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# Determination of Inorganic Bromide Content in Several Vegetable Foods

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**Abstract** The phenol red spectrophotometric method has been studied and applied as an inexpensive screening method for the determination of bromide in vegetables samples. The concentration of bromide ranged from 3.65 to 14.42 mg kg$^{-1}$ in capsicum, 4.50 to 9.30 mg kg$^{-1}$ in potatoes, and 3.63 to 19.02 mg kg$^{-1}$ in fungi. The content of inorganic bromide in the studied vegetables was found to be below the maximum concentration of residues established by Spanish legislation (20 mg kg$^{-1}$).

**Keywords** Inorganic bromide · red phenol method · potatoes · pepper · fungi

## Introduction

Over the last 30 years the use of methyl bromide as a fumigant has become widespread in Europe. Because of the environmental problems related to the use of methyl bromide, global controls have been established in numerous countries (Thomas, 1996; FAO, 1999). In the European Community, its use is regulated for different uses such as in glasshouses and for some types of stored dry foodstuffs and fumigant (Commission Decision of 28 April 2006).

Crops growing in a soil fumigated with methyl bromide contain much more inorganic bromide than those produced in an environment free from this plaguicide (Mino and Yukita, 2005). A contribution to this inorganic bromide is coming from the breakdown product of methyl bromide and brominated fumigants (Di Narda et al., 2001). The presence of elevated dietary levels (above the acceptable dietary intake 1 mg kg$^{-1}$ body weight/day) of bromide has been described as yielding a casual replacement of iodide by bromide during the biosynthesis of thyroid hormones, affecting the status of the thyroid gland (Lober et al., 1983; Mishra et al., 2001; Mina and Yukita, 2005). For these reasons, around 168 countries have agreed to gradually reduce methyl bromide production and use, and to phase out its use in agriculture by the year 2005.

Bromide quantification is of great interest in the foodstuffs for monitoring agricultural sources. Determination of low levels of bromide has traditionally been a difficult task, because of the strong dependence on the nature of the sample and interference caused by other species present in the matrix. Different methods have been reported for the determination of bromide in food: X-ray fluorescence spectrometry (Mino and Yukita, 2005), polarography (Vallon et al., 1980; Di Narda et al., 2003), flow injection analysis (Freeman et al., 1993), ion chromatography (Miyara and Saito, 1994), inductive coupled plasma mass spectrometry (Di Narda et al., 2001; Di Narda et al., 2003), and gas-chromatography mass spectrometry (Mishra et al., 2001). However, spectrophotometric methods have been the most use in government laboratories for quality control of bromide in water (normalized methods of analysis, 1992) because of their low cost and wide availability. Several authors (Basel et al., 1982; Dobolyi, 1984; Jones, 1993; Di Narda, 2001; Di Narda, 2003) have described the use of spectrophotometry for the determination of bromide in water and mushrooms. The phenol red spectrometric method is based on the oxidation of bromide by chloramines-T or Oxone® producing the bromination of phenol red

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to bromophenol blue. The interference of different ions such as ammonium and chloride in the application of phenol red methodology has been discussed by different authors (Dobolyi, 1984; Jones, 1993).

The aim of this paper is the application of the phenol red method as a screening method for the determination of bromide in several vegetable foods.

## Materials and Methods

A total of 62 samples of different vegetables randomly collected in local markets were sampled from November 2005 to May 2006. The foodstuffs were potatoes (*Solanum tuberosum*), $n = 14$; fungi (*Agaricus bisporus*, *Agaricus brunnescens*, and *Pleurotus ostreatus*), $n = 30$; and pepper (*Capsicum annuum*), $n = 14$, from various locations in Spain (Albacete, Castilla La Mancha and Canary Islands). In the case of potatoes, samples from Israel were also analyzed.

All the chemicals used were of analytical reagent grade. In all instances high-purity deionised water (Milli-Q system, Millipore, Bedford, MA, USA) was used as the diluent. Stock standard solutions of potassium bromide (1.49 g $l^{-1}$, equivalent to 1 g $l^{-1}$l of bromide ion) were purchased from Merck (Darmstad, Germany), prepared in Milli-Q distilled water and diluted as and when required. The stock solutions were standardised with a solution of silver nitrate (Merck, Darmstad, Germany), by potentiometric titration. The phenol red (D'Hemio, France) solution was prepared dissolving 0.03 g of the relevant sodium salt in 100 ml of deionised water. Ammonium sulphate was obtained from Merck (Darmstad, Germany) and Oxone® was purchased from Aldrich (Steinheim, Germany). Acetic acid was from Scharlau (Scharlau Chemie S.A, Sentmenat, Spain)

A weighed amount of fresh sample (5.0 ± 0.5 g) was intimately mixed with 1.5 g of sodium carbonate into a small nickel crucible. It was then heated gradually until the temperature of a quiet liquid fusion (~850 °C) was maintained for about 20 min, in a furnace. After it cooled, the nickel crucible was transferred into a 200 ml beaker, and Milli-Q water were added. The beaker was warmed gently until the fusion cake was well disintegrated and the solution obtained was filtered through 0.45 μm filters (25 mm GHP filter, Water Edge). The eluate was transferred to a volumetric flask and Milli-Q water was added to a final volume of 50 ml.

Using the red phenol method (Dobolyi, 1984), 500 μl acetate buffer (pH 4.6–4.7), 200 μl ammonium sulphate (1.16 g·$l^{-1}$), 100 μl of phenol red solution and 0.1 g of Oxone® reagent were added to 5 ml of sample in a Nessler tube. Then the solution was shaken vigorously for 2 min. After 3 min the oxidation was stopped by adding 200 μl $Na_2S_2O_3$ (316.26 g·$l^{-1}$). Finally, absorbance at 590 nm due to the formation of bromophenol blue was measured in a spectrophotometer.

**Table 1** Bromide content of vegetables expressed as mg $kg^{-1}$ on wet weight (*N* = number of samples, SD = standard deviation)

| Sample | *N* | Origin | Bromide (mg $kg^{-1}$) | |
|---|---|---|---|---|
| | | | Mean ± SD | Min. – Max |
| Fungi | 18 | Albacete | 9.60 ± 4.55 | 3.63–19.02 |
| | 12 | Castilla La Mancha | 7.74 ± 2.83 | 5.68–15.21 |
| Potatoes | 12 | Israel | 7.27 ± 1.19 | 4.50–9.30 |
| | 6 | Tenerife | 7.71 ± 0.47 | 7.3–8.5 |
| Capsicum | 6 | Murcia | 10.82 ± 2.39 | 8.61–14.42 |
| | 8 | Tenerife | 3.83 ± 0.20 | 3.65–3.95 |

The absorption spectra were recorded employing a glass cell of 1 cm path length on a Hewlett-Packard Vectra XA spectrophotometer. Under the optimised conditions given above, the calibration graph was prepared using stock standard bromide solutions of potassium bromide (1 g $l^{-1}$ of bromide ion) whose concentrations were between 0.5 to 4.5 mg $l^{-1}$. Correlation coefficients were 0.999–0.998, and the linearity was in the range 0.5–4.5 mg $l^{-1}$. Relative standard deviation (RSD) intra-day or reproducibility after 10 determinations was 5%. Limit of detection was calculated as a signal-to-noise ratio, 3:1, and was found to be 0.25 mg $l^{-1}$. All statistical analyses were performed using STATGRAPHICS® plus V.5 (INST, California, USA), SPSS V. 12.1 (SPSS Inc., Chicago, USA). One-way analysis of variation (ANOVA) was used for the comparison of mean values, assuming that there were significant differences among them when the statistical comparison gives a *p* value lower than 0.05.

## Results and Discussion

The methodology described was applied to the determination of inorganic bromide in several vegetable foods. The influence of the matrix of the vegetable in the determination of bromide was studied using the standard addition method under the experimental conditions described above. In the case of potatoes, the influence of the matrix is detected when the concentration of bromide in solution is greater than 2.5 mg $l^{-1}$.

Table 1 shows the mean and standard deviation values corresponding to the inorganic bromide content expressed as mg $kg^{-1}$ wet weight in the analysed vegetables from the various origins. The content of investigated inorganic bromide in the vegetables was found to be in the range 3.63–19.02 mg $kg^{-1}$, both in samples of fungi, since the bromide content in fungi and vegetables in general is

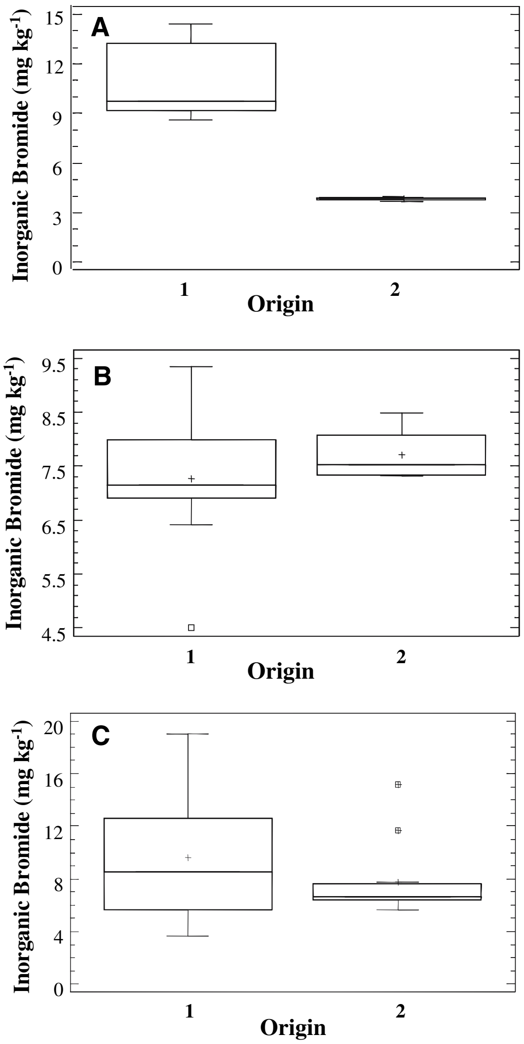

**Fig. 1** Box-and-whisker analysis of the bromide content in A, pepper, B, potatoes, and C, fungi

known to be mainly due to fumigation with methyl bromide. Furthermore, the fumigation of crops with methyl bromide is regulated in the European Community (Commission Decision of 28 April 2006). This relatively high content of bromide may have arisen from the fumigation of green houses and/or soil.

There are a few references in the bibliography to the inorganic bromide content in vegetables. Bromide levels in *Boletus edulis* have been reported in the range 0.2–2 g/kg by Di Narda et al. (2001, 2003). Mino and Yukita (2005) have reported levels of 15 mg kg$^{-1}$ in Shiitake mushroom. In the case of food samples such as fungi, potatoes and capsicum, Spanish legislation has set a maximum limit of residues (MLRs) for inorganic bromide of 20 mg kg$^{-1}$ based on weight of sample ready for consumption. The level of bromide in the studied vegetables was found to be below the MLRs (RD 280/1999).

There were no significant differences between the mean bromide concentrations between the three vegetables foods considered. The maximum bromide content in the samples of capsicum, 14.42 mg kg$^{-1}$, was found in the samples from Murcia. The lowest content was determined as 3.65 mg kg$^{-1}$ in samples from Tenerife. In the case of potatoes, the lowest and highest contents were found in samples from Israel: 4.50 mg kg$^{-1}$ and 9.30 mg kg$^{-1}$, respectively (Table 1). Moreover, samples of potatoes from Tenerife showed a relatively narrow range in the bromide concentrations, between 7.32 mg kg$^{-1}$ and 8.48 mg kg$^{-1}$. The samples of fungi belonging to various species (*Agaricus bisporus*, *Agaricus brunnescens*, and *Pleurotus ostreatus*) (Table 1), presented the lowest and highest bromide contents, 3.63 mg kg$^{-1}$ and 19.02 mg kg$^{-1}$, found in fungi of the species *Agaricus bisporus* from Albacete. Bromide levels in *Agaricus brunnescens* varied between 5.68 mg kg$^{-1}$ and 15.21 mg kg$^{-1}$ in samples from Castilla La Mancha. In the case of samples of *Pleurotus ostreatus*, the lowest and highest bromide concentration values were found as 6.44 mg kg$^{-1}$ and 15.5 mg kg$^{-1}$ in samples from Albacete.

In order to establish if there were significant differences in the bromide contents according to the region of origin for capsicum, potatoes, and fungi, a one-way ANOVA using LSD at a 95% confidence level as a criterion to compare mean values was performed. Significant differences ($p < 0.05$) were found between samples of capsicum from Tenerife and Murcia, while potatoes and fungi did not present significant differences (Table 1). The box-and-whisker plot shows the trends for the studied inorganic bromide in the different region of origin, variability and outliers. The box-plots reveal that there is a statistical difference with respect to the bromide content of the samples of capsicum from regions 1 (Murcia) and 2 (Tenerife) (Fig. 1A). In the case of potatoes (Fig. 1B), box-and-whisker plots indicate that there is a similar bromide distribution, in samples from 1 (Israel) and 2 (Tenerife). Also, there is one sample of potato belonging to Israel that may be considered as a possible outlier. Moreover, the samples of fungi from Albacete displayed a higher variability in bromide content than those from Castilla La Mancha (Fig. 1C).

The spectrophotometric methodology described above is suitable for a routine determination of bromide in terms of labour and precision, being a useful screening method. A single determination can be completed in approximately two hours. A trained person may analyse at least 15 samples without difficulty, including the determination of bromide and calculations, in about eight hours.

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