SPECIAL REPORT
The Challenge of Measuring Sweet Taste in Food Ingredients and Products for Regulatory Compliance:
A Scientific Opinion

Dustin E. Starkey, Zhuzhu Wang, Kommer Brunt, Lise Dreyfuss, Philip A. Haselberger, Stephen E. Holroyd, Kaushik Janakiraman, Prabhakar Kasturi, Erik J.M. Konings, David Labbe, Marie E. Latulippe, Xavier Lavigne, Barry V. McCleary, Salvatore Parisi, Tony Shao, Darryl Sullivan, Marina Torres, Sudhakar Yadlapalli, and Ioannis Vrasidas

1Abbott Nutrition, 3300 Stelzer Rd, Columbus, OH 43219, USA, 2Abbott Nutrition, 1800 South Oak St, Suite 210 Champaign, IL 61820, USA, 3University of Illinois, Department of Food Science and Human Nutrition, 1302 W. Pennsylvania Ave, Urbana, IL 61801, USA, 4Rotating Disc b.v, Spoorlaan 31, 9753HV Haren, The Netherlands, 5SAM Sensory and Marketing International, 46 rue Armand Carrel, 75019 Paris, France, 6Fonterra Research and Development Centre, Private Bag 11029, Palmerston North 442, New Zealand, 7Reckitt Health, 60 Radarweg, 1043NT Amsterdam, The Netherlands, 8PepsiCo R&D, 617, W. Main St, Barrington, IL 60010, USA, 9Société des Produits Nestlé SA Nestlé Institute of Food Safety and Analytical Sciences, EPFL Innovation Park, Bâtimon G, 1015 Lausanne, Switzerland, 10Société des Produits Nestlé SA Nestlé Institute of Material Sciences, Rte du Jorat 57, 1000 Lausanne 26, Switzerland, 11Institute for the Advancement of Food and Nutrition Sciences, 740 15th St NW, #600, Washington DC 20005, USA, 12Abbott Nutrition, Park Lane, Culliganlaan 2B, 1831 Diegem, Belgium, 13Eden Rd, Greystones, Murrumburrah, County Wicklow A63YW01, Ireland, 14Lourdes Matha Institute of Hotel Management and Catering Technology, Kuttichal PO, Thiruvananthapuram, Kerala 695574 India, 15Eurofins Scientific, N2743 Butternut Rd, Pyonette, WI 53955, USA, 16Departamento de Desarrollo de Métodos Analíticos, Laboratorio Tecnológico del Uruguay LATU, Avenida Italia, 6201 11500 Montevideo, Uruguay, 17FirstSource Laboratory Solutions LLP (Analytical Services), First Floor, Plot No- A1/B, IDA Nacharam Cross Rd., Hyderabad 500076 India, 18Purperhoedenveem 94, 1019HM Amsterdam, The Netherlands

*Corresponding author’s e-mail: ivrasidas@gmail.com

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Abstract

The Codex Alimentarius Commission, a central part of the joint Food and Agricultural Organization/World Health Organizations Food Standards Program, adopts internationally recognized standards, guidelines, and code of practices that help ensure safety, quality, and fairness of food trade globally. Although Codex standards are not regulations per se, regulatory authorities around the world may benchmark against these standards or introduce them into regulations within their countries. Recently, the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) initiated a draft revision to the Codex standard for follow-up formula (FUF), a drink/product (with added nutrients) for young children, to include requirements for limiting or measuring the amount of sweet taste contributed by carbohydrates in a product.

Stakeholders from multiple food and beverage manufacturers expressed concern about the subjectivity of sweetness and challenges with objective measurement for verifying regulatory compliance. It is a requirement that Codex standards include a reference to a suitable method of analysis for verifying compliance with the standard. In response, AOAC INTERNATIONAL formed the Ad Hoc Expert Panel on Sweetness in November 2020 to review human perception of sweet taste, assess the landscape of internationally recognized analytical and sensory methods for measuring sweet taste in food ingredients and products, deliver recommendations to Codex regarding verification of sweet taste requirements for FUF, and develop a scientific opinion on measuring sweet taste in food and beverage products beyond FUF. Findings showed an abundance of official analytical methods for determining quantities of carbohydrates and other sweet-tasting molecules in food products and beverages, but no analytical methods capable of determining sweet taste. Furthermore, sweet taste can be determined by standard sensory analysis methods. However, it is impossible to define a sensory intensity reference value for sweetness, making them unfit to verify regulatory compliance for the purpose of international food trade. Based on these findings and recommendations, the Codex Committee on Methods of Analysis and Sampling agreed during its 41st session in May 2021 to inform CCNFSDU that there are no known validated methods to measure sweetness of carbohydrate sources; therefore, no way to determine compliance for such a requirement for FUF.
sweetness of carbohydrate sources and therefore no way to determine compliance for such a provision (2).

The concept of sweetness and its measurement remains an outstanding discussion for the draft CXS for FUF (CXS 156–1987) at CCNFSDU42 in November 2021. This committee must reflect on the conclusions from CCMAS41 and reconsider the provisions that cannot be determined by any known validated methods of measurement. The AOAC Ad Hoc Expert Panel on Sweetness appreciates that a Codex endorsement of a sweetness concept for this revised standard for FUF (CXS 156–1987) could have implications beyond application to this very specific range of products. Thus, the aim of this paper is to present findings and recommendations in relation to the measurement of sweetness, either analytically or by sensory analysis in all types of food products and beverages.

Chemical Theories of Sweetness

Chemoreception

Sweet-Tasting Molecules and Human Perception

Sweet taste perception in humans is a complex mechanism that starts with recognition of sweet-tasting molecules by G-protein-coupled sweet receptors (T1R2/T1R3) on the surface of taste buds located on the tongue. This recognition initiates biochemical cascades involving the stimulation of protein messengers and subsequent transmission of a neurological signal conveying the intensity and quality of the sweet sensation from taste buds through nerves and finally to the brain (3, 4).

Sweet-tasting molecules (5, 6) can be of natural or synthetic/artificial origin and include carbohydrates, sugar alcohols, and high-intensity sweeteners (HIS). Sweet-tasting carbohydrates are generally simple sugars (mono- and disaccharides) that include glucose, galactose, fructose, maltose, lactose, and sucrose. Some trisaccharides (degree of polymerization, DP = 3) are also known to elicit a sweet taste, including raffinose, maltotriose (7), 4-galactosyl-kojibiose, and lactulosucrose (8). Carbohydrates of DP >3 are generally perceived as starchy rather than sweet. Examples include starches (resistant and digestible), gums, fructans, galacto-oligosaccharides, and dietary fiber. Commercially available maltodextrins often have diverse DP profiles and differ markedly in sweetness despite having equivalent dextrose equivalence (DE) values. In other words, it is possible for two different maltodextrins to have the same DE value but significantly different sweetness. Some examples of sugar alcohols are sorbitol, xylitol, mannitol, lactitol, and maltitol. High-intensity sweeteners include saccharin, acesulfame potassium (Ace-K), sucralose, advantame, and cyclamate; some sweet-tasting terpenoids (e.g., steviosides, glycyrrhizin, and mogrosides); dipeptides (e.g., aspartame and neotame); and some sweet proteins (e.g., brazzein, thaumatin, miraculin, monellin, mabinlin, pentadin, and neoculin (curculin)).

Several models have been developed over the last 50 years to explain the sweetness of molecules but no one model is recognized as the best (9, 10). This is because no one model can accommodate the structural diversity of all sweet-tasting molecules. The original AH-B model suggested that sweet-tasting molecules contain a glucophore moiety that consists of two electronegative atoms at a particular distance from each other. The “AH” acts as a hydrogen bond donor and “B” acts as a hydrogen bond acceptor (11). This model was thought to explain the sweetness of structurally diverse compounds such as β-D-fructose, chloroform, alanine, and saccharine (11). The AH-B motif was later updated to a tripartite glycophore, where a hydrophobic binding site X, was included. In the resultant AH-B-X model, these three groups must exist in a triangular geometry with specific bond lengths to bind with receptors effectively. Hydrogen bonds form at A and B, and X acts as a lipophilic region (12). This model explained why for some amino acids their D-isomers are sweet but L-isomers are not. Examples include D-leucine, D-tryptophan, and D-phenylalanine (12). However, although lipophilicity may enhance sweetness, the inclusion of X was an unnecessary extension as it could not explain the sweetness of glycine and other sugar alcohols (9). The α-helix receptor protein theory was also introduced to explain the structural differences between sweet- and non-sweet-tasting amino acids (13). In addition, the direct G-protein interaction (DGI) theory proposed that non-sugar sweeteners with amphiphilic properties activate G-proteins directly under physiological conditions and this mechanism is consistent with their temporal characteristics such as slow taste onset and lingering aftertaste (14).

Finally, the multi-point attachment (MPA) model suggested that a total of eight sites (AH, B, G, D, Y, E1, E2) interact between a sweet-tasting molecule and the receptor, and although attachment at all eight sites is not required, the resulting number of binding sites involved determines the potency of sweetness (15). This MPA theory was adopted to explain the intense sweetness of neotame and aspartame (16).

Relative Potency of Sweet-Tasting Molecules

Quantitating sweet taste is not as simple as determining molecular concentrations and extrapolating to a relative scale. Relative sweetness is often reported as sweetness potency which is calculated as the aqueous concentration of a sweet-tasting molecule to that of another sweet-tasting molecule at equivalent sweetness intensity. Sweetness potency is assessed by human taste panelists and values are most frequently reported relative to sucrose. Using this approach, sucrose is given a value of 1 or 100. A sweetness potency of >1 or >100 is considered as “more potent” than sucrose, since a lower concentration of the sweet-tasting molecule of interest is required to achieve the same sweetness intensity of a specific sucrose concentration. Several methods have been applied to identify equivalent sweetness intensity (17) but the most common are the difference test [e.g., two-alternative forced-choice (2-AFC)], scaling [e.g., Labeled Magnitude Scale (LMS)], and the sucrose-sweetener combined (SSC) method.

The applicability of sweetness potencies reported in the literature is challenged by several practical limitations. Although the relative sweetness values of most sweet-tasting molecules in aqueous solutions are readily available, many of the values are reported as ranges and without noting the temperature of aqueous solution, concentrations of sucrose at which the estimate was made, or which method was applied. Consequently, the determination of sweetness by sensory methods is challenging because it easily leads to ranges of relative sweetness rather than absolute values (Table 1). It is important to understand that the sweetness potency of a sweet-tasting molecule (including sucrose) varies with temperature (Table 2), reference concentration, and evaluation methods (Table 3), and accuracy can be affected by the concentration range evaluated by the panelist (27). Additionally, variation still exists due to the bias of highly trained panelists. Table 3 lists a few examples of sweetness potency of sweet-tasting molecules relative to different sucrose concentrations in aqueous solution at room temperature, as reported by different authors using different methods.
Loosely available is about sweetness potency of sweet-tasting molecules in food and beverage matrices (as compared to aqueous solutions). This is because sweetness is not absolute but rather depends on concentration, pH, serving temperature, matrix effects (e.g., presence of other molecules that affect mineral content and viscosity), and synergistic effects (i.e., presence of other sweet-tasting molecules) (29, 31, 32). In addition, the perception of sweetness intensity varies with time for certain foods and beverages (e.g., lingering sweetness or bitterness of most HIS). As a result, it is unfeasible to determine a sweetness potency value of a sweet-tasting molecule by comparing it to aqueous sucrose solution and applying the value to more complex food and beverage matrices. Instead, different sensory methods with highly trained panelists are needed to assess the sweetness of sweet-tasting molecules in each unique food and beverage matrix.

### Analytical Methods for Quantitating Sweet-Tasting Molecules

To the best of our knowledge, there are no stand-alone analytical methods for determining the sweetness of sweet-tasting molecules in food products and beverages. On the other hand, an abundance of analytical methods for quantitating the composition of these molecules in food products and beverages has been reported in the literature. The most relevant and current analytical techniques and official methods are discussed in this section, with an emphasis on methods for quantitating carbohydrates and relevant sweet-tasting molecules in ingredients and final products.

A variety of analytical techniques are available for both fundamental analytical research and standard routine quantitation (Figure 1). Since most carbohydrates lack a strong UV chromophore, fluorophore, or charge, derivatization/labeling with a suitable molecule may be necessary, depending on the separation and detection techniques applied (figure 2) (33–36). Most state-of-the-art methods use well-accepted analytical instruments to selectively determine a single carbohydrate or multiple carbohydrates (i.e., sugar profile methods that generally include two or more of the most common mono- and disaccharides—glucose, fructose, galactose, lactose, sucrose, and maltose) in ingredients and finished (food) products. Specifically, chromatographic methods like high-performance anion-exchange chromatography with pulsed amperometric (HPAEC–PAD; 37, 38) or mass spectrometric detection (HPAEC–MS; 39), and HPLC with tandem mass spectrometric (HPLC–MS/MS; 40), evaporative light-scattering (HPLC–ELS; 41), refractive index (HPLC–RI; 42), or charged aerosol detection (HPLC–CAD; 43, 44) have been used. These techniques continue to be developed and optimized to extend their applicability to various complex food matrices while providing good accuracy and precision, high sensitivity and resolution, and required LOD and LOQ. In parallel, classical (chemical) methods based on colorimetry (e.g., phenol-sulfuric acid, Lane-Eynon, Luff Schoorl, Somogyi-Nelson, etc.) or calculation (e.g., Method 986.25) of total carbohydrates and sugars are still applied. Alternatively, highly specific enzyme-based methods (45), including enzymatic–amperometric (e.g., Method 2020.01) and enzymatic–polarimetric (e.g., Clerget method, IS 11764:2005/ISO 2911:2004) techniques are also well established and validated for the measurement of individual sugars or groups of sugars. Furthermore, NMR (46) and capillary electrophoresis (CE) have also been suggested as useful tools for carbohydrate determination in food products (47) or beverages (48).

For both natural and artificial sweeteners, a variety of official standard methods are available based on different analytical chemical techniques. Tables 4–9 summarize the most important and currently applied methods. Of these methods, enzymatic–colorimetric and liquid chromatographic techniques are most often applied to the standard analysis of carbohydrates.

Three basic strategies are used for the enzymatic analysis of monosaccharides:

1. phosphorylation of the sugars followed by oxidation of glucose 6-phosphate and concurrent reduction of nicotinamide adenine dinucleotide (NAD\(^+\)) or nicotinamide adenine dinucleotide phosphate (NADPH) to NADH or NADPH, which are measured colorimetrically;
2. direct oxidation of sugars (e.g., galactose or xylose) by dehydrogenase and concurrent reduction of NAD\(^+\) or NADPH to NADP or NADPH, which has also been applied to a range of sugar alcohols such as sorbitol/xylitol and mannitol/arabitol; and
3. oxidation of sugars and concurrent production of hydrogen peroxide, which can be linked to a colorimetric detection system. Disaccharides are usually measured after hydrolysis to constituent monosaccharides with dedicated enzymes (e.g., Method 2020.08, Method 2020.07, Method 2006.06, ISO 26462 | IDF 214).

### Table 1. Summary of relative sweetness ranges in relation to sucrose equal to 100 (18–21)

| Molecule      | Relative sweetness |
|---------------|--------------------|
| Fructose      | 80–180             |
| Glucose       | 50–75              |
| Galactose     | 54                 |
| Lactose       | 15–40              |
| Maltose       | 30–50              |
| Trehalose     | 45                 |
| Saccharine    | 20 000–70 000      |
| Acesulfame-K  | 13 000–20 000      |
| Sucralose     | 40 000–80 000      |
| Steviolide    | 30 000             |
| Aspartame     | 12 000–20 000      |
| Neotame       | 700 000–1 300 000  |
| Thaumatin     | 200 000–300 000    |
| Sorbitol      | 50–70              |
| Xylitol       | 90–100             |
| Mannitol      | 50–70              |
| Lactitol      | 30–40              |
| Maltitol      | 80–90              |
| Erythritol    | 50–80              |

| Concentration of sucrose reference at 22° C | 5% | 10% | 20% |
|--------------------------------------------|----|-----|-----|
| 5°C (± 2°C)                                | 89 | 88  | 92  |
| 37°C (± 1°C)                               | 100| 100 | 100 |
| 50°C (± 3°C)                               | 108| 106 | 108 |

*a* Sweetness of sucrose solutions at room temperature was regarded as 100.

*b* Data were extracted from Hyvonen et al. (29).

**Table 2. Sweetness potency of sucrose at different test temperatures compared to sucrose concentration (5, 10 and 20%) at room temperature**

| Concentration of sucrose reference at 22° C | 5% | 10% | 20% |
|--------------------------------------------|----|-----|-----|
| 5°C (± 2°C)                                | 89 | 88  | 92  |
| 37°C (± 1°C)                               | 100| 100 | 100 |
| 50°C (± 3°C)                               | 108| 106 | 108 |
Different LC systems are often applied in standard analysis protocols for sugars, carbohydrates, carbohydrate derivatives, and artificial sweeteners. In the past, cation-exchange LC in combination with RI detection was applied for the measurement of mono- and disaccharides in relatively simple food matrixes (e.g., Method 980.13, ISO 10504, ISO 11868/IDF 147, ISO 22662 | IDF 198). However, presently, sugar profiles in human foods and animal feeds are measured with HPAEC–PAD [e.g., Method 2018.16, Method 995.13, ISO 22184 | IDF 244, The European Committee for Standardization (CEN) 15754] because of its greatly improved resolution and sensitivity. Additionally, HPAEC–PAD does not require precolumn derivatization/labeling.

| Sweetener | Sucrose, % (w/v) | Sweetness potency | Method | Reference |
|-----------|-----------------|-------------------|--------|-----------|
| **Carbohydrates** | | | | |
| Fructose | 5 | 1.05 | 2-AFC | (23) |
| | 5; 10; 15 | 1.25; 1.36; 1.34 | LMS | (24) |
| Dextrose | 5; 10; 15 | 0.64; 0.64; 0.69 | LMS | (24) |
| Xylose | 5 | 0.63; 0.61 | 2-AFC | (23, 25) |
| Tagatose | 5 | 0.85 | 2-AFC | (23) |
| Allulose | 3; 5; 10; 15 | 0.89; 0.89; 0.90; 0.90 | LMS | (26) |
| | 5; 10; 15 | 0.71; 0.75; 0.80 | LMS | (24) |
| **High-intensity sweeteners** | | | | |
| Sucralose | 5 | 500; 561.8 | 2-AFC | (23, 25) |
| | 2; 8; 9; 16 | 740; 414; 430; 194 | SSC | (27) |
| | 3; 5; 10; 15 | 1896; 954; 376; 218 | LMS | (26) |
| | 5; 10; 15 | 521; 285; 201 | LMS | (24) |
| Aspartame | 2 | 182 | Ranking method | (28) |
| | 5; 10 | 111; 119 | 2-AFC | (23, 29) |
| | 5; 10; 15 | 173; 121; 112 | LMS | (24) |
| Stevia | 5 | 64 | 2-AFC | (23) |
| | 5; 10; 15 | 348; 263; 181 | LMS | (24) |
| Reb A | 5 | 144.93 | 2-AFC | (25) |
| | 2; 8; 9; 16 | 263; 46; 46; 27 | SSC | (27) |
| | 3; 5 | 439; 300 | LMS | (26) |
| Luo han guo extract | 5 | 75.76 | 2-AFC | (25) |
| | 5; 10; 15 | 262; 144; 106 | LMS | (24) |
| Acesulfame-K | 5; 10; 15 | 171; 120; 88.1 | LMS | (24) |
| **Sugar alcohols** | | | | |
| Sorbitol | 9.12 | 0.51 | Rating method | (30) |
| | 5; 10; 15 | 0.80; 0.72; 0.83 | LMS | (29) |
| Xylitol | 5 | 0.83; 0.98 | 2-AFC | (23, 25) |
| | 10; 15 | 1.01; 1.12 | LMS | (24) |
| Erythritol | 5 | 0.53 | 2-AFC | (23) |
| | 3; 5; 10; 15 | 0.50; 0.57; 0.70; 0.78 | LMS | (26) |
| | 5; 10; 15 | 0.72; 0.75; 0.84 | LMS | (24) |
| Maltitol | 5 | 0.67 | 2-AFC | (23) |
| | 5; 10; 15 | 0.93; 0.89; 0.95 | LMS | (24) |
| Mannitol | 9.12 | 0.72 | Rating method | (30) |
| | 5; 10; 15 | 0.58; 0.68; 0.81 | LMS | (24) |

Figure 1. Overview of the most common analytical methods for quantitating natural and artificial sweet-tasting molecules.

Figure 2. Examples of analytical methods for which derivatization/labeling is or is not needed.
making it a strong tool in carbohydrate quantitation (e.g., Method 995.13, Method 2018.16, ISO 11292, ISO 22184 | IDF244, and ISO 22579 | IDF 241). Chromatographic techniques are mainly applied for the quantification of artificial sweeteners in food products [e.g., European Standards (EN) 1378, EN 1379, EN 12856, EN 12857, and CEN 15606], although official gravimetric methods such as Method 957.10 and Method 973.29 for cyclamate and saccharin determination are also available. Generally, methods have been validated and applied specifically for a single or limited number of ingredients and/or food products. For example, methods have been validated for products such as milk/milk products and infant formula (ISO 22184 | IDF 244), foods of low/high protein or sugar matrices (Method 2018.16), fruit/fruit juices (Method 971.18), cereals (Method 982.14), milk chocolate (Method 980.13), and instant coffee (Method 995.13, ISO 11292). In addition to the aforementioned sugar profile methods, there are official methods for quantitating lactose in raw/processed milk (Method 2006.06) and lactose-free or low-lactose dairy products and milk (Method 2020.01), and lactose and sucrose in foods for infants and young children and milk and milk products [GuoBiao Standards (GB)] 5413.5–2010). Furthermore, methods for determining complex carbohydrates (i.e., those that are not particularly sweet) in relevant commodities are also available. These include, among others, fructans in foods, pediatric nutritional formula, and infant formula (Method 997.08, Method 999.03, Method 2016.14, Method 2016.06, ISO 22579 | IDF 241); galactooligosaccharides in foods, cereals, dairy products, and infant formulas (Method 2001.02, Method 2021.01); and β-glucans in barley and oats [Method 995.16/Cereals & Grains Association (AACC) 32–23–01/Codex Type II]).

CXS 234–1999 lists several Type I, II, and III methods for quantitation of natural and artificial sweet-tasting compounds in various commodities. Among these are Type II methods for glucose and fructose determination in fruit juices and nectars [EN 1140/International Fruit and Vegetable Juice Association

| Method | Commodity | Provision | Principle | Codex type |
|--------|-----------|-----------|-----------|------------|
| 923.09 | Sugars and syrups | Invert sugar | Volumetric (Lane-Eynon) | —* |
| 970.58 | Molasses | Invert sugar | Titrimetry | — |
| 971.17 | Cyclamates and artificially sweetened products | Cyclohexylamine | Infrared spectroscopy | — |
| 973.29 | Foods | Saccharin | Gravimetric | — |
| 978.17 | Honey | Corn and cane sugar products | Carbon isotope ratio MS | I* |
| 984.15 | Milk | Lactose | Enzymatic | — |
| 985.09 | Wine | Glucose, fructose | Enzymatic | — |
| 986.25 | Infant formula | Total carbohydrates | Calculation | I |
| 998.12 | Raw cane sugar | Dextran | Colorimetry (Roberts Copper) | — |
| 992.09 | Fruit juices/frozen concentrated orange juice | Syrups/sugar beet-derived syrup | MS | — |
| 995.13 | Instant coffee | Carbohydrates | HPAEC-PAD | — |
| 995.17 | Fruit juices | Beet sugar | NMR spectroscopy | — |
| 996.04 | Cane and beet final molasses | Sugars (glucose, fructose, sucrose) | LC | — |
| 998.12 | Honey | Sugars added (C-4 sugars) | Carbon isotope ratio MS | I* |
| 2000.17 | Raw cane sugar | Trace glucose and fructose | Anion-exchange chromatography | — |
| 2000.19 | Maple syrup | Beet or cane sugar | NMR spectroscopy | — |
| 2006.06 | Milk | Lactose | Spectrophotometric-enzymatic | — |
| 2013.12 | Wine and wine-like products | Total carbohydrates | HPLC–RI | — |
| 2018.16 | Food, dietary supplements, pet food, and animal feeds | Sugar profile | HPAEC-PAD | — |
| 2020.01 | Dairy products/milk | Lactose | LactoSens® Amperometric | — |
| 2020.07 | Cereals and cereal products, dairy products, vegetables, fruits, and fruit products | Available carbohydrates | Enzymatic | — |
| 2020.08 | Low-lactose, lactose-free dairy products, and conventional dairy products | Lactose | Enzymatic | — |

* — Not applicable.

b CXS 234–1999 refers to Method 978.17 which has been replaced by Method 998.12.

c CXS 234–1999 refers to Method 998.18. The authors were not able to identify the Method 998.18 and believe the correct method to be Method 998.12.
**Table 5. Official ISO and ISO IDF methods for quantitative analysis of natural and artificial sweeteners**

| Method          | Commodity                        | Provision                               | Principle                                      | Codex type |
|-----------------|----------------------------------|-----------------------------------------|------------------------------------------------|------------|
| ISO 2911 | IDF35 | Sweetened condensed milk | Sucrose | Polarimetry | IV |
| ISO 5377 | Starch and hydrolysis products | Reducing power and dextrose equivalent | Titrimetry (Lane-Eynon) | I |
| ISO 5548 | IDF 106 | Edible casein products | Lactose | Photometry (phenol and H₂SO₄) | IV |
| ISO 5765–1/2 | IDF 79–1/2 | Whey powders | Lactose | Enzymatic: Part 1—Glucose moiety or Part 2—Galactose moiety | II |
| ISO 10504 | Glucose, fructose-containing and hydrogenated glucose syrups | Glucose, maltose, maltotriose, fructose, sorbitol, mannitol, maltitol, and maltoligosaccharides | HPLC–RI | II* |
| ISO 11285 | IDF 175 | Milk | Lactulose | Enzymatic | —b |
| ISO 11292 | Instant coffee | Free and total carbohydrates | HPAEC–PAD | — |
| ISO 11868 | IDF 147 | Heat-treated milk | Lactulose | HPLC | — |
| ISO 22184 | IDF 244 | Milk and milk products | Galactose, glucose, fructose, sucrose, lactose, and maltose | HPAEC–PAD | — |
| ISO 22579 | IDF 241 | Infant formula/adult nutritionals | Fructans | HPAEC–PAD | — |
| ISO 22662 | IDF 198 | Milk and milk products | Lactose | HPAEC–PAD | — |
| ISO 26462 | IDF 214 | Milk | Lactose | Enzymatic | — |

* — CXS 234–1999 refers to ISO 10504 as Type II method for the commodity sugars (fructose) with either glucose or fructose as provisions.

b — Not applicable.

**Table 6. Official EN/CEN, CEN/TS (Technical Specifications) and NMKL methods for quantitative analysis of natural and artificial sweeteners**

| Method          | Commodity                        | Provision                               | Principle                                      | Codex type |
|-----------------|----------------------------------|-----------------------------------------|------------------------------------------------|------------|
| EN 1140/IFUMA 55 | Fruit juices and nectars | Glucose and fructose | Enzymatic | II |
| EN 12146/IFUMA 56 | Fruit juices and nectars | Sucrose | Enzymatic | III |
| EN 12630/IFUMA 67/NMKL 148 | Fruit juices and nectars | Glucose and fructose | HPLC | III |
| EN 12630/IFUMA 67/NMKL 148 | Fruit juices and nectars | Sucrose | HPLC | II |
| NMKL 122 | Fruit juices and nectars | Saccharin | LC | II |
| NMKL 123 | All foods | Cyclamate | Spectrophotometry | III |
| EN 12856 | All foods | Acesulfame-K, aspartame | HPLC | II |
| EN 12856 | All foods | Saccharin | HPLC | III |
| EN 12857 | All foods | Cyclamate | HPLC | II |
| EN 1376 | Table-top sweeteners | Saccharin | Spectrometric | III |
| EN 1377 | Table-top sweeteners | Acesulfame-K | Spectrometric | II |
| EN 1378 | Table-top sweeteners | Aspartame | HPLC | II |
| EN 1379 | Liquid table-top sweeteners | Cyclamate and saccharin | HPLC | II |
| CEN/TS 14537 | Foodstuffs | Neohesperidin-dihydrochalcone | HPLC | —a |
| CEN 15086 | Foodstuffs | Isomalt, lactitol, maltitol, mannitol, sorbitol, and xylitol | HPLC | — |
| CEN 15606 | Foodstuffs | Acesulfame-K, aspartame, Neohesperidin-dihydrochalcone, and saccharin | HPLC | — |
| CEN/TS 15754 | Animal feeding stuffs | Sugars | HPAEC–PAD | — |
| CEN 15911 | Foodstuffs | Sweeteners | HPLC–ELSD | — |

*a — Not applicable.
Table 7. Official AACC International and International Association for Cereal Science and Technology (ICC) methods for quantitative analysis of natural and artificial sweeteners

| Method          | Commodity                              | Provision                   | Principle                        |
|-----------------|----------------------------------------|-----------------------------|----------------------------------|
| ICC 132         | Cereals and cereal products            | Saccharose                  | Enzymatic                        |
| AACC Method 80–04.01 | Cereals                           | Fructose, glucose, sucrose, maltose, and lactose | HPLC                             |
| AACC Method 80–05.01 | Corn syrups, fructose-containing syrups, corn sugars, and starch hydrolysates | Saccharides                  | LC                               |
| AACC Method 80–10.01 | Sugar mixture                  | Glucose                     | Enzymatic                        |
| AACC Method 80–50.01 | Feeds and feedstuffs             | Sucrose                     |                                  |
| AACC Method 80–60.01 | Flour and semolina               | Reducing and nonreducing sugars |                                  |
| AACC Method 80–68.01 | Prepared bakery mixes       | Reducing sugars             | Titrimetry (Luff Schoorl)        |

Table 8. Official ICUMSA methods for quantitative analysis of natural and artificial sweeteners

| Method          | Commodity                              | Provision                   | Principle                        | Codex type |
|-----------------|----------------------------------------|-----------------------------|----------------------------------|------------|
| GS1–3           | Cane raw sugar                         | Reducing sugars             | Titrimetry (Lane-Eynon)          | —          |
| GS1/37–3        | Sugars (soft white and soft brown sugar) | Invert sugar                 | Titrimetry (Lane-Eynon)          | I          |
| GS1/37–3        | Sugars (plantation or mill white sugar) | Invert sugar                 | Titrimetry (Lane-Eynon)          | I          |
| GS1–4           | Raw sugar                              | Glucose, fructose           | HPAEC                            |            |
| GS1–5           | Cane raw sugar                         | Reducing sugars             | Titrimetry (Luff Schoorl)        |            |
| GS2–4           | White sugar                            | Glucose, fructose           | Enzymatic (hexokinase method)    |            |
| GS2–5           | White sugar                            | Reducing sugars             | Titrimetry (Knight-Allen EDTA method) |            |
| GS2/3–5         | Sugars (powdered sugar)                | Invert sugar                 | Titrimetry                        | I          |
| GS2–6           | White sugar                            | Reducing sugars             | Titrimetry (modified Other method) |            |
| GS4–1           | Molasses                               | Apparent sucrose            | Double polarization method       |            |
| GS4–2           | Molasses, factory products, and cane juice | Sucrose                     | GC                                |            |
| GS4–3           | Cane molasses                          | Reducing sugars             | Titrimetry (Lane-Eynon)          |            |
| GS4/3–3         | Sugars (lactose)                       | Anhydrous lactose           | Titrimetry                        | II         |
| GS4/3–3<sup>b</sup> | Sugars (soft white and soft brown sugar) | Invert sugar                 | Titrimetry (Lane-Eynon)          | I          |
| GS4–5           | Beet molasses                          | Reducing sugars             | Titrimetry (Lane-Eynon)          |            |
| GS4–7           | Molasses and refined syrups after hydrolysis | Total reducing sugars        | Titrimetry (Lane-Eynon)          |            |
| GS4/3–7         | Sugars (soft white and soft brown sugar) | Sucrose plus invert sugar    | Titrimetry                        | I          |
| GS4–9           | Molasses and refined syrups after hydrolysis | Total reducing sugars        | Titrimetry (Luff-Schoorl)        |            |
| GS4–22          | Beet molasses                          | Sucrose and betaine         | HPLC                             |            |
| GS7–22          | Cane juices, syrups, and molasses      | Fructose, glucose, sucrose  | GC                                |            |
| GS7–23          | Cane molasses                          | Fructose, glucose, sucrose  | HPLC                             |            |
| GS7–23          | Beet molasses                          | Sucrose                     | HPLC                             |            |
| GS7–24          | Cane juices, syrups, and molasses      | Glucose, fructose, sucrose  | High performance ion chromatography (HPIC) |            |
| GS7–24          | Beet molasses                          | Sucrose                     | HPIC                             |            |
| GS8–4           | Beet molasses                          | Glucose, fructose           | Enzymatic                        |            |
| GS8–5           | Beet pulp                              | Apparent total sugar content | Titrimetry (Luff-Schoorl)        |            |
| GS4–18          | Beet molasses                          | Total 2-galactosides and raffinose | Enzymatic                      |            |
| GS14–19         | Beet molasses                          | Raffinose                   | HPAEC                            |            |

<sup>a</sup> Not applicable.

<sup>b</sup> Applicable at levels >10% w/w.
Table 9. Official Chinese GB, and China CIQ Import Commodity Inspection Standards (SN) methods for quantitative analysis of natural and artificial sweeteners

| Method* | Commodity | Provision | Principle |
|---------|-----------|-----------|-----------|
| GB/T 5009.7–2008 | Foods | Reducing sugars | Titrimetry |
| GB/T 5009.7–2016 | Foodstuffs | Reducing sugars | Titrimetry |
| GB 5009.8–2016 | Foods | Fructose, glucose, sucrose, maltose, and lactose | HPLC |
| GB 5413.5–2010 | Foods for infants, young children, milk, and milk products | Lactose, sucrose | HPLC |
| GB/T 5513–2008 | Foods | Reducing sugars | Titrimetry |
| GB/T 5513–2019 | Grains | Reducing sugars | Titrimetry |
| GB/T 9656 31–2008 | Meat products | Total sugars | Spectrophotometry and titrimetry |
| GB/T 37493–2019 | Cereals and pulse seeds | Cyclamate, sodium saccharin, acesulfame, aspartame, alitame, and neotame | Titrimetry (Shaffer-Somogyi) |
| SN/T 3538–2013 | Foodstuffs for export | Soluble sugars | LC–MS/MS |

* Codes without T are mandatory, codes with T are recommended.

Methods of Analysis (IFUMA) 55 and sugar (fructose) commodities (ISO 10504:1988); lactose in sugar (lactose) commodities (International Commission for Uniform Methods of Sugar Analysis (ICUMSA) GS 4/3–3 (1994)) and whey powders (ISO 5765–1/2 | IDF 79 1/2); acesulfame-K and aspartame in all foods (EN 12856) as well as table-top sweeteners (EN 1377 and EN 1378 for acesulfame-K and aspartame, respectively); cyclamate in all foods (EN 12857) and liquid table-top sweetener preparations (EN1379); and saccharin in fruit juices/nectars [Nordic Committee on Food Analysis (NMKL) 122] as well as liquid tabletop sweetener preparations (EN1379). Additionally, CXS 234–1999 includes Type III methods for glucose and fructose in fruit juice and nectars (EN 12630/IFUMA 67/NMKL 148); carbohydrates in food for special dietary uses [method described in Codex Alimentarius Commission (CAC) VOL IX–Ed.1, Part III]; cyclamate in all foods (NMKL 123); and saccharin in all foods (EN 12856) and table-top sweeteners (EN 1376).

To our knowledge, there are no official methods for quantitating sweet glycosides such as steviolides and mogrosides in food products. However, various methods for the determination of these sweet-tasting molecules in leaves and fruit extracts and table-top sweeteners have been published. Examples of techniques include enzymatic approaches (49); LC (50–53); near infrared spectroscopy (NIR; 54–56); LC–MS/MS (57, 58); HPTLC (59–61); and hydrophilic interaction LC (HILIC) with charged aerosol and UV detection (62, 63). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommends an HPLC-based method for the estimation of steviol glycosides in stevia (64).

Furthermore, Fayaz et al. (65) described the determination of stevioside and rebaudioside-A in milk, yogurt, chewing gum, jam, and carbonated water by HPLC–UV, while Wald et al. (66) published an HPTLC method for determination of steviosides in food products, stevia leaves, and formulations.

Sensory Evaluation of Sweetness in Foods and Beverages

Sensory Evaluation Principles

Sensory evaluation methods that include discrimination, descriptive, and affective tests have been developed to measure and analyze human responses to foods and beverages as perceived through the sense of sight, smell, touch, taste, and hearing (67, 68). The choice of test methods depends on the purpose of analysis. For example, discrimination tests are used to determine if products are different, while descriptive tests focus on the degree to which products are different with respect to specific sensory characteristics. Affective tests are usually applied when the objective is to determine how well products are liked or which products are preferred. The required number of panelists can vary from 8 to 200 depending on the method. For a scenario in which quantitative descriptive tests are used (69), panelists usually receive extensive training, varying between 10 and 120 hours depending upon the complexity of the product aroma, flavor, and texture profile. Training aims to build a sensory glossary (70) and calibrate panelists on perceived intensity of each attribute, which are quantified on an intensity scale by a panel of experts. Once selected, they are trained to perceive, identify, and rate the perceived intensity of each attribute elicited by the product of interest (71). Extensively trained panelists are qualified as experts (71).

To ensure quality results, sensory evaluation is performed in sensory booths to prevent participants from interacting and influencing each other’s judgments. A randomized and balanced order of product presentation helps statistically suppress carryover effects (i.e., influence of one stimulus on perception of subsequently evaluated stimuli; 69). Finally, the number of stimuli evaluated in a single session is defined per product category to avoid sensory fatigue and saturation.

Sensory Methods for Measuring Sweetness

There are several commonly applied methods for measuring food or beverage perception using trained human panels (72, 73), all of which are based on the identification and quantitation of perceived sensations by adequately trained assessors. Sensory descriptive analysis methods all rely on the consideration that even though perception is subjective and varies from one individual to another, intensive training of panelists reduces this inter-individual variability. A common frame of reference is shared and learned by all panelists during training, so they are able to score the sensations they perceive in a...
similar way. These references are purposely chosen to illustrate, in a qualitative and quantitative manner, the levels of sweetness intensity that can be encountered in the product category under consideration. However, none of these methods are specifically designed for measuring only sweetness in food. Instead, they are designed to cover all sensory characteristics perceived when eating or drinking a product. These characteristics range from appearance to smell, texture, and taste properties. This also includes basic tastes like sweetness as well as flavors perceived by retro-nasal olfaction.

Among available methods, several can be considered the most relevant for measuring sweetness in FUF and other food products and beverages. These include magnitude estimation as described in ISO 11056; studies using a specific measurement scale; the LMS; and quantitative descriptive sensory profile as described in ISO 13299 [including Quantitative Descriptive Analysis® (QDA®) and Spectrum™ methods]. These methods have been developed to identify and quantify the sensory characteristics of a product in its entirety (i.e., all the properties of a product perceived using the five senses). Therefore, according to the objective of the study, the panel would either be restricted to measure the perceived sweetness of different food products or beverages, or would include sweetness and other sensory characteristics that would presumably be interacting with sweetness. Moreover, these methods are designed to discriminate between different products within the same category. A comparison pair might be a newly developed product versus a competitor product, two existing recipes, or for comparison to a fixed reference. This reference would consist of a product with known composition to allow reproduction and validation across tests/comparisons. The reference product would represent the maximum threshold of sweetness that every newly developed product should not exceed.

Although the methods mentioned above are all relevant for measuring perceived sweetness of FUF and other food products and beverages, each requires different investments of time and resources. Therefore, a sensory scientist should consider several criteria to select the most appropriate method for their objectives, including number of panelists to be recruited; screening process requirements; sensory properties to be evaluated; time duration of training; panel performance validation; and duration of panel availability (e.g., how many studies and for how many months).

Magnitude Estimation Method

This method can be considered the “gold standard” for ratio-level measurement of intensity and has historically been the first method used for measuring relative sweetness of different carbohydrate sources in aqueous solutions. The notion of “ratio” refers to the proportionality that two samples may display on a specific sensory property. For example, a carbohydrate source with a sweetness intensity of four can be considered twice as sweet as another carbohydrate source with a sweetness intensity of two.

Labeled Magnitude Scale

The LMS uses a non-linear, continuous scale graduated in a quasi-logarithmic way, with each graduation translating a level of perceived intensity. Scores of perceived sweetness could thus be indicated on this scale from “no sweetness at all” to “the strongest sweetness imaginable.” Empirical data are based on ratio-scaling and similar to those from magnitude estimation.

Quantitative Descriptive Sensory Profile

In the quantitative descriptive sensory profile, assessors evaluate samples on a common list of attributes and score their intensity. There are several methods for establishing a quantitative descriptive sensory profile, among which some techniques have been trademarked. Results shall consist of intensity scores for each attribute that can be submitted to univariate analyses. Empirically derived profiles are panel and product category specific and cannot be interpreted by other groups if no reference standards are given.

Quantitative Descriptive Analysis

This approach is a variant of the quantitative descriptive sensory profile and can be used for a wide variety of purposes, including understanding product similarities and differences, ingredient substitution, new product development, competitive assessments, claims substantiation, advertising, etc. Quantitative Descriptive Analysis uses an unstructured or semi-structured (6 in/approximately15 cm) line scale, anchored 0.5 in. from either end for measuring and scaling perceived differences and intensities. These equal-interval scales are described in psychophysics literature.

Spectrum Method

The Spectrum method is based on a descriptive profiling procedure that includes using documented references for both qualitative attributes and intensity scale points. The method has precise steps and procedures at every stage of development. This includes selection of assessors to panel leadership, panel training, validation, and maintenance of the panel after training is complete. These practices lead to a descriptive panel that produces reproducible and statistically robust data across multiple sessions and categories. Sensory attributes are identified with both physical external references and written definitions, which should allow describing and discriminating among samples in the product category.

The Spectrum method scale is based on a 0- to 15-point intensity scale with the ability to rate in increments of tenths for 150 points of discrimination. This gives the assessors the ability to discriminate using smaller points of difference. The Spectrum scale is universal, covering the entirety of intensities within a scope as large as the global food system.

Human Variability in Sensory Response to Sweet Taste

Even with a well-designed study and use of trained panelists, variability among individual panelists exists in sensory response for perceived sweetness (i.e., in perceived intensity rating) due to inherent physiological and psychological differences. Sweet taste threshold differs between individuals due to genetic differences (74, 75) that are modulated by physiological factors such as hormonal mechanisms and taste bud abundance. See Trius-Soler et al. for a systematic review and meta-analysis on this topic (76). Psychological factors such as mood (77) and emotions (78) can also impact sweet taste perception.

Because of the role of physiological and psychological factors in taste perception variation, sweetness intensity is generally represented by an average value and a within-panel variability statistical estimator such as a confidence interval. Therefore, perceived intensity for a given attribute is not a fixed number
but rather a number plus or minus the variability statistical estimator value.

**Contextual Factors Contributing to Sweetness Perception**

In addition to human variability, contextual factors can also influence perception of sweet taste and increase variability of measured sweetness. Particularly, other taste stimuli generated by food or beverage ingredients such as sourness of organic acids or bitterness of peptides can modulate sweetness perception through binary taste–taste interaction (79). Also, food or beverage sensory modalities such as appearance, smell, and texture (intrinsic contextual factors) or attributes of serving vessels such as cup or plate color, texture, or shape (extrinsic contextual factors) may modulate perceived sweetness through cross-modal perceptual interactions. See Wang et al. for a review (80).

The origin of perceptual interaction is cognitive and built through repeated exposure to sensory stimuli collectively present in beverages or foods. One example is the strawberry aroma and sweet taste experienced during strawberry fruit consumption as well as any sweet foods and beverages flavored with strawberry (81, 82). This so-called associative learning is integrated at a neural level in the orbitofrontal cortex, a brain region responsible for stimulus–stimulus association and integration of sensory perception that helps explain how a strawberry or vanilla odor can enhance perceived sweetness (83, 84).

The magnitude of contextual effects on perception of sweetness differs between people according to their previous food experience. This is true even for a trained panel. As such, associative learning is a resistant phenomenon (85).

**Measuring Sweetness Elicited by an Ingredient in a Finished Food Product or Beverage**

Sensory methods are capable of qualifying and quantifying the perceived sweetness of individual ingredients and finished food products and beverages. However, it is unfeasible to selectively measure the perceived sweetness of an ingredient (e.g., carbohydrate source) in a finished product. Even with a well-designed study and training to limit variability between panelists, it is still impossible to define a standard reference value of perceived sweetness intensity as a QC indicator for a specific ingredient in a finished product; especially one that is identical over time and across global taste panels. This is true for several reasons. First, inherent variability in human sensory response contributes variability in perceived intensity rating. Second, the perceived sweetness of an ingredient dissolved in aqueous solution at a given concentration does not necessarily indicate equivalent sweetness for the same ingredient at the same concentration in a finished product because contextual factors like manufacturing processes and other ingredients may modulate sweetness. Consequently, for Codex, selecting “carbohydrate sources that have no contribution to [food or beverage] sweet taste” and “in no case be sweeter than lactose” (CXS 156–1987, Section B.3.1, footnote 5) cannot be perceptually demonstrated.

**Advancements in Sensory Evaluation**

Novel digital technologies have been implemented in sensory science, combining electronic sensors (e.g., e-tongue and e-nose) and artificial intelligence to predict food sensory properties (86). Commercially available e-tongue devices rely on selective receptors to measure sweetness of known compounds like sucrose. Examples include the Alpha MOS, ASTREE Electronic Tongue (Toulouse, France) and Valiher Swizzle 1.0 (Tel Aviv-Yafo, Israel). However, these devices would not be appropriate for verifying compliance with a sweetness regulation due to the inability to quantify the modulation of sweet taste perception induced by other ingredients in a finished product. Also, they are incapable of achieving the accuracy and precision required of an internationally validated standard method. Hence, to our knowledge, no technical solution exists to measure perceived sweetness solely generated by a source of carbohydrate in food products or beverages.

**Conclusions and Recommendations**

The Codex Alimentarius Procedural Manual includes procedures for the elaboration of Codex standards and related texts as well as presents the format for Codex commodity standards. One of the required chapters in a Codex standard is “Methods of Analysis and Sampling,” which should contain the following language: “For checking the compliance with this standard, the methods of analysis and sampling contained in the Recommended Methods of Analysis and Sampling (CXS 234–1999) relevant to the provisions in this standard, shall be used.” This language is included in the current FUF standard (CXS 156/1987).

In 2013, the Codex Alimentarius adopted a guideline describing principles for the use of sampling and testing in international food trade (CAC/GL 83–2013). These principles are intended to assist governments in the establishment and use of sampling and testing procedures for determination on a scientific basis, whether foods in international trade comply with specifications. To select appropriate sampling and testing procedures, the guideline states that methods should be fit for the intended purposes and applied consistently.

CCNFSDU has proposed requirements for sweetness of FUF in a revised version of the Codex FUF standard (CXS 156/1987). Consequently, questions have been raised about the ability to measure and enforce a requirement for sweet taste objectively.

The results of a thorough review indicate that there are no analytical methods available for objectively determining the sweetness of sweet-tasting molecules in food products and beverages. An abundance of analytical methods are available, however, to quantitate the composition of these molecules in food products and beverages. In the specific area of sensory evaluation, sweet taste can be determined by standard sensory analysis methods. However, it is impossible to define an accurate reference value for sweetness intensity, which makes it impossible to assess sweetness accurately across global taste panels. Furthermore, it is impossible to selectively measure perceived sweetness of carbohydrate sources in food products and beverages, including FUF, due to taste perception of other ingredients in a finished product matrix. CCMAS confirmed that there are no known validated methods to measure sweetness of carbohydrate sources during its 41st session in May 2021.

Novel digital technologies combining electronic sensors and artificial intelligence are in development. However, these will be unable to measure a perceived sweetness solely generated by an ingredient in food products and beverages. Additionally, these technologies are incapable of achieving the accuracy and precision required of an internationally recognized standard method, especially one that would be considered for adoption in CXS 234–1999.

Considering the need for fit-for-purpose testing procedures to enable verification of compliance with specifications to
support international trade, it is not recommended to include any requirement related to “sweetness” or “sweet taste” in a revised Codex standard for FUF or any other food commodities in the future. If, however, there is a need to establish additional requirements beyond those already drafted, considerations should be given on the availability of analytical methods for regulatory compliance verification.

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Conflict of Interest

None declared.

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