Request to amend the list of processing aids for the production of Steviol Glycosides under Australia and New Zealand Food Standard Code Standard 1.3.1– Food Additives and Standard 1.3.4 – Identity and Purity

Executive Summary

This submission requests the addition of an enzyme to the list of processing aids used to produce steviol glycosides by biotransformation to Rebaudioside M and Rebaudioside I. The enzyme is produced by an *E. coli.*

FSANZ approved the use of all steviol glycosides present in the stevia leaf extract in application A1132. That application expanded the definition of steviol glycosides that meet the specification.

In that executive summary of the approval to Broaden Definition of Steviol Glycosides (Intense Sweeteners), FSANZ stated:

"No evidence was found to suggest that the expansion of the definition of steviol glycosides for use as sweeteners to include all steviol glycosides present in the stevia leaf posed any public health and safety concerns. It was expected that all steviol glycosides will be hydrolyzed completely to steviol by gut microflora.

FSANZ concluded that broadening the definition and hence the specification for steviol glycosides preparations to include any mixture of individual steviol glycosides extracted from the stevia leaf was justified. The same analytical methods currently used for steviol glycosides can be used to identify these other minor steviol glycosides. The current ingredient labelling requirements for steviol glycosides added to food as an intense sweetener food additive still apply; it can be listed as 'steviol glycosides' or with the code number '960' (FSANZ, 2017).

In other applications, FSANZ expanded the manufacturing method to include biotransformation and fermentation in addition to extraction.

Manus Bio genetically engineered an E. coli to produce several enzymes to bio-transform stevia leaf extract into Reb M or Reb I that meets all FSANZ's and JECFA's specifications.

The potential allergenicity of all non-native enzymes was confirmed noting the allergens contained in Online Database by Food Allergy Research and Resource Program (FARRP).

The extracellular enzymes convert stevia leaves to Reb M or I. The downstream processing removes all residual protein.

Production of Reb M and Reb I using E. coli were run to confirm specifications such as pesticides and heavy metals.

Manus Bio notified FDA of this new enzyme system and received a letter of no objection dated 26 January 2022 (U.S. FDA, 2022).