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 ~~OF THE INVENTION~~

[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 ~~aspect~~example, the ~~invention~~ 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 ~~embodiment~~ 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 ~~embodiment~~ example the Rebaudioside D, Rebaudioside B, and Rebaudioside A are below the limit of quantification (LOQ) when also quantifying Rebaudioside M. In a further ~~embodiment~~ example Rebaudioside M,

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

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

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

[0008] In yet another aspect, the ~~invention~~ 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 ~~embodiment~~ example the ultrafilter has an ultrafiltration cutoff from about 2kDa to about 100kDa. In another ~~embodiment~~ example the ultrafilter has an ultrafiltration cutoff of about 20kDa. In yet another ~~embodiment~~ example the nanofilter has a nanofiltration cutoff from about 200Da to about 1000Da. In a further ~~embodiment~~ example the nanofilter has a nanofiltration cutoff of about 300Da to about 500Da. In additional ~~embodiments~~ examples the cleared fermentation broth is pH adjusted to have a

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

[0009] In a further aspect, the ~~invention~~ 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 ~~embodiment~~example, the first bulking agent is erythritol. In another ~~embodiment~~ example the second bulking agent is a soluble fiber. In a further ~~embodiment~~ example the soluble fiber is digestion resistant dextrin. In yet another ~~embodiment~~ 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.