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Proceedings of the 4rd Stevia symposium, organised by EUSTAS 2010

Stevia: Science, no Fiction

KULeuven, June 29 and 30 2010

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CHAPTER 1

Sprout culture of *Stevia rebaudiana* Bertoni

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ABSTRACT

Initialisation of *in vitro* sprout culture provides an opportunity to control the level of abiotic stresses on the cultivar. In this system, the cultivar of interest can still maintain the rapid growth combined with sustainable secondary metabolism. *Stevia rebaudiana* Bertoni is a unique plant capable of biosynthesising the sweet tasting compounds. The calorie free steviol glycosides are a product of the secondary metabolism. Their amounts depend on various growing conditions. Certain levels of suboptimal growth conditions can enhance the biosynthesis of steviol glycosides. The sprout culture of *S. rebaudiana* is characterised by rapid growth of plant biomass (up to 400 % within 3 weeks). Introduction of stevia into an *in vitro* sprout culture produced an opportunity to harvest this plant up to 10 times per year, regardless of climatic conditions. The sprout culture of *S. rebaudiana* produces sustainable yields of steviol glycosides and other secondary metabolites. The biosynthesis of steviol glycosides correlates with anthocyanin concentrations in the leaf biomass (Pearson correlation coefficient was 0.7 and 0.8). The radical scavenging capacity (DPPH assay) of water extracts from dried leaves of stevia

CHAPTER 3

Opinion of EFSA on steviol glycosides: what's next?

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ABSTRACT

The European Food Safety Authority has finalised the risk assessment of steviol glycosides: an ADI has been set and the future exposure has been estimated. The next steps have to be taken. The European Commission and the experts on food additives of the Member States started to discuss the need of use in the proposed food groups and the projected levels. It must be ensured that the ADI is not exceeded and that the legislation is clear. Proposed specifications need to be discussed too. Applicants are consulted during the process. Formal draft legislation needs to be written by the Commission, for specifications (amendment of Directive 2008/60/EC), as well as for the authorisation of use as a sweetener (amendment of Regulation (EC) N° 1333/2008). An E-number will be attributed. Further steps include consultation with stakeholders, consultation with the legal service of the Commission, internal consultation in the Commission, notification to the World Trade Organisation, a vote in the standing committee, translation in all official European languages, the scrutiny procedure of Council and European Parliament,

CHAPTER 4

Second EUSTAS round-robin testing of steviol glycosides.

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ABSTRACT

A round-robin testing of a steviol glycoside sample was organised. Ten laboratories participated in the testing. So far, only 7 have sent their results. The sample had a purity of 91.1%. The reported purities of the sample varied between 82.74 and 95.86%. About 3 % purified RebB was added to the sample to check the quality of the analysis of this compound possessing a carboxylic group. The sample contained the following steviol glycosides: Reb D, Reb E, Reb A, ST, Reb F, Reb C, Dul A, Reb G, Rub, Reb B, SB and SM (1 lab). No SV was detected. The number of SVgly analysed in the different laboratories varied between 4 and 11. One lab only analysed ST and Reb A and gave a percentage composition of these compounds.

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. The results of this second round-robin testing are better than those of the first testing (Geuns and Struyf 2009).

CHAPTER 5

Determination of Steviol Glycosides in Various Dairy Matrices and Soy Drink

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ABSTRACT

EFSA recently expressed the opinion that stevia extracts containing at least 95% rebaudioside A and/or stevioside are safe as a food additive. Approval for these stevia extracts as a food additive is expected very soon. It is therefore important that stevia extracts can be accurately and precisely determined in various food matrices.

The objective of this study was to determine steviol glycosides in dairy products and soy drink. Milk, ice-cream, fermented milk drink and soy drink sweetened with

CHAPTER 6

Ultra-High Performance Liquid Chromatography for the analysis of steviol glycosides

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ABSTRACT

When analyzing natural products, such as steviol glycosides, one must always be aware of the fact that the analytical method used might not reveal every compound that is actually present in the sample. For instance, a chromatogram representing the separation of steviol glycosides on two 20 cm C_{18} columns in series (d_p= 5.0 µm) indicates the presence of 12 compounds; four of them not being identified yet. Some compounds may be "invisible" to the instrument, because they are hidden under larger peaks, or because they elute very late and the band broadening renders them undetectable.