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Determination of benzoic acid and sorbic acid in foodstuffs by high performance liquid chromatography with UV detection

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ABSTRACT

Benzoic and sorbic acids are two food additives used to preserve food products by inhibiting microorganism growth. EFSA and FDA recognized both substances as being safe to consumers, however there are some concerns arising from their wide occurrence in food, cosmetics and pharmaceutical products, that can lead to an increase of the daily intake and subsequent risk of exceeding the acceptable daily intake (ADI). That said, occurrence data through exposure assessment studies are essential to ensure consumer safety.

In this study, 23 food products (soft drinks, fruit juices, quince jams, yogurts, sauces, and bakery and cake products) commercially available in the market, were evaluated using a validated HPLC method with UV detection based on European Standard 12856.

Benzoic and sorbic acids were found in concentrations ranging from 94 to 824 mg kg^{-1} or mg L^{-1} and 91 to 1298 mg kg^{-1} or mg L^{-1}, respectively. All samples were within the limits permitted by the legislation.

1. INTRODUCTION

Benzoic acid (BA), sorbic acid (SA) and their sodium and potassium salts (benzoates and sorbates), are commonly used food preservatives as they prevent the growth of bacteria, yeast and moulds [1].

BA inhibits bacterial development, having greater activity for acidic food products (pH ≤4.5), like beverages and sauces. His sodium salt is preferably used due to its greater solubility in water. This preservative can be found in nature in fruits, in spices and in dairy products as a by-product of microbial degradation of hippuric acid [2]. BA exhibits low toxicity, because it is absorbed from the gastrointestinal tract and rapidly metabolized and excreted in urine within 24 hours. However, some concerns arise from studies that revealed some adverse effects, such as metabolic acidosis, hyperactivity in children and allergic reactions in humans and animals [3-5]. In addition, there are reports that refer the formation of benzene in beverages due to the reaction between BA and ascorbic acid [6]. Its acceptable daily intake (ADI) value of 0-5 mg kg body $weight^{-1}$ day^{-1} was established by the European Scientific Committee on Food (SFC).

SA is an antifungal preservative against moulds and yeast being used for food products of higher pH (pH≤6.5). Toxicologically, SA is an unsaturated fatty acid and therefore is

metabolized by β-oxidation pathway [3]. The SFC established an ADI value of 0-25 mg kg body weight^{-1} day^{-1}for this food additive.
In order to ensure consumer safety, reliable analytical methods are crucial to identify and quantify these substances, and estimate intake values.

2. MATERIALS AND METHODS

2.1 Reagents and instrumentation

BA (≥99.9%) and SA (≥99.9%) were purchased from Merck (New Jersey, USA). *Carrez* solutions were prepared with potassium hexacyanoferrate (II) trihydrate and zinc sulphate heptahydrate, purchased from Merck. The binary mobile phase was constituted by HPLC grade acetonitrile and a buffer of potassium phosphate monobasic, both from Merck. All standards and sample solutions were prepared with water type 1 (18.5MΩ, 25ºC), from Mili-Q system. Samples were filtered using PES membrane syringe filter (25 mm, 0.45 µm), from VWR (Pennsylvania, USA). Chromatographic separations were performed on a Grace (Columbia, USA) C18 analytical column (250 x 4.6 mm, 5 µm).
HPLC analyses were carried out on a WATERS (Massachussets, USA) Alliance e2695 separation module and a PDA detector system. Data acquisition and instrument control were performed using Empower software. A pH meter 780 from Metrohm (Herisau, Switzerland) was used to adjust the pH of the mobile phase. All samples and mobile phases were dissolved/degassed using a Branson 3510 sonicator, from VWR. Solid samples were homogenized using two knife mills GRINDOMIX GM 200 and GM 300 from Retsch (Haan, Germany).

2.2 Standard solution preparation

Standard stock solutions were prepared by weighing approximately 50 mg of BA and SA and dissolving them in a 50 mL volumetric flask with 5 mL of acetonitrile and 45 mL of water. From the standard stock solutions, a dilution series of six concentration points (4, 10, 16, 24, 32 and 40 µg mL^{-1}) was performed to prepare the calibration standard solutions.

2.3 Samples and sample solution preparation

A total of 23 samples comprising soft drinks and beverages, quince jam, dairy products, emulsified and non-emulsified sauces, bakery and pastry products were purchased from retailers around Lisbon. Not all samples had the preservatives listed on their label but were analyzed to confirm their absence.
For liquid samples, an aliquot of 10 mL were transferred to a 100 mL volumetric flask containing 50 mL of water. To the previous solution, 2 mL of *Carrez* solution I and 2 mL of *Carrez* solution II were added. The solution was vigorously shaken and allowed to stand for

10 minutes and made up to volume with water. Carbonated beverages were sonicated for 30 minutes to remove dissolved gases.
All solid and semi-solid matrices were homogenized in a knife mill. Then, 10 g of each sample was weighted to a 100 mL volumetric flask containing 50 mL of water. To improve the sample dissolution, an ultrasonic bath step at 40ºC was performed for 20 minutes. After cooling down, the same procedure for the liquid samples was carried out.
All sample solutions were filtered before transferred to the autosampler vials.

2.4 Analytical method

The analytical method used in this study was previously validated and is based on EN 12856. A gradient elution method was performed to detect and quantify other food additives present in the samples. Chromatographic separations were achieved within 55 min at a flow rate of 1.0 ml min^{-1} and column temperature of 37 ºC. The injection volume was 20 µL for standards, and 10-20 µL for samples (to bring analyte concentration within the range of the method). The binary mobile phase was composed of phosphate buffer 0.0125 mol L^{-1} pH 3.5 and acetonitrile. Analytes detection was made at 220 nm and external standard method was used for absolute quantification.

3. RESULTS AND DISCUSSION

All samples were analyzed in duplicate, and a third sample was prepared and spiked to perform recovery studies.
The results obtained are presented in Table 1 in terms of range of concentration for each class of food product, with the respective maximum permitted level (MPL), according to the European regulation.

Table 1. Range of concentration of BA, SA or BA+SA obtained for all food samples and MPLs.

	Range (mg kg^{-1})		MPL (mg kg^{-1})		
	BA	SA	BA	SA	BA+SA
Soft drinks and beverages[1]	94 - 118	91 - 161	150	300	150+250
Quince jams	338 - 824	342 - 491	NA[2]	NA	1500
Dairy products[1]	ND[3]	97 - 117	NA	NA	300
Non-emulsified sauces	394	304 - 641	NA	NA	1000
Emulsified sauces	ND	671 - 1217	NA	2000	NA
Bakery products	ND	1298	NA	2000	NA
Pastry products	105	175	NA	NA	1500[4]

[1] Concentration of liquid samples and their MPLs are presented in mg L^{-1}
[2] NA – Not applicable
[3] ND – Not detected
[4] In Commission Regulation 1129/2011 it is stated a maximum limit of 1500 mg kg^{-1} for the combination of benzoates, sorbates and parabens

Soft drinks and beverage samples presented lower levels of both preservatives. No sample has overlapped the MPLs of 300 mg L^{-1} and 150 mg L^{-1} for SA and BA, respectively, or 250+150 mg L^{-1} when used in combination. Lino and Pena, 2010, did an extensive assessment study for the presence of food sweeteners and preservatives on traditional soft drinks and soft drinks based on mineral waters [7]. The present work showed results like this previous study. Yoghurt samples revealed the presence of both substances at low concentration and lower than the MPL of 300 mg L^{-1} of SA and BA.

Quince jam samples proved to have more complex matrices resulting in more heterogeneous results regarding BA. Comparing the results to those obtained for Ferreira *et al.*, 2000, the same outcome was verified [8]. All samples have BA only and at a concentration lower than the MPL of 1500 mg kg^{-1}.

Regarding emulsified and non-emulsified sauces all concentrations were within the MPLs of 2000 mg kg^{-1} of SA and 1000 mg kg^{-1} of BA. However, one sample presented both BA and SA, a situation that is not predicted in the legislation.

The pastry sample showed a reduce concentration of both preservatives when compared to the MPL of 1500 mg kg^{-1}.

Bakery products have the greatest amount of SA equal to 1298 mg kg^{-1} but within the MPL of 2000 mg kg^{-1}.

Recovery values were between 93% and 105% for BA and 93% and 116% for SA at spiked levels of 50% of the MPL.

4. CONCLUSIONS

The method used in the present work has proved to be suitable for a wide variety of food matrices.

Of the 23 samples analyzed, all were within the limits imposed by Portuguese legislation, and the absence of both preservatives in samples whose label did not mention their presence was also confirmed.

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