



Analytical Methods

Using azomethine-H method determination of boron contents of various foods consumed in Hatay Region in Turkey

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ARTICLE INFO

Article history:

Received 4 April 2008

Received in revised form 21 November 2008

Accepted 30 November 2008

Keywords:

Boron concentration

Food

Fruit

Vegetable

UV-vis spectroscopy

Azomethine-H method

ABSTRACT

In this study, samples of soil and plant (32 species of vegetable and 17 species of fruit) were collected from six different regions of Hatay (Amik Plant, Reyhanlı, Kırıkhan, Samandağ, Dörtöyl and İskenderun). The azomethine-H method was used to determine boron content of samples. Furthermore, the correlation among boron content of soil with boron content of plants was investigated. The boron concentrations in the soil samples, were determined between 32.43 (± 2.90) and 93.43 (± 2.75) ppm. High concentrations of boron were found in thyme (10.44 ± 0.17), mint (6.96 ± 0.15), red cabbage (6.45 ± 0.15), broad-bean (6.28 ± 0.14), quince (5.41 ± 0.11), pomegranate (5.27 ± 0.13) and orange (4.08 ± 0.10). Minimal concentration of boron were found in pumpkin (0.76 ± 0.01), white radish (0.97 ± 0.01), plum (1.16 ± 0.02) and cucumber (1.17 ± 0.01). Most of the foods had boron concentrations in the range of 1.48–3.60 ppm.

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1. Introduction

Boron is known to influence a variety of metabolic actions. It interacts with calcium, vitamin D and magnesium which are all important in bone metabolism (Chapin, Ku, Kenney, & McCoy, 1998). Boron has also been found to increase steroid hormone concentrations in postmenopausal women and to have antioxidant properties which could make it beneficial in preventing atherosclerosis (Nielsen, Hunt, Mullen, & Hunt, 1987). Boron is abundant in nature as boric acid and borate and can be obtained in the diet through the consumption of fruits, vegetables and legumes (Sutherland, Strong, & King, 1998). When boron is increased in the diets of humans, it has been shown by some researchers to increase oestrogen, testosterone and plasma ionised calcium levels and decrease calcium excretion and the negative effects of vitamin D and magnesium deficiency (Nielsen, 1990). According to the WHO's report on environmental health criteria concerning boron, intakes of boron for humans are expected to be 0.44 $\mu\text{g}/\text{day}$ from ambient air, 0.2–0.6 mg/day from drinking water and 1.2 mg/day from the diet (WHO, 1994). For this reason, determination of the

boron content of foods was very important. In the literature, there are a number of studies in this matter. The boron content of selected foods has been published from a number of countries including the USA (Anderson, Cunningham, & Lindstrom, 1994; Anderson, Cunningham, & Mackey, 1990; Dolan & Capar, 2002; Hunt, Shuler, & Mullen, 1991; Rainey et al., 1999; Rainey, Nyquist, Coughlin, & Downing, 2002), Finland (Koivistoinen, 1980), Australia (Naghii, Wall, & Samman, 1996), Singapore (Bloodworth, 1989), Columbia (Sun, Waters, & Mawhinney, 2000) and Poland (Pieczynska et al., 2003). However, there is only one study about the boron content of selected foods consumed in Turkey (Şimşek, Velioğlu, Coşkun, & Saylı, 2003). The objective of this study was determined the boron contents of the soils belong to the same region and various foods consumed in Hatay Region. Boron analyses were performed by UV/vis spectroscopy using azomethine-H method. Furthermore, the relationship between boron content of soil and boron content of plants was investigated.

2. Materials and methods

2.1. Sampling

Hatay is located in the eastern Mediterranean coast of Turkey. The province lies about 30 km inland from the Mediterranean coast on the banks of the Orontes River. Amik Plant, Reyhanlı and Kırıkhan are located in a valley behind the Amanos Mountains.

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Fig. 1. Map of the sampling sites.

Samandağ is the small seaside resort of Hatay. Dörtiyol and İskenderun are located between Amanos Mountains and İskenderun Bay. Sampling sites chosen in this study are shown in Fig. 1.

Soil and plant (32 species of vegetable and 17 species of fruit) samples used in this study were taken from six different regions of Hatay (Amik Plant, Reyhanlı, Kırıkhan, Samandağ, Dörtiyol and İskenderun). The location of samples was reflective of the commercial industry. Plant samples were collected directly from fields. For each sample we attempted to collect 10 plants at random from fields in these regions. Soil samples were taken from surface (0–30 cm). All samples were packed into polyethylene bags.

2.2. Soil analysis

The soil samples were air dried and passed through a 100 mesh sieve. Large stones and roots were removed by hand. About 1 g of powdered samples of soil was taken and ignited for 30 min. About 5 g Na_2CO_3 was added and then this mixture was ignited for 30 min again. After cooling to room temperature, 50 ml water was added. Then, 20 ml of 4 N H_2SO_4 was added in order to ensure complete dissolution of solid. This solution was diluted with ethanol to 100 ml and evaporated to 20 ml at water bath. About 2 ml of buffer solution (25 g ammonium acetate, 1.5 g EDTA disodium salt, 40 ml distilled water, and 12.5 ml acetic acid) and 2 ml of azomethine-H solution (0.45 g azomethine-H in 100 ml 1% ascorbic acid) were added to 1 ml of this solution. The mixture was kept at room temperature for 30 min. The absorbance of solution was measured at 420 nm by UV/vis spectrophotometer. All analyses were made in triplicate. To monitor the reproducibility and sensitivity of the method, standard reference materials of SRM 1573a tomato leaves was used as control and acceptable performance was obtained at each run. All chemicals used were of analytical grade. To avoid contamination no glassware was used. The quantitative determinations were realised using calibration curve. A calibration curve was prepared using different concentrations of aqueous boron standards.

2.3. Plant analysis

The plant samples were cut finely and put in a double polyethylene bag and kept in a freezer at -20°C . They ground using a por-

celain mortar and pestle before analysing. Aliquots of food (0.5 g or 0.5 ml) were digested in polypropylene tubes by the addition of 0.5 ml of concentrated HNO_3 at room temperature for 24 h. After the addition of 0.5 ml of distilled water, boron was determined by using UV/vis spectrophotometer with azomethine-H method as described in soil analysis.

2.4. Calculation of correlation coefficients

Furthermore, correlations between boron concentrations in soil and plants were investigated. The correlation coefficients were calculated by the following equation:

$$r = \frac{\sum x \cdot y}{\sqrt{(\sum x^2) \cdot (\sum y^2)}}$$

where $x = X - \bar{X}$ and $y = Y - \bar{Y}$.

The quantities $\sum x^2$ and $\sum y^2$ are the sums of squared deviations of the X observations and the Y observations, respectively. $\sum x \cdot y$ is the sum of the cross products of the x deviations with the y deviations.

3. Results and discussion

Used as control material SRM 1573a has a certified boron concentration value, $33.3 \pm 0.7 \text{ mg kg}^{-1}$. The value found for the analysis of boron level in SRM 1573a was $33.3 \pm 0.4 \text{ mg kg}^{-1}$. This result showed excellent agreement with the certified value.

The boron concentrations in the soil samples, were determined between $32.43 (\pm 2.90)$ and $93.43 (\pm 2.75)$ ppm. The soil of Kırıkhan had the highest boron concentration (93.43 ± 2.75), followed by the soil of İskenderun (76.95 ± 1.98), the soil of Amik Plant (62.49 ± 2.98), the soil of Dörtiyol (61.77 ± 2.35), the soil of Samandağ (49.32 ± 2.29) and the soil of Reyhanlı (32.43 ± 2.90). According to these results, Hatay soils contain boron at high amounts. The pH values of soil samples were measured between 7.22 and 8.66. The highest pH was found in the soil of Samandağ. The lowest pH was also found in the soil of Kırıkhan.

The boron concentrations of the vegetables and fruits are represented in Table 1. The average boron concentrations of vegetables and fruits belong to six different region of Hatay are also shown in the same table. The results obtained were shown that boron uptake in plants grown in Kırıkhan was higher than other district. High concentrations of boron were found in thyme (10.44 ± 0.17), mint (6.96 ± 0.15), red cabbage (6.45 ± 0.15), broad-bean (6.28 ± 0.14), quince (5.41 ± 0.11), pomegranate (5.27 ± 0.13) and orange (4.08 ± 0.10). Minimal concentration of boron was found pumpkin (0.76 ± 0.01), white radish (0.97 ± 0.01), plum (1.16 ± 0.02) and cucumber (1.17 ± 0.01). On the other hand, the vast majority of samples contained less than 5 ppm boron.

The correlation coefficients calculated are presented in Table 2. As can be seen from Table 2, all the correlation coefficients were found close to 1. The correlation coefficients which are close to 1 or -1 indicates strong correlation (Greenwell, 2002). These values have shown that the strongly correlation can be between soil and plant boron concentrations.

A comparison of boron concentrations obtained from this study with corresponding items in the American, German, Australian and Finnish tables is shown in Table 3. There are several factors such as the pH of the soil, temperature, light intensity, boron, calcium, potassium, humus content and dryness of the soil involved in boron uptake in plants (Marschner, 1995). Therefore, differ-

Table 1
The boron contents of vegetables, fruits and grains^a.

Foods	District						Average boron concentration (mg/1000 g)
	Amik Plant	Reyhanlı	Kırıkhan	Samandağ	Dörtüyl	İskenderun	
	Boron concentration (mg/1000 g) ^a						
<i>Vegetables</i>							
Beans	1.42 ± 0.05	1.08 ± 0.04	2.49 ± 0.12	1.24 ± 0.04	1.38 ± 0.05	1.43 ± 0.05	1.51 ± 0.03
Bell pepper	1.51 ± 0.04	0.88 ± 0.01	5.32 ± 0.32	1.20 ± 0.04	1.43 ± 0.05	1.66 ± 0.05	2.00 ± 0.05
Broad-bean	6.18 ± 0.38	3.02 ± 0.20	11.10 ± 0.44	3.68 ± 0.22	5.05 ± 0.32	8.65 ± 0.40	6.28 ± 0.14
Cabbage	2.86 ± 0.12	1.37 ± 0.03	3.53 ± 0.20	1.52 ± 0.04	2.48 ± 0.10	3.03 ± 0.20	2.46 ± 0.05
Carrot	1.46 ± 0.05	1.02 ± 0.02	1.76 ± 0.06	1.32 ± 0.04	1.43 ± 0.06	1.57 ± 0.08	1.43 ± 0.02
Cauliflower	2.10 ± 0.07	1.24 ± 0.04	2.67 ± 0.10	1.57 ± 0.04	1.84 ± 0.06	2.28 ± 0.08	1.95 ± 0.03
Chard	2.21 ± 0.08	1.39 ± 0.05	3.83 ± 0.24	1.97 ± 0.06	2.19 ± 0.08	2.89 ± 0.10	2.41 ± 0.05
Cucumber	1.18 ± 0.04	0.39 ± 0.01	1.98 ± 0.05	0.98 ± 0.02	1.08 ± 0.04	1.43 ± 0.05	1.17 ± 0.01
Cress	3.05 ± 0.12	1.86 ± 0.06	3.71 ± 0.14	2.08 ± 0.10	2.86 ± 0.12	3.33 ± 0.13	2.81 ± 0.05
Eggplant	2.38 ± 0.12	1.14 ± 0.04	6.30 ± 0.38	1.57 ± 0.04	2.31 ± 0.08	2.49 ± 0.10	2.70 ± 0.07
Garlic	2.47 ± 0.10	1.47 ± 0.05	3.80 ± 0.24	1.66 ± 0.05	2.14 ± 0.08	2.85 ± 0.14	2.40 ± 0.05
Green pepper	1.80 ± 0.05	1.14 ± 0.04	5.48 ± 0.32	1.43 ± 0.05	1.66 ± 0.05	1.98 ± 0.08	2.25 ± 0.06
Green peas	2.21 ± 0.10	1.55 ± 0.05	2.67 ± 0.10	1.94 ± 0.06	1.98 ± 0.06	2.53 ± 0.10	2.15 ± 0.03
Leek	1.43 ± 0.05	0.98 ± 0.01	1.93 ± 0.06	1.12 ± 0.04	1.38 ± 0.04	1.43 ± 0.05	1.38 ± 0.02
Lettuce	2.10 ± 0.06	0.84 ± 0.01	2.42 ± 0.10	1.51 ± 0.05	1.88 ± 0.06	2.25 ± 0.08	1.83 ± 0.03
Mallow	4.23 ± 0.25	1.08 ± 0.03	5.40 ± 0.34	1.93 ± 0.08	4.02 ± 0.26	4.95 ± 0.30	3.60 ± 0.10
Mint	6.48 ± 0.40	3.69 ± 0.22	12.60 ± 0.45	4.72 ± 0.30	6.42 ± 0.40	7.88 ± 0.41	6.96 ± 0.15
Onions	1.87 ± 0.06	1.18 ± 0.04	2.15 ± 0.10	1.36 ± 0.05	1.43 ± 0.05	2.08 ± 0.10	1.68 ± 0.03
Onions, fresh	1.66 ± 0.05	0.92 ± 0.01	2.75 ± 0.10	1.55 ± 0.05	1.62 ± 0.05	2.45 ± 0.10	1.83 ± 0.03
Parsley	4.54 ± 0.29	3.79 ± 0.26	4.85 ± 0.32	4.12 ± 0.26	4.34 ± 0.28	4.63 ± 0.30	4.38 ± 0.12
Potato	2.90 ± 0.12	1.04 ± 0.02	3.08 ± 0.12	1.66 ± 0.06	1.98 ± 0.06	2.98 ± 0.12	2.27 ± 0.04
Pumpkin	0.73 ± 0.01	0.38 ± 0.01	1.47 ± 0.04	0.45 ± 0.01	0.72 ± 0.01	0.81 ± 0.01	0.76 ± 0.01
Radish	2.16 ± 0.08	1.38 ± 0.05	3.08 ± 0.12	1.43 ± 0.06	2.08 ± 0.08	2.46 ± 0.10	2.10 ± 0.03
Red cabbage	6.85 ± 0.40	5.42 ± 0.34	7.62 ± 0.40	5.55 ± 0.34	6.12 ± 0.38	7.16 ± 0.40	6.45 ± 0.15
Red pepper	1.83 ± 0.05	0.84 ± 0.01	5.72 ± 0.34	0.98 ± 0.01	1.39 ± 0.04	1.97 ± 0.05	2.12 ± 0.06
Rocket	4.59 ± 0.30	2.35 ± 0.12	5.94 ± 0.32	3.34 ± 0.16	4.32 ± 0.28	5.03 ± 0.30	4.26 ± 0.10
Spinach	3.24 ± 0.18	2.56 ± 0.14	3.78 ± 0.20	2.98 ± 0.10	3.08 ± 0.15	3.49 ± 0.16	3.19 ± 0.06
Thyme	8.39 ± 0.42	6.12 ± 0.38	17.90 ± 0.45	7.25 ± 0.39	7.95 ± 0.40	15.00 ± 0.42	10.44 ± 0.17
Tomatoes	1.65 ± 0.05	1.02 ± 0.02	2.69 ± 0.10	1.16 ± 0.04	1.38 ± 0.04	1.85 ± 0.06	1.63 ± 0.02
White radish	1.12 ± 0.04	0.38 ± 0.01	1.58 ± 0.05	0.54 ± 0.01	0.96 ± 0.01	1.26 ± 0.04	0.97 ± 0.01
Zucchini	1.42 ± 0.05	1.06 ± 0.04	1.51 ± 0.06	1.08 ± 0.04	1.25 ± 0.04	1.43 ± 0.06	1.29 ± 0.02
<i>Fruits and fruit juices</i>							
Apple	2.06 ± 0.07	0.86 ± 0.01	4.67 ± 0.30	1.19 ± 0.04	1.91 ± 0.05	2.14 ± 0.07	2.14 ± 0.05
Apricot	2.48 ± 0.10	1.12 ± 0.04	4.18 ± 0.28	2.10 ± 0.05	2.23 ± 0.08	2.61 ± 0.06	2.45 ± 0.05
Banana	3.32 ± 0.14	2.52 ± 0.08	5.05 ± 0.30	2.93 ± 0.12	3.06 ± 0.20	3.60 ± 0.22	3.41 ± 0.08
Cherry	3.79 ± 0.23	1.18 ± 0.04	6.23 ± 0.34	1.90 ± 0.05	3.49 ± 0.15	4.73 ± 0.28	3.55 ± 0.09
Grapefruit	3.55 ± 0.16	1.59 ± 0.05	5.19 ± 0.34	1.87 ± 0.06	3.05 ± 0.20	4.96 ± 0.32	3.37 ± 0.09
Grapefruit juice	1.70 ± 0.04	0.78 ± 0.01	2.51 ± 0.08	0.96 ± 0.01	1.62 ± 0.04	1.83 ± 0.05	1.57 ± 0.02
Green almond	3.32 ± 0.13	2.48 ± 0.10	3.81 ± 0.24	3.04 ± 0.12	3.28 ± 0.13	3.44 ± 0.14	3.23 ± 0.06
Lemon	3.44 ± 0.15	1.93 ± 0.08	6.39 ± 0.38	2.30 ± 0.08	3.00 ± 0.12	3.81 ± 0.24	3.48 ± 0.08
Lemon juice	1.16 ± 0.04	0.34 ± 0.01	2.66 ± 0.10	0.76 ± 0.01	1.11 ± 0.04	1.28 ± 0.04	1.22 ± 0.02
Loquat	1.55 ± 0.05	0.41 ± 0.01	2.81 ± 0.10	0.94 ± 0.01	1.46 ± 0.05	1.69 ± 0.06	1.48 ± 0.02
Mandarin	2.96 ± 0.12	1.97 ± 0.06	5.03 ± 0.28	2.25 ± 0.08	2.58 ± 0.06	3.00 ± 0.20	2.97 ± 0.06
Mandarin juice	1.85 ± 0.05	0.82 ± 0.01	2.63 ± 0.06	1.03 ± 0.04	1.26 ± 0.04	1.87 ± 0.05	1.58 ± 0.02
Melon	1.98 ± 0.06	0.63 ± 0.01	2.21 ± 0.08	1.66 ± 0.05	1.92 ± 0.05	2.06 ± 0.07	1.74 ± 0.02
Orange	4.18 ± 0.28	2.01 ± 0.06	7.11 ± 0.40	2.25 ± 0.05	4.02 ± 0.26	4.89 ± 0.30	4.08 ± 0.10
Orange juice	1.83 ± 0.05	0.92 ± 0.01	3.52 ± 0.22	1.19 ± 0.04	1.68 ± 0.05	1.87 ± 0.05	1.84 ± 0.04
Peach	3.44 ± 0.14	1.22 ± 0.04	3.82 ± 0.24	1.57 ± 0.04	3.05 ± 0.12	3.63 ± 0.22	2.79 ± 0.06
Pear	2.56 ± 0.06	1.39 ± 0.04	4.22 ± 0.28	1.66 ± 0.05	2.06 ± 0.05	2.57 ± 0.06	2.41 ± 0.05
Plum	1.33 ± 0.05	0.41 ± 0.01	1.59 ± 0.05	0.94 ± 0.01	1.24 ± 0.04	1.44 ± 0.05	1.16 ± 0.02
Pomegranate	5.55 ± 0.30	2.89 ± 0.10	7.71 ± 0.40	3.55 ± 0.22	5.21 ± 0.34	6.69 ± 0.40	5.27 ± 0.13
Quince	4.48 ± 0.30	1.79 ± 0.05	13.61 ± 0.45	2.85 ± 0.08	4.16 ± 0.28	5.60 ± 0.32	5.41 ± 0.11
Strawberry	2.30 ± 0.04	1.59 ± 0.04	7.07 ± 0.40	1.66 ± 0.05	2.02 ± 0.05	2.69 ± 0.06	2.89 ± 0.07
Watermelon	2.05 ± 0.07	0.57 ± 0.01	5.10 ± 0.30	1.35 ± 0.05	1.98 ± 0.05	2.10 ± 0.07	2.19 ± 0.05
<i>Grains</i>							
Wheat	2.08 ± 0.06	1.36 ± 0.04	2.94 ± 0.08	1.98 ± 0.05	2.02 ± 0.07	2.58 ± 0.08	2.16 ± 0.05
Rice	1.38 ± 0.05	0.52 ± 0.01	1.96 ± 0.05	1.06 ± 0.04	1.36 ± 0.05	1.68 ± 0.05	1.33 ± 0.03

^a N = 3.

ences were observed when data of the other countries were compared. Observed differences from country to country may be reflect boron concentrations in plants grown there. Furthermore, the methods of analysis used in these studies were different from our method. For example, Koivistoinen and Naghii et al. were used colorimetric method for the analysis of boron. Anderson et al.

were determined the boron in foods by neutron capture prompt- γ activation.

As a result, determination of the boron concentration in foods will provide useful information for the assessment of daily intake levels of boron once its nutritional essentiality and health benefits are confirmed.

Table 2
The correlation coefficients.

Foods	Correlation coefficients	Foods	Correlation coefficients	Foods	Correlation coefficients
<i>Vegetables</i>		Onions	0.920	Cherry	0.989
Beans	0.864	Onions, fresh	0.974	Grapefruit	0.956
Bell pepper	0.815	Parsley	0.975	Green almond	0.976
Broad-bean	0.967	Potato	0.905	Lemon	0.935
Cabbage	0.949	Pumpkin	0.932	Loquat	0.977
Carrot	0.987	Radish	0.971	Mandarin	0.903
Cauliflower	0.986	Red cabbage	0.943	Melon	0.878
Chard	0.978	Red pepper	0.847	Orange	0.968
Cress	0.967	Rocket	0.988	Peach	0.907
Cucumber	0.987	Spinach	0.991	Pear	0.931
Eggplant	0.873	Thyme	0.923	Plum	0.946
Garlic	0.970	Tomatoes	0.948	Pomegranate	0.984
Green pepper	0.825	White radish	0.975	Quince	0.893
Green peas	0.973	Zucchini	0.913	Strawberry	0.824
Leek	0.958	<i>Fruits and Fruit juices</i>		Watermelon	0.911
Lettuce	0.948	Apple	0.911	<i>Grains</i>	
Mallow	0.948	Apricot	0.953	Wheat	0.983
Mint	0.954	Banana	0.930	Rice	0.989

Table 3
Comparison of the average boron concentration in Turkish foods (in this study) with corresponding items listed in the American, German, Australian and Finnish food composition tables.

Foods	Boron concentration (ppm)				
	American ^a	German ^b	Australian ^c	Finnish ^d	Turkish ^e
<i>Vegetables</i>					
Carrot	1.90	3.10	3.00	3.20	1.43
Cucumber	0.94	30.63	1.25	1.30	1.17
Lettuce	0.83	0.82	0.90	1.50	1.83
Onion	1.39	17.00	1.90	2.00	1.68
Parsley	–	5.40	5.88	5.40	4.38
Potato	1.39	1.00	1.80	1.40	2.27
<i>Fruits</i>					
Apple	2.73	2.40	3.18	2.10	2.14
Banana	1.04	0.79	1.66	1.70	3.41
Orange	2.17	1.80	2.53	2.85	4.08
Orange juice	0.92	1.00	1.00	1.00	1.84
Peach	4.49	70.00	5.20	6.20	2.79
Pear	–	–	3.20	1.90	2.41
Plum	4.22	3.40	4.50	4.50	1.16

^a Anderson et al. (1994).

^b Souci, Fachmann, and Kraut (1994).

^c Naghii, Wall, and Samman (1996).

^d Koivistoinen (1980).

^e This study.

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