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Estimated intake of sweeteners acesulfame-K and aspartame from soft drinks, soft drinks based on mineral waters and nectars for a group of Portuguese teenage students

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Abstract

A survey of levels of acesulfame-K and aspartame in soft drinks and in light nectars was undertaken, from which the intake of these intense sweeteners was estimated for a group of teenage students. Acesulfame-K was detected in 72% of the soft drinks, with a mean concentration of 72 mg/L and aspartame was found in 92% of the samples with a mean concentration of 89 mg/L. When data on content of these sweeteners in soft drinks was analyzed according to flavour, cola drinks had the highest mean levels for both the sweeteners containing 98 and 103 mg/L for acesulfame-K and aspartame, respectively. For soft drinks based on mineral water, aspartame was found in 62% of the samples, with a mean concentration of 82 mg/L and acesulfame-K was found in 77%, with a mean level of 48 mg/L. All samples of nectars contained acesulfame-K, with a mean concentration of 128 mg/L and aspartame was detected in 80% of the samples with a mean concentration of 73 mg/L. A frequency questionnaire, designed to identify adolescents having high consumption of these drinks, was completed by a randomly selected sample of teenagers (n=65) living in the city of Coimbra, in the year 2007. The estimated daily intakes (EDI) of acesulfame-K and aspartame for the average consumer were below the acceptable daily intakes (ADIs). For acesulfame-K the EDI was 0.7 mg/kg b.w./day for soft drinks, 0.2 mg/kg b.w./day for soft drinks based on mineral waters, and 0.5 mg/kg b.w./day for nectars, representing 8.0%, 2.2%, and 5.8% of the ADI, respectively. A similar situation was observed for aspartame. In this way, the EDI for soft drinks was 1.1 mg/kg b.w./day, representing only 2.9% of the ADI. In respect to nectars, the EDI was 0.2 mg/kg b.w./day, representing 0.5% of the ADI. Soft drinks based on mineral waters showed the lowest EDI values of 0.3 mg/kg b.w./day, accounting for 0.7 % of the ADI.

Keywords: *Estimated intake, sweeteners, acesulfame-K, aspartame, soft drinks, soft drinks based on mineral waters, nectars, Portuguese teenagers*

Introduction

Food additives play a vital role in the modern food industry, and are generally used for maintaining food quality characteristics as well as ensuring food safety. Among them, artificial sweeteners are widely used throughout the world. For many year artificial sweeteners have been commonly used in the food, beverage, confectionary and pharmaceutical industries because of their sweet taste with fewer calories (Zhu et al. 2005). The use of sweeteners is ofsignificance due to increase in obesity problems in developed countries, the general growing awareness of health implications connected to a high calorie diet, to the worldwide increase in the number of people suffering from diabetes, and also to reduce tooth decay (Leth et al., 2007).

Acesulfame-K and aspartame are used in about 100 countries (Zhu et al. 2005). In Portugal, the most common artificial sweeteners used by the food industry are aspartame, acesulfame-K, and sodium cyclamate. Nowadays, in this country, sucralose begins to be introduced in some food items, and the use of saccharin is decreasing. Scrutiny of the labels from soft drinks, soft drinks based on mineral water, and nectars shows that aspartame and acesulfame-K are those preferentially incorporated.

The acceptable daily intake (ADI) values established are 9 and 40 mg/kg b.w./day for acesulfame-K and aspartame, respectively (SCF, 1985; SCF, 2002). Recently, in an update review of the intake estimations, Renwick (2006) showed that few data are available for the population in general, and for teenagers, only two studies from Italy are reported.

As far as we know, this is the first study of sweeteners intake for Portuguese populations, namely adolescents, which is a group with high consumption. The main objective of this study was to check for the presence and concentration of acesulfame-k and aspartame, and verify if below maximum permitted levels in light soft drinks, soft drinks based on mineral waters, and nectars. The data were subsequently used to estimate intake of the sweeteners for a group of Portuguese teenage students aged between 13 and 15.

Materials and methods

Studied population

One public high school was selected in the city of Coimbra (central zone of Portugal). In the school year 2006–2007, students were randomly selected (‘‘Random sample’’) (n=65). All students, aged between 13 and 15 years old, 50.8% males and 49.2% females, were asked to fill in a questionnaire related to the duration and frequency of intake of sugar-free soft drinks and nectars. Students were also asked to record their own personal data, such as age, sex, rural/urban residence area and education level of parents. Permission was obtained from those responsible for their education.

The mean body weight was 56.5 kg ranging from 36.5 to 106.1 kg. The Body Mass Index was calculated, and it was found that 62% of individuals had normal weight, 23% had low weight, and 15% had excess weight or obesity.

The consumption data for the Portuguese population in general were obtained through the annual intake estimated per Portuguese inhabitant in 2006, according the ANIRSF,

the National Association of Soft Drink and Fruit Juice Industry. The estimated mean body weight was 60 kg.

The drink survey

A total of 48 samples were chosen in accordance with products available for sale at the canteen of the school and the consumption of the teenagers. Of these samples, 38 were soft drinks comprising 25 light soft drinks and 13 soft drinks based on mineral waters (a new group of cooled drinks recently introduced in the Portuguese market) and 10 were light nectars. Samples were collected in the school canteen, and in supermarkets between December 2006 and January 2007.

Chemical analysis

Soft drinks were filtered through 0.45 µm membrane filter and ultrasonicated, before HPLC analysis. Nectars were submitted to centrifugation at 3400*g* for 15 min, filtration through membrane filter and ultrasonication. The two sweeteners were separated using reverse phase liquid chromatography (Pump model 305 from Gilson, France, an injection valve with one 20 µL *loop* model 7125 Rheodyne, Cotati, Califórnia, USA) with an Hichrom C18 column (5 µm, 250 x 4.6 mm) and a buffered mobile phase (KH_2PO_4 0.02M / ACN (90:10) / phosphoric acid), at 1 mL/min, which pH was rigorously controlled at 4.2-4.3 for an adequate resolution between aspartame and benzoic acid, since this preservative is present in almost analysed samples. Detection was performed with an UV detector, model 116 from Gilson, France, at 220 nm. External standard method was used for quantification. Integration was performed with an integrator; model SP4290 from Spectra-Physics.

Recovery studies

Recoveries were determined by spiking one soft drink free for the two sweeteners, in triplicate, with known amounts of acesulfame-K and aspartame at final concentrations between 35 and 350 mg/L for the first, and 35 and 600 mg/L for the second sweetener.

Results

The methodology was shown to have adequate linearity, sensitivity, accuracy and precision. Correlation coefficients (r^2) were 0.9906 for acesulfame-K and 0.9973 for aspartame, both between 0.025 µg and 0.4 µg injected. Limits of quantification of acesulfame-k and aspartame were 1.7 mg/L and 3.5 mg/L, respectively. Recovery values were between 91% for acesulfame-K at fortification levels of 350 mg/L and 102% for aspartame at spiked levels of 35 mg/L. Intra-day repeatabilities (% RSD) ranged between 1.0% for aspartame at 35 mg/L and 7.9% for acesulfame-K at 35 mg/L. The inter-day repeatabilities ranged between 7.3 and 11.5% for aspartame at 600 mg/L and acesulfame-K at 100 mg/L, respectively (Table I).

Figure I shows the chromatograms of a standard of acesulfame-K and aspartame containing 0.3 µg of each compound; of a blank sample without sweeteners; and the same sample fortified at 35 mg/L (0.175 µg) with each sweetener.

Table II shows the number of the samples, the range, and the mean of the sweeteners in the different types of analysed beverages. Acesulfame-K was detected in 18 (72%) of the 25 soft drinks analysed, with levels ranging between 7.8 mg/L e 166 mg/L, and a mean concentration of 72 mg/L. Twenty three samples (92%) contained aspartame at

levels between 8.8 mg/L and 339 mg/L. The mean concentration was 89 mg/L (Table II).

When the data on sweeteners soft drinks were analyzed according the flavour, the cola drinks (n=5) had the highest mean levels for both sweeteners, 98 and 103 mg/L for acesulfame-K and aspartame, respectively (Table III). In respect of soft drinks based on mineral waters, aspartame was found in 8 samples (62%), between 19 and 154 mg/L. The mean concentration value was 82 mg/L. Acesulfame-k was detected in ten samples (77%), at levels ranging between 3.7 and 182 mg/L, with a mean level of 48 mg/L (Table II). In five of these ten samples, faulty declarations were found as this sweetener was not declared on the ingredient labels. Those samples represent four Portuguese brands. One of them did not specify the presence of any sweetener, but indicated "without addition of sugar", and four samples only referred to the presence of aspartame.

All samples of nectars showed the presence of acesulfame-K, with levels between 35 and 356 mg/L, being the mean concentration 128 mg/L. Aspartame was detected in 8 (80%) samples, at levels between 39 mg/L and 129 mg/L. The mean concentration was 73 mg/L.

According Portuguese legislation (Decreto-Lei nº 394/98 de 10 de Dezembro de 1998), the maximum permitted level for acesulfame-K in soft drinks and in nectars is 350 mg/L. This level was exceeded in one sample of nectar, with a concentration of 356 mg/L. For aspartame, the maximum permitted level for the same kind of products, 600 mg/L, was not exceeded.

The estimated daily intake (EDI) for each sweetener by the teenager population was calculated by the following equation EDI = ($\sum$c) (CN^{-1} D^{-1} K^{-1}) (Table IV), where $\sum$c is the sum of sweetener concentration in the analyzed samples related to the questionnaire (mg/L), C is the mean annual intake estimated per teenager, N is the total number of analysed samples, D is the number of days in a year, and K is the mean body weight. The estimated intakes of acesulfame-K and aspartame for the average intake, by the consumers were below the acceptable daily intakes (ADIs). For acesulfame-K the EDI was 0.7 mg/kg b.w./day for soft drinks, 0.2 mg/kg b.w./day for soft drinks based on mineral waters, and 0.5 mg/kg b.w./day for nectars, representing 8.0%, 2.2%, and 5.8% of the ADI, respectively (Table IV).

Similar situation was observed for aspartame. The EDI for soft drinks was 1.1 mg/kg b.w./day, representing only 2.9% of the ADI. For soft drinks based on mineral waters 0.3mg/kg b.w./day representing 0.7% of the ADI. In respect to nectars, the EDI was 0.2 mg/kg b.w./day, what represents 0.5 % of the ADI (Table IV).

Three of the well-known international trade mark soft drinks, being two cola type drinks, were responsible for the maximum daily intake of the sweeteners by the studied population, assuming the maximum consumption of the soft drinks. One of them was responsible for the maximum intake of aspartame, with 4.5 mg/kg body weight, and occupying the second place for the acesulfame intake of 1.3 mg/kg body weight. The international trade mark beverage contributed the maximum intake for acesulfame of 1.6 mg/kg body weight. Another soft drink gave an aspartame intake of 2.8 mg/kg body weight. The maximum daily intake for both sweeteners given by nectars was lower than that for soft drinks.

The EDI of the teenager population was higher than the Portuguese population in general. For the latter, the EDIs of acesulfame-K were 0.18 mg/kg b.w./day and 0.057 mg/kg b.w./day when soft drinks and nectars were consumed, respectively, representing 2.0 and 0.6% of the ADI. For aspartame, the EDI values are 0.3 mg/kg b.w./day and 0.026 mg/kg b.w./day for soft drinks and nectars respectively, which means 0.7% and 0.07 % from the ADIs (Table V).

Discussion

The comparison of data on the levels in this study with other countries is difficult, as result of scarcity of data. In what concerns the presence of aspartame in soft drinks, Prodolliet and Bruelhart (1993) found levels of this sweetener of 153 mg/L and 286 mg/L in 2 diet cola drinks in Switzerland. In the same kind of drinks, in Barcelona, the levels of aspartame were 130 mg/L and 676 mg/L (Suárez et al. 1997). A Danish study reported 159 mg/L and 56 mg/L for soft drinks with (n=20) and without (n=43) carbon dioxide, respectively (Leth et al. 2007). The same sweetener was found by Tyler (1984), in USA, in two *diet* cola drinks with 97 mg/L and 92 mg/mL. In USA, Walker et al. (1997) detected aspartame in two soft drinks, one of *diet* coca with 206 mg, and another of diet lemon carbonated soft drink with 185 mg, by scan of 355 mL, which corresponds to 580 mg/L and 521 mg/L, respectively. In China, Zhu et al. (2005) found levels of 7234.6 and 2826.3 mg/L in 2 samples of cola soft drinks. The values in different countries are higher than those found in the samples analysed in the present study, except for soft drinks without carbon dioxide from Denmark.

As far as we know, only one study of nectar samples has been previously reported. In this study, aspartame was found in one exotic fruit nectar at concentrations of 2416

mg/L, in Switzerland (Prodolliet and Bruelhart 1993). One drink of fruit juice, in China, had levels of aspartame and acessulfame-K of 317.2 and 4.5 mg/L, respectively (Zhu et al. 2005). The aspartame level is excessively high when compared with the concentrations found in identical samples involved in the present study, ranging in this case between 39 mg/L and 129 mg/L.

Concerning acesulfame-K levels, Suárez et al. (1997) reported its presence in two cola drinks at 59 mg/L and 146 mg/L. Recently, in Denmark, a study of *light* soft drinks with (n=21) and without (n=60) carbon dioxide showed mean levels of acesulfame-K of 129 mg/L and 62 mg/L, respectively (Leth et al. 2007). Also in China, Zhu et al. (2005) found levels of acesulfame-K of 1105.4 and 1475.2 mg/L, in 2 cola drinks. The levels found in Portuguese study, 56 mg/L e 137 mg/L, are between those reported by Suárez et al. (1997), are higher than those found by Leth et al. (2007), and lower than those referred by Zhu et al. (2005).

The results of the present study are consistent with the percentage of ADI for acesulfame and aspartame reported for other countries. The lowest percentage of ADI for both sweeteners of 0.1%, was found in Italy for a population aged between 13 and 19 years old (Leclercq et al. 1999). Studies carried out in another teenager population, from Italy, had ADI percentages of 0.2 and 0.3% for aspartame and acesulfame-k, respectively (Arcella et al. 2004). The highest values for acesulfame-K, 9%, were found in individuals aged between 3 and 74, in United Kingdom (Wilson et al. 1999). For the aspartame, the highest values were found in one Canadian population, aged between 2 and 6 years, with 10% of the ADI (Devitt et al. 2004).

It should be stressed that youngest population group aged between 1.5 - 4.5 years across different areas of the United Kingdom (Food Standards Agency UK. 2003), and 2-6 years in Toronto, Canada (Devitt et al. 2004), had some of the highest percentages of the ADI for aspartame and acesulfame-K. In the UK those values are 6% and 8% for acesulfame and aspartame, respectively, and in Canada represented 4% and 10% for the same sweeteners. The first European conference to review the safety of aspartame, held in Paris in 2006, showed that the intakes are about 10% of the acceptable daily intake, even by high consumers of aspartame (Renwick and Nordmann 2007).

Conclusions

The analytical methodology is well-suited for quality control, providing adequate linearity, sensitivity, accuracy and precision for the determination of both sweeteners in soft drinks and nectars. Moreover, the speed of analysis and simple sample preparation allows for its use in a quality control laboratory. In this study it was demonstrated that nectars showed 100% frequency of detection and the highest mean concentration of 128 mg/L for acesulfame-K, and soft drinks contained aspartame in 92% of the samples with mean levels of 89 mg/L. The highest estimated daily intakes of both sweeteners for the teenage population was for soft drinks, giving 0.7 and 1.1 mg/kg b.w./day, being far below their respective ADI-values. It was also shown that EDIs of both sweeteners by the general Portuguese population is lower than the teenager population. It can be concluded that, with the current observed consumption patterns and occurrence levels, the risk of an excessive intake of intense sweeteners by Portuguese teenagers is low.

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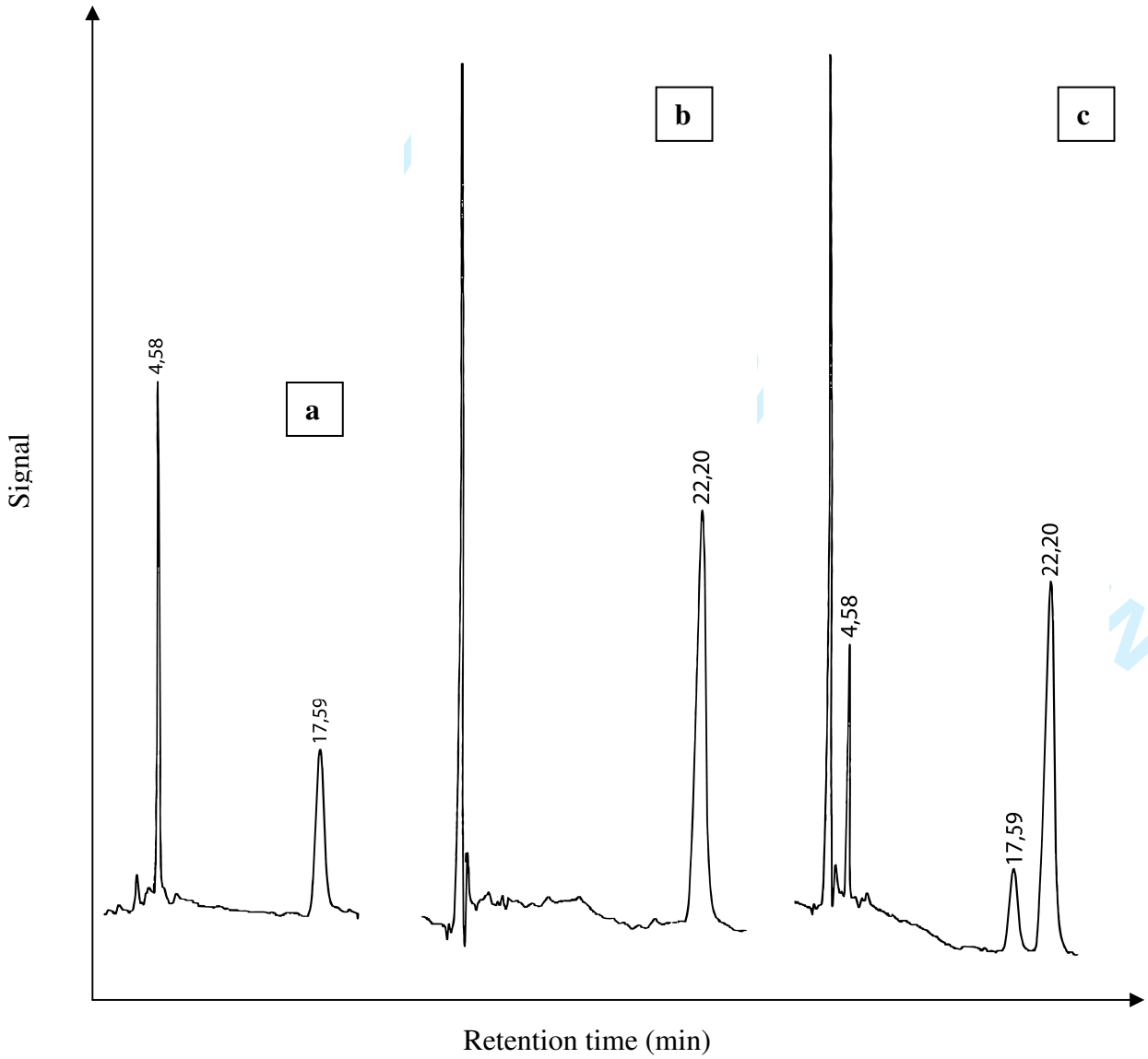
a
b
c
4,58
17,59
22,20
4,58
22,20
17,59
Signal
Retention time (min)

Figure I. Chromatograms of: **a)** standard of acesulfame-K (rt= 4.58) and aspartame (rt=17.59) containing 0.3 μg of each compound; **b)** blank (sample without sweeteners); **c)** sample fortified at 35 mg/L (0.175 μg) with each sweetener. The rt=22.20 represents benzoic acid.

Table I. Recovery, intra and inter-day repeatability (% RSD) for soft drinks spiked at different levels of acesulfame-K and aspartame

Sweeteners	Fortification level (mg/L)	Exactitude*	Intra-day repeatability	Inter-day repeatability
Acesulfame-K	35	92	7.9	8.2
	100	94	1.1	11.5
	350	91	5.3	9.7
Aspartame	35	102	1.0	8.5
	100	85	1.7	9.9
	600	89	2.8	7.3

*n=3

Table II. Frequency, range, and mean concentration[1] of the sweeteners in the different types of analysed beverages.

Type of sample	Analysed samples	Acesulfame-K (mg/L)			Aspartame (mg/L)		
		Positive samples	Range	Mean	Positive samples	Range	Mean
Soft drinks	25	18	7.8 - 166	72[1]	23	8.8 - 339	89[1]
Soft drinks based on mineral waters	13	10	3.7 - 182	48[1]	8	19 - 154	82[1]
Nectars	10	10	35 - 356	128	8	39 - 129	73[1]

[1] Mean concentration for samples with the sweeteneer

Table III. Mean levels (mg/L) of acesulfame-K and aspartame in *light* soft drinks according to the flavor.

Samples	Acesulfame-K	Aspartame
Orange (n=3)	-	70
Cola (n=5)	98	103
Lemon (n=2)	70	87
Pineapple (n=3)	51	52
Soy (n=2)	57	49

Table IV. Estimated daily intake (EDI)[a] of acesulfame-K and aspartame by scholar teenagers through soft drinks, soft drinks based on mineral water, and nectars and respective comparison with acceptable daily intake (ADI)

	Soft drinks			Soft drinks based on mineral water		Nectars	
Sweeteners	ADI[b] (mg/kg b.w./day)	EDI (mg/kg b.w./day)	ADI (%)	EDI (mg/kg b.w./day)	ADI (%)	EDI (mg/kg b.w./day)	ADI (%)
Acesulfame-K	9	0.7	8.0	0.2	2.2	0.5	5.8
Aspartame	40	1.1	2.9	0.3	0.7	0.2	0.5

[a]EDI was calculated using the equation EDI = $(\sum c)$ $(CN^{-1}$ D^{-1} $K^{-1})$, where $\sum c$ is the sum of sweetener concentration in the analyzed samples related to the questionnaire (mg/L), C is the mean annual intake estimated per teenager (288 L for soft drinks, 111.3 L for soft drinks based on mineral water, and 84 L for nectars), N is the total number of analysed samples, D is the number of days in a year, and K is the mean body weight, which was considered 56.5 Kg (mean of body weight of the studied population).

[b]ADIs proposed by SCF (1985).

Table V. Estimated daily intake (EDI)[a] of acesulfame-K and aspartame by Portuguese population in general through soft drinks, , and nectars and respective comparison with acceptable daily intake (ADI)

		Soft drinks		Nectars	
Sweeteners	ADI[b] (mg/kg b.w./day)	EDI (mg/kg b.w./day)	ADI (%)	EDI (mg/kg b.w./day)	ADI (%)
Acesulfame-K	9	18×10^{-2}	2.0	57×10^{-3}	0.6
Aspartame	40	0.3	0.7	26×10^{-3}	7×10^{-2}

[a]EDI was calculated using the equation EDI = $(\sum c)$ $(CN^{-1} D^{-1} K^{-1})$, where $\sum c$ is the sum of sweetener concentration in the analyzed samples related to the questionnaire (mg/L), C is the annual intake estimated per Portuguese inhabitant in 2006 (77.6 L for soft drinks, and 9.8 L for nectars according the ANIRSF, 2007), N is the total number of analyzed samples, D is the number of days in a year, and K is the mean body weight, which was considered 60 Kg (mean of body weight of the Portuguese population).

[b]ADIs proposed by SCF (1985).