



**JOINT FAO/WHO FOOD STANDARDS PROGRAMME  
CODEX COMMITTEE ON FOOD ADDITIVES  
Fiftieth Session  
Information from International Stevia Council (ISC)**

The Alternative Technologies for the Production of Steviol Glycosides

**The Alternative Technologies for The  
Production of Steviol Glycosides**

By Ashley Roberts  
PhD, Senior Vice President, Food & Nutrition  
Health, Environmental & Regulatory Services - Intertek

Technical Expert Advisor to ISC


Xiamen, China - 27 March 2018



1

**Agenda**


- 1) Alternative Technologies:
  - Enzyme Modified Glycosides
  - Bioconversion
  - Steviol Glycosides *via* fermentation
- 2) Safety of steviol glycosides
- 3) Regulatory status of alternative technologies



2

**Enzyme modified steviol glycosides**

- Manufactured using *S. rebaudiana* plant extracts
- Starts with purified steviol glycosides (≥95%) and an enzyme system is used to transfer sugar/glucose units to the steviol glycosides - glycosylation
- End product contains a combination of steviol glycosides some of which may not be present in the leaf
- Final product ≥95% steviol glycosides
- The enzymes may be from a GM-modified source or a non-GM modified source





3



**Bioconversion**

- Involves the enzymatic conversion of steviol glycosides extracted from the plant (e.g. Reb A) to different steviol glycosides such as Reb D and M by adding one or more glucose units from a sugar source, using enzymes expressed by genetically modified organisms
- The various enzymes produced through this technology are isolated and then reacted with the plant extract
- The resulting end products are identical to those steviol glycosides found in the stevia leaf but are not directly extracted from the stevia plant
- These products meet the current JECFA purity specification for steviol glycosides

4



### Stevia Glycosides via Fermentation

- Steviol glycosides are produced directly from GMM such as *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, via fermentation
- The GM organisms are modified to produce rebaudiosides A, D and M
- Produced through fermentation procedures
- No involvement of the plant extract
- These products meet the current JECFA purity specification for steviol glycosides



5



### Steviol Glycoside - Safety

- Several *in vitro* studies have confirmed the ability of the gut microflora from animals and humans to hydrolyze steviol glycosides completely to steviol
- Human fecal homogenate experiments have shown all glycosides independent of the attached sugar moiety are hydrolyzed to steviol (Purkayastha *et al.*, 2016)
- Studies indicate that the individual sugar moieties may be cleaved sequentially from the steviol backbone
- No gender or ethnic differences
- The major gut bacteria responsible for the hydrolysis process are the *Bacteroides* family (Renwick and Tarka, 2008)
- The safety of steviol glycosides produced through the new technologies are similarly covered using the metabolic paradigm



6



### Alternative Technologies: Regulatory Status

Manufacturers are beginning to obtain international regulatory clearance for these new manufacturing methods:

#### Enzyme Modified Glycosides

- Currently approved in Japan, Malaysia, S. Korea and have generally recognized as safe (GRAS) in the United States (U.S.) (GRN# 337,375,448,452,607,662)

#### Bioconversion

- Products have gained GRAS status in the U.S. (GRN #667 and #715) and food additive approval in Canada, Mexico, and Ecuador.

#### Steviol Glycosides via fermentation

- JECFA safety opinion for rebaudioside A produced from *Yarrowia lipolytica* (JECFA, 2016) and food additive approval in Mexico
- Two products currently have GRAS status in the U.S. (GRN#626 - Steviol glycosides produced in *Saccharomyces cerevisiae* and #632 - Rebaudioside A from *Yarrowia lipolytica*)



7



Thank you



8