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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.

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 maintaining 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 (Lobstein 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; Nielsen and Popkin, 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; Nielsen and Popkin, 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

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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, behavioural 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 vander-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, Chapter 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 a Hichrom C18 column (5 µm, 250 × 4.6 mm) and a buffered mobile phase (KH_2PO_4 50 mM/ACN (85:15, v/v)/phosphoric acid to control pH at 4.2–4.3), at 0.7 mL/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.