Acta Pharm. Sci. Vol 63:(1), 2025
Original article
DOI: 10.23893/1307-2080.APS6313

Determination of boron levels in tea samples: A preliminary study

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ABSTRACT

The best sources of boron are fruits, vegetables, drinking tea and water. It is known that insufficient or toxic levels of boron intake may lead to disruptions in various metabolic pathways. Hence the increase of tea consumption in Türkiye more than other countries day by day, the boron intake from tea has become important too. This study aimed to determine the amount of boron in the most consumed teas in Türkiye and its health impact. Black bulk and bagged tea samples were used. All teas were pulverized to a certain size. Then, the teas were brewed under certain conditions and centrifuged. The boron content in pulverized and brewed tea samples was determined by the carminic acid method. The average boron level in dried tea samples was 10 ppm, while in brewed tea samples it was about 3.3 ppm. The boron levels transferred to the brewed tea sample decreased by approximately 3-4 times to around 30%. In conclusion, considering the daily upper limit level, our research supports the safety of tea consumption in terms of boron levels. It also helps fulfill our boron intake without surpassing the daily upper limit, thereby maintaining homeostasis and potentially helping with many health problems.

Keywords: boron, black tea, brewed tea, health, Türkiye

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(Received 23 Feb 2024, Accepted 18 Apr 2024)

INTRODUCTION

Tea is obtained by processing the buds and the upper two leaves as much as possible of the green-leafed *Camellia sinensis* (L.), belonging to the Theaceae family, plant with various methods. It turns into a commercial beverage through fermentation[1]. Unfermented tea is green tea, semi-fermented tea is oolong tea, and fully fermented tea is black tea[2]. Another type of tea is white tea, which is produced by being protected from oxidation, but differently contains a high amount of bud leaves[3]. The most produced tea type worldwide is black tea, with a share of 78%[4]. During the fermentation step, polyphenol compounds found in fresh black tea leaves undergo various hydrolysis, oxidation, transformation and polymerization reactions and transform into theaflavins and thearubigens that give black tea its color[5,6]. Apart from polyphenol oxidase, which provides taste and color formation, the activities of many enzymes such as alcohol dehydrogenase enzyme, which provides the formation of some alcohols that contribute to aroma, transaminase enzyme, which provides the transformation of amino acids and the formation of terpenes, pectinase enzyme, which provides the breakdown of pectin substances, and peptidase enzyme, which provides the hydrolysis of proteins, play important roles in shaping the characteristic properties of black tea[7].

In Türkiye, tea is traditionally consumed widely at breakfast, after meals, in social environments, when hosting guests, during picnics, celebrations, evening conversations, and many other occasions. As a result, Türkiye surpasses other countries in terms of worldwide tea consumption. Consumption of tea bags and cup tea bags instead of bulk tea brewed in a teapot is becoming more common because it is more practical[8,9]. Although the teas in bulk tea and tea bags go through the same processes, they are different in terms of quality because they pass through different sieves just before packaging[10].

Tea content is quite complex, with more than 2000 chemical components, and even among the same type of tea, different content can be observed depending on soil structure, climate characteristics, and storage conditions[11]. The amount of water in the dry form of fresh tea leaves, which contain approximately 25% water when fresh, is approximately 5%. Other components mainly include phenolic compounds, flavonols, amino acids + proteins, caffeine, simple carbohydrates, polysaccharides, and minerals.

Boron is an essential element for plants as it plays an important role in the growth and development of plant cells, the synthesis of the cell wall and the continuity of its structure, the transport of necessary ions and various metabolites through membranes, and various enzymatic reactions[12]. Boric acid, which can dissolve very well in the acidic soil where tea grows with the contribution of rain, is the main source of boron in tea. Thanks to its high membrane permeability, boric acid infiltrates the roots and reaches the uppermost leaves[13]. It is known that as the acidity of tea soil increases, the amount of boron absorbed by the roots increases. Boron fertilizers, which are known to have a positive effect on the growth of tea, and boron drugs used to prevent pest formation are unnatural boron sources of tea[14,15].

There is an increase in tea consumption in Türkiye compared to other countries, making the amount of boron taken with tea important. Therefore, this study aimed to determine the amount of boron in teas consumed most in Türkiye and its health impact.

METHODOLOGY

Chemicals

The chemicals used in this study were of analytical grade and were obtained from Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA) and Fluka (Buchs, Switzerland) companies.

Tea samples

The seventeen (six black bulk and eleven black bagged teas) frequently consumed teas were purchased from large markets with high sales circulation, and their packages were checked. The quantities to be used were separated and numbered. Then, the tea samples were ground into powder using a blender. They were labeled.

Modified carminic acid method

The modified carminic acid method was used to determine the boron concentration[16,17]. Carminic acid and concentrated sulfuric acid were used to prepare the carmine solution (0,4 mM). Boric acid was used to prepare boron standard solutions (1-10 ppm). All solutions were freshly prepared. In this method, the absorbance at 585 nm of the colored complex formed as a result of the reaction between boron and carminic acid in a sulfuric acid medium was measured. The boron concentration in the sample was determined using the boron standard curve. The lowest boron concentration that could be measured with this method was 0.25 ppm.

Boron determination in dry and brewed tea samples

A dry tea sample (0.125 g) was placed into porcelain crucibles. Due to the high volatility of boron at low pH values, sodium hydroxide (100 µL, 1 M) was added and the mixture was left to dry in an oven overnight at 85°C. The dried sample was then burned in an ashing furnace at 550°C for 4 h. After cooling in a desiccator, the sample was acidified with hydrochloric acid (0.4 mL, 6 M). Then it was diluted with distilled water to 1 mL and centrifuged at 4000 rpm for 10 min. The supernatant was used for the carminic acid assay. Concentrated HCl (10 μL), concentrated H_2SO_4 (1 mL), and carmine solution (1 mL, 0,4 mM) were added to supernatant (0.2 mL) and to water blank (0.2 mL), respectively, and mixed. After incubation for 45 min at room temperature, the absorbance was measured at 585 nm wavelength in a spectrophotometer (Rayleigh-UV-1800). Measurements were repeated for two times.

To determine the amount of boron in brewed teas, each powdered tea sample was weighed 0.5 g and brewed with 50 mL of hot water for 20 minutes. After 20 minutes, the liquid portion was immediately separated from the tea and centrifuged at 4000 rpm for 10 minutes. Supernatants were aliquoted and stored in the deep freezer (at −20°C). The amount of boron in the supernatants was determined using the carminic acid method as described for dried tea samples in the paragraph above. However, this time a sample blank was used instead of a water blank. To prepare a sample blank, concentrated HCl (10 μL) and concentrated H_2SO_4 (2 mL) were added to the supernatant (0.2 mL). Carminic acid was not added.

Statistical analysis

The results were statistically evaluated using via GraphPad Prism 9.0. The values were expressed as means ± standard deviation. The Mann Whitney test was used for pairwise comparisons. A $p<0.05$ was considered statistically significant.

RESULTS and DISCUSSION

The main sources of boron in the human body include drinking water, tea, mineral water and food. Additionally, various industrial products such as antiseptics, preservatives, some drugs containing boron due to its plasticizer or flame retardant properties, cosmetic products, detergents, pesticides, adhesives, and carpets also contribute to boron intake. Results obtained from studies suggest that boron, as a dynamic trace element, affects a broad range of biological functions[18]. This emphasizes the necessity of determining boron levels in biological samples, particularly those relevant to human nutrition.

Although the metabolism of boron is not yet fully understood, it is thought to be absorbed entirely by the human body through the digestive system and can also be absorbed through the respiratory tract and skin contact.

Boron can accumulate in various organs and tissues of the human body at different rates. It is reported that boron, found at levels of 3-20 mg in the body, accumulates mostly in the heart (28 ppm), followed by the bones (4.3-17.9 ppm) and liver (2.3 ppm). Although daily boron intake varies by country, it is generally considered to be 1-3 mg[19,20].

In the present study, the average amount of boron in dried tea samples was found to be 10.21 and 13.06 ppm for black bulk tea and black bag tea, respectively. There was a significant difference between the boron amount ($p<0.05$) (Table 1). However, this significant difference may not be meaningful due to the use of black teas from different brands.

Table 1. Comparison of boron contents of black bulk and bagged teas

Boron (ppm)	Black bulk tea (n=6)	Black bagged tea (n=11)	p
Mean	10.21	13.06	<0.05 Mann Whitney test
Standard Deviation	0.80	3.66	

No significant difference was detected between the amount of boron in brewed black bulk and brewed bagged teas ($p>0.05$, Table 2)

Table 2. Comparison of boron contents of brewed black bulk and bagged teas

Brewed Tea Boron (ppm)	Black bulk tea (n=6)	Black bagged tea (n=11)	p
Mean	3.29	3.26	>0.05 Mann Whitney test
Standard Deviation	0.88	0.86	

The amount of boron in the brewed tea samples was about 3.3 ppm. The amount of boron transferred to the brewed tea sample decreased by approximately 3-4 times to around 30%.

In a study, boron concentrations have been determined in water-soluble and acid-soluble fractions in black teas and fruit teas available on the Polish Market using inductively coupled plasma-atomic emission spectrometry. The potential human health risk has been investigated. The mean total content of boron ranged from 8.31 to 18.40 mg/kg (ppm) in black teas. The degree of extraction of boron in black tea ranged from 8% to 27%. It has been concluded that it may not produce any health risks for human consumption, if other sources of metal contaminated food are not taken at the same time. The findings of Frankowska's study were similar to this study[21]. The difference between this study and theirs was the method. They used the ICP method, while we used the spectrophotometric method (carminic acid method). However, the boron concentrations determined by ICP and by the carminic acid method were correlated with each other[16].

The boron level of black tea found in our study was similar to the boron concentration mentioned as between 3.10–57.8 ppm in a review of surveyed the elemental analysis of teas[22]. It was also similar to the boron concentration mentioned in another review article about micronutrients (B, Co, Cu, Fe, Mn, Mo, and Zn) content in made tea and tea infusion[23].

In the study examining the mineral nutrient composition of tea plants harvested at different shoot periods, changes in the composition of many elements, including boron, and their relationship with soil properties were examined. It was found that the boron content in tea leaves varied between 10.63-31.58 ppm[24]. Our results are consistent with the findings of this research.

In a study conducted in Türkiye, the boron concentrations in brewed teas (black tea, chamomile tea, apple tea, rosehip tea, sage tea, linden tea, and green tea produced by Lipton) have been determined using Optical Emission Spectroscopy. The boron content in the tea infusions after five minutes have been varied between 0.084 and 2.023 ppm[25]. This amount was lower than our results for brewed teas. The variation in infusion time, 20 minutes in our study and 5 minutes in theirs, could explain this difference.

Other factors that affect boron intake include age, gender, and metabolic rate. Considering these factors, the daily boron intake recommended by the European Food Safety Authority (EFSA) is as follows: 3 mg for ages 1-3; 4 mg for 4-6 years old; 5 mg for ages 7-10; 7 mg for ages 11-14; 9 mg for ages 15-17; and 10 mg for adults. According to the World Health Organization (WHO), although the safe dose range for adults was defined as 1-13 mg boron/day, this value has been revised to 28 mg boron/day for an adult weighing 70 kg. These calculations change over the years according to countries and organizations, and as a result, they have an impact on the limits imposed on the amount of boron in drinking water, tea and mineral water[26,27,28]. Assuming an individual consumes five glasses of tea (500 mL) daily, the average daily boron intake from bulk or brewed tea is 5.25 mg or 1.75 mg, respectively.

In conclusion, when considering the daily upper limit level, our research supports the safety of tea consumption in terms of boron levels. It also helps fulfill our boron intake requirements without surpassing the daily upper limit. Therefore, it has the potential to contribute to the maintenance of homeostasis and the prevention and treatment of many diseases.

STATEMENT OF ETHICS

This study does not require any ethical approval.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Design: AY, BAT. Acquisition of Data: AC, ABOK, BAT. Analysis of Data: AC, AY. Drafting of the Manuscript: AC, ABOK, BAT, AY. Critical Revision of the Manuscript: AC, ABOK, BAT, AY. Statistical Analysis: AY. Supervision: AY.

FUNDING SOURCES

No funding or other financial support was received.

ACKNOWLEDGMENTS

Not applicable.

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