

1. Carl Horn: Some socio-economic and environmental aspects of the Stevia industry in developing countries. Is the Stevia supply chain an opportunity in the stabilization of democracies?

National and regional, European and American foreign aid agencies have been increasingly frustrated over the lack of significant results of multi billion dollar foreign aid programmes. It is easy to diagnose how some well intended aid programmes end up in "greedy pockets".

It is becoming brutally clear that there will be no stabilization of democracy without successfully addressing education. There is no way of addressing education without addressing poverty.

2. John C. Fry: Practical aspects of steviol glycosides from the perspective of a product development scientist.

Product developers in the West are focusing enthusiastically on steviol glycosides as these natural high-potency sweeteners become more widely accepted. These potential users of steviol glycosides require specific information on potency, taste quality and stability to guide their development efforts. This presentation deals with these three prerequisites using rebiana as an example.

3. Peter Grosser: EUSTAS Quality Assurance for Stevia Raw Products.

In the European Union, approval for the steviol glycosides from *Stevia rebaudiana*, as a natural and calorie-free sweetener, is expected in 2010. European consumers expect this new food additive to be healthy and safe, and without negative side-effects. This paper describes a concept by which the quality of steviol glycosides can be guaranteed.

4. Troy Rhonemus & Bruce Furlano: CURRENT STATUS OF STEVIOL GLYCOSIDE ANALYTICAL METHODS.

The importance of well developed and validated analytical methods for rebiana (synonym for >95% rebaudioside A), other glycosides, and non-glycoside impurities have grown over the last year with the FDA no objection letter for GRAS approval in food and beverages. With the growing acceptance of rebiana as a natural high-potency sweetener the industry requires methods that can analyze all aspects of rebiana. These methods will provide the needed information for physical property characteristic, production data, food application rebiana concentrations, and rebiana final product quality. The industry has inconsistencies in the methods used and the

discussion will focus on these differences, method changes, and issues that need to be addressed.

5. Jan M.C. Geuns & Tom Struyf: EUSTAS Round-Robin Testing of Steviol Glycosides.

A round-robin testing of 2 steviol glycoside samples was organised. Ten laboratories participated in the testing. The first sample had a purity of 96.2%. The second sample was a 4/5 dilution of sample 1 with NaHCO₃. This way, the drying process itself could be checked. The purity of sample 2 was 82.35%. The reported purities of sample 1 varied between 79.8 and 96.2%, those of sample 2 varied between 58.1 and 81.8%. To improve the accuracy of analysis, different suggestions can be made, such as controlling the drying process of samples and standards, purity of standards, injection of sufficient material and use of solvent gradients to shorten run time and reduce integration errors.

6. Benjamine Geeraert¹, Florence Crombé¹, Maarten Hulsmans¹, Nora Benhabilès¹, Jan Geuns², Paul Holvoet¹: Natural sweetener stevioside inhibits atherosclerosis by increasing the antioxidant defense in obese, insulin-resistant mice

Objective - Stevioside is a non-caloric natural sweetener that reduces glucose by increasing insulin in non-obese animals. Obesity is frequently associated with insulin resistance and increased oxidative stress. Therefore, we investigated its effects on insulin resistance and oxidative stress related to atherosclerosis in obese, insulin-resistant and hyperlipidemic mice.

Research Design and Results - Twelve-week old mice were treated with stevioside (10 mg/kg, orally; n=14) or placebo (n=17) for 12 weeks. Stevioside had no effect on weight, but lowered fasting glucose (-18%), insulin (-34%), and cholesterol (-21%). Stevioside treatment increased *Lxra*, *Fabp4*, and *Glut4*, *Irs1*, *Irs2*, and *Insr* in white visceral adipose tissue, supporting increased adipocyte differentiation and improved insulin signaling. Increased adipose tissue differentiation was associated with an increase in adiponectin (+98%). Stevioside reduced plaque volume in the aortic arch (-22%) by decreasing the macrophage (-23%), lipid (-21%) and oxidized LDL (-44%) content of the plaque. Stevioside treatment was associated with an increase in the antioxidative defense in the vascular wall, as evidenced by increased *Sod1*, *Sod2*, and *Sod3*, which was associated with a decrease in oxidized LDL in the

aorta. Furthermore, we found a relation between adiponectin, insulin signaling and oxidative stress in the aorta of stevioside-treated mice.

Conclusion - Stevioside treatment inhibited the atherosclerotic plaque development and was associated with improved insulin signaling and antioxidative defense in the vascular wall.

7. Amal A.A. Mohamed, Jan M.C. Geuns, Wim Van den Ende & Marc De Ley : Molecular aspects of early steps in the steviol biosynthesis.

The first committed steps in the shared pathway for synthesis of the gibberellins and steviol glycosides are controlled by *ent-copalyl diphosphate synthase* and *ent-kaurene synthase*. The expression of these genes was followed by RT-Q-PCR in *Stevia rebaudiana*. Plants were subjected to different light conditions: 12 h light/dark and 18 h light/6 h dark in both full and shady illuminations. Samples from both old and young leaves were collected weekly, immersed in liquid nitrogen and kept at -80 °C for further extractions of RNA, steviol and steviol glycosides.

To maximise accuracy, housekeeping genes *18S rRNA* and *actin* were both used as a reference to normalize the data in RT-Q-PCR. The results of normalized data were similar for both genes.

The expression patterns of the two genes were considerably variable depending on both the age of the leaves and the light conditions under which the plants were grown.

8. Annelies Smedts*, Stijn Ceunen, Ruis Amery*, Jan M.C. Geuns** and Boudewijn Meesschaert*/***Bacterial consortia from Paraguayan soil samples with β -glucosidase activity degrading stevioside to steviol.**

Steviol glycosides are natural sweeteners known to have pharmacological effects in animals when administered in high doses (3×250 mg/day). Its possible active component is steviol glucuronide. Steviol glucuronide can be chemically synthesized from steviol. In this study, an attempt is made to produce steviol with a high yield by incubating stevioside with soil samples from a Paraguayan *Stevia* plantation. Different incubation conditions resulted in a different yield of steviol, a different rate of stevioside degradation and steviol formation, and different ways of hydrolysis of

stevioside to steviol. The influence of parameters like the concentration of yeast extract, pH, stirring and temperature was analyzed.

9. Nico Moons, Wim M. De Borggraeve and Wim Dehaen: Stevioside and (iso)steviol as starting materials in organic synthesis

In this review, we offer an overview of the more important papers containing derivatisations of stevioside, steviol and isosteviol. Biotransformations as well as chemical reactions will be discussed. The current review has literature coverage up to May 2009 and can be seen as an introduction to current work in our laboratories. We do not aim to be comprehensive but have rather focused on the more recent research.

10. Joël PERRET: Status of Steviol Glycosides in France

The present situation concerning the authorization to use steviol glycosides extracted from *Stevia rebaudiana* Bertoni plant as a sweetener in France is presented in this paper. The choice of protocol, the history of the different steps, the evolution and the regulatory status of these products at the date of redaction are discussed.

11. Amy Boileau, Leslie Curry: Stevia update – regulatory status in the United States - May 2009

It's been a year since Cargill submitted a GRAS notification for rebaudioside A (common name rebiana) to the U.S. Food and Drug Administration. Much has happened in the past year and the purpose of this presentation is to two-fold. First is to describe the process that Cargill undertook with partner The Coca-Cola Company to bring a characterized Stevia-based sweetener, rebiana, to the U.S. market; and second to recap the events that have occurred in the first year of GRAS status.

12. Frank Jaksch, CEO and President: The Importance of Well Characterized Analytical Reference Standards for the Stevia Industry

Stevia based sweeteners are a growing industry in North America and worldwide. While the economic opportunities are relatively obvious, the underlying challenges for quality control of stevia based sweeteners are not readily apparent. Often difficult to analyze, both stevia based ingredients as well as finished products present a huge

challenge to in-house and contract analytical laboratories. When considering a laboratory test result the question must be asked, "*What reference standards or reference materials were used to produce this analytical result?*" Certificates of Analysis are often used to describe quality control data for a particular lot/batch of reference standard or material. These materials are only as good or reliable as the methodologies and tools used to define them. This lecture will acquaint attendees with the differences between reagent chemicals and chemicals that are reference materials. All reference materials are not created equal, and the primary differences lie in the analytical data and documentation contained within the supporting the CofA. It is not uncommon, within the food industry to discover the use of unqualified reference materials that have no supporting documentation, a practice which needs to change.