ABSTRACT – Many non-carbonated soft drinks include additives, mostly benzoic and ascorbic acids, to prolong their shelf life. However, over time these compounds tend to react, producing benzene, a known carcinogen. This study analysed, in Rio de Janeiro, the regular and low-calorie versions of three brands of commercial non-carbonated guarana soft drinks, assessing their chemical quality. Benzoic acid and benzene quantification was carried out using, respectively, high-performance liquid chromatography (HPLC) and gas chromatography coupled to solid phase micro-extraction headspace (HS-SPME-GC). In some samples, benzoic acid and benzene concentrations are above the thresholds established by ANVISA, in Brazil: 500 µg.mL\(^{-1}\) in soft drinks and 5 µg.L\(^{-1}\) in potable water, respectively.

1. INTRODUCTION

Interactions involving sodium benzoate and ascorbic acid have been target of research, as it results in benzene formation, a substance which chronic exposure can cause from vomiting to leukemia (Weisel, 2010). This reaction, due to benzoate ion decarboxylation by hydroxyl radicals formed by the reduction of oxygen in the presence of ascorbic acid, catalysed by traces of metal ion, is accelerated with exposure to heat, such as during the transportation and storage of beverages (Gardner; Lawrence, 1993).

Considering many instances of benzene in non-alcoholic beverages reported in the literature (Sanchez \textit{et al.}, 2012), studies about quantification of the preservative precursor and identification of benzene in food matrices are of general concern for public health.

2. MATERIALS AND METHODS

Regular and low-calorie versions of three brands (A, B and C) of non-carbonated commercial guarana soft drinks were analyzed. For each soft drink, four different batches were tested, three samples for batch. All analyzes were performed with the drinks before the expiration date, totalizing 72 sample units analyzed. The sample units were polypropylene (PP) cups heat- and pressure-sealed with aluminum foil with capacity for 290 mL.
Benzoic acid determination and quantification: The benzoic acid analysis was performed using high-performance liquid chromatography (HPLC) according to the methodology proposed by Mota et al. (2003). Reverse phase column Hypersil ODS (4.6 X 250 mm, 5 µM) was used. Mobile phase consisted of 0.005 M acetate buffer (pH = 4.4) and methanol (65:35) with a flow rate of 0.8 mL.min⁻¹ and UV detection at 235 nm. The standards were benzoic acid solution in HPLC grade methanol at concentrations ranging from 50 to 1000 µg.mL⁻¹.

Preceding the analysis, samples of guarana soft drink were centrifuged for 20 min at 3000 rpm and purified with C18 cartridge previously activated. The activation was performed by passing 2 mL of methanol followed by 4 mL of water, and for purification, 1 mL of the supernatant obtained after centrifugation of the drink was passed through the cartridge, cleaned with 4 mL of n-hexane and then eluted with 3 mL of methanol. The resulting methanol extract was filtered through a 0.45 µM membrane.

Benzene determination and quantification: The determination of benzene was carried out by gas chromatography coupled to solid phase micro extraction headspace (HS-SPME-GC) following the methodology proposed by Sanchez et al. (2012) with modifications. The chromatograph used was GC-2010 Plus (Shimadzu), associated with FID and MS detectors. The mass spectrometer (MS) was used to identify and later the ionization detector (FID) was used for the quantification of benzene.

The CAR-PDMS (carboxen-polydimethylsiloxane) fiber was used for the adsorption of benzene in the matrix and subsequent desorption in GC injector at 300 °C for 5 minutes. The standards were solutions with concentrations ranging from 0.5 to 15 µg.L⁻¹ of benzene in water prepared from benzene in methanol ampoule (200 µg.mL⁻¹). A volume of 10 mL of both standards and samples was added to 3 g of sodium chloride (NaCl) and placed in vials of 20 mL. The vials were submitted to an ice bath at 0 °C for 15 minutes while the fiber was exposed to vial’s vapor phase, rich in benzene.

The injector temperature was maintained at 300 °C, the capillary column used was Restek RTX-1 (30 m X 0.25 mm X 0.25 µM) with helium gas flow rate of 5.5 mL.min⁻¹. The temperature was raised from 40 °C to 100 °C at a rate of 10 °C.min⁻¹, followed by 15 °C.min⁻¹ until 180 °C. These conditions were maintained in analysis using MS and in analysis using FID.

3. RESULTS AND DISCUSSION

Figure 1 shows the concentrations of both benzoic acid and benzene for three brands of guarana based soft drinks in regular and low-calorie versions. Benzoic acid limit allowed in soft drinks is 500 µg.mL⁻¹ (Brazil, 2007), and from analytical decision limit calculation (CCₐ = MRL + 1.64.sreproLMR) (ISO, 2000), all values above of 501.76 µg.L⁻¹ can be considered over the maximum allowed. Benzene limit in water is 5 µg.L⁻¹ (Brazil, 2011), and after the calculation of analytical decision limit, all values above 6.64µg.L⁻¹ can be considered over the limit.
Figure 1. Benzoic acid and benzene concentrations for guarana soft drinks samples and their limits

Brands A, B and C – regular versions.
Brands Adiet, Bdiet and Cdiet – low-calorie versions.
Numbers 1, 2, 3 and 4 on y axis refer to batches.

In six batches, the benzoic acid concentration was above Brazilian regulatory limits (Brazil, 2007). The fact that industries use benzoic acid in excess demonstrates the lack of control in weighing the supplies, which reflects directly on microbial growth and, probably also in the formation of benzene. Weighing of components, including preservatives, is usually performed manually in this industry and should, therefore, be considered a critical point in the process as it depends on the precision of the operator.

In four batches of guarana soft drinks benzene concentrations were above the levels allowed for drinking water by regulations of both the Brazilian Ministry of Health and the FDA (Brazil, 2011; FDA, 2009). Three of those batches also had benzoic acid levels above regulatory limits, which is significant as benzene is formed from benzoic acid decarboxylation. This reaction is favored at higher temperatures, suggesting that the presence of benzene above the threshold in some batches may have been due both to problems in production (excess benzoic acid) and in storage or commercialization (storage at too high temperatures) (Nyman et al., 2008). Neither the United States nor Brazil have specific legislation for benzene content in non-alcoholic beverages other than water.

4. CONCLUSION

By the methodology applied, taking into account the limits of decision (CCα inherent to analytical procedures and sample standard deviations) seven batches, representing 29.17% of total batches, presented benzoic acid concentration above 500 µg.mL⁻¹, which is the maximum
allowed by law. Regarding benzene, four samples showed concentration value above 5 µg.L⁻¹, maximum established for drinking water.

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