

# Assessment of Benzoic Acid, and Benzene in “Pimenta-Da-Terra” Red Hot Pepper (*Capsicum Sp*) Processed Traditionally

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## Abstract

Traditionally made red hot pepper products (“pimenta-da-terra”), from ten different producers were assessed as potential sources of benzene, benzoic acid or sodium benzoates, to find whether traditional producers comply with the established regulations concerning these preservatives. Benzene was absent in all non-cooked and cooked samples, and the values for benzoic acid ranged in 887-1685 mg kg<sup>-1</sup>, which are below maximum values (i.e., 2000 mg kg<sup>-1</sup>) established by the European Commission for this preservative in vegetable food products excluding olives. Forty percent of the samples showed complete absence of both substances. Our results suggest that producers comply in general with the established limits in using benzoic acid as a preservative in PT products. However, considering the observed wide range of values, it may be concluded that traditional processors use this preservative in a “*quantum satis*” non-standardized fashion, as long as it is below the established regulations.

Because these preservatives may be added to many other food products, more data – including the average consumer uptake of specific foods - is required to estimate average daily benzoic acid intake for Azorian consumers.

**Keywords:** Benzoic acid; Hot pepper; Safety; Traditional foods; HPLC; GC

## Introduction

Benzoic acid (E210) or its sodium benzoate (E211) is a common ingredient added as preservative in many foodstuffs, toothpastes and cosmetics creams, and values ranging in 226 to 1776 mg kg<sup>-1</sup> have been reported, in beverages, processed fruits and vegetable products [1]. However, benzoic acid may as well occur naturally in plant and animal products at levels not exceeding 40 mg kg<sup>-1</sup> [2].

After oral uptake, both additives are rapidly absorbed from the gastrointestinal tract and metabolized in the liver, resulting in the formation of hippuric acid, which, in general, is rapidly excreted via the urine [3] thus, its toxicity to humans is considered to be very low. However, they may cause non-immunological contact reactions exacerbated in patients with frequent urticaria or asthma, while there are also carcinogenic concerns [4]. Benzoic acid itself is only slightly soluble in water, thus sodium benzoate — which, under acid conditions, converts to undissociated benzoic acid - is often used instead. The European Commission Regulation states in its Article 27 that “Member States shall maintain systems to monitor the consumption and use of food additives on a risk-based approach and report their findings with appropriate frequency” [5], while responsible authorities are strongly encouraged to characterize risk on the basis of locally measured or predicted exposure scenarios [3].

In many products a trend of increasing formation of benzene has been associated to the presence of sodium benzoate and ascorbic acid, after exposure to heat and or light and low pH [6,7]. Recall that benzene is confirmed as a carcinogenic substance, and consumption of foods containing high levels of benzene can result also in symptoms such as vomiting, irritation of the stomach, dizziness, sleepness, convulsions, rapid irregular heartbeat and death [8].

“Pimenta-da-terra”, (PT) hot pepper products are popular for cooking, as well as for processing traditional food products by the local industry in Azores. In preliminary studies we characterized the traditional processing method, the microbial and chemical composition

of PT hot sauce produced in Azores, and we showed that producers add high amounts of salt (10-15%) and benzoic acid as main preservatives. PT products can be ingested directly (without heat treatment), or via food products subject to cooking, in which case there are concerns that benzene may be formed. The increasing popularity of many traditional foods, such as PT products, produced with little sophistication and likely less control, may lead to a potentially increased consumer exposure to these substances, making their monitoring an important issue in public health.

The goal of this work was to assess the levels of benzoic acid/sodium benzoate and benzene in “pimenta-da-terra” products produced in Azores to find whether traditional producers comply with the established regulations.

## Materials and Methods

Different pepper products (n=10) were obtained from the local market and transported to our laboratory for analysis. All samples were analyzed before and after cooking for 10 min in a closed pan.

## Determination of benzene

Benzene in PT samples was analysed by Gas Liquid Chromatography, (Trace GC Ultra, Thermo Electro Corporation), with Mass Spectrometry detection (Polaris Q, Thermo Electro Corporation)

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after a pre concentration step in a Purge and Trap system (Purge and Trap concentrator, Hewlett Packard).

Internal standard fluorobenzene, was added to the sample placed in the Purge Trap vessel, heated at 70°C and purged for 15 min, followed by desorption of the volatile adsorbed to the trap with helium at a 225°C for 5 min. The chromatographic separation was performed in a 60 m long DB23 column, 0.25 i.d. and 0.25 µm film thickness. The oven was with a ramp rate of 5°C/min from 35°C to 150°C. The injector temperature was set at 275°C. The carrier gas (helium) was set at a constant pressure of 70 KPa and split at 20 mL/min. The MS transfer line was at 200°C, the ionization was by electronic impact of 70 eV, mass range was from 35 to 350 amu, and the ions source was at 200°C. Identification of peaks was based in relative retention times and matching of mass spectral data from sample with data bases from the equipment libraries.

After the validation process a limit of quantification of LQ = 0,1 ng g<sup>-1</sup>, was reached.

### Determination of benzoic acid

Benzoic acid and sodium benzoate were determined by a RP-HPLC method, ISO 9321:2008.

The extraction of preservatives was made by adding NaOH 0.1 M to a weighed sample amount, followed by pH adjustment to 8.0 and addition of Carrez solutions to promote the precipitation of protein and fats of the sample. The resultant solution was diluted with methanol and the supernatant liquid was filtered (Minisart RC, 0.45 µm, Sartorius). Benzoic acid was separated by HPLC (Waters, Alliance including Empower software), on a reversed-phase C18 column (Waters Bondapak C18 10 µm, 3.9×300 mm), measuring the absorbance at 227 nm (Waters 996 PDA detector). A solution of methanol/phosphate buffer was used as eluent in an isocratic mode and quantification was made by standard external method (benzoic acid reagent, 99.5%, Sigma-Aldrich).

### Statistical analysis

The means of detected preservatives in non-cooked and cooked samples were compared via Analysis of Variance (ANOVA) at 0.05 significance level, using the software MicroCal Origin v 2.8 (Northampton, MA, USA)

### Results and Discussion

The results of the analysis of PT products are show in Table 1. Benzoic acid was detected in 60% of the samples ranging in 887-1685 mg kg<sup>-1</sup>, while the remaining of the samples did not show presence of this preservative. These values are below the limit of 2000 mg kg<sup>-1</sup> established by the EU Commission regulation, for vegetable food products [5] while most samples showed values in the range of those reported elsewhere [1]. Our work also included the assessment of benzoic acid in a sample of ready to eat lutein beans, (a very popular appetizer in Portugal), which showed the lowest value (75 mg kg<sup>-1</sup>) of this preservative.

Although the mean value of benzoic acid in cooked samples was lower than in non-cooked samples (1251 vs 1318 mg kg<sup>-1</sup>, respectively) the difference was not significant, meaning said difference cannot be statistically attributed to cooking the samples. Finally, we were able to monitor the presence of benzene, a substance less frequently determined in food products, due to the expertise required [9,10] and concluded that no sample had detectable amounts of benzene. In our preliminary studies we reported pH values of 3.5 for PT products, a value in the

Food Product	Benzen [mg kg <sup>-1</sup> ]	Benzoic acid [mg kg <sup>-1</sup> ]	
		Before cooking	After cooking
A	ND	994	968
B	ND	ND	ND
C	ND	1609	1460
D	ND	921	887
E	ND	1351	1320
F	ND	1716	1620
G	ND	ND	ND
H	ND	ND	ND
I	ND	ND	ND
J	ND	83	105

ND – Not Detected

**Table 1:** Quantification of benzoic acid and benzene in red hot pepper products processed traditionally.

range likely to promote formation of benzene when a food product containing benzoic acid and ascorbic acid is subject to heat treatment. Such was not the case in the present work as we did not detected benzene in any of PT products analyzed, even after heat treatment. On the other hand we were also unable do detect benzoic acid in four out of ten samples, meaning that either the producers do not use this preservative or they were used in amounts that were below the limit of detection of our method (1 mg kg<sup>-1</sup>), as product labels did not include reference to this preservative. Our results suggest that producers comply in general with the established limits in using benzoic acid as a preservative in PT products. However, considering the observed wide range of values, as well as the value found for lutein beans it may be concluded that traditional processors use this preservative in a “*quantum satis*” non-standardized fashion, as long as it is below the established regulations.

Because benzoic acid is used in many other food products – namely in soft drinks [11,13] the exposure to this preservative can only be adequately assessed after consideration of the average daily consumption of all products containing benzoic acid [11-14]. Therefore more data is required to fully estimate the consumer’s exposure to benzoic acid.

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