THE NEW LOW CALORIE SWEETENER

REVIEW

OGNEAN Claudia Felicia, DARIE Neli, OGNEAN Mihai

"Lucian Blaga" University of Sibiu

Abstract: This review summarizes information relate to new low-calorie sweetener: erythritol, difructose anhydride, monellin, mabinlin, pentadin, and stevioside. In addition, important developments in food applications are reported.

INTRODUCTION

Dietary and health demands are continuing to expand the marked for sweeteners as alternative to sucrose. Low-calorie sweeteners are important for persons affected by diseases linked to the consumption of sugar, e.g. diabetes, hyperlipemia, caries, overweight, obesity. Most sweet compounds, including all popular sweeteners, are small molecular weight compounds of widely different chemical nature, but there are also sweet macromolecules, both synthetic and natural.

ERYTHRITOL

Erythritol is a 4-carbon sugar alcohol. Erythritol is a new potential sweet food additive. Erythritol occurs widely in nature and has been found to occur naturally in several foods including wine, sake, beer, water melon, pear, grape and soy sauce at levels up to 0,13% (w/v) (Dubernet et al., 1974, Shindou et al., 1989, Sponholz et al., 1986 quoted by Munro et al., 1998). Evidences indicate that erythrol also exists endogenously in the tissues and body fluids of humans and animals (Goosees and Röper, 1994; Noda et al., 1994).

Owing to its properties, erythritol is intended for use as a low-calorie sweetener. Its intended uses principally include confectionery, chewing gum, and beverage and bakery products.

It has sweetness 60-80% of sucrose. It is produced from corn or wheat starch by enzymatic hydrolysis yielding glucose which is fermented by safe and suitable food-grade osmophilic yeast, either *Moniliella pollinis* or *Trichosporonoides megachliensis.* Once erythritol is separated from the

fermentation broth, it is purified to result in a crystalline product that is more than 99% pure.
The safety database on erythritol provides no evidence that erythritol has any carcinogenic, mutagenic or teratogenic potential. In addition, no effects on reproductive performance or fertility have been reported in the studies. The available studies demonstrate that erythritol is readily absorbed, is not systemically metabolized, and is rapidly excreted unchanged in the urine. Both animal toxicological studies and clinical studies have consistently demonstrated the safety of erythritol, even when consumed on a daily basis in high amounts (Munro et al., 1998).

DIFRUCTOSE ANHYDRIDE

Difructose anhydride is a new potential sweet food additive. The disaccharide is derived from the vegetable storage compound inulin. It is a non-reducing sugar which is very well soluble in water and has melting point of 162^0C. Due to an intramolecular dioxane ring (Fig. 1), the molecule is very stable and is not hydrolysed in the stomach. It has approximately half sweetness of sucrose, is not cariogenic, and is not metabolised by the human body (Jahnz et al., 2003). As is known from oligofructoses, it influences the composition of the intestinal flora positively as was shown in feeding experiments with rats (Saito and Tomita, 2000). Likewise, in rats an increased uptake of calcium could be observed in the intestine after nourishing on difructose anhydride-enriched food. The authors conclude that the risk of osteoporosis could be lowered by such a diet (Suzuki et al., 1998).

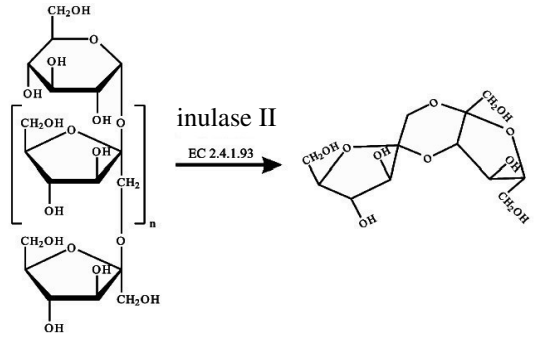

Fig. 1 - Chemical structure of inulin and the enzymatic product difructose dianhydride III

So far, difructose anhydride is not produced on a commercial scale as a food additive. Difructose anhydride can be produced either chemically or enzymatically from inulin. The prior method uses diluted sulfuric acid or pyrolysis conditions and yields of up to 40% can be found in inulin-derived caramels (Richards, 1996). The enzymatic route is more specific. The

enzyme inulase II (EC 2.4.1.93) accomplished the difructose anhydride formation by an intramolecular transfructosylation. This enzyme was first described in *Arthrobacter ureafaciens* and has meanwhile been found in various other bacteria (Kawamura et al., 1998, Yokota et al., 1991, Kim and Lee, 2000). However, the cited enzymes are not long-term stable at elevated temperature which is eligible for a process on industrial scale. To produce difructose anhydride in technical and industrial scale, large amounts of incapsulated enzyme are needed.

MONELLIN

Monellin was first purified in 1972 by Morris and Cagan from the fruit of the *Dioscoreophylum cumminsii* grown in West Africa. The fruit of 1 cm length is found in grapelike clusters on the stem of the plant. The active ingredient is isolated with sodium chloride solution and the extract is then ultrafiltered and freeze-dried. It consist of two non-covalently associated polypeptide chains, an A-chain of 44 residues and B-chain of 50 residues. These chains have been completely sequenced by Frank and Zuber and Komura et al. (Gibbs et al., 1996).

The molecular weight is 11 086 Da (5 251 from the A chain and 5 835 from the B chain). The chains do not contain disulfide bonds or histidine. The isoelectric point is between 9-9,4. The A chain does not contain any cysteine while he B chain has one cysteine residue. Modification of the individual A and B chains by replacement of peptides with cis-proline was found to increase the thermostability of monellin (Kim and Lim, 1996).

Monellin is 100 000 times sweeter than sugar on a molar basis and several thousand times on a weight basis. Monellin maintains its sweet taste between pH 2,4-9,6, but it loses its taste at pH 10 due to unfolding of the tertiary structure. Its activity is restored upon acidification. Denaturants such as urea and sodium dodecyl sulfate lead to a complete loss in sweet taste. Subjecting the protein to heat (pH 2) also caused a loss in sweetness but was recovered by reneutralization. Since the sweet taste lasts for a long time, it has been postulated that the binding of monellin to taste receptors is strong. In addition, lactose and sucrose were found to inhibit binding (Konno et al., 1999).

The native conformation is important for the sweet taste (Mizukoshi et al., 1997). The commercial feasibility of monellin is very low despite its intense sweetness. It is very costly to produce because the plant cannot be grown outside of its natural environment. Its stability is limited. In addition, if monellin is added to a carbonated drink such as cola, within a few hours, the

sweetness will decrease rapidly (Suami et al., 1996). The taste properties are also not ideal. Sweetness decline for up to an hour. In addition, proteolytic enzymes decrease sweetness activity by creating peptides without sweetness. However, modification of the protein may lead to a potentially commercial product. Kim et al., (1998) were able to increase the sweetness of the protein and stability towards pH and temperature changes by fusing the two chains into one. This was accomplished by using several linkers from monellin which were copied and then transplanted. The modified protein renaturated after heating at low pH to 100^0C.

MABINLIN

In 1986, several sweet proteins were extracted from the seed of a plant found in the south of China. The plant, *Capparis masaikai*, bears fruit as large as apples and the natives have eaten the seeds. The proteins were called mabinlins. Mabinlin was found to be 375 times sweeter than sucrose (Hu, 1986). These five homologous proteins were difficult to separate by conventional techniques. This included extraction with sodium chloride and ammonium sulphate, followed by ion-exchange and gel filtration chromatography.

One of the proteins, designated mabinlin II, was found to be extremely heat stable. It did not lose its sweetness after being subjected to 100^0C for 48 hours. It is also the most abundant in seeds, 1,4 g being obtained from 100 g of seeds. The molecular weight has been estimated at 14 kDa and the isoelectric points as 11.3. This protein consist of two polypeptide chains, chain A comprising 33 amino acids residues and chain B with 72 residues. There is one disulfide bridge in chain A and 3 bridges in chain B. The chains are non-covalently linked; the chain A contains mostly hydrophilic residues while the Bcontains mostly hydrophobic. No homology was found between mabinlin II and other sweet proteins (Liu et al., 1993).

In 1994, mabinlins I, III and IV were purified by Nirasawa et al. All Homologs were stable when heated at 80^0C for 1 hour with the exception of mabinlin I which lost its sweetness. An effort was then made to determine what the difference was between mabinlin I and the others by amino acid sequences and evaluation of the disulfide bridge position. The disulfide bridges were not responsible for the heat stability of the protein since they were the same in mabinlin I and mabinlin II. However, there were some differences in the amini acid sequences of the homologous proteins. It appears that the heat stability is due to the B-chain residue at position 47which is arginine in the heat stable proteins but is replaced by glutamine in

the unstable homolog. The arginine at position 47 may form a salt bridge with the C-terminal of the A or B-chains (Nirasawa et al., 1994).

PENTADIN

The most recently documented sweet protein was discovered in 1989 and named pentadin. Microgram quantities were isolated from the extract of the pulp of the plant *Pentadiplandra brazzena* Baillon. This plant found in tropical Africa (especially Gabon) bears red globular berries approximately 2 inches in diameter. These berries contain one to five seeds surrounded by a thick layer of pulp where the sweet protein resides. Ultrafiltration membrane was used to eliminate the low molecular weight compounds. Then, gel filtration was used to fractionate a series of proteins, one of which had an intense sweet taste (Van der Wel, et al., 1989).

The substance was estimated to be 500 times as sweet as sucrose with a similar taste profile to sweet proteins. The onset of sweetness due to sweet proteins is relatively slow with a slight liquorice aftertaste. The sensation is not only limited to the front of the tongue but over a large portion of the tongue. The sweetness profile differs from sucrose and thus will probably be used in combination with other sweeteners such as saccharin to mask the bitter aftertaste of the latter (Gibbs et al., 1996).

Structurally, pentadin is believed to be a protomer whose smallest unit is approximately 12 000 daltons molecular weight. Amino acid composition revealed a high proline content, a residue associated with sweet taste. Other dominant amino acids included aspartic acid, glutamic acid, tyrosine, lysine and arginine. When are chemically denaturated, it retains its sweetness. It did not lose its potency even after exposure to 100^0C for 5 hours (Van der Wel, et al., 1989). The presence of disulfide bonds was detected and may be responsible for the protein's stability. Because of the limited quantity isolated, the protein was not further characterized.

STEVIOSIDE

Stevioside, a high intensity non-nutritive sweetener, is extracted from the leaves of *Stevia rebaudiana* Bertoni, a sweet plant native to north-eastern Paraguay. It is a white, crystalline, odourless powder which is approximately 300 times sweeter than sucrose (0,4% solutions). Structures of the sweet components of *Stevia* occurring mainly in the leaves are given in Fig. 2. Their content varies between 4 and 20 % of the dry weight of the leaves depending on the cultivar and growing conditions. Stevioside 3 is the main sweet component. Other compounds present but in lower concentrations are:

steviolbioside 2, rebaudioside A 4, B 5, C 6, D 7, E 8, F 9 and dulcoside A 10 (Starrat et al., 2002).

Stevia plant, its extracts, and stevioside have been used several years as a sweetener in South America, Asia, Japan, China, and in different countries of the EU. In Brazil, Korea and Japan *Stevia* leaves, stevioside and highly refined extracts are officially used as a low-calorie sweetener (Kim et al., 2002). In the USA, powdered *Stevia* leaves and refined extracts from the leaves have been used as a dietary supplement since 1995. In 2000, the European Commission refused to accept *Stevia* or stevioside as a novel food because of a lack of critical scientific reports on *Stevia* and the discrepancies between cited studies with respect to possible toxicological effects of stevioside and especially its aglycone steviol (Geuns, 2003).

The advantages of stevioside as a dietary supplement for human subjects are mainfold: it is stable, it is non-calorific, it maintains good dental health by reducing the intake of sugar and opens the possibility for use by diabetic and phenylketonuria patients and obese persons. The stability of the low calorie sweetener stevioside during different processing and storage conditions, as well as the effects of its interactions with the water-soluble vitamins ascorbic acid, thiamin, ribiflavin, pyridoxine and nicotinic acid, the organic acids aceticacid, citric acid, tartric acid and phosphoric acid, the other common low calorie sweeteners saccharin, cyclamate, aspartame, acesulfame, neohesperidin dihydrochalcone, and caffeine in coffee and tea, were evaluated. Incubation of solid stevioside at high temperatures for 1 h showed good stability up to 120^0C, whilst forced decomposition was noticed at temperatures exceeding 140^0C. In aqueous solutions stevioside was remarkably stable in a pH range of 2-10 under thermal treatment up to 80^0C; however, under strong acidic conditions (pH 1), a significant decrese in the stevioside concentration was detected. Incubation up to 4 h of with individual water-soluble vitamins in aqueous solutions at 80^0C showed no significant changes with regard to stevioside and the B-vitamins, whereas a protective effect of stevioside on the degradation of ascorbic acid was observed, resulting in a significant delayed degradation rate. In the presence of other individual low calorie sweeteners, practically no interaction was found at room temperature after 4 month of incubation in aqueous media. Stability studies of stevioside in solutions of organic acids showed a tendency towards enhanced decomposition of the sweetener at lower pH values, depending on the acid medium. In stevioside-sweetened coffee and tea, very few significant chances in caffeine content or in stevioside content were found (Kroyer et al., 1999).

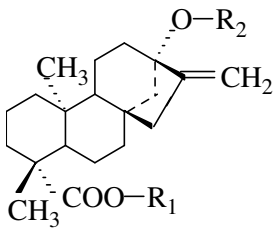

Fig. 2 – Structures of stevioside and related compounds

Table .1. Related compounds of stevioside

Compound name	R_1	R_2
1. steviol	H	H
2. steviolbioside	H	β-glucose-β-glucose (2→1)
3. stevioside	β-glucose	β-glucose-β-glucose (2→1)
4. rebaudiosite A	β-glucose	β-glucose-β-glucose (2→1) | β-glucose (3→1)
5. rebaudiosite B	H	β-glucose-β-glucose (2→1) | β-glucose (3→1)
6. rebaudiosite C (dulcosite B)	β-glucose	β-glucose-α-rhamnose (2→1) | β-glucose (3→1)
7. rebaudiosite D	β-glucose-β-glucose (2→1)	β-glucose-β-glucose (2→1) | β-glucose (3→1)
8. rebaudiosite E	β-glucose-β-glucose (2→1)	β-glucose-β-glucose (2→1)
9. rebaudiosite F	β-glucose	β-glucose-β-xylulose (2→1) | β-glucose (3→1)
10. dulcosite A	β-glucose	β-glucose-α-rhamnose (2→1)

REFERENCES

1. B.F.Gibbs, and colab., *Sweet and taste-modifying proteins: a review,*1996,Nutrition Research, vol. 16, nr. 9, pg. 1619-1630;
2. J.M.C.Geuns, *Stevioside*, 2003, Phytochemistry, nr.6;
3. J.Goossens, and H.Röper, *Erythritol: A new bulk sweetener,* 1994, International Food Ingredients, vol. 1/2, pp. 27-33;
4. Z.Hu, *The sweet proteins from the seeds of Capparis masakai,* 1986, Proceedings of the Munchen Shanghai Symposium: Peptide and Protein Chemistry;
5. U.Jahnz,et al., *Process for producing the potential food ingredient DFA III from inulin: screening, genetic engineering, fermentation and immobilisation of inulase II,* 2003,

6. International Journal of Pharmaceutics, 7424, pg. 1-8;
7. M.Kawamura, S.Takahashi, T.Uchiyama, *Purification and some properties of inulin fructotransferase (depolymeriying) from Arthrobacter ilicis* – Agric. Biol. Chem., vol. 52, pp. 3209-3210;
8. S.H.Kim, et al., *Redesigning a sweet protein: increased stability and renaturability,* 1989, Protein Eng, vol. 2, pp. 571-575;
9. G.E.Kim, S.Lee, *Efficient production of DFA III (di-D-fructofuranose-dianhydride) from chicory root,* 2000, Abstracts of the World Congress on Biotechnology 2000, vol. 272, Berlin;
10. I.H.Kim, K.J.Lim, *Large-Scale Purification of Recombinant Monellin from Yeast*, 1996, Journal of Fermentation and Bioengineering, vol. 82, nr. 2, pp. 180-182;
11. J.Kim, et al., *Use of stevioside and cultivation of Stevia rebaudiana in Korea,* 2002, Medicinal and Aromatic Plants – Industrial Profiles, vol. 19, pp. 196-202;
12. T.Konno, et al., *Amyloid-like aggregates of a plant protein: a case of a sweet- asting protein, monellin,* 1999, FEBS Letters, vol. 454, pp. 122-126;
13. G.T.Kroyer, *The Lo7w Calorie Sweetener Stevioside: Stability and Interaction with Food Ingredients,* 1999, Lebensm.-Wiss. u. Technol., vol. 32, pg. 509-512;
14. X.Liu, et al., *Purification, complete amino acid sequence and structure characterization of heat-stable sweet protein, mabinlin II,* 1993, Eur J Biochem, vol. 211, pp. 281-288;
15. T.Mizukoshi, et al., *Structure and dynamic studies by NMR of the potent sweet protein monellin and a non-sweet analog,* 1997, FEBS Letters, vol. 413, pp. 409-416;
16. I.C.Munro, et al., *Erythritol: An Interpretive Summary of Biochemical, Metabolic, Toxicological and Clinical Data,* 1998, Food and Chemical Toxicology, vol., 36, pp. 1139-1174;
17. S.Nirasawa, et al., *Structures of heat-stable and unstable homologues of the sweet protein mabinlin. The difference in the heat stability is due to replacement of a single amino acid rezidue,* 1994, Eur. J. Biochem, vol. 223, pp. 989-995;
18. K.Noda, K.Nakayama, and J.Modderman, *Fate of erythritol after single oral administration to rats and dogs*, 1996, Regulatory Toxicology and Pharmacology, vol., 24, pp. S206-S213;
19. G.N.Richards, *Production of fructose dianhydride products from inulin,* 1996, US-patent applications US525190;
20. K.Saito, F.Tomita, *Difructose anhydrides: their mass-production and physiological functions,* Biosci. Biotech. Biochem., vol. 64, pg. 1321-1327;
21. A.N.Starrat, et al., *Rebaudioside F, a diterpene glycoside from Stevia rebaudiana,* 2002, Phytochemistry, vol. 59, pp. 367-370;
22. T.Suami, et al., *Molecular mechanism of sweet taste. Part 6: the sweet protein, Monellin,* Food Chemistry, vol. 56, nr. 3, pp. 275-281;
23. T.Suzuki, et al., *Effects of difructose anhydride III on calcium absorbtion in small and large intestin of rats,* 1998, Biosci. Biotech. Biochem., vol. 62, pp. 837-841;
24. H.Van der Wel, et al., *Isolation and characterization of pentadin, the sweet principle of Pentadiplandra brazzeana Baillon,* 1989, Chemical Senses, vol. 14, pp. 75-79;
25. A.Yokota, et al., *Production of inulin fructotransferase (depolymeriying) by Arthrobacter sp. H65-7 and preparation of DFA III from inulin by the enzyme,* 1991, J. Ferm. Bioeng., vol., 72, pp. 258-261