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UNIVERSIDADE DO PORTO

Patrícia Daniela Martins da Silva

EVALUATION OF SUGARS AND ARTIFICIAL SWEETENERS IN SOFT DRINKS:

a decade of evolution

Dissertation of the 2th Cycle of Studies Leading to the Master's Degree in Quality Control
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Performed under the supervision of:
Prof. Doctor Susana Isabel Pereira Casal Vicente
MSc Rebeca Cruz

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Patrícia Daniela Martins da Silva

AVALIAÇÃO DE AÇÚCARES E EDULCORANTES EM REFRIGERANTES:
uma década de evolução

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Prof. Doutora Susana Isabel Pereira Casal Vicente
Mestre Rebeca Cruz

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ABSTRACT

High consumption of free sugars (sucrose, glucose and fructose) represents a current health concern on a global scale. In Portugal, its consumption, particularly through soft drinks, seems to be at the top of the list of the main factors responsible for overweight and obesity that characterizes our population, especially children and adolescents. In 2017, the Special Consumption Tax (IEC) was created to cover sugary drinks with contents higher than 8 g/100mL. This measure emerged to answer to this public health issue, though new taxes are still being implemented (for sugar levels above 2.5 g/100mL and 5 g/100mL), which are expected to drive even better results. However, the reduction of sugars in soft drinks occurs mostly at the expense of non-nutritive sweeteners addition that, while are reducing caloric intake not risk-free and do not contribute to sensory education as they do not seek to change the perception of sweet taste.

The current study aimed to verify how soft drink reformulations were made by the industries, evaluating the amount and type of free sugars and non-nutritive sweeteners used and to verify if these modifications could have other health implications. For this purpose, in January 2019, 68 of the most consumed soft drinks in Portugal were purchased in local supermarkets and their free sugar content was determined by refractive index HPLC and artificial sweeteners by light scattering detector HPLC. The results were further compared with analytical data from 2008.

For sugar drinks (N = 48), only a small fraction of the samples (15%) had a total sugar content above 8 g/100mL, some of them were just below this mark (25%) and most of them near to 5 g/100 mL (39%). This trend indicates that there will be no difficulties in further reducing the amount of added sugar for this portion of the market. Among groups of drinks, the profile and quantity of sugars were distinct, with the group of “colas” and “juice drinks” showing the highest variability in sugar content, mostly represented by fructose and glucose. On the other hand, the group of “iced teas” and “lemon flavoured drinks” revealed a more consistent sugar content between samples, mainly comprising sucrose. Regarding the brand, apparently there is a greater resistance in the reformulation of the so-called “original” brands, with the “own brands” presenting very similar formulations, except in the “colas”. In terms of sweeteners, they are present in the vast majority of samples (85%) (N=58), most often in mixtures and very dependent on the type of drink, although there are already some samples with sucrose only (27%) or steviol glycosides (10%). In a quantitative perspective, the main non-nutritive sweeteners were cyclamate, aspartame and acesulfame K, with the former approaching the recommended daily dose for younger children (under 8 years of age) through the consumption of two artificially sweetened drink cans. Considering these evidences plus the use of sweeteners in other types of food, special attention should be given by health entities.

Based on the evolution of beverage composition over the last decade, it is clear that sugar content has been reducing, especially in “juice-based drinks”, together with a reduction of aspartame in these food products and an increase in cyclamate, sucralose and steviol glycosides. Unfortunately, the reformulations have apparently not been accompanied by an attempt to reduce sweet taste intensity which, if gradual, could constitute a long-term educational measure.

Keywords: soft drinks; free sugars; non-nutritive sweeteners; artificial sweeteners; taxation; HPLC

RESUMO

O elevado consumo de açúcares livres (sacarose, glucose e frutose) representa uma preocupação de saúde atual à escala global. Em Portugal, o seu consumo, particularmente através de refrigerantes, parece estar no topo da lista dos principais responsáveis pelo excesso de peso e obesidade que caracterizam a nossa população, principalmente nas crianças e adolescentes. Em 2017, foi criado o Imposto Especial sobre o Consumo (IEC) que incide sobre bebidas adicionadas de açúcar com teores superiores a 8 g/100mL. Esta medida surgiu para responder a este flagelo da saúde pública, estando ainda em implementação novas tributações (para teores de açúcar superiores a 2,5 g/100mL e a 5 g/100mL) que se antevêm impulsionadoras de melhores resultados. Contudo, a redução de açúcares nos refrigerantes é feita maioritariamente à custa da adição de edulcorantes não-nutritivos que, apesar de reduzirem o aporte calórico, não são isentos de risco e não contribuem para uma educação sensorial uma vez que procuram não modificar a perceção do sabor doce.

O presente estudo pretendeu verificar de que forma foram feitas as reformulações das bebidas pelas indústrias, avaliando a quantidade e tipo de açúcares livres e edulcorantes utilizados, e verificar se estas alterações poderão ter outras implicações na saúde. Para o efeito, em janeiro de 2019, foram adquiridos 68 dos refrigerantes mais consumidos em Portugal, tendo sido determinado o seu teor em açúcares livres por HPLC com detetor índice de refração e edulcorantes artificiais por HPLC com detetor de dispersão de luz. Os resultados foram ainda comparados com dados analíticos de 2008.

No que respeita às bebidas com açúcares (N = 48), apenas numa pequena fração das amostras (15%) apresentava um teor de açúcares totais acima de 8 g/100mL, estando uma parte próxima desta marca (25%) e a maioria muito próxima de 5 g/100 mL (39%). Esta aproximação é indicativa de que não haverá dificuldade em reduzir ainda mais a quantidade de açúcar adicionado. Entre grupos de bebidas, o perfil e quantidade de açúcares é distinto, com o grupo das “colas” e “bebidas de sumo” a apresentarem maior variabilidade no teor de açúcares (maioritariamente representado por frutose e glucose), e o grupo dos “ice tea” e “bebidas de aroma de limão” com teores mais consistentes entre amostras, utilizando principalmente a sacarose. Relativamente à marca, aparentemente verifica-se uma maior resistência na reformulação das marcas ditas “originais”, com as “marcas próprias” a apresentarem formulações muito semelhantes entre elas, exceto nas “colas”. Em termos de edulcorantes, eles estão presentes na grande maioria das amostras (85%) (N=58), mais frequentemente em misturas e bastante dependentes do tipo de bebida, apesar de se verificarem já algumas amostras apenas com sucrose (27%) ou glicosídeos de esteviol (10%). Em termos quantitativos os principais edulcorantes não-nutritivos são o ciclamato, o aspartame e o acessulfame K, com o primeiro a aproximar-se da dose diária recomendada nas crianças mais jovens (idade inferior a 8 anos) através do consumo de duas doses de refrigerantes com este edulcorante. Esta evidência aliada à utilização de edulcorantes noutros tipo de de alimentos deverá merecer alguma atenção por parte de entidades do sector da saúde.

Considerando a evolução da composição das bebidas na última década, é clara a redução no teor em açúcares, principalmente nas “bebidas de sumo”, bem como uma redução na utilização do aspartame e aumento de ciclamato, sucralose e glícosideos de esteviol nesta classe de produtos. Infelizmente as reformulações não têm sido aparentemente acompanhadas de uma tentativa de redução da intensidade de sabor doce que, se gradual, poderia constituir uma medida educativa a longo prazo.

Palavras Chave: refrigerantes; açúcares livres; adoçantes não nutritivos; edulcorantes; rótulo, imposto; HPLC

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ABBREVIATIONS

ADI – Acceptable Daily Intake

COSI – European Childhood Obesity Surveillance Initiative

EFSA – European Food Safety Agency

ELSD – Evaporate Light Scattering Detector

EPA – Environmental Protection Agency

FAO – Food and Agriculture Organization

FDA – Food and Drug Administration

GBD – Global Burden Disease

GC – Gas Chromatography

GI – Glycaemic Index

GL – Glycaemic Load

HFCS – High Fructose Corn Syrup

HPAEC – High Performance Anion-Exchange Chromatography

HPLC – High Performance Liquid Chromatography

IAN-AF – National Food and Physical Activity Survey

IEC – Special consumption tax

LOD – Limit of Detection

LOQ – Limit of Quantification

MS – Mass spectrometry

NCDs – Noncommunicable Diseases

NIRS – Near-Infrared Spectroscopy

NMR – Nuclear Magnetic Resonance

NNSs – Non-nutritive Sweeteners

OECD – Organization for Economic Co-operation and Development

PAD – Pulsed Amperometry Detector

PROBEB – Portuguese association of non-alcoholic refreshing beverages

RID – Refractive Index Detector

RSD – Coefficient variation

RT – Retention Time

TEA – Triethylamine

SEC – Size-Exclusion Chromatography

SP – Sweetening Power

SPE – Solid Phase Extraction

UV – Ultra-Violet

WHO – World Health Organization

THEORETICAL PART

1. Introduction

1.1. Free Sugars Health Concern

The ingestion of high amounts of sugars (particularly mono and disaccharides) through processed foods has been under the main agenda of food and health authorities. High intake of sugars is commonly associated with “poor” diets, obesity and overweight, glucose homeostasis, dental caries and risk of noncommunicable diseases (NCDs, such as diabetes, heart disease and cancer) (1), all representing major causes of death worldwide. Free sugars[1] represent the carbohydrate class of highest concern to human health (2). In 2010, the EFSA (European Food Safety Authority) has issued a scientific opinion on reference values for carbohydrates and dietary fibre, which also included sugars, but no limits for the daily intake of total or added sugars were defined (3). However, the World Health Organization (WHO) recommends that free sugars should not exceed 10 % of total caloric energy (4).

One of the biggest health concerns associated with free sugars is their high intake by children and teenagers, particularly through the consumption of soft drinks, which usually comprise sucrose and high fructose corn syrup. (5) Scientific evidences linked the consumption of this type of drinks with weight gain as well as to an absence of nutritional benefits, thereby providing only water and energy (6). Also, when compared to sugary solid foods, these drinks have a lower satiety effect as sugar absorption and digestion is faster than in solid foods (6). Also, excess calories are converted to body fat and stored in the body, which can lead to overweight and obesity (5, 6).

According to the Organization for Economic Co-operation and Development (OECD), childhood obesity is one of the biggest concerns of the 21st century for the various health organizations worldwide, as the number of children with this health problem has been increasing in the last decade in several European countries (7). As a result, several countries have been taking actions to reduce the consumption of sugary drinks (8). Among other measures implemented, taxation of sugary drinks is widely applied with the aim of reducing consumption and leading to reformulation of this type of beverages by the industries. In fact, taxes on sugary drinks are already in place in nineteen countries worldwide, including France, Portugal, Mexico, Chile and six cities in the United States of America. In Mexico, for example, this tax was applied in 2013 and, only one year after its implementation there was already a decrease in drinks sales and an increase in bottled

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[1] According to WHO, free sugars refers to simple sugars that are added to foods and beverages by the manufacturer or the consumer, particularly sucrose, glucose and fructose and the sugars naturally present in honey, syrups and fruit juices (2).

water sales (9). A similar trend was observed in numerous European countries after the implementation of such taxes. For example England has two tax levels for soft drinks with total sugar amounts between 5 and 8 grams per 100 mL and another for drinks with higher than 8 grams per 100 mL (6). However, robust studies are needed to assess, for example, the effect of these measures in the short and long term or the possibility of regression (6).

The main objective of the implementation of these taxes is to lead consumers to reduce the purchase and consumption of sugary drinks and, above all, to educate consumers with less literacy and less income to choose healthier and more profitable options. It is also necessary to decrease the availability and accessibility of these products in public spaces such as schools or hospitals. Also, the promotion and availability of water is needed as a healthier alternative (6).

The soft drink industries are one of the major sectors of the food industry with frequent and intense marketing strategies that strongly influence the consumption of this type of beverages. For example, the United States of America has spent more than 700 million dollars on soft drink advertising (9). Also, these products are often low priced as they are almost always subjected to promotions and other commercial strategies, thus stimulating their purchase (6). Therefore, another major goal of taxes is to lead the industries to reformulate sugary drinks in order to reduce the amount of added sugar (6). However, the industries use other strategies so that sales are not affected by the rates applied.

1.2. The Portuguese panorama

According to the 2018 Health Portrait report (8), chronic diseases are responsible for about 80 % of deaths, mainly due to lifestyle factors such as poor eating habits, sedentary lifestyle, smoking, and alcoholism.

Portugal is no exception in terms of market offer and pursue, revealing the same problems as the rest of the world in terms of overweight and obesity. The main factors that can lead to these conditions include lack or reduced exercise as well as inadequate high-calorie diet, which is high in fat and sugars (8).

According to the National Food and Physical Activity Survey (IAN-AF) (10), 5.9 million Portuguese suffer from total overweight and only 41.8 % practice some kind of physical or sports activity. According to the same report, only about 36 % of young people are considered physically active (10).

Within these concerns, childhood obesity is a public health concern with Portuguese children having an average of overweight at different ages (11-13 and 15 years-old) of 28.5 %, above the OECD average countries, with 24.6 % (7, 8). According to the WHO European Childhood Obesity Surveillance Initiative (COSI) study, in 2016, 30.7 % of the Portuguese children (6-8 years old) were overweight and obese (Table 1) (11). However, the number of overweight and obese children has been declining since 2008, although the figures are still high (12).

Table 1. Overweight and obese Portuguese children from 2008 to 2016.

	2008	**2010**	**2013**	**2016**
Overweight	22.6%	21.0%	17.7%	19.0%
Obesity	15.3%	14.6%	13.9%	11.7%
Total	37.9%	35.6%	31.6%	30.7%

Data regarding children aged 6 to 8 years-old, according to (11).

According to the IAN-AF (10), the age group with the highest prevalence of obesity is the 5-9 years group and is also the one with the highest obesity values (12.5 %), which comprises a higher level than the one reported by COSI study (11).

According to the same report (10), when it comes to teenagers and children (under 15 years old), about 43 % is considered non-active, spending 9 hours on sedentary behaviours, which increases even further with age. However, over 60 % of children between the ages of 3 and 14 are engaged in scheduled sports, predominantly soccer and swimming for boys and swimming, dance and expression activities for girls (10).

Although the lack of exercise contributes widely for the overweight and obesity figures in Portugal, “poor” diet is also a determinant factor. Consumption of high-calorie foods, with high amounts of sugar and/or fat lead to an estimated loss of 15.4 % of healthy living years in the Portuguese population, according to the 2018 Health Portrait Report (8).

The widespread of inadequate eating habits of the Portuguese population is a major concern for the national health authorities, namely the high salt intake and the high consumption of free sugars. According to a study by the Global Burden of Disease (GBD) (12), these eating habits were the second largest risk factors contributing to early mortality due to cardiovascular disease and cancer in 2016. The main foods that contribute to the intake of free sugars in the Portuguese population were described in the IAN-AF report as being soft drinks, natural or concentrated fruit juices, cakes, crackers, cookies, breakfast cereals (8, 12). Among these foodstuffs, particular attention should be given to soft drinks as they contribute to 11.9 % of the intake of free sugars, only preceded by table sugar (21.4 %) and candy-type sweets (16.7 %) (12). According to the IAN-AF report (10), about 17 % of the Portuguese population drinks at least one soft drink a day, together with a low consumption of fresh fruit and vegetables (56 % of the Portuguese population does not eat the amount recommended by WHO) (8). Also, the average amount of water ingested by the

Portuguese population is less than 1 L per day, with children drinking even less (about 500 mL per day).

According to the Generation XXI cohort study, which accompanies 8,647 children in northern Portugal since birth, about 35 % of these children at 2 years of age have already consumed soft drinks at least once per week, a behaviour that remained in 88 % of these children up to 4 years old (13). Also, it was found that 52 % of 4-year-olds consumed soft drinks and nectars daily, essentially tea-based soft drinks that are the most consumed in Portugal. Their diet was also associated with low consumption of fruits and vegetables, which do not sustain the high energy levels of this type of drinks (13).

Because of this panorama, the Portuguese governmental authorities decided to follow the example of other countries and implemented a tax on drinks with added sugars. The Special Consumption Tax (IEC) on sugary drinks was implemented in 2017 (Regulation No.42/2016, of 28 December), and focused on beverages with added sugar or other sweeteners (Table 2). The beverages included fall under the Combined Nomenclature code 2202 and include soft drinks, energy drinks, flavoured waters and syrup or powdered beverage concentrates (14).

Table 2: Tax evolution in Portugal for added sugars on soft drinks (14, 15).

2017 (Reg. 42/2016)		**2019** (Reg. 71/2018)	
Sugar (g/L)	**Tax** (€/hL)	**Sugar** (g/L)	**Tax** (€/hL)
< 80	8.22	< 25	1
≥ 80	16.46	> 25 < 50	6
		> 50 < 80	8
		≥ 80	20

As a result, based on data from the Portuguese Association of Non-Alcoholic Refreshing Beverages (PROBEB), sales of sugar-added beverages decreased by 4.3 % during 2017 compared to previous years. In addition, according to data from the Tax and Customs Authority (16), there was a reduction of almost 50 % in the consumption of drinks with more than 80 g/L. The main reason for this variation is that some industries with high sugar beverages have reformulated in their products through the addition of artificial sweeteners (16). However, other factors may have influenced these changes, such as consumer choices, marketing and corporate advertising. Although data are still preliminary, taxation schemes have been shown to have a positive impact on reducing sugar in beverages and consumer consumption even in the short term, as has already happened in other countries. In addition, the results also showed that the implementation of the sugar tax in 2017 had a greater impact than the education and self-regulation mechanisms implemented between 2013 and 2016 (16). Because of these factors, and the fact that the decrease in beverage consumption was more impactful in the upper tax than in the lower one, it was decided to split the lower tax, into two further classes and the implementation was approved by Regulation No. 71/2018, of 31 December (Table 2) (15, 16).

Further developments are expected soon since the new limits have been applied, but it is expected that there will be further reformulations on sugary drinks, with the addition of non-caloric sweeteners as sugar substitutes, as they provide an equally sweet and pleasant taste without adding any energy value.

1.3. Label interpretation

The distinction between these terms is particularly important because they bring many doubts to consumers when reading a label. "Total sugar" is the information that appears on the food label and refers to all sugars present from any source, namely naturally-occurring sugars and those ones that are added to food (17). Naturally-occurring sugars are the intrinsic sugars that are incorporated within the structure of intact fruit and veggies and the sugar present in milk (18, 19). But where the biggest differences arise is in the distinction of added sugars and free sugars. The main difference between the two is that free sugars include the sugars naturally present in the food. WHO prefers the term "free sugar" to refer simple sugars that are added to foods and beverages by the manufacturer or the consumer, particularly sucrose, glucose and fructose and the sugars naturally present in honey, syrups and fruit juices (4). Other institutions like U.S. Food and Drug Administration (FDA) and EFSA prefer the term “added sugars” (19, 20). For example, in the United States of America, "added sugar" means sugars or syrups added to foods during their preparation and processing, in this case it does not include sugars that are naturally present in milk (lactose) and in fruit (fructose) (20). In 2009, EFSA was even more specific and defined that "added sugar" is the addition of sucrose, fructose, glucose, starch hydrolysates such as glucose syrup and high-fructose corn syrup, and other isolated sugar preparations during the preparation and manufacture of foods (20). However, there are still some incongruities in the definition of free and added sugars, so several definitions may arise (17).

2. Sweeteners

A sweetener is a natural or synthetic substance that gives sweet taste to foods and beverages (19). Depending on their caloric value, sweeteners can be classified as nutritive or non-nutritive (NNSs) (Figure 1).

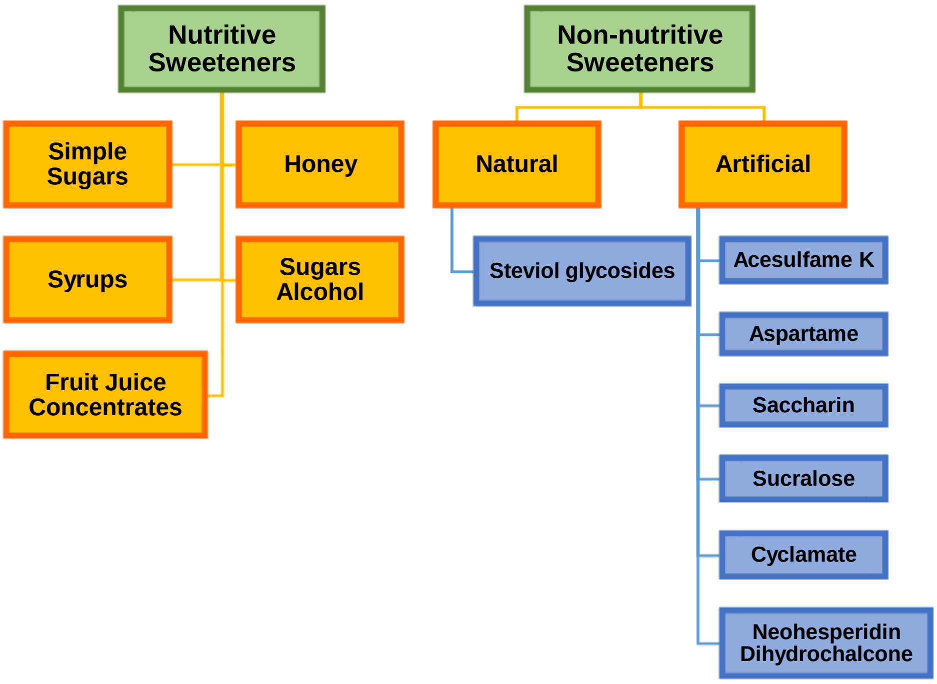


Figure 1. Classification of the most common sweeteners used in foods.

There is a wide variety of sweeteners on the market, from sugars (sucrose, glucose and fructose), to sugars alcohols (xylitol, mannitol, etc.) or to artificial sweeteners that are increasingly found in soft drink formulations as an alternative to sugar.

Nutritional or caloric sweeteners are mostly represented by sugars (sucrose, glucose and fructose), honey, syrups and concentrated fruit juices. (19) On the other hand, non-nutritive sweeteners or high-intensive sweeteners can sweeten foods even in small amounts by adding little or no calories and have a huge chemical diversity. Furthermore, unlike sugars, they might not be totally metabolised or even excreted without any metabolization and do not cause a glycaemic response in the body. Non-nutritive sweeteners can be further divided into natural and artificial sweeteners, where the former come mostly from plants (e.g., stevioside obtained from leaves of *Stevia rebaudiana*), and artificial ones that are synthetically obtained (e.g., acesulfame K) (19, 21).

2.1. Nutritive Sweeteners

The term “sugars” is used to describe the smallest molecules belonging to carbohydrates, namely mono and disaccharides. Sugar synthesis begins with plants and algae photosynthesis, which is an endothermic reductive condensation of carbon dioxide, requiring light energy and the pigment chlorophyll, thus giving rise to glucose, the most important sugar molecule. Other important sugars include sucrose, fructose, galactose, lactose and maltose (Table 3) (20).

Sugars have a high commercial value for the food industries, since they can provide the most varied properties to foods at a low cost, with high digestibility, being used in the most diverse types of food, from infant formulas to nutritional supplements for the elderly (22).

The sweet taste is innate to humans since we are already born with their perception (23). In addition, when added to foods and beverages, sugars are not only intended to sweeten them, they have other functions capable of improving the appearance, consistency and taste of the food (23). In addition to acting as a bulking agent to improve the texture of food, add viscosity and consistency to food, sugars can also be used as a preservative, reducing the water activity in food. They also improve the taste and colour of the food through Maillard's reactions that produce a brown colour and reproduce a very pleasant caramel aroma (19, 23).

2.1.1. Glucose

Glucose is one of the most important monosaccharide, as it works as a carrier and an energy supplier for living beings. In addition, it is also widely used in the food industry as a sweetener or as a bulking agent in beverages, confectionery and in the production of chewing gums. In terms of sweetness, glucose is about 31 to 36% less sweet than sucrose (Table 3) and is fully digested by the human body (22, 24).

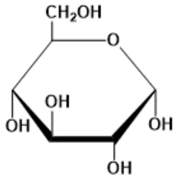
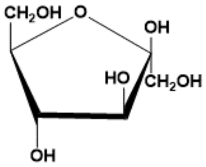
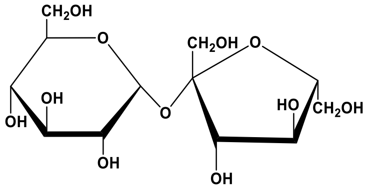
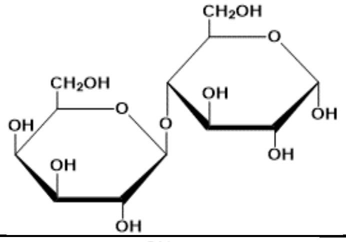
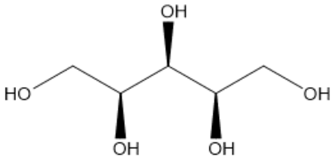
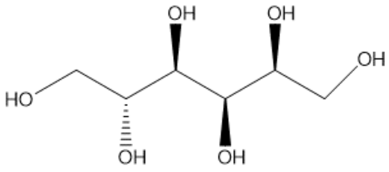
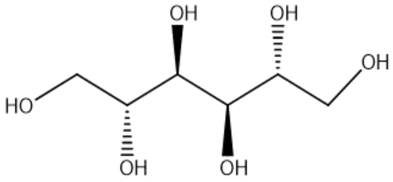
The human body has mechanisms for production and transport of glucose through the blood, and the ingestion of glucose in high amounts can lead to dysregulation and damage of the biochemical mechanisms of the cells (22). It is possible to estimate the impact of a certain carbohydrate on circulating glucose through the glycaemic index (GI) (22). The GI is not characteristic of foods, but of the carbohydrates present in the foods. In addition, this value is independent of the amount of food or the amount of carbohydrate ingested. Foods that have a high GI, are rapidly absorbed, digested, and metabolized by the body, giving rise to high levels of blood sugar (glucose), inducing the pancreas to produce a high amount of insulin to maintain normal levels of sugar in the body (22). An increase in insulin leads to a lower sensation of satiety, leading to the consumption of more food, which can cause an increase in weight and an insulin resistance that can later lead to the onset of diseases such as diabetes. For the body, carbohydrates with a lower GI are more advantageous, as the increase in blood glucose is more gradual and the production of insulin is made more slowly. In general, foods that have low and medium GI include fruits, vegetables and legumes, while processed and sweet foods have a higher GI (25). Glucose has the highest glycaemic index (100) and serves as a reference for the others (Table 3) (26).

2.1.2. Fructose

Fructose or fruit sugar is also a very relevant monosaccharide and is naturally present in abundance in fruits and, at a lower extent, in some vegetables, such as potatoes and onions (27). In the food industry, fructose is a very important component in sugar syrups, such as invert sugar syrups (hydrolysed sucrose), isoglucose syrups and high fructose syrups (HFCS), with variable fructose amounts (42, 55 and up 90 % fructose) (22). In addition, high purity crystalline fructose (98 % fructose at least and a maximum of 0.5 % glucose) can also be obtained. This type of sweetener is not commonly used in Europe because of the prevalence of table sugar (sucrose) but is increasingly seen in the biological products section in supermarkets, being regarded as a healthier sugar derived from its association with fruit. In 2011, EFSA issued a positive scientific opinion on the consumption of fructose instead of glucose and sucrose (28).

This is partly because of fructose lower GI compared to glucose and sucrose. Some studies have proven that fructose had beneficial effects on diseases such as diabetes since it provides lower glycaemic responses. EFSA reported that fructose reduced the postprandial glycaemic response, unlike glucose and sucrose, from tests performed after ingestion of several sweetened foods with different types of sugar, without affecting body weight, blood pressure and uric acid (28, 29). However, although fructose has more health benefits against glucose and sucrose, it should not be consumed uncontrollably, since it is still a free sugar. The measures advocated by EFSA are the substitution of high GI products for those with lower GI, such as fructose. Fructose is primarily used in the ice-cold product industry, such as ice creams, cold soft drinks and yogurts, because of its water holding capacity at low temperatures. In terms of sweetness, fructose is about 114 to 117% sweeter than sucrose (Table 3).

Table 3: Properties of major sugars and sugars alcohols (polyols) (22, 24, 30, 31).

Name	Structure	Class	Glicemic Index	Relative Sweetness	Calories (kcal/g)
Glucose		Monosaccharides	100	69	4
Fructose			19-23	114	4
Sucrose		Disaccharide (glucose+fructose)	61-65	100	4
Lactose		Disaccharide (glucose+galactose)	46	39	4
Xylitol		Sugar alcohols	13	100	2.5
Sorbitol			9	50-70	2.5
Mannitol			0	50-70	1.5

2.1.3. Sucrose

Sucrose, or table sugar (Table 3), is the most important disaccharide and the most used in everyday life, being associated with about 55 % of the total sugar consumption. Among other properties, sucrose has sweetness and is a bulking agent capable of performing the most diverse functions in various types of foods (cookies, biscuits, ice cream, etc) (22). Sucrose is usually extracted from sugar cane (*Saccharum officinarum*) and sugar beet (*Beta vulgaris*) and treated by the industry leading to a high purity (> 99.9 %) product with a low production cost and making it one of the most used organic compounds in the food industry. Thus, sucrose has been extensively exploited in the industry and is also the raw material for the production of several other products, as the fermentation ones, and even synthetic production of D-glucose and D-fructose (22).

2.1.4. Lactose

Lactose, a disaccharide, is the energetic source present in mammalian milk produced in mammary epithelial cells. When lactose enters the body, it is hydrolysed by the enzyme lactase, giving rise to galactose and glucose that are absorbed in the duodenum. In addition, lactose has a low sweetness (about 15 % sucrose) (Table 3). By replacing other sugars with lactose, there is a reduction in the appetite and lack of sweet foods on the part of the children, as well as being less cariogenic than the other sugars (32, 33). In the industry, lactose is primarily used in baby food, cakes and biscuits, chocolate and chocolate products, confectionery, soups and sauces (32).

Over the last few years, lactose intolerance has been much discussed. This type of intolerance occurs when the activity of the enzyme lactase decreases in the intestine and can occur in both healthy adults and children in any population. What happens is that the lactose present in dairy products is not hydrolysed in the small intestine (due to lactase deficiency) and is fermented to lactic acid by the microflora, giving rise to diarrhea. Several studies have stated that these low levels of activity are normal for most of the world's population, as the activity of lactase decreases throughout age. However, the population of northern Europe does not present major problems in lactose absorption, showing that this intolerance is highly related to ethnic and is very dependent on age. Because lactose intolerance has no cure, the only way not to manifest it is by reducing or stopping the consumption of milk and dairy products, such as cheese and yogurts (32), or to follow some alternatives, as the ingestion of pills or capsules containing lactase from yeast (*Kluyveromyces lactis*) or fungal (*Aspergillus niger, A. oryzae*), as they have been shown to be efficient in the digestion of lactose, or the consumption of lactose-free products increasingly available in the market. In these products, added lactase is capable of hydrolysing about 70 to 100 % of the lactose. Usually these hydrolysed products are sweeter due to the release of galactose and glucose (34).

2.1.5. Sugar alcohols

As an alternative to simple sugars, sugar alcohols or polyols have been added to foods because they have a lower calorific value and since they are absorbed in the intestine more slowly and incompletely. These compounds result from the reduction of simple sugars and the most important in food processing are xylitol, sorbitol, glucitol and mannitol (Table 3) (22). Some alditols are very important in food processing, as xylitol, sorbitol, glucitol and mannitol that appear in nature in many fruits (e.g. pears, apples and plums). These sugar alcohols are used to replace sugars in foods to reduce water activity in intermediate stages of food processing, are used as softeners, are also used as inhibitors of crystallization and can also be used to keep in good condition dehydrated foods, ensuring their rehydration (24, 30). Sorbitol and mannitol are widely used in chewing gums, toothpastes, compressed tablets and diet chocolate (22).

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2.2. Non-nutritive Sweeteners

NNSs have been increasingly present in foods and beverages as sugar substitutes to reduce caloric intake and free sugars content, contributing to reduce overweight, obesity and ultimately diabetes, as they are not or are only partially metabolized by the human body. The main advantage of these compounds over sugars is that they give foods a sweet taste without adding calories or glycaemic effects. This happens because NNSs are about 30 to 20,000 times sweeter than sugar, so smaller amounts of these products are needed (23, 35). However, other differences are evident, particularly as regards sensory properties, as sweeteners have metallic mouthfeel and a bitter taste. Within NNS, there are natural and artificial ones, the latter being the most used in the food industry for their lowest price and for having a better flavour profile (23, 36).

NNSs are used in foods and beverages as a substitute for sugars to decrease the number of calories present. However, this replacement is not that simple. When removing sugar from food or drink by an artificial sweetener, it is necessary to add other bulk compounds that do not greatly modify the original product. This situation can lead to a negligible reduction in the calories of food, and in some may even increase them. For example, when sugar is removed it is usually replaced by another carbohydrate-based bulking agent such as maltodextrin which has the same caloric density and so will contribute in the same way. In addition, fat is often added as a bulking agent and to improve mouthfeel but the problem remains since the reduced calorie count is negligible (less than 10%) (19, 23). Therefore, several other ingredients must be added for sugar substitution (high potency sweeteners, fat, bulking agents, thickeners and flavourings) which will cause the number of additives on the food label to increase and be regarded as negative for some consumers. In addition, the use of artificial sweeteners is still seen as negative, mainly due to several previous studies linking saccharin with cancer and the fact that aspartame cannot be consumed by some individuals due to the presence of phenylalanine (19). However, NNSs are currently the most effective strategy to replace added sugars in the food industry. In addition, these compounds can help achieve the similar characteristics of sugary foods without adding calories. Nevertheless, a recent Scientific Report of the 2015 Dietary Guidelines publication noted that there is insufficient evidence to state that the use of NNSs is an effective long-term weight loss strategy and that sugars should not be substituted for foods and drinks for this kind of food (19). However, for now, NNSs are the best solution for sugar substitution and furthermore it is unlikely that new alternatives will be found in the next decade (19).

Figure 2 shows the main sweeteners, i.e. aspartame, acesulfame K, cyclamate, saccharin, neo-hespiridin dihydrochalcone, sucralose and the main steviol glycosides (stevioside and rebaudioside A), whose properties will be detailed in the following sections.

2.2.1. Saccharin

Saccharin (SAC), or 1,1-Dioxo-1,2-benzisothiazol-3(2H)-one, was the first artificial sweetener to be discovered in 1878 and the first to be commercialized (21). In relation to sucrose, it is about 300 to 500 times sweeter, but its taste is not the most pleasant one, having a bitter and metallic taste. However, if used with other sweeteners, this taste is minimized, particularly in the presence of cyclamate and aspartame (37). Saccharin has a synergistic effect with these artificial sweeteners due to the cooperative binding these compounds make at different sites from the sweetener receptors. In addition, it is normally coupled to a sodium or calcium molecule since saccharin alone is poorly soluble in water (38). Sodium saccharin is a very stable molecule, unaffected by temperature and pH and is therefore widely used in the manufacture of food and beverages, especially dietetic, bakery and confectionery (37, 38). Due to the synergistic effect with other artificial sweeteners, the commercialized drinks have in their composition saccharin sodium with aspartame, cyclamate and sucralose.

However, saccharin has already faced several controversies as it was associated with the onset of cancer in laboratory rats in 1960 (21, 38). Fortunatelly, after further studies it was found that the mechanism causing cancer in male rats was not confirmed in humans. In addition, no other study has shown that saccharin consumption presented health risks when consumed at normal doses, i.e., bellow the Acceptable Daily Intake (ADI) of 5 mg/kg body weight per day (37, 38). It was not until December 2010 that the United States Environmental Protection Agency (EPA) ceased to regard saccharin as a potential hazard to human health. According to Commission Regulation (EU) No. 1129/2011 of 11 November 2011 up to 80 mg/L may be added to products with low energy value or without added sugar (39) (Table 4).

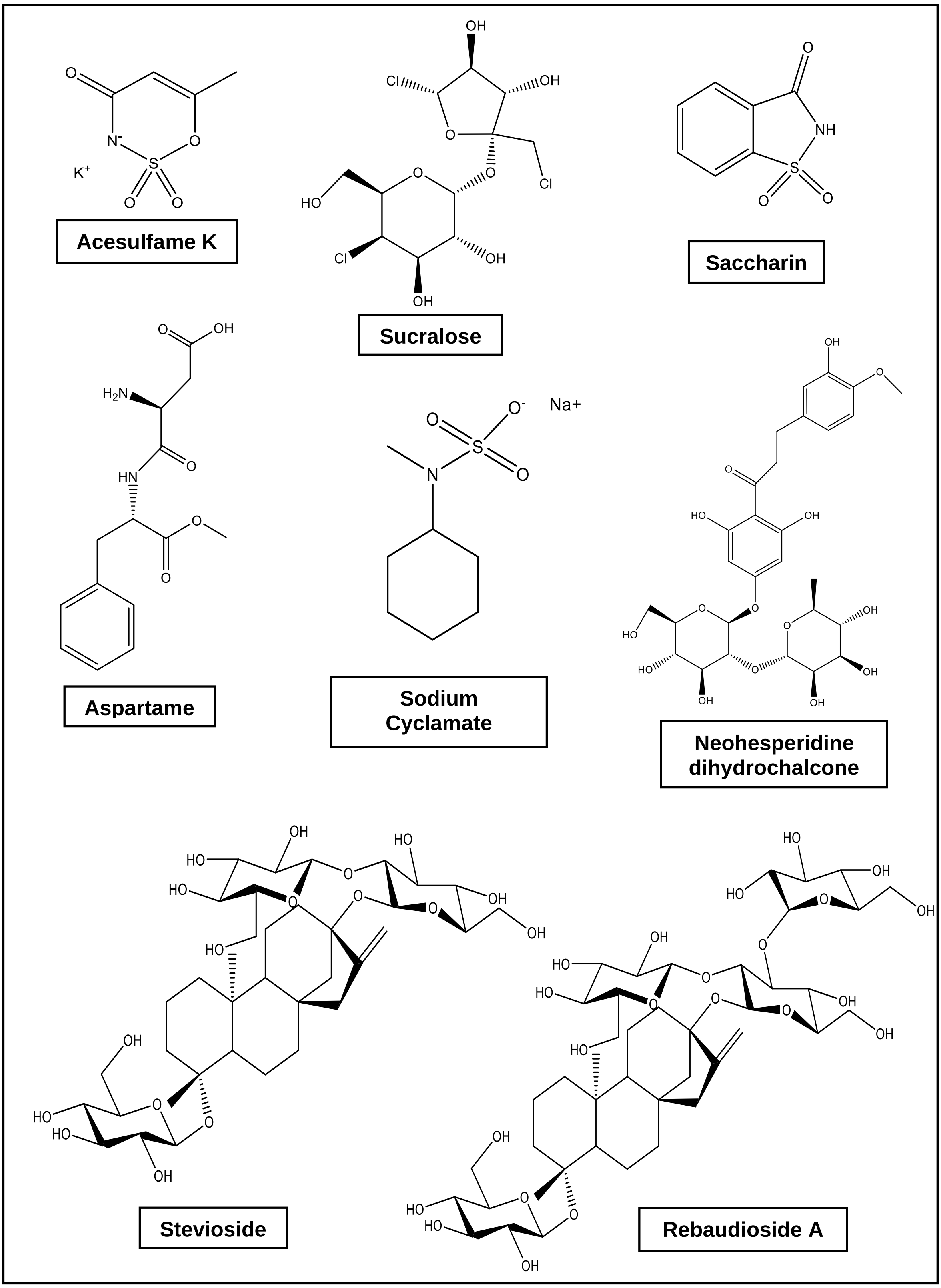

Figure 2: Most commonly used non-nutritive sweeteners in foods and beverages (Chemdraw 18.0).

Table 4: Properties of artificial and natural intense sweeteners (21, 38-40).

Name	Source	Year	Relative Sweetness (sucrose = 1)	Calories (kcal/g)	Legislation limit (mg/L)	ADI (mg/kg/day)	Aftertaste
SAC (E954)	Synthetic	1878	300-600	0	80	5	Bitter, metallic
CYC (E952)		1937	30-40	0	250	11	Prolonged sweeteness
ASP (E951)		1965	160-220	4	600	50	Prolonged sweeteness
ACS K (E950)		1970	150-200	0	350	15	Slightly bitter
SUC (E955)		1976	400-800	0	300	5	Not unpleasant
NHDC (E959)	Semi-synthetic	1950	1000-2000	2	30	5	Very bitter
Steviol Glycosides	Natural	1970	300	0	242	4	Very bitter

2.2.2. Cyclamate

Cyclamate (CYC), or cyclohexylsulfamic acid, is commonly found as sodium salt and was discovered in 1937. In addition, it may be in the form of calcium salt and thus may be used in low sodium diets (37). Sodium cyclamate is not one of the sweetest artificial sweeteners as it is only 30 times sweeter than sucrose. Moreover, its taste when used alone is bitter, but, it has a good synergistic effect with saccharin as already mentioned (38). Their use in mix has the advantage of better taste and quality of products (21). In terms of chemical properties, solid cyclamate is very stable and in solution leads to slow formation by hydrolysis of cyclohexylamine and inorganic sulphate (38). To understand whether their use is safe or not, the FDA studied the presence of these compounds in foods and found low levels of cyclohexylamine. Since their levels did not increase after four months at room temperature in cola beverages, so they concluded that cyclamate sweeteners are quite stable for use in various foods such as beverages, bakery goods and confectionery (37, 38). However, some studies have also been conducted on the possible exposure of cyclohexylamine from cyclamate metabolism in humans following long term consumption. This study found that long-term consumption is toxicologically relevant to a cyclamate ADI establishment. Therefore, the recommended ADI is 11 mg/kg body weight per day (37). However, cyclamate consumption has been banned in the United States, Canada, and the United Kingdom since 1970, following the discovery in 1969 that high amounts of cyclamate caused bladder cancer in laboratory rats. Still, over 90 countries worldwide, including European countries and China, allow the consumption of cyclamate as a sweetener (23).

According to Comission Regulation (EU) No. 1129/2011 of 11 November 2011 up to 250 mg/L cyclamate and its sodium and calcium salts may be added to products with low energy value or without added sugar (Table 4) (39).

2.2.3. Aspartame

Aspartame (ASP), or L-Aspartyl-L-phenylalanine methyl ester, is a dipeptide composed of two amino acids: phenylalanine and aspartic acid, the most commonly used artificial sweetener in Portugal and around the world (21, 41). The appearance of aspartame is a white crystalline powder, is 200 times sweeter than sucrose and is generally used in cold desserts, jellies, drinks and chewing gum (37, 38). Aspartame can be used alone or mixed with other sweeteners as it has a clean sweet taste, unlike saccharin, for example. However, it is most commonly found in a mixture of sweeteners to mask the flavours of saccharin, acesulfame K and cyclamate. In addition, it has the ability to enhance flavours, particularly citrus flavours (38). However, aspartame should not be used in foods with prolonged heating as is the case with confectionery and cooking products, as high temperatures lead to decomposition of amino acids. At a pH around 4.3, aspartame becomes highly stable and can be used in acidic solutions such as soft drinks, while its application at neutral pH products is not advised (21, 37). When ingested by the body, aspartame decomposes into aspartic acid, phenylalanine, methanol and even formaldehyde, formic acid and diketopiperazine (21). The only problem reported for aspartame is that it contains phenylalanine, an amino acid that cannot be ingested by people suffering from phenylketonuria (21). As with any artificial sweetener, carcinogenic effects were tested, and one study reported an increased incidence of cancer in transgenic mice exposed to aspartame. However, more than 100 regulatory agencies from various countries (including the UK Food Standards Agency and EFSA) believe that aspartame is safe for human consumption and its recommended ADI is 50 mg/kg body weight per day in the United States of America (37, 38). According to Commission Regulation (EU) No. 1129/2011 of 11 November 2011 up to 600 mg/L of aspartame may be added to products with low energy value or without added sugar, being the highest value compared to other artificial sweeteners (Table 4) (39).

2.2.4. Acesulfame K

Acesulfame K (ACS K), the potassium salt of 6-methyl-1,2,3-axathiazine-4(3H)-one 2,2-dioxide, is a crystalline white powder, about 150 to 200 times sweeter than sucrose with good solubility in water (37). As most sweeteners have a bitter taste when used alone, it is preferably used with aspartame and sucralose, giving a mixture that is sweeter than each of its components (38). Acesulfame K is very stable at high temperatures, pH and light exposure and is widely used in long shelf life, baked goods and candies. Acesulfame K together with aspartame is the most commonly used sweeteners in Portugal for soft drinks and nectars (21, 41). These two artificial sweeteners have high synergistic effects (about 30%), leading to reduced costs and improved taste of foods and beverages. In terms of toxicity studies, acesulfame K does not appear to pose major problems because it is not metabolised by the human body (38). However, acetoacetamide is one of the products resulting from the decomposition of acesulfame K which is toxic in large quantities. But as the amount to which humans are exposed is so small, the consumption of acesulfame K presents no danger (21, 37). The recommended ADI for acesulfame K is 15 mg/kg body weight per day in the United States of America (38). According to Commission Regulation (EU) No. 1129/2011 of 11 November 2011 up to 350 mg/L of acesulfame K may be added to products with low energy value or without added sugar (Table 4) (39).

2.2.5. Sucralose

Sucralose (SUC), 1,6-dichloro-1,6-dideoxy-beta-D-fructofuranosyl 4-chloro-4-deoxy-alpha-D-galactopyranoside, or chlorinated sugar, appeared in the year 1976 and results from a sucrose or raffinose molecule from which three hydroxyl groups were removed and replaced by three chlorine atoms (37). In terms of properties, sucralose is a crystalline solid and highly water-soluble. Because of its good light stability and wide range of pH stability, it can be used in numerous foods and beverages (21). In terms of sweetness it is about 450 to 650 times sweeter than sucrose and has a pleasant sweet taste and quality and durability very close to that of sucrose (21). Sucralose can be used alone or in a mixture with other artificial sweeteners, having a moderate synergy with other nutritive sweeteners. Although sucralose is obtained from sugar, the human body does not recognize it as such because the enzymes are unable to open its ring structure for further metabolization. Thus, only 15 % of sucralose is absorbed and then excreted in the urine, while the rest is eliminated directly in faeces (21, 38).

In terms of toxicity, extensive animal and human studies have been reviewed by the FDA and no reproductive and neurological carcinogenic effects have been found (21). For this reason, its consumption has been approved in a recommended ADI of 5 mg/kg body weight per day by the FDA and can be used as a general-purpose sweetener (38). However, there are still some questions about how sucralose is metabolized by the human body (37). In Europe, its consumption is also approved and in accordance with Commission Regulation (EU) No. 1129/2011 of 11 November 2011 up to 300 mg/L of sucralose may be added to products with low energy value or without added sugar (Table 4) (39).

2.2.6. Neohesperidin Dihydrochalcone

Neohesperidin dihydrochalcone (NHDC) was discovered in 1950 and is a semi-synthetic sweetener. NHDC is a flavone glucoside, obtained from narigin by several chemical processes (42). In terms of sweetness, this compound is 100 times sweeter than sucrose. It is usually found in sweetener mixtures since its taste is very bitter and its sweetness development is much slower than that of sucrose. It has high stability at various pH, so it can be used in a wide variety of foods. However, its consumption is prohibited in the United States of America, but in Europe is allowed. According to Commission Regulation (EU) No. 1129/2011 of 11 November 2011 up to 30 mg/L of NHDC may be added to products with low energy value or without added sugar (Table 4) (38).

2.2.7. Steviol Glycosides

This substance can be found in the leaves of *Stevia rebaudiana*, a native plant of Paraguay, being responsible for the characteristic sweet taste of the leaves of this plant. Nevertheless, other species from Mexico, China and Japan may also produce them (43). For several years it was believed that stevioside was the only glycoside of steviol present in Stevia leaves. The most commonly found steviol glycosides are stevioside (5-10%) and rebaudioside A (2-5%). However, there are others such as rebaudioside B, C, D, E and F, steviolbioside and dulcoside A (43).

The great advantage of steviol glycosides is that they are natural, provide no calories and are about 300 times sweeter than sucrose. However, it was not until 2011 that its use in food and drink was approved in Europe, although it is already widely used in South America and Japan (43, 44). Following the publication of a positive scientific opinion by EFSA in 2010, where it reported that steviol glycosides are non-carcinogenic, genotoxic and have no reproductive/developmental toxicity that is now used in Europe (29). In 2011, the European Parliament issued Regulation (EU) No. 1131/2011 of 11 November 2011, an ADI for steviol glycosides of 4 mg/kg body weight per day, expressed in equivalents of steviol (Table 4) (40).

3. Analytical methods

The determination and quantification of added sugars is a challenge as there are no analytical techniques to distinguish them from naturally occurring sugars, since there is no chemical difference between them, so on the label we find the value of the total sugar present in the food (19). However, there are several analytical techniques capable of determining and quantifying free sugars (glucose, sucrose and fructose) in various types of foods.

Regarding artificial sweeteners, most methods are dedicated to their individual quantification. However, analytical techniques have been developed in the last decade that allow the detection and quantification of all substances in a single run (45).

The following sections describe the main analytical methods for the detection and quantification of free sugars and artificial sweeteners.

3.1. Free Sugars

Despite the emergence of several newer techniques for separation of sugars, HPLC remains one of the most widely used ones since it provides sensitive, reliable and rapid results for separating and detecting the composition or the addition of this type of compounds in plants or foods (46). For the quantification and identification of sugars, the most commonly used detectors are Pulsed Amperometry Detector (PAD), Refractive Index Detector (RID), Evaporate Light Scattering Detector (ELSD) and Mass Spectrometry (MS) (47). Currently, the method of separation of carbohydrates adopted for most food matrices is that of separation by anionic chromatography (HPAEC), mainly due to the presence of hydroxyl groups which, in alkaline medium, form oxyanions, thus allowing their separation (48). This type of separation is combined with PAD, since this type of detector is not very sensitive to changes in the mobile phase, and as this type of chromatography generally uses a NaOH concentration gradient, it makes it a very suitable detector for the analysis of carbohydrates (49). In addition, this type of separation can be conjugated to size exclusion chromatography (SEC). This method shows good linearity and satisfactory detection limits, showing that it can be easily applied in daily analyses of sugars in fruit juices in the industry (50). In the analysis of carbohydrates in several matrices, RID is used in conjunction with reverse phase chromatography or size-exclusion. The major disadvantage of this detector is that no gradient elution can be performed, which is sometimes very much used in chromatographic techniques to improve the separation of the compounds (47). In addition, it is not a selective method and it also varies greatly with the flow rate. In terms of sensitivity, it is less sensitive than PAD, however it has a better sensitivity in the quantification of major sugars (51). Despite all these disadvantages, RID is still characterized as being an universal detector and does not require any derivatization for the quantification of sugars, being a cost-effective method (49, 52). Although similar to RID in its universality, ELSD has very different characteristics for the detection and quantification of sugars. ELSD is not dependent on factors such as composition, flow rate of the mobile phase and temperature, since the detection method is based on the ability of the particles to cause photon scattering. It presents precise, accurate results and some sensitivity in the analysis of fructose, glucose and sucrose in fruits (53). Also, it does not require derivatization and constitutes a good approach for the detection and quantification in samples of plants, foods and drink products. ELSD has more advantages than RID, one of which is that it is possible to use an elution gradient because it does not give rise to any baseline drift since this problem is caused by the mobile phase and temperature, which are factors that are not well denoted in this type of detector (46, 52).

Another technique that can be used for sugar separation is gas chromatography (GC), however, it is not widely used due to the need for derivatization of sugars, since they are not volatile compounds (54).

Other more modern techniques for sugar analysis have emerged, namely Near-Infrared spectroscopy (NIRS) and Nuclear Magnetic Resonance (NMR), as they are non-destructive techniques, require simple or no sample preparation, are selective and can quantify and measure multiple sample properties at the same time. NIRS has proven to be able to quickly detect adulteration in beverages and in various products and is still able to analyse glucose, fructose and sucrose at the same time in juice samples, which makes this technique very suitable for use in a wide range of industries to obtain faster, cost-effective and more efficient analyses (55). In the study of carbohydrates, NMR was responsible for supplying most of the experimental data in complex mixtures of reducing sugars, and as over time the compounds marked with C^{13} were increased, even the minor components became visible and this allowed the development of the technique (56). However, for the success of these techniques a good calibration is necessary, and it is essential to create a database with reference values of each sample, which at the beginning can make the technique time consuming and expensive. Despite these restrains, in the long term, the application in industries can be an advantage over the spectrometers, since the use of reagents and the time of analysis is reduced (57, 58).

3.2. Artificial Sweeteners

Artificial sweeteners comprise a huge diversity of chemical compounds and therefore their collective quantification is not easy. For the separation of artificial sweeteners, the most commonly used technique is reverse phase HPLC and the most commonly used detectors for its detection and quantification are usually the ultraviolet (UV) detector, spectrophotometer, MS and ELSD, among others. Artificial sweeteners can be individually detected, such as aspartame which can be detected and quantified by spectrophotometric detector-conjugated RP-HPLC at a given wavelength (45). On the other hand, cyclamate cannot be detected by any spectrophotometric detector (e.g. UV), because there are no chromophores in the molecule without prior pre-treatment (59).

However, preferred techniques are those that allow simultaneous detection and quantification of various sweeteners. The reverse phase HPLC technique with UV detector allowed the separation and quantification of seven artificial sweeteners (ASP, SAC, CYC, ACS K, SUC, alitame and dulcin). The ELSD detector was used in one study to determine nine artificial sweeteners (ACS K, alitame, ASP, CYC, dulcin, neotame, NHDC, SAC and SUC) in various foods and the applicability of this method was proven in an interlaboratory study (60). In another study, HPLC technique combined with an MS detector it was possible to detect and quantify six artificial sweeteners (ACS K, SAC, SUC, CYC, ASP and dulcin) and three natural sweeteners (glycyrrhizic acid, stevioside and rebaudioside) in various foods (45).

Gas chromatography may also be used for the separation of artificial sweeteners. However, it is not widely used due to the low volatility of these compounds. As with sugars, artificial sweeteners must be converted into volatile compounds through derivatization which makes the technique time-consuming and inaccurate (45).

Non-destructive techniques such as Raman and infrared spectroscopy have also been applied in the analysis of various foods. However, because databases are required for fast and accurate results. That is why these techniques are the most promising for use in quality control laboratories (45).

4. AIM OF THE WORK

Based on the evaluation of free sugar content in soft drinks during the past few years, this work aimed to determine the type and amounts of free sugars and artificial sweeteners in the most consumed soft drinks by the Portuguese population. Also, it aimed to see how the tax on sugary drinks influenced the the sugars and artificial sweeteners profile.

Therefore, the data available in this dissertation was obtained by evaluating:

(i) the amounts of free sugars and artificial sweeteners present in soft drinks;

(ii) the position of the sugar-free quantities of soft drinks within the limits of the IEC, and

(iii) the results of the quantities of free sugars and artificial sweeteners obtained compared to the quantities present in soft drinks analysed in 2008.

EXPERIMENTAL

5. Materials and Methods

5.1. Standards and Reagents

Anhydrous D(+)-Glucose standard was purchased from Merck (Darmstadt, Germany) and D(-)-Fructose and Sucrose were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ribose, arabinose, melezitose, maltotriose and raffinose of diverse purity and suppliers were tested as possible internal standards (IS) for sugars analysis.

Sodium cyclamate (CYC), sodium saccharin di-hydrate (SAC) and sucralose (SUC) were a gift from previous collaborative work with sweeteners (60). Aspartame (ASP), acesulfame K (ACS K) and neohespiridin dihydrochalcone (NHDC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid was purchased from AppliChem Panreac (Spain) and triethylamine, used to adjust buffer's pH, was from Fisher Scientific (United Kingdom). Methanol was acquired Honeywell Riedel-de Haën^{TM} (Harvey St., MI, USA) and acetone was purchased from Fisher Scientific (United Kingdom).

Deionized water of 0.055 $\mu S.cm^{-1}$ was obtained with a Seralpur Pro 90CN purification system from Seral (Ransbach-Baumbach, Germany).

5.2. Sampling

Soft drinks (N = 68) were purchased in January 2019 on several local commercial places as detailed in Table 5. Among them, 10 contained only added sugars, 20 only intense sweeteners and 38 were composed by both added sugars and intense sweeteners. All samples were identified and grouped as "Colas" (N = 16), "Juice drinks" (N = 28), "Iced teas" (N = 13) and "Lemon-flavoured" soft drinks (N = 11). Also, sampling included brands (N=29) and own brand (N= 39) drinks based on market representativeness. At the laboratory, soft drinks were homogenized, and a 30 mL aliquot was collected into plastic flasks, degassed and stored at -18 °C until analysis.

Table 5: Samples classification.

Brand	Group	Added Sugars	Intense Sweeteners	Both (Sweetners + added sugars)	Total
Classical Brand	Colas	2	3	0	5
	Juice Drinks	5	2	12	19
	Iced Teas	1	0	2	3
	Lemon flavoured drinks	0	1	1	2
Own Brand	Colas	2	5	4	11
	Juice Drinks	0	3	6	9
	Iced Teas	0	4	6	10
	Lemon flavoured drinks	0	2	7	9
Total		**10**	**20**	**38**	**68**

5.3. Analytical Methodologies

5.3.1. Free Sugar analysis

5.3.1.1. Sample preparation

After defrosting, samples (500 µL) were transferred to 1.5 mL-centrifuge tubes and the IS (raffinose, 50 mg/mL, 100 µL) was added. After vortex-mixing (30 sec.), samples were centrifuged (13.000 rpm, 5 min.). An accurate portion of the supernatant (150 µL) was diluted up to 1 mL with deionized water in a second 1.5 mL-centrifuge tube and centrifuged again. Finally, the extract and stored at 4 ºC until analysis. Extracts were prepared in duplicate.

5.3.1.2. Standards Preparation

All standards (i.e., sucrose, fructose and glucose) were individually prepared at 200 mg/mL) in water. Six-level calibration curves (0 g/100 mL; 0.1 g/100 mL; 0.6 g/100 mL; 1.6 g/100 mL; 3.2 g/100 mL; 4.8 g/100 mL; 6.0 g/100 mL) were prepared using these aqueous stock solutions and further prepared for HPLC analysis as described above for samples (*5.3.1.1. Sample preparation).*

5.3.1.3. Chromatographic Analysis

Instrumental analysis was carried out in an HPLC system equipped with a quaternary pump (PU-1580, Jasco, Japan), coupled to a manual injector (Rheodyne, USA) with a 20 µL loop. Chromatographic separation was achieved with a SupelcoGel Ca^+ column for ion chromatography (300 x 7.8 mm; Supelco, Sigma-Aldrich, USA), at 85 ºC (oven, Model 7981, Jones Chromatography), in isocratic mode using deionized water as mobile phase at 0.5 mL/min. Detection was accomplished by a refractive index detector (RID) (Gilson).

5.3.2. Artificial Sweeteners Analysis

5.3.2.1. Sample preparation

Samples (500 µL) were diluted with methanol (500 µL), vortexed (30 sec.) and centrifuged (13,000 rpm, 5 min.). Then, formic acid buffer (500 µL, pH = 4.5) was added to an accurate portion of the supernatant (500 µL), vortexed and stored at 5 ºC until analysis. Samples without fruit pulp (500 µL) were directly diluted with buffer (1.5 mL), vortexed (30 sec.) and stored at 5 ºC until analysis. Extracts were prepared in triplicate.

The protocol used for the tentative isolation and concentration of sweeteners by SPE used 500 mg and 2 mg C_{18} SPE Bond Elut® (Varian, USA), and 1g end-capped C_{18} SPE columns from Telos®, United Kingdom), using a solid phase extractor device - Visiprep™ DL Supelco® (USA).

5.3.2.2. Standards Preparation

Each sweetener was individually prepared in methanol:water (50:50, V/V) at 200 mg/L. Four-level calibration curves (25 mg/L; 50 mg/L; 75 mg/L; 100 mg/L) were prepared for each individual sweetener using these stock solutions and further prepared for HPLC analysis as described in *5.3.2.1. Sample preparation*.

5.3.2.3. Chromatographic Analysis

Instrumental analysis was carried out in an HPLC system equipped with two pumps (PU-2080 plus, Jasco, Japan), coupled to an AS-2057 plus autosampler (Jasco, Japan) with a 20 µL loop. A reversed-phase C_{18} column (150 x 4.6 mm; 5 µm particle size, 110 Å pore size; Gemini, Phenomenex, USA), preceded by a C_{18} guard column from the same brand and supplier was used. Samples were eluted (0.5 mL/min) for 30 min, at room temperature, with a linear gradient from as detailed in Table 6. Detection was achieved by light scattering (ELSD) (Sedex 75, Sedere, France), operated at 40 ºC, at an air pressure of 2.5 bar and at gain of 12.

Collection and processing of chromatographic data in both chromatographic systems were performed with Borwin™ PDA Controller Software 1.50 (JMBS, France).

Table 6: HPLC gradient for the separation of artificial sweeteners.

Time (min)		0	4	11	23	24	26	36
Mobile Phase (% V/V/V) (Methanol:Buffer:Acetone)	**A** (69:24:7)	0	0	53	100	100	0	0
	B (11:82:7)	100	100	47	0	0	100	100

5.3.3. Quality Assurance/Quality Control

The reliability of the optimized procedures was assessed through multilevel calibration with six calibration levels for sugars and four calibration levels for sweeteners with well-distributed concentrations throughout the linear range. Calibration curves were generated by the least squares' linear regression model, plotting the peak area (or peak area ratios of target compound and their respective internal standard) *versus* the concentration or amount of each target substance.

The measurement of LOD and LOQ performed with real sample extracts was based on the standard deviation (multiplied by 3 or 10, respectively) of baseline noise divided by the slope of each regression equation.

Repeatability was assessed over duplicate injection of several samples and accuracy were assessed with recovery assays in two different concentration levels (three replicates).

5.3.4. Statistical Analysis

All statistical analyses were performed by SPSS software, version 24.0 (IBM Corporation, New York, USA) at 5 % significance level.

Normal distribution of the residuals and the homogeneity of variances were evaluated through the Shapiro-Wilk's test (sample size N < 50) and the Levene's test, respectively. If normal distribution was confirmed, all dependent variables were studied using a one-way ANOVA, subjected or not to Welch correction, depending if the requirement of the homogeneity of variances was verified or not. Furthermore, if a statistically significant effect was verified, post hoc tests, Tukey's or Dunnett's T3 test, were also applied for means comparison, depending if equal variances were assumed or not.

When normal distribution of residuals was not verified, analysis of variance was performed by Kruskal-Wallis test. Furthermore, if a statistically significant effect was verified, multiple pairwise comparisons were performed using Mann-Whitney's test.

6. Results and Discussion

6.1. Optimization of the analytical methodologies to quantify free sugars and artificial sweeteners

6.1.1. Sample preparation

6.1.1.1. Free Sugars

Initially, samples were prepared by adding to 500 µL sample in a centrifuge tube, 200 µL internal standard and methanol to a final volume of 2 mL (1:4 dilution). By adding methanol, a precipitation of interferences was attempted. This solution was centrifuged (13,000 rpm, 5 min.), and then 500 µL of supernatant was diluted with 500 µL of deionized water (1:2), in a final 1:8 dilution factor. However, it was understood that, in accordance with the HPLC column characteristics, the amount of methanol could be deteriorating the column (as it only supports up to 10 % methanol in the injected solution). Hence, for subsequent extractions, water was added instead of methanol, without any modification perceived in the chromatograms or column working pressure. Methanol was only used occasionally on samples with some turbidity but in lower proportions.

Several sugars were tested to be used as IS, including ribose, arabinose, melezitose, maltotriose and raffinose, due to their similar structures to the sugars analysed. However, raffinose was the chosen one due to its non-coeluting retention time with expected sugars and its absence in all samples.

6.1.1.2. Artificial sweeteners

The initial method tested was based on the one described by Wasik *et al.* (60). However, when testing diverse SPE columns, including the end-capped C_{18} column (1 g) indicated in the original procedure, it was found that the artificial sweeteners standards were being eluted in the column washing process, even when using buffered standards. In order to increase the interaction of the sweeteners with SPE column, the composition of the buffer solution was changed but pH was the same (4.5), using N,N-diisopropylethylamine (61), a tertiary amine, instead of TEA. N,N-diisopropylethylamine has higher aliphatic chains than TEA, which provides a greater interaction between sweeteners and sorbent, increasing the efficiency of the extraction of some sweeteners, like CYC and ACS K. However, even after this modification, sweeteners were still being leached during the SPE washing step, therefore not being adequately retained in the sorbent. Afterwards, the pH of the extraction buffer was reduced to 3.5, maintaining TEA. In this case, the losses were reduced with small peaks being detected in the HPLC system when compared with non-extracted standards.

Two other SPE C_{18} columns from different brands, one of 500 mg and another one of 2 g without end-capping, were further tested. In both columns, the same extraction procedure was used, though, for the former, the amounts of reagents and sample were reduced to keep the same proportion. It was found that the washed phase had no traces of sweeteners, however, in the eluted extract the concentration of sweeteners was lower than expected, which is indicative of retention in the SPE sorbent. After confirming that other sample ingredients, like caffeine, sugars and other additives did not coelute with any artificial sweetener standard, it was decided to inject the samples without the SPE cleaning and concentration step. As detailed in *5.3.2.1. Sample preparation* section, samples were either precipitated with methanol and further diluted with buffer (pH = 4.5) or were just diluted in the buffer.

6.1.2. Chromatographic conditions

6.1.2.1. Free Sugars

The separation principle of the chromatographic column used (ion interaction and size exclusion), together with the use of a RID, do not allow the use of gradient elution. Therefore, the retention times (RT) were closed to the ones expected from the column manufacturer and some co-elution might occur. Based on the list of RT provided by the manufacturer (available at www.sigmaaldrich.com), no co-elutions were expected. An example of standards and sample chromatograms is detailed in Figure 3.

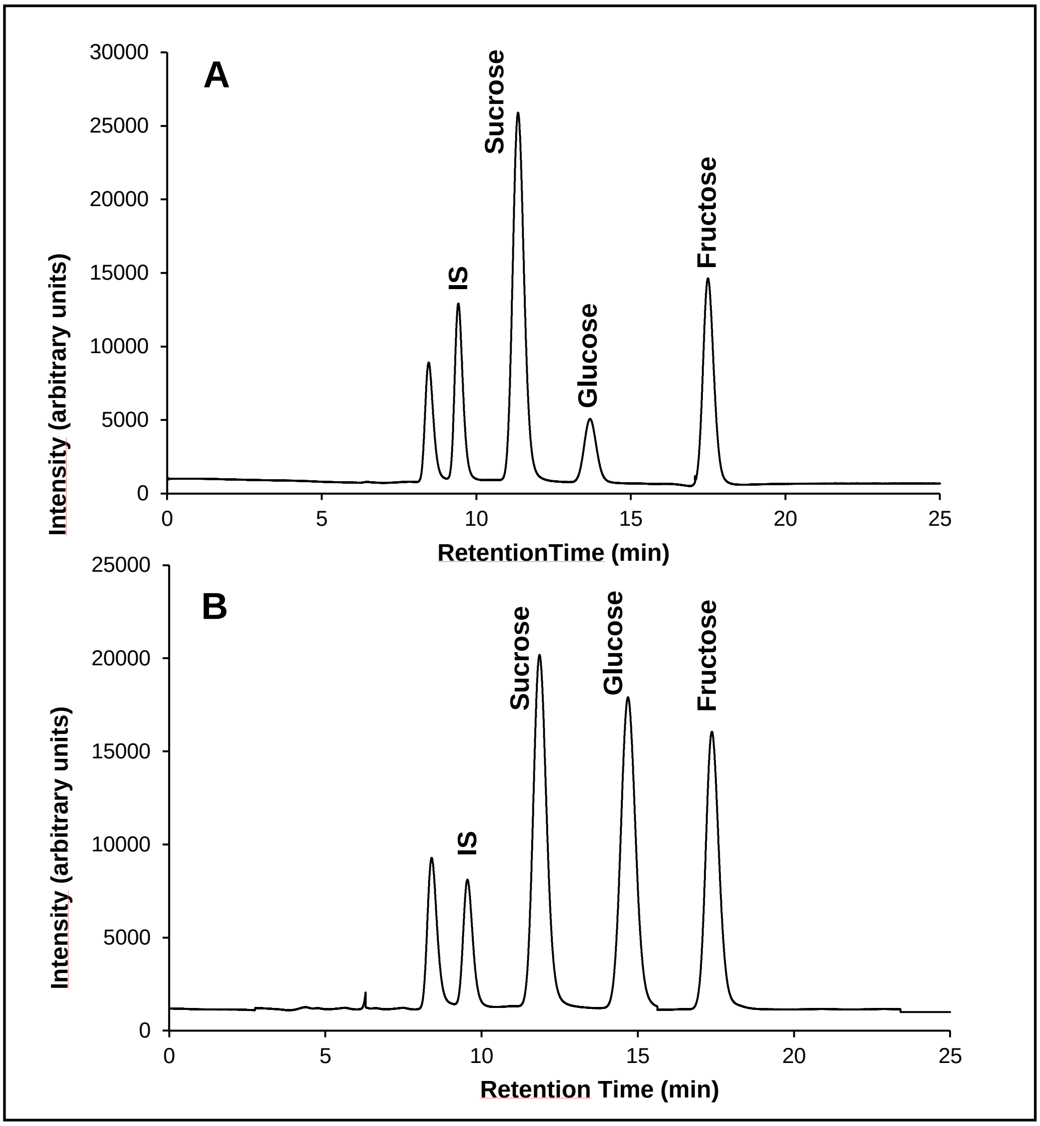

Figure 3: Examples of a standard (A) and a sample (B) chromatogram of free sugars.

6.1.2.2. *Artificial sweeteners*

Chromatographic conditions were also based on the method described by Wasik *et al.* (60), including the separation gradient, though the nebulization temperature, pressure and detector's gain were adjusted to maximize responses, particularly imposed by the absence of the concentration SPE step. After performing preliminary tests at several temperatures (30, 35 and 40 °C) combined with different pressures (1.5, 2.5, 3.5 bar) and gains (6, 10 and 12) (data not shown), it was found that the best separation conditions were obtained at a temperature of 40 °C, at a pressure 2.5 bar and with a gain of 12. Also, the injection volume of 20 µL was maintained for most samples as described by the method (60). An example of a standard and sample chromatogram is given in Figure 4.

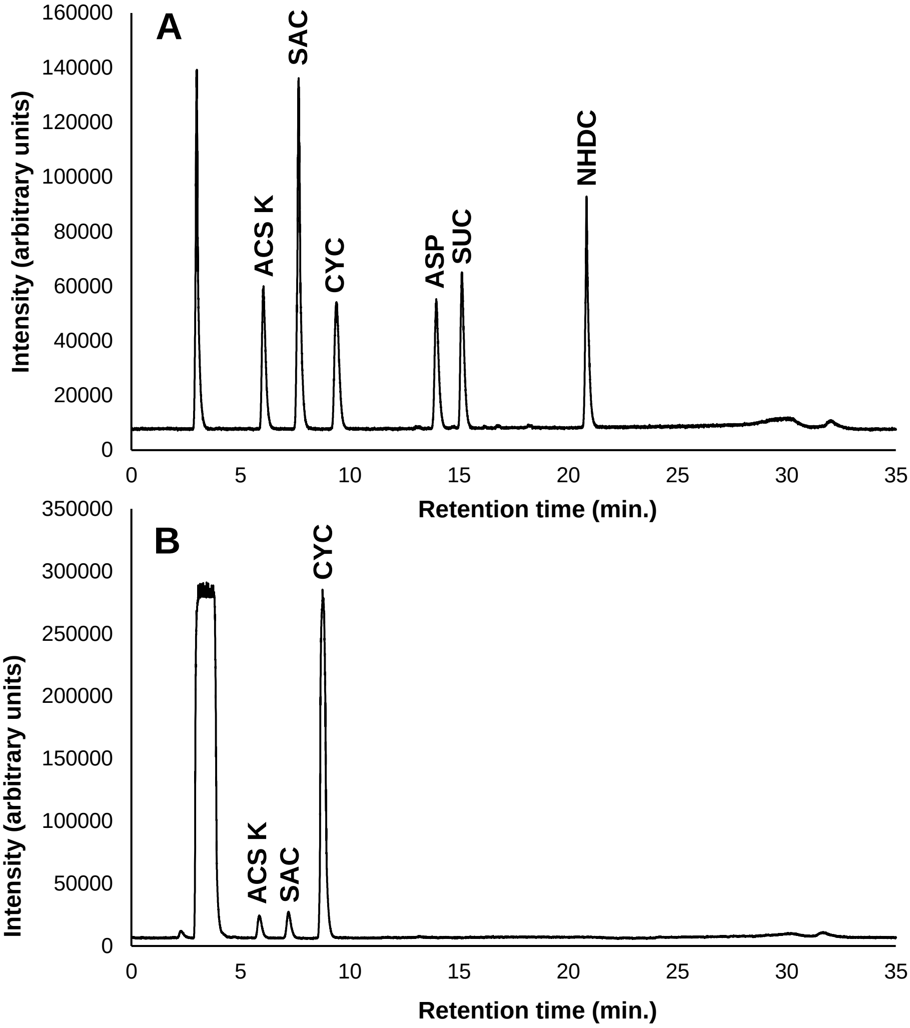

Figure 4: Examples of a standard (A) and a sample (B) chromatogram of artificial sweeteners.

6.2. Validation

Validation is required when developing an analytical method, adapting or implementing it to assess its efficiency in the laboratory so that it can be routinely reproduced. Owing to the changes made in the determination of sugars and sweeteners, it was necessary to evaluate certain analytical parameters to understand if these methods could be used for the same type of analysis in the laboratory. In this work, the parameters evaluated were linearity, working range, precision, accuracy and limits of detection and quantification.

6.2.1. Linearity and working range

Linearity corresponds to the ability of the analytical method to generate proportional results to the analyte concentration within a specific working range of the component under analysis (62).

In the case of sugars, different concentrations of standard solutions were prepared from the individual sucrose, glucose and fructose stock solutions. Standard solutions were initially prepared over a wider concentration range, but linearity was observed up to 30 to 35 mg of individual sugar per mL at the time of injection. Linearity was assessed using the correlation coefficients obtained from the calibration curve of the injected standard solutions (Figure 5). It is found that correlation coefficients in the three sugars were greater than 0.99, demonstrating that there was no large dispersion of results in the preparation of the samples.

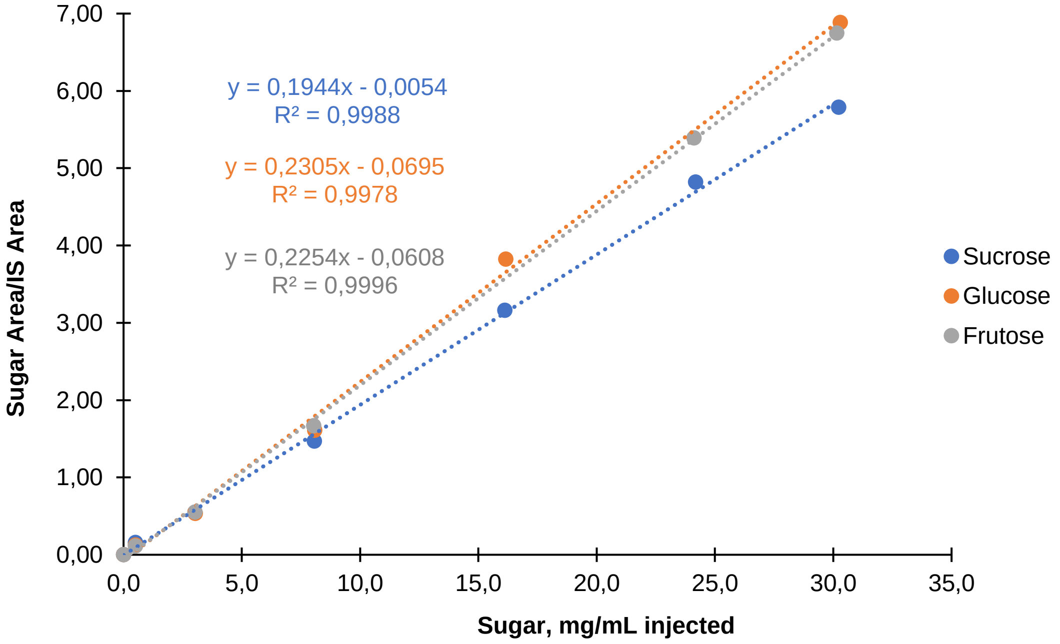

Figure 5: Example of free sugars calibration curve.

The concentration range for each of the standard artificial sweetener solutions was only tested between 25 and 100 mg/L, in accordance with the supporting reference (60). Figure 6 shows the $\log_{10}$ calibration curves of standard solutions of the six sweeteners analysed. Some sweeteners had correlation coefficients below 0.99, indicating that the dispersion of results is greater than expected. This may be due to the lack of an internal standard and the fact that the detector provided some area variations, probably derived from fluctuations in the air flow or correct dispersion of the nebulized eluent in the heating/detection chamber.

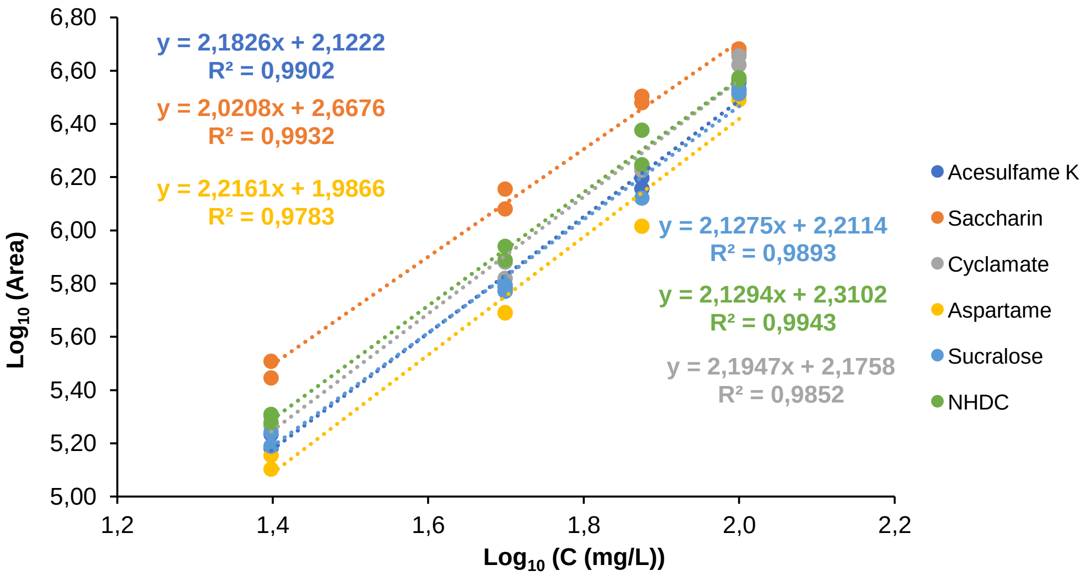

Figure 6: Example of artificial sweeteners calibration curve.

6.2.2. Limits of Detection and Quantification

The limit of detection (LOD) and quantification (LOQ) correspond to the lowest concentration of a given analyte that is detected or quantified, respectively, with acceptable accuracy and precision under the adopted experimental conditions (62). The LOD and LOQ obtained in the analysis of sugars and sweeteners are detailed in Table 7. In the case of free sugars, the concentrations obtained for LOD are lower than those obtained by Chinnici *et al.* (50). When comparing the detection and quantification limits obtained for the six sweeteners with those of the study by Wasik *et al.* it is verified that the values obtained in this work are similar (60).

Table 7: Working range, LOD and LOQ for free sugars and artificial sweeteners.

Analytes		Working range	LOD	LOQ
Sugars (g/100mL)	Sucrose	0.1-6	0.03	0.08
	Glucose		0.08	0.13
	Fructose		0.07	0.12
Artificial Sweeteners (mg/L)	Acesulfame K	25-100	14	23
	Saccharin		9	16
	Cyclamate		11	20
	Aspartame		11	19
	Sucralose		10	17
	NHDC		11	18

6.2.3. Repeatability and Accuracy

Repeatability is a parameter that evaluates the proximity between the various measurements made for the same sample. Repeatability was calculated from duplicate sample preparations. The results are presented in Table 8 and it can be verified that the obtained coefficients of variation (RSD) are satisfactory (below 2 %) for sugars and artificial sweeteners.

Accuracy is the correlation between the actual analyte value in the sample and the value estimated by the analytical procedure. This parameter can be assessed by: (i) using reference material; (ii) comparison with a reference method; (iii) recovery tests, and (iv) inter-laboratory testing. Accuracy allows expressing the systematic error that occurs in situations where there is sample loss in an extraction process, inaccurate volumetric measurements and the presence of interfering substances in the sample (62). In this work, accuracy was determined by recovery testing with two recovery levels tested for sugars (25 % and 50 %) and sweeteners (50 % and 100 %). The recovery test consists of fortifying a sample already containing the compounds under test with a certain volume of a solution of well-known concentration (standard solution) (62). In general, the recoveries were equivalent for sugars and sweeteners, all within the 96-99% range (Table 8).

Table 8: Repeatability (RSD) and average recovery (%) of free sugars and artificial sweeteners.

Analytes		Repeatability (RSD)	Recovery (average ± standard deviation)
Sugars	Sucrose	2	99 ± 4
	Glucose	2	97 ± 4
	Fructose	2	96 ± 3
Artificial Sweeteners	Acesulfame K	0	99 ± 2
	Saccharin	2	98 ± 3
	Cyclamate	1	99 ± 2
	Aspartame	1	97 ± 4
	Sucralose	1	97 ± 2

Based on the parameters evaluated here, it can be said that both techniques used for the determination of sugars and sweeteners have satisfactory precision and sensitivity and are adequate for the analytical purposes of the present work.

6.3. Free Sugars Analysis and Interpretation

6.3.1. Label information

The label is the first contact the consumer has with the product, so it is very important to know how to interpret it and how to make conscious and adequate choices. Each label has, among other mandatory information, the indication of the type of beverage, a nutritional table, where the free sugars are detailed separately, and a list of ingredients, from where the type of free sugars present in the beverage can be deduced.

Consumers are increasingly concerned about the product's ingredient list and the amounts of sugar, fat and salt added. Consumers prefer a shorter ingredient list because of the associated perception that a product is more "natural", with less addition of additives (19). In the case of soft drinks, consumers are mainly concerned with the presence or absence of sugars and the amount that they contain. Unfortunately, the opposite is also relevant, and several consumers do not take into consideration the amount of free sugars or caloric value, but mostly just the price, taste or even brand.

Therefore, each sample label was initially inspected from the total sugar’s amounts declared on the nutritional label, corresponding to total free sugars. From the 68 samples acquired, only 48 contained free sugars on the label, and therefore only these are discussed in this part. In general, a high variability was observed, varying from 0.8 g/100 mL to 11 g/100 mL (Annex I).

Figure 6 shows the boxplots of total free sugars declared in the nutritional labels of the analysed beverages, grouped by type of beverage. From the analysed groups it can be observed that "Colas" and "Juice drinks" were the groups with the larger dispersion of concentrations. In the "Colas" group there is a huge dispersion of total sugar values, with the largest amount of sugar being 10.6 g/100 mL. In the "Juice drinks" group, although total sugars ranged between 2 and 11 g/100 mL, most samples were grouped between 5-7 g/100 mL. In addition, it appears that for "Juice drinks" there are more isolated extreme cases, so the “whiskers” are wider, unlike the group of "Colas" that have a greater dispersion of values, but the samples are well distributed, that is why the “whiskers” are almost imperceptible. In opposition, the "Iced Teas" group, with 13 samples, was highly homogeneous, ranging from 4.5-5 g/100 mL with the exception of only one sample, with 7.8 g/100 mL that, despite being interpreted as an outlier, might be of relevance for consumers that have this beverage on a regular basis. The group of “Lemon flavoured drinks” behaved similarly to the group “Iced Teas”, but the amounts of total sugars are higher (7-8 g/100 mL), also with an outlier with less than half of the average sugar content of the group (2 g/100 mL). These samples include samples using sugars and those using sugars and artificial sweeteners, so this dispersion of sugars content is expected.

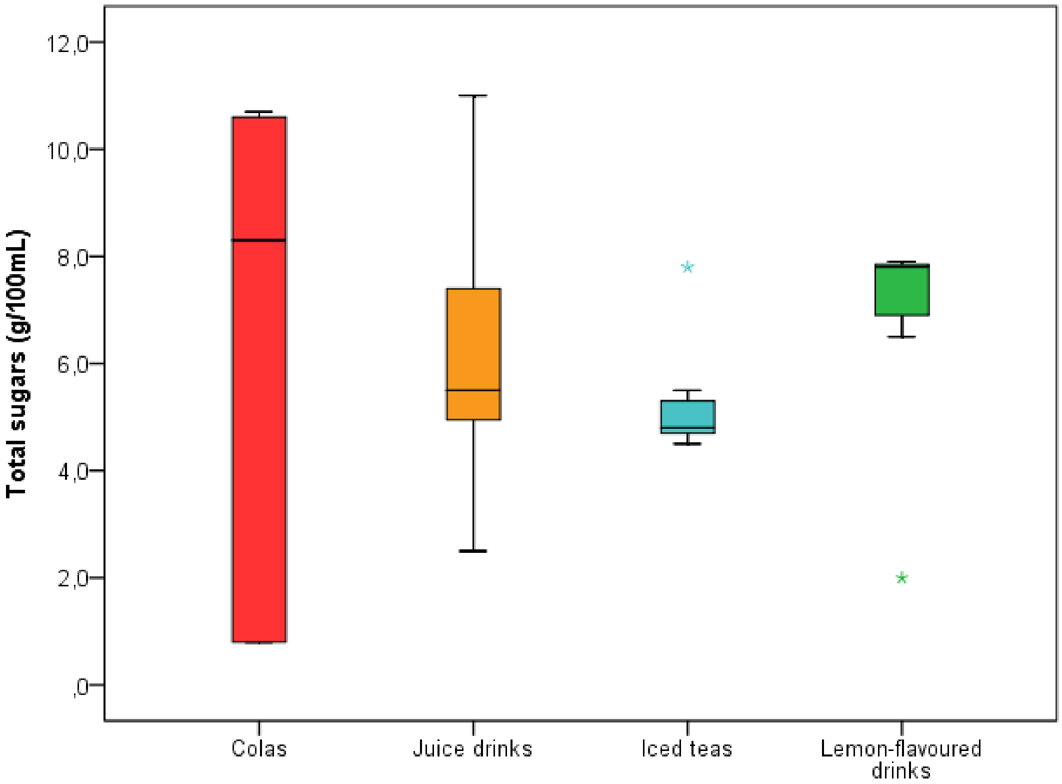

Figure 7: Boxplots with labelled “total sugar” amounts (g/100mL).

Each beverage group can be further divided into branded or own-brand beverages as shown in Figure 8. The number of branded and own branded drinks with added sugars is quite similar (Table 5) but it appears that each group has different concentrations of free total sugars when it comes to branded or own branded beverages, as shown in Figure 8. For example, the group branded “Colas” contained only two samples, and both have a concentration of around 10.6 g/100 mL, while the own-brand groups contained 6 samples, ranging from around 1 to 10.6 g/100 mL.

In the case of “Iced Teas”, brands had very dispersed values, while own brand beverages (Figure 7B) presented less dispersion of values but higher free total sugar concentrations. Indeed, except for the “Colas” group, own brand beverages have a smaller dispersion of values and a lower concentration range than branded ones. This might be an indication that despite being sold under different names, some of these beverage samples might indeed be prepared in the same industries.

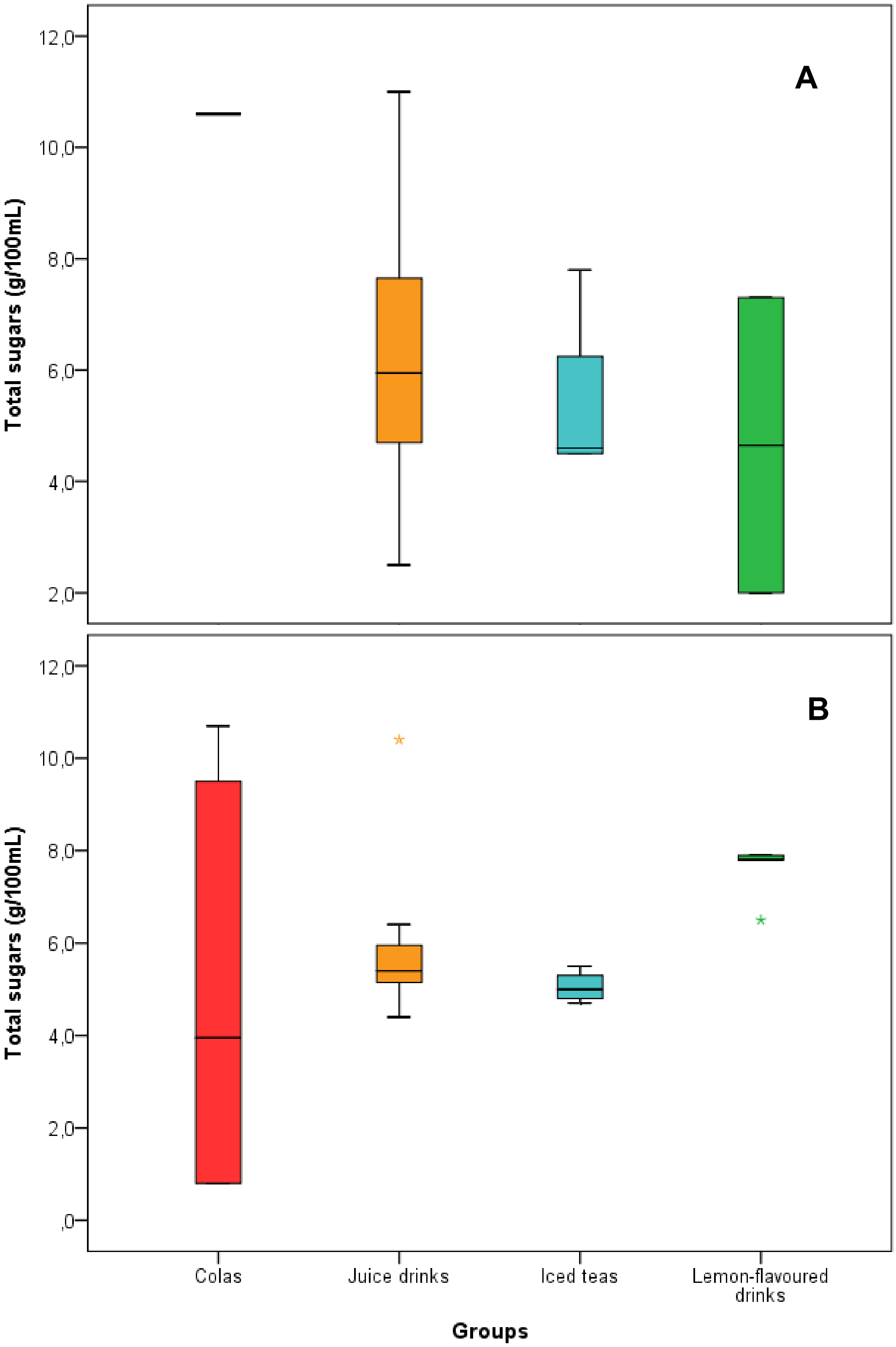

Figure 8: Boxplots with labelled “total sugars” values grouped by branded (A) and own brand (B) beverages.

As discussed in the introduction, the special tax on sugary drinks began to be applied in 2017 with the aim of reducing consumption and leading to a reformulation of this type of beverages by industries. After several market and consumption surveys (8, 16) the results were found to be very satisfactory. For this reason, in 2019 new levels were implemented for this tax, in order to see if these outcomes could further improve. However, for this work, beverages were purchased prior to the implementation of the new 2019 taxation levels and therefore should be interpreted on the basis of the 2017 tax, with 8 g/100 mL as reference. Figure 9 details all the 48 soft drinks examined for labelled total sugars.

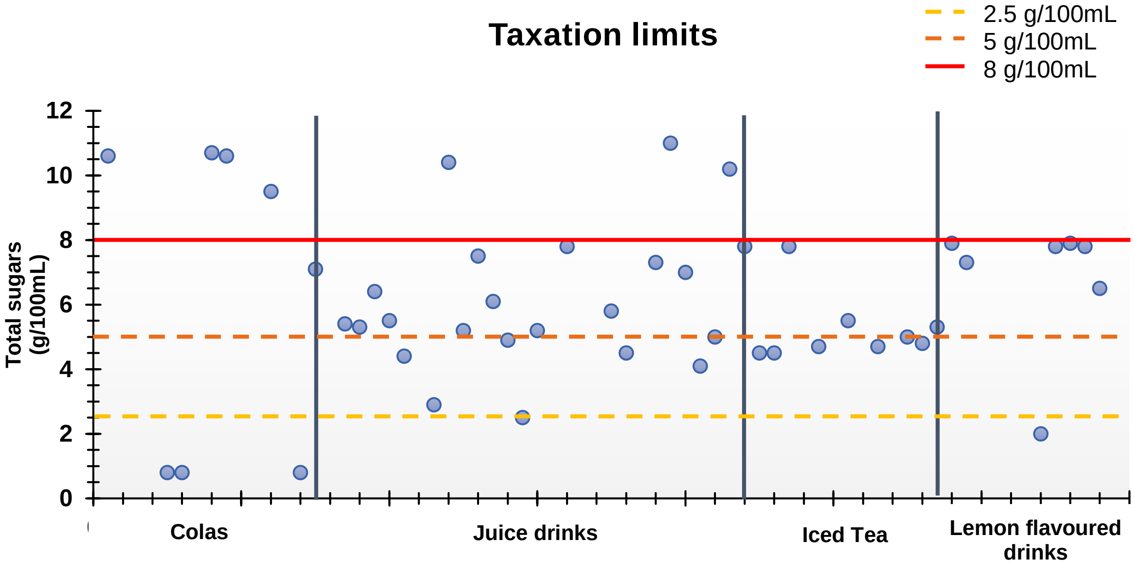

Figure 9: Results for the free sugars on the analysed beverages (g/100 mL).

Figure 9 shows that most beverages are below 8 g/100 mL, the 2017 taxation line, with only seven above this limit. It also shows that 7 samples are positioned exactly in the 8 g/100 mL line and other 5 sample just below it. The group "Colas" has simultaneously the higher and lower amounts of sugars compared to the other groups, with two clearly distinct patterns. The group "Juice drinks" includes all beverages prepared from juice concentrate and powdered or syrup preparations for further preparation. Within this group, there are also drinks of distinct flavours including orange, apple and pineapple. In this group, it is found that most drinks are below 8 g/100 mL, namely in the range 5-8 g/100 mL. In both the "Iced Teas" and "Lemon flavoured drinks" groups all drinks are below 8 g/100 mL with most drinks are found to be around 5 g/100 mL.

As the tax worked and led to a reformulation by the industries, it was decided to create more strict limits to lead to an even greater decrease in the amount of sugar in added sugar drinks. The dotted lines represent these new limits introduced in the legislation in 2019 (2.5 and 5.0 g/100 mL) and it is expected that drinks in limit zones or in larger quantities will change the formulations to a lower sugar amount. (Figure 9). The creation of two more limits may lead to further reformulations and to a decrease in the number of beverages present in the 5-8 g/100 mL concentration range, currently where most soft drinks are located. This does not mean that beverages will be less sweet, as the amount of sugar removed will be compensated by the addition of low or non-caloric sweeteners, such as artificial sweeteners and other additives, which provide the same sweet and pleasant flavour that consumer expects for that drink and that will be discussed ahead.

6.3.2. Individual sugars

In addition to the label information, it is important to understand the type of sugars consumed when drinking soft drinks and this information is not labelled. It can be partially deducted from the ingredients list, but not quantified. The main types of sugar added to soft drinks are sugar (sucrose) and glucose-fructose syrup. The list of ingredients for the analysed soft drinks is in Annex III and the type of added sugar is mentioned there.

Therefore, the individual amounts of sucrose, fructose and glucose present in the beverages with added sugar were analysed and their relative percentage and absolute amounts calculated as shown in Table 9.

Regarding the relative percentage, indicative of the sugar source, the "Colas" and "Juice drinks" groups are significantly different from the other groups ($p < 0.05$), since they contain higher amounts of glucose and fructose and lower amount of sucrose. The similarity between the amounts of glucose and fructose is a clear indication of the use of glucose-fructose syrup. The "Iced Teas" and "Lemon flavoured drinks" groups have the highest amounts of sucrose, as indicated in the ingredients list (Annex III) and smaller amounts of glucose and fructose, although no glucose-fructose syrup was added to any of the "Iced teas" and "Lemon flavoured drinks" groups (with one exception). This may be because they have natural aromas and fruit juices that naturally contain these sugars. In addition, sucrose when present in acidic beverages, such as soft drinks, can be converted to glucose and fructose, and this may also justify the presence of these sugars even if not directly added (63).

Table 9: Relative percentage and average concentration of sucrose, glucose and fructose in each of the beverage groups analysed with added sugars.

Groups	N	Fructose (%)	Glucose (%)	Sucrose (%)	Fructose (g/100 mL)	Glucose (g/100 mL)	Sucrose (g/100 mL)
Colas	8	42.4^a (17.1-45.8)	46.1^a (32.8-52.3)	9.8^a (5.5-43.6)	2.8 (0.3-5.8)	3.4 (0.4-6.3)	2.0^a (0-5.7)
Juice drinks	23	33.6^a (8.2-43.7)	37.0^b (7.1-51.5)	29.3^b (7.1-84.7)	2.0 (0.6-5.5)	2.1 (0.5-5.5)	2.7^a (0.3-7.3)
Iced Teas	9	15.9^b (10.5-18.1)	16.0^c (10.8-18.7)	68.1^c (64.2-78.6)	0.9 (0.5-1.2)	0.9 (0.5-1.2)	4.0^b (3.3-5.8)
Lemon flavoured drinks	7	12.3^b (1.9-39.0)	12.1^c (1.4-36.7)	75.6^c (24.3-96.6)	1.2 (0.2-3.0)	1.2 (0.1-2.8)	5.2^b (1.1-8.1)

Different letters in a column correspond to statistically significant (*p* <0.05) differences between medians.

The GI is a very important property related to carbohydrates in foods and beverages. It is associated with the digestion, absorption, metabolism and transformation into glucose and ultimately to the amounts and velocity that it achieves the bloodstream. Glucose is the sugar with the highest GI (100) (Table 3) and serves as a reference value for the remaining sugars. Sucrose has a GI of about 65, while fructose has the lowest index of the three types of sugars with about 20. As a consequence, EFSA has given a favourable scientific opinion about fructose as a substitute for sugars, like glucose and sucrose (28). Moreover, aware that fructose has a higher sweetening power (SP) than glucose and sucrose, it can be used in smaller amounts to attain the same sweetness intensity. Owing to this and its lower price, glucose-fructose syrup has been increasingly used in Europe in substitution of sucrose (63).

By calculating the Glycemic Load (GL) it is possible to understand the amounts of a certain carbohydrate in a portion of food (64). The formula for the calculation is as follows:

$$\text{Glycemic Load (GL)} = \frac{\text{GI}_{\text{Food}} \times \text{amount (g) of available carbohydrate}_{\text{Food}}\ \text{per serving}}{100}$$

A GL value greater than 20 is considered “high”, between 11-19 “intermediate” and “low” is less than 10 (64). For each of the beverage groups, whenever GL is calculated by the sum of the concentration of free sugars in a 330 mL can, all groups have values below 20. For instance, the “Colas” group have the highest GL (17), being inserted in the intermediate level of GL. “Iced Teas” have the lowest GL value of the three groups (12), followed by “Juice drinks” with a GL of 14, and “Lemon flavoured drinks” that presented a GL value of 16. The fact that "Colas” revealed a higher GL is due to the higher amount of glucose in comparison to the other groups. The "Iced teas" showed the lowest concentration of glucose and fructose, thus leading to a lower GL.

However, with the emergence of taxation in several countries, including Portugal, the amount of sugars added to sugary drinks has been decreasing but mostly at the expenses of added low calorie artificial sweeteners, with no GI value and high sweetening power. Conscious about the increase of NNS due to taxation of total sugars, the inexistence of detailed amounts in the food label as well as their potential health effects, the concentration of artificial sweeteners in beverages from the Portuguese market was determined.

6.4. Non-nutritive sweeteners

6.4.1. Label information

NNS are increasingly used to sweeten the most diverse types of foods. These are mainly used as an alternative to sugars in “light” foods or beverages to reduce caloric intake while preserving sweeteness (23). In the case of soft drinks, NNS are currently present in both sugar-free and sugar-added beverages, with a huge diversity of NNS being used, most frequently even more than one per beverage. This is confirmed by the information in figure 10, extracted from the ingredients list, which shows the NNSs present in the soft drinks ingredients list.

When the different beverage groups are analysed (Figure 10A) some patterns can be depicted, with some NNS or NNS combinations being more frequently used in a specific type of beverage. is found that there are non-nutritive sweeteners more frequently used by each type of drink. For example, in the “Colas” group, aspartame and acesulfame K are the most commonly mentioned, as found in the study by Lino *et al* (41) and all samples of this beverage group (Figure 10B) have both aspartame also acesulfame K, probably due to their synergistic effect between (63) and most have also cyclamate, with the exception of one that uses only sucralose.

In the “Juice drinks” group, the most common artificial sweeteners are sucralose and acesulfame K (Figure 10A), followed by aspartame and cyclamate, with steviol glycosides added to only one sample (Figure 10C). In this group, sucralose is often used alone, as its taste is not as unpleasant compared to the rest, revealing a similar taste to sucrose, apparently a more recent market trend, but is also used frequently in combination with acesulfame K. The “Iced Teas” are the group with the largest number of steviol glycoside samples (Figure 10D), whose presence is increasing in the market because it is a natural origin and is already one of the most used sweeteners in the world (63). Although the use of saccharin is decreasing, it is found that it is one of the most common artificial sweeteners in this type of beverages (63). Saccharin shares the most commonly used place with sucralose and acesulfame K (Figure 10A). In the “Lemon flavoured drinks” group, the most commonly used artificial sweetener is sucralose which appears as the only sweetener in several samples (Figure 10E). In addition, all beverages in this group have NNS and this is the only group where neo-hesperidin dihydrochalcone appears in one sample (Figure 10E). The samples with aspartame-acesulfame K and saccharin-cyclamate always appear together, probably due to the synergistic effects.

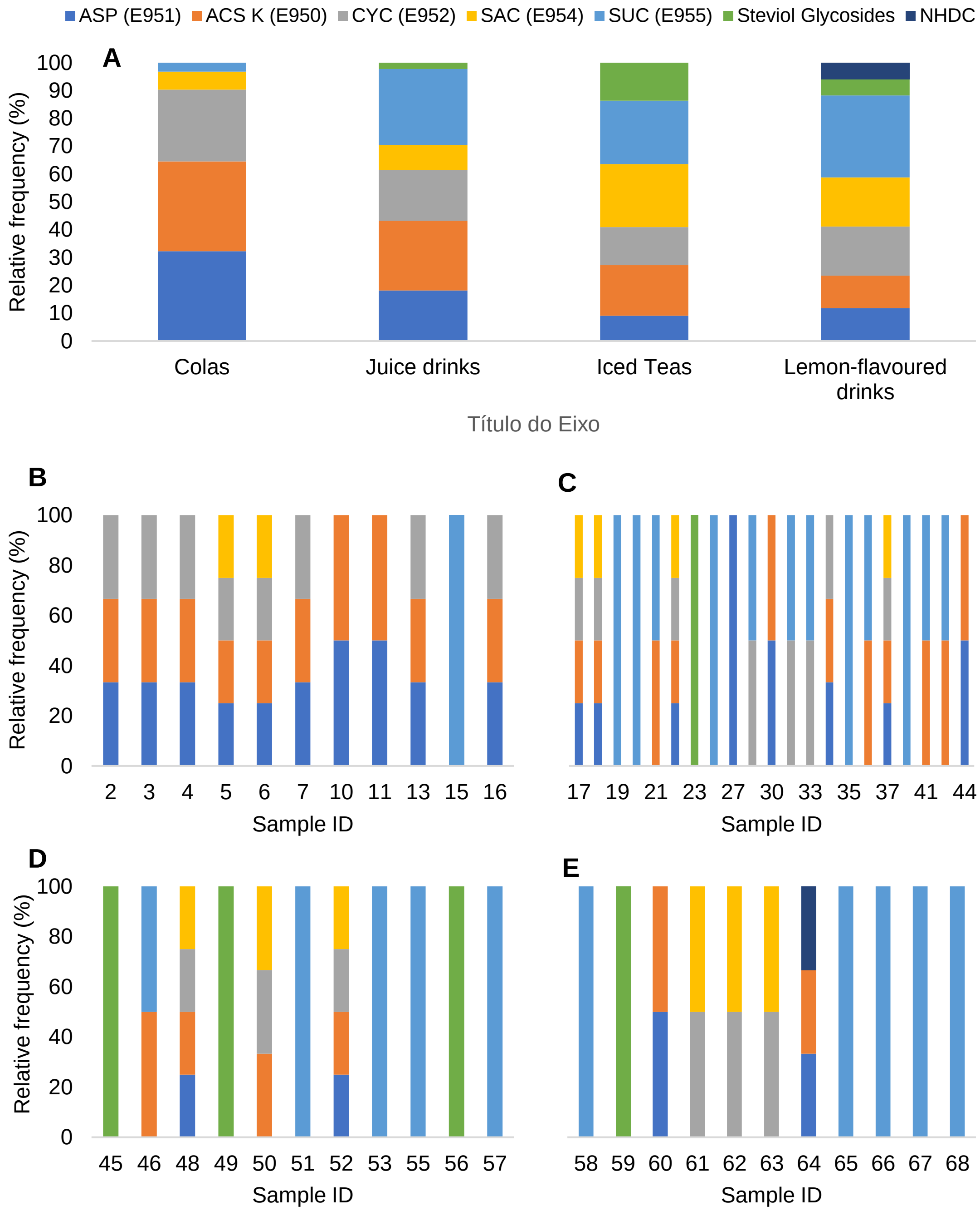

Figure 10: Relative frequency of use of each artificial sweetener per beverage group (A) and individually for Colas (B), Juice drinks (C), Iced Teas (D) and Lemon flavoured drinks (E).

Aware that each NNS has its own potency and, particularly, maximum limits of used (Table 4), it is also important to understand the amount of non-nutritive sweeteners consumed daily. Artificial sweeteners are still at the centre of some controversy, but they are also present in more and more foods as sugar substitutes. Through analytical methodologies it was possible to determine artificial sweeteners added to soft drinks, allowing to understand their mass amounts on marketed beverages.

6.4.2. Individual artificial sweeteners

The average results for each individual sweetener are presented in the table below (Table 10), again grouped by beverage type. Individual results can be consulted in Annex I. It is important to mention that none of the sweeteners analysed exceeded the limits of the imposed legislation (39).

Generally speaking, in most groups, the largest quantities of artificial sweeteners belong to aspartame, acesulfame K and cyclamate.

In "Colas" group, cyclamate is present in higher amounts, followed by acessulfame K and aspartame. Although saccharin was present in two drinks, it was not possible to quantify it as it was below LOQ.

In the "Juice drinks" group, all the artificial sweeteners analysed are present cyclamate amounts are more relevant, and in fact, when present, it is the most commonly found in beverages. This is because it is the least sweet sweetener (30-40) and therefore a larger amount of this compound are required. The amounts of acesulfame K and aspartame are similar, this is because they are usually found together and in similar amounts, perhaps due to the fact that the best synergistic effects between these compounds are in a 50:50 amount (63). In “Colas” group, cyclamate was significantly higher than the other NNS, and very similar to the concentration present in the “Juice drinks” group.

In "Iced Teas" group, the artificial sweetener that is in greater quantity is once again cyclamate. The remaining artificial sweeteners are in very similar amounts and relatively low relative to the other groups. In comparison with the former groups, “Ice teas” contain the lowest amounts of all sweeteners, except cyclamate that is similar to “Juice drinks” and sacharin that was absence also in the “Cola” group.

In “Lemon flavoured drinks” group it is found that the artificial sweetener present in much greater quantity than the others is cyclamate again, as in “Iced teas” however it is not the most used in this group. Sucralose is present in several samples, however its amount is much lower than that of cyclamate and this may be due to the fact that it is sweeter (Table 3).

Table 10: Average concentrations (mg/L) of aspartame, acesulfame K, cyclamate, saccharin and sucralose of each beverage groups.

Sweetener	Group	N	Average	Standard deviation
Colas	Aspartame	10	76	21
	Acesulfame K	10	98	6
	Cyclamate	8	203	4
	Saccharin	2	<LOQ	0
	Sucralose	1	(69)	
Juice drinks	Aspartame	8	45	11
	Acesulfame K	11	79	9
	Cyclamate	8	198	17
	Saccharin	4	53	6
	Sucralose	12	59	5
Iced Teas	Aspartame	2	<LOQ	3
	Acesulfame K	4	57	4
	Cyclamate	3	177	10
	Saccharin	3	47	3
	Sucralose	5	35	4
Lemon flavoured drinks	Aspartame	2	144	67
	Acesulfame K	2	97	13
	Cyclamate	3	249	2
	Saccharin	3	28	1
	Sucralose	5	62	5

As mentioned, none of the analysed beverages exceeded the maximum limits of the legislation, though artificial sweeteners have defined values of ADI. Table 11 shows a health risk assessment for the artificial sweeteners analysed for various age groups with an estimation based on the consumption of two cans of 330 mL soft drinks in one day, using the highest contents of each NNS found. The weights for each age group were taken from a reference table (65).

As mentioned, artificial sweeteners are increasingly present in various types of foods mostly due to sugar reduction. Thus, if two cans of soda are consumed, an 8-year-old might have ingested 72.5% of the ADI for cyclamate. Since other foods like yoghurt, cookies and breakfast cereals also have sweeteners in their composition, the ADI might be achieved. This NNS is of particular relevance since it is prohibited in some countries, including the USA. A high percentage of the ADI is also observed for sucralose, with 60%, and this NNS is being increasingly incorporated in foods and beverages due to its technological and sensorial properties. The situation is not so worrying when it comes teenagers or adults.

For the other NNS the amounts are relatively far from the ADI. Increasing the presence of these compounds in various types of food in concentrations unknown to the consumer might be a concern for the health of children.

Table 11: Maximum acceptable consumption per day and respective health risk assessment for each artificial sweetener in several age groups, based on the consumption of 330 mL soda cans with the highest NNSs content in the study.

	Weight (kg)	**Consumption per day** (mg/day)*					**Health Risk** (%)**				
		ASP (E951)	**ACS K** (E950)	**CYC** (E952)	**SAC** (E954)	**SUC** (E955)	**ASP** (E951)	**ACS K** (E950)	**CYC** (E952)	**SAC** (E954)	**SUC** (E955)
Child (4-8 years)	22	1100	330	242	110	110	14	29	73	41	60
Child (9-13 years)	40	2000	600	440	200	200	8	16	40	23	33
Teenagers (14-18 years)	60	3000	900	660	300	300	5	10	27	15	22
Adults (19-30 years)	70	3500	1050	770	350	350	4	9	23	13	19

*Calculated based on ADIs of 50,15,11, 5, 5 mg/kg per day for ASP, ACS K, CYC, SAC, SUC, respectively.
**Two cans of 330 mL soft drinks.

6.5. Sweeteners evolution over a decade (2008-2019)

With the emergence of the tax on sugary drinks in 2017 some industries reformulate the composition of soft drinks to reduce the amount of sugar, at expenses of other non-nutritive sweeteners. Since there was information available on the amount of total free sugars and artificial sweeteners prior from a study conducted at the Laboratory of Bromatology in 2008 (labels, percentage of free sugars and non-caloric sweeteners), with 34 common samples, a comparison was attempted aiming to understand the market evolution of these beverages.

Figure 10 details the data in 2008 and 2019, with samples grouped in accordance with trend of evolution observed. Figure 10 a) shows the samples where the amount of sugar was maintained over the last decade and b) those where a free sugar reduction was observed.

From the total of 34 matched samples, 8 (24%) kept an equivalent amount of free sugars. Moreover, some of these even added non-caloric sweeteners without reducing the free sugar amounts which seems senseless as it would increase the sweet taste intensity.

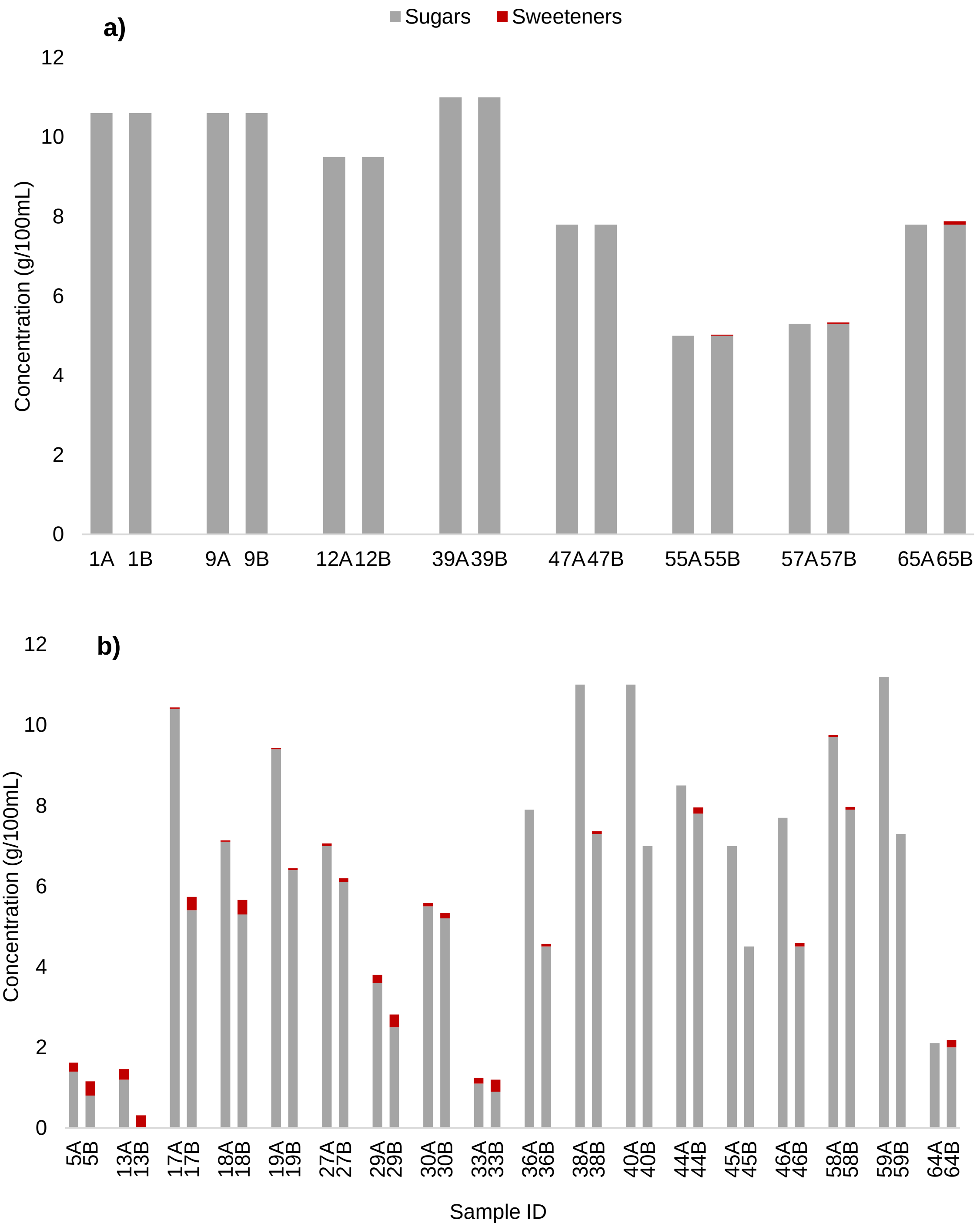

Figure 11: Total sugar content of soft drinks purchased in A) 2008 and B) 2019. a) Samples without total sugar content variation and b) Samples with total sugar content reduction.

On the other hand, in Figure 11 b), the majority of samples (N=18; 53%) reduced the amount of sugars, adding or increasing the amount of non-caloric sweeteners, and therefore effectively reducing their caloric value without reducing sweetness (Figure 11 b)). Three samples were exceptions, 40, 45 and 59, which reduced their sugar content without adding any non-nutritive sweetener (7-11 g/100 mL). In addition, it is curious that some of the samples reduced their sugars just to below 8 g/100mL, which was the only limit imposed at the time of the study. That being so, it can be confirmed that the tax on soft drinks really works.

For NNS, their prevalence increased and the amount maintained or increased, but this mass proportion is highly dependent on the type of sweeteners, as they have different potencies. Sample 13, for example, has removed all sugar from its composition and contains only artificial sweeteners.

In a separate group (Figure 12), there are also samples that were already ”added sugar-free” in 2008, preserving this classification, but with some variation in the profile of the intense sweeteners used and their amounts. Usually, all samples contain 2 to 3 different sweeteners. It turns out that in some samples (61, 62 and 63) the number of sweeteners indicated in the ingredients list has decreased, but the amount of one of them compensates the withdrawal. In addition, sample 4 decreased the amount of two sweeteners, but one increased (cyclamate). This figure confirms what was mentioned in the study conducted in 2008 (40) and those from within the laboratory taken as reference here, that the most commonly used artificial sweeteners in Portugal were acesulfame K, aspartame and cyclamate, a trend that seems to continue to these days (41)

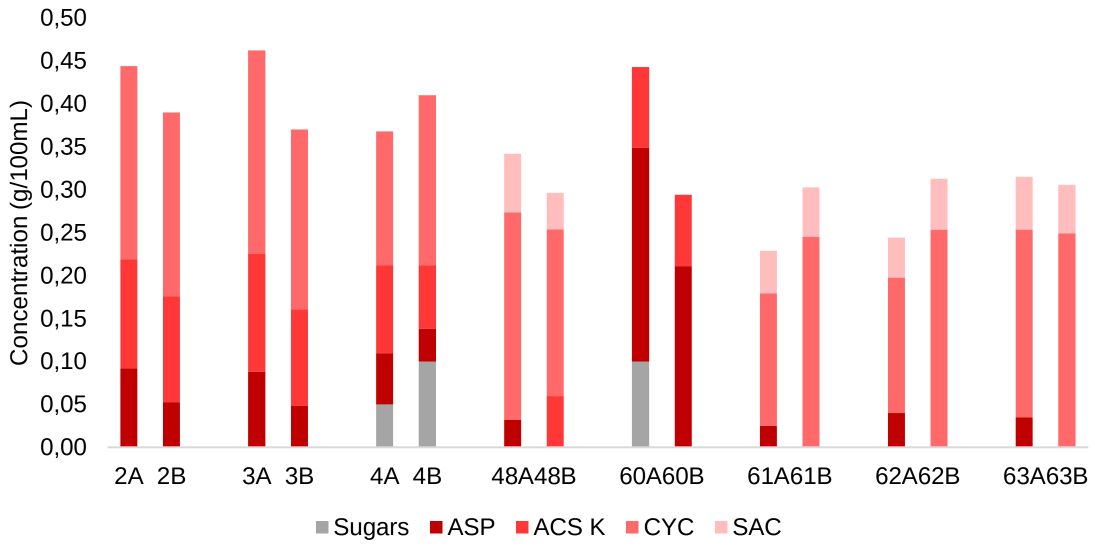

Figure 12: Type of artificial sweeteners present in some samples purchased in A) 2008 and B) 2019.

Whitin these samples, “colas “(2, 3, 4, 5, 6, 13) one sample preserved the exact same formulation, as it was an “original” brand product, while the others were already mostly sugar-free. All “Juice drinks” (17, 18, 19, 27, 29, 30 and 33) had their sugar amounts decreased at expenses of an increase in NNS. In the “Iced teas”, one sample (45) reduced its sugar content while other was already free from sugar and altered only slightly its NNS formulation (48).

In the “Lemon flavoured” beverages (58, 60, 61, 62 and 63) most were already sugar free with aspartame being removed from its formulations.

While the taxes on sugar impose their reduction, these changes could also be regarded as an oportunity to change habits, namely an adaptation to less sweet beverages. To understand if changes in the amount of sugar and NNS altered the sweetness intensity, we have estimated a “Sweetening Power” for some of the drinks using the following formula, being the results obtained presented in Table 12:

$$\text{Sweeteness Power (SP)} = \frac{\text{Sweeteness } \times \text{Concentration (g/100mL)}}{1000}$$

Table 12: “Sweetening power” of soft drinks from the Portuguese market.

Groups	Sample	Sweetening Power		
		2008	2019	Variation (%)
Colas	2	5.2	4.3	83
	3	5.3	3.9	74
	4	3.8	2.9	76
	5	3.2	3.8	119
	13	3.8	3.3	87
Juice drinks	17	2.3	4.6	200
	18	2.1	5.8	276
	19	1.6	3.4	213
	27	2.1	2.6	124
	29	1.1	4.3	391
	30	2.1	3.3	157
	33	2.4	3.9	163
Iced Teas	48	4.2	3.6	86
Lemon Flavoured drinks	58	3.3	4.7	142
	60	6.8	5.9	87
	61	3.0	3.2	107
	62	3.2	3.3	103
	63	3.9	3.1	79

From the results (Table 12) it can be seen that in the "Colas" group only one sample increased its sweetening power, a sample that decreased the amount of sugars and increased the amount of artificial sweeteners #5 (Figure 11 b)). Samples #2, #3 and #4 decreased their amount of sweeteners (Figure 12) and their sweetening power decreased (Table 12).

In the "Juice drinks" group all samples increased the sweetening power, this was because all of these samples decreased the amount of sugars in exchange for the increased amount of sweeteners (Figure 11 b)). The same situation happened in the groups "Iced Teas" and "Lemon flavored drinks". Samples #61 and #62, although not having added sugar, increased their amount of sweeteners (Figura 12) so their sweetening power also increased (Table 12). In sample 63 the opposite happened, as the sample decreases its amount of artificial sweeteners (Figure 12), its sweetening power decreased (Table 12). This proves that replacing sugars with artificial sweeteners in addition to the calorie benefits also adds more sweeteness to beverages.

7. General Conclusions

The survey presented in this dissertation focused on the most consumed soft drinks in Portugal (January 2019) and showed a reduction of the sugars amounts in comparison with those from 2008. This evidence clearly reveals an effectiveness of the sugar tax imposed in 2017, with most samples close or below the 8 g/100 mL limit. Moreover, it showed that the type and amount of sugars and NNS are highly dependent on the beverage type. Overall, the percentage of samples using NNS increased since 2008, as well as the amounts of additives in order to reformulate these products, which was highly perceivable in the "juice-based" group.

From a health safety perspective, the cumulative ingestion of some NNS, particularly cyclamate, can reach doses close to the ADI for younger children. The emergence of natural-based intense sweeteners, as steviol glycosides, is of particular interest in this regard, since they help to reduce the ingestion of artificial sweeteners. However, so far they have only been found in few samples, probably derived from its cost. Artificial sweeteners are not only present in soft drinks, but also in other foods such as yoghurt, cookies and breakfast cereals as an alternative to sugar. Expansion into a wide range of foods can present a long-term problem as these compounds are increasingly being consumed on a daily basis.

Several worldwide campaigns have been developed for sensitizing and educating consumers to ingest less salt, thus a similar trend should be followed for sugars and sweet taste, rather than looking for alternatives that may not be that healthy in the long run. These taxation measures should also be accompanied by educative measures, helping consumers to interpret the information on the food labels for more healthy and conscious choices. Unfortunately, most of these beverages are plain solutions of artificial sweeteners and flavours, without any nutritional value, contributing solely for an increased ingestion of these food additives. A concerted strategy to reduce the sweet intensity in beverages could bring more consistently long term effects, as a taste education, as well as a valorization of other healthier beverages.

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Annex I

Table 13: Individual results of sugars and artificial sweeteners analysed in 2019.

Group	**Sample**	**Label** (g/100mL)	**F** (%)	**G** (%)	**S** (%)	**ASP** (mg/L)	**ACS K** (mg/L)	**CYC** (mg/L)	**SAC** (mg/L)	**SUC** (mg/L)	**NHDC** (mg/L)
Colas	1	10.6	44	45	12	None					
	2	0	No added sugars			52	123	214			
	3	0				49	112	209			
	4	0.1				38	74	198			
	5	0.8	42	52	6	57	95	206	<LOQ		
	6	0.8	43	51	6	58	103	216	<LOQ		
	7	0	No added sugars			<19	94	204			
	8	10.7	43	44	13	None					
	9	10.6	17	42	41	None					
	10	0	No added sugars			231	73				
	11	0				171	85				
	12	9.5	46	47	7	None					
	13	0	No added sugars			48	88	178			
	14	0.8	41	51	8	None					
	15	7.1	24	33	44					69	
	16	0.01	No added sugars			36	125	201			
Juice Drinks	17	5.4	44	48	8	<LOD	69	216	51		
	18	5.3	43	48	9	31	63	195	68		
	19	6.4	41	52	7					48	
	20	5.5	39	50	11					75	
	21	4.4	42	51	7		142			56	
	22	0	No added sugars			<LOD	60	216	42		
	23	2.9	23	22	55	None					
	24	10.4	31	40	29	None					
	25	5.2	43	43	13					42	
	26	7.5	13	14	73	None					
	27	6.1	31	35	34	99					
	28	4.9	44	38	19	None					
	29	2.5	24	23	53			266		52	
	30	5.2	12	11	77	67	70				
	31	1.8	No added sugars					129		77	
	32	7.8	34	38	29	None					
	33	0.9	No added sugars					248		50	
	34	0.7				55	91	173			

(Continuation)

	35	5.8	32	34	35					99	
	36	4.5	38	38	24		32			31	
	37	0	No added sugars			12	61	145	51		
	38	7.3	8	7	85					65	
	39	11	37	37	27			None			
	40	7	21	21	58						
	41	4.1	37	11	52		89			47	
	42	5	41	46	14		94			71	
	43	10.2	9	9	82			None			
	44	7.8	18	17	65	63	94				
Iced Teas	45	4.5	17	16	67			None			
	46	4.5	11	11	79		51			32	
	47	7.8	15	15	71			None			
	48	0	No added sugars			<LOQ	60	194	42		
	49	4.7	12	12	76						
	50	0	No added sugars				50	161	53		
	51	5.5	16	17	67					38	
	52	0	No added sugars			18	66	175	47		
	53	4.7	18	18	64					46	
	54	0.2	No added sugars					None			
	55	5	14	14	72					24	
	56	4.8	17	19	64			None			
	57	5.3	16	16	68					34	
Lemon flavoured drinks	58	7.9	12	12	76					65	
	59	7.3	7	7	86			None			
	60	0	No added sugars			211	83				
	61	0						245	57		
	62	0						253	59		
	63	0						249	57		
	64	2	27	27	46	77	110				<18
	65	7.8	26	27	46					78	
	66	7.9	2	1	97					58	
	67	7.8	8	9	83					58	
	68	6.5	39	37	24					51	

Annex II

Table 14: Individual results of sugars and artificial sweeteners from 2008**.**

Group	Sample number	Label (g/100mL)	F (%)	G (%)	S (%)	ASP (mg/L)	ACS K (mg/L)	CYC (mg/L)	SAC (mg/L)	SUC (mg/L)
Colas	1	11						None		
	2	0		No added sugars		92	127	225		
	3	0				88	137	237		
	4	0				59	102	156		
	5	1	31	51	18	33		140	48	
	9	11				No information				
	12	10								
	13	1	30	52	17	50		158	53	
Juice Drinks	17	10	33	55	12				37	
	18	7	38	62					37	
	19	9	21	31	48				29	
	27	7	39	53	8	42			16	
	29	4	19	23	58			190	7	
	30	6	29	30	41	30	58			
	33	1	32	32	36	46		70	29	
	36	8						None		
	38	11						None		
	39	11						None		
	40	11						None		
	44	9				No information				
Iced Tea	45	7	30	31	39			None		
	46	8						None		
	47	8						None		
	48	0		No added sugars		32		242	68	
	55	5				No information				
	57	5								
Lemon flavoured drinks	58	10	12	12	76				58	
	59	11				No information				
	60	0	0	0	0	249	94			
	61	0	0	0	0	25		154	50	
	62	0	0	0	0	40		158	47	
	63	0	0	0	0	35		219	62	
	64	2				No information				
	65	8								

Annex III

Table 15: Ingredients list of beverages analysed in 2019.

Group	Sample number	Name	Ingredients
Colas	1	Plant extract Soft Drink	Water; **Sugar;** Carbon Dioxide; Caramel Dye (E150d); Acidifier (E338) and Natural Flavour (including Caffeine).
	2	Plant extract Soft Drink	Water; Carbon Dioxide; Caramel Colouring (E150d); **Sweeteners (Aspartame; Acesulfame K; Cyclamate)**; Acidifier (Phosphoric Acid); Natural Flavours (including Caffeine) and Acidity Regulator (Sodium Citrate).
	3	Plant extract Soft Drink	Water; Carbon Dioxide; Caramel Colour (E150d); **Sweeteners (Aspartame; Acesulfame K and Cyclamate)**; Acidifiers (E338); Flavourings and Acidity Regulator (E331).
	4	Refreshing Flavoured Drink	Carbonated Water; Dye (E150d); Acidifier (E338; E330); **Sweeteners (Aspartame; Cyclamate and Acesulfame K);** Preservative; Flavourings (including Caffeine).
	5	Refreshing Flavoured Drink	Carbonated Water; **Glucose-fructose Syrup;** Dye (E150d); Acidifier (E338); **Sweeteners (Cyclamate; Acesulfame K; Aspartame; Saccharin);** Flavourings (including Caffeine).
	6	Refreshing Flavoured Drink	Carbonated Water; **Glucose-fructose Syrup;** Dye (E150); Acidifier (E338); **Sweeteners (E952; E954; E951; E950);** Flavourings (including Caffeine) and Preservatives (E211).
	7	Zero Cola	Carbonated Water; Dye (E150d); Acidifier (E338); Acidity Regulator (E-331III); **Sweeteners (E952; E950; E951);** Preservative (E211) and Flavourings (including Caffeine).
	8	Refreshing Flavoured Drink	Carbonated Water; **Sugar;** Dye (E150d); Acidifiers (Orthophosphoric Acid; Citric Acid); Caffeine; Natural Aroma.
	9	Plant extract Soft Drink	Water; **Sugar;** Carbon Dioxide; Colouring (E150d); Acidity Regulator (Phosphoric Acid); Caffeine and Aroma.
	10	Refreshing Flavoured Drink	Carbonated Water; Dye (E150d); Acidity Regulator (Phosphoric Acid; Sodium Citrate and Citric Acid); **Sweeteners (Aspartame and Acesulfame K)**; Aromas (Caffeine; Extracts and Natural Flavours).
	11	Refreshing Flavoured Drink	Carbonated Water; Dye (E150d); Acidifiers: Orthophosphoric Acid; Citric Acid; **Sweeteners: Aspartame and Acesulfame K;** Acidity Regulator: Sodium Citrates; Preservative: Sodium Benzoate; Caffeine; Natural aroma.
	12	Carbonated plant extract Soft Drink	Carbonated Water; **Sugar**; Dye (Ammonium Sulphide Caramel); Acidity Regulator (Phosphoric Acid); Flavourings; Caffeine.
	13	Refreshing Flavoured Drink	Carbonated Water; Dye (Ammonium Sulphite Caramel); **Sweeteners (Sodium Cyclamate, Acesulfame K, Aspartame);** Acidifier (Phosphoric Acid); Acidity Regulator (Sodium Citrate); Caffeine Aroma and Natural Aroma.
	14	Carbonated Flavoured Soft Drink	Water; **Glucose-fructose Syrup;** Carbon Dioxide; Dye (E150d); Acidifier (E338); **Sweeteners (E952; E950; E951; E954);** Preservative (E211) and aroma (caffeine)
	15	Carbonated plant extract Soft Drink	Carbonated Water; **Sugar; Glucose-fructose Syrup;** Colouring (E150d); Acidifier (E338); Caffeine Flavour; **Sweetener (E955)** and Natural Flavour.
	16	Carbonated Flavoured Soft Drink	Carbonated Water; Dye (E150d); Acidifier (E338); Acidity Regulator (E331iii); **Sweeteners (E951; E950; E952);** Caffeine Aroma and Natural Aroma.

(continuation)

Juice Drinks	17	Orange Juice Soft Drink	Carbonated Water; Orange Juice obtained from a concentrated product (8%); **Glucose-fructose Syrup;** Acidifying (Citric Acid); **Sweeteners (E952; E950; E951; E954);** Flavourings; Stabilizers (E410; E440; E414 ; E445); Preservatives (E202); Antioxidant (Ascorbic Acid) and Dye (E160d).
	18	Orange Juice Soft Drink	Carbonated Water; Orange Juice obtained from a concentrated product (8%); **Glucose-fructose Syrup**; Acidifying (Citric Acid); **Sweeteners (Sodium Cyclamate; Saccharin Sodium salt; Acesulfame K and Aspartame);** Aroma; Stabilizers (E440; E410; E414; E445); Preservative (E202); Antioxidant (Ascorbic Acid) and Dye (E160a).
	19	Carbonated orange Juice Drink	Carbonated Water; Concentrated Orange Juice (10%); **Glucose-fructose Syrup;** Acidifying (Citric Acid); Stabilizers (Pectin; Gum Arabic; Locust Bean Gum and Glycerol Esters of Rosin); Flavours; Antioxidant (Ascorbic Acid); Preservative (Potassium Sorbate); Colouring (Beta-carotene) and **Sweetener (Sucralose).**
	20	Pineapple Juice Drink	Water; Concentrate-based Pineapple Juice (8%); **Glucose-fructose Syrup;** Acidifier (Citric Acid); Flavours; Stabilizers (Gum Arabic and Rosin Glycerol Esters); Preservatives (Potassium Sorbate and Dicarbonate Dimethyl); Antioxidant (Ascorbic Acid); **Sweetener (Sucralose)** and Dye (lutein).
	21	Pineapple Juice Drink	Water; Pineapple Juice obtained from a concentrated product (6%); **Glucose-fructose Syrup;** Acidifier (E330); Flavourings; Stabilizers (E414 and E445); Preservatives (E242 and E202); Antioxidant (E300); **Sweeteners (Acesulfame K and Sucralose)** and Dye (E161b).
	22	Pineapple Flavoured Soft Drink	Water; Concentrate Pineapple Juice; Acidity Regulator (Citric Acid); **Sweeteners (Cyclamate; saccharin; Acesulfame K and Aspartame);** Flavouring; Preservatives (Potassium Sorbate and Sodium Benzoate); Stabilizers (Arabic Gum and Glycerol Esters Rosin); Antioxidant (Ascorbic Acid) and Dye (Beta-carotene).
	23	Lemon Soft Drink	Water; Lemon Juice (7%): concentrate based; **Cane Sugar;** Citrus Pulp (0.7%); Natural Aromas and **Sweetener: Steviol Glycosides.**
	24	Fruit Juice Soft Drink	Water; **Sugar;** 6% Lemon Juice obtained from a concentrated product; **Glucose-fructose Syrup;** Acidifier: Citric Acid.
	25	Orange and Peach Drink concentrate	Water; Orange (19%) and Peach (6%) Juice from concentrate; **Sugar**; Acidity Regulator (Citric Acid; Potassium Citrate); Stabilizer (Guar Gum); Natural Flavourings; Preservative (Potassium Sorbate); Antioxidants (Sulphur Dioxide; Ascorbic Acid); **Sweetener (Sucralose)**; Colour (Beta-carotene).
	26	Refreshing Concentrated Orange Drink	**Sugar;** Concentrated Orange Juice (50%); Acidity Regulator (Citric Acid); Milk Flavourings; Stabilizers (Pectin; Cellulose Gum and Guar Gum); Vitamin C; Antioxidant (Sulfur Dioxide) ; Dyes (Beta-carotene and Beta-apo-8'-carotenal).
	27	Soft Drink with Orange Juice	Water; **Glucose-fructose Syrup; Sugar;** Concentrated Orange Juice (8%); Acidifier (Citric Acid); Flavour; **Sweetener (Aspartame)** and Colourings (E160a; E160e).
	28	Soft Drink with Apple Juice	Water; **Glucose-fructose Syrup**; Concentrated Apple Juice (16%); **Sugar;** Acidity Regulators (Malic Acid and Sodium Citrate); Aroma; Preservatives (E211; E202); Antioxidant (Ascorbic Acid); Dye (E150d); **Sweeteners (Aspartame and Acesulfame K).**
	29	Fruits Juice Soft Drink	Water; Orange Juice from concentrate (8.1%); **Sugar;** Acidifier (Citric Acid); Stabilizers (Gum Arabic and E445); Preservative (E202); **Sweeteners (E952 and E955)**; Flavourings; Vitamins C; Colourings (Carotenes; E160E).
	30	Orange Juice Soft Drink	Water; **Sugar;** Concentrated Orange Juice (10%); Acidity Regulator (Citric Acid); Flavourings; Antioxidant (Ascorbic Acid); Colouring (E160a) and **Sweeteners (Aspartame and Acesulfame K).**

(continuation)

31	Fruits Juice Soft Drink	Water; Apple Juice from concentrate (16%); Natural Flavours; Acidifiers: Citric Acid and Malic Acid; Barley Malt extract; **Sweeteners (E952 and E955).**
32	Fruits Juice Soft Drink	Water; Orange Juice from concentrate (10%); **Glucose-fructose Syrup; Sugar;** Acidifiers: Citric Acid; Natural Orange Flavour with other Natural Flavours; Stabilizer (Pectin); Colouring (Carotenes); Vitamin C.
33	Fruits Juice Soft Drink	Water; Orange Juice from concentrate (10%); Acidity Regulators (Citric Acid and Trisodium Citrate); Natural Flavours; **Sweeteners (E952 and E955)**; Stabilizer (Pectin); Colouring (Carotenes).
34	Orange Juice Soft Drink	Carbonated Water; 8% Orange Juice obtained from a concentrated product; Acidifying (Citric Acid); Acidity Regulator (Sodium Citrates); **Sweeteners (Cyclamate; Acesulfame K; Aspartame)**; Preservative (Potassium Sorbate); Natural Aroma Orange; Antioxidant (Ascorbic Acid); Stabilizers (Pectins; Locust Bean Gum); Dye (Beta-carotene).
35	Orange Juice Soft Drink	Water; **Glucose-fructose Syrup; Sugar**; Concentrated Orange Juice (8%); Carbon Dioxide; Acidifier: Citric Acid; Antioxidant: Ascorbic Acid; Aroma; Stabilizers: E440; E410; Dye: E160a
36	Refreshing Flavoured Drink	Water; **Sugar;** Acidifiers: Citric Acid and Malic Acid; Flavour enhancers: Sodium Chloride; Potassium Phosphate and Calcium Phosphate; Acid Regulator: Sodium Citrate; Antioxidant: Ascorbic Acid; Stabilizers: E414 and E445; **Sweeteners: Sucralose and Acesulfame K**; Natural Aromas of Citrus and Carotene Dye.
37	Refreshing Flavoured Drink	Water; Orange Juice obtained from a concentrated product (0.45%); Acidifier (E330); **Sweeteners (E952; E954; E950; E951);** Stabilizers (E414; E 445); Preservatives (E202; E211); Antioxidant (E300); Flavourings and Dyes (E160aii; E160e).
38	Orange Juice Drink	Water and Carbon Dioxide; Orange Juice and Pulp from concentrate; **Sugar;** Flavouring; Acidity Regulators (Citric Acid; Malic Acid and Sodium Citrate); Antioxidant (Ascorbic Acid) and **Sweetener (Sucralose).**
39	Soft Drink	Water; **Sugar;** Carbon Dioxide; Concentrated Orange Juice (3%); Acidifier (Citric Acid); Preservatives (E202 and E211); Antioxidant (Ascorbic Acid); Stabilizers (E414; E410 and E445); Aroma; Dye (E160e).
40	Soft Drink	Water; **Sugar;** Carbon Dioxide; Concentrated Orange Juice (3%); Acidifier (Citric Acid); Preservatives (E202 and E211); **Sweeteners (Cyclamate; Saccharin; Acesulfame-K and Aspartame);** Antioxidant (Ascorbic Acid); Stabilizers (E414; E410 and E445); aroma; Dye (E160e)
41	Soft Drink with fruit Juice and carrot Juice	Water; Fruit and Vegetables Juices from concentrates (15%) (Orange (5.5%); Carrot (4%); Lemon; Apple); **Sugar; Fructose;** Vitamins C; E and Provitamin A (Beta-carotene); Acidity Regulator: Citric Acid; Preservative: Potassium Sorbate; **Sweeteners: Acesulfame K and Sucralose**; Flavours.
42	Soft Drink tropical and carrot	Water; Juice from concentrates (17%) (Orange (11.8%); Carrot (3.1%); Pineapple (1.3%); Passion Fruit (0.8%); **Glucose-fructose Syrup;** Acidifying (E330); Vitamins (Provitamin A (Beta-carotene); C; E); Antioxidant (E300); Stabilizer (E440; E410); Flavours; Preservatives (E242); E202); **Sweeteners (E950; E955).**
43	Soft Drink with Pineapple Juice	Water; **Sugar;** 5% Concentrated Pineapple Juice; Carbon Dioxide; Acidity Regulators (E330 and E331); Modified Starch; Preservative (E202); Stabilizers (E444 and E445); Antioxidant (Ascorbic Acid) and Dye (E160b).
44	Orange Juice Soft Drink	Water; 8% Orange Juice from concentrate**; Sugar;** Carbon Dioxide; Acidifiers (Citric Acid and Malic Acid); Stabilizers (E414; E444; E445); Preservative (E202); **Sweeteners (E950 and Aspartame);** Antioxidant (Ascorbic Acid); Natural Orange and other Natural Flavourings and Colouring (Beta-carotene).

(continuation)

Iced Tea	45	Soft Drink Tea Extract with Peach Juice	Tea: Water and Black Tea Extract (4.7%); **Sugar;** Acidity Regulator (Citric Acid; Sodium Citrate); Concentrated Peach Juice (0.1%); Flavours; Antioxidant (Ascorbic Acid); **Sweetener (Steviol Glycosides).**
	46	Refreshing Beverage of Extracts	Water; **Sugar**; Acidity Regulators (Citric Acid and Sodium Citrate); Tea Extract; Concentrated Lemon Juice (0.1%); Antioxidant (Ascorbic Acid); **Sweeteners (Acesulfame-K and Sucralose)** and Natural Flavours.
	47	Refreshing Beverage of extracts	Water; **Sugar;** Acidity Regulators (E330 and E331); Concentrated Peach Juice (0.1%); Tea Extract; Natural Flavours and Antioxidant (E330).
	48	Refreshing Flavoured Drink	Water; Acidifier (E330); Acidity Regulator (E331iii); **Sweeteners (E952; E954; E950; E951)**; Tea and Peach Flavourings; Preservatives; (E202 and E211); Colouring (E150d).
	49	Refreshing Beverage of Extracts	Water; **Sugar;** 0.12% Tea Extract; Acidifier (Citric Acid); 0.1% Lemon Juice obtained from a concentrated product; Natural Aroma; Acidity Regulator (Sodium Citrates); Antioxidant (Ascorbic Acid) ; **Sweetener (Steviol Glycosides).**
	50	Refreshing Beverage of Extracts	Water; Acidifier (Citric Acid); 1% Lemon Juice obtained from a concentrated product; 0.12% Tea Extract; Flavourings; Acidity Regulator (Sodium Citrates); **Sweetener (Sodium Cyclamate; Sodium Saccharin; Acesulfame-K).**
	51	Soft Drink Tea Extract with Peach Flavour	Water**; Sugar;** Concentrated Peach Juice (0.25%); Acidifier (Citric Acid); Tea Extract (0.12%); Flavour; **Sweetener (Sucralose)** and Acidity Regulator (trisodium citrate).
	52	Tea and Peach Flavoured Soft Drink	Water; Acidifier (E330); Acidity Regulator (E331iii); **Sweeteners (Cyclamate; Acesulfame K; Aspartame and Saccharin)**; Tea and Peach Flavourings; Preservatives (Sodium Benzoate and Potassium Sorbate) and Colouring (Ammonium Sulfite Caramel).
	53	Plant extract Soft Drink	Water; **Sugar;** Acidity Regulators (Citric Acid and sodium citrate); Flavourings; Lemon Juice (0.3%) (concentrate based); Tea Extract (0.05%); Colouring (E150d); **Sweetener (E955).**
	54	Soft Drink Tea Extract with Lemon Juice	Water; Lemon Juice from concentrate (1%); Acidifier (E330); Tea Extract (0.14%); Flavourings; Acidity Regulator (E331iii) and **Sweeteners (E950; E951).**
	55	Soft Drink Tea Extract with Lemon Juice	Water; **Sugar;** Lemon Juice from concentrate (1%); Acidifier (E330); Tea Extract (0.12%); Flavouring; Acidity Regulator (E331iii) and **Sweeteners (E955).**
	56	Soft Drink Tea Extract with Lemon Juice	Water; Sugar; **Glucose-fructose Syrup;** Acidifier (Citric Acid); Antioxidant (Ascorbic Acid); extracts (0.12%); Concentrated Lemon Juice (0.1%); Lemon Flavour with other Natural Flavours; **Sweetener (Steviol Glycosides).**
	57	Soft Drink Tea Extract with Peach Juice	Water**; Sugar;** Acidity Regulators (Citric Acid; trisodium citrate); Concentrate-based Peach Juice (0.25%); Tea Extract (0.1%); Flavour; **Sweetener (Sucralose).**

(continuation)

Lemon Flavoured Drinks	58	Carbonated Flavoured Soft Drink	Carbonated Water; **Sugar**; Acidifiers (E330; E296); Flavourings; Acidity Regulator (E331iii); Preservative (E202) and **Sweetener (Sucralose).**
	59	Soft Drink with Natural Lemon and Lime Flavours	Carbonated Water; **Sugar;** Acidifiers (Citric Acid and Malic Acid); Natural Lemon and Lime Flavours; Acidity Regulator (Sodium Citrate); **Sweetener (steviol glycosides).**
	60	Soft Drink with Natural Lemon and Lime Flavours	Carbonated Water; Acidity Regulators (Citric Acid; Malic Acid and Sodium Citrate); Natural Lemon Lime Flavours; **Sweeteners (Aspartame and Acesulfame K)**; Preservative (E211).
	61	Carbonated Flavoured Soft Drink	Carbonated Water; Acidifier (E330); **Sweeteners (E952; E954)** and Flavourings.
	62	Flavoured Soft Drink	Carbonic Water; Acidifying: Citric Acid; **Sweeteners: sodium Cyclamate; Saccharin; aromas.**
	63	Carbonated Soft Drink	Carbonated Water; Acidulant (E330); **Sweeteners (E952 and E954)** and Lemon Flavour.
	64	Soft Drink with Natural Lemon and Lime Flavours	Water; **Sugar;** Carbon Dioxide; Acidity Regulators (Citric Acid and Sodium Citrate); **Sweeteners (Acesulfame K; Aspartame and Neo-hesperidin DC)** and Natural Lemon and Lime Flavours.
	65	Carbonated Lemon Extract Flavoured Soft Drink	Carbonated Water; **Sugar (7.6%)**; Acidity Regulators (Citric Acid; Sodium Citrate; Malic Acid); Flavour; **Sweetener (Sucralose).**
	66	Lemon and Lime Soft Drink	Carbonated Water; **Sugar**; Acidifier (E330; E296); Acidity Regulator (E331 iii); Flavour; Preservative (E202) and **Sweetener (E955).**
	67	Carbonated lemon Lime Flavoured Soft Drink	Carbonated Water; **Sugar;** Acidifier: Citric Acid; Natural Lemon-lime Flavouring and other Natural Flavourings; Acidity Regulator: Sodium Citrates; **Sweetener: Sucralose**; Antioxidant: Ascorbic Acid.
	68	Flavoured Soft Drink	Carbonated Water; **Sugar;** Acidifiers (E330; E296); Flavourings; Acidity Regulator (E331); Preservative (E202); **Sweetener (E955).**