Low Calorie High-Intensity Sweeteners

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ABSTRACT: The review on low-calorie, High-intensity Sweeteners (HIS), synthetic and natural, will focus particularly on those sweeteners which have already received approval as food additives from the European Food Safety Authority (EFSA), the Federal Drug Administration (FDA) or have achieved Generally Recognized as Safe (GRAS) status.

In the Europe Union, the approved HIS are given a particular E number, shown in bracket; the following sweeteners are approved by the EFSA for use in food, drink and medicine: acesulfame potassium also called Acesulfame K, (E950), Aspartame (E951), Cyclamate (E952), Saccharine (E954), Sucralose (E955), Thaumatin (E957), Neohesperidine dihydrochalcone (E959), Neotame (E961), Steviol glycosides (E2960), and Mogroside; likewise, in the USA, the FDA, has approved all the above sweeteners for application in food, drink and medicinal formulations (see Table 1).

This review will describe their history, chemical structure and composition, taste profile, process of manufacture, brief comments on their market position. The historical note will reflect and highlight the role of the inventors responsible for their discoveries of low-calorie sweeteners, which has resulted in new and profitable global business ventures and has provided health and economic benefit to the society at large.

KEYWORDS: Low-calorie sweeteners, Sucralose, Stevioside, Aspartame, Acesulfame K, Saccharine, Cyclamate.

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I. SUCRALOSE (SPLENDA)

In response to the growing obesity and consequential diseases such as diabetes and heart conditions in the world in general and the West in particular, the use of low-calorie sweeteners in processed foods, soft drinks and pharmaceuticals has increased considerably. According to Global Opportunity Analysis and Industry Forecast, 2017-2023, the global market of these sweeteners was valued at \$1,807 million and is expected to reach at the \$2,134 million by 2023, registering a Compound Annual Growth Rate of 2.3% from 2017 to 2023.

Historical Background

For any invention to happen there has to be an appropriate environment they don't just jump out from nowhere, sucralose invention or discovery is one example of that scenario.

In 1965, Dr Leslie Hough, an eminent carbohydrate chemist and a Reader at the University of Bristol, received a research funding from the *International Sugar Research Foundation (ISRF)*, based in the United States, whose Director General, Dr John Hickson, was a man of vision and believed that sucrose (sugar) has great potential as a feedstock for products of commercial significance, and he trusted that that objective can be

achieved by the Bristol Group. It just so happened that Riaz Khan, who was an organic chemistry lecturer at the University of Sind in Pakistan, applied to Dr Hough to do Ph.D., with him, his application was accepted and he received the ISRF research position to carry out specifically research in the field of sucrose chemistry (in short, *Sucrochemistry*). That was beginning of the research venture in *Sucrochemistry*. Then came the involvement of the second group of key players in the *Sucrochemistry* game; Dr A. J. (Chuck) Vlitos and Dr Kenneth John Parker, Director of Research and General Manager, respectively, of Philip Lyle Memorial Research Laboratory (PLMRL), University of Reading. Riaz Khan had already joined the Chuck's Group (1970) as the Project Leader of the *Sucrochemistry* research. And Dr Hough had also moved from Bristol University to Queen Elizabeth College (QEC), London, as a full Professor. In addition to the common objectives i.e., *Sucrochemistry* of the two Groups, the favourable geographic location coupled with the encouragement of the visionary Greek New Yorker, Chuck Vlitos, played an important role in maintaining close research collaboration between PLMRL and QEC. The appropriate environment for invention was created and that led to the discovery of the intense sweetness of Sucralose® (Splenda®).

Yes, there too was an element of serendipity in the discovery of the sweetness of the 4,6,1',6'-tetrachloro-

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4,6,1',6'-tetradeoxygalactosucrose (TCS). However, the story of the discovery of Sucralose as presented in the Wikipedia, "Shashikant Phadnis was told to "test" a chlorinated sugar compound. Phadnis thought Hough asked him to "taste" it, so he did", does not correspond with the story published by Les Hough, his Ph.D., supervisor and one of the key inventors, in the *New Scientist* [1], which states, "Curiously, these substances (one of them 4,6,1',6'-tetrachloro-4,6,1',6'-tetradeoxygalactosucrose) might still have been waiting to be tested for sweetness had not Shashi Phadnis, a graduate researcher, misheard a telephone call requesting sample of the chlorinated sugars for **testing**. As the caller came from a large sugar company it is perhaps understandable that Phadnis thought that the company had requested them for **tasting** and so he tried them himself."

Professor Hough decided to withhold the names of the caller and the large sugar company; that gap in the story will be filled by naming them: the caller was Riaz Khan and the sugar company was Tate & Lyle. At the time of the discovery Khan's team was working on the effect of chlorine substituent's on the stability of the sucrose molecule against hydrolytic enzymes, for this he needed a pure sample of TCS, Khan knew that Les Hough's group had that compound and therefore he called Les for a small sample of pure TCS, who suggested to contact his Ph.D., student, Shashi Phadnis; Khan called Phadnis and asked to send him a small sample of his pure TCS, Phadnis enquired, *why do you want it?* Without going much in detail, he briefly answered, "it is for some test". It transpired later that Phadnis heard his **test** as **taste**, and as luck will have, he put a small sample of TCS on his tongue, thus the discovery of the sweetness of TCS was made.

Professor Bert Fraser-Reid in his book entitled, "From Sugar to Splenda", describes that event more elegantly, "that one Indian could have mistaken the accent of a fellow Indian, is the basis of a modern-day legend and it underscores the role of "luck" in scientific discoveries" [2].

It is of interest to note that the actual compound, Sucralose (Splenda), that was finally chosen as the candidate for further biological evaluation and marketing was synthesised in Khan's Group two years earlier, sitting on the shelf, waiting to be discovered and marketed [3-5].

Chemical Structure, Nomenclature, Physical Properties, and Taste Profile

Its systematic nomenclature is, 4,1',6'-trichloro-4,1',6'-trideoxygalactosucrose [1], it is registered with the trade names of *Sucralose*® and *Splenda*®, a crystalline compound, melting point 125°C, soluble in water, it is 600 times sweeter than sucrose, the sweet taste is similar to that of sucrose. Sucralose retains its sweetness over a wide range of temperatures and storage conditions in variety of applications, such as canned

fruit, low-calorie fruit drinks, baked goods, sauces and syrups. Sucralose can also be used as a sweetener in nutritional supplements, pharmaceuticals, vitamins, and mineral supplements.

Fig. 1 Structure of *Sucralose*® (*Splenda*®)

Process for the Production of Sucralose®

In the literature, Sucralose has been prepared in a number of ways [6-9], in this article only the most cost-effective processes will be described which may bear some similarity with the current manufacturing processes by different producers. Out of the eight hydroxyl groups in the sucrose (sugar) molecule, the chlorination reaction must take place selectively at the 4-secondary hydroxyl group in the glucose ring and at the two C-1' and C-6' primary hydroxyl groups in the fructose ring. The primary hydroxyl groups (6,1',6') are more reactive than the secondary hydroxyl groups (2,3,4,3',4') in sucrose towards halogenation; the reactivity of the secondary hydroxyl groups towards chlorination is in the order of 4 > 3 > 2, 3',4'. In order to achieve chlorination selectively at the C-4, 1', 6' positions to afford Sucralose, the three steps followed are: first production of Sucrose 6-acetate, second selective chlorination of the sucrose 6-acetate at the 4, 1', 6' position, and finally removal of the acetate (OCOCH₃) group from the 6-position using a base.

The Processes for the Production of Sucrose 6-acetate

Treatment of sucrose with 1,1,1-trimethoxyethane [6] or alternatively 1,1-dimethoxyethene [7] in DMF in the presence of toluene-*p*-sulphonic acid gave the 4,6-orthoestersucrose, which on hydrolysis, using aqueous acetic acid, proceeded with C4 → C-6 acetyl migration, to yield Sucrose-6-acetate in high yield. The sucrose-6-acetate on chlorination with the *Vilsmeier Reagent*, followed by deacetylation gave *Sucralose*® in overall high yield (Scheme 1).

Polymer-supported butyltin (IV) dichloride or 1,3-di(hydrocarbyloxy)-1,1,3,3 tetra (hydrocarbyl) distannoxane catalysed acetylation of sucrose afforded 6-O-acetylsucrose in high yield [8,9].

Sucrose 6-acetate has been prepared in high yield using *Polysaccharide-Scaffold-Bioreactor* embedded with an esterification catalytic enzyme [10,11].

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Final Step is the Chlorination of Sucrose 6-Acetate followed by Deacetylation to Afford Sucralose. Treatment of Sucrose 6-acetate with chloroformiminium chloride salt, tertiary amide, and acetic acid at a temperature below about 65°C, affording O-alkylformiminium chloride adduct, and then heating between about 85°C and about 95°C to sustain the chlorination afforded, after quenching the reaction, predominantly 4,1',6'-trichloro-4,1',6'-trideoxygalactosucrose-6-acetate. Deesterification of the 4,1',6'-trichloro-Sucrose-6-acetate with catalytic amount of a base, such as sodium methoxide, in methanol afforded Sucralose [12].

Scheme 1: Synthesis of Sucralose® via 4,6-orthoester

Sucralose (Splenda): Market Position

Tate & Lyle, a British-based multinational food ingredient company, originally one of the largest sugar companies in the world, was first producer of Sucralose in the world on an industrial scale. Today, there are two new big producers of Sucralose from China: JK Sucralose, the second largest producer of Sucralose in World, followed by Niutang.

Tate & Lyle PLC

Tate & Lyle is a British-based multinational food ingredients provider. It's well-known as the first manufacturer to produce and market Sucralose in the world. The company posted in the 2018 financial year report: Sucralose (SPLEND) sales volume change to be 12% and adjusted profit of £146 million.

Because of over-supply and other problems, Tate & Lyle has re-structured its Splenda Sucralose business to further focus on and strengthen its Specialty Food Ingredients. In April 2016, Tate & Lyle closed the Singapore facility, and consolidated all Splenda Sucralose production into one facility in Alabama, US.

Sucralose Inc.

JK Sucralose is the largest sucralose manufacturer in China, and only second to Tate & Lyle in the world. In 2009, JK Sucralose intervened in the ITC investigation, and successfully won the ITC Case against Tate & Lyle's Asserted Patents. After that, with several of its own Sucralose processing patents in the United State and China, JK Sucralose has taken great advantage in the field of sweeteners. However, in 2016, the production JK Sucralose has been limited because of environmental problem.

Niutang Changhai Food Additive Co. Ltd.,

They claim to be the China's leading manufacturer of high-intensity sweeteners, including Sucralose and aspartame.

The sales and profit figures for Sucralose from these two big Chinese Sucralose producers are not available.

II. STEVIOL GLYCIDES SWEETENER

Stevia rebaudiana Bertoni of the family *Asteraceae*, a member of the daisy family, is a perennial herb, a native of Mambay and Iguacu on the border of Brazil and Paraguay, where it has historically been used as medicine and as a high potency sweetener [13]. In the late 1990's, China was the major country that cultivated Stevia for the Japanese sweetener market. The current growing interest world-wide in natural, low-calorie, sweeteners, has led to the cultivation of stevia in many parts of the world, such as Europe, Asia, India, Australia, Brazil, China, Japan, Korea, New Zealand.

Since the approval of Stevia sweeteners in the US by the FDA in 2008, and by the European Union (E960) in 2011, industrial interest has risen considerably and stevioside and rebaudioside A extracted from Stevia leaves are now more or less widely used in Japan, South Korea, China, South-East Asia and South America, as a sweetener in a wide variety of foods. However, its economic impact, particularly in the developing country, is yet to be realised.

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Chemical Composition and Nomenclature of Steviol Glycosides

According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 84th meeting 2017, sweeteners from stevia plant are defined as, "Steviol glycosides consist of a mixture of compounds containing a steviol, diterpenoid backbone, conjugated to any number or combination of the principal sugar moieties (glucose, rhamnose, xylose, fructose, arabinose, galactose and deoxyglucose) in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni."

The steviol (**Fig. 2**; **R1 = R2 = H**) is the aglycon of the steviol glycoside; out of various steviol glycosides extracted from the dry leaves of *stevia rebaudiana*, of particular interest from their sweetness characteristics are the glycosides of diterpenoid ent-kaurene type. The eight steviol glycosides isolated and characterised are: dulcoside A (50-120 times sweeter than sucrose), rebaudioside A (250-450 x), rebaudioside B (300-500 x), rebaudioside C (50-120 x), rebaudioside D (240-450 x), rebaudioside E (100-125), steviobioside (100-125 x), and stevioside (300 x) [14-16].

Fig. 2 STEVIOL (R1 = R2 = H)

The most abundant sweetener in the dry leaf of stevia is stevioside (4-13%) with sweetness power of 300 times that of sucrose (sugar), the next in sweetness intensity is rebaudioside A (2-4%) followed by rebaudioside B and C (1-2%), D, F and steviobioside. It is of interest to note that the sweetness profile of rebaudioside A is better than the stevioside, which is a bit astringent.

The systematic nomenclature of some of the steviol glycosides as follow:

- Steviol monoside (SVG1); R1 = H, R2 = Glc β1- : 13-[(β-D-(-glucopyranosyl) oxy] kaur-16-en-18-oic acid.
- steviol monoside A (SVG1); R1 = Glc β1, R2 = H: 13-(hydroxy) kaur-16-en-18-oic acid, β-D-glucopyranosyl ester.

- Rubuside (SVG2); R1 = R2 = Glc β1: 13-[(β-D-(-glucopyranosyl) oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester.
- Steviobioside (SVG2); R1 = H, R2 = Glc β (1-2) Glc β1- : 13-[(β-D-(2-O-glucopyranosyl - β-D-glucopyranosyl) oxy] kaur-16-en-18-oic acid.
- Stevioside (SvG3); R1 = Glc β1, R2 = Glc β (1-2) Glc β1- : 13-[(2-O- β -D-glucopyranosyl- β -D-glucopyranosyl) oxy] kaur-16-en-18-oic acid β-D-glucopyranosyl ester.
- Stevioside A or Rebaudioside KA (SvG3); R1 = Glc β (1-2) Glc β1-, R2 = Glc β1- : 13-[(2-O-β-D-glucopyranosyl) oxy] kaur-16-en-18-oic acid, -O- β-D-glucopyranosyl-deoxy-(1,2)- O-[β-D-glucopyranosyl] ester.
- Dulcoside A: 13 - [2-O- α-L-rhamnopyranosyl- β -D-glucopyranosyl] oxy] kaur-16-en-18-oic acid β -D-glucopyranosyl ester.
- Stevioside B (SvG3); R1 = Glc β- (1-3) Glc β1-, R2 = Glc β1- : 13-[(2-O-β-D-glucopyranosyl) oxy] kaur-16-en-18-oic acid, -O- β-D-glucopyranosyl-deoxy-(1,3)- O-[β-D-glucopyranosyl] ester.
- Rebaudioside A: 13- [(2-O- β -D-glucopyranosyl-3-O-β -D-glucopyranosyl- β -D-glucopyranosyl) oxy] kaur-16-en-18-oic acid β -D-glucopyranosyl ester.
- Rebaudioside B (SvG3); R1 = H, R2 = Glc β (1-2) [Glc β1-3] Glcp1- : 13-[(2-O-β-D-glucopyranosyl)-3-O-β -D-glucopyranosyl- β -D-glucopyranosyl) oxy] kaur-16-en-18-oic acid.
- Rebaudioside C: 13-[(2-O- α -L-rhamnopyranosyl-3-O-β -D-glucopyranosyl- β -D-glucopyranosyl)oxy] kaur-16-en-18-oic acid β -D-glucopyranosyl ester.

Fig. 3 REBAUDIOSIDE A

Extraction Process of Steviol Glycosides

The extracts of leaves of *Stevia rebaudiana* Bertoni generally contain a high percentage of the glycoside diterpenes stevioside and rebaudioside A. However, the purity and the yield of the product are dependent on the quality of the raw material. In the literature there are many reports describing the steviol glycosides extraction using water, acidified water, organic solvents, ion-exchange resins, and super critical fluid extraction methodology. As a general procedure, the leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and

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concentrate the component steviol glycosides; the resin is washed with a solvent alcohol to release the glycosides and the product is recrystallized from methanol or aqueous ethanol; ion exchange resins may be used in the purification process; and the final product may be spray-dried. The two standard processes, Paraguay and Japanese, described in the FAO WHO reports, for the isolation of sweetener principles of *Stevia rebaudiana* leaves will be illustrated here [17].

The **Paraguay Process** involves: (Step a), treatment of the dry leaves with hot water for about half an hour, the water containing the sweetener principles, pigments and other water-soluble components are filtered (drained), cooled to room temperature, insoluble material removed such as by centrifuge; (Step b), the sweetener and pigments are partitioned from the aqueous phase by shaking with water insoluble organic solvents such as butanol, isobutanol, hexane, benzene or chloroform; and (Step c), the organic phase containing the sweetener is concentrate and crystallised from hot methanol, the crystalline product is washed with cold methanol, and recrystallized from methanol and water to afford steviol glycosides of 97-98% purity with about 4% of water.

The Japanese Process involves: (Step a) the dry stevia leaves are extracted two or three times with hot water (50 -60°C); (Step b) treated with calcium hydroxide and aluminium sulphate to effect flocculation; (Step c) treated with macroporous adsorption resin to adsorb the steviol glycoside, then released using alcohol; (Step d) the sweetener in aqueous solution was deionised using ion exchange resin mix, water concentrated under reduced pressure, passed through a microfilter (1 µm), sterilized at 120° C for 1 min, and spray dried.

Product Description and Analysis

Commercial steviol glycosides preparations are white to light yellow powders and are freely soluble in ethanol and water (50:50) mixtures. The powders can be odourless or have a slight characteristic odour. Its sweetness intensity in water solution is 200 to 300 times that of sucrose. *Stevia* has had comparatively extended market exposure and there is now abundance of commercially accessible *Stevia* products worldwide [18-24].

Different methods of analytical techniques have been used to determine the composition of the steviol glycosides in the mixture; of these techniques, the infrared (IR), liquid chromatographic, and ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) have been developed and validated to evaluate steviol glycosides in commercial samples e.g., *Truvia*[®] [25-27].

III. ASPARTAME

Aspartame, N-(L-α-Aspartyl)-L-phenylalanine 1-methyl ester, (Fig. 4) is one of the old synthetic high-

intensity sweeteners. It was discovered in 1965 by James M. Schlatter, an organic chemist working on a peptide based anti-ulcer drug candidate, which involved the peptide synthesis, for G.D. Searle & Company. During his research one day Schlatter serendipitously licked his finger and discovered the intense sweetness of the aspartame (Ref. 28).

Aspartame has received considerable exposure in the literature and therefore only some key issues will be elaborated in this review, such as its safety profile, selected enzymatic, chemical and microbial processes of its preparation.

Safety Status of Aspartame

Aspartame was first approved by the FDA in 1982 for use in dry foods and then in 1983 it received approval for use in carbonated beverages, and ten years later for other beverages, baked goods, and confections. In 1996, the FDA removed all restrictions. And today, it has been approved by almost every country in the world. It is marketed worldwide with such trade names as, Equal, NutraSweet, and Canderel. It is commonly used in diet soft drinks, and as table-top sweetener.

Aspartame has been deemed safe for human consumption by over 100 regulatory agencies in their respective countries, including the FDA, the UK Food Standards Agency, the European Food Safety Authority (EFSA) and Canada's Health Canada. In 2013 EFSA published its full risk assessment on aspartame with the conclusion that it is safe for general population including infants, children and pregnant women. The Chair of the ANS Panel, Dr A. Mortensen stated that, "this opinion represents one of the most comprehensive risk assessments of aspartame ever undertaken..." [29,30].

The Acceptable Daily Intake (ADI) of 40 mg/Kg body weight/day is considered safe; however, patients suffering from medical condition phenylketonuria (PKU) the ADI is not applicable, as they required strict adherence to a diet low in phenylalanine, an amino acid component of aspartame.

Fig. 4: Aspartame N-(L-α-Aspartyl)-L-phenylalanine 1-methyl ester

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Manufacture of Aspartame**An Enzyme Catalysed Synthesis of Aspartame**

An enzyme catalysed synthesis of Aspartame has been described involving three components of the process: (a) development of enzyme catalysed deformylation formylmethionyl peptide deformylase activity with a co-factor group 5 to 11 bivalent metal ions, (b) selective

preparation of aspartame from a mixture N-formyl- α - and β -L-aspartyl-L-phenylalanine compounds, and finally (c) one-pot enzymatic synthesis aspartame from N-formyl-L-aspartic acid and L- or D, L-phenylalanine methyl ester [31].

Chemical Synthesis of Aspartame (APM)

Scheme 2: APM Synthesis

An elegant synthesis of aspartame has been described involving a number protecting and deprotecting strategy of alpha amino, alpha carboxyl and side chain groups. The reaction sequence takes the following steps: (a) the NH_2 group of the aspartic acid is first protected by carboxybenzyl (Cbz) group by treatment with benzyloxy carbonyl chloride in the presence of a base; (b) then the two carboxylic groups are benzylated with Benzyl alcohol (BnOH) in the presence p-toluenesulphonic acid (TsOH) as catalyst in refluxing toluene; (c) the third step involves selective hydrolysis of one of the two benzyl ester groups, using lithium hydroxide (LiOH), to afford the required intermediate i.e., the free carboxylic group to link with the phenylalanine methyl ester in the last step; (d) this free carboxylic group is activated by treatment with TsOH catalyst to afford the corresponding 2,4,6-trichlorophenol ester for the final coupling reaction; (e) this active ester derivative of aspartic acid is coupled with phenylalanine methyl ester

hydrochloride in the presence of a base to give the dipeptide derivative; which finally (f) on hydrogenation in the presence of palladium catalyst gives the sweetener aspartame[31,32].

Microbial Process for Aspartame

Aspartame has been produced using a cloned microorganism. A DNA which codes for a large stable peptide comprised of the repeating amino acid sequence (Asp-Phe) has been inserted into a cloning vehicle, which in turn is introduced into a suitable host microorganism. The host microorganism was cultured and the large peptide containing the repeating Asp-Phe sequence was harvested therefrom. The free carboxyl group of the large peptide was benzylated and then hydrolysed to benzyl Asp-Phe dipeptides. This dipeptide was methylated and then debenzylated to form aspartame [32].

IV. NEOTAME

Neotame, a neoheptyl derivative of aspartame, is a high-intensity sweetener with sweetness potency of neotame is 60,00-10,000 times that of sucrose and as compared to aspartame (APM) it is 30-60 times sweeter. It has many other advantages over aspartame, such as it is significantly more stable in high temperatures, high pH conditions and neutral medium, providing extensive shelf life. Neotame has improved taste profile than APM and has flavour enhancing properties. However, as compared to sucrose, neotame has slight lingering sweet after taste, which can be overcome by blending with other sweeteners. Neotame (**Fig. 5**) blends well with other high-intensity sweeteners, such as APM, acesulfame K, sucralose, saccharine, and cyclamate. It is soluble in water, ethanol and ethyl acetate. Its favourable solubility pattern in different solvents makes it an attractive candidate for food and beverage producers.

Chemical Structure

Fig. 5: Neotame N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester

Synthesis of Neotame

Scheme 3

A low cost, high purity, synthesis of neotame, a derivative of N-(L-α-Aspartyl)-L-phenylalanine 1-methyl ester (APM), has been achieved by catalytic hydrogenation of a mixture of APM and 3,3-dimethylbutyraldehyde with a

catalyst such as Palladium on carbon, as shown in the following reaction **Scheme 3** [33].

Safety and Regulatory Status

Neotame has been thoroughly assessed and determined safe by international and national authorities; it has not been associated with any adverse health effects.

Neotame has been approved by Codex as a high-intensity sweetener in various foods. Over sixty countries/areas such as Mainland China, Australia, New Zealand, USA, Canada, and the European Union (E961) have approved neotame as a sweetener.

V. ALITAME

Alitame is a dipeptide of L-aspartic acid and D-alanine with a terminal N-substituted tetra methylthietanyl-amine moiety (**Fig. 6**). It was developed by Pfizer in the early 1980s and currently marketed in some countries under the brand name of **Aclame**. Its sweetness potency is 2000 times that of sucrose and has no aftertaste. Its stability profile is considerably better than aspartame, its half-life under hot or acidic conditions is twice as long.

Incorporation of D-alanine as the second amino acid, in place of L-phenylalanine as in aspartame, and the addition of the novel terminal amide in alitame, formed from 2, 2, 4, 4-tetramethyl-3-thietane, provides increased steric and lipophilic bulk resulting in such high sweetness potency, and also its heat stability as compared to aspartame.

Alitame is a crystalline powder, odourless and non-hygroscopic; It is soluble in water and forms clear solutions; it is also soluble in polar solvents such as methanol, ethanol and propylene glycol; it is not soluble in nonpolar solvents such as fats, oils or chloroform [34-37].

Chemical Structure

Fig. 6 ALITAME L-α-aspartyl-N-(2, 2, 4, 4-tetramethyl-3- thietanyl)-D-alaninamide

Synthesis of Alitame

Scheme 4

Alitame is a dipeptide of L-aspartic acid and D-alanine with a terminal N-substituted tetra methylthietanyl-amine moiety; it can be prepared by a number of ways. One of the routes described here (*see Reaction Scheme 4*) starts from diisopropyl ketone (DIPK), which on treatment with sulphur dichloride (SCl_2) affords compound **2**, successive treatment of **2** with potassium tertiary butoxide (t-BuOK) followed by reaction of the resulting **2**, 2, 4, 4-tetramethylthietanyl ketone compound **3** with amine hydroxide (NH_2OH) in the presence sodium boron hydride (NaNBH_4) afforded 2, 2, 4, 4-tetramethylthietan-1-amine (**4**), which on treatment with D-alanine gave 3-(D-alaninamido)-2,2,4,4-tetramethylthietane (**5**). A protected derivative of aspartic acid (**6**), L-aspartic acid N-thiocarboxyanhydride (**7**), obtained by treatment with trifluoroacetic anhydride in *N,N*-dimethylformamide (DMF). Compound **7** was then treated with compound **4**, in aqueous in aqueous solution with vigorous stirring; maintain the pH of 8.5-9.5. The pH was adjusted to 5.5 and p-toluenesulphonic acid monohydrate was added over a period of one hour. The p-toluenesulphonate salt of alitame was collected, deionised using liquid anion exchange resin, and partitioned between water and dichloromethane, the aqueous layer containing alitame was treated with carbon, clarified by filtration, cooled crystallised to give (**Fig. 6**, Alitame) [38-42].

Regulatory Profile

JEFCA reviewed safety data on alitame in 2002 and the committee concluded that there was no evidence that alitame is carcinogenic. Alitame has already been approved in Mexico, India, Colombia and China as well as Australia and New Zealand. Danisco has withdrawn its petition for using alitame as a sweetening agent or flavoring in food in USA [40].

VI. ADVANTAME

Advantame is a new ultrahigh-intensity noncaloric sweetener derived from aspartame and has recently (2014) been approved by the United States FDA for human consumption. Its sweetness potency has been described as being approximately 20,000 times as sweet as sucrose, which **means** that a very low concentration of advantame matches the sweetness potency of a dilute (3%) sucrose and has a clean sensory taste. It is produced and marketed by Ajinomoto of Japan. [I. Bishay I, R.G. Bursey RG, in *Alternative sweeteners*, p. 31-45 (2012), L.O. Nabors, editor.; Boca Raton, CDC Press]. It is produced and marketed by Ajinomoto of Japan.

Chemical Composition and Structure

Advantame is derived from aspartame and is composed three components: 3-hydroxy-4-methoxyphenylpropane, aspartic acid and phenylalanine methylester; its IUPA name is: N-[N-[3-(3-hydroxy-4-

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methoxyphenyl)-propyl]-L- α -aspartyl]-L-phenylalanine-1-methyl ester (Fig. 7).

Fig. 7 Advantame

Synthesis of Advantame

The production of advantame involves three chemical steps: (a) preparation of the principal intermediate, 3-hydroxy-4-methoxycinnamaldehyde (HMCA) from isovanillin by treatment with sodium hydroxide in aqueous methanol, (b) followed by selective reduction to afford the HMCA to afford 3-(3-hydroxy-4-methoxyphenyl) propionaldehyde (HMPA), finally (c) N-alkylation of aspartame with HMPA to yield advantame [43-45].

Safety and Regulatory Status

Advantame has been found to be safe for consumption in foods and beverages by the general population; it is a safe alternative for people with diabetes, since consumption of advantame does not impact blood glucose levels.

Advantame has been approved as a general sweetener in the US FDA (2014), Mexico, Canada, Europe, Japan, Israel, Turkey, Australia, New Zealand, Singapore and many others that are being added regularly. INS No. 969 has been assigned to advantame at the 45th Session of the CCFA in 2013 (FAO/WHO, 2013); New tentative specifications were prepared at the 77th JECFA (2013). Interestingly, advantame has also been approved as an artificial flavour [46].

VII. ACESULFAME K

The low-calorie, high-intensity sweetener abbreviated as acesulfame K, is the potassium salt of 6-methyl-1,2,3-oxathiazine-4(3H)-one 2,2-dioxide. It was developed after the accidental discovery by Karl Clauss and Harald Jensen in 1967 [47-53].

Acesulfame K is about 200 times the sweetness of sucrose. Like saccharin, at higher concentration, it has a little bitter after taste; for this reason and for its synergist characteristics, it is normally blended with

other artificial sweeteners, such as sucralose and aspartame. In addition, acesulfame K has heat and chemical stability, which offers considerable advantage to the food industry. Acesulfame K is not metabolised by the body and is excreted completely unchanged; hence, it has no caloric value.

Fig. 8 6-methyl-1,2,3-oxathiazine-4(3H)-one 2,2-dioxide

Synthesis

Synthesis of Acesulfame K has been described using the following sequence of reactions (*see Scheme 1*): (a) Reaction of trialkylammonium amidosulfonic acid (1) with diketene (2) in an inert solvent in the presence of triethyl amine resulted into acetoacetamide salt (3), the key starting intermediate in the synthesis of sweetener; in the final step (b) compound 3 is treated with a cyclising agent to create the dihydrooxathiazinone ring system, which on treatment with potassium hydroxide gave acesulfame K. This method lends itself to produce the sweetener on a large scale using a continuous process [53,54].

Safety and Regulatory Profile

Acesulfame K (E 950) has been widely approved as a food additive by various independent agencies such as Safety Committee of Food (SCF) in 2000, now the EFSA; FDA in 2003; and JECFA in 1990. Acesulfame K has been approved for a variety of uses as a sweetener in more than 100 countries, including USA, Japan, Norway, Switzerland, Australia, The ADI level of acesulfame K has been fixed at 15 mg/body weight by JECFA and the FDA, and European level has been determined at 9 mg/body weight [55].

VIII. THAUMATIN

Thaumatococcus (Talin®) is isolated from the Katemfe fruit (Thaumatococcus daniellii.) found in the West African

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rainforest. In the 1970s, Tate & Lyle research institute, Philip Lyle Memorial Research Laboratory, based at the University of Reading, explored thaumatin as a natural David Snodin and John Daniel. The work carried out on thaumatin included: extraction of the protein from the Katemfe fruit purification, characterisation of the proteins, toxicological studies, and food formulations. Tate & Lyle were aiming to the market the sweetener but

low-calorie sweetener; this work was led by a team comprising of Drs Wilson Nicol, John Higginbotham,

they had another high-intensity sweetener, sucralose, under development at the same time. When the crunch came to which one to take to the market, the decision went for sucralose, and work on Thaumatin was left behind [56].

Scheme 5 6-Methyl-3,4-dihydro-1,2,3-oxathiazin-4-one 2,2-dioxide

Preparation and Properties

Thaumatococcus is purified by selective ultrafiltration. However, small amounts of organic non-protein impurities and some carbohydrate constituents of plant gums and mucilages remain in the commercial product.

Thaumatococcus proteins have also been prepared by genetic engineering. In 1990, researchers at Unilever reported the isolation and sequencing of the two principal proteins found in thaumatococcus, which they dubbed **thaumatococcus I** and **thaumatococcus II**. These researchers were also able to express thaumatococcus in genetically engineered bacteria. More recently, Japanese scientists have obtained Thaumatococcus I using an expression vector, which possesses three copies of thaumatococcus gene containing the 22-amino acid pre-sequence. The expression yield was three -fold higher than when the α -factor secretion signal from *saccharomyces cerevisiae* [57,58].

The molecular weights of the thaumatococcos are approximately 22,000. There are no unusual side-chains, atypical peptide linkages, or end-groups. Extensive disulfide cross-linking is crucial in maintain its tertiary structure, thermal stability, resistance to denaturation, and its sweetness. Some proteins in thaumatococcus family of sweeteners are 2000 times the sweetness of sucrose;

thaumatococcus taste is different from the taste of sugar; perception of its sweetness lasts a long time, leaving a liquorice-like after taste; it is highly soluble in water and

is stable under acidic conditions. Thaumatococcus is used in foods not only for its sweetness but also as flavour modifier.

Safety and regulatory Profile

Thaumatococcus has been extensively evaluated in animals and humans and has shown no adverse effect to health. It has been approved as a sweetener in the European Union (E957) and elsewhere. In the US, it is a Generally Recognized as Safe flavoring agent (FEMA GRAS 3732). Thaumatococcus was approved by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1985. Similar approval exists in Switzerland, Canada, Israel, Hongkong, Singapore, Japan, Australia, New Zealand, and South Africa.

IX. MONK FRUIT SWEETENER

Siraitia grosvenorii (Luo han guo) monk fruit is a native of southern China and Thailand, it belongs to the herbaceous perennial vine of the Cucurbitaceae (gourd) family, it is a cultivated plant and is rarely found in the wild. Historically, its extract has been used by the locals as a sweetener in food and medicines.

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The primary sweet components of the Monk fruit extracts are cucurbitane glycosides, which are generally defined as mogrosides; there are at least eleven types of mogrosides in monk fruit extract they are defined as mogrosides II, III, IV, V, and VI, Mogroside II E, Mogroside III E, Mogroside A, Neomogroside, Siamenside I, and 11-Oxo-Mogroside V. Mogroside IV is a component with no sweet taste. Mogroside A, Mogroside II, and Neomogroside are bitterness substance. Siamenside I is the sweetest component, whose sweetness is approximate 500-600 times of sucrose but its content in the fruit is very low. The mogroside V content is highest,

as compared to other mogrosides, in Monk fruit extracts, its sweetness potency is approximate 250-350 times that of sucrose [59].

Mogroside has not yet gained wide usage because it has a cost factor double that of Stevia (Stevioside and Rebaudioside A); there are fewer orchards that can grow monk fruit as compared to stevia plants; in addition, it has not yet gained approval in Europe.

Fig. 9 MOGROSIDE V

Production Process

A simple process for the extraction of Monk fruit sweetener has been developed, which involves the following steps: (a) The fresh fruit is crushed to release its natural sweet juice; (b) the resulting juice is mixed with hot water to make a sweet infusion; (c) the extract is then filtered and passed through a separation process and to further extract the sweet components, mogrosides; (d) the extract is purified and decolourised by treatment with ion exchange resin such as a macroporous **polymeric** adsorbent with aqueous ethanol; and (e) the resin is eluted with 50% ethanol and the eluate solution is concentrated and the concentrate is sprayed dried to produce a fine powder of mogroside with sweetness around 160 – 200 times sweeter than sugar. Further purification can be performed with reverse phase silica gel column chromatography using 30% ethanol [60, 61].

Safety and Regulatory Status

Monk fruit has already received regulatory approval in Asia, the United States and **some** Latin American countries, so looking at its use in those markets may provide some clues to its promise in Europe. In 2010, the U.S., monk fruit was granted GRAS (generally recognized as safe) status [62].

X. SACCHARIN

Sodium saccharin (benzoic sulfimide) is the oldest synthetic sweetener. It was first discovered and synthesised by Remsen and Fahlberg in 1879 [63]. It is about 300-400 times as sweet as sucrose but it has a bitter or metallic aftertaste at high concentration. It is widely used in food industry in soft drinks, baked goods, jams, chewing gum, canned fruits, candy, salad dressings and pharmaceutical products. In 1959 this sweetener was classified by FDA as GRAS; from 1907 onwards, saccharin was used as a replacement for sugar in foods for diabetics. A recent market report revealed that saccharin market would reach a value of US\$ 260 Million in 2017 [64].

The chemical formula for saccharin is $C_7H_5NO_3S$ and its IUPAC nomenclature is: 2H-1λ6,2-benzothiazol-1,1,3-trione. The molar mass for saccharin is 183.19 grams per mole. Structurally, saccharin is made up of two connected rings; the first ring is a phenyl ring and the second is a 5 membered ring with nitrogen, a carboxyl group, and a sulfone group beside the nitrogen. Saccharin has been produced in different ways. Remsen & Fahlberg's original route starts with toluene but yields from this starting point are small. An improved synthesis was developed at the Maumee Chemical Company of Toledo, Ohio, in 1950. Anthranilic acid successively reacts with nitrous acid, sulphur dioxide and chlorine followed by the treatment of ammonia to yield saccharin. Another route begins with *O*-chlorotoluene [65].

Safety and Regulatory Status

Saccharin was banned in 1981 because it was suspected that it may cause urinary bladder cancer but a renewed reinvestigation in 1990 by Ellwein and Cohen in rats when fed at high doses in two-generation studies the results did not support the assertion that sodium saccharin is the urinary bladder carcinogenic, similar results were observed with mice, hamsters or monkey; and numerous epidemiological studies provide no clear or consistent evidence to support the assertion that sodium saccharin increases the risk of bladder cancer in the human population: Saccharin is deemed "hazardous" In United States and abroad [65-69].

Fig. 10: SACCHARINE

XI. CYCLAMATE

Cyclamate, the second low-calorie was discovered in 1937 by Michael Sveda at the University of Illinois, USA. The patent for cyclamate was initially bought by DuPont, who later sold to Abbott Laboratories, which undertook the necessary studies and submitted a New Drug Application in 1950. In 1958, it was given GRAS status by the FDA for use as a food additive.

Cyclamate is a salt of cyclohexylsulfamic acid; sodium cyclamate is used as non-nutritive sweetener and the analogous calcium salt used specially in low sodium diets; cyclamate is 30 times sweeter than sucrose, it has a slow of sweetness and higher concentration a distinct sweet-savour lingering after taste. It exhibits good sweetness synergy with saccharin. Cyclamate is soluble in water and its solubility can be increased by preparing the sodium or calcium salt; it has good stability in soft drink formulations within the pH range 2-10.

Structure and Synthesis

Fig. 11 CYCLAMATE

Cyclamate is prepared by the sulfonation of cyclohexylamine with either sulfamic acid or sulphur trioxide; it is used as the sodium or calcium salt.

Safety and Regulatory Status

Cyclamate itself shows very low toxicity but its metabolite, cyclohexylamine, continues to raise questions on its overall safety status during the long-term administration. It is permitted for use as a sweetener in over 130 countries. The acceptable daily intake (ADI) for cyclamate of 0-7 mg/Kg bw has been calculated by both the scientific committee of food (SCF) and the joint expert committee on food additives (JECFA) based on the "no observed adverse effect level" (NOAEL).

The metabolism of cyclamate to cyclohexylamine in humans during long-term administration continues to be a concern. Recent studies have provided new data on the extent to which individuals convert cyclamate to cyclohexylamine during long term consumption. This study gives first true indication of possible exposure to cyclohexylamine from cyclamate metabolism in humans over a period that is toxicologically relevant to reevaluate the ADI of cyclamate. As cyclamate is only about 40 times the sweetness of sugar, commercial interest in this sweetener is not high [70-72].

TABLE: Some Properties of Low Calorie High intensity Sweeteners

Sweetener	m.p. (°C)	Solubility in Water (%)	Relative Sweetness	Regulatory Status E Number & US FDA Approval (GRAS)	ADI Values (mg/Kg body weight)
Sucralose (Splenda)	125	25.7	500	E955 GRAS	0-15
Steviol rebaudia	198	0.125	300	E 960	0-4
Aspartame	246	1.0	200	E 951 GRAS	0-40
Neotame	80.9 – 83.4	1.26	6,000 – 10,000	E 691 GRAS	0.2
Alitame	136 – 147	1.3	2,000	E 956 GRAS	0-1
Advantame	99.3 – 101.5	0.99	20,000	GRAS	32.8
Acesulfame-K	200	27.0	200	E 950 GRAS	0-9
Neohesperidin dihydrochalcone	156 – 158	0.05	250	E 959 GRAS	0-5
Saccharin (Na ⁺)	228.8 – 229.7	83.3	500	E 954 GRAS	0-5
Cyclamate (Na ⁺)	169 – 170	19.5 ^a	40	E 952 GRAS	0-7
Thaumatococin	-	60.0	2500	E 957 GRAS	
Monk Fruit	-	100	150 – 200	GRAS	

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XX Sucrose = 1 (relative to a 10% sucrose solution)
Source: R. Khan and P.A. Konowicz, SWEETENERS in *Encyclopedia of Analytical Science*, 5085-5090, 1995; M.A. Godshall, The Expanding World of Nutritive and Non-Nutritive Sweeteners, *Sugar J.*, 69, 12-20, 2007.

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