Analytical methodologies for determination of artificial sweeteners in foodstuffs

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Artificial high-intensity sweeteners are used increasingly frequently for food production. The food industry tends to highlight beneficial aspects of their use (e.g., tooth friendliness, increasing the quality of life of those suffering from different forms of diabetes and the possibility of weight control without anyone sacrificing their favorite "unhealthy" drinks or snacks). However, some consumers are deeply concerned about the safety of artificial sweeteners and claim that the food industry is replacing natural beet sugar or cane sugar for purely economic reasons.

Most of these food additives have a maximum usable dose or a maximum allowable concentration specified for a given type of food. In order to assure consumer safety, it is necessary to control the content of sweeteners in foodstuffs. Analytical methods (including high-performance liquid chromatography, ion chromatography, thin-layer chromatography, gas chromatography, capillary electrophoresis, flow-injection analysis, electroanalysis and spectroscopy) can determine sweeteners individually and simultaneously in mixtures. This review focuses on the application of some popular analytical procedures for determination of artificial sweeteners in food.

Keywords: Artificial sweetener; Capillary electrophoresis; Electroanalysis; Flow-injection analysis; Foodstuff; Gas chromatography (GC); High-performance liquid chromatography (HPLC); Ion chromatography (IC); Spectroscopy; Thin-layer chromatography (TLC)

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1. Introduction

Artificial high-intensity sweeteners (also called non-nutritive sweeteners) form an important class of food additives, which are commonly used in the food, beverage, confectionery and pharmaceutical industries. They provide the sensation of sweetness, but with little or no intake of food energy. There are a large number of known intense sweeteners, but only very few are allowed to be used in modern food industry. The list of authorized artificial sweeteners varies from country to country. For example, there are six artificial high-intensity sweeteners authorized for use in European Union (EU) (acesulfame-K, aspartame, cyclamic acid and its salts, saccharin and its salts, sucralose and neohesperidine dihydrochalcone) [1], whereas, in the USA, the corresponding list does not include cyclamates and neohesperidine dihydrochalcone, instead one can find neotame there [2].

The food industry is heavily promoting its artificially-sweetened products (frequently called "diet" or "light"), highlighting their benefits. Low-calorie or reduced-calorie food products and beverages can help in treatment of obesity, maintaining body weight and management of diabetes. Last, but not least, artificial sweeteners are not fermented by the microflora of the dental plaque, which makes them tooth-friendly.

Sweeteners may be used separately or in combination with other sweeteners, as so-called blends. Nowadays, the common trend in food industry is to use sweetener blends, because some of the sweeteners impart side tastes and aftertastes that can limit their applications in foods and beverages [3]. It was found that mixing such a problematic sweetener with another frequently yields a blend not only lacking unwanted side or aftertastes but also sweeter than the algebraic sum of the components. A very well-known example of such a mixture is saccharin-cyclamate (1:10) blend. The bitter aftertaste of saccharin is masked by cyclamate and the unpleasant aftertaste of cyclamate, sensed

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by some people, is masked by saccharin. Simultaneously (due to synergistic effect), the sweetening power of the mixture increases. Properly formulated sweetener blends can precisely reproduce the texture and the sweetness profile of traditional sugar-containing products, create new products characterized by an original sweetness profile and improve taste stability [4].

Artificial high-intensity sweeteners, intensely promoted by the food industry are among the most controversial food additives due to suspicions of adverse health effects [5]. These allegations include causing dermatological problems, headaches, mood variations, behavior changes, respiratory difficulties, seizures, allergies and cancer.

Many experiments have been performed on the safety of saccharin. Some associated saccharin with bladder cancer when fed at high doses to rats. However, results from subsequent carcinogenicity studies showed no consistent evidence of association between saccharin consumption and cancer in test animals. Another suspicion about saccharin is connected with the possibility of allergic reactions in people who do not tolerate sulfa drugs [4,6].

In the case of cyclamate, the issue is more complicated because different people metabolize this sweetener in different ways [5]. One study conducted in 1966 indicated that cyclamate can be metabolized by some intestinal bacteria resulting in formation of cyclohexylamine, a compound suspected to have some chronic toxicity in animals. Another study from 1969 linked cyclamate consumption with increased risk of bladder cancer in rats. In 2000, the European Food Safety Agency (EFSA) published its opinion on safety of cyclamate, stating that available epidemiological data revealed no indications of harmful effects on human reproduction parameters of cyclamate used as a food additive [7].

Aspartame is probably the most controversial artificial high-intensity sweetener on the market, with adverse medical effects attributed to it including brain tumors, multiple sclerosis, systemic lupus, and methanol toxicity, causing blindness, spasms, shooting pains, seizures, headaches, depression, anxiety, memory loss, birth defects, leukemia and death. The European Ramazzini Foundation (ERF) of Oncology and Environmental Sciences published, in 2006 and 2007, results of two studies [8,9] on aspartame toxicity. Both studies linked aspartame with cancer, lymphomas and leukemias in tested rats. In March 2009, the EFSA discounted the results of these studies and found no indications of any genotoxic or carcinogenic potential of aspartame [10]. Nevertheless, people with phenylketonuria should eliminate foods containing aspartame, because excess intake of phenylalanine (one of the aspartame's metabolites) can lead to brain damage.

Safety concerns pertaining to sucralose are mainly caused by the presence of three chlorine atoms in its molecule, which make it an organochloride. Many organochlorides are toxic or carcinogenic (e.g., pesticides and dioxins) and this is probably the reason for the mistrust of sucralose. However, studies in human beings and animals have shown that this sweetener did not pose carcinogenic, reproductive or neurological risk to people [5].

The content of sweeteners in foodstuffs is limited by country-specific regulations. In the EU, sweeteners are thoroughly assessed for safety by the EFSA before they are authorized for use. EU Directives 94/35/EC [11], 96-83/EC [12], 2003/115/EC [13], 2006/52/EC [1] define, which sweeteners have approval to be added to food products and beverages. Considering medical and legal aspects, the determination of these artificial sweeteners has economic and social relevance [14].

Due to consumer safety, it is necessary to control the content of sweeteners in foodstuffs. To obtain this information, reliable quantitative methods of analysis are required to measure levels of sweeteners in a broad range of food matrices [15]. A number of analytical methods based on different principles are available for their determination. The aim of this review is to present and to compare the available analytical methods for determination of artificial sweeteners in foodstuffs.

2. Artificial sweeteners

High-intensity sweeteners can be divided into three categories: synthetic, semi-synthetic and natural. They comprise a wide variety of organic molecules (e.g., carbohydrate derivatives, salts of organic acids, terpenoids and even proteins [16]). The majority of sugar substitutes approved for use in food chemistry are artificially-synthesized compounds, so we do not consider naturally intense sweeteners in this review. The most popular artificial sweeteners are: acesulfame-K (ACS-K), aspartame (ASP), cyclamate (CYC), saccharin (SAC), sucralose (SCL), alitame (ALI), neotame (NEO) and neohesperidine dihydrochalcone (NHDC), which is a semi-synthetic sweetener. Table 1 shows the chemical structures and the basic characteristics of aforementioned sweeteners.

3. Sample preparation

Sample preparation is an essential stage in the analytical process, and food samples are among most difficult matrices, due to the great variability in their composition (e.g., preservatives, colors, thickeners, vitamins, proteins, lipids and minerals). All of the components can interfere with the determination of sweeteners. Sample preparation procedure must be tailored to the method of final determination, considering the instrumentation