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REVIEW ARTICLE

# Sweetener food additives: a synoptical overview on their chemical properties, applications in food products and side effects

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## ABSTRACT

The increase of obesity and its metabolic comorbidities have led to a growing consumption of sugar-free products, where sucrose is replaced by low-calorie sweeteners. Since the discovery of the synthesis of saccharin, progressively more non-nutritive sweeteners have been produced and consumed, as they release none or only very small amounts of energy. In this context, traditional synthetic food sweeteners are largely used, because they have a higher sweetener power than most of the sweeteners of natural origin. Yet, due to their potential risks, some of them are being replaced by those obtained from natural origins with high sweetening power, such as thaumatins and neohesperidin DC, or by synthetic sweeteners with an even greater sweetener power, with advantame as the one with the highest sweetening power. Yet, numerous side effects of synthetic sweeteners have been reported (namely, stomach and liver problems, allergic reactions, nausea, vomiting, changes in behaviour, cognitive problems, genotoxicity and carcinogenic effects), whereas those naturally derived seem to have less important health problems associated. Moreover, some sweeteners of natural origin, such as polyols, have a low sweetening power, which makes them less effective, but have other functions in food processing. To further understand the implications of using synthetic and natural-derived food sweeteners, this review aims to provide a synoptical approach on chemical characteristics, properties, uses and side effects of those which are currently allowed and applied during food processing mostly considering the authorized sweeteners in European Union.

**Keywords:** Side effects of sweeteners; Sweetener food additives; Sweeteners of natural origin; Sweeteners of synthetic origin; Sweeteners applications

## INTRODUCTION

The rising prevalence of obesity and its associated metabolic disorders has resulted in a rise in the use of sugar-free products, which substitute low-calorie sweeteners for sugar. Due to their potential effects on body weight, glucose tolerance, hunger, and taste perception, they are ingested by both the general public and those with diabetes. (Wojtus et al., 2022). Sucrose and other natural sugars such as fructose, glucose and corn syrup provide energy by breaking chemical bonds and are therefore commonly classified as energy sugars and nutritive sugars. Moreover, low-calorie sweeteners have a special value in developed countries when the aim is to reduce the amount of sugar consumed (Lidon and Silvestre, 2007; Lidon and Silvestre, 2010). It is strongly advised by the World Health Organization to cut back on sugar consumption in the diet to less than 10%, ideally as low as 5%. (WHO, 2015; WHO, 2018).

Sweetener food additives are products of natural or synthetic origin, with a sweet taste, and without significant energy value, which are used to replace energetic sugars. Since the discovery of the synthesis of saccharin, in 1878, progressively more non-nutritive sweetener food additives have been prepared that release only very small amounts of energy. Nevertheless, this research is not easy since the relationship between structure and sweet taste is not well known, being difficult to find the most efficient sweeteners that do not have harmful side effects on health. Besides, it is necessary to consider its solubility and stability at different temperatures, as well as if its sweet taste is not accompanied by other unpleasant flavours and if it is cheap enough to

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be used replace sucrose (Lidon and Silvestre, 2007; Lidon and Silvestre, 2010).

Sweeteners can be classified by their origin, being of natural or of synthetic origin. Some sweeteners of natural origin can occur in nature and be obtained by processes that can be consider to some extend natural, namely as neo-hesperidin from grapefruit. Other sweeteners can be produced through hydrogenation or fermentation of natural sugars, like the preparation of polyols, which are sugar alcohols, an important group of sweeteners of natural origin (Commission Regulation (EU) Nª 231/2012). Sweeteners of synthetic origin are obtained by chemical synthesis, from organic compounds.

In the framework of the evaluation of safety risks, sweetener food additives must be approved by specialized entities, such as the Joint FAO/WHO, the Expert Committee on Food Additives (JEFCA), the European Food Safety Authority (EFSA), and the Food and Drug Administration (FDA). This evaluation considers the establishment of acceptable daily intakes (ADI), which according to the Joint FAO/WHO, is defined as the "amount of a food additive that for a human, expressed on the basis of body weight, can be consumed daily even during all of life, without risk". In the European Union (EU), the use of sweeteners, like to other food additives, is regulated by specific laws, being report applications to food products, maximum usable quantities, chemical characterizations, and purities (Commission Directive 2008/60/EC; Commission Directive 2010/37/EU; Commission Regulation (EU) Nº 1129/2011; Commission Regulation (EU) Nº 1130/2011; Commission Regulation (EU) Nº 1131/2011; Commission Regulation (EU) Nª 231/2012; Commission Regulation (EU) Nº 1049/2012; Commission Regulation (EU) Nª 1050/2012; Commission Regulation (EU) Nº 724/2013; Commission Regulation (EU) Nº 913/2013; Commission Regulation (EU) Nº 497/2014; Commission Regulation (EU) Nº 1092/2014; Commission Regulation (EU) 2015/647; Commission Regulation (EU) 2015/1832; Commission Regulation (EU) 2016/479; Commission Regulation (EU) 2016/1776; Commission Regulation (EU) 2018/97; Commission Regulation (EU) 2017/335; Commission Regulation (EU) 2018/677; Commission Regulation (EU) 2018/1497; Commission Regulation (EU) 2021/1156; Commission Regulation (EU) 2023/447). In EU each food additive has a code, which includes the letter E (for Europe), and three or four digits. The numbering scheme follows the International Number System (INS), as determined by the Codex Alimentarius.

Sweeteners can be used in foods, namely dietetic or low-energy foods (specially "light", "zero" "no sugar added" sweet products), such as some jams and jellies, dairy products, low-energy sweetened yogurts, soft drinks, and various others confectionery products. Most of these sweeteners are much more powerful than sucrose, and only very small amounts are required to sweeten food products. This is not the case of polyols whose sweetness is less than that of sucrose.

Food products, particularly bakery goods, are impacted when 100% sugar is replaced with non-nutritive sweeteners from a sensory, physical, and physiological perspective. For example, in this situation, pastry made with sugars retain a notable distinction from non-caloric sweeteners, and food goods made with sugars have a longer shelf life than those made with sweeteners. In bakery products produced using sugars, differences in texture, aroma, colour and flavour were also detected, relatively to those prepared with sweetener (Quitral et al., 2019). Besides, as unlike sucrose, sweeteners do not provide texture or viscosity to food products, the addition of bulking agents, such as polydextrose (E1200), becomes necessary.

Despite some published controverse information, some health side effects have been identified from the use of sweeteners in food products, specially related with those of synthetic origin. Accordingly, considering the importance, of safety limits in food processing, this review aims to provide information about general proprieties, applications in food products and human health effects of sweetener food additives, mostly considering the authorized sweeteners in Europe.

## APPLICATIONS, PROPERTIES AND SIDE EFFECTS OF SWEETENERS

### Sweeteners food additives of natural origin

#### *Sorbitol*

With the generic name of sorbitol, two polyol food additives can be distinguished: sorbitol, also known as D-sorbitol or D-glucitol (with the chemical formula $OHCH_2(CH(OH))_4CH_2OH$), which constitutes the food additive with the code E420(i), and sorbitol syrup, also named D-glucitol syrup, which constitutes the food additive with the code E420(ii).

Although sorbitol exists in its natural state, E420(i) is obtained by hydrogenation of D-glucose, and it is mainly (not less than 91%) composed of D-sorbitol (Fig. 1). The fraction of the products which are not D-sorbitol are composed of related substances such as mannitol, iditol and maltitol. This food additive can be obtained as white hygroscopic powder, crystalline powder, flakes, or granules, being very soluble in water and slightly soluble in ethanol (Commission Regulation (EU) Nª 231/2012; FAO, 2002).

E420(i) can have several technological functions, such as sweetener, humectant, sequestrant, texturizer stabilizer and bulking agent (FAO, 2002). As a sweetener, D-sorbitol has 0.6 times the sweetening power of sucrose.

Sorbitol syrup (E420(ii)), prepared by hydrogenation of glucose syrup, is composed of D-sorbitol, D-mannitol, and hydrogenated saccharides. Hydrogenated oligosaccharides are synthesised through hydrogenation of glucose syrup (commonly used as raw material) or by mannitol (Commission Regulation (EU) Nª 231/2012). This food additive is in the form of a clear, colourless, sweet-tasting aqueous solution, and can be used with several functions, such as sweetener, humectant, sequestrant, texturizer or as bulking agent (FAO, 2001a).

Sorbitol and sorbitol syrup can be used in most food products, namely in desserts, ice creams, jams, jellies, chewing gum, breakfast cereals, confectionery, pastry, fine bakery products with low energy value or no sugar, decorations, coatings and fillings without added sugar. Besides, these food additives can also be used with other purposes, than sweetening, in unprocessed frozen or deep-frozen fish, crustaceans, molluscs and cephalopods (Commission Regulation (EU) Nº 1129/2011).

Concerning to health effects, in high doses these additives are laxative and cause swelling in the abdomen, diarrhea and intestinal pain (Voss, 2011; Jain et al. 1985). Animal experiments also revealed the development of kidney stones and tumours when used in high doses (Voss, 2022). Additionally, a possible association of sorbitol with genotoxicity and shifts in the metabolism of rats was reported (Ruiz-Ojeda et al., 2019). A study related with the consumption of sorbitol further concluded that many diabetics are intolerant to sorbitol and that regular consumption may explain their "idiopathic" diarrhea (Badiga et al. 1990). Irritable bowel syndrome (IBS) patients also showed negative gastrointestinal responses to polyols, particularly sorbitol and mannitol (regardless of each molecule's patterns of absorption). (Ruiz-Ojeda et al., 2019). Even in healthy people, the laxative effects of sorbitol in humans when consumed in high doses are also well known (Yao et al., 2014), and some studies also showed malabsorption after consumption of this sweetener, as well as gastrointestinal symptoms (Hyams, 1983; Corazza, 1988).

### *Mannitol*

There are two polyol food additives with the generic designation of mannitol, or D-mannitol (with the chemical formula $OHCH_2(CH(OH))_4CH_2OH$, related to E421 code): "Mannitol by hydrogenation" E421(i) (Commission Regulation (EU) Nº 724/2013), and "Mannitol manufactured by fermentation" E421(ii) (Commission Regulation (EU) Nª 231/2012; (Commission Regulation (EU) Nº 724/2013). In the structural formula of mannitol, the main component of E421, is a structural isomer of sorbitol (E420) (Fig. 2).

E421(i) is manufactured by catalytic hydrogenation of carbohydrate solutions containing glucose and/or fructose, whereas E421(ii) is produced by discontinuous fermentation, under aerobic conditions, using conventional strains of the yeast *Zygosaccharomyces rouxii* (Commission Regulation (EU) Nª 231/2012; (Commission Regulation (EU) Nº 724/2013). These food additives have a sweetening power 0.5 times greater than sucrose and exist in the form of white odourless crystalline powders (FAO, 2001b). In addition to their sweetening functions, these additives can also be used as anti-caking agents and transport agents.

Mannitol can be applicate in general foodstuffs, with emphasis in desserts, jams and jellies, breakfast cereals, liqueurs, chewing gum, several confectionery products, pastries and fine bakery products with low energy or no added sugar, and also some frozen fish products, crustaceans, molluscs and unprocessed cephalopods, frozen, sauces, mustard and baby foods (as a carrier for vitamin $B_{12}$) (Commission Regulation (EU) Nº 1129/2011).

As health site effects of mannitol, when used in high doses, laxative effects with nausea, vomiting and diarrhea, and

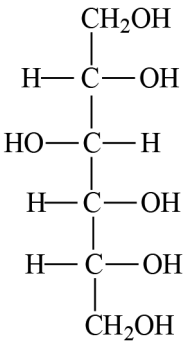


**Fig 1.** Structural formula of D-sorbitol, the main component of (E420i).

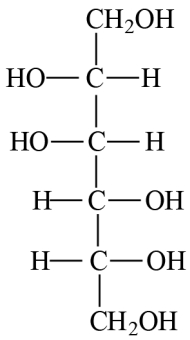


**Fig 2.** Structural formula of D-mannitol, the main component of E421.

hypersensitivity reactions have been reported (Voss, 2011). Patients with irritable bowel syndrome (IBS) also have adverse gastrointestinal reactions to mannitol, in similitude with other polyols (Ruiz-Ojeda et al., 2019).

### *Isomalt*

Isomalt, also named hydrogenated isomaltulose, is a polyol food additive (E953) that consists of a mixture of hydrogenated mono- and disaccharides (Fig. 3), whose main components are disaccharides 6-O-alpha-D-glucopyranosyl-D-sorbitol (1,6-GPS) (with the chemical formula $C_{12}H_{24}O_{11}$) and 1-O-alpha-D-glucopyranosyl-D-mannitol dihydrate (1,1-GPM) (with chemical formula $C_{12}H_{24}O_{11}{\cdot}2H_2O$) (Commission Regulation (EU) Nª 231/2012).

E953 food additive has 0.5 – 0.6 times the sweetness of sucrose and, in addition to sweetening, has other functions such as anti-caking agent, coating agent and transport agent. This sweetener is white, crystalline in appearance and slightly hygroscopic, being synthesised by enzymatic conversion of sucrose with nonviable cells of *Protaminobacter rubrum,* followed by catalytic hydrogenation (Commission Regulation (EU) Nª 231/2012). E953 is poorly soluble in water and insoluble in ethanol. It possesses a clean and pleasant taste profile, as well as gelling properties. As it is stable at high-temperatures and does not have aftertaste, it can be used in baking products (Lidon and Silvestre, 2010).

The E953 sweetener can be used in a large number food products, namely in chewing gum, ice creams, desserts, dairy drinks, food supplements, jams and jellies, breakfast cereals, flavoured fermented milk products (including heat-treated products), cocoa and chocolate, some decorations, coatings and fillings, pastry and fine bakery products, sauces, mustard, dietary foods (for special medical purposes and for weight control), table-top sweeteners and foods products suitable for people intolerant to gluten. Furthermore, E953 can be used in frozen and deep-frozen unprocessed fish, crustaceans, molluscs and cephalopods for other purposes than sweetening (Commission Regulation (EU) Nº 1129/2011).

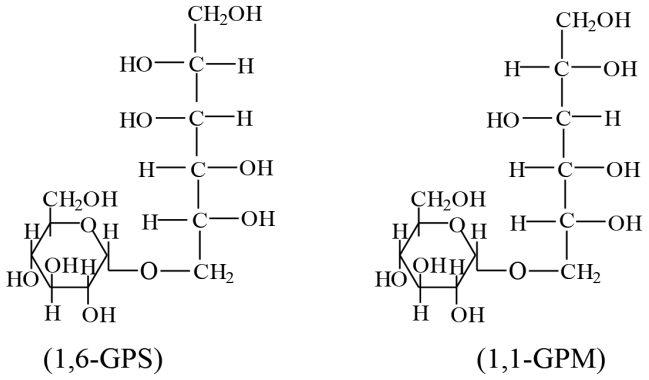


**Fig 3.** Structural formulas of the two main components of isomalt (E953).

Some side effects have been described when consumed in high doses, namely vomiting, diarrhea, nausea and laxative effect (Voss, 2011; Pereira et al., 2021). Moreover, Gostner et al. (Gostner et al., 2006) showed that isomalt is bifidogenic in healthy volunteers, being a prebiotic carbohydrate candidate that might contribute to a healthy function gut flora.

### *Thaumatin*

Thaumatins (I and II) are sweet-tasting proteins that can be extracted from berry arils of *Thaumatococcus daniellii* (Benth), by aqueous phase extraction (pH 2.5-4). The sweetener with the designation thaumatin (with the code E957) consists of two proteins and small amounts of vegetable materials from the raw material (Commission Regulation (EU) Nª 231/2012). This sweetener is an odourless cream-coloured powder, being very soluble in water (Commission Regulation (EU) Nª 231/2012). The sequence of 207 amino acids of thaumatin I is very well know.

E957, as a sweetener food additive is particularly relevant importance, due to its high solubility (it can be easily used in aqueous products), and because is about 2000 to 3000 times sweeter than sucrose (Commission Regulation (EU) Nª 231/2012). Although the thaumatin additive has a high sweetening power, studies have been carried out to increase sweetness perception through additive manufacturing in foods (Burkard, 2023). This food additive can also be used as a flavour enhancer, as it can augment the existing umami and savoury taste, making several food products more appealing for human taste (Commission Regulation (EU) 2018/677).

Thaumatin does not seem to have any side effects, and it is considered that there is no safety concern for thaumatin (EFSA, 2021), being used as a sweetener, or as a flavour enhancer, in chewing gum, flavoured water-based drinks (non-alcoholic), confectionery of various types (namely cocoa-based or nuts), edible ices (with no added sugar or low energy value), food supplements (that are part of dietary diets) and dairy or non-dairy desserts. As a flavour enhancer is use in flavoured fermented milk products (including heat-treated products), as well as in sauces and potato, cereal and flour or starch-based snacks, because it improves the organoleptic properties of savoury food products (Commission Regulation (EU) 2018/677). It is also used as a table-top sweetener (Commission Regulation (EU) Nº 1129/2011) and in some food supplements (Commission Regulation (EU) 2018/1497).

### *Neohesperidine dihydrochalcone*

Neohesperidine dihydrochalcone, also named neohesperidin dihydrochalcone, neohesperidin DC, or NHDC, is a sweetener (with the code E 959) with the chemical formula $C_{28}H_{36}O_{15}$, obtained by catalytic hydrogenation of neohesperidin (Commission Regulation (EU) Nª 231/2012), which is naturally occurring, and can be isolated by alcohol extraction from bitter oranges (*Citrus aurantium*) (EFSA, 2022). The study and structural characterization of neohesperidin DC (Fig. 4) is already known (Kwon et al., 2022). E959 is an off-white, crystalline, odourless powder with an intense and characteristic sugary taste ranging between 1000 and 1800 times as sweet as sucrose. This sweetener has high solubility in hot water, but very low solubility in cold water (Commission Regulation (EU) Nª 231/2012), which limits its use in cold watery food products.

E959 is applied in many low-energy foodstuffs, including some soft drinks, dairy drinks, fiber-rich breakfast cereals, jams and jellies, preserves, desserts, confectionery, fine baked goods, chewing gum, ice cream, soups, meat products, snacks, margarine and other spreads (Commission Regulation (EU) Nº 1129/2011; Commission Regulation (EU) Nº 913/2013), and in some food supplements (Commission Regulation (EU) 2018/1497).

In 2022, EFSA reevaluated neohesperidine dihydrochalcone (E 959) as a food additive and reported an ADI of 20 mg/kg bw, concluding that dietary exposure under this level would not raise safety concerns (EFSA, 2022).

Kwon et. al. (Kwon et al., 2022) published a study with mice and concluded that neohespiridin dihydrochalcone and neohespiridin dihydrochalcone-O-glicoside attenuates subcutaneous fat and lipid accumulation by regulating the PI3K/AKT/mTOR pathway. These results are of relevant importance considering the growing increase in obesity and associated diseases, such as diabetes mellitus, cardiovascular diseases, and cancer (Kwon et al., 2022).

### *Steviol glycosides*

Steviol glycosides additive (with the code E 960) has been obtained by aqueous extraction of leaves of *Stevia rebaudiana* Bertoni, followed by purification. This food additive contains several steviol glycosides (Fig. 5), with special relevance to stevioside ($C_{38}H_{60}O_{18}$) and rebaudioside A ($C_{44}H_{70}O_{23}$) (Commission Regulation (EU) Nº231/2012). Amending of European Union regulation, in July 2021 renamed the authorized food additive "steviol glycosides (E 960) to "steviol glycosides from Stevia (E 960a)" and included the food additive "Rebaudioside M produced via enzyme modification of steviol glycosides (E 960c)" (Commission Regulation (EU) 2021/1156). More recently (in 2023) E 960c changed its name to "Enzymatically produced steviol glycosides" and the new food additive "Glucosylated steviol glycosides" (E 960d) was authorized (Commission Regulation (EU) 2023/447).

Steviol steviosides E 960a, c, d are white to light yellow powder, soluble in water, about 100 - 350 times sweeter than sucrose (Commission Regulation (EU) Nª 231/2012; Commission Regulation (EU) 2021/1156; Commission Regulation (EU) 2023/447). These additives can be used in a large number of foodstuffs with low energy value, or with no added sugar, namely freshening throat pastilles and breath-freshening micro-sweets (Commission Regulation (EU) 2017/335; Commission Regulation (EU) 2021/1156; Commission Regulation (EU) 2023/447), flavoured fermented dairy products, ice cream, sweet and sour fruit preserves and fruit preparations, jams, jellies and marmalades, spreads made from dried fruits, confectionery products, chewing gum, decoration and coating products, breakfast cereals, fine bakery products, sweet and sour preserves of fish, crustaceans and molluscs, table top sweeteners, soups and broths, diet foods, fruit nectars, flavoured drinks, beer, snacks, desserts, food supplements (Commission Regulation (EU) Nº 1131/2011; Commission Regulation (EU) 2021/1156; Commission Regulation (EU) 2023/447), in certain fruit or vegetable spreads (Commission Regulation (EU) Nº 913/2013; Commission Regulation (EU) 2021/1156; Commission Regulation (EU) 2023/447), coffee, tea and herbal infusion beverages, flavoured instant coffee and instant cappuccino

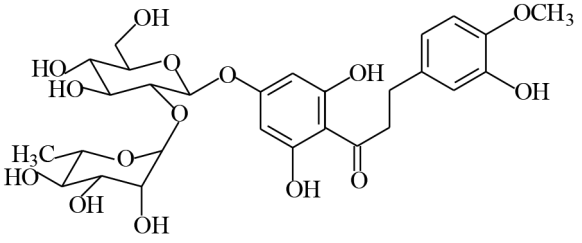

**Fig 4.** Structural formula of neohesperidine DC (E959).

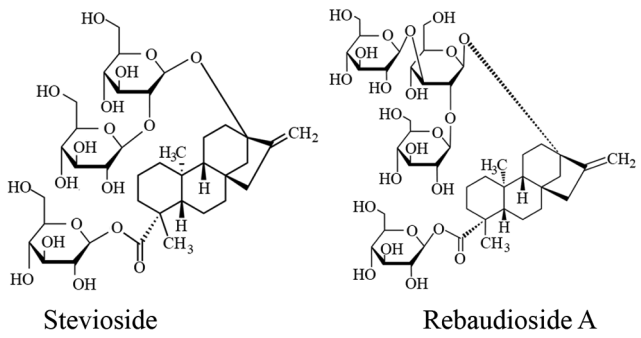


**Fig 5.** Structural formula of stevioside and rebaudioside A, two of the main components of steviol glycosides (E960).

products and malt-based, as well as chocolate/cappuccino flavoured drinks energy-reduced or with no added sugars (Commission Regulation (EU) 2016/479; Commission Regulation (EU) 2021/1156; Commission Regulation (EU) 2023/447).

Using sucrose-sweetened ice cream as a control, a recent study (Muenprasitivej et al., 2022) assessed the sensory qualities and acceptability of ice cream sweetened exclusively with three steviol glycosides (rebaudioside (Reb) A, D, and M). The findings demonstrated that rebaudioside D and rebaudioside M, the two most commonly used glycoside in the food business, had superior tastes and improve consumer impressions over rebaudioside A. (Muenprasitivej et al., 2022).

The safety of steviol glycosides (E 960), as a food additive, was evaluated by EFSA in 2010, and was considered an Acceptable Daily Intake (ADI) of 4 mg/kg body weight bw, expressed as steviol equivalents. A subsequent EFSA assessment in 2015 (EFSA, 2015), further included rebaudioside D and M in the specifications for steviol glycosides (E 960). Recently the European Food Safety Authority evaluated the safety of the three steviol glycosides E 960a, E 960c and E960d and concluded that the existing Acceptable Daily Intake (ADI) of 4 mg/Kg bw per day can also be applied (Commission Regulation (EU) 2021/1156; Commission Regulation (EU) 2023/447).

Al-Dujaili et al. (Al-Dujaili et al, 2017) reported a study with humans, pointing that short periods of stevia intake can trigger small but significant increases in blood pressure, possibly due to the increase of cortisol levels and modifications of 11B-HSD type 1 and enzymes activities. The authors further concluded that consumers should avoid the intake of stevia for longer periods of time. Moreover, the same authors studied the effect of stevia on the body weight and body mass of volunteers and reported that no significant effects were found (Al-Dujaili et al, 2017). A Scientific Opinion, published by EFSA, in 2020, relatively to a proposed amendment for specifications concerning steviol glycosides (E 960) as a food additive (to expand the list of steviol glycosides to all the 60 identified in the leaves of *Stevia rebaudiana* Bertoni), was not totally conclusive (EFSA, 2020).

Recently, it was reported that the use, as a sweetener, of stevioside at recommended doses of 4 mg/kg is not teratogenic or cancerogenic, and that neither stevioside nor rebaudioside A are absorbed in the human gastrointestinal tract, and converted to free steviol through microbial action (Orellana-Paucar, 2023). These authors also stated that when steviol is absorbed it is rapidly eliminated in the urine as steviol glucuronide. Accordingly, these authors concluded that these pharmacokinetic aspects constitute a significant advantage, since a quicklier elimination of this metabolite decreases the probability of side effects. Thus, stevioside can be considered a safe, noncaloric, noncariogenic, nonallergenic, and natural alternative to sucrose (Orellana-Paucar, 2023). Current evidence has also pointed out that stevioside and rebaudioside A, administered at higher doses than ADI, might improve treatments related with cardiovascular disorders, diabetes, cancer, inflammation, diarrhea, and oxidative processes (Orellana-Paucar, 2023). Nevertheless, it should be noted that anti-cancer properties of *Stevia rebaudiana* have been reported but it was mentioned that further research is required (Iatridis et al., 2022).

### *Polyglycitol syrup*

Polyglycitol syrup is a mixture, mainly consisting of maltitol and sorbitol and, in smaller amounts, of hydrogenated oligosaccharides and polysaccharides and maltrotriitol, being used as a food additive with a sweetening function (with the code E964). Thus, E964 is essentially made up of a mixture of sorbitol: D-glucitol ($C_6H_{14}O_6$) and maltitol - ($\alpha$)-D-glucopyranosyl-1,4-D-glucitol ($C_{12}H_{24}O_{11}$). E964 is produced through a catalytic hydrogenation of a mixture of starch hydrolysates, consisting of glucose, maltose and higher molecular weight glucose polymers, similar to the catalytic hydrogenation process used for the synthesis of maltitol syrup. The resulting syrup is desalted by ion exchange and concentrated to the desired level (Commission Regulation (EU) Nª 1050/2012). This additive, which comes in the form of a viscous, clear, colourless, and odourless liquid, is very soluble in water.

E964 has been use in several categories of low-energy, or no-sugar food products, including ice cream, jams, jellies, chestnut cream, fruit and vegetable-like spreads, breakfast cereals, fine bakery wares, desserts, several confectionery products, including breath refreshening microsweets and chewing gum (Commission Regulation (EU) Nº 1049/2012).

### *Maltitol*

With the generic name of maltitol, two additives can be distinguished: maltitol (with the chemical formula $C_{12}H_{24}O_{11}$), also known as D-maltitol (Fig. 6), or hydrogenated maltose, which constitutes a polyol food additive with the code E965(i), and maltitol syrup (also called hydrogenated glucose syrup), which is the food additive with the code E965(ii). The sweetener E965(i) is obtained by hydrogenation of D-maltose, and it is mainly composed of D-maltitol, while E965 (ii) is synthetised by the catalytic hydrogenation of high maltose-content glucose syrup, or by hydrogenation of its individual components (Commission Regulation (EU) Nª 231/2012).

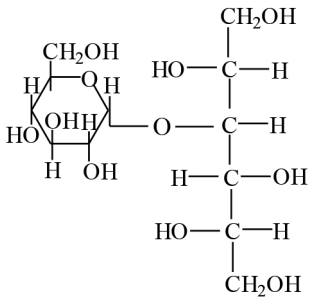

**Fig 6.** Structural formula of D-maltitol, the main component of E965(i).

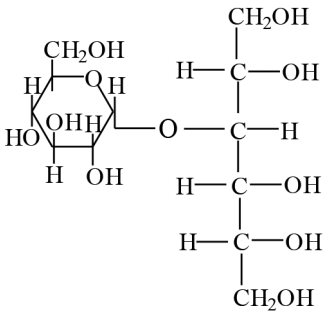

**Fig 7.** Structural formula of Lactitol (E966).

D-maltitol is a white, crystalline powder, very soluble in water, and has 0.8 times the sweetening power of sucrose. Maltitol syrup is a colourless, odourless, viscous liquid, or a white crystalline mass.

E965 can have various technological functions, such as sweetening, emulsifying, stabilizing, and transporting agent functions. The sweetness of these food additives resembles the flavour of sugar (about 90% if sucrose is considered), and its solubility and hygroscopicity is similar to sucrose. For these reasons maltitol is the sugar of choice for production of no-sugar-added-labelled chocolate (Joshi et al., 2016).

Like other polyol sweeteners, these additives can be used in most food products, namely in desserts, confectionery products, including cocoa and chocolate products, pastry and fine bakery products, flavoured fermented milk products including heat-treated products, some decorations, coatings and fillings, jams and jellies, breakfast cereals, ice cream and chewing gum, sauces, mustard, dietary foods for special medical purposes and for weight control, table-top sweeteners and foods suitable for people intolerant to gluten. It can also be used in unprocessed, frozen, and deep-frozen fish, crustaceans, molluscs, and cephalopods but with non-sweetener functions (Commission Regulation (EU) Nº 1129/2011).

As side effects, is reported that maltitol is fermented in the colon, by the gut microbiota, at a very slow digestion rate (Lidon and Silvestre, 2010). Studies with volunteers, using chocolate with maltitol, highlighted prebiotic benefits, but further research is needed to determine the specific effect of the maltitol on gut microbiota (Ruiz-Ojeda et al., 2019). Linked with the ingestion of high amounts of maltitol, laxative side effect, nausea, vomiting and diarrhea have been reported (Kwon et al., 2022).

### *Lactitol*

Lactitol, lactit, lactositol and lactobiosit are the common names of the polyol food additive E966, which has the chemical name of 4-O-β-D-galactopyranosyl-D-glucitol, and the formula $C_{12}H_{24}O_{11}$ (Fig. 7). E966 is manufactured via catalytic hydrogenation of lactose, being a crystalline powder with a sweet taste, or a colourless solution (being very soluble in water) (Commission Regulation (EU) Nª 231/2012; FAO, 2001c). As a food additive, it can have several technological functions, highlighting its sweetening function (0.4 times the sweetening power of sucrose), thickening and transport agent. The sweetening power of E966 is limited, even when compared with other polyols and, consequently, it is usually applied in combination with other sweeteners having a more intense flavour (Ruiz-Ojeda et al., 2019).

The E966 additive has similar applications to other polyol additives and can be used in most sweet food energy-reduced products (with emphasis in some desserts), confectionery products, fine bakery products, cocoa and chocolate products, jams, jellies, marmalades, breakfast cereals or cereal-based products, edible ices, fruit and vegetable preparations, and chewing gum, decorations, coatings and fillings, sauces, flavoured fermented milk products including heat-treated products, dietary foods for special medical purposes or for weight control diets, food supplements, and table-top sweeteners (Commission Regulation (EU) Nº 1129/2011). It can also be used in unprocessed, frozen or deep-frozen fish, crustaceans, molluscs and cephalopods (Commission Regulation (EU) Nº 1129/2011).

Health side effects, like those reported for E965, have been reported for E966, namely laxative effects after high intakes (Ruiz-Ojeda et al., 2019). However, some data suggest that lactitol is useful as prebiotic for enhancing the gut microbiota, as well as noncariogenic, and having mild sweetness (Ruiz-Ojeda et al., 2019).

### *Xylitol*

Xylitol is a polyol food additive (with the code E967), obtained from the xylan of birch wood, corn cobs, sugar cane bagasse or straw. This food additive is mainly composed of D-xylitol ($C_5H_{12}O_5$) (Fig. 8). The fraction that is not D-xylitol is composed of related substances, such as L-arabinitol, galactitol, mannitol, sorbitol (Commission

Regulation (EU) Nª 231/2012). This sweetener is a white, crystalline, and practically odourless powder. E967 is produced by hydrogenation of dxylose, being the sweetest of all polyols (*i.e.,* equivalent to sucrose) (Lidon and Silvestre, 2010).

In addition to its important sweetening function, this additive can have other technological functions, such as humectant, stabilizing, thickening, emulsifying and transport agent. Like other polyol sweeteners, it can be used in most food products, with emphasis in breakfast cereals, confectionery products, including cocoa and chocolate products, pastry and fine bakery products, jams and jellies, desserts and chewing gum, flavoured fermented milk products (including heat-treated products), some decorations, coatings and fillings, ice creams, sauces, mustard, dietary foods (for special medical purposes and for weight control), table-top sweeteners and foods suitable for people intolerant to gluten. When used for other functions than sweetener, it can be applied in unprocessed, frozen, and deep-frozen fish, crustaceans, molluscs, and cephalopods (Commission Regulation (EU) Nº 1129/2011).

The health side effects reported for this additive are similar to those pointed for polyol sweeteners. Meyer-Gerspach et al. (Meyer-Gerspach et al., 2021) reported that xylitol and erythritol have a unique combination of properties, namely no calories, virtually no effect on glucose and insulin, while promoting the release of gut hormones, and impacting appetite-regulation. This could contribute to prevention and treatment of obesity and its complications, such as type 2 diabetes (Meyer-Gerspach et al., 2021). Bordier et al. (Bordier et al., 2021) also reported that chronic intake, close to real-life conditions, of the natural sweeteners xylitol and erythritol, does not affect intestinal glucose absorption in humans with obesity. In this study, the dosage, and the time points when participants took the substances, closely corresponded to how much and when added sugar is consumed in everyday life. The authors also concluded that xylitol and erythritol can be consumed safely and can be used as sugar alternatives, even by patients with diabetes, as they do not affect glucose homeostasis (Bordier et al., 2021).

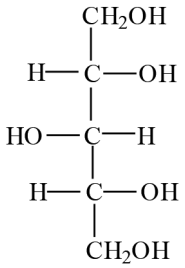


**Fig 8.** Structure formula of xylitol, the main component of E967.

### *Erythritol*

Since 2003, the EU Scientific Committee on Food (SCF) considers the use of erythritol ($HOCH_2(CH(OH))_2CH_2OH$) (Fig. 9) as a sweetener. This polyol sweetener (with the code E968) exists naturally in some fruits, mushrooms, fermented food, and cheeses, but can be obtained by fermenting carbohydrates sources by osmophilic yeasts, such as *Moniliella pollinis* or *Moniliella megachilensis*, followed by purification and drying (Commission Regulation (EU) Nª 231/2012).

Erythritol (E968) consists of white, odourless, non-hygroscopic, heat-stable crystals, that are freely soluble in water, and with a sweetness of about 60-80 % (relatively to sucrose) (Commission Regulation (EU) Nª 231/2012). This food additive is being used as sweetener in several categories of low-energy, or no-sugar food products, including breakfast cereals, some confectionery products, ice cream, jams, jellies, chestnut cream, fruit and vegetable-like spreads and chewing gum (Commission Regulation (EU) Nº 1129/2011). E968 can also be used as a flavour enhancer in energy-reduced, or with no added sugars, in flavoured drinks (Commission Regulation (EU) 2015/1832).

Erythritol does not seem to have laxative effects. Yet, there is a safety concern relatively to gastrointestinal tolerability in beverages, at a maximum use level of 2.5% for non-sweetening purposes (EFSA, 2010). Nevertheless, it was reported (EFSA, 2013a) that the use of erythritol for soft drinks, with a margin of safety of 1.54 protects children (3-9 years) adequately. Based on new data, in 2015, it was further concluded that the acute bolus consumption of erythritol via non-alcoholic beverages, at a maximum level of 1.6 %, would not raise concerns for laxation (EFSA, 2015). Accordingly, erythritol does not affect glucose homeostasis and can be used as a sugar alternative, even by patients with diabetes (Bordier et al., 2021).

## Sweetener food additives of synthetic origin

### *Acessulfame K*

Acesulfame *K* is the potassium salt of acesulfame, with the chemical name 6-methyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide potassium salt and chemical formula $C_4H_4KNO_4S$ (Fig. 10). This sweetener (with the code E950) is about 200 times sweeter than sucrose (Commission Regulation

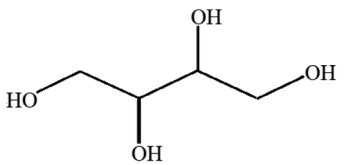


**Fig 9.** Structural formula of erythritol (E968).

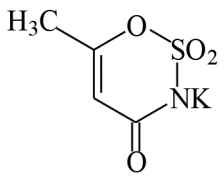

**Fig 10.** Structural formula of acesulfame K (E950).

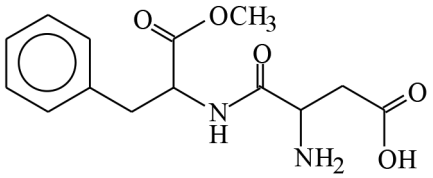

**Fig 11.** Structural formula of aspartame (E951).

(EU) Nª 231/2012), being an odourless, white, crystalline powder, very soluble in water and slightly soluble in ethanol (Commission Regulation (EU) Nª 231/2012).

Acesulfame K is used as a tabletop sweetener and in most sweet foodstuff energy-reduced or with no added sugar, namely soft drinks, desserts, dairy products, breakfast cereals, jam, jellies and marmalades, flavoured fermented milk products (including heat-treated products), edible ices, sweet-sour preserves of fruit and vegetables, canned or bottled fruit and vegetables, beer, soups, a large diversity of confectionery products (including cocoa or milk or dried fruits), chewing gums and breath-freshening micro-sweets, pastry and fine bakery products (Commission Regulation (EU) Nº 1129/2011), and food supplements (Commission Regulation (EU) 2018/1497).

E950 has no carcinogenic activity (Voss, 2011), however, there are reports pointing chronic health problems, namely thyroid tumours in rats, clastogenic effects, and genotoxicity (if high doses are consumed) (Poshaba, 2020). Although sweeteners are often used to prevent or reduce obesity, and thus contribute to minimise various health problems (such as diabetes and cardiovascular diseases), a study with mice concluded that acesulfame K augmented HCD-induced dyslipidemia through an increment in lipogenesis and a decrement in lipolysis (Lin et al. 2021). Accordingly, these effects might further exacerbate HCD-induced atherosclerosis in ApoE$^{-/-}$ mice (Lin et al. 2021). Besides, acesulfame K might also disturb lipid metabolism in the liver, further contributing to lipid dysregulation, thereby enhancing the progression of atherosclerosis (Lin et al. 2021). Recent studies by Shinohara et al. (2022) showed that Acesulfame K inhibits the growth of Porphyromonas *gingivalis*, a pathogen of periodontal disease, which constitutes an advantageous side effect for this food additive.

### *Aspartame*

Aspartame is the common name for N-methyl ester of N-L-α-aspartyl-L-phenylalanine ($C_{14}H_{18}N_2O_5$) (Fig. 11). This sweetener (with the code E951) is widely used to replace sugar and enhance the flavour of sweet food products. Aspartame is a white, odourless, crystalline powder, being 200 times sweeter than sucrose, but is poorly soluble in water and alcohol (Commission Regulation (EU) Nº 724/2013).

E951 is widely used to replace sugar and enhance the flavour of sweet food products, in energy-reduced, namely in flavoured fermented milk products (including heat-treated products), edible ices, fruit and vegetables in vinegar, energy-reduced or with no added sugar fruit nectars and flavoured drinks, cider and perry, some alcoholic drinks, oil, or brine, some fruit and vegetable preparations, jams, jellies and marmalades and similar products, and sweetened chestnut purée, confectionary products (including cocoa and chocolate products), and other confectionery including breath freshening micro-sweets, chewing gum, decorations, coatings and fillings, except fruit-based fillings, sauces, mustard, ready-to-eat savouries and snacks, breakfast cereals, fine bakery products for special nutritional uses, energy-reduced soups, some dietary foods, sweet-sour preserves and semi-preserves of fish and marinades of fish, crustaceans and mollusc, as table-top sweeteners (Commission Regulation (EU) Nº 1129/2011), energy-reduced fruit or vegetable spreads and dried-fruit-based sandwich spreads, energy-reduced or with no added sugar (Commission Regulation (EU) Nº 1092/2014), and food supplements (Commission Regulation (EU) 2018/1497).

Aspartame is rapidly, and completely, hydrolysed in the gastrointestinal tract to phenylalanine, aspartic acid, and methanol (EFSA, 2013b). Consequently, on labels of foodstuffs containing aspartame, it is mandatory to inform the consumer that this product is a source of phenylalanine, since phenylketonuria patients (PKU) have a markedly reduced capacity for phenylalanine metabolism (EFSA, 2013b). For normal consumers (without PKU disease) the current ADI for aspartame of 40 mg/kg bw (EFSA, 2013b).

Aspartame (with the code E951) has stability problems in products that must be heated for a long time, and the formation of harmful substances by heating has been described (Tsau and Young, 1987). However, the protection of aspartame salts and its metal complexes has been achieved by fats, thus allowing its use in baking processes (Tsau and Young, 1987). The heating stability problems does not seem to exist in cold and hot beverages, such as tea and coffee. Moreover, allergic reactions, headache, visual disturbances, and the existence of harmful impurities when used in high concentrations were detected (Voss, 2011). It has also been reported that consumption of

aspartame can cause dry mouth, dizziness, nausea, vomiting, thrombocytopenia, mood swings, as acute problems (Poshaba, 2020). Additionally, several reports pointed problems of genotoxicity or carcinogenicity triggered by aspartame (Poshaba, 2020; Yılmaz, S. and A. Uçar, 2014). Although it has been considered that further studies are needed (Czarnecka et al., 2021), it was reported that aspartame also causes long-turn changes in behaviour, genotoxic proprieties, mental stress, several effects in children and foetuses, affects learning skills and memory, elevates plasma corticosterone level and plasma adrenocorticotropic level. In studies with pregnant women volunteers (Liu et al., 2022), it was a quantitative detection of serum aspartame was positively associated with maternal insulin resistance index, total cholesterol, and LDL cholesterol during pregnancy. As upon ingestion, aspartame is metabolized quickly in the intestine, including aspartic acid, phenylalanine, and methanol, it was suggested that these findings may be the first report of circulating aspartame levels in pregnant women (Ahmad et al., 2020), and that despite the metabolism of aspartame in the intestine, another possible pathway into the human body is absorption through the mouth (Liu et al., 2022). Studies with mice (Finamor et al., 2017) also showed that a chronic aspartame intake can cause changes in the trans-sulphuration pathway, triggering glutathione depletion and liver damage.

In another study (Abu-Taweel et al., 2014), forty male albino mices were exposed to monosodium glutamate and aspartame, through drinking water for one month, to identify possible cognitive and biochemical effects. Although the authors pointed that more studies are required to achieve a conclusion, it seems that with a combined dose, a more significant and synergistic toxicity prevails (Abu-Taweel et al., 2014).

Recent evaluation of aspartame by the International Agency for Research on Cancer (IARC) and Joint FAO/WHO Expert Committee on Food (JECFA) was carried out. IARC classified aspartame as possibly carcinogenic to humans on the basis of limited evidence for cancer in humans and that was limited evidence for cancer in experimental animals and limited evidence related to possible mechanisms for causing cancer (Liu et al., 2022). JECFA concluded that the data evaluated indicated no sufficient reason to change previously established ADI of 0-40 mg/Kg body weight for aspartame (IARC and JECFA, 2023).

### *Cyclamic acid and its Na and Ca salts*

Cyclamic acid or cyclohexylsulfamic acid (with the code E952(i)), its sodium salt, sodium cyclamate, (E952(ii)) and its calcium salt, calcium cyclamate, (with the code E952(iii)) (Table 1, Fig. 12) are almost colourless sweeteners, being white crystalline powders or white crystals and soluble in water. Besides, only the acid form has significant solubility in alcohol (Commission Regulation (EU) Nª 231/2012). The synthesis of cyclamic acid is based on sulfonation of cyclohexylamine (Belitz et al., 2009).

Relatively to saccharin, cyclamic acid and cyclamates have a lower sweetening intensity (but are 30-40 times sweeter than sucrose) and do not have any bitter aftertaste. However, its flavour is considered less pleasant than that of saccharin.

E952 are used in a wide range of energy-reduced food products, such as micro-confectionery products for breath freshener, desserts, dairy drinks, confectionery, pastry and fine bakery products, jam, jellies and marmalades and similar products, ice cream, chewing gum, flavoured fermented milk products, canned or bottled fruit and vegetables, fruit and vegetable preparations**,** fruit and vegetables spreads and food supplements (Commission Regulation (EU) Nº 1129/2011; Commission Regulation (EU) Nº 913/2013; Commission Regulation (EU) Nª 1050/2012; Commission Regulation (EU) 2018/1497).

Some studies indicate carcinogenic effects as health side effects (Voss, 2011; Renwick et al., 2004). Although controverse prevails until today, in 1969, were banned in the United States of America, United Kingdom, and several other countries, due to reports concerning to increases on the incidence of bladder tumours in male rats (Renwick et al., 2004). Cyclamate itself shows very low toxicity, but it is metabolized by the gut bacteria to cyclohexylamine (Drasar et al., 1972), which has high toxicity (Bopp et al., 1986). In a study with 14 humans, were selected from 261 volunteers, in a 1-week screening, it was found that they were able to metabolize cyclamate to cyclohexylamine (>0.2% of a daily dose). Accordingly, an acceptable daily intake (ADI) of 0 – 11 mg/kg bw was suggested (Renwick et al., 2004). More recent studies suggest that sodium cyclamate can seriously inhibit the proliferation and differentiation of osteoblasts (Chen et al., 2019).

### *Saccharin and its Na, K and Ca salts*

The chemical name of saccharin is 3-oxo-2,3dihydrobenzo(d)isothiazol-1,1-dioxide. Saccharin, together with cyclamates, is the most important sweetening substance, being mainly used in the form of sodium and calcium salts (Table 2; Fig. 13) (Commission Regulation (EU) Nª 231/2012). Saccharin, initially discovered in

**Table 1: Cyclamic acid and its salts used as sweeteners**

| Additive code | Designation | Chemical formula |
|---|---|---|
| E 952 (i) | Cyclamic acid | $C_6H_{13}NO_3S$ |
| E 952 (ii) | Sodium cyclamate | $C_6H_{12}NNaO_3S$<br>$C_6H_{12}NNaO_3S.H_2O$ |
| E 952 (iii) | Calcium cyclamate | $(C_6H_{12}NO_3S)_2Ca.{}_2H_2O$ |

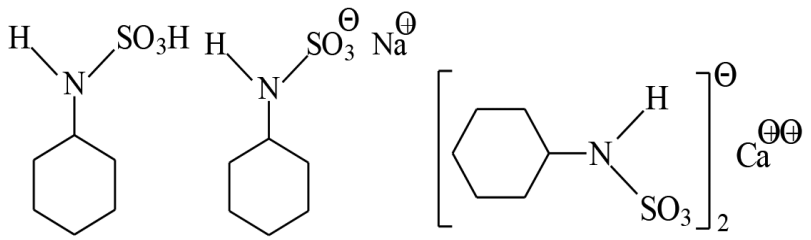

**Fig 12.** Structural formula of cyclamic acid (E952-I), sodium cyclamate (E952-II) and calcium cyclamate (E952-III).

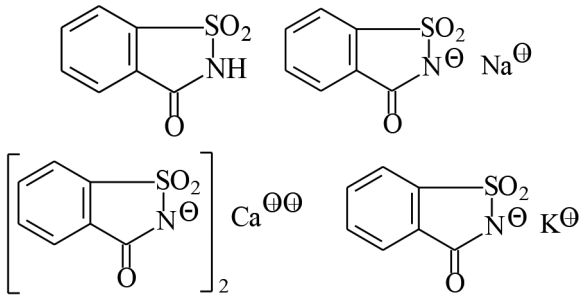

**Fig 13.** Structural formulas of (respectively A to D) saccharin (E954 (i)), saccharin sodium salt (E954(ii)), saccharin calcium salt (E954(iii)) and saccharin potassium salt (E954(iv)).

1878, by Ira Remsen and Constantine Fahlberg, was the first commercially recognized sweet-tasting agent more potent than sucrose.

Saccharin is used as a food additive (with the code E954) in a large number of foodstuffs with a low energy value, such as soft drinks, dairy drinks, desserts, confectionery and fine bakery products, chewing gum, jams, sweets and jellies, ice creams, preserved fruits, fruit and vegetables spreads, and even as a table sweetener (Commission Regulation (EU) Nº 1129/2011; Commission Regulation (EU) Nº 913/2013), as well as in some food supplements (Commission Regulation (EU) 2018/1497).

Saccharin is a white crystal, or a white crystalline powder, odourless or with a faint, aromatic odour, being about 300 - 500 times sweeter than sucrose. Is also slightly soluble in water, soluble in basic solutions, whereas potassium salts are freely soluble in water (Commission Regulation (EU) Nª 231/2012). At high concentrations saccharin produces a slightly metallic to bitter solutions, sparingly soluble in ethanol (Belitz et al., 2009).

Some health effects have been described for the use of saccharin and its salts, namely the development of cancerous tumours, inhibition of carbohydrate and protein digestion and allergic reactions (Voss, 2011). A study with rats showed that saccharin harmfully altered biochemical markers in liver and kidney at higher as well as lower doses

**Table 2: Saccharin and its salts used as sweeteners**

| Additive code | Designation | Chemical formula |
|---|---|---|
| E 954 (i) | Saccharin | $C_7H_5NO_3S$ |
| E 954 (ii) | Sodium saccharin | $C_7H_4NN_aO_3S._2H_2O$ |
| E 954 (iii) | Calcium saccharin | $(C_7H_4NO_3S)_2C_{a\cdot 3}$ 1/2H2O |
| E 954 (iv) | Potassium saccharin | $C_7H_4NKO_3S.H_2O$ |

(Amin et al., 2016). A survey conducted to gather available information and providing an overall perspective on the genotoxicity and carcinogenicity of saccharin, showed that there is still some controversy about its use (Uçar1 and Yilmaz, 2015). Nevertheless, possible nausea, vomiting and diarrhea, as well as low birth weight, bladder cancer and hepatoxicity were reported as chronic problems (Poshaba, 2020).

### *Sucralose*

Sucralose also called 4,1',6'-trichlorogalactosucrose is an artificial sweetener (with the code E955) with the chemical formula $C_{12}H_{19}Cl_{13}O_8$ (Fig. 14). Sucralose has a sweetening power 600 times higher than sucrose and is usually in the form of a white to off-white crystalline powder, being practically odourless (Commission Regulation (EU) Nª 231/2012; FAO, 2004).

E955 can be used in a relatively large number of food products with low energy value, or without added sugars, including some desserts and similar products, confectionery energy-reduced or with no added sugar products, bread spreads made from cocoa, milk, flavoured fermented milk products, fruit products, fats, throat fresheners, chewing gum, certain types of beer, edible ices, energy-reduced jams, jellies and marmalades, fruit and vegetable preparations, sweet and sour preserves and semi-preserves of fish and marinades of fish, crustaceans and molluscs, bakery products, diet foods, non-alcoholic water-based and milk-based beverages, derived products or fruit juices (Commission Regulation (EU) Nº 1129/2011; Commission Regulation (EU) Nº 913/2013), and in some food supplements (Commission Regulation (EU) 2018/1497). E955 can also be used as a flavor enhancer in chewing gum with added sugars or polyols (Commission Regulation (EU) 2016/1776).

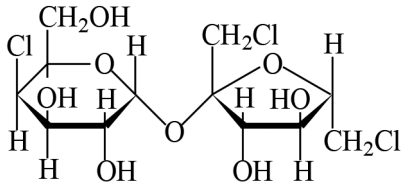

**Fig 14.** Structural formula of sucralose (E955).

The use of sucralose seems to have several advantages over other sweeteners, such as high solubility in water and in foods and it does not become transformed in our body. Although it was reported that the administration of sucralose in male mice induced hematopoietic neoplasia (Soffritti et al., 2016), the available data is not conclusive (EFSA, 2017). Diarrhea, dizziness stomach pain, thymus shrinkage and enlargement of cecal chronic problems in rodents were also reported (Poshaba, 2020). Recent studies showed that sucralose showed bactericidal activity for planktonic Porphyromonas *gingivalis*, higher than that observed for several other additives, which constitutes an advantageous side effect for this food additive (Shinohara et al, 2022).

### *Neotame*

Neotame ($C_{20}H_{30}N_2O_5$), with the chemical name N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester, is a food additive with the code E961 (Fig. 15), synthesised through the reaction, under hydrogen pressure, of aspartame with 3,3-dimethylbutyraldehyde, in methanol and in the presence of a palladium/carbon catalyst (Commission Regulation (EU) Nª 231/2012). Neotame is a white to off-white powder, sparingly soluble in water and very soluble in ethanol (Commission Regulation (EU) Nª 231/2012; JECFA, 2003).

Neotame can be use as flavour enhancer and a sweetener, with a sweetness factor about 7000 to 13000 times higher than sucrose and, depending upon the food application, 30 to 60 times greater than aspartame (EFSA, 2007). Neotame shows more stability than aspartame when submitted heat treatments in food processing (Kumari et al., 2023), which is another advantage of neotame.

E961 is used in a large number of energy-reduced, or sugar-free foodstuffs, namely jams and jellies, dried fruit spreads, cocoa-based spreads, flavored tablets, chewing gum, sauces, breakfast cereals, ice cream biscuits, fine baked goods, sweet and sour preserves and semi-preserved fish and fish marinades, crustaceans and mollusks, tabletop sweeteners, salads, soups and broths, diet foods for medicinal purposes, nectars and flavoured drinks (Commission Regulation (EU) Nº 1129/2011), confectionery products (Commission Regulation (EU) Nº 1129/2011; Commission Regulation

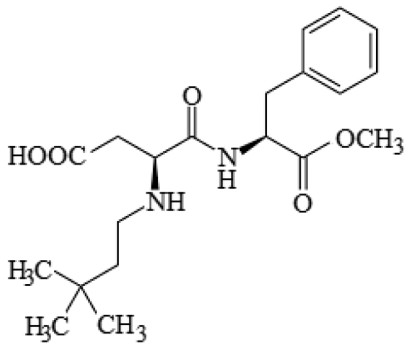

**Fig 15.** Structural formula of Neotame (E961).

(EU) 2015/647), and energy-reduced fruits or vegetables spreads and dried-fruit-based sandwich spreads, energy-reduced or with no added sugar (Commission Regulation (EU) Nº 1092/2014), as well as in some food supplements (Commission Regulation (EU) 2018/1497).

Some studies concluded that neotame is not carcinogenic, genotoxic, or associated with any reproductive/developmental toxicity (EFSA, 2007). Studies in humans demonstrated that daily doses of neotame up to 1.5 mg/kg bw were well tolerated (EFSA, 2007). However, health side effects of neotame have been reported, namely acute problems (like headache, hepatotoxic at high doses), and chronic problems (like lower birth rate, weight loss, cancer in offspring) and hepatotoxicity (Poshaba, 2020). Studies with mice showed effects of neotame on the Gut microbiome (Chi et al., 2018). Recently was developed a new method for determination of neotame in various foods by high-performance liquid chromatography coupled with ultraviolet and mass spectrometric detection, which can be useful in the quality control of food products containing neotame (Zhu et al., 2023).

### *Salt of aspartame-acesulfame*

Salt of aspartame-acesulfame, has the chemical name 6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide salt of L-phenylalanyl-2-methyl-L-α-aspartic acid and the chemical formula $C_{18}H_{23}O_9N_3S$. This food additive (with the code E962) is prepared by heating a solution, in an approximately 2:1 ratio (w/w), of aspartame and acesulfame K, at acidic pH and allowing crystallisation to occur (being potassium and moisture eliminated) (Commission Regulation (EU) Nª 231/2012). E962 is a white, odourless, crystalline powder, sparingly soluble water, slightly soluble in ethanol, and more stable than aspartame alone (Commission Regulation (EU) Nª 231/2012). Nevertheless, the salt dissociates into an anion (a negatively charged acesulfame) and a cation (a positively charged aspartame) in saliva and in contact with water. As the two ions released by the aspartame-acesulfame salt are the same two ions derived from the individual sweeteners, the salt raises no additional

toxicological or environmental risks beyond those of the two separate substances (Lewis and Tzilivakis, 2021).

The salt of aspartame and acesulfame (E962), can be used as sweetener in a relatively large number of food products, with low energy value, or without added sugars, of which some desserts (and similar products), as well as some confectionery products can be highlighted. E962 can further be used in spreads made from cocoa, milk, flavoured fermented milk products including heat-treated products, nuts or fats, refreshing throat lozenges, chewing gum, certain types of beer, edible ices, canned or bottled fruit and vegetables, breakfast cereals, jams, jellies and marmalades, fruit and vegetable preparations, sweet-sour preserves and semi-preserves of fish and marinades of fish, crustaceans and molluscs, crustaceans and molluscs, fine bakery products for special nutritional uses, mustard, soups, sauces, diet foods, food supplements, non-alcoholic water-based and milk-based beverages (and derived products) or fruit juices, tabletop sweeteners (Commission Regulation (EU) Nº 1129/2011), energy-reduced fruit or vegetable spreads and dried-fruit-based sandwich spreads, energy-reduced or with no added sugar (Commission Regulation (EU) Nº 1092/2014).

### *Advantame*

Advantame, with the formula $C_{24}H_{30}N_2O_7 \cdot H_2O$ (Fig. 16), is a sweetener (with the code E969) obtained by chemical synthesis in a three-step process (production of 3-hydroxy-4- methoxycinnamaldehyde, followed by hydrogenation to form 3-(3-hydroxy-4-methoxyphenyl) propionaldehyde, and combination with aspartame to produce the imine that under selective hydrogenation forms advantame). A crystallization followed by recrystallization triggers the synthesis of advantame crystals (Commission Regulation (EU) Nº 497/2014).

E969 is approximately 100 times sweeter than aspartame, and approximately 37000 times sweeter than sucrose, being a white to yellow power, very slightly soluble in water (at 25ºC) and sparingly soluble in ethanol (although with solubility increasing in both solvents with heating) (FAO, 2015).

E969 are used in a large number of energy-reduced, or sugar-free foodstuffs, namely jams and jellies, dried fruit spreads, confectionery products, cocoa-based spreads, flavored tablets, chewing gum, sauces, breakfast cereals, ice cream biscuits, fine baked goods, sweet and sour preserves and semi-preserved fish and fish marinades, crustaceans and mollusks, tabletop sweeteners, salads, soups and broths, diet foods for medicinal purposes, nectars and flavored drinks (Commission Regulation (EU) Nº 497/2014). It was determined a ADI for advantame of 5 mg/kg bw/day.

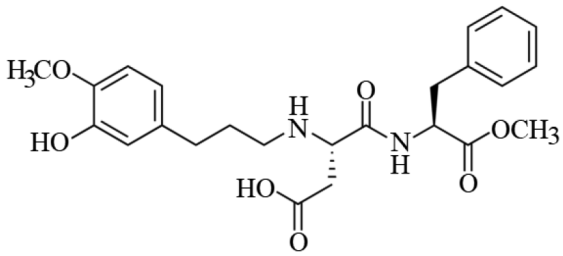


**Fig 16.** Structural formula of advantame (E969).

## CONCLUSION

Increased obesity and its metabolic comorbidities in many countries have led to a growing consumption of sugar-free products, where sugars are replaced by low-calorie sweeteners. In this context, the development of new processed foods with the use of sweetener food additives is increasing. The choice of sweeteners to apply in each food category depends on specific legislation and on several factors such as solubility, sweetness, and stability. In general, sweeteners of synthetic origin, such as acesulfame K, aspartame, saccharin, and sucralose have higher sweetness than sucrose, which allows the use of small amounts of these additives. The different solubility in water further determines their application in a wide variety of foodstuffs. However, some sweeteners of synthetic origin have recognized effects on human health, namely tumours, allergic reactions, diarrhea and hepatoxicity. Alternatively, sweeteners of natural origin are being used in large number of foodstuffs with low energy value, or with no added sugar, which seems to have less significant side effects on health. Polyols can cause diarrhea, nausea and laxative effects when consumed in high amounts and have low sweetening power. Moreover, other sweeteners of natural origin, such as thaumatin, neohespiridin DC and steviol glycosides, have much higher sweetness. Advantame is a new sweetener of synthetic origin with an even greater sweetening power than the others, which reduces the amount needed to be added to food. Although there is a significant number of studies, given the existing controversies, more studies are needed to unequivocally clarify possible side effects of each authorized sweetener, in the amounts of food additive permitted by law.

### Author's contributions

All authors contributed equally to the writing of the paper and were involved in the overall planning and supervision of the work. All authors have read and agreed to the published version of the manuscript.

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### Conflicts of Interest

The authors declare no conflict of interest.

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