Fig. 1 Determination of the optimum levels of sucrose (S) and lemon juice (L) in ice-tea

ice-tea A. Sample D with 60 % sucrose replacement by RA also differed significantly in these descriptors from the reference sample A and from sample B and C. Additionally, sample D was evaluated to be significantly ($p < 0.05$) more sweet and astringent and less refreshing as the other investigated varieties of ice-tea (Fig. 3). The overall quality score was 6.76 for sample A, 6.20 for sample B, 6.19 for sample C and 4.47 for sample D. The only statistically significant difference was observed between reference sample A and sample D with 60 % sucrose replacement by rebaudioside A.

Hedonic tests

In the ranking test of preference conducted with three samples A, B and C, sample B with the rank sum 208 was the most preferred among the evaluated ice-tea varieties, followed by reference sample A (rank sum 213) and sample C (rank sum 227) (Fig. 4). However, the preferences were not statistically significant.

To investigate to what extent the evaluated samples A (reference) and B and C with the 20 % and 40 % sucrose replacement by RA, respectively, were accepted by consumers, the monadic acceptance test was applied. The results of this test showed that the acceptance of the reference sample A with 70 g/l sucrose, sample B with 20 % and sample C with 40 % replacement of sucrose by

Fig. 2 Determination of the sucrose equivalence at 7 % by rebaudioside A in ice-tea

rebaudioside A was similar: 85 %, 82.5 % and 80 % of the 120 respondents rated these samples with at least 6 points on the 9-point-scale, respectively. The differences were not statistically significant.

Discussion

The consumers participating in the present study considered 7 % sucrose as the optimum level of sucrose to produce the most preferred sweetness in ice-tea, which was close to the 8.3 % identified for iced and hot instant tea (Cardoso et al. 2004) but lower than the 10 % level in peach nectar reported by Cardoso and Bolini 2007. However, the authors stated that the concentration was higher than obtained by other researches in similar experiments, e.g. by evaluation of mango (7.5–8 %) and pineapple juices (8.5 %) (Cardoso and Bolini 2007). The ideal sweetness for instant or roasted ground coffee was achieved by adding 9.5 % or 12.5 % of sucrose, respectively (Trevizam Moraes and Bolini 2010). The sweetness differences in the evaluated samples are probably due to the interaction among ingredients as suggested by the authors, but to our opinion the consumers' expectation towards the product also is of relevance. Fruit juices have a more sour taste and, therefore, the sucrose level to achieve an acceptable taste and flavor is different from that of an ice-tea with 4 % lemon concentrate.

Unfortunately, no studies with exact specification of steviol glycosides or rebaudioside A content were available. Comparison with those studies where insufficiently characterized extracts of stevia leaves were used, is not possible with our results. The sweetness potency of rebaudioside A in our study was calculated to be 200, but it has to be noted that this is highly dependent on the food system used and may therefore vary in different food products (Cardello et al. 1999).

Because of the undesirable attributes, rebaudioside A as a non-caloric sweetener of natural origin should be blended with carbohydrates such as sucrose (Prakash et al. 2008). High concentration of rebaudioside A (more than 6 % sucrose equivalency) was reported to exhibit a strong bitter taste and black licorice flavor (Prakash et al. 2008). In a study on peach nectar containing Stevia extract at the equivalent sweetness of sucrose at a concentration of 10 % sucrose showed, among others, a distinct bitter taste as well as residual sweetness and bitterness (Cardoso and Bolini 2008). In the ice-teas of our study with 20 % and 40 % sucrose replacement by RA, sweet and bitter aftertaste as well as some unpleasant flavor attributes, such as artificial sweetness, licorice-like and metallic were significantly higher compared to the reference ice-tea A, but the hedonic tests did not show any significant differences in the consumers preferences or acceptance between these three varieties of ice-tea.

Fig. 3 Sensory profile of evaluated ice-tea varieties

Samples B and C had lower energy content than reference sample A only containing sucrose as source of sweetness (22 and 17 kcal/100 ml, resp., vs 29 kcal/100 ml) and was accepted by most consumers who participated in the study.

In order to replace or reduce sucrose by non-caloric sweeteners, testing by sensory studies is required, because for each type of beverage the concentration of sweetener may be different (Trevizam Moraes and Bolini 2010) as their sweetness potencies in each of the food depends on the food or beverage matrix (Palazzo et al. 2011). Therefore, the development of low-calorie products requires further investigation and individual adjustment.

Fig. 4 Ranking test for preference of evaluated ice-tea varieties

Conclusion

In view of the results of our study, it can reasonably be stated that a replacement of 20 % (sample B) or 40 % (sample C) sucrose by RA in an ice-tea is achievable.

Both samples B and C showed, except some flavor attributes, such as artificial sweetness, licorice-like and metallic, very similar sensory profiles compared with the reference sample A. In the hedonic tests there were no statistically significant differences between samples with 20 % and 40 % RA and the reference.

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