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Determination of boron in hazelnut (*Corylus avellana* L.) varieties by inductively coupled plasma optical emission spectrometry and spectrophotometry

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Abstract

Boron content of 16 Turkish hazelnut (*Corylus avellana* L.) cultivars were determined by using inductively coupled plasma optical emission spectrometry (ICP-OES) and Azomethine H. spectrophotometric method. The results varied between 13.8 and 22.2 mg/kg. No significant difference was observed between the results by the two methods ($P > 0.05$). However, the difference among boron content of hazelnut varieties was found to be significantly important ($P < 0.01$). The highest boron content was in Mincane (20–22.2 mg/kg). Results revealed that the Turkish hazelnut is a good natural source of boron.
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1. Introduction

Turkey is the generic origin of hazelnut for wild species and cultivated varieties. Furthermore, the land has suitable ecological conditions and high quality hazelnut varieties (Ayfer, Türk, & Eriş, 1997). Hazelnut production in Turkey utilizes over 540,000 ha of land and annual production is approximately 450,000 t, affecting the lives of nearly eight million people. Hazelnut exportation provides about 12% of Turkey's foreign trade earnings. Moreover, Turkey is capable of achieving 65–70% of world hazelnut production and 70–85% of world export (Anonymous, 2001).

Hazelnut plays a major role for human nutrition because of its very special nutrient contents such as proteins, carbohydrates, fats, vitamins and minerals. A hundred grams of hazelnut provides energy of 600–650 kcal. Ash content in hazelnuts varies between 1 and 3.4% (Ackurt, Ozdemir, Biringen, & Looker, 1999; Mehlenbacher, 1990). In spite of known major mineral composition of hazelnuts of Turkey, there are no data available for boron content. The boron content of hazelnuts in "*Food Composition and Nutrition Tables*" have been given in only one sample which contains 2.2 mg boron in a 100-g sample (Souci, Fachmann, & Kraut, 2000).

Boron is an essential micronutrient for plants. However, when optimal doses are exceeded, it becomes a herbicide. Boron is an important mineral in human nutrition, especially under conditions of nutritional metabolic stress; it helps in maintaining cell membrane function and stability, and is involved in enzymatic reactions. Boron functions closely with calcium and vitamin D in the preservation of bone mass and the prevention of bone demineralization and stimulates immune system and inflammatory and hormonal responses. Therefore it may have significant roles in the prevention and treatment of osteoporosis and osteoarthritis. It positively affects calcium and magnesium metabolisms and may be needed for energy-substrate use (Hunt, 1996; Nielsen, 1998). Taking continuously 1.8 mg/day boron decrease prostate cancer for men (Lucich & Lucich, 2002). In addition, boron as a modulator of steroid hormone pathway has importance for animals and humans. Peanut butter, wine, raisins, dried

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fruits, legumes, fresh vegetable and fruits, peanut and other nuts are important boron contributors (Naghii, Wall & Samman, 1996; Nielsen, 1998; Rainey, Nyquist, Christensen, Strong, Culver, & Coughlin, 1999). However, high doses of boron is known to cause atrophy and degeneration in testicles (Chapin, 1994). Amounts greater than 500 mg/day may occur nausea, vomiting and diarrhea. Daily intake of up to 40 mg has not caused any toxic reaction in humans because the mineral is rather poorly assimilated (Anonymous, 2000). Boron deficiency symptoms have been shown in especially related to bone and immune systems and inflammatory and hormonal responses (Hunt, 1994; Sheng, Taper, Weit, Rian, Ritchey, & Low, 2001). World Health Organization (WHO) has established a 1–13 mg/day safe and adequate range of boron intake for healthy individuals replacing the earlier value, 1 mg/day (WHO, 1998).

Many methods are described in the literature for the determination of boron. Inductively coupled plasma spectrometry has become one of the most attractive detection systems for the determination of trace elements in plant materials and other biological samples. The limiting factor for use of this system is high equipment costs. As numerous colored complexes with boron exist, a variety of colorimetric methods have been studied. Well known sensitive photometric reagents are carminic acid and curcumine. However, the fact that determination with these reagents only works in concentrated sulphuric acid medium, makes them very inconvenient for easy practical use. The azomethine H method working in a buffered system, is a good alternative with high sensitivity (Evans & Krahenbühl, 1994).

2. Materials and methods

2.1. Materials

Sixteen hazelnut cultivars (*Corylus avellana* L.) used in this study, were obtained from different regions in Turkey's main growing areas (Ordu, Giresun, Trabzon), at harvest of July 2001. Total sample quantity for all the species was 3.0 kg each; from this batch 100 g was taken by random selection. After removing hulls, seed samples were ground to a powder using a grinder and stored at −20 °C in a polyethylene bag until time of analysis.

All chemicals used were of analytical grade. In order to avoid contamination no glassware was used; solutions were kept in containers manufactured from HDPE. Boron standard solution (1000 mg/l) was used for standardization. The dilutions were made with deionized water (Millipore Water Purification System).

2.2. Methods

2.2.1. Ashing procedure

Three grams of powdered hazelnut were weighed in crucibles. Due to the high volatility of boron in acidic solutions, pH was increased up to approximately 7.0 using 0.1 M NaOH solution. Samples were dried in oven for 12 h at 75 °C. Crucibles then were taken into a muffle furnace and temperature was gradually raised to 525 °C. Samples were kept in furnace until completely ashed. 2 M HNO_3 (10 ml) were added and the contents were heated on a hot plate, avoiding any boiling. After dissolution, the contents were filtered through Whatman No. 42 filter paper and diluted to a final volume of 50 ml with deionized water. This final solution was used for analysis. All analyses were made in triplicate. When relative standard deviation was found to be higher than 5%, analyses were repeated.

2.2.2. Spectrophotometric method

Boron concentrations were measured in 1.00-cm sample cells, at 420 nm, according to Azomethine H method (John, Chuah, & Neufeld, 1975; Wolf, 1971).

2.2.3. Instruments

A Shimadzu double beam UV-VIS spectrophotometer, Model UV 1601, was used for Azomethine H method. Leeman Labs Inc., model DRE with axial view configuration was used in ICP-OES analyses. The operation conditions for ICP-OES were as follows:

Frequency: 40 MHz; power RF: 1.1 kW; plasma gas flow rate: 15 l/min; auxiliary gas flow rate: 0.5 l/min; sample uptake: 0.6 ml/min; integration time: 1 s; replicates: 3; emission line: B(I) 249.773 nm. Other lines, 208.893, 208.959, 249.678 nm were also used to confirm the results in analyses.

2.2.4. Statistical methods

"Paired Comparison *t*-Test" was used to evaluate the effect of using different methods. Considering the rather low number of replicates ($n=3$), the possibility of a non-Gaussian distribution was considered. The data set were subjected to a non-parametric (Wilcoxon) test; the same results as compared to parametric test were obtained. We have, however, preferred to give the results of parametric paired *t*-test. Differences of among varieties were determined by analysis of variance (completely randomized design ANOVA). Significant means compared according to Duncan's multiple comparison test (Sokal & Rohlf, 1995). For the statistical analysis of data, a MINITAB Statistical Package Version 12.0 (Anonymous, 1998) was employed.

3. Results and discussion

In this study, two different analytical methods have been applied for determination of boron in Turkish hazelnut varieties. The results obtained by ICP-OES at 249.773 nm and spectrophotometric Azomethine H methods have been compared by using ''paired comparison *t*-test''. Mean values and standard deviations by these two methods and other parameters for comparison are given in Table 1.

The range of results for the boron contents found by spectrophotometry and ICP-OES were 13.9–22.2 mg/kg and 13.8–20.6 mg/kg, respectively. In all the cases by applying the test of significance the calculated P values were found to be larger than 0.05, indicating that the difference between the results from two methods is not significant at a confidence level of 95%. The two methods, therefore, can be trusted to give results with the same level of accuracy. Regarding the absolute accuracy, SRM-1573a, tomato leaves were used; the certified value for B is 33.3 mg/kg. The values found by spectrophotometry and ICP-OES was 30.5 ± 3.6 and 32.7 ± 1.2, respectively; indicating that both methods provide sufficiently accurate results. In order to eliminate any possible errors due to spectral interferences, other analytical lines for B, namely, 208.893, 208.959 and 249.678 nm were also used; the results were found to be not significantly different than those reported for both the samples and SRM-1573a using wavelength of 249.773 nm. In addition, all peak profiles were examined and no spectral interferences were observed.

The analysis results given in Table 1 show that Turkish hazelnut varieties have rather rich boron content and therefore can be used as nutritional source for this element. Regarding boron content, the richest and poorest species are Mincane (20.6–22.2 mg/kg) and Kalınkara (13.8–14.1 mg/kg), respectively.

On the other hand, some species that are commercially important, namely Tombul, Palaz and Foşa, contain 17–19 mg/kg boron, a rather moderate value among the others. These species constitute approximately 60% of the hazelnut exportation annually. According to ANOVA analysis the effect of species on boron concentration has been found to be significant ($P < 0.01$).

A comparison of results by Duncan's multiple comparison test is given in Table 2. According to this test, the species Mincane is the richest one (A), while some species that are rather in a wild form and having no commercial value, namely Kalınkara, Kan, Kargalak, Kuş and Yassıbadem, have the lowest boron content (F). The remaining species with high commercial value are rated in between these two extremes (B–E).

From nutritional point of view, it is known that the hard-crusted fruits are the most important boron sources (~12 mg/kg); these are followed by fruits (~4 mg/kg) and then vegetables (~1.7 mg/kg) to complete the sequence (Anderson, Cunningham, & Lindstrom, 1994). The results found in this study that the minimum level for boron content is 14 mg/kg. Therefore, Turkish hazelnut is a rich and important source for boron in human nutrition.

Table 1
Paired comparison *t*-test for spectral and ICP-OES methods ($P > 0.05$)

Varieties	n	Boron content (mg/kg)				P[a]
		Spectrophotometric method		ICP-OES method		
		Mean	S.E. of means	Mean	S.E. of means	
Cavcava	3	18.2	0.7	17.2	0.2	0.30
Çakıldak	3	17.4	0.5	16.8	0.4	0.14
Foşa	3	18.7	0.3	18.4	0.3	0.48
Incekara	3	16.3	0.4	16.6	0.2	0.52
Kalınkara	3	14.1	0.1	13.8	0.5	0.54
Kan	3	14.6	0.7	14.1	0.1	0.54
Kara	3	17.6	0.7	17.8	1.1	0.89
Kargalak	3	14.5	0.6	14.5	0.03	0.95
Kuş	3	14.3	0.4	14.3	0.4	0.97
Mincane	3	22.2	0.5	20.6	0.1	0.058
Palaz	3	17.0	0.4	17.5	0.4	0.62
Sivri	3	16.3	0.6	15.7	1.0	0.73
Tombul	3	17.3	0.5	17.3	0.2	0.96
Uzunmusa	3	17.8	0.3	18.3	0.033	0.17
Yassıbadem	3	13.9	0.7	14.2	1.0	0.77
Yuvarlakbadem	2	19.0	0.3	18.4	0.3	0.14

[a] Values greater than 0.05 indicates no difference between two methods.

Table 2
Duncan's multiple comparison test for hazelnut varieties ($P < 0.01$)

Varieties	n	Boron content (mg/kg)		Results of Duncan's test[a]
		Mean	S.E. of means	
Cavcava	6	17.7	0.409	BCD
Çakıldak	6	17.1	0.311	CDE
Foşa	6	18.6	0.177	BC
Incekara	6	16.4	0.186	DE
Kalınkara	6	13.9	0.247	F
Kan	6	14.4	0.323	F
Kara	6	17.7	0.593	BCD
Kargalak	6	14.5	0.247	F
Kuş	6	14.3	0.254	F
Mincane	6	21.4	0.416	A
Palaz	6	17.2	0.293	CDE
Sivri	6	16.0	0.529	E
Tombul	6	17.3	0.259	CDE
Uzunmusa	6	18.1	0.184	BC
Yassıbadem	6	14.1	0.570	F
Yuvarlakbadem	5	18.8	0.242	B

[a] A and F shows the highest and lowest values. Other notations indicate the values between these two extremes.

4. Conclusion

Both spectrophotometric Azomethine H method and ICP-OES have adequate sensitivity and accuracy for boron determination in hazelnuts. While ICP-OES is presently one of the most common and powerful techniques for B determination, the results show that spectrophotometric Azomethine H method can provide similar results with considerably low cost. UV-VIS spectrophotometers are easily available in most laboratories and can be confidently and economically used for boron determination in hazelnuts. The results and their evaluations have also illustrated that Turkish hazelnut varieties have relatively rich boron content and thus can be used as a nutritional source for this element.

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