

## Food and Nutritional Analysis I Sweeteners<sup>☆</sup>

MC Yebra-Biurrun, University of Santiago de Compostela, Santiago de Compostela, Spain

© 2013 Elsevier Ltd. All rights reserved.

### Introduction

Sweeteners are defined as food additives that are used or intended to be used either to impart a sweet taste to food or as a tabletop sweetener. Tabletop sweeteners are products that consist of, or include, any permitted sweeteners and are intended for sale to the ultimate consumer, normally for use as an alternative to sugar. Foods with sweetening properties, such as sugar and honey, are not additives and are excluded from the scope of official regulations. Sweeteners are classified as either high intensity or bulk (Figure 1). High-intensity sweeteners possess a sweet taste, but are no caloric, provide essentially no bulk to food, have greater sweetness than sugar, and are therefore used at very low levels. On the other hand, bulk sweeteners are generally carbohydrates, providing energy (calories) and bulk to food. These have a similar sweetness to sugar and are used at comparable levels.

### High-Intensity Sweeteners

High-intensity sweeteners (also called nonnutritive sweeteners) can offer consumers a way to enjoy the taste of sweetness with little or no energy intake or glycemic response and they do not support growth of oral cavity microorganisms. Therefore, they are principally aimed at consumers in four areas of the food and beverage markets: treatment of obesity, maintenance of body weight, management of diabetes, and prevention and reduction of dental caries. There are several different high-intensity sweeteners. Some of the sweeteners are naturally occurring, while others are synthetic (artificial) or semisynthetic. Most of the more commonly available high-intensity sweeteners and/or their metabolites are rapidly absorbed in the gastrointestinal tract. For example, acesulfame-K and saccharin are not metabolized and are excreted unchanged by the kidney. Sucralose, stevioside, and cyclamate undergo degrees of metabolism, and their metabolites are readily excreted.

|            |                                   |                         |                                                 |                                           |                                   |                                               |
|------------|-----------------------------------|-------------------------|-------------------------------------------------|-------------------------------------------|-----------------------------------|-----------------------------------------------|
| Sweeteners | High-intensity<br>(non-nutritive) | Synthetic<br>artificial | Acesulfame<br>Alitame<br>Aspartame<br>Cyclamate |                                           | Neotame<br>Saccharin<br>Sucralose |                                               |
|            |                                   | Semisynthetic           | Heohesperidine dihydrochalcone                  |                                           |                                   |                                               |
|            |                                   | Natural                 | Glycyrrhizin<br>Stevioside<br>Thaumatococin     |                                           |                                   |                                               |
|            | Bulk<br>(nutritive)               | Caloric                 | Sucrose<br>Molasses<br>Honey and maple syrup    |                                           |                                   |                                               |
|            |                                   |                         | Starch-derived sweeteners                       |                                           | Glucose<br>Fructose               |                                               |
|            |                                   | Low-caloric             | Sugar<br>alcohols                               | Monosaccharides                           |                                   | Erythritol<br>Mannitol<br>Sorbitol<br>Xylitol |
|            |                                   |                         |                                                 | Disaccharides                             |                                   | Isomalt<br>Lactitol<br>Maltitol               |
|            |                                   |                         |                                                 | Hydrogenated starch<br>hydrolyzates (HSH) |                                   |                                               |
|            | Tagatose                          |                         |                                                 |                                           |                                   |                                               |

Figure 1 Classification of sweeteners.

<sup>☆</sup>Change History: March 2013. MC Yebra-Biurrun updated Figure 2, Table 2, Further Reading, and Relevant Websites.  
This is a reprint of M.C. Yebra-Biurrun, Sweeteners, Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, Elsevier, 2013.

**Table 1** Sweetness characteristics of high-intensity sweeteners (the scale uses sucrose as a sweetness of 1, and compares the sweetness of other sweeteners to sucrose).

| Sweetener                      | Relative sweetness (sucrose = 1) | Aftertaste                                                                  |
|--------------------------------|----------------------------------|-----------------------------------------------------------------------------|
| Acesulfame-K                   | 150–200                          | Very slight bitter                                                          |
| Alitame                        | 2000–3000                        | No unpleasant                                                               |
| Aspartame                      | 160–220                          | Prolonged sweetness                                                         |
| Aspartame-acesulfame salt      | 350                              | –                                                                           |
| Cyclamate                      | 30–40                            | Prolonged sweetness. At high concentrations a distinct sweet-sour lingering |
| Glycyrrhizin                   | 50–100                           | Prolonged sweetness (liquorice)                                             |
| Neohesperidine dihydrochalcone | 1000–2000                        | Lingering menthol-liquorice                                                 |
| Neotame                        | 7000–13 000                      | No unpleasant                                                               |
| Saccharin                      | 300–600                          | Bitter metallic                                                             |
| Stevioside                     | 250–300                          | Bitter and unpleasant                                                       |
| Sucralose                      | 400–800                          | No unpleasant                                                               |
| Thaumatococin                  | 2000                             | Liquorice                                                                   |

**Table 2** Characteristics and selected physical properties of high-intensity sweeteners.

| Sweetener     | Molecular formula                                               | Molecular weight | m.p. (°C)   | Solubility in H <sub>2</sub> O at 20 °C (%) | ADI (mg kg <sup>-1</sup> bodyweight)                                            |            |
|---------------|-----------------------------------------------------------------|------------------|-------------|---------------------------------------------|---------------------------------------------------------------------------------|------------|
|               |                                                                 |                  |             |                                             | JECFA                                                                           | SCF/EFSA   |
| Acesulfame-K  | C <sub>4</sub> H <sub>4</sub> NO <sub>4</sub> SK                | 201.2            | 200         | 27                                          | 15                                                                              | 9          |
| Alitame       | C <sub>14</sub> H <sub>25</sub> N <sub>3</sub> O <sub>4</sub> S | 331.43           | 136–147     | 14.3 (pH 7)                                 | 1                                                                               | 0.1        |
| Aspartame     | C <sub>14</sub> H <sub>18</sub> N <sub>2</sub> O <sub>5</sub>   | 294.31           | 246         | 1                                           | 40                                                                              | 40         |
| A-A salt      | C <sub>18</sub> H <sub>23</sub> O <sub>9</sub> N <sub>3</sub> S | 457.46           | –           | –                                           | covered by the ADI values previously established for aspartame and acesulfame-K |            |
| Cyclamate     | C <sub>6</sub> H <sub>13</sub> NO <sub>3</sub> S                | 179.24           | 169–170     | 7.7<br>Na salt: 19.5                        | 11                                                                              | 7          |
| Glycyrrhizin  | C <sub>42</sub> H <sub>62</sub> O <sub>16</sub>                 | 822.93           | –           | –                                           | Not established                                                                 | 5          |
| NHDC          | C <sub>28</sub> H <sub>36</sub> O <sub>15</sub>                 | 612.6            | 156–158     | 0.05                                        | Not established                                                                 | 5          |
| Neotame       | C <sub>20</sub> H <sub>30</sub> N <sub>2</sub> O <sub>5</sub>   | 378.46           | 80.9–83.4   | 1.3                                         | 2                                                                               | 2          |
| Saccharin     | C <sub>7</sub> H <sub>5</sub> NO <sub>3</sub> S                 | 183.18           | 228.8–229.7 | 0.3<br>Na salt: 83                          | 5                                                                               | 5          |
| Stevioside    | C <sub>38</sub> H <sub>60</sub> O <sub>18</sub>                 | 804.9            | 198         | 0.125                                       | 4                                                                               | 4          |
| Sucralose     | C <sub>12</sub> H <sub>19</sub> O <sub>8</sub> Cl <sub>3</sub>  | 397.63           | 125         | 25.7                                        | 15                                                                              | 15         |
| Thaumatococin | –                                                               | ~22 000          | –           | 60                                          | Not established                                                                 | Acceptable |

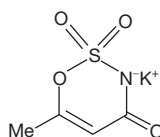
A-A salt, Aspartame-acesulfame; NHDC, Neohesperidine dihydrochalcone; m.p., melting point; ADI, acceptable daily intake; JECFA, Joint Expert Committee on Food Additives of the Agriculture Organization/World Health Organization; SCF, Scientific Food Committee of the European Community; EFSA, European Food Safety Authority

Acesulfame-K, aspartame, and saccharin are permitted as intense sweeteners for use in food, virtually worldwide. In order to decrease cost and improve taste quality, high-intensity sweeteners are often used as mixtures of different, synergistically compatible sweeteners. Sweetness characteristics of high-intensity sweeteners are shown in [Table 1](#).

The more important properties of the high-intensity sweeteners that are permitted for use in food and drink applications are shown in [Table 2](#).

### Acesulfame-K

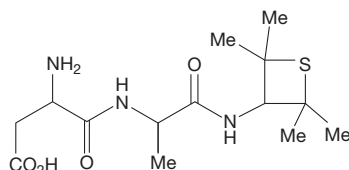
Acesulfame-K [I] has an excellent stability under high temperatures, and good solubility which makes it suitable for numerous products. It is approved for use in food and beverage products in ~90 countries, including the USA, Switzerland, Norway, UK, Canada, Australia, and the European Union (EU) where it is known under the E number (additive code) E950.



Acesulfame-K. Systematic name: 6-methyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide, potassium salt.

## Alitame

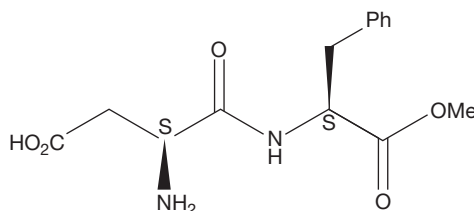
Alitame [II] is nutritive, but due to its intense sweetness, the amounts used are small enough for it to be considered and classified as a nonnutritive sweetener. Alitame is formed from the amino acids L-aspartic acid and D-alanine with a novel amide moiety (formed from 2,2,4,4-tetramethylthienylamine). Alitame exhibits superior stability under a variety of conditions because of its unique amide group. Alitame has been approved for use in some countries such as Australia, Mexico, New Zealand, and China, but not in the USA or EU.



Alitame. Systematic name: L-aspartyl-N-(2,2,4,4-tetramethyl-3-thietanyl)-D-alaninamide.

## Aspartame

Aspartame [III] is made from two amino acid components, L-aspartic acid and L-phenylalanine. Although nutritive, containing 4 kcal g<sup>-1</sup> like any other protein substance, due to its intense sweetness, the amounts used are small enough for aspartame to be considered and classified as a nonnutritive sweetener. In the dry form, the stability of aspartame is good and little decomposition is observed if the moisture content is kept below 8%. However, in solution, and under heat, it is not stable and undergoes hydrolysis to the free dipeptide and methanol, and cyclodehydration to its diketopiperazine derivative, in both cases with loss of sweetness. People suffering from the metabolic disorder phenylketonuria are unable to metabolize L-phenylalanine resulting from the hydrolysis of the dipeptide and are advised to avoid this sweetener. Hydrolysis of the ester functionality affords methanol but in insufficient quantities to be harmful. Aspartame is approved in more than 90 countries (the EU ((additive code: E950), the USA, Canada, South America, Australia, Japan, etc.) for use in numerous foodstuffs.



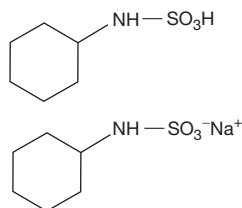
Aspartame. Systematic name: N-L-aspartyl-L-phenylalanine 1-methyl ester.

## Aspartame–Acesulfame Salt

The salt is prepared by heating a ~2:1 ratio (w/w) of aspartame and acesulfame-K in solution at acidic pH. Aspartame–acesulfame dissolves completely in saliva and gastric juice. Although this salt it mainly consists of the two approved sweeteners, it is considered as a separate compound, which requires specific approval in certain countries. It was approved for use as an artificial sweetener in the European Parliament and Council Directive 94/35 EC as amended by Directive 2003/115/EC in 2003 (additive code: E962). It is approved in the USA, Australia, Canada, Mexico, New Zealand, Russia, and China.

## Cyclamate

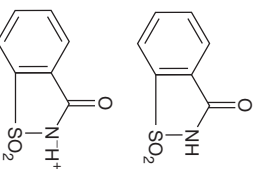
Cyclamate [IV] is generally used in the form of a sodium salt [V] because it is more soluble in water than the free acid. The calcium salt is also used as a sweetener, but, for some applications, it is not suitable as it can cause gelation and precipitation. Sodium cyclamate exhibits good stability in the solid form and is also stable in soft drink formulations within the pH range 2–10. Cyclamate is permitted in several countries (EU (additive code: E952), Australia, Canada, New Zealand, etc.). However, it has been banned in the USA after controversial toxicity studies.



Cyclamate. Systematic name: cyclohexylsulphamic acid.

### Glycyrrhizin

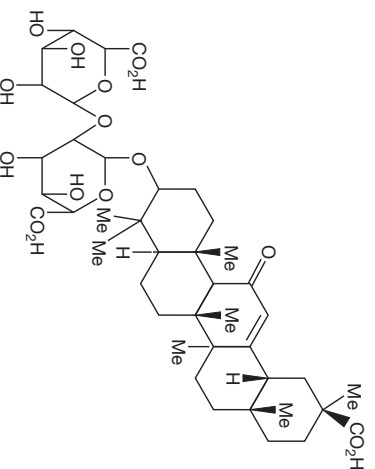
Glycyrrhizin [VI] is a terpenoid glycoside and is isolated from the liquorice root plant *Glycyrrhiza glabra* L. Glycyrrhizin as the ammonium salt is soluble in both hot and cold water and is stable in its dry form. Glycyrrhizin is used in Japan and in other countries as sweetening agent. In the USA, it is approved for use as a flavor and flavor enhancer.



Glycyrrhizin. Systematic name: -D-Glucopyranosiduronic acid, (3R,20R,28R,30R,32R,34R,36R,38R,40R,42R,44R,46R,48R,50R)-3-O-oxo-30-norolean-12-en-3-yl 2-O-D-glucopyranuronosyl.

### Neohesperidine Dihydrochalcone

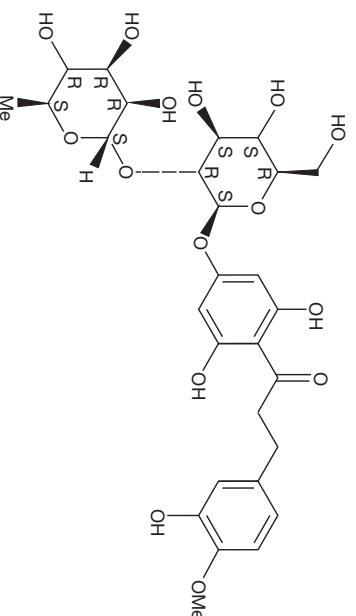
Neohesperidine dihydrochalcone [VII] is a semisynthetic sweetener prepared from neohesperidin or naringin, two flavanones extracted from citrus peel. In aqueous solutions, neohesperidine dihydrochalcone is stable in the pH range 2.5–3.5. Neohesperidine dihydrochalcone is currently allowed for many applications within the EU (additive code: E959). In the USA, it has not been approved as a sweetener.



Neohesperidine dihydrochalcone. Systematic name: 3,5-dihydroxy-4-(3-hydroxy-4-methoxy-hydrocinnamoyl) phenyl-2-O- (6-deoxy-L-mannopyranosyl)-D-glucopyranoside.

### Neotame

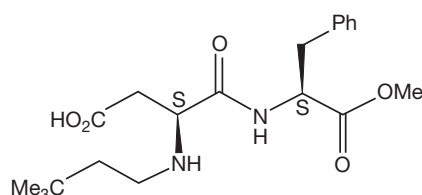
Neotame [VIII] is a derivative of the dipeptide composed of the amino acids aspartic acid and phenylalanine. The optimum pH for maximum stability is ~4.5. Neotame has been approved in the EU as a flavor enhancer (E961), the USA, Australia, Mexico and New Zealand.



Neotame. Systematic name: L-phenylalanine, N-(3,3-dimethylbutyl)-L-aspartyl-, 2-methyl ester.

## Saccharin

This is the oldest high-intensity sweetener. It is commercially available in three forms: acid saccharin [IX], sodium saccharin [X], and calcium saccharin. Sodium saccharin is the most commonly used form because of its high solubility and stability. Saccharin and its salts in their solid form show good stability under conditions present in soft drinks. However, at low pH they can slowly hydrolyze to 2-sulfo benzoic acid and 2-sulfamoyl benzoic acid. Saccharin continues to be used in food and drink formulations in at least 90 countries despite controversy over its safety. In the EU, saccharin is also known by the additive code E954. Many studies have shown that there is no significant risk of cancer in humans associated with consumption of large quantities of saccharin. However, in the USA, an accompanying warning label was required until 2000. In 2000, after more than 20 years of scientific studies and further research, legislation was passed giving saccharin a clean bill of health and the warning label was allowed to be removed.

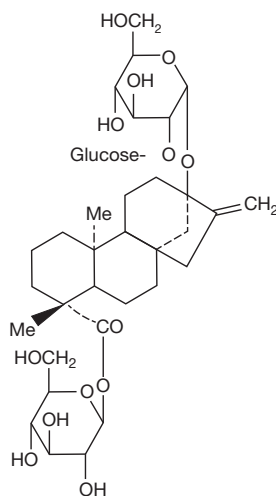


Saccharin. Systematic name: 1,2-benzisothiazol-3(2H)-one-1,1-dioxide.

## Stevioside

Stevioside or stevia [XI] is the name given to a group of sweet diterpene glycosides extracted from the leaves of *Stevia Rebaudiana* plant (native of South America). Steviosides show good stability in the solid form. They are also quite stable in acidic condition beverages at 22 °C.

Stevioside is widely used as a sweetener in Japan, and it is also available in several countries as sweetener, as a dietary supplement or as both a sweetener and dietary supplement (EU (additive code E960), USA, among others).

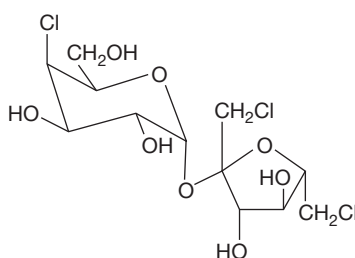


Stevioside. Systematic name: 13-[(2-O-D-glucopyranosyl-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid -D- glucopyranosyl ester.

## Sucralose

Sucralose [XII] is the common name for a sweetener derived from ordinary sugar through a multistep patented manufacturing process that selectively substitutes three atoms of chlorine for three hydroxyl groups on the sugar molecule. One advantage of sucralose for food and beverage manufacturers and consumers is its exceptional stability. Under forcing conditions in acidic solution it slowly hydrolyzes to its constituent chlorinated monosaccharides 4-chloro-4-deoxy-D-galactose and

1,6-dichloro-1,6-dideoxy-D-fructose. Following a lengthy safety evaluation it has been approved in the USA and in more than 35 countries around the world. In the EU, sucralose is also known by the additive code E955.



Sucralose. Systematic name: 1,6-dichloro-1,6-dideoxy-D-fructofuranosyl 4-chloro-4-deoxy-D-galactopyranoside.

### Thaumatococcus

Thaumatococcus is a group of intensely sweet basic proteins isolated from the fruit of *Thaumatococcus danielli* (West African Katemfe fruit). It consists essentially of the proteins Thaumatococcus I and Thaumatococcus II. Thaumatococcus is a taste-modifying protein that functions as natural sweetener or flavor enhancer. Thaumatococcus is stable in aqueous solutions between pH 2.0 and 10 at room temperature. As occurs with aspartame it is nutritive, containing 4 kcal g<sup>-1</sup>, but due to its intense sweetness, the amounts used are small enough for thaumatococcus to be considered and classified as a nonnutritive sweetener. Thaumatococcus is approved for a number of uses in UK, Japan, Australia, the EU (E957), and in many other countries. In the USA, it is approved as a flavor enhancer.

### Analytical Methodology

Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are useful techniques for structural elucidation of unknown compounds but the results obtained are difficult to quantify. Liquid chromatography (LC) methods have been used extensively for the determination of highly intense sweeteners because in many instances the sample matrices from which they are to be determined may be complex. In addition, a sweetener may be used in combination with other sweetener(s). Nevertheless, in recent years, capillary electrophoresis methods were developed that compete successfully with LC methodologies. Figure 2 shows the most common techniques used for the determination of high-intensity sweeteners, and it can be seen that LC is the most widely used technique for all. There are only a few methods for neotame, alitame and thaumatococcus. Most methods for the determination of thaumatococcus involve immunochemical assays (IC) and measurement in an enzyme-linked immunosorbent assay reader. On the other hand, the largest number of methodologies has been proposed for the determination of saccharin because it is the oldest known and used high-intensity sweetener. Automated continuous determinations by flow injection analysis (FIA) have been developed for acesulfame-K, aspartame, cyclamate saccharin, and stevioside; generally, these procedures involve spectrophotometric or electroanalytical detections.

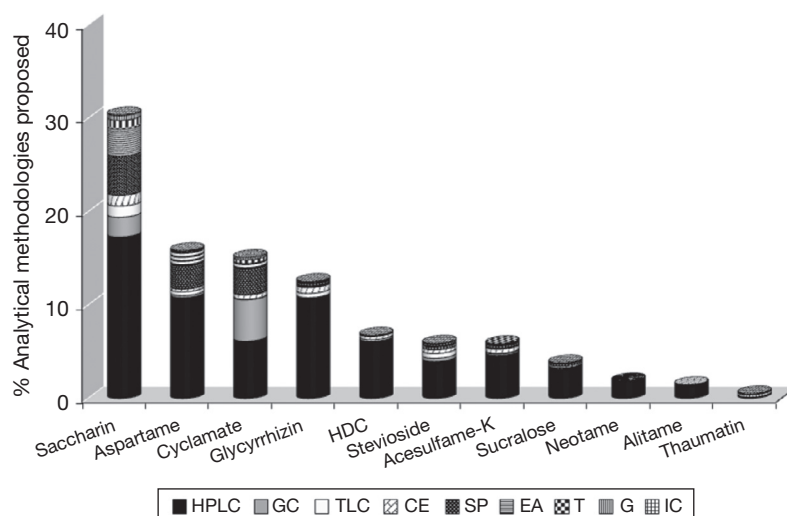


**Figure 2** Analytical methodologies for high-intensity sweeteners determination. NHDC, neohesperidine dihydrochalcone; HPLC, high performance liquid chromatography; GC, gas chromatography; TLC, thin-layer chromatography; CE, capillary electrophoresis; SP, spectrophotometric; EA, electroanalytical; T, titrimetry; G, gravimetry; IC, immunochemical. Source of information: Chemical Abstracts (until February 2013).

### Sample preparation

Sample preparation for the determination of high-intensity sweeteners is relatively simple. Carbonated soft drinks are degassed prior to analysis. Liquid beverages and tabletop sweeteners are diluted or dissolved in water. Sweeteners in complex foods are extracted with water or an appropriate solvent. This extraction can be assisted pressurized liquid extraction or by ultrasounds. Then, the extract can be clarified, centrifuged, or cleaned by using solid-phase extraction techniques.

### Liquid chromatography

LC using amino, ion-exchange, or reversed-phase columns allows the separation of a sweetener from other sweeteners and additives present in foods in a single chromatographic run. The mobile phase can be methanol–water, methanol–acetic acid, methanol–phosphate buffer, methanol–ammonium citrate, or acetonitrile–phosphate buffer, when reversed-phase columns (RP-C18) are used. Methanol–phosphoric acid and  $\text{Na}_2\text{CO}_3$  or  $\text{NaOH}$  solutions were used with amino and ion-exchange columns, respectively. Acesulfame-K, alitame, aspartame, glycyrrhizin, neohesperidine dihydrochalcone, saccharin, and stevioside can be determined by ultraviolet (UV) absorbance (192–282 nm), and by amperometric or conductimetric detection in the case of ion-chromatographic procedures. Aspartame can be detected by fluorimetry ( $\lambda_{\text{EX}} = 205$  nm,  $\lambda_{\text{EM}} = 284$  nm). Cyclamate and sucralose are poorly detected by UV absorbance due to the absence of a chromophore. These sweeteners can be detected by UV absorbance if converted into a derivative possessing strong UV absorption: sucralose by treatment with *p*-nitrobenzoyl chloride, and cyclamate by conversion to dichlorohexylamine for UV detection or to a fluorescence derivative for fluorimetric detection. An alternative for the determination of these sweeteners is the postcolumn ion-pair extraction where the eluted sweetener is mixed with an appropriate dye (methyl violet or crystal violet) being detected by visible absorption. Furthermore, sucralose and cyclamate can be detected directly by refractive index. In the last years, high-performance liquid chromatography with mass spectrometric detection methodologies are proposed for the determination of artificial sweeteners mixtures due to its simplicity, separation efficiency, and excellent sensitivity and selectivity.

### Gas chromatography

Acesulfame-K, aspartame, cyclamate, saccharin, stevioside and sucralose are determined by gas chromatography, but the main drawback of this technique is that a derivatization is required. Acesulfame-K is methylated with ethereal diazomethane, aspartame is converted into its *N*-(2-methylpropoxycarbonyl) methyl ester derivative, menthol and isobutyl chloroformate are used to convert aspartame to 3-[(isobutoxycarbonyl)amino]-4-[[ $\alpha$ -(methoxycarbonyl)phenethyl]amino]-4-oxobutyric acid, cyclamate is determined as cyclohexene resulting from the reaction with nitrite, saccharin is converted to *N*-methylsaccharin, and stevioside is hydrolyzed. The sucralose is converted into its trimethylsilyl ether. Detection is carried out utilizing flame-ionization, flame-photometric electron-capture detectors or nitrogen–phosphorus detection.

### Thin-layer chromatography and paper chromatography

Qualitative, semiquantitative, and quantitative methodologies have been described for acesulfame-K, alitame, aspartame, cyclamate, glycyrrhizin, neohesperidine dihydrochalcone, saccharin, stevioside, and sucralose. Quantification is achieved using scanning densitometry.

### Capillary electrophoresis

Capillary electrophoresis techniques, such as capillary zone electrophoresis and micellar electrokinetic capillary chromatography have been used to analyze high-intensity sweeteners in foods. These methods are rapidly gaining acceptance for the determination of sweeteners because they are comparable in resolution and precision to LC, but are faster and less expensive to operate. Aspartame, saccharin, acesulfame-K, alitame, and the other food additives are well separated in less than 12 min using an uncoated fused-silica capillary column with a buffer consisting of sodium deoxycholate, potassium dihydrogenorthophosphate, and sodium borate operating at 20 kV. In the micellar electrokinetic chromatographic mode, carbonate buffer at pH 9.5 is used as the aqueous phase and sodium dodecyl sulfate is used as the micellar phase. The determination can be performed by direct or indirect (cyclamate) UV detection, or by potentiometric detection with coated-wire ion-selective electrodes.

### Electroanalytical techniques

Polarographic procedures are described for acesulfame-K, cyclamate, and saccharin. Cyclamate was decomposed by heating with sodium nitrite and the sulfate liberated is precipitated as lead sulfate, and the excess of  $\text{Pb}^{2+}$  is the specie measured. Sensors including biosensors such as enzyme electrodes or filter-supported bilayer lipid membranes are proposed for acesulfame-K, aspartame, cyclamate, saccharin, and sucralose. A fluoride-selective electrode is used for the kinetic potentiometric monitoring of the reaction between 2,4-dinitrofluorobenzene and aspartame. Ion-selective electrodes based on ion associates, liquid membrane electrodes based on crystal violet and brilliant green, and silver electrodes are used for the potentiometric titration of saccharin.

### Spectrophotometric techniques

Extractive spectrophotometric techniques using colorimetric reagents (oxazine dye, Sevron blue 5G for acesulfame-K; *p*-dimethylaminobenzaldehyde, 1,4-benzoquinone, ninhydrin for aspartame; picryl chloride, *p*-quinone for cyclamate; vanillin for glycyrrhizin; Nile Blue, Azure A, B, or C, Sevron blue 5G, Brilliant cresyl blue for saccharin; and anthrone for steviosides) are used for the direct determination of the high-intensity sweeteners. Indirect spectrophotometric methodologies are also proposed. UV absorbance

detection is possible for aspartame, cyclamate, glycyrrhizin, and saccharin. Nevertheless, as cyclamates are not readily detected by spectroscopic techniques, a chemical derivatization is performed; generally to convert it to cyclohexylamine. Fluorimetric determinations are suggested for aspartame, where its reaction product with fluoescamine is detected, and for saccharin since this forms a fluorescent complex with sodium carbonate. Flame atomic absorption spectrometry can be used for the indirect determination of saccharin and cyclamate.

### Titrimetric methods

Titrimetric assays have been developed for acesulfame-K (titrated with sodium methoxide in benzene), aspartame, sodium cyclamate, and sodium saccharin (titrated with perchloric acid), and for saccharin (acid form) with potassium hydroxide as titrant. Precipitation, chelatometric, and redox titrations are proposed for the determination of cyclamate. The oldest methods for saccharin involve its determination by means of a Kjeldahl procedure.

### Gravimetric methods

Cyclamate, saccharin, glycyrrhizin, and stevioside can be determined by gravimetric procedures. Those for cyclamate and saccharin are including in the method book of the Association of Official Analytical Chemists (AOAC).

## Bulk Sweeteners

Bulk sweeteners, defined as those delivered, in solid or liquid form, for use in sweeteners per se or in foods in quantities greater than 22.5 kg, are disaccharides and monosaccharides of plant origin. Sucrose from sugarcane and sugar beet and starch-derived glucose and fructose from maize (corn), potato, wheat, and cassava are the major sweeteners sold in bulk to the food and beverage manufacturing industry, or packers of small containers for retail sale. Relative sweetness of bulk sweeteners and their caloric values are shown in Figure 3.

### Caloric Sweeteners

#### Sucrose

Sucrose is composed of one molecule of glucose and one molecule of fructose,  $\alpha$ -D-glucopyranosyl-D-fructofuranoside. It is the traditional sweetener, table sugar. It is highly water soluble and is present in most fruits, some root vegetables, many trees, and grasses. It is more concentrated in sugarcane, *Saccharum officinarum*, and the sugar beet, *Beta vulgaris*, the latter in temperate zones. From either crop, juice is extracted, purified, and concentrated to syrup. Sucrose is crystallized from the syrup by serial crystallization; the residual viscous syrup is molasses. From both crops comes a range of white sugar products: solid granulated, powdered, cubes, liquid sucrose, and invert syrups (blends of sucrose, glucose, and fructose). Primarily from sugarcane come brown sugars, edible molasses, golden syrups, and dark cane syrups.

#### Molasses

Molasses, the residual material from sugarcane and sugar beet processing, is traded in bulk, primarily for animal feed where it is mixed with fibrous residues, and serves to increase caloric value and palatability. It is not sold as a sweetener, although it is blended with other sweeteners to make special syrups.

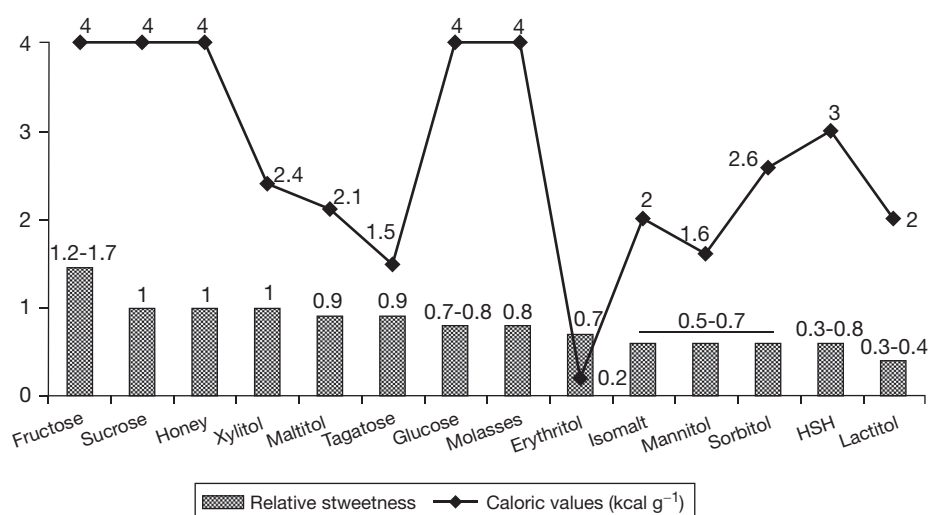


**Figure 3** Relative sweetness for bulk-sweeteners and their caloric values. HSH, hydrogenated starch hydrolizates.

### Honey and maple syrup

Honey, produced by honey-bees (*Apis mellifera* and *Apis dorsata*) is a liquid product, ~80% solids, containing a mixture of simple carbohydrates: 25–45% fructose, 25–45% glucose, 2–12% maltose, and 0.5–3% sucrose, with traces of many other sugars depending on the bees' floral source.

Maple syrup and sugar, made from sap of the sugar maple tree (*Acer saccharum*) through concentration and crystallization, are, like honey, mixtures of simple sugars. Freshly made maple syrup, generally ~65% solids by weight, contains 50–63% sucrose and 0–8% combined glucose and fructose, but no maltose. As maple syrup is stored, the sucrose will invert to glucose and fructose.

## Starch-Derived Sweeteners

### Glucose

Glucose, commercial name dextrose, in the aldohexose form  $\alpha$ -D-glucose ( $C_6H_{12}O_6$ ), is the major product from starch hydrolyzed by acid and/or enzymes. The major starch source in the USA and Japan is corn (*Zea mays*) and in Europe, wheat and potato. There is some starch and starch hydrolyzate production from cassava in the tropics. Glucose is sold as anhydrous dextrose; more commonly as dextrose monohydrate, as glucose syrup or corn syrup.

### Fructose

Fructose, in the ketohexose form  $\beta$ -D-fructose ( $C_6H_{12}O_6$ ), is produced from glucose by an isomerase enzyme (glucose–fructose isomerase), which converts glucose to fructose, and subsequent enrichment of the fructose fraction (equilibrium conversion is ~50%), or isolation of fructose and crystallization. Products are high-fructose corn syrup, the most widely used monosaccharide sweetener, at 42%, 55%, and 90% fructose (with glucose, the other major component) and crystalline fructose.

## Low-Caloric Sweeteners

### Sugar alcohols, polyhydric alcohols, or hydrogenated sugars

Sugar alcohols or polyols differ from sugars in that the aldehyde or ketone function of the sugar molecule is reduced to an alcohol. They can also be categorized as sugar substitutes because they can replace sugar sweeteners. Most common as bulk sweeteners are sorbitol, mannitol, xylitol, and erythritol (monosaccharides); isomalt, maltitol, and lactitol (disaccharides); and hydrogenated starch hydrolyzates (HSH, a mixture of sugar alcohols). Many sugar alcohols are found in nature, but it is not commercially feasible to isolate and concentrate them from their sources. Sorbitol, mannitol, maltitol, and the HSH are produced by enzymatic hydrolysis of a starch. Lactitol, xylitol, and isomalt are produced in a similar manner, except that they are not derived from starch. Erythritol has been commercially produced by a fermentation process. All retain sweetness through heating and their bulking properties are similar to those of sucrose. All polyols are absorbed slowly and incompletely from the intestine by passive diffusion. Therefore, these sweeteners provide low energy and offer potential health benefits (e.g., reduced glycemic response and reduced dental caries risk). All these sweeteners do not represent a hazard to health and the Joint Expert Committee on Food Additives of the Agriculture Organization/World Health Organization (JECFA) deemed it not necessary to assign a numerical value for ADI, but instead assigned the most favorable term 'not specified'. Furthermore, in the USA they are considered Generally Recognized as Safe. Nevertheless, an excess consumption of mannitol may have a laxative effect, and for this reason, JECFA has allocated a temporary ADI of 50mgperkg.

### Tagatose

This is a new low-calorie sweetener. It is a bulk sweetener, as it is used where the bulk (mass) of sugar is important to the final product qualities. It is a monosaccharide sugar derived from lactose. Therefore, tagatose is a naturally occurring sugar derived from dairy whey.

## Analytical Methodology

Analysis of bulk sweeteners falls into two categories: (1) chromatographic methods, used in starch hydrolyzate production and for most sweetener-containing products, and (2) polarimetric and wet chemical methods used in sucrose production. There are some UV–visible spectrophotometric methods for sugar analysis; these are generally used in clinical or biological sugar analysis, and not for bulk sweeteners, and so will not be further considered here. FIA methodologies have been developed for a large number of methods for the determination of bulk sweeteners, above all for reducing sugars. The most recent methodologies involve automation of enzymatic determinations and biosensors. Sample preparation procedures are shown in [Table 3](#).

### Polarimetric methods

These methods generally determine some overall feature of the bulk sweeteners such as total carbohydrates. Polarimetry, using a flow-through polarimeter at 589 nm, is the general method of sucrose analysis in bulk raw and white sugars. Traditional polarimeters are being replaced by polarimeters using light of longer wavelengths,  $\lambda=880$  nm, which can be used for monitoring a colored (though not a turbid) solution, and so decreases solid waste generated in the clarification step required by traditional polarization.

**Table 3** Sample preparation procedures for bulk sweeteners analysis.

| <i>Technique</i>      | <i>Sample preparation procedures</i>                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Polarimetry (589 nm)  | White sugar: do not require clarification.<br>Raw sugar, brown and yellow sugars: addition of lead subacetate, along with a diatomaceous earth filter aid, with filtration to obtain a clear solution of little color. Subacetate can be replaced by an aluminum salt or ultrafiltration, but for raw cane sugar these reagents do not provide satisfactory clarification.                                                                                                         |
| Polarimetry (~880 nm) | Addition of diatomaceous earth before filtration.                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Titrimetry            | Samples other than molasses require not pre-treatment.                                                                                                                                                                                                                                                                                                                                                                                                                             |
| GC                    | Sucrose, glucose, fructose, syrups and sugar alcohols can all be analyzed with pre-treatment derivatization of the sugars as either aldonitrile or trimethylsilyl derivatives.                                                                                                                                                                                                                                                                                                     |
| HPLC                  | Simple sugars and sweetened beverages: generally require only filtration through at least a 0.5 µm filter to remove suspended solids.<br>Fats and proteins present in food must be removed. Fats by extraction with an organic solvent (petroleum ether) and extraction of sugars with water or water/ethanol mixtures; proteins by precipitation with a solution of zinc acetate-potassium hexacyanoferrate(II)-water.<br>Extraction with cartridges of an appropriate adsorbent. |
| Enzymatic methods     | For methodologies supplied in kit form: filtration of the dissolved sample.<br>For biosensors containing immobilized enzymes: generally requires only filtration. Time may also be required for treatment with enzymes other than the detecting enzyme, e.g. mutarotase treatment before glucose oxidase addition.                                                                                                                                                                 |

GC, Gas chromatography; HPLC, High performance liquid chromatography.

### Titrimetric and other methods

Titration with a copper(II) salt (Lane–Eynon titration) is the standard method for the determination of reducing sugars (glucose and fructose) in bulk raw and white sugars.

Moisture in solid sugars is determined generally by oven drying; in liquid products, by Karl Fischer titration. Inorganic content is determined by either conductivity in solution, or sulfated ash gravimetric procedures.

In syrups, the solids content (Brix, or refractometric dry solids (RDS)) is determined by refractive index measurement. Tables correlating refractive index with sucrose and invert content are published in the International Commission for Uniform Methods of Sugar Analysis and AOAC methods books.

### Gas chromatography

This was the first quantitative chromatographic system for sugars analysis, but it has been replaced by liquid chromatographic methods, which are simpler, less expensive, and require no derivatization, except in the fermentation industries, where the ease of analysis of alcohols and other fermentation products along with starting (glucose, sucrose, molasses) and intermediate materials has helped in the continuation of the use of this technique.

### Liquid chromatography

Four LC systems are in general use for analysis of bulk sweeteners and sugar alcohols (all systems require a sample with sugars concentration from < 1% to 10% and the detection of separated peaks is usually by differential refractometry):

1. Separation on a cation-exchange column, in the calcium or sodium form, with an aqueous mobile phase. Cation-exchange columns separate by a combination of liquid exchange and size exclusion; higher molecular weight sugars elute earlier than the smaller sugar molecules, but after ionic inorganic components, because the smaller molecules complex more strongly with cations on the column. Sugar alcohols may be separated on these columns, but only with pulsed amperometric detection and long separation times.
2. Separation on an amino-bonded silane column with an acetonitrile–water mobile phase. These were the first LC systems utilized for the separation of carbohydrates. The main drawback is the use of acetonitrile as solvent, but its expense and hazard can be decreased by recycling the mobile phase over a desugaring adsorbent. The column separates sugars because of their varying affinities in an aqueous/acetonitrile solvent for the bonded amino groups on the packing material. Monosaccharides (glucose, then fructose) elute first, followed by di- and trisaccharides. This system is operated at room temperature. Analysis for trisaccharides or sugar alcohols can add 40 min to the normal 10–15 min analysis time.
3. Separation on reversed-phase columns with an aqueous mobile phase. This rapid and inexpensive system is used where only separation of mono- and disaccharides is required without further separation within either group. The non-polar column packing is silica particles coated with octadecyl (C-18) groups. Separation occurs in order of decreasing polarity and, therefore, for bulk sweeteners, in order of molecular weight.
4. Separation on anion-exchange columns with an alkaline aqueous mobile phase. This system for sugar analysis, originally called ion chromatography, is now, for carbohydrates, more selectively called high-performance anion-exchange-pulsed amperometric detection. It has gained use in the general analysis of bulk sweeteners because of its versatility and selectivity. It is the preferred system for analysis of low levels of bulk sweeteners.

The selectivity and retention time may vary by changing the pH and ionic strength of the mobile phase. The responses of the detector, a gold electrode, may be set at specific potentials to analyze for specific carbohydrates. Sugar alcohols can be analyzed along with sugars in a single analysis; isocratic elution is usually satisfactory for food and beverage products, but gradient elution is available for more complex mixtures.

In recent years, high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) is applied for the simultaneous determination of several saccharides in foods. This methodology provides high selectivity, low quantitation limits (LOQs) and accurate analysis. Meanwhile, the LOQ of the HPLC methodologies are too restrictive and cannot be useful in trace quantification of reducing sugars.

### Enzymatic method

Enzymatic methods are available for the analysis of bulk sweeteners in food and beverages; enzyme electrodes and detection kits are available for several sweeteners (e.g., sucrose, glucose, etc.). The usage of enzyme methods is determined by conditions under which the enzyme is viable, i.e., heat, substrate concentration, water availability, and interferences. This methodology is a popular alternative to chromatographic methods due to its speed, portability, and wide range of application.

### Capillary electrophoresis

This technique has a high capability in separation of bulk sweeteners. Since bulk sweeteners lack both a charge and a strong UV chromophore, several derivatization reactions have been proposed (e.g., interaction with oxoacid or metal ions). An alternative for bulk sweeteners is their separation in a fused-silica capillary using indirect fluorescence detection or indirect UV detection. Electrokinetic micellar chromatography has extended the applicability of electrophoretic techniques even to neutral molecules such as carbohydrates.

### Stable isotope ratio analysis

Adulteration of fruit juices or honey by addition of bulk sweeteners can be detected by stable isotope ratio mass spectrometric analyses, because the naturally occurring carbon isotope ratio of  $^{14}\text{C}$  to  $^{12}\text{C}$  in honey and fruit is different from that in corn or sugarcane although not from that in sugar beet. The oxygen isotope ratio may be used for beet sugar addition.

### Near-infrared spectrometry

This is an alternative for the process analysis of bulk sweeteners, e.g., raw sugar.

## Further Reading

- Association of Official Analytical Chemist (AOAC). *Official Methods of Analysis*, 19th ed.; AOAC: Arlington, TX, 2012.
- International Commission for Uniform Methods of Sugar Analysis (ICUMSA). *ICUMSA Methods Book*, Bartens: Berlin, 2011.
- Capitan Vallvey, L. F. Intense Sweeteners. In *Handbook of Food Analysis*, Vol. 3, Nollet, L. M. L., Ed.; 2nd ed.; Vol. 3; CRC Press: Boca Raton, FL, 2004; pp 1643–1739.
- Kokotou, M.; Asimakopoulos, A. G.; Thomaidis, N. S. Sweeteners. In Nollet, L. M. L., Toldrá, F., Eds.; 3rd ed. CRC Press: Boca Raton, FL, 2012; pp 493–514.
- Larsen, J. C. *Nutrafoods* **2012**, 11, 3–9.
- Nollet, L. M. L.; Toldra, F. *Handbook of Analysis of Active Compounds in Functional Foods*, CRC Press: Boca Raton, FL, 2012; pp. 847–876.
- O'Brien, N. *Alternative Sweeteners*, 4th ed.; CRC Press: Boca Raton, FL, 2012.
- O'Donnell, K.; Kearsley, M. Sweeteners and Sugar Alternatives. In *Food Technology*, 2nd ed.; Wiley: Chichester, 2012.
- Priya, K.; Gupta, V. R. M.; Srikanth, K. *Journal of Pharmacy Research* **2011**, 4, 2034–2039.
- Salminen, S.; Hallikainen, A. Sweeteners. In *Food Additives*; Branen, A. L., Davidson, P. M., Salminen, S., Thorngate, J. H., Eds., 2nd ed.; Dekker: New York, 2002; pp 477–500.
- Von Rymon Lipinski, G. Sweeteners. In *Food Chemical Safety*, Vol. 2, Watson, D., Ed.; Vol. 2; CRC Press: Boca Raton, FL, 2002; pp 228–248.
- Yebrá-Biurrun, M. C. *Food Additives and Contaminants* **2000**, 17, 733–738.

## Relevant Websites

- Authorized sweeteners (summaries of EU legislation): [http://europa.eu/legislation\\_summaries/other/i21069\\_en.htm](http://europa.eu/legislation_summaries/other/i21069_en.htm).
- European Food Safety Authority (EFSA): <http://www.efsa.europa.eu/>.
- International Commission for Uniform Methods of Sugar Analysis (ICUMSA): <http://www.icumsa.org/>.
- International Sweeteners Association (ISA): <http://www.info-edulcorants.org/>.
- Joint FAO/WHO Expert Committee on Food Additives (JECFA): <http://www.who.int/foodsafety/chem/jecfa/en/>.
- U.S. Food and Drug Administration: <http://www.fda.gov/Food/IngredientsPackagingLabeling/default.htm>.