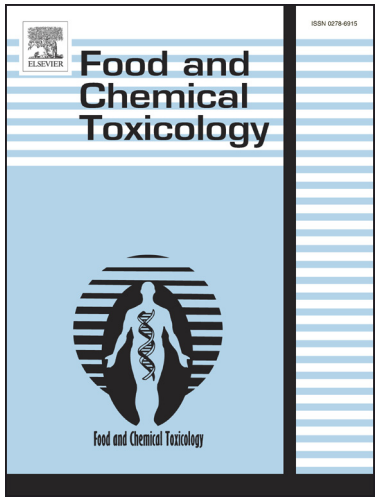

Risk assessment of additives through soft drinks and nectars consumption on Portuguese population: a 2010 survey

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PII: S0278-6915(13)00644-3
DOI: http://dx.doi.org/10.1016/j.fct.2013.09.006
Reference: FCT 7592

To appear in: *Food and Chemical Toxicology*

Received Date: 15 February 2013
Accepted Date: 6 September 2013

Please cite this article as: Diogo, J.S.G., Liliana Oliveira, S., Pena, A., Lino, C.M., Risk assessment of additives through soft drinks and nectars consumption on Portuguese population: a 2010 survey, *Food and Chemical Toxicology* (2013), doi: http://dx.doi.org/10.1016/j.fct.2013.09.006

Risk assessment of additives through soft drinks and nectars consumption on Portuguese population: a 2010 survey

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Abstract

This study investigated whether the Portuguese population is at risk of exceeding ADI levels for acesulfame-K, saccharin, aspartame, caffeine, benzoic and sorbic acid through an assessment of dietary intake of additives and specific consumption of four types of beverages, traditional soft drinks and soft drinks based on mineral waters, energetic drinks, and nectars.

The highest mean levels of additives were found for caffeine in energetic drinks, 293.5 mg/L, for saccharin in traditional soft drinks, 18.4 mg/L, for acesulfame-K and aspartame in nectars, with 88.2 and 97.8 mg/L, respectively, for benzoic acid in traditional soft drinks, 125.7 mg/L, and for sorbic acid in soft drinks based on mineral water, 166.5 mg/L.

Traditional soft drinks presented the highest acceptable daily intake percentages (ADIs%) for acesulfame-K, aspartame, benzoic and sorbic acid and similar value for saccharin (0.5%) when compared with soft drinks based on mineral water, 0.7, 0.08, 7.3, and 1.92 % versus 0.2, 0.053, 0.6, and 0.28 %, respectively. However for saccharin the highest percentage of ADI was obtained for nectars, 0.9%, in comparison with both types of soft drinks, 0.5%.

Therefore, it is concluded that the Portuguese population is not at risk of exceeding the established ADIs for the studied additives.

Keywords: additives, beverages, risk assessment, Portuguese population

1. Introduction

Initially, food preservation consisted mainly of techniques such as salting and smoking- and later, by the use of potassium nitrate, by the Egyptians. With the evolution of science and technology, new substances were developed, which play different role in food, from preservatives, antioxidants, sweeteners (EUFIC, 2012). These substances are intentionally added during manufacture, processing, preparation, treatment, packaging, transport or storage (WHO, 2010) of foods, in order to keep the quality as well as ensure food security (Lino et al., 2008).

Food additives play a vital role in food industry. Some of the most common additives used in food industry include: artificial sweeteners, such as saccharin, acesulfame-potassium and aspartame; preservatives, such as benzoic and sorbic acid; and the flavoring agent, caffeine.

Artificial sweeteners were initially added to foodstuffs for diabetics, to reduce their sugar content. However, the shortage of sugar during the World War II, and the change in the concept of aesthetics, which promoted leanness, encouraged to resort to the use of artificial sugar substitutes. Just a small concentration of these sugar substitutes is adequate to provide food with the original sweetness, but without or reduced calories. Thus, the concept that diet products were "for use only in people who must limit sugar intake" was eventually substituted for the concept that they are available "for use in people who desire to limit sugar intake" (Yang, 2010).

Usually, most consumers that select low-calorie foodstuffs and beverages added of artificial sweeteners aim at decreasing or controlling calorie intake, as means of maintaning or reducing body mass index (BMI). These products are, nevertheless, still used to control certain health or medical conditions such as diabetes.

During the past 40 years, obesity has become a pressing public health, with important current and future health consequences (Lobestein et al., 2004). Obesity may occur as a result of several causes, but is usually associated to an imbalance between consumed and expended energy (Brownell et al., 2009; Nielsen et al., 2002, 2003). This imbalance may be contributed too by sedentarism and intake of large portion meals, snacking, away-from-home meals, and consumption of sugar-sweetened beverages (Brownell et al., 2009; Nielson et al., 2003).

The consumption of sweetened beverages has been positively correlated with the incidence of obesity, due to their high caloric density. Also in recent years, many reports have shown that the intake of sugar-sweetened beverages (mainly soda and juices) is strongly and positively correlated with the increased incidence of metabolic syndrome, that includes diabetes type 2, hypertension and cardiovascular disease (Chen et al., 2009; Fung et al., 2009; Dhingra et al., 2007; Dubois et al., 2007).

Despite the benefits that some food additives apparently have, such as the reduction of the calorie intake in soft drinks and sugared fruit juices by the use of sweeteners, several studies report the existence of adverse reactions, such as allergies, behavioral changes and carcinogenicity (Sugimura and Wakabayashi, 2003; Willett, 2003; Evangelista, 2000; Schilderman et al., 1995; Poulsen, 1993; Pollock, 1991).

Allergic reactions and the occurrence of certain pathological conditions have been described as resulting from excessive intake of food additives from soft drinks (Hendriksen et al., 2011). These potential harmful effects are also considered to be dependent on the consumption frequency, as well as its amounts in kg^{-1} body weight (Polônio and Peres, 2009). However, the possible health risks associated with eating food additives are still controversial.

Many studies have reported excitotoxic effects of some additives, such as the destruction of central neurons (Rothman and Olney, 1995; Olney, 1988) and an acute neuronal degeneration of retina and brain of neonatal animals (Olney et al., 1972; Olney and Ho, 1970). It is also described that aspartame may trigger or aggravate chronic diseases, and could also mimic or exacerbate some diseases, such as multiple sclerosis (Gurney et al., 1996). Furthermore, the intake of aspartame by patient with phenylketonuria (PKU) condition, can lead to dangerously high levels of phenylalanine in the brain, which may be lethal (Garriga and Metcalfe, 1988; Janssen and Heijden, 1988).

Caffeine, a trimethylated xanthine, is almost certainly the most widely consumed psychoactive substance in the world. Caffeine-containing beverages are popular in part due to effects of decreasing fatigue, increasing mental activity and improving cognitive functioning following the intake of moderate doses. Although moderate amounts of caffeine are not harmful to human health, the possibility that caffeine consumption can have adverse effects on human health was assessed based on results of published human studies. It is thus imperative for consumers to be knowledgeable about the caffeine content of these beverages and evaluate the potential daily average intake of caffeine (Pena et al., 2005).

Preservatives are substances which prolong the shelf-life of foods by protecting them against deterioration caused by micro-organisms and/or which protect against growth of pathogenic micro-organisms (Official Journal of the European Union, 2008). Preservatives having antimicrobial properties are permitted as food additives in various food products to prevent the growth of yeasts, moulds, and bacteria in food and beverages. Sorbates (E200, E202–203) and benzoates (E210–213) are generally used in a great variety of foodstuffs (Official Journal of the European Union, 2011, Mota et al., 2003), being the preservatives most often used for beverages such as soft drinks (Dong and Wang, 2006; Ochiai et al., 2002; Techakriengkrai and Surakarnkul, 2007; Wen et al., 2007) These preservatives are allowed by legislation that establishes the maximum levels in each type of

food (Official Journal of the European Union, 2011). However, their presence at levels higher than permitted safety levels can be harmful to human health. Some adverse effects, such as metabolic acidosis, convulsions, hyperpnoea, allergic reactions in experimental animals and in humans are described (Tfouni and Toledo, 2002; Wen et al., 2007).

Maximum levels are adjusted for additives to avoid consumers from a higher intake than the acceptable daily intake (ADI) (Leth et al., 2007). ADI of all approved additives is the daily ingestion over a lifetime, without appreciable health risk, and is allocated by European Food Safety Authority (EFSA, 2009). Several studies bring in the question about the safety of food additives, mainly artificial sweeteners, reporting a similar, and sometimes stronger, relationship between the consumption of diet soda and the prevalence of metabolic syndrome, when compared to the consumption of regular soda (Nettleton et al., 2009; Fowler et al., 2008; Lutsey et al., 2008).

The aim of this study was evaluate the degree of exposure of Portuguese population to six additives and the subsequent risk assessment through soft drink and nectar consumption in 2010. In order to obtain a good analytical performance, several experimental conditions, such as the mobile phase composition, flux proportion, and wavelengths were primarily optimized using high performance liquid chromatography (HPLC) with UV detection. Afterwards, the occurrence and levels of additives in drinks were determined, in order to verify the compliance with European legislation regarding maximum permitted levels.

2. Material and methods

2.1. Sampling

A total of 78 samples were purchased in accordance with the market availability, in the central zone of Portugal. Samples were collected in supermarkets between October and December 2010. The studied commodities were 59 soft drinks (traditional soft drinks and based on mineral waters), 3 energetic drinks, and 16 nectars. Labels of the packaging contained only qualitative information about the additives, without mention of their concentrations.

2.2. Calculation of estimated daily intake

Estimated Daily Intake (EDI) was calculated through a deterministic method (IPCS, 2009, chap. 6) using the equation EDI = $(\sum c)$ $(CN^{-1}\ D^{-1}\ K^{-1})$, where $\sum c$ is the sum of additive concentration in the analyzed samples (mg/L), C is the mean annual intake estimated per person, N is the total number of analyzed samples, D is the number of days in a year, and K is the body weight. The latest assessment of the soft drinks, corresponding to 2010, is of 807.9 millions of litres (Probeb, 2012), which

correspond to 80.79 L/inhabitant, distributed by 77.5 L for traditional soft drinks and 9.8 L for soft drinks based on mineral waters and nectars. Mean body weight for the adult Portuguese population was considered 69 kg, from data retrieved from Arezes et al. (2006).

2.3. HPLC conditions and sampling preparation

Analytical separation of the additives was carried out by reverse phase liquid chromatography (Pump model 305, from Gilson, France, an injection valve with 20 µl loop model 7125 Rheodyne, Cotatim Califórnia, USA) with an Hichrom C18 column (5 µm, 250 x 4.6 mm) and a buffered mobile phase (KH_2PO_4 50mM/ACN (85:15, v/v)/phosphoric acid to control pH at 4.2-4.3), at 0.7mL/min. An UV detector, model 116 from Gilson, France, at 235 nm, was used. External standard method was used for quantification. Integration was performed with an integrator model SP4290 from Spectra-Physics, San Jose, Califórnia, USA).

Soft drinks and nectars were prepared according to Lino and Pena (2010).

2.4. Standards and standard curves preparation

Saccharin (SAC), caffeine (CAF), benzoic acid (BA) and sorbic acid (SA) were purchased from Merck, and acesulfame-K (ACE) and aspartame (ASP) from Sigma. Acetonitrile was obtained from Riedel-de Haen. Stock solutions were prepared at 1000 mg/L with mobile phase. Standard curves were done using the standard concentrations at 10, 20, 100, and 200 mg/L for SA, 10, 50, 100, and 200 mg/L for BA and CAF, and 25, 50, 100 and 200 mg/L for ACE, SAC, and ASP.

2.5. Recovery studies

Recoveries were determined by spiking a soft drink known to be free of all additives, in triplicate, with known amounts of acesulfame-K, saccharin, caffeine, aspartame, benzoic acid and sorbic acid at final concentrations between 35 and 350 mg/L for acesulfame-K, 40 and 100 mg/L for saccharin, 60 and 250 mg/L for caffeine, 75 and 600 mg/L for aspartame, 25 and 150 mg/L for benzoic acid, and 50 and 300 mg/L for sorbic acid. Limits of quantification (LOQs) were determined through spiking of blank samples with additive standard solutions. The lowest concentration which originated repeatable precision and trueness was considered the LOQ.

3. Results

3.1. Analytical performance

Several experimental conditions were tested in order to obtain adequate resolution between the studied additives (Table 1 and Figure 1). Good analytical performance was obtained using as mobile

phase KH_2PO_4 50mM:ACN (85:15) with a flow proportion of 0.7mL/min, and a wavelength of 235 nm.

The analytical methodology showed adequate linearity, sensitivity, accuracy and precision. Correlation coefficients (r^2) were 0.9763 for benzoic acid, 0.9806 for caffeine, 0.9908 for saccharin, 0.9927 for sorbic acid, 0.9942 for aspartame and 0.9949 for acesulfame-K.

Limits of quantification varied between 1.5 mg/L for acesulfame-K and 6.0 mg/L for aspartame and sorbic acid (Table 2).

Recovery values were between 66.5% for acesulfame-K at fortification levels of 100 mg/L and 109.4% for aspartame at spiked levels of 75 mg/L. Intra-day repeatability oscillated between 0.3% for aspartame at 75 mg/L, and 10.3% for caffeine at 60 mg/L (Table 2).

Figure 1 shows chromatograms of acesulfame-K, saccharin, caffeine, aspartame, benzoic acid and sorbic acid standards, and of one sample containing acesulfame-K, aspartame, benzoic acid and sorbic acid.

3.2. Occurrence of additives in drinks

The developed method was successfully applied to the determination of the six additives in 78 samples, comprising 59 soft drinks, 3 energetic drinks, and 16 nectars.

Table 3 shows the number of the positive samples, the range, the mean concentration, and the samples exceeding maximum permitted level (MPL) in traditional soft drinks, energetic drinks, soft drinks based on mineral water, and nectars, and for the all samples. For traditional soft drinks the results show high number of positive samples for the six additives involved in the present study.

For all analyzed samples, the caffeine levels ranged between 2.0-306.5 mg/L, and the mean concentration was 87.9 ± 88.2 mg/L. Twenty seven per cent of the traditional soft drinks have caffeine, which levels oscillated between 3.0 and 164.6 mg/L, with a mean concentration of 70.6 mg/L. Two samples of nectars had caffeine, although at low levels, 3.1 and 6.5 mg/L. As expected, all energetic drinks had caffeine with a mean concentration of 293.5 mg/L (ranging between 280.0 and 306.5 mg/L).

Saccharin levels oscillated between 3.2 and 80.5 mg/L, with a mean concentration of 51±26 mg/L. The occurrence and mean levels were higher in traditional soft drinks, with 15.4 % and 18.4 mg/L, respectively. Acesulfame was present in all four different types of beverages, in a frequency of 24% in traditional soft drinks, 21 % in nectars, 12% in soft drinks based on mineral water, and in one sample of energetic drinks. Nectars showed higher mean levels, 88.2 mg/L. The levels of acesulfame ranged between 1.59 and 283.7 mg/L, and the mean concentration was 82 ± 64 mg/L. Aspartame was mainly present in traditional soft drinks and in nectars, both with 10%, however nectars presented the

highest mean concentration, 97.8 mg/L. For all samples, the range was 6.10 - 568.3 mg/L, and the mean concentration 154.8 ± 141 mg/L.

Benzoic acid and sorbic acid were not detected in energetic drinks and nectars. BA and SA only were present in both types of soft drinks, traditional, 17% and 23%, and based on mineral water, 8% and 3%, respectively, with mean concentrations of 121.3 ± 19.6 mg/L (range 69 - 150 mg/L) for BA, and 148.4 ± 87 mg/L for SA (range 30 to 319.8 mg/L). Mean concentration for the sum of both preservatives was high, 288.9 ± 98.6 mg/L. The simultaneous presence of these only occurred for the two types of soft drinks analyzed, nine for traditional and one based on mineral waters.

According to the Portuguese legislation (Decreto-Lei no. 394/98 de 10 de Dezembro de 1998) the maximum permitted level (MPL) for saccharin in soft drinks and nectars is 80 mg/L. This level was exceeded in one sample of traditional soft drink with a concentration of 80.5 mg/L. For acesulfame-K and for aspartame, the MPL for the same kind of products (350 mg/L and 600 mg/L, respectively) was not exceeded. The limit of 150 mg/L for benzoic acid and 300 mg/L for sorbic acid if they are used separately, 150 and 250 mg/L, respectively, if they are used in combination, and one maximum limit of 400 mg/L for the sum of both preservatives (EU, Directive 95/2/EC, 1995; Decreto-Lei no 363/98, 1998). Tree samples exceeded the MPL for sorbic acid, and two exceeded for the sum of both. Maximum permitted level of saccharin, caffeine, sorbic acid and for the sum of both preservatives (benzoic and sorbic acid) were overlapped in 1, 3, 3 and in 2 samples, respectively.

3.3. Estimated daily intake by Portuguese consumers and risk assessment

Based on the data described before (Section 2.2), the estimated daily intake (EDI) of different additives by Portuguese adult population was comprised between 6.8×10^{-5} mg/kg body weight/day for saccharin, when nectars are ingested, and 0.903 mg/kg body weight/day for caffeine as a result of energetic drinks consumption (Table 4).

The EDIs of six additives were below the acceptable daily intakes (ADIs) established by WHO/JECFA/SCF committees (SCF, 2000, EFSA, 2006, WHO Joint FAO/WHO, 2009). The EDIs in the present study represent an ADI % oscillating between 0.02% for aspartame in nectars and 7.3% for BA in traditional soft drinks (Table 4).

Giving this oversight, none of the additives in different groups of beverages represents a risk to this population.

4. Discussion

The analytical performance of analytical methodology is adequate according to the international parameters established for accuracy and precision (Report of the AOAC/FAO/IAEA/IUPAC Expert, 1999).

A previous study in Portugal on the presence of BA and SA in soft drinks, performed by Lino and Pena (2010) showed a similar high number of positive samples for both preservatives with similar concentration ranges and mean concentrations. Survey on presence of BA in soft drinks in USA, conducted by Walker et al. (1997), revealed that the range found was higher than those reported in the present study. However, studies in Spain (Suárez et al., 1997), and China (Wang et al., 2006) revealed similar results with the studies made in Portugal, for benzoic acid and sorbic acid, respectively. Only for benzoic acid the results were in agreement with the study accomplished in United States by Ree and Stoa (2011).

The results found for saccharin were below than those found previously in Portugal (Lino and Pena, 2010), Spain (Suárez et al., 1997), and USA (Tyler, 1984), and were higher than those observed in Denmark (Leth et al., 2007). However, the results of this study were similar to that conducted by Ree and Stoa (2011) in United States. In this study, caffeine occurred in a larger number of samples, when compared with the results reported by Lino and Pena (2010), but in similar concentrations. The levels for caffeine in USA (Walker et al., 1997) and in Spain (Suárez et al., 1997) are higher.

Aspartame was detected mainly in traditional soft drinks and nectars (10%). There is few published data to compare our results, however the levels for aspartame in soft drinks were similar to those found by Ree and Stoa (2011). Concerning the presence of aspartame in soft drinks, in Portugal, Lino et al. (2008) found levels between 19 and 154 mg/L in soft drinks based on mineral water, lower than those obtained in the present study (50-568.3 mg/L). In Barcelona the levels found by Suárez et al. (1997) were between 130 and 676 mg/L. These results are similar with those found in the present study. In Denmark, one study reported levels of 159 and 56 mg/L for soft drinks with and without carbon dioxide, respectively (Leth et al., 2007). In China, Zhu et al. (2005) found levels of 7234.6 and 2826.3 mg/L in two samples of cola soft drinks. The values in these countries are higher than those found in the present study, except for soft drinks without carbon dioxide from Denmark.

The results found in the present study for acesulfame were below to those found by Lino et al. (2008), in Portugal. As far as we know, the present study is the third study of nectar samples, being the two previous studies carried out in Portugal (Lino et al., 2008, 2010), and Switzwerland

(Prodolliet and Bruelhart, 1993). Nectar samples from this country, exhibit aspartame in one exotic fruit nectar at concentration of 2416 mg/L. In the Portuguese study (Lino et al., 2008), aspartame was found in concentrations between 39 and 129 mg/L and acesulfame between 35 and 356 mg/L. The results of the present study are far below than the results from Switzerland, but similar with levels previously found in Portugal (Lino et al., 2008).

As regards acesulfame-K levels, the results found in the present study for the totality of samples are similar with those found by the other study in Portugal (Lino et al. 2008), but are higher than those found by Leth et al (2007) in Denmark, and lower than those referred to by Zhu et al. (2005) in China. In Denmark, a study of light soft drinks with and without carbon dioxide showed mean levels of acesulfame-K of 129 and 62 mg/L, respectively (Leth et al., 2007). In China, Zhu et al. (2005) found levels of acesulfame-K of 1105.4 and 1475.2 mg/L in two cola drinks.

The results obtained in this study concerning the MPLs for SA are similar to those observed by Lino and Pena (2010), in which one sample of each type of soft drinks exceeded that limit.

Due to the paucity of data about the risk assessment resulting from additives consumption in this kind of drinks, a comparison between results of this study and from other countries is difficult. Moreover, the Portuguese consumption data by age is not available by (Probeb, 2012) and this fact can be considered as a limitation.

Regarding the results of this survey, the estimated daily intake of saccharin, acesulfame-K and aspartame, and its comparison with the respectively ADI, were below than the results obtained by Lino et al. (2008) and Lino and Pena (2010), for soft drinks, traditional and based on mineral water consumed in Portugal. These studies showed EDIs oscillating between 26 x 10^{-3} mg/kg b.w./day for ASP through the nectar consumption (Lino et al., 2008) and 0.32 mg/kg b.w./day for BA in soft drinks based on mineral waters (Lino and Pena, 2010), representing 0.07 and 6.4% of the ADI, respectively. As far as we know, the highest values were found for ASP, in a Canadian population aged between 2 and 6 years, with 10% of the ADI (Devitt et al. 2004), and for BA in Brazil oscillating between 18% of the ADI (IBGE data) or 54% (Datamark data) (Tfouni and Toledo, 2002). As observed by Tfouni and Toledo (2002) for benzoates, in the present study traditional soft drinks contribute substantially to the intake of the six studied additives.

Conclusions

The developed analytic method showed to be suitable for quality control, provide adequate linearity, sensitivity, accuracy and precision for the routine monitoring of saccharin, acesulfame-K, aspartame, caffeine, benzoic acid and sorbic acid.

The levels found in these four different types of drinks analyzed, were not of concern for consumer health, because the estimated daily intake (EDIs) values are much lower than the respective ADIs for each additive. However, there is a need to ensure compliance with additives legislation. Since maximum permitted level of some additives were overlapped, manufactures have taken measures to avoid reoccurrence of the problems related to levels above statutory limits.

Acknowledgements

This study was supported by FCT through the project vPEst-OE/SAU/UI0177/2011.

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Figure caption:

Figure 1. Chromatograms of (a) acesulfame-K (rt=5.14), saccharin (rt=5.67), caffeine (rt=7.56), aspartame (rt=14.68), benzoic acid (rt=25.69) and sorbic acid (rt=32.21) standards containing 2 µg of each compound, (b) one sample containing aspartame (rt=14.63), benzoic acid (rt=25.62) and sorbic acid (rt=32.17).

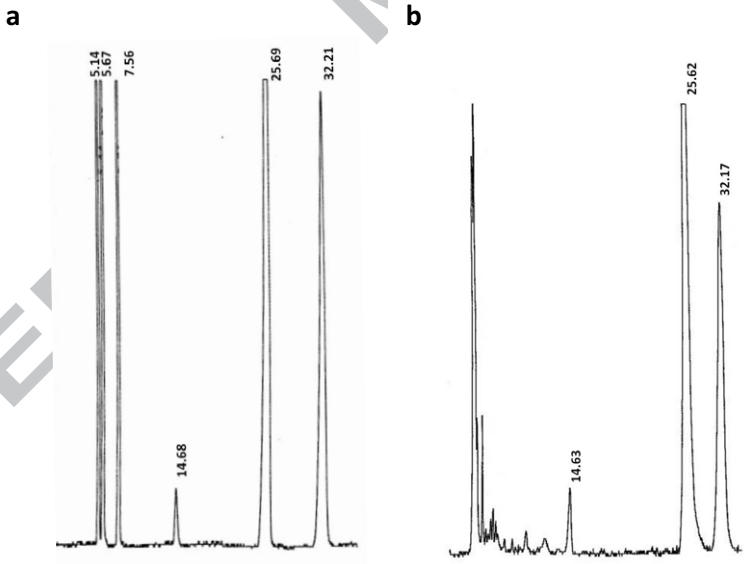

Table 1. Several experimental conditions

Conditions	Composition of mobile phase	Wave-lenght (nm)	Flow (mL/min)	Conclusions
1	KH_2PO_4 50 mM:ACN 60:40	255	1	Didn't have separation between SAC and ASP
2	KH_2PO_4 50 mM:ACN 80:20	255	1	ASP not detected or not elute
3	KH_2PO_4 50 mM:ACN 80:20	235	1	ASP not detected or not elute
4	KH_2PO_4 50 mM:ACN 90:10	235	0,8	ACE rt = 7.08 SAC rt = 9.59 ASP - n.d.
5	KH_2PO_4 50 mM:ACN 85:15	245	0,8	ACE rt = 7.06 SAC rt = 9.59 ASP - n.d.
6	KH_2PO_4 50 mM:ACN 80:20	235	0,7	ACE rt = 4.46 SAC rt = 4.87 CAF rt = 6.07 ASP rt = 9.57 AB rt = 22.51 AS - n.d.
7	KH_2PO_4 50 mM:ACN 82:18	235	0,7	ACE rt = 5.19 SAC rt = 5.48 CAF rt = 6.67 ASP rt = 12.59
8	KH_2PO_4 50 mM: ACN 85:15	235	0,7	ACE rt = 5,14 SAC rt =5.67 CAF rt = 7.56 ASP rt = 14.68 AB rt = 25.69 AS rt = 32.21

Table 2. Validation studies of the analytical method.

Additives	Fortification level (mg/L)	Exactitude (n = 3)	Precision (%)	LOQ (mg/L)
ACE	35	102.1	5.2	1.5
	100	66.5	8.3	
	350	87.5	1.1	
SAC	40	93.8	5.4	3.0
	80	74.9	6.7	
	100	88.2	7.3	
CAF	60	105.1	10.3	2.0
	120	104.9	5.1	
	250	92.6	3.4	
ASP	75	109.4	0.3	6.0
	200	95.3	2.0	
	600	99.6	1.7	
BA	25	103.6	7.6	3.0
	80	96.3	2.2	
	300	97.9	4.1	
SA	50	94.6	2.4	6.0
	200	87.8	0.8	
	300	80.0	5.0	

Table 3. Occurrence, range, mean concentration, and number of samples exceeding MPL of additives in traditional soft drinks, energetic drinks, soft drinks based on mineral water and nectars.

	Traditional soft drinks	Energetic drinks	Soft drinks based on mineral waters	Nectars		Totality of samples									
	Occurrence (%)	Range (mg/L)	Mean (mg/L)	Occurrence (%)	Range (mg/L)	Mean (mg/L)	Occurrence (%)	Range (mg/L)	Mean (mg/L)	Occurrence (%)	Range (mg/L)	Mean (mg/L)	Range (mg/L)	Mean (mg/L)	> MPL
Saccharin (SAC)	12 (15.4)	26.5 - 80.5	18.4	0	-	n.d.	1 (1.3)	0-3.20	0.2	1 (1.3)	0-13.6	0.8	3.20 - 80.5	51 ± 26	1
Acesulfame-K (ACE)	19 (24)	18.2 - 178.5	38	1 (1.3)	0-198.8	66.3	9 (12)	3.2 - 95.5	34.1	16 (21)	47.1-283.7	88.2	1.59 - 283.7	82 ± 64	0
Aspartame (ASP)	8 (10)	26.4 - 236.7	16.3	1 (1.3)	0-100	33.3	4 (5)	50-568.3	56.3	8 (10)	6.10-493.5	97.8	6.10 - 568.3	154.8 ± 141	0
Caffeine (CAF)	21 (27)	3-164.6	70.6	3 (3.8)	280-306.5	293.5	-	-	-	2 (3)	3.1 - 6.5	4.8	2.0 - 306.5	87.9 ± 88.2	3

Benzoic Acid (BA)	13 (17)	83.5-150	125.7	0	-	-	6 (8)	69-130	108.5	0	-	-	69.0 - 150.0	121.3 ± 19.6	0
Sorbic Acid (SA)	18 (23)	30-319.8	146.4	0	-	-	2 (3)	94.5-238.5	166.5	0	-	-	30.0 - 319.8	148.4 ± 87.0	3
BA + SA	9 (12)	165.5-443.6	275.3	0	-	-	1 (1.3)	-	*	0	-	-	165.6 - 443.6	288.9 ± 98.6	2

*only one sample presented BA+SA with 224.5 mg/L

Table 4. Estimated daily intake (EDI) of the six additives by Portuguese population through soft drink, energetic drink and nectar consumption, and the respective comparison with acceptable daily intake (ADI).

Additives	Traditional soft drinks		Soft drinks based on mineral water	Energetic drinks		Nectars				
	ADI[ab]	EDI[a]	ADI(%)	EDI[a]	ADI(%)		EDI[a]	ADI(%)	EDI[a]	ADI(%)
Saccharin	5	0.024	0.5	1.26×10^{-4}	0.5		-	-	6.8×10^{-5}	0.9
Acesulfame-K	9	0.07	0.7	0.02	0.2		-	-	7.0×10^{-3}	0.07
Caffeine	-	0.43	-	-	-		0.903	-	-	-
Aspartame	40	0.03	0.08	0.03	0.053		-	-	0.008	0.02
Benzoic acid	5	0.365	7.3	0.03	0.6		-	-	-	-
Sorbic acid	25	0.48	1.92	0.07	0.28		-	-	-	-

[a] calculated in mg/kg b.w./day.
[b] ADIs proposed by SCF (2000)
[c] EDI was calculated using the equation EDI = $(\sum c)$ $(CN^{-1}D^{-1}K^{-1})$, where $\sum c$ is the sum of additive concentrations in the analyzed samples (mg/L), C is the mean annual intake estimated per Portuguese inhabitant in 2010 (77.5 L for soft drinks, and 9.80 for soft drinks based on mineral water and nectars according the Probeb, 2012), 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 69 kg (mean of body weight of the Portuguese population from data retrieved from Arezes, Barroso, Cordeiro, Costa, and Miguel, 2006).

Highlights

► Six additives in different drinks collected in Portugal were investigated

► Traditional soft drinks showed the highest mean levels for saccharin and benzoic acid

► Dietary exposure to additives of the Portuguese adult population was assessed

► The results show that the Portuguese population is not at risk of exceeding the ADIs