DLG Expert report 14/2015

Steviol Glycosides – Compact Knowledge





1. Introduction

Since the end of 2011 steviol glycosides have been authorised and can thus be used as highly intensive sweeteners in the EU. Already in the run-up to the authorisation, the sweetener, frequently called "Stevia", attracted a great deal of attention in the media. This article aims to provide an overview of the properties and possible uses of this relatively new sweetener of natural origin.

2. Properties/raw material qualities

Stevia rebaudiana Bertoni – the complete botanical name – is a plant which originates from Paraguay. There the leaves of the plant have been used already for centuries for sweetening and are brewed like tea leaves. Their sweetening components are known as steviol glycosides. These occur naturally in the leaves of the plant. The term "steviol glycosides" does not comprise any chemically defined individual substance, but instead a mixture of substances with changing proportions of different steviol glycosides [1]. This is an essential difference by comparison with the sweeteners of synthetic origin, which are built up from defined individual substances. Among the steviol glycosides, stevioside and rebaudioside A are essentially responsible for the sensory properties of the extracts produced from the Stevia leaves. Fundamentally the relative sweetening power of steviol glycosides, as in the case of other highly intensive sweeteners, depends distinctly on the quantity used. For example stevioside has a sweetening power of 60 to 180 times more depending on whether it is compared with a 10% (w/v) or 2.5% (w/v) sucrose solution. In the case of rebaudioside A, we speak of 125 to 380 times the sweetening power, also dependent on whether a sugar equivalent of 10% (w/v) or 2.5% (w/v) is taken as a basis for comparison. [2]

It is very frequently mentioned that rebaudioside A leaves the cleanest sweetening impression and also displays the most advantageous relative sweetening power. Accordingly very highly purified steviol glycoside extracts with a rebaudioside-A component of 95% or even 98% (related to the total quantity of steviol glycosides) are also offered on the market. However, it should be noted that purification of the extract involves additional production outlay, which is necessarily reflected in the costs of such an extract. Generally a good basis for product development can also be found with an extract which contains for example 60% rebaudioside A and 40% stevioside.

3. Legal situation

In the European Union the use of steviol glycosides is regulated in EU Regulation 1131/2011 of 11.11.2011. Since 02.12.2011 it has been possible to use steviol glycosides in many foods, provided the name or the E number E 960 is quoted [5].

The bases for the specification of the steviol glycosides were set out already substantially earlier by the JECFA, the joint FAO/WHO Expert Committee on Food Additives. The purity standard states that a Stevia extract must contain at least 95% steviol glycosides. Furthermore, the steviol glycosides must be obtained from the leaves of the plant *Stevia rebaudiana* Bertoni [1]. The authorisation of the additive in the EU is based on this specification, which states the following with regard to production of the extract:

The manufacturing process comprises two main phases: the first involving water extraction of the leaves of the



Fig. 1: Stevia plant

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Category	Designation	Maximum level [mg/kg] or [mg/l] steviol equivalent
01.4	Flavoured fermented milk products including heat treated products	100
03	Edible ices	200
04.2.2	Fruit and vegetables in vinegar, oil, or brine (only sweet-sour preserves of fruit and vegetables)	100
04.2.4.1	Fruit and vegetable preparations excluding compote	200
04.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EC	200
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/ EC	200
04.2.5.3	Other similar fruit or vegetable spreads	200
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	270
05.2	Other confectionery including breath refreshening microsweets:	
	only cocoa or dried fruit based, energy reduced or with no added sugar	270
	only cocoa, milk, dried fruit or fat based sandwich spreads, energy-reduced or with no added sugar	330
	only confectionery with no added sugar	350
	only breath-freshening micro-sweets, with no added sugar	2.000
	only strongly flavoured freshening throat pastilles with no added sugar	670
05.3	Chewing gum (only with no added sugar)	3.300
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	
	only confectionery with no added sugar	330
	only cocoa or dried fruit based, energy reduced or with no added sugar	270
06.3	Breakfast cereals (only breakfast cereals with a fibre content of more than 15%, and containing at least 20% bran, energy reduced or with no added sugar)	330
07.2	Fine bakery products (only essoblaten - wafer paper)	330
09.2.	Processed fish and fishery products including molluscs and crustaceans (only sweet-sour preserves and semi preserves of fish and marinades of fish, crustaceans and molluscs)	200
11.4.1	Table Top Sweeteners in liquid form	Quantum satis
11.4.2	Table Top Sweeteners in powder form	Quantum satis
11.4.3	Table Top Sweeteners in tablets	Quantum satis
12.5	Soups and broths (only energy-reduced soups)	40
12.6	Sauces (except soy-bean sauce , fermented and non-fermented)	120
	only soy-bean sauce, fermented and non-fermented	175
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	330
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	270
14.1.3	Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products	100
14.1.4	Flavoured drinks	80
14.2.1	Beer and malt beverages (only alcohol-free beer or with an alcohol content not exceeding 1,2% vol; "Bière de table/Tafelbier/Table beer" (original wort content less than 6%) except for 'Obergäriges Einfachbier'; beers with a minimum acidity of 30 milli-equivalents expressed as NaOH; Brown beers of the 'oud bruin' type	70
14.2.8	Other alcoholic drinks including spirits with less than 15% of alcohol and mixtures of alcoholic drinks with non-alcoholic drinks	150
15.1	Potato-, cereal-, flour- or starch-based snacks	20
15.2	Processed nuts	20
16	Desserts excluding products covered in category 1, 3 and 4	100
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms	670
17.2	Food supplements supplied in a liquid form	200
17.3	Food supplements supplied in a syrup-type or chewable form	1.800

Table 1: Authorised possible uses of steviol glycosides in the EU

Stevia rebaudiana Bertoni plant and preliminary purification of the extract by employing ion exchange chromatography to yield a steviol glycoside primary extract, and the second involving recrystallisation of the steviol glycosides from methanol or aqueous ethanol resulting in a final product containing not less than 95% of stevioside and/or rebaudioside A.

No specific ratio of the steviol glycosides contained in the extract is required, but stevioside and rebaudioside A are stated as the main components [6].

An unusual feature prior to the general authorisation in the EU was the fact that the use of certain steviol glycoside extracts had been authorised already since August 2009 in one individual EU Member State, namely in France. Producers were allowed to market foods there that were sweetened with a Stevia extract that displayed a steviol glycoside content of 95%, and 97% of this had to consist of rebaudioside A. This authorisation was provisional for a period of two years and was replaced by the EU-wide authorisation in 2011. However, this then led to a remarkable constellation in which products could be purchased in France that contained a (sweetening) substance that was not authorised in the rest of Europe.

It should be noted that a restricted authorisation for certain food groups has been issued for steviol glycosides. Generally the additive is only admissible in energy-reduced foods. The end product must thus contain 30% fewer calories than a conventionally formulated product. Table 1 provides an overview of the authorised possibilities for use [5].

In the year 2015, following an enquiry for extension of the authorisation of steviol glycosides, EFSA published a new expert opinion [8]. Following the positive finding, nothing stands in the way of authorisation in the categories summarised in Table 2:

Category	Designation	Maximum level [mg/kg] or [mg/l] steviol equivalent
14.1.5	Coffee, tea, herbal and fruit infusions, chicory; extracts of tea and herbal and fruit infusions and of chicory; tea, herbal and fruit infusions and cereal preparations for infusions, as well as mixes and instant mixes of these products	
	Tea beverages and instant coffee and instant cappuccino products	29
	Coffee and herbal tea beverages	29
14.1.5.2	Others	
	Malt-based and chocolate/cappuccino flavoured drinks	20

Table 2: Anticipated expanded authorisation of steviol glycosides in the EU

In the authorisation issued in the EU Regulation 1131/2011, the legislator uses the term "steviol equivalents" for stating the maximum levels. The steviol equivalents are practically the "smallest common denominator" of the very different composition of extracts containing steviol glycosides. The steviol equivalent can be calculated for each steviol glycoside and allows a uniform comparison related to the central molecule steviol. The EU law states the conversion factors for the different steviol glycosides in accordance with Table 3 [6].

It is therefore important for product development that the steviol equivalent be stated on the specification of the respective steviol glycoside extract. Only in this way can the quantity of extract used be compared with the admissible maximum quantity. Two model calculations are set out below, taking as an example the admissible maximum level for soft drinks (max. 80 mg/l steviol equivalent):

Trivial name	Conversion factor	
Steviol	1.00 (Aglycon)	
Stevioside	0.40	
Rebaudioside A	0.33	
Rebaudioside C	0.34	
Dulcoside A	0.40	
Rubusoside	0.50	
Steviolbioside	0.50	
Rebaudioside B	0.40	
Rebaudioside D	0.29	
Rebaudioside E	0.33	
Rebaudioside F	0.34	

Table 3: Conversion factors of steviol glycosides reflecting the central molecule "Steviol"

At 80 mg steviol equivalent level, a steviol glycoside extract with 98% rebaudioside-A component, 2% stevioside component and 95% purity ("Reb A98") can be used up to a quantity of 254 mg:

- 98% of the 80 mg steviol equivalent (78.4 mg) from the rebaudioside A (F=0.33) = 237.6 mg
- 2% of the 80 mg steviol equivalent (1.6 mg) from the stevioside (F=0.40) = 4.0 mg
- 241.6 mg (237.6 mg+4.0 mg) correspond (purity 95%) to an extract quantity of 254.3 mg

A steviol glycoside extract with 60% rebaudioside-A- and 40% stevioside component and also of 95% purity ("Reb A60") can be used up to a concentration of 237 mg/l:

- 60% of the 80 mg steviol equivalent (48 mg) from the Rebaudioside A (F=0.33) = 145.4 mg
- 40% of the 80 mg steviol equivalent (32 mg) from the stevioside (F=0.40) = 80.0 mg
- 225.4 mg (80 mg+145.4 mg) correspond (purity 95%) to an extract quantity of 237.3 mg

The following section explains to what extent this also has effects on the maximum sweetening equivalent that can be replaced.

4. Sensory analysis/sweetness profile

The above remarks make it clear that "not all Stevia is the same" – the quality of this sweetener depends on the composition of the extract and the proportions of the individual steviol glycosides. The individual steviol glycosides display sensory differences, and this influences the sensory analysis of the standard commercial "Stevia extracts". Even in the case of compositions "of the same specification", different sensory profiles are possible from extract to extract. Depending on the production and composition, the product can display undesirable notes and give off the frequently cited liquorice-like or bitter taste notes, but this is not fundamentally the case.

Like many highly intensive sweeteners, the sweetness profile of steviol glycosides is also characterised by a relatively long-lasting sweetness taste. This is perceived very differently from individual to individual. It varies in its intensity and also on the basis of the composition of the Stevia extracts already described and depends on the product matrix.

The specific sensory properties of the steviol glycosides need great know how for application in the final product. A simple exchange of a sweetener consisting of a single molecule in an existing product concept for steviol glycosides will hardly be possible. The selection of the right steviol glycoside extract requires care and attention. Furthermore, strategies and technologies for masking the long-lasting aftertaste and possible bitter or metallic secondary notes may be necessary.



Fig. 2: Diverse possible uses of Stevia, e.g. Chewing gum lozenges

There are functional aromas that can mask the undesirable secondary notes and at the same time improve the profile of the "sweetness of natural origin". Generally the producer will also adapt this functional aroma to the respective product application. Furthermore, it must be noted whether sugar substitutes such as erythritol, which is often used in combination with steviol glycosides, are admissible for the planned application [3].

As already mentioned, the sweetening power of steviol glycosides depends on their concentration in use. When a low quantity is used, the sweetening power is up to 300 times higher than that of sucrose. If the quantity used is increased related to the same product quantity, the sweetening power drops to around 100 times that of sucrose. Even slight modifications of the recipe have a much stronger effect with steviol glycosides than with synthetic sweeteners. Special attention must be paid to this in product development.

As already mentioned, the individual steviol glycosides differ in their sweetening power and this has perceptible effects depending on the share of rebaudioside A in the extract. For example, a "rebaudioside A 60" extract with a share of 60% rebaudioside A and 40% stevioside in the total steviol glycosides has a relative sweetening power of about 220 times that of 5% (w/v) sucrose. An almost pure rebaudioside A with 98% purity has a relative sweetening power of about 240 times 5% (w/v) sucrose.

If the corresponding maximum quantities used are now applied to the above model calculations, we obtain the following calculated sweetening power equivalent:

- 254 mg "Reb A98" extract with a sweetening power of 240 times corresponds to approx. 61 g sucrose
- 237 mg "Reb A60" extract with a sweetening power of 220 times corresponds to approx. 52 g sucrose

Thus with a highly purified extract it is possible to replace significantly higher quantities of sugar. Thus in certain product developments a few grams of sugar equivalent more are possible.

The combination with sweet carbohydrates is particularly advantageous for the sensory analysis of products containing steviol glycosides. The additional use of sugar types such as for example invert sugar, sucrose, glucose or fructose in the formulation is very advantageous for the taste. Depending on the quantity of sweetener allowed, this will also be necessary. Thus the legally admitted use of 80 mg/l steviol equivalents in soft drinks does not allow any complete replacement of the customary sugar quantity of 10% (w/v) in this application. In so far, partial sweetening with other sweeteners or sweet carbohydrates is necessary.

Prominent producers of steviol glycosides offer products with different shares of rebaudioside A so that developers can choose the sweetening system in a manner which best suits their products and cost concepts. Direct access of such suppliers to high quality Stevia extracts is just as helpful as international experience in product development.

5. Stability

Alongside the sensory properties, the stability of the sweetener is a key quality feature of the end product. There are many experience re-

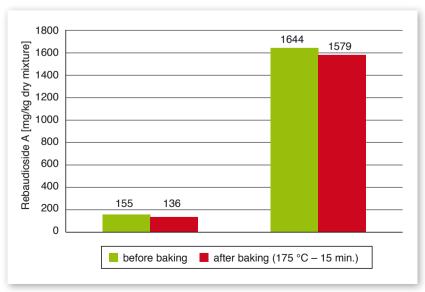


Fig. 3: Stability of rebaudioside A in baking

ports on the stability of steviol glycosides that can be read in the relevant expert literature. Although analyses can show losses at low pH values, the sensory alterations are generally negligible. Thus steviol glycosides can be considered as stable sweeteners for use in sour soft drinks or at high process temperatures (baking, confectionery production) as well. Figures 3 to 6 show a few examples from practice of the stability of steviol glycosides in different applications.

A good overview of the stability in further applications such as milk beverages, fermented milk beverages, yoghurt, soy products, ice cream, jam and biscuits is shown in [7]. The authors come to the conclusion that no loss of steviol glycosides could be shown in any of the applications tested. The use of steviol glycosides is thus possible without problems in a broad spectrum of foods.

6. Conclusion

To summarise it can be said that the steviol glycosides open up new options for product development as very interesting highly intensive sweeteners that are to be taken seriously. When selecting steviol glycoside extracts, it should be noted that large sensory differences can occur with practically the same specification. That is why particular attention is to be devoted to this point in practice.

Conceptually, steviol glycosides as sweeteners of natural origin are suitable for use as sole sweetener in the development of products. Given careful selection of raw materials and incorporating the experience of international producers of aromas and basic materials, the frequently cited taste deficits will not play any role.

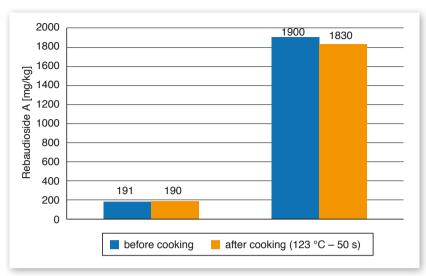


Fig. 4: Stability of rebaudioside A in the production of jelly products

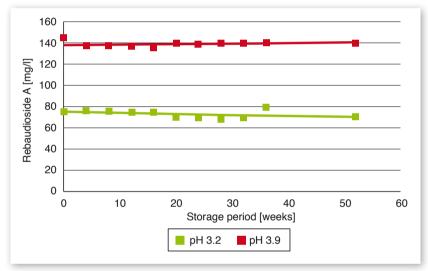


Fig. 5: Stability of rebaudioside A at different pH values in beverages

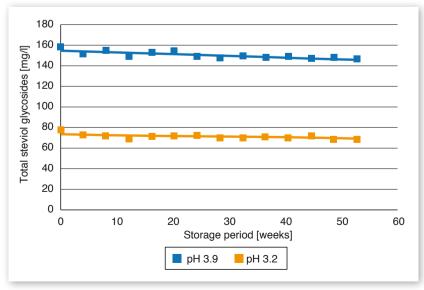


Fig. 6: Stability of steviol glycosides in in model beverages at different pH values

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