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Possibilities and limitations of sugar reduction by steviol glycosides in yoghurt

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Summary

Steviol glycosides – commonly known as stevia – are calorie-free and are not cariogenic. Rebaudioside A has been approved in the EU since 2011 and can taste sweet or bitter, depending on the concentration. In the present sensory study, the calorific value of yoghurt was reduced by replacing 50 % of the sucrose by rebaudioside A, the main component of the approved steviol glycosides. This did not impair the taste of the yoghurt in any way. However, if 75 to 100 % of the sucrose was replaced by rebaudioside A, the sweetness intensity was reduced. Moreover, at these levels of rebaudioside A, the yoghurt tasted bitter and astringent. The sweetener stevia is approved as food additive in the form of steviol glycosides (E 960), but not (yet) as a food ingredient or "novel food" – for example as a calorie-free sweetener –, as its safety has not yet been confirmed by EFSA.

Keywords: stevia, steviol glycosides, yoghurt, sugar reduction, sugar consumption

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Introduction

Excessive levels of sugar are consumed in Western industrial countries and this is thought to be linked to several diseases of civilization. One possible way of reducing the level of sugar in diet would be to use the natural sweetener stevia. The present study shows that stevia can be used to halve the amount of sucrose added to yoghurt, without significantly changing the taste of the fresh product.

The plant *Stevia rebaudiana* Bertoni

It may be possible to replace sucrose with the sweetener stevia isolated from the plant *Stevia rebaudiana* Bertoni (SRB) and this has encouraged a great deal of interest in the food industry, in dietetics and in the media. It is therefore essential to investigate and assess this sweetener and the plant from which it is extracted. SRB is a plant of subtropical areas with semi-humid climate and is a member of the sunflower family (*Asteraceae*) (◆ Figure 1). It grows as a perennial herbaceous bush, which can reach a height of 80 cm. This plant is also known as "sweet leaf" or "honey leaf", as it accumulates sweet substances in its leaves [1, 2]. SRB originates from the highlands of Amambay in northeast Paraguay. For several hundred years, indigenous peoples have been using it in green herbal teas, for its sweetness and healing powers [2, 3].

Stevia was first cultivated in 1908 in Paraguay. Between 1968 and 1971, the Japanese dug up almost all the stock of wild plants in Paraguay and transported them to Japan, where they were cultivated and studied. As a consequence, the first stevia products went on sale in Japanese supermarkets in 1975. After the successful marketing in Japan, cultivation and production were moved to China for commercial reasons. It has been estimated that China now produces 15,000 tons of stevia annually, corresponding to ca. 95 % of the world production [4].

The sweetener stevia

The sweet ingredient of the leaves of SRB is commonly referred to as stevia. Stevia is a glycoside (glycosides very commonly occur in plants) and constitutes the subgroup of steviol glycosides (SGs), in which the aglyone consists of the diterpene de-

rivative steviol ([5ß,8α,9ß,10α,13α]-13-hydroxykaur-16-en-18-oic acid) [5]. The SGs differ with respect to the type, number and arrangement of the sugar molecules bound to the aglycone (◆ Figure 2) [6, 7]. As a consequence of cultivation, 30 different SGs are currently known [8]; 10 of them were approved in the EU in November 2011 as food additives (E960) in specific foods in various food categories under specific conditions of use: stevioside, rebaudioside A–F, steviol bioside, dulcoside A and rubusoside [9] (◆ Table 1). Relative to the dry weight of steviol leaves, stevioside and rebaudioside A (RebA) are the dominant components, with 5–10 % and 2–4 %, respectively. An application for approval of stevia as a novel food is pending.

For use as food additives, commercial SG products must fulfil the criteria of the European Commission and be at least 95 % pure relative to the dry mass [11]. Because of the prescribed manufacturing process, the final product must contain at least 75 % stevioside and/or RebA; a further 20 % can be made up of the rebaudiosides B, C, D, E and F, together with dulcoside A, rubusoside and steviol bioside. The remaining 5 % consists of substances that have not been unambiguously defined [4]. New varieties and modern production techniques may modify the composition of the SGs, so that commercial products may have different properties. This may even be the case for commercial products with the same RebA content. For this reason, the SG content is normally given as equivalents. RebA is a white or slightly yellowish powder with 200- to 300-times the sweetness of sucrose (◆ Figure 2).

Fig. 1: ***Stevia rebaudiana*** **Bertoni**

Glossary

ADI value (Acceptable Daily Intake) = the weight of a substance that can be ingested in the course of a lifetime without causing damage to health

allosteric site = specific region in an enzyme, to which an effector binds and thus causes a change in enzymic activity

glycaemic index = measure of the blood sugar concentration after ingestion of a carbohydrate-containing food

pyranose = stable, six-member ring form of monosaccharides (semiacetal formation)

sweetness = measure of the relative sweetness of a substance. The reference value is 1 for sucrose (saccharose or domestic sugar)

Nutritional physiology and nutritional toxicology

After oral administration, SGs are not absorbed in the stomach or small intestine. Within the colon, the SGs are subject to bacterial hydrolysis. The bacteria in this section of the intestine split the SGs into steviol (scaffold) and the monosaccharide units. Steviol is transported into the liver, where it is glucuronidated to give water soluble steviol glucuronides and then eliminated in bile into the small intestine (enterohepatic circulation) or directly excreted through the kidney [12, 13].

In order to exclude acute and/or chronic toxic effects in man, the European Food Safety Authority (EFSA) has laid down that the Acceptable Daily Intake (ADI) of SGs is 4 mg/kg body weight (calculated as steviol equivalents) [10]. It has been shown that SGs have no carcinogenic, mutagenic or teratogenic effects under this ADI value [14, 15]. For RebA, calculating the sterol equivalents in SG gives an ADI that is about 3-fold greater, i.e. the ADI value of 4 mg/kg body weight is changed to ca. 12 mg/kg body weight (◆ Table 1) [13]. In comparison, the ADI for aspartame is 40 mg/kg body weight [4].

SGs are not cariogenic and have no glycaemic effect. One study found that they can be hypotensive in man [16]. Other studies have failed to find a scientific explanation for this finding [17, 18].

Significant reductions in systolic and diastolic blood pressures have been found in patients with hypertension [19, 20]. The explanation may be that SGs have diuretic and natriuretic effects, as well as inhibiting calcium influx into vascular smooth muscle cells [21].

Sensory properties

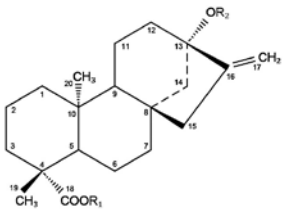
The sensory evaluation of SGs concentrates on their sweetness and quality of taste [22].

In 5 % aqueous solutions, the two most important SGs – stevioside and RebA – are 120-fold or 250-fold sweeter, respectively, than sucrose [23, 24]. The sweetness depends on the purity and composition of the two SGs. In addition, the sweetness depends on the pH and temperature of

Food Category	Maximum quantity as SG-equivalents (mg/L or mg/kg)	Maximum quantity as RebA (mg/L or mg/kg)
aromatic drinks (reduced calorific value or without added sugar)	80	240
beer and malt drinks (alcohol-free beer or beer with maximal alcohol content of 1.2 %)	70	210
alcoholic drinks (< 15 % alcohol)	150	450
aromatic fermented milk products (reduced calorific value or without added sugar)	100	300
ice cream (reduced calorific value or without added sugar)	200	600
jams, jellies, marmalade (reduced calorific value)	200	600
sauces (excepting soy sauce)	120	360
cocoa and chocolate products (reduced calorific value or without added sugar)	270	810
sweets, without added sugar	350	1 050
chewing gum, without added sugar	3 300	9 900
table-top sweeteners in tablet form	q. s.	q. s.

Tab. 1: **Maximum quantities of steviol glycosides as additives to specific foods (extract)** [10]
q. s. = *quantum satis* („as much as necessary"); RebA = rebaudioside A; SG = steviol glycosides

the relevant food [3]. The sweetness of stevioside and RebA initially increases at increasing concentrations. However, it decreases again after reaching a maximum (> 1–2 mM RebA in 5 mL water) [25]. It is therefore thought that stevioside and RebA bind to the allosteric receptors of the sweetness receptors and inactivate these, so that the perception of sweetness decreases. In addition, at rising SG concentrations, a bitter liquorice-like secondary or after-taste becomes increasingly apparent [25].



Structure	name	R_1	R_2	sweetness
	steviol	H	H	–
	stevioside	β-Glc	β-Glc-β-Glc	150–250
	rebaudioside A	β-Glc	β-Glc-β-Glc | β-Glc	200–300
	rebaudioside D	β-Glc-β-Glc	β-Glc-β-Glc | β-Glc	220
	dulcoside A	β-Glc	β-Glc-α-Rha	30

Fig. 2: **Structural formulas of important steviol glycosides and their aglycones** [6, 7]
sweetness, with reference to sucrose = 1
Glc = D-glucopyranose, Rha = L-rhamnopyranose, R1/2 = rest 1/2

At higher SG concentrations, the bitter taste may become dominant and mask the sweet taste that is dominant at lower concentrations (so-called "quality change") [26].
Each SG with its specific structure has a characteristic taste profile with a different degree of sweetness, and the residues bound to C13 and C18 play an important role here (◆ Figure 2). It is assumed that the sweetness is enhanced by β-D-glucopyranose [25]. The more β-D-glucopyranose units are coupled to the scaffold, the greater is the perceived sweetness of the SG. On the other hand, binding α-L-rhamnopyranose decreases sweetness. The double bond between C16 and C17 in the SG structure also contributes to sweetness. Indirect evidence of this is that when stevioside und RebA are converted to the corresponding ketones they are no longer sweet [27].
Because of their structures, rebau-

dioside D and RebA have the most pleasant taste profiles. Both SGs include several bonds to β-D-glucopyranose residues, but no bonds to α-L-rhamnopyranose residues (◆ Figure 2). It is therefore sensible mainly to isolate rebaudioside D and RebA from the plant and to use these two SGs for sweetening. RebA is present in the leaf at comparably high concentrations and can therefore be exploited economically.

As stevia products are very sweet, only very low quantities are needed for sweetening and their effects on colour and consistency are negligible. For example, an aqueous solution of only 0.05 % RebA gives the same sweetness as 10 % aqueous sucrose. Moreover, SGs are highly soluble in water, stable to heat and compatible with the organic acids in fruits and vegetables [28].

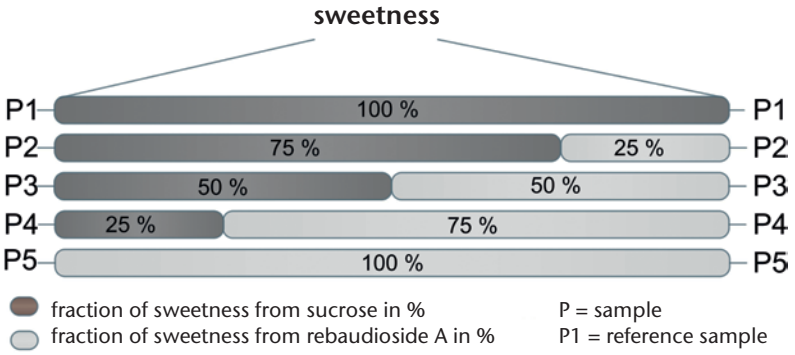


Fig. 3: **Study design (fractions of sweetness from sucrose and RebA)**

Own studies

The aim of the present studies was to gradually replace the fraction of sucrose that is normally present in yoghurt with stevia, without a change in the yoghurt's taste properties.

Materials and methods

Organically produced yoghurt (3.8 % fat) was sweetened with sucrose, so that its sucrose concentration corresponded to that in commercial products (6–12 %). This sweetened yoghurt served as reference sample

sweetness[1]	P1 (control) (0 %)	P2 (25 %)	P3 (50 %)	P4 (75 %)	P5 (100 %)
	sweetness intensity (none to extreme)				
0.5[2]	5.6^{bc}	5.4^{c}	4.6^{cd}	2.8^{de}	2.0^{e}
1.0[2]	8.4^{a}	8.2^{a}	7.6^{ab}	6.4^{abc}	5.4^{c}
	sweetness onset (none/develops slowly/to develops rapidly)				
0.5	7.2^{ab}	6.6^{abc}	4.4^{bcd}	3.2^{de}	1.4^{e}
1.0	8.6^{a}	8.4^{a}	6.6^{abc}	3.8^{cde}	4.6^{bcde}
	sweetness duration (brief to persistent)				
0.5	7.8^{a}	5.8^{ab}	4.2^{abc}	2.2^{bc}	1.4^{c}
1.0	5.6^{ab}	5.8^{ab}	7.2^{a}	6.4^{a}	5.8^{ab}
	astringency (none to intense)				
0.5	1.0^{c}	1.6^{bc}	3.2^{bc}	3.0^{bc}	5.0^{ab}
1.0	2.2^{bc}	1.8^{bc}	2.8^{bc}	4.4^{abc}	7.0^{a}
	bitter tone (none to intense)				
0.5	0.8^{c}	1.4^{bc}	2.8^{bc}	1.8^{bc}	4.4^{b}
1.0	1.8^{bc}	0.8^{c}	2.6^{bc}	3.6^{bc}	7.6^{a}

Tab. 2: **Sensory evaluation of the characteristics sweetness intensity, sweetness onset, astringency and bitter tone of yoghurt, in which the added sucrose was replaced by RebA (in brackets) in 2 sweetness levels (0.5 or 1.0), using a 10-point scheme (0 = no perception, up to 10 = most intense perception)**

[1] Significance of the factors sweetness and stevia fraction in the analysis of variance with 2-fold classification ($p < 0.05$) for all characteristics (interaction sweetness x stevia fraction not significant)

[2] 0.5 corresponds to 5.26 g and 1.0 corresponds to 11.11 g sucrose in control P1

[a, b, c, d, e] Different indices characterise significant differences between the mean values ($p < 0.05$)

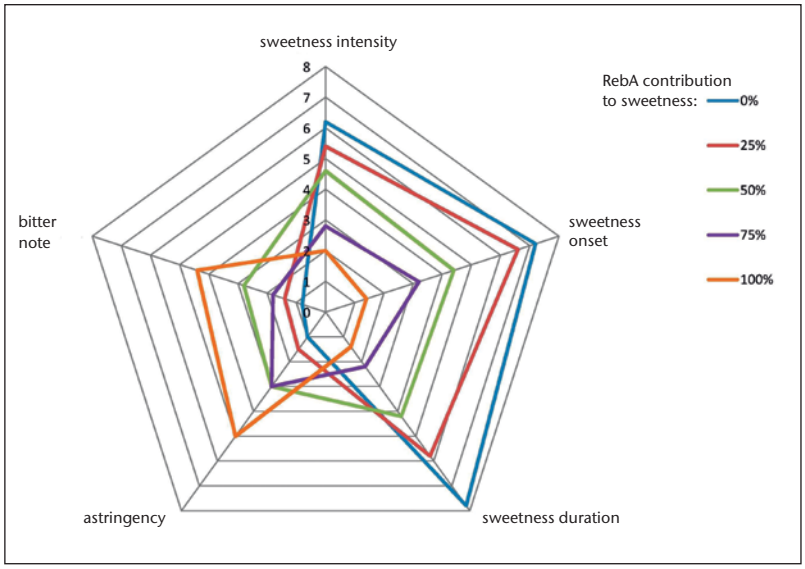


Fig. 4: **Test series A – Taste profile of the five samples P1–P5 with graduated sweetness composition (reference sample P1: sweetness intensity 0.5 = 5.26 g sucrose per 100 g yoghurt)**

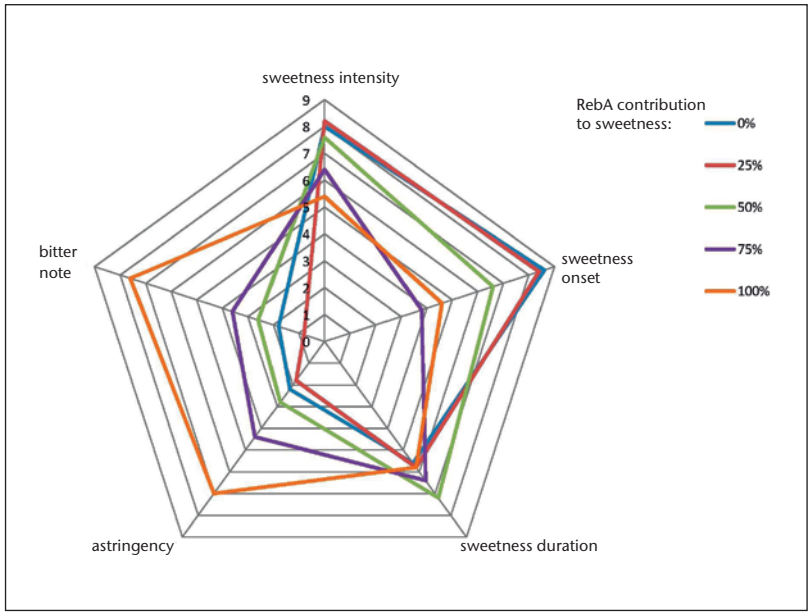


Fig. 5: **Test series B – Taste profile of the five samples P1–P5 with graduated sweetness composition (reference sample P1: sweetness intensity 1.0 = 11.11 g sucrose per 100 g yoghurt)**

(P1). In four additional samples (P2–P5), the sucrose was gradually replaced by RebA (◆ Figure 3). The source of RebA was a product from NP Sweet A/S (Copenhagen, Denmark), that contains at least 97 % RebA and not more than 3 % of other SGs (pure granulate, sweetness 190 in comparison to a 10 % sucrose solution, product specification PS 236740-1.2EN). The four step substitution was performed in two test series (A and B), with different levels of sweetness (A: sweetness 0.5, and B: sweetness 1.0). The samples were anonymised within the two test series A and B. They were then tested and evaluated blind by a sensory panel consisting of five trained examiners, including three with DLG certification. Even though DIN 10967 [29] lays down that a panel should contain at least 6 examiners, it appeared to be justified to use only 5, as the changes in the level of sucrose substitute were large (0–100 % in four steps), together with the large difference in sweetness between the two test series (100 %). Unsweetened natural yoghurt and water were provided to the sensory testing panel for neutralisation.

In accordance with DIN EN ISO 13299, a conventional profile was established on the basis of five test properties: sweetness intensity, sweetness onset, sweetness duration, bitter note and astringency [30]. The sensory study and the evaluation of sweetness intensity, bitter note and astringency were performed in accordance with method 00.90–DIN 10967 (profile test [29]). The parameters "sweetness onset" and "sweetness duration" were measured in accordance with DIN 10970 (time intensity test [31]), but were modified, as the necessary software was not available.

For each sample, the five test parameters were rated on a 10-point scale. For each test parameter, the scale ranged from the lowest point (0 = no perception) up to the highest point (10 = most intense perception).

The ratings awarded by the examiners were recorded in an Excel table and evaluated with the SPSS statistics program. Two-fold analysis of variance (ANOVA) was carried out with factors 1 (sweetness intensity in steps 0.5 and 1.0) and factor 2 (successive substitution of sucrose with RebA, where 0 % corresponds to "only sucrose" and 100 % to "only RebA"). Means were compared using Duncan's method.

Results

In both test series, the intensity, onset and duration of sweetness

decreased with the increasing RebA fraction, or decreasing sucrose fraction. At the same time, the astringent feeling and bitter taste increased (◆ Table 2, Figures 4 and 5). In test series A (sweetness 0.5), there were significant differences between 75 % RebA (25 % sucrose) and control (100 % sucrose) with respect to sweetness intensity and sweetness duration (◆ Table 2, Figure 4). In contrast, in test series B (sweetness 1.0), there was only a significant difference in sweetness and sweetness duration when sucrose was totally substituted by RebA (◆ Table 2, Figure 5).

In both test series, 50 % substitution of sucrose with RebA gave a significant delay in sweetness onset. For astringency, there were only significant changes in the two test series after sucrose had been totally substituted by RebA. In a similar manner, the difference from the control in the bitter note was only significant at 100 % RebA (◆ Table 2, Figures 4 and 5).

Discussion

The results for sweetness intensity, sweetness onset and sweetness duration show that 50 % of the sucrose in fresh yoghurt can be substituted by RebA without any change in taste. With the parameters astringency and bitter note, a reduction of 75 % was even possible. However, 75 % and 100 % substitution of sucrose with RebA weakened sweetness intensity (◆ Figures 4 and 5), but enhanced the bitter note and astringency. This change in quality is confirmed by significant differences in comparison to the reference sample. In spite of the modified evaluation, the results for sweetness onset and sweetness duration in accordance with DIN 10970 (time intensity test) are plausible and may therefore be regarded as indicating significant differences.

The results for test series A and B may be explained with the concentration-dependent affinity of RebA to both the sweet and bitter receptors. The sweetness intensity decreased with increasing RebA content (at 75 % and 100 %). A bitter note was detected at the same time, which indicates that the RebA molecules bind to bitter receptors (◆ Figures 4 and 5). The attenuated sweetness intensity is particularly detectable in the samples that were only sweetened with RebA. In these samples, the bitter note was dominant, whereas sweetness was only a secondary component. This change in quality is confirmed by the conclusions of Hellfritsch et al. [25]. If the ratio of the sweeteners is balanced, with 50 % RebA and 50 % sucrose, it can be assumed that the sweet receptors are not yet inactivated and that there are no changes in the perception of sweetness. Both adults and children are thought to ingest stevia mainly in non-alcoholic aromatic drinks (refreshment beverages) and aromatic fermented milk products. Thus, persons who consume high levels of these foods may repeatedly exceed the ADI value over an extended period. For this reason, in November 2011, the EFSA issued a revised estimate of the exposure to SGs when it is used as an additive [9]. It was concluded that the maximum quantities for 16 foods should be reduced by factors of 1.5 to 3.0 in comparison with the previous EFSA estimate [10]. Some of the greatest reductions were for milk drinks, fruit juices and non-alcoholic aromatic drinks. The different manufacturing procedures may give rise to different isomers and breakdown products, so that products from different manufacturers may have different taste properties, in spite of having the same specification. In addition, the type and purity of the SG, the type of food and the period of storage may influence the taste properties of the final products. Different SG samples may have different tastes even if the purity is the same.

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Conflict of Interest
The authors declare no conflict of interest according to the guidelines of the International Committee of Medical Journal Editors.

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