Ad hoc meeting with industry representatives – Steviol glycosides (E960)

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Background information

- EFSA evaluated three application dossiers (Morita Kagaku Kogyo Co., EUSTAS and Cargill) on the safety of steviol glycosides to be authorised as a new food additive.
- In April 2009, EFSA received a “Roadmap” petition combining the information from the three application dossiers
- Information on the identification and quantification of up to nine steviol glycosides was submitted.
- Following the EFSA opinion (2010), the European Commission authorised steviol glycosides (E 960) as a food additive in the EU. Specifications based on the information submitted by the applicants were included in Regulation 231/2012 for E960.

Background information

- EFSA evaluated in 2015 a new application dossier (PureCircle Ltd.) requesting to include rebaudioside M in the food additive E960.
- EFSA evaluated in 2018 a new application dossier (PureCircle Ltd.) requesting to amend the existing EU specifications for steviol glycosides (E960) to allow the inclusion of all steviol glycosides identified in *Stevia rebaudiana* Bertoni leaves, including both 'major' and 'minor' glycosides, that may comprise the assay value of not less than 95% total steviol glycosides.
- In this latest application dossier, over forty steviol glycosides were elucidated by liquid chromatography–mass spectrometry (LC–MS) and nuclear magnetic resonance

Background information - JECFA

→ The presence of kaurenoic acid as an impurity of 'Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica* is acknowledged by JECFA (2016)

"Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica* will contain minor amounts of rebaudioside D, rebaudioside B, rebaudioside M, and stevioside and may contain minor amounts of steviol and kaurenoic acid which are intermediates in the biosynthetic pathway of steviol glycosides."

Background information

In the previous application dossiers submitted for evaluation by EFSA, the presence of kaurenoic acid was never reported by any of the applicants.

→Consequently, no limits for kaurenoic acid are currently included in the specifications for the food additives steviol glycosides (E 960) according to Regulation (EU) No. 231/2012

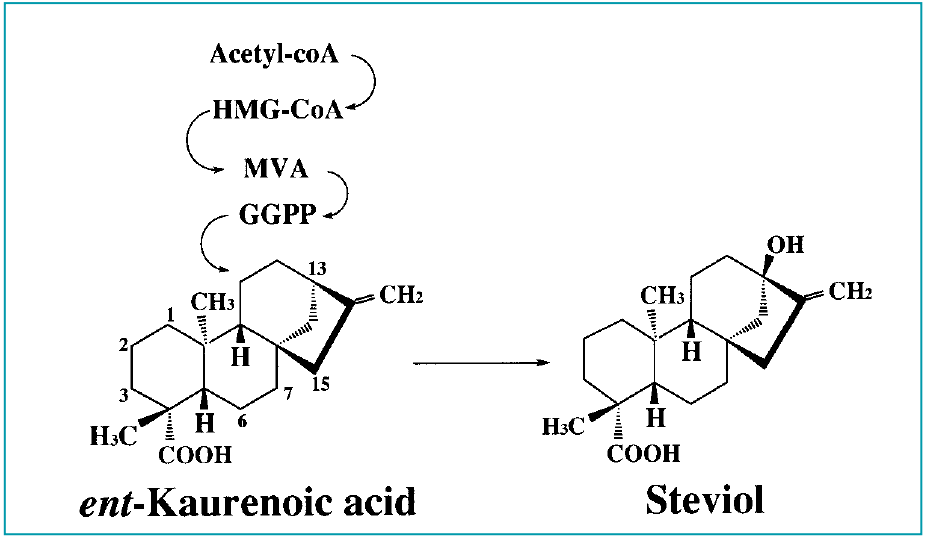
Background information

EFSA is currently evaluating an application dossier : “Request for EFSA's scientific opinion as regards a proposed amendment of the specifications of the food additive Steviol glycosides (E 960)” EFSA-Q-2016-00689

→new production process that covers fermentative rebaudioside A produced by a strain of *Yarrowia lipolytica*, genetically engineered to express the steviol glycoside metabolic pathway of the plant Stevia Rebaudiana Bertoni

Background information

Kaurenoic acid is described in literature as being the immediate precursor in the biosynthetic pathway of the steviol aglycone



From Kim et al. (1996). Archives of Biochemistry and Biophysics. 332(2), 223-230

The presence of kaurenoic acid as a residual in the food additive E 960 produced by water extraction of the leaves of *Stevia Rebaudiana* Bertoni would also seem plausible

Background information

Additionally, kaurenoic acid can be glycosylated (by UDP-glucosyltransferases) to kaurenoic acid glycosides that are expected to be metabolized to kaurenoic acid

EFSA Guidances (food additives – botanicals)

Specifications: define the requirements concerning the identity, the purity and the limits of any impurity present in the additive (EFSA ANS Panel, 2012)

In the case of food additives derived from botanical sources:

→Limits for specific undesirable/toxic substances known to be present in the plant should be specified (EFSA, 2009); accordingly, validated methods should be provided for the analysis

Questions to participants/discussion

- Do you analyse the presence/quantification of kaurenoic acid (or kaurenoic acid glycosides) in the steviol glycosides (E 960)?

- If yes, do you have a validated method of analysis for the determination and quantification of kaurenoic acid in the steviol glycosides (E 960)?

Next steps

EFSA will be finalising the assessment on the new production process by fermentation for the food additive steviol glycosides (E 960)

Based on all the information available, EFSA may recommend adding limits for kaurenoic acid to the specifications for E960.