REBAUDIOSIDE M SWEETENER COMPOSITIONS

FIELD OF THE INVENTION

[0001] The present disclosure relates to sweetener compositions containing high purity rebaudioside M and methods of making the sweetener compositions.

BACKGROUND

[0002] Reduced-calorie sweeteners derived from natural sources are desired to limit the health effects of high-sugar consumption. The stevia plant (*Stevia rebaudiana* Bertoni) produces a variety of sweet-tasting glycosylated diterpenes termed steviol glycosides. Of all the known steviol glycosides, rebaudioside M has the highest potency (~300 times sweeter than sucrose) and has the most appealing flavor profile. However, rebaudioside M is only produced in minute quantities by the stevia plant and is a small fraction of the total steviol glycoside content (<1.0%), making the isolation of rebaudioside M from stevia leaves impractical. Alternative methods of obtaining rebaudioside M are needed. One such approach is the application of synthetic biology to design microorganisms (e.g. yeast) that produce large quantities of rebaudioside M from sustainable feedstock sources. In addition, given the high-intensity sweetness of rebaudioside M, usable sweeteners like table-top sweeteners and sugar substitutes containing rebaudioside M are needed that dilute the high-intensity sweetness without introducing off flavors are needed.

SUMMARY

[0003] Provided herein are high-intensity sweeteners that contain greater than 95% Rebaudioside M, methods of producing the high-intensity sweeteners, and sugar substitutes containing the high-intensity sweetener and one or more bulking agents.

[0003a] In one aspect, the disclosure provides a purified high-intensity sweetener comprising at least 95% by weight Rebaudioside M and less than 20,000 ppm combined amount of Rebaudioside D, Rebaudioside B, and Rebaudioside A.

[0004] In one example, the disclosure provides a purified high-intensity sweetener containing at least 95% by weight Rebaudioside M and less than 5000 ppm Rebaudioside D, less than 4000 ppm Rebaudioside B, and less than 2000 ppm Rebaudioside A.

[0005] In an example the Rebaudioside D is less than 3200 ppm, the Rebaudioside B is less than 2000 ppm, and the Rebaudioside A is less than 1000 ppm. In another example the Rebaudioside D, Rebaudioside B, and Rebaudioside A are below the limit of quantification (LOQ) when also quantifying Rebaudioside M. In a further example Rebaudioside M,

Rebaudioside D, Rebaudioside B, and Rebaudioside A amounts are measured using high performance liquid chromatography (HPLC).

[0006] In another aspect, the disclosure provides a table-top sweetener comprising the purified high-intensity sweetener provided herein. In an example the table-top sweetener contains a bulking agent. In another example the bulking agent is selected from erythritol, dextrin, inulin, polydextrose, and maltodextrin.

[0007] In another aspect, the disclosure provides a sugar substitute comprising the purified high-intensity sweetener described herein. In an example the sugar substitute contains one or more bulking agents. In another example the bulking agents are selected from erythritol, soluble fiber, dextrin, inulin, polydextrose, and maltodextrin. In yet another example the sugar substitute has the same level of sweetness on a per weight basis as sucrose. In an example the sugar substitute contains from about 85% to about 90% erythritol by weight, from about 9% to about 15% soluble fiber by weight, and from about 0.1 % to about 1.0% of the purified high-intensity sweetener described herein. In another example the sugar substitute contains about 90% erythritol by weight, about 9.5% soluble fiber by weight, and about 0.5% of the purified high-intensity sweetener described herein. In another example the soluble fiber is selected from beta-glucans, glucomannan, pectin, gum guar, inulin, fructo-oligosaccharide, digestion resistant dextrin, and polydextrose. In a preferred example the digestion resistant dextrin is NUTRIOSE FM10. In additional examples the high-intensity sweetener is agglomerated with the one or more bulking agents.

[0008] In yet another aspect, the disclosure provides a method of preparing the purified high-intensity sweetener described herein involving the steps of obtaining a cleared fermentation broth comprising rebaudioside M; filtering the cleared fermentation broth with an ultrafilter to generate a ultrafiltration permeate; filtering the ultrafiltration permeate with a nanofilter to generate a nanofiltration flow-through; washing the nanofiltration flow-through; and spray drying the washed nanofiltration flow-through to obtain the purified high-intensity sweetener described herein. In an example the ultrafilter has an ultrafiltration cutoff from about 2kDa to about 100kDa. In another example the ultrafilter has an ultrafiltration cutoff of about 20kDa. In yet another example the nanofilter has a nanofiltration cutoff from about 200Da to about 1000Da. In a further example the nanofilter has a nanofiltration cutoff of about 300Da to about 500Da. In additional examples the cleared fermentation broth is pH adjusted to have a

pH greater than pH7. In another example the cleared fermentation broth has a pH of about pH10. In further examples the nanofiltration flow-through is washed after being acidified with an acid solution. In an example the acid solution comprises citric acid.

[0009] In a further aspect, the disclosure provides a method of making the sugar substitute described herein involving the steps of adding a first bulking agent to a mixer; precoating the mixer with the first bulking agent; adding a second bulking agent, and the purified high-intensity sweetener described herein; mixing the first bulking agent, second bulking agent, and high potency sweetener; adding water to the mix; mixing the first bulking agent, second bulking agent, high potency sweetener, and water; and drying the mixture. In an example, the first bulking agent is erythritol. In another example the second bulking agent is a soluble fiber. In a further example the soluble fiber is digestion resistant dextrin. In yet another example the digestion resistant dextrin is NUTRIOSE FM10.

BRIEF DESCRIPTION OF THE FIGURES

[0010] FIG. 1 is a diagram showing the biochemical pathway from the precursor farnesyl pyrophosphate (FPP) to steviol.

[0011] FIG. 2 is a diagram showing the biochemical pathway from the precursor isoprenoid backbone steviol to many of the known steviol glycosides including rebaudioside M.

[0012] FIG. 3 is a diagram showing the scale-up of the fermentation process for the production of rebaudioside M.

[0013] FIG. 4 is a flow diagram of the purification process used to produce the high-intensity sweetener comprising rebaudioside M.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[0014] As used herein “high-intensity sweetener” refers to sucrose alternatives that are at least several times sweeter than sucrose on a per weight basis. In addition, high-intensity sweeteners are low or zero calorie and do not impact blood sugar levels. Illustrative examples of a high-intensity sweeteners includes the steviol glycosides produced by the plant *Stevia rebaudiana*. A preferred high-intensity sweetener is one predominantly comprising the steviol glycoside Rebaudioside M.

[0015] As used herein “sugar substitute” refers to a food additive that provides a sweet taste like that of sucrose yet contains significantly fewer calories on a per weight basis than sucrose.