International Journal of Peptide Research and Therapeutics (2022) 28:158
https://doi.org/10.1007/s10989-022-10464-4

A Review of Natural Peptide Sweeteners

Fariba Sharififar[1] · Anis Ashrafzadeh[2] · Mahboubeh Kavirimanesh Khanaman[2]

Accepted: 23 September 2022 / Published online: 31 October 2022

Abstract
The increasing prevalence of diseases caused by sugar consumption has become a threat to human health, and various studies have reported the relationship between high sugar consumption and the risk of various cardiovascular diseases, obesity, type 2 diabetes. Sugar-free products such as low-calorie sweeteners, especially peptide types, are very popular today due to the production of fewer calories. These sweeteners often have a protein structure and have a wide variety in terms of taste and dosage. Although extensive studies consider sweeteners to be safe and suitable substitutes for sugar, studies show that artificial types of these sweeteners can cause oxidative stress, metabolic syndrome, nervous system diseases, changes in the gastrointestinal microflora. Despite these conflicting studies, food safety organizations such as the FDA, FAO, EFSA limit the consumption of sweeteners to the acceptable daily intake (ADI) for all people, except for cases such as phenylketonuria. The purpose of this study is to briefly introduce natural peptide sweeteners (NPSs) that are good candidates to replace sugar and artificial sweeteners. The most important NPSs discussed in this summary include thaumatin, brazzein, monellin, curculin, miraculin, mabinlin, pentadin, whose safety, dosage and toxicity are discussed. Among the NPSs, thaumatin has been approved by FDA. This protein offers sweetness about 2000 times more than sucrose while produces only 4 kcal/g. NPSs generally show fewer side effects than synthetic types. The use of other NPS is also currently legal as a flavor enhancer and sugar substitute, but there are still challenges to their approval by the FDA.

Keywords Natural peptide sweeteners · Protein structure · Low calories sweeteners · Diseases caused by sugar

Introduction

One characteristic of the human species is preference for sweet taste at a range of intensities, so that even, the newborn infant is able to respond favorably to sweetened solutions because the taste buds are developed by the 16th week of gestation. It is acknowledged that extra sugar (a natural sweetener that provides 4 cal/g) increases energy intake in ingestion, which can lead to weight gain and chronic diseases associated with weight problems and enamel decay. Given that sugar consumption is essential, the need for sugar substitutes that can help reduce calorie intake, especially in obese people, is inevitable (Dafaalla et al. 2020; Piekara et al. 2020) and the call for brand new opportunity "low calorie" sweeteners for dietetic and diabetic functions has been expanded worldwide (Kim and Kinghorn 2002).

Sweetness is usually measured relative to the reference sugar sucrose. Biologically, the perception of sweetness takes place via the receptors T1R1 and T1R2 (taste receptor types 1 and 2) that form a part of C class of proteins coupled to G proteins (Jiao and Wang 2018).

Nonnutritive sweeteners (NNSs) are described as sweetening factors which have a upper sweetening potency and lower calorie content per weight compared with caloric or nutritive sweeteners for example sucrose or corn syrup (Chattopadhyay et al. 2014). NNSs may be of artificial or herbal origin, the latter being more and more consumed. Low-calorie sweeteners (LCSs), along with polyols or sugar alcohols and different new sugars, are low digestible carbohydrates derived via way of the hydrogenation in their sugar or syrup sources (Ruiz-Ojeda et al. 2019).

Now a day sugar loose foods are very famous. So, food enterprise makes use of numerous synthetic sweeteners that

✉ Anis Ashrafzadeh
ashrafzadeh.anis@gmail.com

[1] Department of Pharmacognosy, Pharmaceutical Research Center, School of Pharmacy, Institute of Neouropharmacology, Kerman University of Medical Science, Kerman, Iran

[2] Herbal and Traditional Medicines Research Center, School of Pharmacy, Kerman University of Medical Science, Kerman, Iran

are low in calorie content material rather than excessive calorie sugar. Today, the primary aim of diabetes control is to control blood glucose. So, consumers have the right for a loose sugar preference of food products. They have to select the proper foods to conform with nutritional suggestions and food enterprise can substantially contributes to this variation by presenting tailored meals products. This led food factory to find out numerous types of alternative excessive sweeteners, that have made viable to provide purchaser the sweet flavor without the calories. Sugar can't actually get replaced through those form of extreme sweetener due to the fact the question of bulk, quality, intensity of sweetness and physical characteristics (Chattopadhyay et al. 2014). Hence demand significantly improved for herbal sweetening agents, in particular for non-sacchariferous sweetening agents, due to the fact they're notably potent, useful, secure and low-calorie sugar alternatives (Kumar et al. 2021).

Ideal Properties of Sweetening Agents

Sweeteners should be effective at low concentrations, maintain their stability and strength at different temperatures, have very low or non-calorific value, be compatible with different components in the formulations, do not show batch-to-batch variations, should be readily available and inexpensive (Priya et al. 2011).

Classification of Sweetening Agents

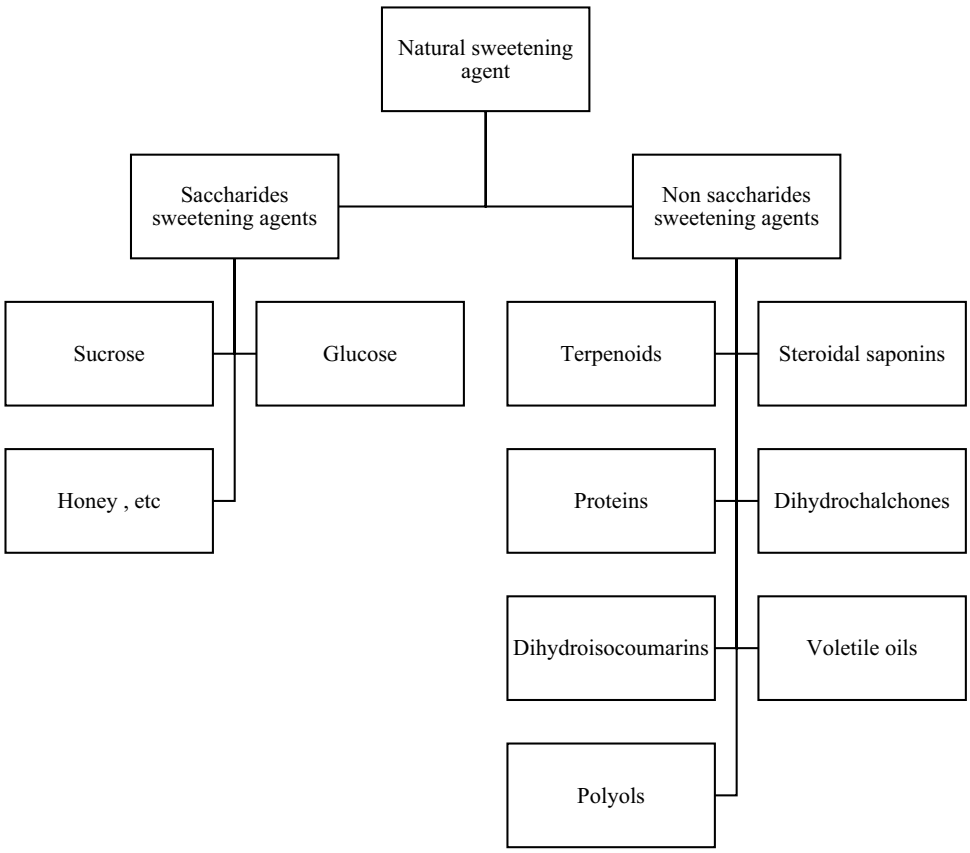
In general, sweeteners can be divided into two categories: natural sweeteners of plant origin and artificial sweeteners (Kumari et al. 2019).

Natural Sweeteners

Among the most important advantages of natural sweeteners over synthetic types, we can point out the less adverse effects of the first category on health, low caloric generation, non-toxicity, and their very sweet nature, which can overcome the problems of sucrose and artificial sweeteners and be a useful alternative to sugar in diabetic patients (Priya et al. 2011) (Flowchart 1).

Artificial or Synthetic Sweeteners

Artificial sweeteners are alternatives that have fewer calories and much more intense sweetness than sugar can be a good substitute for it because they add almost no calories to the diet. Artificial sweeteners, also known as intense sweeteners, are synthetic sugar substitutes that are many times sweeter than sugar (Chattopadhyay et al. 2014) and information about these sweeteners, including precursors, sweetness,



Flowchart 1 Classification of natural sweetening agent (Priya et al. 2011)

other names, ADI and biological effects are given in Table 1. Eight artificial sweeteners (aspartame, saccharin, sucralose, neotame, acesulfame-K and stevia, swingle fruit extract, and advantame) have been approved by the US Food and Drug Administration and the safety of different sweeteners is reviewed in Table 2. These sweeteners cause weight loss and are especially safe for diabetics. Although these compounds are less metabolized in the body, there are concerns about the toxicity of "unmetabolized" compounds (Whitehouse et al. 2008). The carcinogenicity of some of these compounds such as cyclamate, the possibility of increasing the risk of coronary heart disease and chronic kidney disease and the relationship between these compounds and weight gain are among their possible toxic effects (Fung et al. 2009; Lin and Curhan 2011) For instance, safety studies show that metabolic products of aspartame are more harmful to the body than aspartame itself. Aspartame consumption affects obesity, glucose and insulin intolerance (Czarnecka et al. 2021) (Flowchart 2).

Peptide Sweeteners

Several sweet proteins have been identified so far that are much sweeter than sucrose. Some of them are of vegetable origin and are used commercially as non-carbohydrate sweeteners without calorie load and as sugar substitutes. Many cases of these proteins have been expressed in transgenic plants or bacteria because the purification of these proteins from natural sources is expensive and not economical. Each of these proteins appears to interact with and stimulate the human taste receptor, but the exact mechanisms are only partially elucidated. There are seven naturally sweet and flavor modifying plant proteins, namely brazzein, thaumatin, monellin, curculin, mabinlin, miraculin and pentadin whose important characteristics are listed in Table 3. All of these proteins are extracted from plants that grow in tropical rainforests. Among the applications of these proteins, we can mention the low-calorie sweeteners industry and, in the cola, snack, food and chocolate industries. The property that makes these sweeteners significantly different from sugar is the slow taste of sweet proteins (Gnanavel and Peddha 2011) (Flowchart 3).

Natural Peptide Sweeteners (NPSs)

Thaumatin

Thaumatins are a mixture of proteins isolated from the fruit of Katemfe (*Thaumatoccus daniellii* Benth.) (Maranthaceae) in West Africa. Thaumatin is a low-calorie peptide sweetener that is often used for its sweetening and taste modifying properties. It's sweetness is different from sugar and develops very slowly. This protein is very soluble in water and is stable acidic conditions, is non-caloric and non-toxic. Thaumatin is composed from the mixture of two proteins (thaumatin I and II) (BeMiller 2019; de Jesús-Pires et al. 2020). It characterizes with sweetness about 2000 times higher than sucrose (on a weight basis). As it is protein, it undergoes the same digestion in the human organism and supplies 4 kcal/g, but due to such high sweetness it is used in extremely small amounts, thus, their caloric values in food is negligible(Bassoli and Merlini 2003). Thaumatin is stable in freeze-dried form and is soluble in water and aqueous alcohol. An unusual feature of thaumatin II is its high resistance to heat under acidic conditions so that its sweet taste is fully retained for 30 min at 80 °C in a pH 2.0 buffer. Thaumatin has a shelf life of at least 36 months when stored at cool temperature (BeMiller 2019; Majie 2021).

Uses Thaumatin have laxative and emetic effect and are suitable sweetener for diabetic patient. Thaumatin is used as flavoring agent in foods and drinks. The properties of thaumatin II have been comprehensively studied and its safety has been approved. It is allowed as a sugar substitute in the European Union and Japan, as a taste modifier and sweetener in USA (Firsov et al. 2021; Additives et al. 2021).

Safety According to Joint FAO/WHO Expert Committee on Food Additive (JECFA) thaumatin is safe as a sweetener with no specified acceptable daily intake (ADI), and there is no information on its mutagenic, allergenic, or teratogenic effects (Bassoli and Merlini 2003). It has shown no adverse reactions in animal and human studies. Thaumatin is classified as generally recognized as safe by the FDA (Majie 2021). The recommended range of thaumatin use is 1 to 5 mg/kg that is safe for all animal species. Consequently, thaumatin can be taken through food or water. Thaumatins are highly digestible proteins and therefore do not leave a residue in the tissues/food products (Panel 2011; Kelada et al. 2021).

Brazzein

This sweet-tasting protein is derived from the fruit of the West African climbing plant of *Pentadiplandra brazzeana* Baillon and was first isolated by the University of Wisconsin-Madison in 1994. Brazzein is found in the extracellular region, in the pulp tissue around the seeds and it is the second peptide sweetener discovered in this plant, like other vegetable proteins. Brazzein taste is similar to sucrose with no metallic after taste. It effectively reduces the aftertaste of other sugar substitutes when used in combination (Barre et al. 2015; Lufulwabo et al. 2018). Brazzein is 500 times sweeter when compared to 10% sucrose solution and 2000 times sweeter when compared to 2% sucrose solution (Neiers et al. 2021). It is stable over a broad pH and heat

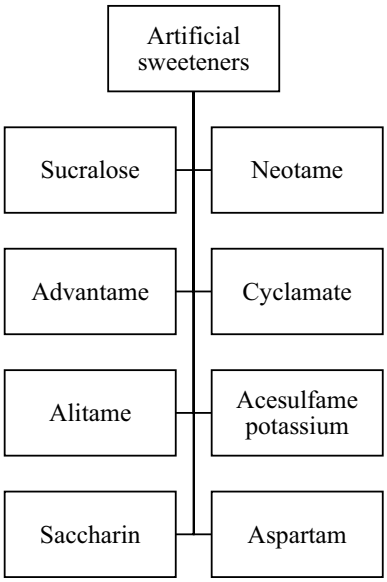
Table 1 Precursor, sweetness, other names, ADI, and biological effects of some synthetic sweeteners (Ruiz-Ojeda et al. 2019; Farag et al. 2022; Wilk et al. 2022)

Name	Other names	Precursor	Sweetness	ADI* (mg kg^{-1} day^{-1})	Biological effects	References
Acesulfame K	Ace-K; Sunette; Sweet & Safe; Sweet One	N-sulfonyl amide	200	15	Acesulfame K undergoes metabolization by the human body. No effects on body weight or glucose tolerance	Chattopadhyay et al. (2014)
Aspartame	NutraSweet, Equal	Aspartyl-phenylalanine methyl ester	180–200	40	Connected through methyl ester bonds, is rapidly absorbed. This compound is safe and without toxicity in gene mutations	Chattopadhyay et al. (2014)
Neotame	_	Derivative of aspartame, more stable than aspartame	7000–13,000 Avg 8000	2	It is safe for patients with phenylketonuria, but also safe for diabetics. With regard to its metabolization, half of the ingested neotame is not absorbed and excreted through the feces, whereas the other half is excreted in the urine as de-esterified neotame	Ruiz-Ojeda et al. (2019)
Advantame	_	obtained through chemical synthesis from aspartame and isovanillin and is a source of phenylalanine	20,000	5	This compound is nontoxic or carcinogenic and there are no risks of its consumption as a food additive	Chattopadhyay et al. (2014)
Cyclamate	Sucaryl, Sugar Twin	Sulfamic acid Na or Ca salt	30–50	11	Cyclamate is prepared by the sulfonation of cyclohexylamine (toxic compound). The FDA completely banned it in 1970. No effects on body weight or glucose tolerance	Chattopadhyay et al. (2014)
Saccharin	Sweeten' low	N-sulfonyl amide	300	5	Saccharin is excreted through urine and is not metabolized in the body, although it can cross the placenta and can be transferred through breast milk	Ruiz-Ojeda et al. (2019)
Sucralose	Splenda	Trichlorinated derivative of sucrose	600	5	Approximately 11–27% of ingested sucralose is absorbed from the gut and is excreted in the kidneys. Sucralose is safe	Chattopadhyay et al. (2014)

*ADI** acceptable daily intake

Table 2 Investigation the safety of different sweeteners (Castro-Muñoz et al. 2022; Kumar et al. 2021; Ambawat et al. 2021; Zeece 2020)

Sweetener name	Safety
Aspartame	There are reports of mood disorders, depression, premature birth, memory and learning disorders, and the risk of premature menstruation in humans, as well as allergic reactions, weight gain, and autism in infants Aspartame approved in 1980 by FDA
Neotame	The FDA reviewed data on the effects of neotame on the reproductive, nervous, and immune systems in animal and human studies, and as a result, this sweetener was approved by the FDA
Advantame	Advantame does not have any systemic toxicity and animals and humans can consume advantame because the results of animal and human toxicology show that the use of Advantame in food is safe. Advantame approved in 2014 by FDA
Alitame	Alitame is not carcinogenic and does not show reproductive toxicity. This sweetener has been approved in Australia, New Zealand, China, Mexico and Colombia
Thaumatin	Thaumatin has no mutagenic, allergenic or teratogenic effects and has not shown any adverse reactions in animal and human studies. As a result, Thaumatin has been approved by the FDA (Kelada et al. 2021)
Brazzein	Brazzein has been studied by the FDA for its use in food, but is not currently approved for use. Brazzein and other novel proteins have the potential to cause food allergies
Monellin	Safety of monellin has not been studied and is not approved for use as a sweetener
Curculin	Curculin has not approved in the USA or the EU (Świąder et al. 2019)
Miraculin	FDA and EFSA have not approved miraculin as a sweetener
Mabinlin	Mabinlin is not used as a sweetener in food production because mabinlin has not approved with the FDA
Pentadin	FDA and EFSA have not approved Pentadin as a sweetener



Flowchart 2 Classification of artificial sweetening agent (Chaudhry and Dutta 2021)

so no changes where said about behavior of electrophoresis and sweetness (the stability of brazzein is retained after at 80 °C for 4 h or 0.05% brazzein for 2 h at 98 °C in buffers at pH 2, 4, 6 and 8 (Fry 2012). It's small in size and molecular mass is 6.4 kDa and isoelectric point is 5. It's a monomer protein that consisting of 54 amino acid residues with 8 cysteines and four disulfide bonds. It's solubility in water at least 5% (Stanhope 2016; Izawa et al. 2010).

Uses For brazzein, an efficient bacterial production system has been developed and it is also possible to express brazzein protein in maize seed embryos, thus opening the interesting possibility of producing pre-sweetened cereals with 'no added sugar' (Lamphear et al. 2005).

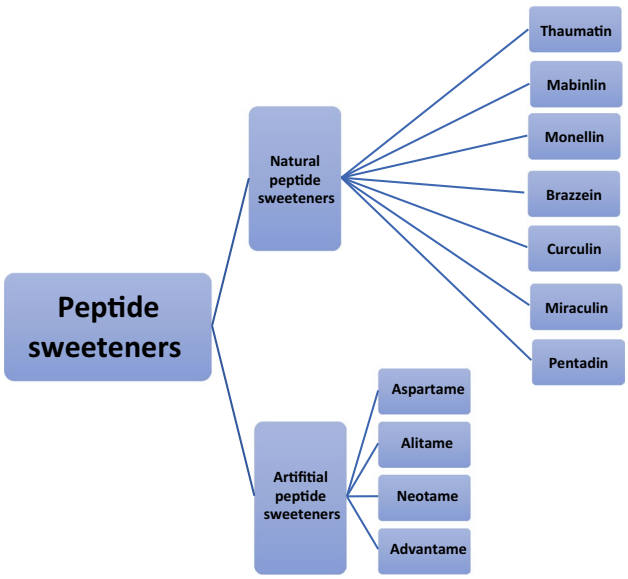
Safety Brazzein is being studied by FDA for its potential use in food, but it is not currently approved for use. Brazzein and other new proteins have the potential to cause food allergies and this shows that these proteins should be investigated before they are considered safe (Zeece 2020; Farag et al. 2022).

Monellin

Monellin is a sweet protein from a family of proteins that has no structure or sequence homology and can create a sense of sweetness in humans through interaction with the receptor (Leone et al. 2016). It was isolated from the fruit of *Dioscoreophyllum cumminsii* (Stapf) Diels, which is known as the serendipity berry and is native to West Africa (Izawa et al. 2010). A gene encoding monellin has been introduced in tomatoes and lettuce (*Lactuca sativa*) using the 35S and E8 promoters, but the level of the protein was too low to detect changes in flavor. However, increased sweetness of fruit and vegetables using the monellin and thaumatin genes (either native or genetically modified) has been reported in several patents (Bernadac et al. 2004). Unlike most globular proteins, monellin is made up of two non-covalently linked subunits held together only by secondary forces (Yasui et al. 2021). The sequence of monellin, like all sweet proteins,

Table 3 Important characteristics of seven sweet proteins (Kabore et al. 2022; Chawla et al. 2017):

Peptide sweetener	Thaumatin	Monellin	Mabinlin	Pentadin	Brazzein	Curculin	Miraculin
Geographic distribution	West Africa	West Africa	China	West Africa	West Africa	Malaysia	West Africa
Source	*Thaumatococcus danielli* Benth	*Dioscoreophyllum cumminsii* Diels	*Capparis masakai* Levi	*Pentadiplandra brazzeana* Baillon	*Pentadiplandra brazzeana* Baillon	*Curculingo latifolia*	*Richadella dulcifica*
Family	Maranthaceae	Menispermaceae	Capparidaceae	Pentadiplandraceae	Pentadiplandaceae	Hypoxidaceae	Sapotaceae
Plant parts	Fruit	Fruit	Fruit seed	Fruit	Fruit	Fruit	Fruit
Sweetness factor (than 2%sucrose solution-weight basis)	3000	3000	100	500	2000	550	–
Molecular mass	22.2	10.7	12.4	12.0	6.5	24.9	98.4
Amino acids	207	45 (A chain) 50 (B chain)	33 (A chain) 72(B chain)		54	114	191
Active form	Monomer	Dimer (A + B)	Dimer (A + B)		Monomer	Dimer (A + A)	Tetramer (A + A + A + A)



Flowchart 3 Classification of peptide sweetening agent (Kashani-Amin et al. 2021)

bears no significant similarity to that of any of the other sweet proteins (Esposito et al. 2006). Monellin is a heterodimer of two non-covalently linked subunits chain A and B, which loses its sweetness on denaturation. Reddy et al. are synthesized Purified recombinant monellin protein retained its sweet flavour at 70 °C and pH 2 (Reddy et al. 2015).The A chain contains of 44 amino acid residues and the B chain of 50 residues. Two different primary structures have been reported for each of these chains, the difference lying in positions 22, 25 and 26 of the A chain, and 49 and 50 of the B chain. The natural monellin is structurally different from its synthetic form, which can be identified through tryptic peptide mapping (Farag et al. 2022). Monellin is 1500–2000 times sweeter than 7% sucrose solution and 800 times sweeter than 5% sucrose solution, and but it is less sweet than thaumatin, and brazzein (Temussi 2011). The sweet sensation of the protein comes slowly and has a lingering taste. Monellin has no sweetness at a pH lower than 2 and higher than 9. Perception lasts for more than 1 h and leaves an after taste and it loses its sweetness when heated over 50 C at low pH (Izawa et al. 2010). Monellin use is limited by low stability and high aggregation propensity at neutral PH. It has hydrophilic properties and therefore easily dissolves in water (Hobbs et al. 2007; Delfi et al. 2021).

Uses Monellin can be used as a sweetener in some foods and beverages especially for diabetic patients, but due to degradation under high temperature conditions, has limited uses in processed foods (Hobbs et al. 2007).

Safety Safety of monellin has not been studied and is not approved for use as a sweetener (Zeece 2020).

Curculin

Curculin or neoculin is a heterodimeric protein with high intensity sweetness and consists of two monomers with 114 identical amino acids and a molecular mass of 12 kDa (The molecular weight determined by low-angle laser light scattering was 27,800) and an isoelectric point of 7.1. It has

both sweet-tasting and taste-modifying activities that was isolated with 0.5 M NaCl from whole fruit (contain 1–3 mg) of Malaysian plant of *Curculigo latifolia* and purified by ammonium sulfate in 1990 (Delfi et al. 2021). This protein has two different subunits with two disulfide bridges that this structure is necessary for its activities (Kinghorn et al. 2010). The sweetness of curculin lasts for several minutes and is 35,000 times sweeter than sucrose on a molar basis and 430 times sweeter on a weight basis. The sweetness of curculin increases with increasing concentration and reaches saturation at 10 µM, but after a short time the sweet taste in the mouth diminishes and the sweet taste is re-induced as water, especially the deionized form, washes away those cations. Curcoline reacts with divalent cations (Ca^{2+} and Mg^{2+}) in saiva and reduces the sweet taste (Iwaniak et al. 2016). Sour substances induce a stronger sense of sweetness because the taste-modifying protein strongly binds to the membrane surfaces of the taste cells in the presence of an acid such as citric acid (Izawa et al. 2010; Castro-Muñoz et al. 2022). Curculin is stable between pH 3 and 11, and heating at 55 °C for 1 h. and it is dissolves in water (Iwaniak et al. 2016; Esposito et al. 2006).

Uses Curcoline has a taste- modifying activity like miracoline, so that after taking curcoline, sour solutions also have a sweet taste. But unlike miracoline, it also has a sweet taste by itself (Castro-Muñoz et al. 2022).

Safety Curculin has not approved in the USA or the EU (Świąder et al. 2019), however, it is recognized as a safe food additive in Japan and is included in the list of food additives allowed by the Ministry of Health and Welfare of this country (Bahadur and Pal 2020).

Miraculin

Miraculin is a taste-modifying glycoprotein that was extracted from the miracle fruit of *Synsepalum dulcificum* or *Richadella dulcifica* which is a native shrub to tropical West Africa. The fruit is a small bright red berry containing a single seed (also called miracle berry or sweet berry) (Firsov et al. 2021). Miraculin was isolated by two independent research groups in 1968 (Tafazoli et al. 2019). The molecular mass of the glycoprotein is 24.6 kDa that including 3.4 kDa (13.9% of the weight) of sugar consisting (on a molar basis) of glucosamine (31%), mannose (30%), fructose (22%), xylose (10%) and galactose (7%) that it is a single polypeptide with 191 amino acid residues having 2 glycosylated residues, Asn-42 and Asn-186, cross-linked by a disulfide bond. (Demesyeux et al. 2020; Ohkura et al. 2018). The expression of these proteins in *E. coli*, yeast and tobacco has not been successful, but it has been produced in transgenic lettuce and tomato (Izawa et al. 2010; Castro-Muñoz et al. 2022). Analysis of the composition of cystine-containing peptides isolated by high-performance liquid chromatography (HPLC) has shown that there are three intrachain disulfide bridges and one inter-chain disulfide bridge in this composition. Miraculin is a tetramer (98.4 kDa), where within each dimer, two miraculin glycoproteins are connected by an intramolecular disulfide bridge. Miraculin by itself is not sweet, but tastes sweet at acidic condition. A sweet response 400,000 times sweeter than sucrose on a molar basis (Tafazoli et al. 2019; Haddad et al. 2020). This protein binds directly to the sweet-taste receptors, hT1R2-hT1R3, without activating them, and it is only when subjected to an acidic pH activates these receptors. It modifies the sweet receptor in such a way that it can be stimulated by acid (Haddad et al. 2020). Therefore, Miraculin has an unusual property and changes sour taste to sweet taste, which is caused by the binding of sweet taste receptors when acids are present at the same time. Miraculin is a relatively heat-stable protein (Matsuyama et al. 2009; Farag et al. 2022) and is an easily water- soluble protein (Matsuyama et al. 2009; Kabore et al. 2022).

Uses Miraculin has taste modifying activity and it is used to make acidic foods and drinks palatable, as well as to help improve the taste and reduce the bitterness of foods and drinks (Matsuyama et al. 2009).

Safety FDA and European Food Safety Authority (EFSA) have not approved miraculin as a sweetener. Pure form is a tetramer of a 25 kDa peptide, and native miraculin in the crude form or denatured, non-reduced miraculin is the pure form of the peptide dimer. Both the miraculin tetramer and the native miraculin dimer in the crude state have taste modifying activity. The encoded precursor of miraculin was composed of 220 amino acid residues, including a possible signal sequence of 29 amino acids (Świąder et al. 2019). Miraculin is not approved for use in the United States, but has been given novel food status in the European Union. (Tafazoli et al. 2019).

Mabinlin

Mabinlins are sweet-tasting proteins, mabinlin are derived from the matured seeds of Chinese plant of *Capparis masaikai* Levi. (local name mabinlang) (Haddad et al. 2020; Tafazoli et al. 2019). Among the four identified homologous proteins (mabinlin I–IV), mabinlin II is the most studied., This protein has 2 chains of A chain with 33 amino acid residues and B chain composed of 72 residues with two intramolecular disulfide bonds and loses sweetness under cleavage with dithiothreitol (Kulik and Waszkiewicz-Robak 2019). There is no intron in the mabinlin II gene and difficult to extract from the Capparis but biotransformation studies

via *E. coli* and Lactococcus lactis provide availabilities to produce mabinlin in wide spectrums for food applications (Hung et al. 2019). Mabinlin ll produced in potatoes has an astringent and sweet taste. It is least intense sweet-tasting protein mabinlin possesses four isoforms and form II, the sweetest isoform (400 times that of sucrose on weight basis) (Świąder et al. 2019; Haddad et al. 2020). Mabinlin II is the only, sweet-tasting protein, compound with the highest known thermostability. It shows the most pronounced heat stability and acid resistance of any of the six known types of plant sweet proteins. Its heat stability is due to the presence of the four disulfide bridges. The sweetness of Mabinlin-II is unchanged after 48 h. incubation at boiling point and of Mabinlin-III and -IV are unchanged after 1 h. at 80 °C (Kant 2005). Mabinlins are readily soluble in water (Kabore et al. 2022).

Uses Mabinlin II has an astringent-sweet taste, so the sweetness characteristics of mabinlin allow the use of this protein to mask the bitterness of plant compounds (Yusuf 2021).

Safety It is not used as a sweetener in food production because mabinlin has not approved with the FDA in the United States (Świąder et al. 2019).

Pentadin

This sweet-tasting protein was extracted from the pulp of *Pentadiplandra brazzenna* Baillon, a climbing shrub found in tropical Africa (especially Gabon). The plant bears red globular berries approximately 2 inches in diameter which contain one to five seeds surrounded by a thick layer of pulp. This sweet protein was first isolated, identified and named pentadin in 1989 by Vandervel et al. (Zhao et al. 2021). The molecular weight of pentadin is 12 kDa and its sweetness intensity was estimated based on the weight of about 500 times that of sucrose and more similar to monellin than thaumatin. Pentadin is obtained from the same plant from which brazzein is extracted, with the difference that pentadin is extracted from the heat-dried fruit, but brazzein is extracted from fresh fruit. Pentadin appears to be the non-native cross-linked form of brazzein with a molecular weight nearly twice that of brazzein, and its sweetness is significantly reduced compared to that of brazzein (Faus 2000; Farag et al. 2022). Pentadin contains mostly of amino acids of aspartic acid, glutamic acid, tyrosine, lysine, arginine and proline, the dominant amino acid of which is proline, and this protein does not lose its strength after being exposed to a temperature of 100 degrees Celsius for 5 h (Kashani-Amin et al. 2021). It also consists of subunits coupled by disulfide bonds.pentadin is soluble in water (Kashani-Amin et al. 2021).

Uses Pentadin is a low-calorie natural sweetener that would be a useful substitute for some sugars, especially in diabetic patients (Gnanavel and Peddha 2011).

Conclusion

Sugar consumption and related diseases have increased globally in recent years and many people are suffering from these diseases. But today, public awareness of the adverse effects of excessive sugar consumption on health has increased and has caused concerns. For many people, alternative sweeteners, both natural and artificial, have become the preferred alternatives. But artificial sweeteners get a bad reputation because of the safety issue. Among the natural sweeteners, sweet proteins with a peptide structure are much sweeter than regular sugar, are easier to produce than other sweeteners and are potential replacements for high-calorie sweeteners. These sweeteners also improve taste and reduce bitterness. The sweeteners described in this review are types of high-intensity natural peptide sweeteners that can be used in the production of new food and pharmaceutical products in the future, although some of them have not yet been approved by the FDA for use. Contrary to the challenges that still exist for the FDA approval of natural peptide sweeteners, these compounds generally show fewer side effects than synthetic types. The reports about the serious side effects of some types of synthetic sweeteners make the need for safer alternatives inevitable, and perhaps natural sweeteners can be good candidates in this field. The problems that exist with natural peptide sweeteners mainly include their instability in different conditions of temperature and pH, or the interactions they have with other sweeteners, which increases the need for further processing of this category of compounds. Among the natural peptide sweeteners, thaumatin has exhibited no mutagenic, allergenic, or teratogenic effects, and no adverse reactions have been reported for it in animal and human studies (at the same time, it is the only natural sweetener approved by the FDA). This protein offers sweetness about 2000 times more than sucrose while, produces only 4 kcal/g. In addition to giving a good taste to foods and beverages, natural peptide sweeteners have a positive effect on the color, shelf time, caloric value and texture of the products that are added to them. This category of sweeteners is of interest due to their low caloric value and especially the ability to be used in health problems such as diabetes and even the protective effects of teeth. These sweeteners known legal as flavor enhancers and sugar substitute in various foods and beverages in a number of countries, and there are no reports of their serious side effects in the literature.

Declarations

Conflict of interest The authors declare no competing interests.

References

Ambawat S, Tiwari M, Sharma A, Chauhan NS (2021) Natural non-nutritive but healthier sweeteners. Med Plants-Int J Phytomed Relat Ind 13(3):378–387

Bahadur B, Pal AK (2020) Natural sweetening plants: a global review. LS Int J Life Sci 9(2):134–157

Barre A, Caze-Subra S, Gironde C, Bienvenu F, Bienvenu J, Rougé P (2015) What about the allergenicity of sweet-tasting proteins? Rev Francaise Allergol 55(5):363–371

Bassoli A, Merlini L (2003) SWEETENERS | intensive. In: Caballero B (ed) Encyclopedia of food sciences and nutrition, 2nd edn. Academic Press, Oxford, pp 5688–5695. https://doi.org/10.1016/B0-12-227055-X/01172-X

BeMiller JN (2019) Carbohydrate and noncarbohydrate sweeteners. Carbohydr Chem Food Sci 3:380–383

Bernadac A, Latché A, Li Z, Bouzayen M, Pech J-C (2004) Postharvest physiology: genetic engineering for postharvest quality. Academic Press, Cambridge

Castro-Muñoz R, Correa-Delgado M, Córdova-Almeida R, Lara-Nava D, Chávez-Muñoz M, Velásquez-Chávez VF et al (2022) Natural sweeteners: sources, extraction and current uses in foods and food industries. Food Chem 370:130991

Chattopadhyay S, Raychaudhuri U, Chakraborty R (2014) Artificial sweeteners–a review. J Food Sci Technol 51(4):611–621

Chaudhry S, Dutta A (2021) A short communication on sweeteners and their relative profiles. J Pharm Qual Assur Qual Control

Chawla R, Goyal S, Doal RK, Rana K, Arora A, Sahoo U (2017) Recent trends of some natural sweet substances from plants. Int J Pharm Sci.

Czarnecka K, Pilarz A, Rogut A, Maj P, Szymańska J, Olejnik Ł et al (2021) Aspartame—true or false? Narrative review of safety analysis of general use in products. Nutrients 13(6):1957

Dafaalla MH, Khidir AM, Osman EA, Saeed AA, Mousnad MA (2020) Awareness of pharmacists towards aspartame side effects in Khartoum City, Sudan. Univ J Pharm Res. https://doi.org/10.22270/ujpr.v5i3.410

de Jesús-Pires C, Ferreira-Neto JR, Pacifico Bezerra-Neto J, Kido EA, de Oliveira Silva RL, Pandolfi V et al (2020) Plant thaumatin-like proteins: function, evolution and biotechnological applications. Curr Protein Peptide Sci 21(1):36–51

Delfi M, Emendato A, Leone S, Lampitella EA, Porcaro P, Cardinale G et al (2021) A super stable mutant of the plant protein monellin endowed with enhanced sweetness. Life 11(3):236

Demesyeux L, Brym M, Valdes D, Collazo C, Chambers AH (2020) Yield and miraculin content of nine miracle fruit (*Synsepalum Dulcificum*) morphotypes. Euphytica 216(11):1–12

EFSA Panel on Food Additives and Flavourings (FAF), Younes M, Aquilina G, Castle L, Engel KH et al (2021) Re-evaluation of thaumatin (E 957) as food additive. EFSA J 19(11):e06884

Esposito V, Gallucci R, Picone D, Saviano G, Tancredi T, Temussi PA (2006) The importance of electrostatic potential in the interaction of sweet proteins with the sweet taste receptor. J Mol Biol 360(2):448–456

Farag MA, Rezk MM, Elashal MH, El-Araby M, Khalifa SA, El-Seedi HR (2022) An updated multifaceted overview of sweet proteins and dipeptides as sugar substitutes; the chemistry, health benefits, gut interactions, and safety. Food Res Int 162:111853

Faus I (2000) Recent developments in the characterization and biotechnological production of sweet-tasting proteins. Appl Microbiol Biotechnol 53(2):145–151

Firsov A, Shaloiko L, Kozlov O, Vainstein A, Dolgov S (2021) Tomatoes expressing thaumatin II retain their sweet taste after salting and pickling processing. J Sci Food Agric 101(12):5286–5289

Fry J (2012) Natural low-calorie sweeteners. In: Baines D, Seal R (eds) Natural food additives, ingredients and flavourings. Elsevier, Amsterdam, pp 41–75

Fung TT, Malik V, Rexrode KM, Manson JE, Willett WC, Hu FB (2009) Sweetened beverage consumption and risk of coronary heart disease in women. Am J Clin Nutr 89(4):1037–1042

Gnanavel M, Peddha MS (2011) Identification of novel sweet protein for nutritional applications. Bioinformation 7(3):112

Haddad SG, Mohammad M, Raafat K, Saleh FA (2020) Antihyperglycemic and hepatoprotective properties of miracle fruit (*Synsepalum dulcificum*) compared to aspartame in alloxan-induced diabetic mice. J Integr Med 18(6):514–521

Hobbs J, Munger S, Conn G (2007) Monellin (MNEI) at 1.15 Å resolution. Acta Crystallogr Sect F Struct Biol Cryst Commun 63(3):162–167

Hung C-Y, Cheng L-H, Yeh C-M (2019) Functional expression of recombinant sweet-tasting protein brazzein by *Escherichia coli* and *Bacillus licheniformis*. Food Biotechnol 33(3):251–271

Iwaniak A, Minkiewicz P, Darewicz M, Hrynkiewicz M (2016) Food protein-originating peptides as tastants—physiological, technological, sensory, and bioinformatic approaches. Food Res Int 89:27–38

Izawa K, Amino Y, Kohmura M, Ueda Y, Kuroda M (2010) 4.16-human–environment interactions-taste. Compr Nat Products II 4:631–671

Jiao Y, Wang Y (2018) The effects of sweeteners and sweetness enhancers on obesity and diabetes: a review. J Food Bioact 4:107–116

Kabore I, Diao M, Guenne S, Metuor-Dabire A, Sama H, Ciobica A et al (2022) Phytochemistry and alternative use of sweeteners in metabolic diseases. Ann Acad Romanian Sci Ser Biol Sci 11:109

Kant R (2005) Sweet proteins–potential replacement for artificial low calorie sweeteners. Nutr J 4(1):1–6

Kashani-Amin E, Faraji H, Nouriyengejeh S, Ebrahim-Habibi A (2021) Structure-sweetness relationship of sweet proteins: a systematic review on "sweet protein" studies as a sub-group of "sweetener" investigations. Mosc Univ Biol Sci Bull 76(4):175–190

Kelada KD, Tusé D, Gleba Y, McDonald KA, Nandi S (2021) Process simulation and techno-economic analysis of large-scale bioproduction of sweet protein thaumatin II. Foods 10(4):838

Kim N-C, Kinghorn AD (2002) Highly sweet compounds of plant origin. Arch Pharm Res 25(6):725–746

Kinghorn AD, Chin Y-W, Pan L, Jia Z (2010) Natural products as sweeteners and sweetness modifiers. In: Comprehensive natural products II, vol. 3, pp. 269–315

Kulik K, Waszkiewicz-Robak B (2019) Sweet nutraceuticals in plants. Polish J Appl Sci 4(2):65–71

Kumar S, Tyagi PK, Gola D, Mishra AK, Arya A (2021) Plant-based sweeteners and their applications in modern lifestyle. In: Husen A, Bachheti RK (eds) Non-timber forest products. Springer, Berlin, pp 75–103

Kumari N, Kumar S, Sharma SB (2019) Low calorie natural sweeteners: a boon to society. Today & Tomorrow's Printers and Publishers, New Delhi, p 359

Lamphear BJ, Barker DK, Brooks CA, Delaney DE, Lane JR, Beifuss K et al (2005) Expression of the sweet protein brazzein in maize for production of a new commercial sweetener. Plant Biotechnol J 3(1):103–114

Leone S, Pica A, Merlino A, Sannino F, Temussi PA, Picone D (2016) Sweeter and stronger: enhancing sweetness and stability of the single chain monellin MNEI through molecular design. Sci Rep 6(1):1–10

Lin J, Curhan GC (2011) Associations of sugar and artificially sweetened soda with albuminuria and kidney function decline in women. Clin J Am Soc Nephrol 6(1):160–166

Lufulwabo GL, Bongo GN, Konga DW (2018) Pentadiplandra brazzeana Baill.(Pentadiplandraceae): chemical screening assessment and a mini-review on its Bioactivity and Phytochemistry. J Adv Med Life Sci. https://doi.org/10.5281/zenodo.1250435

Majie A (2021) Natural sweeteners and its use in pharmaceutical industry. ScienceOpen Posters. https://doi.org/10.14293/S2199-1006.1.SOR-.PPCRINY.v1

Matsuyama T, Satoh M, Nakata R, Aoyama T, Inoue H (2009) Functional expression of miraculin, a taste-modifying protein in Escherichia coli. J Biochem 145(4):445–450

Neiers F, Belloir C, Poirier N, Naumer C, Krohn M, Briand L (2021) Comparison of different signal peptides for the efficient secretion of the sweet-tasting plant protein brazzein in *Pichia pastoris*. Life 11(1):46

Ohkura S-I, Hori M, Saitoh K, Okuzawa T, Okamoto I, Furukawa N et al (2018) Structural and functional analysis of miraculin-like protein from *Vitis vinifera*. Biochim Biophys Acta Proteins Proteomics 1866(11):1125–1130

Panel E (2011) Scientific opinion on the safety and efficacy of thaumatin for all animal species. EFSA J 9(9):2354

Piekara A, Krzywonos M, Szymańska A (2020) Sweetening agents and sweeteners in dietary supplements for children-analysis of the polish market. Nutrients 12(8):2387

Priya K, Gupta VRM, Srikanth K (2011) Natural sweeteners: a complete review. J Pharm Res 4(7):2034–2039

Reddy CS, Vijayalakshmi M, Kaul T, Islam T, Reddy MK (2015) Improving flavour and quality of tomatoes by expression of synthetic gene encoding sweet protein monellin. Mol Biotechnol 57(5):448–453

Ruiz-Ojeda FJ, Plaza-Díaz J, Sáez-Lara MJ, Gil A (2019) Effects of sweeteners on the gut microbiota: a review of experimental studies and clinical trials. Adv Nutr 10(suppl_1):S31–S48

Stanhope KL (2016) Sugar consumption, metabolic disease and obesity: the state of the controversy. Crit Rev Clin Lab Sci 53(1):52–67

Świąder K, Wegner K, Piotrowska A, Tan F-J, Sadowska A (2019) Plants as a source of natural high-intensity sweeteners: a review. J Appl Bot Food Qual 92:160–171

Tafazoli S, Vo TD, Roberts A, Rodriguez C, Viñas R, Madonna ME et al (2019) Safety assessment of miraculin using in silico and in vitro digestibility analyses. Food Chem Toxicol 133:110762

Temussi PA (2011) New insights into the characteristics of sweet and bitter taste receptors. Int Rev Cell Mol Biol 291:191–226

Whitehouse CR, Boullata J, McCauley LA (2008) The potential toxicity of artificial sweeteners. AAOHN J 56(6):251–261

Wilk K, Korytek W, Pelczyńska M, Moszak M, Bogdański PJN (2022) The effect of artificial sweeteners use on sweet taste perception and weight loss efficacy: a review. Nutrients 14(6):1261

Yasui N, Nakamura K, Yamashita A (2021) A sweet protein monellin as a non-antibody scaffold for synthetic binding proteins. J Biochem 169(5):585–599

Yusuf EH (2021) An overview of biotransformation for the sustainability of sweet tasting proteins as natural sugar replacers. Chemistry proceedings

Zeece M (2020) Chapter six—flavors. In: Zeece M (ed) Introduction to the Chemistry of Food. Academic Press, Cambridge, pp 213–250. https://doi.org/10.1016/B978-0-12-809434-1.00006-2

Zhao X, Wang C, Zheng Y, Liu B (2021) New insight into the structure-activity relationship of sweet-tasting proteins: protein sector and its role for sweet properties. Front Nutri. https://doi.org/10.3389/fnut.2021.691368