

Highly anticipated Stevia approval for the EU

The International Stevia Council gives a timely update on the purification, safety and use of natural stevia sweeteners for which EU approval has just taken place

Introduction

French consumers and consumers in many other parts of the world have had access for some time now to steviol glycoside sweeteners, a new zero-calorie natural sweetener from the stevia plant. The rest of Europe is on the verge of joining them as the highly anticipated EU approval of steviol glycoside sweeteners took place on 11 November 2011. Table-top stevia sweetener products and a small number of food and beverage products were launched shortly after stevia was permitted for sale in the USA in late 2008 and the same is expected across Europe.

Steviol glycosides – purification and safety

Stevia sweeteners are derived from a plant that is native to Paraguay, *Stevia rebaudiana* (Bertoni) (1). Stevia plants (Fig.1) can be grown in many places around the world, but most of the commercial stevia cultivation occurs in China. Steviol glycosides are found primarily in the plant leaves that can contain up to 20% by weight of the sweet-tasting glycosides and details of the extraction process likely vary across manufacturers. In the first stage, stevia leaves are extracted with hot water, which is followed by a flocculation or filtration step to remove plant material. This is followed by a second stage in which the cleared solutions can be further purified to meet regulatory specifications. The extract may then pass through one or more re-crystallisation steps using various food grade alcohol solutions to enhance the purity of one or more specific steviol glycosides.



Figure 1. Stevia.

Name	Formula	Conversion Factor to Steviol Equivalents
Steviol	$C_{20}H_{30}O_3$	1.00
Stevioside	$C_{38}H_{60}O_{18}$	0.40
Rebaudioside A	$C_{44}H_{70}O_{23}$	0.33
Rebaudioside B	$C_{38}H_{60}O_{18}$	0.40
Rebaudioside C	$C_{44}H_{70}O_{22}$	0.34
Rebaudioside D	$C_{50}H_{80}O_{28}$	0.29
Rebaudioside E	$C_{44}H_{70}O_{23}$	0.33
Rebaudioside F	$C_{43}H_{68}O_{22}$	0.34
Dulcoside A	$C_{38}H_{60}O_{17}$	0.40
Rubusoside	$C_{32}H_{50}O_{13}$	0.50
Steviolbioside	$C_{32}H_{50}O_{13}$	0.50

Table 1. Steviol and the ten steviol glycosides permitted in stevia sweetener products in the EU.

mouth and digestive tract unchanged until bacteria in the colon metabolise them. Bacterial metabolism releases steviol, which is excreted, and the sugars that are not absorbed are presumably consumed by bacteria in the colon. The safety of purified stevia extracts and their degradation products for human consumption have been reviewed elsewhere (2). Noteworthy among the safety studies are two clinical studies that demonstrated the safety of steviol glycosides in people with type-2 diabetes and people with low to below-normal blood pressure. The approval process for stevia as a food ingredient in the USA and Europe has been controversial in the past because it appeared to some interested consumers that regulatory officials were being arbitrary in their refusal to permit its use. However, prominent food safety and regulatory agencies were quite clear for many years about the scientific gaps they wanted to see filled (3, 4, 5).

Many of these gaps were only recently filled in, based on studies completed in 2008, including the two clinical studies described above. This work allowed the FAO/WHO Joint Expert Committee on Food Additives (JECFA) to set a permanent acceptable daily intake (ADI) of 4 mg steviol equivalents/kg body weight/day. These studies also facilitated the attainment of GRAS status for steviol glycosides in the USA and the positive safety review recently issued by the European Food Safety Agency (EFSA)

that enables EU-wide approval (1, 6, 21).

Specifications key to the approval process

Steviol glycosides are a group of compounds with a common core called steviol (Table 1). The sweeteners differ primarily in the number and conformation of glucose moieties attached to the steviol core. In order to compare stevia sweeteners with various glycosides formulations, individual glycoside amounts are converted to steviol equivalents based on their molecular weight. The acceptable daily intake (ADI) is based not on any one individual steviol glycoside, but on all steviol glycosides and is expressed in steviol equivalents.

Many early studies on the safety, metabolism and sensory attributes of stevia used ground leaves or semi-purified extracts with a wide variety of specifications, so these studies were difficult to compare (2). This was another barrier to the wider approval of stevia sweeteners in many countries where stevia was still considered a relatively unknown herb. At its 68th

meeting in 2007, JECFA established final specifications for stevia sweeteners used as food (7). The 2010 JECFA specifications require that the total steviol glycoside content of the purified stevia product to be at least 95% among nine of the ten individual glycosides shown in Table 1. However, stevia sweeteners currently on the global market consist of mostly rebaudioside A due to its superior taste attributes and relative abundance in the plant. Smaller amounts of the other steviol glycosides, especially stevioside, are also present in most products. France currently permits only 97% rebaudioside A, but the EU approval will permit specifications similar to those of JECFA. Steviol glycoside sweeteners meeting the EU specifications have been assigned the number E-960 for labeling purposes (21).

Rebaudioside - stable in most food applications

Rebaudioside A and stevioside as dry powders are very stable when stored in polyethylene bags at ambient temperature and controlled humidity. Rebaudioside A is stable for at least 3 years and stevioside is stable for at least 2 years under these conditions (1). Rebaudioside A in solution is stable between pH values of 4 and 8, but less stable below pH 2 (8). Degradation products in both dry powder and in acidic solutions consist of other steviol glycosides and related steviol hydrolysis products (8, 9). Some degradation products have actually been found to be sweet (1). Stevioside and rebaudioside A are both photo-stable when exposed to light for prolonged periods (10).

Rebaudioside A has been reported to be stable in a variety of foods and beverages including soft drinks, chewing gum, plain yogurt, white cake (baked at 180°C for 25 minutes), cereals and other products (8). Dry stevioside has been shown to be stable for an hour at temperatures of 40° to 120°C. At 140°C for an hour, degradation was minimal, but at 200°C there was complete degradation (11, 12). Esmat Abou-Arab *et al.* (12) raised concerns that dry powder instability at higher temperatures might not support the utility of steviol glycosides in baking applications, but dry powder stability and stability in baking applications are very separate issues. Due to the presence

of moisture and other food ingredients, the internal temperature of baked goods generally rises to only 100°C (13, 14, 15). Even dry baked goods such as cookies and pastry contain significant amounts of water (4%) to keep them palatable (16). At these internal temperatures, virtually no thermal decomposition of steviol glycosides would be expected. The surface of bakery goods cooked at high temperatures would likely attain temperatures well above 100°C, but even sucrose traditionally used in baked goods begins to decompose at temperatures of >120°C (17). Fry *et al.* (18) reported on the stability of rebaudioside A in white cake, cookies, muffins and banana bread baked at temperatures ranging from 182° to 205°C. Rebaudioside A was recovered from the finished baked goods at levels of 95.5% for the banana bread that was baked for almost an hour at 182°C to 100% for muffins and white cake baked for 25 minutes.

Some limitations to use levels in EU

Stevia has been consumed as dried leaves (mostly as tea) or an extract for many years in countries such as Japan and Paraguay (1). Stevia has been used as a dietary supplement in many other countries, but its predominant use has been as a sweetener regardless of how or where it was sold. New table-top sweetener products that are predominantly rebaudioside A are among the more successful stevia products currently on the market in countries where stevia has recently been approved. Improved purification processes have eliminated the licorice off-taste and lingering aftertaste that was a problem with semi-purified products that were common on the market 10 or more years ago. The importance of commercially viable purification processes that have produced high-purity stevia products cannot be over-emphasised as an important factor in the recent success of stevia in the USA, Australia, France and elsewhere.

Beverages, both powdered and liquid, have been one of the more popular product categories for the introduction of stevia. In most countries, stevia can be used as the sole sweetener in water-based beverages at rebaudioside A levels up to 600 ppm (approximately 200 ppm steviol equivalents). However, due to

concerns about intake levels in the EU, water-based flavoured drinks (e.g., carbonated soft drinks) will be limited to 80 ppm steviol equivalents or 240 ppm rebaudioside A. This will allow beverage producers to replace less than half the sweetness-equivalence (depending on product formulation) of a caloric sweetener used in the non-reduced calorie version. Few, if any, zero-calorie beverage products sweetened only with rebaudioside A will be possible under the new EU regulation. It is unclear how this will impact on innovation in the soft drink category where most zero-calorie sweeteners are used. In order to maintain the natural positioning of stevia-containing products, rebaudioside A will likely be used in blends with sucrose or a similar caloric sweetener that will produce a mid-calorie or reduced-calorie beverage.

Milestones for EU approval

Three independent submissions requesting approval for steviol glycosides use in food were submitted to the EU in 2007 and 2008. These were later combined for a more efficient review process. In late 2009, France's food safety agency, AFSSA, approved the use of 97% rebaudioside A for a limited - up to two-year - period, pending EU approval. This was recently extended because of the up-coming EU approval. In April, 2010 the European Food Safety Agency (EFSA) finalised their safety review and set an ADI paving the way for final EU approval (6). The EFSA review confirmed several important conclusions from the earlier JECFA review that was finalised in 2008. First, all steviol glycosides are metabolised to a common metabolite (steviol) and have similar excretion pathways, so studies performed on stevioside can be used to establish the safety of rebaudioside A and other steviol glycosides. Second, the complete body of safety studies available indicates that steviol glycosides are not carcinogenic, genotoxic or associated with reproductive or developmental toxicity. This is important because some earlier studies reported genotoxicity and reproductive toxicity that were later found to be erroneous, but such information persists on the Internet. Third, that a 100-fold safety factor applied to the no-effect-level from the two-year carcinogenicity study

can be used to establish the ADI of 4 mg steviol equivalents/kg body weight/day. This is equivalent to 12 mg/kg/day for rebaudioside A.

However, comments from EFSA about the potential for the ADI to be exceeded at the maximum use levels originally proposed resulted in several intake reassessments in 2010 and early 2011. These reassessments eliminated some food categories for steviol glycosides use and reduced the permitted use levels in several others, including water-based flavoured beverages (food category 14.1.4). Based on these reassessments, a representative from the Directorate General for Health and Consumers (DG SANCO) recently stated in June that final approval for steviol glycosides would come by the end of 2011, including the review and approval by the European Parliament (19). The approval happened on 11 November 2011 and afterwards the Commission Regulation 1131/2011 was published in the EU's Official Journal on 12 November. The appearance of stevia products on an EU-wide basis can occur shortly after this in early December 2011. Given the controversy surrounding the potential for some consumers to exceed the ADI with the original requested use-levels, the European Commission will ask food manufacturers to report actual use levels following approval. Based on this information, there may be a re-evaluation of the current intake estimates (21).

Steviol glycosides are approved under EC regulation 1333/2008 on food additives that replaced the "Sweeteners Directive". The new regulation harmonises and combines the authorisation process for all food additives, including sweeteners, along with enzymes and flavourings. Approved additives are included in Annex II, a list of authorised ingredients and their conditions of use. Despite it being a new Directive, much of the regulatory process, required evaluations (e.g., a safety review) and information submissions (e.g., specifications and labeling) remain unchanged.

Stevia's natural origin appealing to food manufacturers

New stevia sweetener products provide food manufacturers with an attractive alternative that meets new

consumer desires for "natural" or "pure" products. In 2009, Mintel reported that 23% of new food and drink launches across Europe in 2008 featured some sort of "natural" reference (20). Mintel also reported that natural claims appeared on a third (36%) of new food and drink launches in the UK in 2008 while the use of many previously popular fortification claims fell dramatically. While technically-oriented food technologists can disagree with the scientific relevance of natural claims, no one can deny the attractiveness of naturally-derived ingredients to consumers.

Conclusions

Stevia sweeteners have already proven their value in countries that have recently permitted stevia use where they have been incorporated into many new products. The approval of stevia in the EU is being closely watched by the food industry as the new naturally sourced zero-calorie sweetener is expected to generate widespread consumer interest. Hopefully, use limitations in water-based flavoured beverages and several other categories can be overcome in time so that European consumers will be able to enjoy the full range of stevia-sweetened products expected to be available globally.

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