ARTIFICIAL SWEETENERS IN VARIOUS FOOD PRODUCTS - QUANTIFICATION AND INTAKE ASSESSMENT

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ABSTRACT

The aim of the study is to determine the content of artificial sweeteners in 133 different food products. The analytical quantification was performed by the application of a reversed-phase, validated, gradient HPLC method, with simultaneous determination of aspartame, acesulfame K and Na-saccharin content. Supelcosil 150 x 4.6 mm, 5μm was used as the stationary phase and mobile phase (buffer 0.1 M NaH_2PO_4, pH = 2.5 and acetonitrile), at temperature = 26 °C and flow = 1.5 mL/min. The method was applied on: non-alcoholic beverages, chewing gums, sweets, chocolates, confectionery, dietary products, food supplements, fruit yogurt, etc. Depending on the structure of the food, different extraction methods were applied. The assessment of food safety showed irregularity in 21 % of the products, of which 50 % were imported. Some of the products` declarations were not completely translated nor properly labelled. Of the irregular domestic products, 21 % exceeded the maximum permitted quantities of added sweeteners and the rest had irregular labelling. The analysis showed that the most commonly used sweetener was Na-saccharin, which along with aspartame and acesulfame K are most frequently utilized in non-alcoholic beverages. Intake of artificial sweeteners was estimated according to the mean body weight for children, adolescents and adults, under the supposition that the products contained the maximum permitted quantities. The theoretical maximum daily intake of different food products in different population groups indicates the existence of a potential health risk only with continuous "large" intake of products that contain the maximum allowable amounts of artificial sweeteners, especially for the youngest populations.

Keywords: ADI, acesulfame K, aspartame, HPLC method, Na-saccharin.

INTRODUCTION

Food additives are substances added intentionally to foods to perform certain technological functions, for example for color, to sweeten, or to preserve. Artificial sweeteners are a class of food additives that provide sweet taste without increasing the caloric intake (Ebrahimzadeh et al., 2018). They are generally considered as substances which on a weight basis are substantially sweeter than the common carbohydrate sweeteners such as sucrose. Their sweetness intensities range from about 30 times to several thousand times that of a sucrose. Accordingly, they can be used at much lower concentrations in foods, giving a lower energy value to the final product (RAS, 2003). Sweeteners can be found in almost all products, including soft drinks and table-top sweeteners, dairy products, such as yoghurt and ice cream, desserts, chewing gums and sweets, condiments such as salad dressings, mustards and sauces, and many other products (Zygler et. al., 2011). Artificial sweeteners are synthesized in laboratories and these are also called non-nutritive sweeteners, high-intensity sweeteners, and low caloric sweeteners (Ebrahimzadeh et al., 2018). Many national health organizations have implemented public health initiatives for sugar reduction (Popkin & Hawkes, 2016). Many manufacturers therefore offer beverages with low and no-calorie sweeteners (Hafner et. al., 2021). The most common artificial sweeteners used in food products, which are found on Macedonian market, are: aspartame (E951), Na-saccharin (E954) and acesulfame K (E950). It is common to use several sweeteners in combination to provide a better taste to food and drinks (NSCFS, 2014). Chemically, aspartame is N-L-α-aspartyl-L-phenylalanine methyl ester and is a white, crystalline powder substance without odor. It is found as an ingredient in about 6,000 food products and beverages consumed worldwide. Aspartame is about 180 times sweeter than sugar in typical concentration and has a caloric value of 4 kilocalories (17 kilojoules) per gram. It has an ability to prolong and intensify the taste, and increases the aroma of the food. The aspartame is not used in food that is exposed to thermal processing, cooking, sterilization, etc., because its degradation under heating and loss of its sweetness. According to International and national regulations, it is mandatory for the aspartame to be labelled on the food products. People with the genetic disorder phenylketonuria (PKU) must watch out for aspartame intake as an additional source of phenylalanine, so foods containing aspartame must be labelled as containing a source of phenylalanine (Nollet, 2000; Magnuson et al., 2007). Chemically, acesulfame K is the potassium salt of 6-methyl-1,2,3-oxathiazine-4(3X)-one-2,2-dioxide and is a white crystalline, powdery substance. Acesulfame K is 125-250 times sweeter than 3 % sucrose solution. At higher concentrations, the relative sweetness decreases. It has a slightly bitter aftertaste, especially in high concentrations (Nollet, 2000). Acesulfame K is stable at high temperatures and under moderately acidic or basic conditions, which allows its use in baking and in products that have a longer period of use. In carbonated soft drinks, acesulfame K is almost always used in combination with another sweetener, such as aspartame or sucralose (Lawrence, 2003). Chemically, saccharin is 2,3-dihydro-3-iobenzisosulfonazole, and its salts Ca saccharin and Na-saccharin are also known. Na-saccharin has an undesirable bitter or metallic aftertaste, especially when used in high concentrations. Na-saccharin is stable under the influence of temperature, and in the presence of acids, it does not chemically react with other food ingredients. Otherwise, it is a crystalline, colorless substance and is about 300 times sweeter than sucrose. Mixing Na-saccharin with other sweeteners is often used to compensate for any weakness in the sweetener (Nollet, 2000). Na-saccharin is not metabolized in the human body and is quickly excreted in the urine (as unchanged). The discovery of Na-saccharin was of great importance, especially for people with diabetes because of the fact that it passes directly through the human digestive system without being processed, does not affect the level of insulin in the blood, and has no nutritional value (Kroger et al., 2006). Samples with artificial sweeteners can be analyzed by High-Performance Liquid Chromatography (HPLC). In most food products it is often necessary to perform extractions for isolation certain compounds for analysis. The methods of applied extraction and purification is necessary and depends on the complexity of the sample matrix (Di Pietra et. al., 1990; Nollet, 2000).

The main goal of modern HPLC is to achieve high separation efficiency in a sufficiently short time (Kavrakovski, 1997). The development of the method for simultaneous detection and quantification of artificial sweeteners, acesulfame K, Na-saccharin, aspartame, is of great importance for their regular quantification and requires optimization of the conditions. After establishing the conditions of a given analytical method, it is necessary to carry out its validation, which actually represents statistical processing of data, in order to confirm and justify its application. Validation shows how good and applicable a given method is for a given analysis under certain analytical conditions, which today represents a basic activity of analytical laboratories. This should be done very carefully to ensure that the new method gives accurate and conclusive results (Kastelan-Macan, 2003; Van Vondel, 2005).

In the European Union (EU) legislation on food additives is governed by Regulation 1333/ 2008 of the European Parliament and Council, which includes general guidelines for the use of food additives and where are prescribed their maximum permitted quantity. Also, there is Regulation (EU) No 1129/2011, amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council, with the Union list of food additives. Prior to their authorization, sweeteners are evaluated for their safety by the European Food Safety Authority (EFSA). The list of authorized sweeteners is revised regularly by the European Commission in line with the opinion of EFSA.

In North Macedonia, the maximum allowed amounts of artificial sweeteners for each group of products are determined by the Regulation on Additives used in food production (Official Gazette of R. Macedonia no. 31/2012). With the Regulation on food related information (Official Gazette of R. Macedonia no. 150/2015), all used artificial sweeteners in food products should be properly and regularly labelled, with all other notice what are prescribe in this Regulation.

International bodies such as the European Food Safety Authority (EFSA), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have established values for the acceptable daily intake (ADI) of artificial sweeteners. The ADI value is estimation of the amount that may be ingested daily over a lifetime, without appreciable health risk. The ADI is therefore expressed as the maximum acceptable intake, usually in term of mg/kg body weight (b.w.) (NSCFS, 2014). A body weight of 60 kg is usually taken to represent the average weight of the population. It is also important to determine whether an individual has a certain potential intake of a sweetener that could exceed the ADI value for it (CCFAC). The value of ADI for aspartame is 0-40 mg/kg of body weight, per day (EFSA Panel, 2013). The safety of acesulfame K was also estimated with the ADI value of 0-15 mg/kg of body weight or 900 mg per person per day (SCF, 2000). The ADI value for Na-saccharin is 5 mg/kg of body weight (SCF,1997).

The safety of chronic consumption of larger quantities of artificial sweeteners throughout life is also not well researched. This could be problematic for some diabetics who have been consuming sweeteners regularly for decades. Another point of concern is the possibility for exceeding ADI levels in children, where the quantity of consumed soft drinks can be high, while their body mass is low (Hafner et. al., 2021). According to the European regulation on food additives, member states should establish a monitoring system for the consumption of additives. The subject of this monitoring is the consumption of food additives, to ensure that their use does not exceed the value of acceptable daily intake (ADI), determined for each additive (FAO/WHO CA-IFS, 192-1995).

The main goal of the study is to quantified the content of aspartame, acesulfame K and Na-saccharin, as the artificial sweeteners in different food products. For assessment the safety of analyzed products in accordance to the regulations, the validated HPLC method was used, for simultaneously determination of the examined sweeteners. Based on the obtained results, it will be estimated the intake of the artificial sweeteners (to the mean body weight) for different population groups, for children, adolescents and adults, under the supposition that the products contained the maximum permitted quantities of the analyzed artificial sweeteners.

MATERIALS AND METHODS

The research in this article were made on 133 food products, as nonalcoholic drinks, chewing gums, candies, chocolates, confectionery products, dietary products, food supplements, fruit yogurt etc. Due the different composition content of food matrix, have been used different appropriate extraction methods. For the sample preparation, it is important that the sample of food are appropriate and well homogenized. For solid products, 2 to 10 g were used, measured with an accuracy of 0.1 mg. The liquid samples were used by diluting in a ratio of 1:2, with prior degassing for carbonated soft drinks. According to the sample composition, different sample preparations were applied. For the chewing gum it was necessary to disperse the gum in a solvent with volume ratio, (chloroform) : (water) : (acetic acid), 25:50:1. Sample extracts were purified by solutions of Carrez I (1.5 g of potassium hexacyanoferrate in 10 mL distilled water) and Carrez II (3 g of zinc sulfate in 10 mL distilled water) solutions for products with protein content, and food products with fats need use of ethanol for extraction from the sample, etc.

The quantification of the content of these artificial sweeteners (by *Supelco*): aspartame (99 %), acesulfame K (99.9 %) and Na-saccharin (99.5%) was performed by simultaneous analytical reversed-phase, HPLC method (Shimadzu type). The separation was obtained on stationary phase – Supelcosil 150 x 4.6 mm, 5µm, mobile phase (buffer 0.1 M NaH_2PO_4, pH = 2.5 and acetonitrile, gradient grade), temperature = 26 ^{o} C and flow = 1.5 mL/min. The method was validated according to the laboratory procedure.

For assessment the safety of food products that have been analyzed, the comparison was made of the obtained results with the maximum allowed quantities for each analyzed sweetener in accordance to the Regulation on Additives used in food production (Official Gazette no. 31/2012). Also, in accordance to the Regulation on food related information (Official Gazette no. 150/2015), assessment was made on the presence of irregularities in the analyzed groups of products.

Under the assumption that food products contain the maximum allowed amount of sweetener, an estimation of theoretical daily intake of sweeteners was made by applying mathematical operations, by dividing the total intake by the average body wight (mg/kg), for children, adolescents and adults. So therefore, different daily amounts of food products were taken that could be consumed, in order to determine the deviation from value of the ADI, for each sweetener and in each population group.

RESULTS AND DISCUSSION

The development of the method for the simultaneous detection and quantification of artificial sweeteners, acesulfame K, Na-saccharin, aspartame, is of great importance for their regular, quick control by the authorized institutions, but requires an optimization of conditions of the applied method.

In this research were used 133 different food products, divided in 11 groups: refreshing carbonated drinks A - 9 samples; refreshing non-carbonated drinks B – 11 samples; soft drinks C – 6 samples; instant drinks D – 18 samples; fruit syrups E – 4 samples; other drinks (flavored tea, energy drinks, etc.) F – 13 samples; dietary supplements G – 20 samples; food supplements H – 20 samples; chewing gums and candies without sugar I – 13 samples; chocolates, chocolate products, biscuits and confectionery J – 6 samples; and other food (fruit yogurt, pudding, mustard, etc.) K - 3 samples. The percentage representation of each group of samples in relation to the total number of samples is presented in Figure 1.

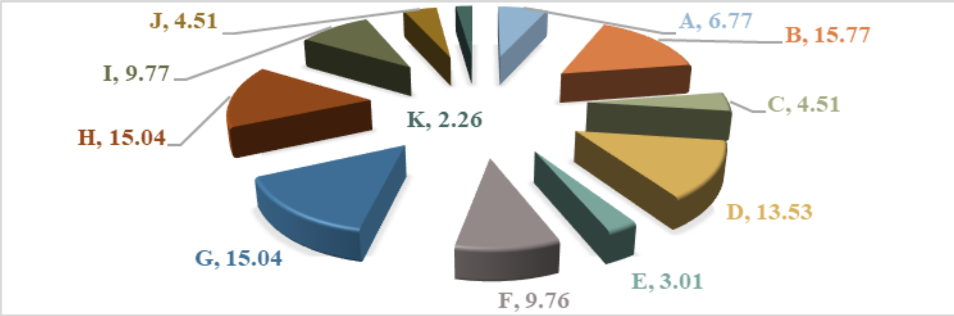

Figure 1. Percentage (%) of each group of examined samples

Depending on the structure of the food, different extraction methods were applied. The analysis for the content of artificial sweeteners, aspartame, acesulfame K and Na-saccharin in different food products was performed by validated HPLC method. Developed analytical HPLC method was reversed-phase, gradient, with simultaneously quantification of acesulfame K, Na-saccharine and aspartame. The separation was obtained on stationary phase – Supelcosil 150 x 4.6 mm, 5µm, mobile phase (buffer 0.1 M NaH_2PO_4, pH = 2.5 and acetonitrile, gradient grade), temperature = 26 °C and flow = 1.5 mL/min, at a wavelength of 210 nm. The length of the run was 8 minutes.

To determine the selectivity of this method, a comparison was made of the chromatograms of a combined standard solution containing all three analytes, Na-saccharin, acesulfame K and aspartame (Figure 2a) and the chromatograms of a combined standard solution containing all analytes, with the injection of another sample containing the three analytes (Figure 2b).

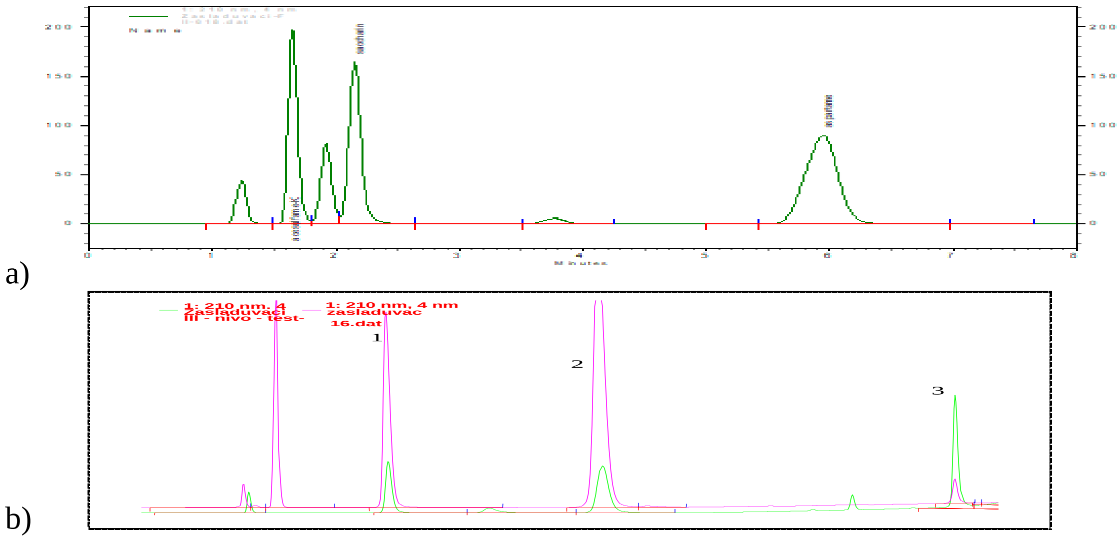

Figure 2. Analytical chromatograms obtained by HPLC method analysis of:

a) a mixture of standards acesulfame K, Na-saccharin, aspartame)

b) comparison of a mixture of standards (the green line) and beverages (the purple line) (1-acesulfame K, 2-Na saccharin, 3-aspartame)

The Figure 2b, shows a comparison of chromatograms obtained during the analysis of a standards mixture (green), and of a non-alcoholic beverage (purple). The chromatograms show that there is no interference between the basic peaks of the analytes Na-saccharin, acesulfame K and aspartame and the solvent. A good resolution was achieved between each of the analyte peaks. By comparing the spectra obtained from the analyzed samples, with those from the standards, the identification of the analytes was successfully confirmed. An "ideal" matching of spectra for each analyzed sweetener was obtained, therefore the identification was successful.

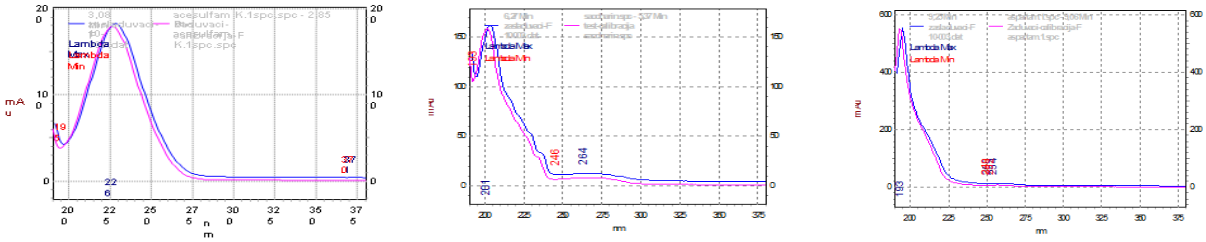

Figure 3. Matching of spectra by HPLC (UV-DAD) analysis of acesulfame K, Na-saccharin and aspartame

An overview was made of the individually representation (%) of each analyzed sweetener, in each group of food products, and the results are presented in the Figure 4.

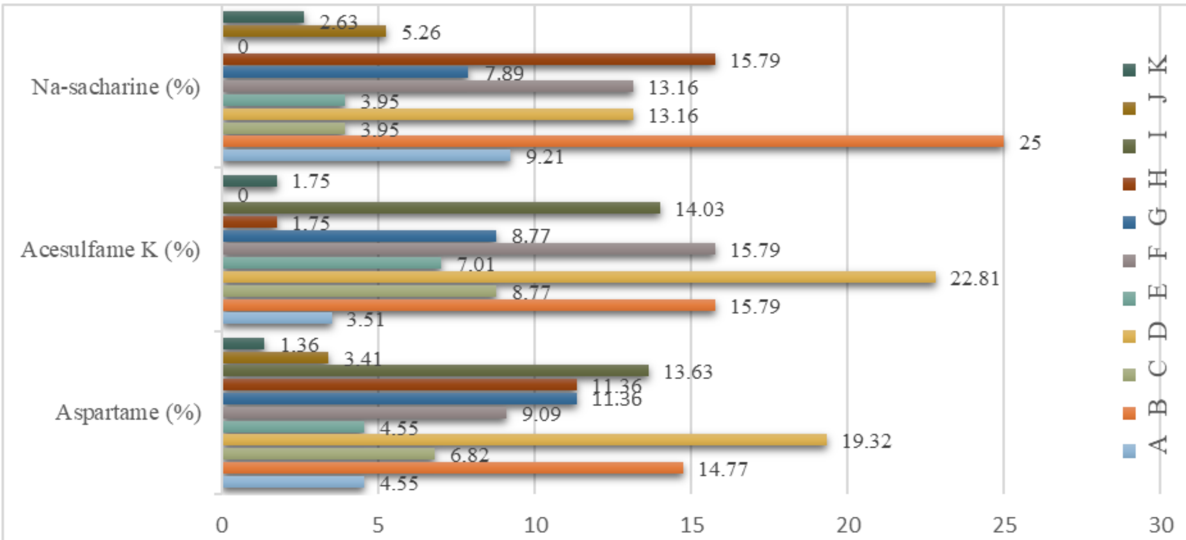

Figure 4. The representation (%) of each of analyzed sweeteners in analyzed groups of products

According to the results obtained for origin of production, 23 (17.29 %) products of total groups of products were domestic production, mostly as low- calorie beverages and 110 (82,71 %) products of total groups of products were imported from different foreign countries, with 60,87 % of them were low- calorie beverages. Figure 5 presents the percentage of representation of each product groups based on the product origin (domestic and imported).

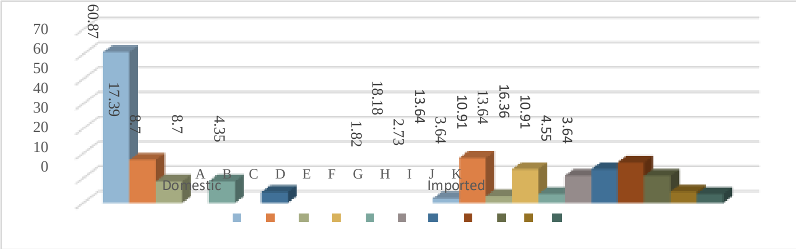

Figure 5. Percentage of each group of products according to the product origin

The data for the content of analyzed artificial sweeteners were used to determine the presence of each of the sweeteners in the different groups of food products, both domestic and foreign. It is noticeable a presence of one sweetener in 46.62 % of all analyzed products, a combination of two sweeteners in 33.08 %, and three sweeteners in 20.30 %. The most used sweetener is Na saccharin, which, with aspartame and acesulfame K is most utilized in beverages. According to the Regulation on Additives used in food production (Official Gazette of R. Macedonia no. 31/2012), the highest maximum allowable content is: for acesulfame K 2500 mg/kg, for aspartame 6000 mg/kg, and for Na-saccharin 3000 mg/kg – all for refreshing sugar-free micro-candies. At the same time, the range of the maximum allowed amount in mg/kg in different food products is different and ranges within the following limits: for aspartame, 25 - 6000 mg/kg(L); for Na-saccharin, from 80 - 3000 mg/kg(L) and for acesulfame K, 25 - 2500 mg/kg(L). The labeling of the analized groups of products was checked in order to determine their compliance according to the Regulations of food related information (Official Gazette R. Macedonia no. 150/2015). According to these regulations, the food products containing one or more of the permitted sweeteners should be labelled "with artificial sweetener" which should be listed together with the names of the sweeteners or the E numbers. Also, the labelling should be defined as "low-energy" or "light", that is, with a reduced energy value of at least 30 % in compared to the original food product where the sugar is added. The declaration should clearly state a warning about the presence of some sweeteners, that is, when labelling products that contain aspartame as sweeteners, it must contain the warning "contains a source of phenylalanine".

By analyzing the examined products, irregularities were discovered in reference to: 1) the product is not marked as "with low-energy" or "light", "with sweetener" or "with sweetener and sugar"; 2) incorrect declaration of sweeteners in relation to the maximum allowed quantities; 3) incorrect declaration of sweeteners regarding their presence in the ingredients list, together with the name of the sweetener or the E number; 4) not including the warning on the product/translation

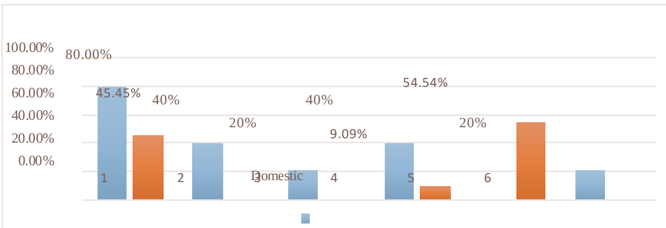

Figure 6. The types of irregularities in products of domestic and foreign origin in relation to the prescribed labelling in Regulations

of the declaration "contains a source of phenylalanine" for products in which aspartame has been added as a sweetener; 5) the translation of the declaration about the presence and/or the warning about sweeteners/aspartame is incomplete; 6) the expiration date is not easily legible. The representation of the types of irregularities in products of domestic and foreign origin in relation to the prescribed labelling in Regulations are presented in the Graph 4. In order to assess the safety of the food products that were previously analyzed, a comparison was made of the obtained results with the maximum allowed amounts of sweetners for each group of products, as well as the assessment on the presence of irregularities in terms of proper way of labeling. There was 21 % of products with labelling irregularities, out of which 50 % were imported products. The irregularities were in terms of incomplete translation of the labelling or irregularities of labelling of the products in general. For the domestic products, in 21 % of the products were exceeding the maximum permitted quantities of any individual sweetener and others had irregularities with labelling of the food products.

Regulation 1333/ 2008 of the European Parliament and Council, includes monitoring the use and consumption in different Member States in view of potential legal action if intake levels frequently exceed the advised acceptable daily intake (ADI). The estimation of theoretical daily intake of sweeteners was made by applying mathematical operations, using data on average body weights for children, adolescents and adults, under the assumption that the food products contain the maximum allowed amount (ADI) of sweetener. The results are presented at the Table 1.

Table 1. Theoretical daily intake of each of the artificial sweeteners in food, at maximal allowed value of ADI (mg/kg body weight), for different population of consumers

Age	Average body mass (kg)	Aspartame ADI 40 (mg/kg b.w./d)	Acesulfame K ADI 15 (mg/kg b.w./d)	Na saccharine ADI 5 (mg/kg b.w./d)
Children				
<1	7.3	292	109.5	35.5
1-3	13.4	536	201	67
4-6	20.2	808	303	101
7-9	28.1	1124	412.5	140.5
Men adolescent				
10-12	36.9	1476	553.5	184.5
13-15	51.3	2052	769.5	256.5
16-19	62.9	2516	943.5	314.5
Women adolescent				
10-12	38.0	1520	570	190
13-15	49.9	1996	748	249.5
16-19	54.4	2176	816	272
Adult men (with average physical activity)				
>20	65.0	2600	975	325
Adult women (with average physical activity)				
>20	55.0	2200	825	275

*Data taken from reports of Joint FAO/WHO Ad Hoc Expert Committee

Based on the Table 1, it can be noted that for adult men, the highest theoretical intake of aspartame was calculated at 2600 mg/kg b.w./d, due to the highest estimated value of ADI for aspartame. The lowest amount of theoretical intake for Na-saccharin was determined to be achieved for children less than 1 year old, due the value of 5 mg/kg b.w. for ADI. But taking into account the legal normative according to which, the use of sweeteners is prohibited in food for children under one year of age, this would mean that the possible consumption of food containing artificial sweeteners, which is not intended for this population, could only be controlled by the guardians of those children.

Some products have been regrouped, that is, low-energy soft drinks are taken as one group, with a single maximum allowed amount for all groups from A-F, except for Na-saccharin, due to its differences in the maximum limits for carbonated and non-carbonated soft drinks. Groups G and H will not be taken into account, because as food supplements and as dietary supplements, it is considered that they are consumed according to the recommendations of the manufacturer (both for daily doses and for the preparation of meals) or according to a medical recommendation. The product group I is divided into two sub-groups, Im and Ib, due to the different maximum allowed amounts for chewing gum and candies. In group K, fruit yogurt is taken as a more attractive product for consumption, which is especially interesting for the youngest population.

In Table 2, Table 3 and Table 4 are presented the calculated percentage (%) of the maximum ADI value that is achieved by consuming the different quantities of the product, with the maximum allowed amount of aspartame, acesulfame K and Na-saccharin, respectively, for different population groups (mg/kg b.w.). There are 5 population groups according to their age ranges and each group is divided for male and female. For each sweetener respectively, are presented the amounts of sweetener used to achieve max. value of ADI (mg), according to which was calculated what a quantity of group of products it will be necessary to be achieved that value. Then, there are presented different amounts from each product groups, according to which was calculated the percentage in relation to the theoretical maximum of ADI.

Table 2. Percentage of the maximum ADI value that is achieved by consuming different quantities of the product with the maximum allowed amount of aspartame, for different population

Age, gender, and body mass (kg)	4-6 male (20.2)	4-6 female (19.4)	7-8 male (25.97)	7-8 female (24.89)	10-11 male (40.43)	10-11 female (40.23)	15-16 male (63.6)	15-16 female (54.4)	>20 male (79)	>20 female (66)
Aspartame in different products group	Amount of sweetener used to achieve max. value of ADI (mg)									
	808.0	776.3	1039	995.6	1617.2	1609	2544	2176	3160	2640
Groups (A-F) and K for aspartame (600 mg/L)										
200 mL	14.85	15.5	11.6	12.1	7.42	7.5	4.7	5.5	3.8	4.5
330 mL	24.5	25.5	19.1	19.9	12.2	12.3	7.8	9.1	6.3	7.5
500 mL	37.1	38.7	28.9	30.1	18.6	18.6	11.8	13.8	9.5	11.4
1000 mL	74.26	**77.3**	57.6	60.3	37.1	37.3	23.6	27.6	19.0	22.7
Group Im for aspartame (5500 mg/L)										
1 g	0.7	0.7	0.5	0.6	0.3	0.3	0.2	0.3	0.2	0.2
10 g	6.8	7.1	5.3	5.5	3.4	3.4	2.2	2.5	1.7	2.1
50 g	34.0	35.4	26.5	27.6	17.0	17.1	10.8	12.6	8.7	10.4
100 g	68.1	**70.9**	52.9	55.2	34.0	34.2	21.6	25.3	17.4	20.8
Group Ib for aspartame (2000 mg/L)										
1 g	0.24	0.26	0.19	0.20	0.12	0.12	0.01	0.09	0.06	0.08
10 g	2.5	2.6	1.9	2.0	1.2	1.2	0.8	0.9	0.6	0.8
50 g	12.4	12.9	9.6	10.0	6.2	6.2	3.9	4.6	3.2	3.8
100 g	24.8	**25.8**	19.3	20.1	12.4	12.4	7.9	9.2	6.3	7.6
Group J for aspartame (1000 mg/L)										
10 g	1.2	1.3	1.0	1.0	0.6	0.6	0.4	0.5	0.3	0.4
50 g	6.2	6.4	4.8	5.0	3.1	3.1	2.0	2.3	1.6	1.9
100 g	12.4	12.9	9.6	10.0	6.2	6.2	3.9	4.6	3.2	3.8
200 g	24.8	**25.8**	19.3	20.1	12.4	12.4	7.9	9.2	6.3	7.6

Table 3. Percentage of the maximum ADI value that is achieved by consuming different quantities of the product with the maximum allowed amount of acesulfame K, for different population

Age, gender, and body mass (kg)	4-6 male (20.2)	4-6 female (19.4)	7-8 male (25.97)	7-8 female (24.89)	10-11 male (40.43)	10-11 female (40.23)	15-16 male (63.6)	15-16 female (54.4)	>20 male (79)	>20 female (66)
Acesulfame K in different products group	Amount of sweetener used to achieve max. value of ADI (mg)									
	303	291	389.6	373.4	606.5	603.5	954	816	1185	990
Groups (A-F) and K for acesulfame K (350 mg/L)										
200 mL	23.1	24.1	18.0	18.74	11.54	11.6	7.3	8.6	5.9	7.1
330 mL	38.12	39.7	29.6	30.9	19.04	19.1	12.1	14.2	9.7	11.7
500 mL	57.8	60.1	44.9	46.9	28.9	29.0	18.34	21.4	14.8	17.7
1000 mL	**115.1**	**120.3**	89.8	93.7	57.7	58.0	36.7	42.9	29.5	35.4
Group Im for acesulfame K (2000 mg/L)										
1 g	0.66	0.68	0.51	0.54	0.33	0.33	0.21	0.25	0.17	0.2
10 g	6.6	6.9	5.1	5.4	3.3	3.31	2.1	2.5	1.7	2.0
50 g	33.0	34.4	25.7	26.8	16.5	16.6	10.5	12.3	8.4	10.1
100 g	66.0	**68.7**	51.3	53.6	33.0	33.1	21.0	24.5	16.9	20.2
Group Ib for acesulfame K (500 mg/L)										
1 g	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
10 g	1.65	1.7	1.3	1.3	0.8	0.8	0.5	0.6	0.4	0.5
50 g	8.25	8.6	6.4	6.7	4.1	4.1	2.6	3.1	2.1	2.5
100 g	16.5	**17.2**	12.8	13.4	8.2	8.3	5.2	6.1	4.2	5.1
Group J for acesulfame K (1000 mg/L)										
10 g	3.3	3.4	2.6	2.7	1.6	1.7	1.0	1.2	0.8	1.0
50 g	16.5	17.2	12.8	13.8	8.2	8.3	5.2	6.1	4.2	5.1
100 g	33.0	34.4	25.7	26.8	16.5	16.6	10.5	12.3	8.4	10.1
200 g	66.0	**68.7**	51.3	53.6	33.0	33.0	21.0	24.5	16.9	20.2

Table 4. Percentage of the maximum ADI value that is achieved by consuming different quantities of the product with the maximum allowed amount of Na-saccharin, for different population

Age, gender, and body mass (kg)	4-6 male (20.2)	4-6 female (19.4)	7-8 male (25.97)	7-8 female (24.89)	10-11 male (40.43)	10-11 female (40.23)	15-16 male (63.6)	15-16 female (54.4)	>20 male (79)	>20 female (66)
Na-saccharin in different products group	Amount of sweetener used to achieve max. value of ADI (mg)									
	101	97.0	129.9	124.5	202.2	201.2	318	272	295	330
Groups (B-F) for Na-saccharin (80 mg/L)										
200 mL	15.8	16.5	12.3	12.8	7.9	8.0	5.0	5.9	5.4	4.8
330 mL	26.1	27.2	20.3	21.2	13.1	13.1	8.3	9.7	8.9	8.0
500 mL	39.6	41.2	30.8	32.1	19.8	19.9	12.6	14.7	13.6	12.1
1000 mL	79.2	**82.5**	61.6	64.3	39.6	39.8	25.2	29.4	27.1	24.2
Group Im for Na-saccharin (1200 mg/L)										
1 g	1.2	1.2	0.9	1.0	0.6	0.6	0.4	0.4	0.4	0.4
10 g	11.9	12.4	9.2	9.6	6.0	6.0	3.8	4.4	4.1	3.6
50 g	59.4	62.9	46.2	48.2	29.7	29.8	18.9	22.1	20.3	18.2
100 g	**118.8**	**123.7**	92.4	96.4	59.34	59.7	37.7	44.1	40.7	36.4
Group Ib for Na-saccharin (500 mg/L)										
1 g	0.49	0.51	0.38	0.40	0.25	0.25	0.16	0.18	0.17	0.15
10 g	5.0	5.2	3.8	4.0	2.5	2.5	1.6	1.8	1.7	1.5
50 g	24.8	25.8	19.2	20.1	12.4	12.4	7.9	9.2	8.5	7.6
100 g	49.5	**51.5**	38.5	40.2	24.7	24.9	15.7	18.4	16.9	15.2
Group J for Na-saccharin (200 mg/L)										
10 g	2.0	2.7	1.5	1.6	1.0	1.0	0.6	0.7	0.7	0.6
50 g	9.9	10.3	7.7	8.0	4.9	5.0	3.1	3.7	3.4	3.0
100 g	19.8	20.6	15.4	16.1	9.9	9.9	6.3	7.4	6.8	6.1
200 g	39.6	**41.2**	30.8	32.1	19.8	19.9	12.6	14.7	13.6	12.1
Groups A + K for Na-saccharin (100 mg/L)										
200 mL	19.8	20.6	15.4	16.1	9.9	9.9	6.3	7.4	6.8	6.1
330 mL	32.7	34.0	25.4	26.5	16.3	16.4	10.4	12.1	11.2	10.0
500 mL	49.5	51.5	38.5	40.2	24.7	24.9	15.7	18.4	16.9	15.2
1000 mL	99.0	**103.1**	77.0	80.3	49.5	49.7	31.4	36.8	33.9	30.3

Based on the results presented in Table 2, Table 3 and Table 4, it can be noted that for female children aged 4 - 6 years, to achieve the prescribed maximum ADI value for each sweetener respectively need the smallest intake amount from products with maximum content of sweeteners. Therefore, in this population there is a potential theoretical risk of reaching the maximum prescribed ADI value, but it should be taken into account that the maximum ADI values are estimated for an average body weight of 60 kg. For this population, it was noticed a potential exceeding of the ADI values for Na-saccharin in product groups of carbonated soft drinks and fruit yogurts (assuming a consumption of 1000 mL of product/day), as well as in the group of sugar-free chewing gums (assuming a consumption of 100 g product/day), where was calculated over 100 % of ADI. For acesulfame K, such an exceeding of maximum ADI was determined for groups A-F (under assumption that 1000 mL of product/day are being consumed). However, the existence of this potential theoretical risk is not always justified, taking into account that the artificial sweeteners contained in the products are not always with the maximum allowed amounts and not always the "most at risk" population consumes high amounts of products that would exceed the ADI values. Although, according to the obtained results, there is a theoretical potential health risk for the youngest population by consuming the given amounts of products, this does not mean that for the rest of the population ages there is no possibility of such a risk, especially for those who are " large" consumers of products containing artificial sweeteners.

CONCLUSIONS

In this researching a new analytical, reversed-phase, gradient, validated HPLC method was used for simultaneous determination of aspartame, acesulfame K and Na-saccharin. This method is applicable for different food products, with using different sample preparation methods of extraction. It was analyzed 133 different food products, to estimate their safety according to the national regulation. The half of the estimated irregularities were in domestic food products, mainly in non-alcoholic drinks, where was determined exceeding of the maximum permitted quantities of added sweeteners or irregularity in labelling of products. According to the origin of production, 17.29 % products were domestic production and 82,71 % products were from different foreign countries, mostly as low- calorie beverages. The estimation of theoretical daily intake of sweeteners was made by applying mathematical operations, using data on average body weights for children, adolescents and adults, under the assumption that the food products contain the maximum allowed amount (ADI) of sweetener. For adult men there was a lowest health risk for consuming food products, with prediction that it contains the maximum ADI values content of Na-saccharin. In contrast, for young females, 2-4 years old, was assessed potential theoretical health risk for consuming food products, with prediction that it contains the maximum ADI values content of Na-saccharin, but it should be taken into account that the maximum ADI values are determined for an average body weight of 60 kg. However, the existence of this potential theoretical risk is not always justified, taking into account that the artificial sweeteners contained in the products are not always with the maximum allowed amounts and not always the "most at risk" population consumes high amounts of products that would exceed the ADI values.

Recommendation: There is a need for a greater awareness of the consumers about the role and function of artificial sweeteners in food products. It is necessary for North Macedonia to implement continuous monitoring of intake of artificial sweeteners for wide range of population groups.

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