FORMULATION FOR IMPROVING SWEETNESS OF THE RARE SUGARS WITHOUT ALTERING THE TEXTURE AND FLAVOUR AND TO THE METHOD THEREOF

TECHNICAL FIELD OF THE INVENTION:

The present invention relates to a formulation comprising sweet protein with carbohydrate and/ or non-carbohydrates for improving the sweetness without changing the texture and flavours and to the method for improving the sweetness of the rare sugars. More particlularly, the present invention relates to method for improving the sweetness of the rare sugars without changing the texture and flavours of the primary sugars.

The present invention further relates to addition of sweet protein such as Brazzein with Liquid Rare sugars before crystallization or at pre caramelization stage or post caramelization stage of rare sugars at different tempertures to increase the sweetness. Brazzein increases the sweetness of Rare sugars not limited to D-Allulose, D-tagatose, D-Allose, Isomaltulose, Trehalulose etc.

BACKGROUND AND PRIOR ART OF THE INVENTION:

Rare sugars and their derivatives rarely exist in nature. These sugars possess the properties like sweeteners but lack calorific value. Therefore, these could be used as an alternative to other sweeteners. Rare sugars are not metabolized as natural sugars by the body.

There is currently increased demand for sugar substitutes because many harmful effects of sugar have been reported. Some of these detrimental effects include the increased risk of coronary heart disease, contribution to obesity and diabetes, and suppression of the immune system. Alternative sugars can be divided into two classes of compounds, natural and artificial.

The synthetic sugar substitutes include acesulfame potassium, aspartame, neotame, saccharin, and sucralose. The Food and Drug Administration from various countries has strictly regulated artificial sweeteners as food additives due to the controversial health risks of synthetic sugar substitutes.

In addition to synthetic sugar substitutes, consumers are exposed to many natural sugars as sugar alternatives including isomaltulose, tagatose, allulose, trehalulose, turanose, allose which are referred as rare sugars.

Isomaltulose, also known by the trade name Palatinose and chemical name 6-O-α-D-glucopyranosyl-D-fructose, is a disaccharide carbohydrate formed from glucose and fructose. It can be found naturally in honey and sugarcane extracts, and it is also manufactured industrially from sucrose, serving as a substitute for sugar.

Tagatose, a hexose monosaccharide, can be found in small amounts in various foods and has garnered interest as a as a potential alternative sweetener. It is generated during the heating process of milk, particularly in dairy products. It closely resembles sucrose (table sugar) in texture and appearance, being 92% as sweet, but containing only 38% of the calories.

D-allulose or simply allulose is a low-calorie isomer of the monosaccharide fructose. It is utilized by several prominent food and beverage companies as a low-calorie sweetener. Initially discovered in wheat during the 1940s, allulose occurs naturally in small amounts in specific food items.

Trehalulose is a disaccharide consisting of one fructose molecule linked to one glucose molecule. It serves as a structural isomer of sucrose and is found in minor amounts in honey. This compound constitutes 50% of the sugars found in the honeydew produced by silverleaf whiteflies and is synthesized from sucrose by certain bacteria, including Protaminombacter rubrum.

Allose is classified as an aldohexose sugar and found in the form of a 6-O-cinnamyl glycoside within the leaves of the African shrub Protea rubropilosa. Additionally, this sugar is present in extracts from the freshwater alga Ochromas malhamensis, although its absolute configuration remains unidentified. Furthermore, it is identified as a C-3 epimer of glucose.

Rare sugars are monosaccharides with a limited availability in the nature. Therefore, the research was focussed on industrial synthesis of the rare sugars using various chemical, enzymatic and microbial processes and thus reduces their production costs and availability of these molecules thereby making research on these molecules more accessible. Thereafter, a number of studies on the medical/clinical applications of rare sugars carried out by various studies and consequently, raresugars are emerged as potential candidates to replace conventional sugars in human nutrition due to their beneficial health effects. Further, the use of rare sugars in agriculture was also reported recently. Above mentioned rare sugars do not have equivalent sweetness like sucrose. For example, D-tagatose is 8% less sweet than sucrose but contains only 1.5 kcal/g [Torrico, D.D.; Tam, J.; Fuentes, S.; Viejo, C.G.; Dunshea, F.R. D-tagatose as a sucrose substitute and its effect on the physicochemical properties and acceptability of strawberry-flavored yogurt. Foods 2019, 8, 256].

US20080182752A1 discloses an agricultural composition comprising a rare sugar selected from the group consisting of D-psicose, a mixture of D-psicose and D-fructose, D-allose, D-altrose and L-galactose as an active ingredient, which has an effect of inducing systemic acquired resistance in a plant.

US9717267B2 discloses a low-calorie carbonated soft drink beverage having sugar-sweetened characteristics comprising D-psicose and Rebaudioside A, as sweetness enhancer. Sweet proteins have the potential to replace these artificial sweeteners, by acting as natural, good, low calorie sweeteners, as we know that proteins do not trigger a demand for insulin in these patients whereas sucrose does.

So far there are seven known sweet and taste-modifying proteins, namely Brazzein, Thaumatin, Monelin, Curculin, Mabinlin, Miraculin and Pentadin. All of these sweet proteins have been isolated from plants that grow in tropical rainforests.

Brazzein is first disclosed in WO1994019467A1 as a protein sweetener that has been isolated from Pentadiplandra brazzeana Baillon. This publication further dscloses that the Brazzein is thermostable, lysine rich and has a relative long-lasting taste. WO2008112475A2 and PCT/KR2009/004855 discloses variants of Brazzein, and nucleotide sequences capable of expressing them.

WO2010030999A1 discloses codon-optimized brazzein coding sequences and the expression of brazzein and variants thereof using yeast expression systems. This publication further discloses methods of expression of proteins to enhance the sweetness taste profile of foods and/or beverages.

WO2018056746A1 discloses a brazzein multiplex variant having antiallergic and antioxidative activities and, more specifically, to a brazzein multiplex variant capable of simultaneously exhibiting both an antioxidative effect through GST and tyrosinase activities and an antiallergic effect through an inhibitory activity against the release of β-hexosaminidase, which is a degranulation-indicating substance, released with histamine. Accordingly, the brazzein multiplex variant according to the present invention can be usefully employed in a pharmaceutical composition for allergic disease prevention or treatment, an antiallergic cosmetic composition, an antioxidative composition, an antioxidative cosmetic composition, and a health functional food.

The above literature shows that there is a continued commercial interest in rare sugars and sweet proteins such as brazzein. However, since the early days of brazzein discovery and rare sugars in the late 1980s, there appears no industrial solution for the provision of successful compositions of rare sugars with brazzein or any technical solution published with the potential to meet the market needs.

In the light of the foregoing, there remains a need in the art to improve the sweetness index of the naturally available rare sugars by employing sweet proteins such as Brazzein to meet the growing health care market needs.

Summary of the invention:

In line with the above, the present invention relates to a formulation comprising sweet protein with carbohydrate and/ or non-carbohydrates for improving the sweetness without changing the texture and flavours.

In one aspect, the sweet protein is selected from the group consisting of brazzein, curculin/neoculin, egg white lysozyme, mabinlin, miraculin, monellin, pentadin, and thaumatin, and the like. . The present invention relates to a formulation comprising sweet protein such as Brazzein with rare sugars for improving the sweetness of the rare sugars with low glycemic index without changing the texture and flavours of said rare sugars

In an aspect, the sweet protein used to improve the sweetness of the rare sugars is brazzein.

In another aspect, the sweet protein Brazzein can also be used in formulation containing rare sugars and/or sugar substitutes and/ or non-nutritive sweeteners such as and not limited to aspartame, acesulfame-K, Cyclamate, Neotame, Saccharin, Sucralose, Stevia etc.,

In one aspect, the invention describes the formulation to include the sweet protein either in native or modified form, wherein the modifications include functional and non-functional changes in sweet protein. The sweet protein in native form also includes the major form of brazzein

One aspect of the invention describes that the sweet proteins such as Brazzein and Thauamtin exist in 2 forms, as major and minor form, which differ by one or few aminoacids in N-terminal region. For example, major form of Brazzein starts with QDKC sequence, whereas the monor form starts as DKC sequence. The minor form is twice the sweetness when compared to the major form. Similar to Brazzein, thaumatin also exists as major and minor form as Thaumatin I and II sequence. Both of them have same number of amino acids, but differ by amino acid changes at 4 positions at 68^{th}, 85^{th}, 89^{th} and 98^{th} positions.

One embodiment of the invention describes the importance of major form and minor form of protein, for increased or decreased/ reduced sweetness. For example, the minor form of Brazzein (53 amino acid protein) having a more sweetness give lingering sweetness taste. In applications, where the lingering taste is not required and less sweetness is preferred, the major form of Brazzein (54 amino acid protein) with 2x reduced sweetness will be the preferable sweet protein

In yet another aspect, the invention focuses on the sweet protein in formulation, wherein the said sweet protein also can include their variants with amino acid modifications to enhance the binding capacity to taste recepotors.

Another major aspect describes the functional modifications in sweet protein include but not limited to modifications involving amino acid changes pertaining to (i) enhanced thermostability, (ii) enhanced pH stability, (iii) Differential binding capacity (increased or decreased) with taste receptors. Modifications of the amino acids in sweet protein , which are not involved in sweetness, can impact on generation of variants with amino acid alterations for increased stability. Bioinformatic studies and docking studies suggested point mutations in amino acid sequence of Brazzein, which improves the effective binding of Brazzein with tongue recepotors. For example, Brazzein protein with amino acid changes at D29 position enhances the binding capacity and sweetness feeling of Brazzein, wheras modificaions at K6 and E36 positions reduces the binding capacity and hence sweetness feeling

One major aspect of the invention describes the non-functional modifications in sweet protein includes but not limited to the modifications in aminoacids pertaining to the stability of sweet protein in monomeric form and/ or multimeric forms, suitable for the liquid formulations and in solid/ crystalline formulations, respectively.

In another aspect, invention discloses the stable variants of sweet protein including the monomeric form and the multimeric form are achieved by modifications in the aminoacid(s) involved in hydrogen bonding between the monomeric peptides. The amino acid modifications in the sweet protein, in particular to Brazzein, involves modifications of amino acids such as and not limited to Gln 17, Ala 19, Asn 20, Cys 37, Try 24, Glu 36 and combinations thereof.

In one another aspect, the invention describes a formulation comprising non-carbohydrate, such as and not limited to polyphenols, phenolicac acids, tannin, and one or more saccharides including polysaccharides/ oligosaccharides/ trisaccharides with raresugars, wherein sweetness is enhanced/ compensated by inclusion of brazzein.

In one major aspect, the invention describes the formulation to comprise rare sugars which include monosaccharides such as allulose, tagatose, sorbose, etc., disaccharides such as isomaltulose, trehalulose, turanose, leucrose, etc..

In another major embodiment, the invention details the trisaccharides in the formulation includes but not limited to melezitose, erlose, esculose, theanderose, and the like.

In an aspect, Brazzein is used at a concentration ranging between 10µg to 100mg per 100g of the rare sugar.

In an aspect, appropriate amounts of Brazzein can be used as the sole sweetener or added to rare sugars and/or sugar substitutes and/ or non-nutritive sweeteners depending on the practical application of the formulation, wherein the purpose of Brazzein is for enhancement of sweetness and/ or compensation for bitterness.

The rare sugars are selected from the group including but not limited to D-Allulose, D-Tagatose, D-Allose, Isomaltulose, Trehalulose, Leucrose, Turanose and the like.

In another aspect, the present invention provides a method for improving the sweetness of the rare sugars without changing the texure and flavours of the rare sugars comprising adding brazzein to the rare sugar at pre caramalization stage or post caramalization stage to increase the sweetness.

More particlularly, the present invention discloses a method for improving the sweetness of the rare sugars without changing the sweetness profile, texture and flavours of the primary sugars by adding Brazzein at pre carmalization stage or post caramalization stage of rare sugars.

According to the present invention, addition of Brazzein at pre Caramelization stage or post Caramelization stage of the synthesis of the rare sugars increases the sweetness of Rare sugars but not limited to D-Allulose, D-tagatose, D-Allose, Isomaltulose, Trehalulose etc.

In another aspect, the formulation of the present invention can be applied to food applications such as and not limited to addition in beverages, in chocolates, in cookies, and the like. The processing of the liquid to obtain solid form of sugar and protein together can be achived by the methods selected from methods such as and not limited to co-crystallization, lyophilization, spray drying, etc.

In one aspect, the invention describes the advantages of sweet protein formulation with carbohydrates, as

1. The present process improves the sweetness of the carbohydrates, especially the rare sugars with low glycemic index without changing the texture and flavours of said rare sugars.

2. Sweet protein such as Brazzein can be mixed with rare sugars like, D-Allulose, Isomaltulose, Turanose, Trehalulose etc. which have low Glycemic index (GI) and low caloric value.
3. Addition of Brazzein to the rare sugars enhances the sweetness index of rare sugar more than sucrose.

One major aspect of the invention, defines the formulation to contain sweet protein at a concentration ranging between 10µg to 100 mg per 100g of the carbohydrate.

Certain aspect of the invention includes a list of carbohydrates in formulation with sweet protein, wherein carbohydrates include monosaccharide, disaccharides, trisaccharides, oligosaccharides, polysaccharides, sugar alcohols, and their derivatives, including isomer/ epimers

(i) monosaccharides and their derivatives including allulose, tagatose, sorbose, allose, gulose, galactose, mannose, fucose, rhamnose, xylose, xylulose, etc.

(ii) isaccharides and their derivatives including isomaltulose, trehalulose, turanose, leucrose, maltose, trehalose, etc.

(iii) trisaccharides including melezitose, erlose, esculose, theanderose, raffinose, fucosyl lactose, sialyl lactose, etc.

(iv) oligosaccharides including malto oligosaccharides, fructo oligosaccharides, isomalto oligosaccharides, galacto oligosaccharides, lacto oligosaccharides, etc.

(v) polysaccharides including glucan, dextran, maltodextrin, pullulan, starch, etc.

(vi) sugar alcohols including but not limited to sorbitol, mannitol, xylitol, galactitol, arabitol, etc.

In certain other aspect, the invention includes a list of non-carbohydrates in formulation with sweet protein, wherein non-carbohydrates include include phenolic compounds, cinnamic acids, methylxanthines, trogonelline, catechins, stilbenes, nitrogen containing compounds like amino acids both synthetic and natural, proteins and thickener compounds, lipids and fatty acids, alcohols.

(i) phenolic compounds include phenolic acids and derivatives such as raspberry ketone, tyrosol, hydroxy tyrosol, salicylic acid, gallic acid, naringenin, tannic acid, ellagic acid, ellagitannins, gallotannins, ellagitannins, anthocyanins, the anthocyanidins, flavones, flavans, flavanols, their salts and cocrystals

(ii) cinnamic acids and derivates including cinnamic acid, p-coumaric acid , chlorogenic acids, rosmarinic acid, caffeic acid, ferulic acid, sinapic acid, cinnamaldehyde, their salts and cocrystals

(iii) methylxanthines and derivates including caffeine, theacrine, methylliberine, theobromine, theophylline, their salts and cocrystals

(iv) catechins and derivates including but not limited to catechin, quercetin, luteolin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG), their salts and cocrystals

(v) stilbenoids include pterostilbene, resveratrol, piceatannol, combretastatin their salts and cocrystals

(vi) proteins and peptides including microbial proteins/peptides such as peptone, single cell protein; plant proteins/ peptides such as soy products, legumes, pulses, grains, nuts, seeds, pezadeftide, defensins, thionins, cyclotides; animal proteins/ peptides including milk proteins such as lactalbumin, lactoglobulin, lactoferrin, whey protein, casein, egg protein like ovalbumin, egg lipo proteins like phosvitin, livetin, porcine, soy protein, leghemoglobin, and the like.Protein/peptides obtained from Fish, shrimp, sponges, oysters, insects, meat

(vii) aminoacids including natural and unnatural aminoacids such as and not limited to Glycine, Phenylalanine, Valine, Tryptophan, Threonine, Isoleucine, Methionine, Histidine, Leucine, Serine, Proline, Glutamine, Glutamic acid, para-nitro-L-phenylalanine (pN-Phe), hydroxyproline (Hyp), beta-alanine, citrulline (Cit), ornithine (Orn), norleucine (Nle), 3-nitrotyrosine, nitroarginine, pyroglutamic acid (Pyr), Amino Benzoic acid and derivatives, α-hydroxy/ α-thio aminoacids, N-formyl -L-α-

aminoacids like N-formyl methionine, N-formyl phenylalaine, piperazinic acid (α-hydrazino acid).

(viii) thickening agents including but not limited to glycerol, agar-agar, sodium alginate, glycerol monostereate, gums such as xanthan, gum arabic, starch, modified starch, gelatin, polyols such as sorbitol, mannitol, xylitol, erythritol, and the like.

(ix) Lipids including cationic, anionic and neutral lipids, mono/di/tri glycerides.

(x) Fatty acids including short chain, medium chain and long chain fatty acids, esters of fatty acids

(xi) Alcohols including ethanol, edible alcohol.

One major aspect describes the use of sweet protein with carbohydrates and non-carbohydrates for improving sweetness or altering sweetness in various food application and pharma application such as not limited to usage in beverages, chocolates, cookies, ice cream, pharmaceutical excipients, including syrups, gels, and the like

Detailed description of the invention:

The invention will now be described in detail in connection with certain preferred and optional embodiments, so that various aspects thereof may be more fully understood and appreciated.

The term "rare sugar" or "primary sugar" used herein interchangeably relates and means the rare sugars D-Allulose, D-tagatose, D-Allose, Isomaltulose, Trehalulose, Turanose, Leucrose, Sorbose used in the present invention.

In an embodiment, the present invention provides a formulation comprising sweet protein with carbohydrate and/ or non-carbohydrates selected from rare sugars and/or sugar substitutes and/ or non-nutritive sweeteners for improving the sweetness without changing the texture and flavours.

In another embodiment, the sweet protein is selected from the group consisting of brazzein, curculin/neoculin, egg white lysozyme, mabinlin, miraculin, monellin, pentadin, thaumatin, and the like..

In one embodiment, the sweet protein used to improve the sweetness of the rare sugars is brazzein.

In a preferred embodiment, the present invention relates to a formulation comprising Brazzein with rare sugars for improving the sweetness of the rare sugars with low glycemic index without changing the texture and flavours of said rare sugars.

In one embodiment, the invention describes the formulation to include the sweet protein either in native or modified form, wherein the modifications include functional and non-functional changes in sweet protein. The sweet protein in native form also includes the major form of brazzein

One embodiment describes that the sweet proteins such as Brazzein and Thauamtin exist in 2 forms, as major and minor form, which differ by one or few aminoacids in N-terminal region. For example, major form of Brazzein starts with QDKC sequence, whereas the monor form starts as DKC sequence. The minor form is twice the sweetness when compared to the major form. Similar to Brazzein, thaumatin also exists as major and minor form as Thaumatin I and II sequence. Both of them have same number of amino acids, but differ by amino acid changes at 4 positions at 68^{th}, 85^{th}, 89^{th} and 98^{th} positions.

One embodiment of the invention describes the importance of major form and minor form of protein, for increased or decreased/ reduced sweetness. For example, the minor form of Brazzein (53 amino acid protein) having a more sweetness give lingering sweetness taste. In applications, where the lingering taste is not required and less sweetness is preferred, the major form of Brazzein (54 amino acid protein) with 2x reduced sweetness will be the preferable sweet protein

In yet another emboniment, the invention focuses on the sweet pritein in formulation, wherein the said sweet proteinalso can include their variants with amino acid modifications to enhance the binding capacity to taste recepotors.

Another major embodiment describes the functional modifications in sweet protein include but not limited to modifications involving amino acid changes pertaining to (i) enhanced thermostability, (ii) enhanced pH stability, (iii) Differential binding capacity (increased or decreased) with taste receptors. Modifications of the amino acids in sweet protein , which are not involved in sweetness, can impact on generation of variants with amino acid alterations for increased stability. Bioinformatic studies and docking studies suggested point mutationsi in amino acid sequence of Brazzein, which improves the effective binding of Brazzein with tongue recepotors. For example, Brazzein protein with amino acid changes at D29 position enhances the binding capacity and sweetness feeling of Brazzein, wheras modificaions at K6 and E36 positions reduces the binding capacity and hence sweetness feeling

One major embodiment of the invention describes the non-functional modifications in sweet protein includes but not limited to the modifications in aminoacids pertaining to the stability of sweet protein in monomeric form and/ or multimeric forms, suitable for the liquid formulations and in solid/ crystalline formulations, respectively.

In another embodiment, invention discloses the stable variants of sweet protein including the monomeric form and the multimeric form are achieved by modifications in the aminoacid(s) involved in hydrogen bonding between the monomeric peptides. The amino acid modifications in the sweet protein, in particular to Brazzein, involves modifications of amino acids such as and not limited to Gln 17, Ala 19, Asn 20, Cys 37, Try 24, Glu 36 and combinations thereof.

In one major embodiment, the invention describes the impact of sweet protein in formulation with sugar, whrein the brazzein addition reduced the level of addition

of sugar to an extent. As brazzein compensates for the sweetness, sugar level can be reduced to certain extent without altering the sweetness and calorie of the formulation

In one another embodiment, the invention describes a formulation comprising non-carbohydrate, such as and not limited to polyphenols, phenolicac acids, tannin, and one or more saccharides including polysaccharides/ oligosaccharides/ trisaccharides with raresugars, wherein sweetness is enhanced/ compensated by inclusion of brazzein.

In one major embodiment, the invention describes the formulation to comprise rare sugars which include monosaccharides such as allulose, tagatose, sorbose, etc., disaccharides such as isomaltulose, trehalulose, turanose, leucrose, etc..

In another major embodiment, the invention details the trisaccharides in the formulation includes but not limited to melezitose, erlose, esculose, theanderose, and the like.

In an embodiment, Brazzein is used at a concentration ranging between 10µg to 100 mg per 100g of the rare sugar.

In an embodiment, appropriate amounts of Brazzein can be used as the sole sweetener or added to rare sugars and/or sugar substitutes and/ or non-nutritive sweeteners depending on the practical application of the formulation, wherein the purpose of Brazzein is for enhancement of sweetness and/ or compensation for bitterness.

The rare sugars are selected from the group including but not limited to D-Allulose, D-Tagatose, D-Allose, D-Sorbose, Isomaltulose, Trehalulose, Turanose, Leucrose and the like.

In another embodiment, the sweet protein Brazzein can also be used in formulation containing rare sugars and/or sugar substitutes and/ or non-nutritive sweeteners such as and not limited to aspartame, acesulfame-K, Cyclamate, Neotame, Saccharin, Sucralose, Stevia etc.,

In another embodiment, the present invention provides a method for improving the sweetness of the rare sugars without changing the texure and flavours of the rare sugars comprising adding brazzein to the rare sugar at pre caramalization stage or post caramalization stage.

In another embodiment, the sweet protein used in the method is selected from the group conistsing of brazzein, curculin/neoculin, egg white lysozyme, mabinlin, miraculin, monellin, pentadin, thaumatin, and the like.

In another embodiment, the said primary sugars are the raresugars which are also altenate diet sugars such as and not limited to D-Allulose, D-Tagatose, D-Allose, Isomaltulose, Trehalulose, Sorbose, Turanose, Leucrose and the like.

In another embosiment, the process includes processes of sweet protein addition to the primary sugars suchas and no limited to addition at pre carmalization stage or post caramalization stage of the synthesis of the rare sugars at different tempertures to increase the sweetness.

More particlularly, the present invention discloses a method for improving the sweetness of the rare sugars without changing the flavours of the primary sugars by adding Brazzein at pre caramalization stage or post caramalization stage of the synthesis of the rare sugars at different tempertures to increase the sweetness.

According to the present invention, addition of Brazzein at pre carmalization stage or post caramalization stage of the synthesis of the rare sugars increases the sweetness of Rare sugars but not limited to D-Allulose, D-tagatose, D-Allose, Isomaltulose, Trehalulose etc.

According to one embodiment, to increase the sweetness of the rare sugars, alternative naturally available sweetner, having more sweetness index than sucrose, like sweet protein selected from the group conistsing of brazzein, curculin/neoculin, egg white lysozyme, mabinlin, miraculin, monellin, pentadin, and thaumatin is formulated with rare sugar by in house developed method.

Compared to other sweet proteins, brazzein is attractive as a candidate sweetener as the same has other health benefits also such as controlling obesity, oral health, and diabetic management, in addition to its potential sweetness, sugar-like taste. Brazzein was isolated in two forms from the fruit of the West African *Pentadiplandra brazzeana Baillon* plant. The major form contains a pyroglutamate at its N-terminus, while the minor form lacks this N-terminal pyroglutamate. The major form has an intrinsic sweetness 500 times sweeter than sucrose in weight-weight comparisons (9500 times higher on a per-molecule basis).

The minor form Brazzein is about 5,890 Daltons in size and is about 2,000 times sweeter in 2% solution than a 2% sucrose solution. Thermostability tests according to the invention indicate that this protein has desired heat stability characteristics (thus making it much more suitable for use in connection with formulating or baking). In this regard, the protein still tasted very sweet after two hours of incubation at 98°C.

Thus, Brazzein is especially suitable to being mixed with other known rare sugars like, D-Allulose, Isomaltulose, Turanose, Trehalulose etc. which has low Glycemic index (GI) and low caloric value and thus introduction of Brazzein with these rare sugars will enhance the sweetness index of rare sugar more than sucrose.

In another embodiment, the formulation of the present invention can be applied to food applications such as and not limited to addition in beverages, in chocolates, in cookies, and the like.

The processing of the liquid to obtain solid form of sugar and protein together can be achived by the methods selected from methods such as and not limited to co-crystallization, lyophilization, spray drying, etc.

The present invention has the following advantages:

1. The present process improves the sweetness of the rare sugars with low glycemic index without changing the texture and flavours of said rare sugars.
2. Brazzein can be mixed with rare sugars like, D-Allulose, Isomaltulose, Turanose, Trehalulose etc. which have low Glycemic index (GI) and low caloric value.
3. Addition of Brazzein to the rare sugars enhances the sweetness index of rare sugar more than sucrose.

Certain embodiment of the invention includes a list of carbohydrates in formulation with sweet protein, wherein carbohydrates include monosaccharide, disaccharides, trisaccharides, oligosaccharides, polysaccharides, sugar alcohols, and their derivatives, including isomer/ epimers

(i) monosaccharides and their derivatives including allulose, tagatose, sorbose, allose, gulose, galactose, mannose, fucose, rhamnose, xylose, xylulose, etc.

(ii) disaccharides and their derivatives including isomaltulose, trehalulose, turanose, leucrose, maltose, trehalose, etc.

(iii) trisaccharides including melezitose, erlose, esculose, theanderose, raffinose, fucosyl lactose, sialyl lactose, etc.

(iv) oligosaccharides including malto oligosaccharides, fructo oligosaccharides, isomalto oligosaccharides, galacto oligosaccharides, lacto oligosaccharides, etc.

(v) polysaccharides including glucan, dextran, maltodextrin, pullulan, starch, etc.

(vi) sugar alcohols including but not limited to sorbitol, mannitol, xylitol, galactitol, arabitol, etc.

Certain other embodiment of the invention includes a list of non-carbohydrates in formulation with sweet protein, wherein non-carbohydrates include include phenolic compounds, cinnamic acids, methylxanthines, trogonelline, catechins, stilbenes, nitrogen containing compounds like amino acids both synthetic and natural, proteins and thickener compounds, lipids and fatty acids, alcohols.

(i) phenolic compounds include phenolic acids and derivatives such as raspberry ketone, tyrosol, hydroxy tyrosol, salicylic acid, gallic acid, naringenin, tannic acid, ellagic acid, ellagitannins, gallotannins, ellagitannins, anthocyanins, the anthocyanidins, flavones, flavans, flavanols, their salts and cocrystals

(ii) cinnamic acids and derivates including cinnamic acid, p-coumaric acid , chlorogenic acids, rosmarinic acid, caffeic acid, ferulic acid, sinapic acid, cinnamaldehyde, their salts and cocrystals

(iii) methylxanthines and derivates including caffeine, theacrine, methylliberine, theobromine, theophylline, their salts and cocrystals

(iv) catechins and derivates including but not limited to catechin, quercetin, luteolin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG), their salts and cocrystals

(v) stilbenoids include pterostilbene, resveratrol, piceatannol, combretastatin their salts and cocrystals

(vi) proteins and peptides including microbial proteins/peptides such as peptone, single cell protein; plant proteins/ peptides such as soy products, legumes, pulses, grains, nuts, seeds, pezadeftide, defensins, thionins, cyclotides; animal proteins/ peptides including milk proteins such as lactalbumin, lactoglobulin, lactoferrin, whey protein, casein, egg protein like ovalbumin, egg lipo proteins like phosvitin, livetin, porcine, soy protein, leghemoglobin, and the lik; Protein/peptides obtained from Fish, shrimp, sponges, oysters, insects, meat

(vii) aminoacids including natural and unnatural aminoacids such as and not limited to Glycine, Phenylalanine, Valine, Tryptophan, Threonine, Isoleucine, Methionine, Histidine, Leucine, Serine, Proline, Glutamine, Glutamic acid, para-nitro-L-phenylalanine (pN-Phe), hydroxyproline (Hyp), beta-alanine, citrulline (Cit), ornithine (Orn), norleucine (Nle), 3-nitrotyrosine, nitroarginine, pyroglutamic acid (Pyr), Amino Benzoic acid and derivatives, α-hydroxy/ α-thio aminoacids, N-formyl -L-α-aminoacids like N-formyl methionine, N-formyl phenylalaine, piperazinic acid (α-hydrazino acid).

(viii) thickening agents including but not limited to glycerol, agar-agar, sodium alginate, glycerol monostereate, gums such as xanthan, gum arabic, starch, modified starch, gelatin, polyols such as sorbitol, mannitol, xylitol, erythritol, and the like.

(ix) Lipids including cationic, anionic and neutral lipids, mono/di/tri glycerides.

(x) Fatty acids including short chain, medium chain and long chain fatty acids, esters of fatty acids

(xi) Alcohols including ethanol, edible alcohol.

(xii) Vitamins including Vitamin A (carotenoid, retinol), vitamin C (Ascorbic acid), vitamin D, Vitamin K, vitamin B and its isoforms/ derivatives like thiamine, thiamine pyrophosphate, riboflavin, niacin, pantothenic acid, biotin, folate, vitamin B6 (pyridoxine), vitamin B12 (cobalamine), P5P, Methylcobalamin, Hydroxycobalamine, THF, Coenzyme, etc.)

(xiii) Mineral ions including Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Potassium (K^{+}), Sodium (Na^{+}) and Chloride (Cl^{-}).

One major embiment describes the the se of sweet protein with carbohydrates and no- for improving sweetness or altering sweetness in various food application and pharma application such as not limited to usage in beverages, chocolates, cookies, ice cream, pharmaceutical excipients, including syrups, gels, and the like.

The invention can be better understood by the following non-limiting examples. The examples given are mere an illustration of the instant invention and should not be construed as limiting the scope of the present invention in any manner.

EXAMPLES

Example 1: Assessment of sweetness of Allulose sugar solution by addition of Brazzein

In the natural form Allulose has 70% sweetness as compared to sucrose.
The sweetness lacking in Allulose solution was compensated by adding Brazzein to the the Allulose.

Brazzein preparations of different concentrations (5 to 25 mg) were added to 100g Allulose solution. Sweetness was assessed by tasting analysis and was recorded on a sweetness scale of 0 – 10.

Note:
On a sweetness scale of 0-10: where 0 represents no sweetness and 10 represent the highest level of sweetness.

S.No.	Allulose	Brazzein	Sweetness
1	100 g	0 mg	+++
2	100 g	5 mg	+++
3	100 g	10 mg	++++
4	100 g	20 mg	+++++
5	100 g	25 mg	++++++

Results: Addition of 5 to 25 mg Brazzein to 100g Allulose compensated for the lacking sweetness, and enhanced the sweetness.

Conclusion:

It can be concluded that the addition of brazzein to Allulose resulted in significant increase in sweetness.

Example 2: Assessment of Sweetness of Tagatose sugar solution by addition of Brazzein

In the natural form Tagatose has 90% sweetness as compared to sucrose.
The sweetness lacking in Tagatose solution was compensated by adding brazzein to the Tagatose solution before caramalisation.

Brazzein preparations of different concentrations (2 to 20 mg) were added to 100g Tagatose solution. Sweetness was assessed by tasting analysis and was recorded on a sweetness scale of 0 – 10.

Note:
On a sweetness scale of 0-10: where 0 represents no sweetness and 10 represent the highest level of sweetness.

S.No.	Tagatose	Brazzein	Sweetness
1	100 g	0 mg	+++
2	100 g	2 mg	+++
3	100 g	10 mg	++++
4	100 g	20 mg	+++++
5	100 g	20 mg	++++++

Addition of 2 to 20 mg Brazzein to 100g Tagatose compensated for the lacking sweetness, and enhanced the sweetness.

Conclusion:
It can be concluded that the addition of brazzein to Tagatose resulted in significant increase in sweetness.

Example 3: Reduction in sugar addition due to brazzein formulations without altering sweetness.

Brazzein formulations with sugars such as sucrose as example is given here. As Brazzein sweetness is determined based on comparison with sweetness of 10% sucrose solution, here we have assessed the possibility of reducing the sucrose content in formulations and compensating the sweetness by addition of required quantity of Brazzein.

S.No.	Sucrose	Brazzein	Sweetness
1	100 g	0 mg	++++++
2	90 g	5 mg	++++++
3	80 g	10 mg	++++++
4	70 g	15 mg	++++++
5	60 g	20 mg	++++++
6	50 g	25 mg	++++++

Addition of Brazzein to Tagatose in appropriate amount to compensate for the sweetness, based on 2000 times sweetness index of brazzein over 10% sucrose. To compensate for the loos of 10g sucrose and S.No.2 sample, 5mg Brazzein was added (5mg of brazzein must be equivalent to 10g sucrose in sweetness). Similarly other ratios of sucrose: brazzein also resulted in same sweetness.

Conclusion:

It can be concluded that the addition of brazzein in appropriate amounts to compensate the sweetness of sucrose formulations, helps in reduced addition of sucrose but retaining the same sweetness.

Although the invention has been described in detail in the foregoing for the purpose of illustration, it is to be understood that such detail is solely for that purpose and that variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention except as it may be limited by the claims.

WE CLAIM,

1. A formulation comprising sweet protein and/or its variants in monomeric or multimeric forms, with carbohydrate and/ or non-carbohydrates for improving the sweetness without changing the texture and flavours.
2. The formulation as claimed in Claim 1, wherein the sweet protein includes both proteins and peptides including brazzein, curculin/neoculin, egg white lysozyme, mabinlin, miraculin, monellin, pentadin, thaumatin, and the like.
3. The formulation as claimed in Claim 2, includes the sweet protein either in native or modified form, wherein the modifications results in functional and non-functional changes.
4. As claimed in claim 3, the sweet protein in native form also includes the major form of brazzein.
5. As claimed in claim 3, functional modifications includes amino acid changes pertaining to (i) enhanced thermostability, (ii) enhanced pH stability, (iii) differential binding capacity (increased or decreased) with taste receptors.
6. As claimed in claim 3, the non-functional modifications includes the modifications in the aminoacid sequence pertaining to the stability of sweet protein in monomeric form and/ or multimeric forms, suitable for the liquid and solid formulations.
7. As claimed in claim 6, the stable variants of sweet protein including the monomeric form and the multimeric form are achieved by modifications in the aminoacid(s) involved in hydrogen bonding between the monomeric peptides.
8. As claimed in claim 3, the functional modifications are also included in the said monomeric and/ or multimeric forms of the sweet protein
9. As claimed in claim 7, the amino acid modifications in the sweet protein, in particular to Brazzein, involves modifications of amino acids such as and not limited to Gln 17, Ala 19, Asn 20, Cys 37, Try 24, Glu 36 and combinations thereof.

10. The formulation as claimed in Claim 1, wherein the sweet protein is used at a concentration ranging between 10µg to 100 mg per 100g of the carbohydrate.

11. The formulation as claimed in Claim 1, wherein the said carbohydrates include monosaccharide, disaccharides, trisaccharides, oligosaccharides, polysaccharides, sugar alcohols, and their derivatives, including isomer/ epimers

    (i) monosaccharides and their derivatives including allulose, tagatose, sorbose, allose, gulose, galactose, mannose, fucose, rhamnose, xylose, xylulose, etc.

    (ii) isaccharides and their derivatives including isomaltulose, trehalulose, turanose, leucrose, maltose, trehalose, etc.

    (iii) trisaccharides including melezitose, erlose, esculose, theanderose, raffinose, fucosyl lactose, sialyl lactose, etc.

    (iv) oligosaccharides including malto oligosaccharides, fructo oligosaccharides, isomalto oligosaccharides, galacto oligosaccharides, lacto oligosaccharides, etc.

    (v) polysaccharides including glucan, dextran, maltodextrin, pullulan, starch, etc.

    (vi) sugar alcohols including but not limited to sorbitol, mannitol, xylitol, galactitol, arabitol, etc.

12. A method for improving the sweetness of the rare sugars by addition of sweet proteins pre or post Caramelization stage, wherein the said raresugars includes but not limited to allulose, tagatose, allose, sorbose, gulose, idose, isomaltulose, trehalulose, turanose, leucrose and the like.

13. The formulation as claimed in claim 1, wherein the said non-carbohydrates include phenolic compounds, cinnamic acids, methylxanthines, trigonelline, catechins, stilbenes, nitrogen containing compounds like amino acids both synthetic and natural, proteins and thickener compounds, lipids and fatty acids, alcohols, vitamins, mineral ions.

(i) phenolic compounds including phenolic acids and derivatives such as raspberry ketone, tyrosol, hydroxy tyrosol, salicylic acid, gallic acid, naringenin, tannic acid, ellagic acid, ellagitannins, gallotannins, ellagitannins, anthocyanins, anthocyanidins, flavones, flavans, flavanols, their salts and cocrystals

(ii) cinnamic acids and derivates including cinnamic acid, p-coumaric acid , chlorogenic acids, rosmarinic acid, caffeic acid, ferulic acid, sinapic acid, cinnamaldehyde, their salts and cocrystals

(iii) methylxanthines and derivates including caffeine, theacrine, methylliberine, theobromine, theophylline, their salts and cocrystals

(iv) catechins and derivates including but not limited to catechin, quercetin, luteolin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG), their salts and cocrystals

(v) stilbenoids including pterostilbene, resveratrol, piceatannol, combretastatin their salts and cocrystals

(vi) proteins and peptides including microbial proteins/peptides such as peptone, single cell protein; plant proteins/ peptides such as soy products, legumes, pulses, grains, nuts, seeds, pezadeftide, defensins, thionins, cyclotides; animal proteins/ peptides including milk proteins such as lactalbumin, lactoglobulin, lactoferrin, whey protein, casein, egg protein like ovalbumin, egg lipo proteins like phosvitin, livetin, porcine, soy protein, leghemoglobin, and the like.Protein/peptides obtained from Fish, shrimp, sponges, oysters, insects, meat

(vii) aminoacids including natural and unnatural aminoacids such as and not limited to Glycine, Phenylalanine, Valine, Tryptophan, Threonine, Isoleucine, Methionine, Histidine, Leucine, Serine, Proline, Glutamine, Glutamic acid, para-nitro-L-phenylalanine (pN-Phe), hydroxyproline (Hyp), beta-alanine, citrulline (Cit), ornithine (Orn), norleucine (Nle), 3-nitrotyrosine, nitroarginine, pyroglutamic acid (Pyr), Amino Benzoic acid and derivatives, α-hydroxy/ α-thio aminoacids, N-formyl -L-α-

aminoacids like N-formyl methionine, N-formyl phenylalaine, piperazinic acid (α-hydrazino acid).

(viii) thickening agents including but not limited to glycerol, agar-agar, sodium alginate, glycerol monostereate, gums such as xanthan, gum arabic, starch, modified starch, gelatin, polyols such as sorbitol, mannitol, xylitol, erythritol, and the like.

(ix) Lipids including cationic, anionic and neutral lipids, mono/di/tri glycerides.

(x) Fatty acids including short chain, medium chain and long chain fatty acids, esters of fatty acids

(xi) Alcohols including ethanol, edible alcohol.

(xii) Vitamins including but not limited to Vitamin A, C, D, E, K and their derivatives, vitamin B- complex and its isoforms/ derivatives like thiamine, thiamine pyrophosphate, riboflavin, niacin, pantothenic acid, biotin, folate, vitamin B6 (pyridoxine), vitamin B12 (cobalamine), P5P, Methylcobalamin, Hydroxycobalamine, THF, Coenzyme, choline etc.)

(xiii) Mineral ions including Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Potassium (K^{+}), Sodium (Na^{+}) and Chloride (Cl^{-}), Nickel, cobalt, Iodine, Manganese, Strontium, copper, Zinc, carbonates, silica, boron, sulphur.

14. The formulations as claimed in claim 1, wherein the inclusion of sweet proteins reduces the sugar addition in the formulations.

15. Use of sweet protein with rare sugars as claimed in any one of the preceding claims for improving sweetness in beverages, in chocolates, in cookies, , ice cream, pharmaceutical excipients, including syrups, gels, and the like.