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Evaluation of the macro, essential micro, and toxic element compositions of commercial red beetroot juice samples using ICP-MS

Çiğdem Tuncay[1] and Hatice Aybuke Karaoglan[2*]

[1] Instute of Science, Sivas Cumhuriyet University, Sivas, Türkiye
[2] Department of Nutrition and Dietetics, Faculty of Health Sciences, Sivas Cumhuriyet University, Sivas, Türkiye

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ABSTRACT

Red beetrootjuice (RBJ) is known for its health benefits. However, red beetroot is a soil-grown crop; thus, water sources and materials used in vegetable juice production are particularly vulnerable to toxic elements. This study investigated the concentrations of macro, essential micro, and toxic elements in RBJ samples and their compliance with relevant regulations. The analysis revealed that the RBJ samples contained average concentrations of 1179 mg 100 mL^{-1} sodium (Na), 124 mg 100 mL^{-1} potassium (K), 21.18 mg 100 mL^{-1} calcium (Ca), 13.37 mg 100 mL^{-1} magnesium (Mg), and 13.21 mg 100 mL^{-1} phosphorus (P). The average levels of essential microelements were 634 µg 100 mL^{-1} iron (Fe), 85.8 µg 100 mL^{-1} manganese (Mn), 25.71 µg 100 mL^{-1} zinc (Zn), 9.68 µg 100 mL^{-1} copper (Cu), 0.345 µg 100 mL^{-1} cobalt (Co), 9.05 µg 100 mL^{-1} selenium (Se), and 1.197 µg 100 mL^{-1} molybdenum (Mo). Although RBJ is a moderate source of K, Mn, and Se, it contributed less significantly to daily intake levels of Ca, Mg, and Zn. In terms of toxic elements, high levels of aluminum (Al; mean: 6369 µg L^{-1}), arsenic (As; 28.66 µg L^{-1}), and chromium (Cr; 142 µg L^{-1}) were found, exceeding the maximum allowable limits set by standards. Cadmium (Cd), lead (Pb; detected in 5 samples), Co (exceeding limits in 3 samples), and nickel (Ni) were also detected but generally remained within acceptable thresholds. The findings of this study emphasize the dual role of RBJ as both a source of beneficial micronutrients and a potential carrier of toxic elements.

1. Introduction

Red beetroot (*Beta vulgaris* L.) is a plant species belonging to the Amaranthaceae family. It is native to the Mediterranean region but is now cultivated in various parts of the world, including the Americas, Europe, and India (Zohary et al., 2012; Chawla et al., 2016). Türkiye possesses significant red beetroot production potential. In 2022, the country's annual red beetroot yield increased by 125.4% compared to the previous year, reaching 23,453 tonnes (TÜİK 2021). Compared to the other subspecies, such as *Beta vulgaris* subsp. *vulgaris var. altissima* (commonly known as sugar beet), red beetroot contains approximately 50% less sugar (Wruss et al., 2015). As a result, it is primarily used in the production of pickles, salads, and vegetable juices, rather than for sugar extraction (Janiszewska-Turak et al., 2022a; Janiszewska-Turak et al., 2022b). Red beetroot is also a rich source of bioactive compounds, particularly betalains and polyphenols, which contribute significantly to its antioxidant capacity (Clifford et al., 2015).

Assessing the quantity and nutritional importance content of bioactive compounds, particularly in plant-based foods, is of considerable importance. Moreover, determining the macro- and micro elements content of foods is crucial, as it provides insights into potential toxic effects arising from the excessive accumulation of heavy metals. The evaluation of food quality often depends on both the variety and concentration of these elements. Approximately 30 components are essential for life survival. While certain elements like calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), and sodium (Na) are necessary in large amounts, copper (Cu), iron (Fe), zinc (Zn), and cobalt are vital in fewer amounts. Elements such as cobalt (Co), selenium (Se), molybdenum (Mo), and manganese (Mn) are necessary in micro/trace amounts (Cindrić et al., 2011). Although these elements are commonly referred to as heavy metals, in fact, they are essential for human life and have a significant impact on biological processes in various amounts (Handique et al., 2017; Mitra et al., 2022). However, excessive intake can lead to toxicity. For instance, while Fe is essential for many physiological functions, prolonged overexposure has been linked to adverse health outcomes, including the development of Parkinson's disease (Powers et al., 2003).

*Corresponding author
E-mail address: akaraoglan@cumhuriyet.edu.tr

Similarly, higher Mn intake may lead to Mn-induced Parkinsonism, and excessive Zn levels can impair growth and reproductive functions (Powers et al., 2003; Handique et al., 2017).

Conversely, certain non-essential components can be poisonous even when present in small amounts (Cindrić et al., 2011). Gold (Au), Cd nickel (Ni), Cr, indium (In), lithium (Li), platinum (Pt), vanadium (V), strontium (Sr), mercury (Hg), and microelements such as Pb, Al, As, antimony (Sb), barium (Ba), and beryllium (Be) have toxic effects even at extremely low concentrations (Peralta-Videa et al., 2009; Waldbauer et al., 2017). Toxic elements are an important class of pollutants because of their hypertoxicity, persistence, and bioaccumulation, and they pose a major environmental threat to water security (Wei et al., 2022). The risk assessment for toxic elements is predicated on the hypothesis that these toxins may possess either non-carcinogenic or carcinogenic characteristics following exposure via consumption of water or food, or through skin contact (Wei et al., 2022).

Contamination of agricultural soils with toxic elements is a major environmental concern, as it can reduce crop yield and compromise the safety of vegetable juices derived from plants grown in contaminated areas (Kabata-Pendias & Mukherjee, 2007). Therefore, it is essential to assess the levels of toxic elements and evaluate potential tolerance thresholds before human exposure occurs. Several organizations regulate toxic element limits and food safety standards, including the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the World Health Organization (WHO), the United States Environmental Protection Agency (USEPA), the European Commission (EU Directive), the Republic of Türkiye Ministry of Agriculture and Forestry (TMAF), and the Republic of Türkiye Ministry of Health (TMH). These institutions establish various guidelines and permissible limits for toxic elements in food products.

This study examined the macro- and microelement compositions - both essential and potentially toxic - of red beetroot juice (RBJ) (Kyung et al., 2005; Georgiev et al., 2010; Ravichandran et al., 2013; Sawicki et al., 2016; Barutçu Mazi et al., 2018; Guneser, 2021; Ozcan et al., 2021; Durukan et al., 2024). Although similar studies have been conducted globally (Nizioł-Lukaszewska & Gawęda, 2016; Pohl et al., 2019; Sentkowska & Pyrzynska, 2020), there remains a lack of region-specific research on this topic in Türkiye. Given that factors such as soil characteristics, climate conditions, plant functional types, and environmental contamination levels significantly influence the elemental composition of vegetables and their juices (Peralta-Videa et al., 2009; Han et al., 2011; Stagnari et al., 2014), it is essential to conduct such studies at the regional level. Comprehensive evaluations of representative samples can help assess the potential health risks associated with elemental exposure. Furthermore, these findings can be compared with results from other geographic regions, enhancing our understanding of elemental profiles in plant-based beverages.

The aim of this study is to provide the first detailed assessment of the elemental composition of RBJ samples collected from different regions in Türkiye, thereby addressing a significant gap in the national literature. This research offers novel insight into the local variability of both beneficial and toxic elements in RBJ, contributing to public health awareness and regional food safety monitoring.

2. Materials and Methods

2.1. Sample collection

In August 2023, ten red beetroot juice samples from different brands were collected from various companies via local markets in Ankara (Türkiye), and online shopping platforms across Türkiye. The products have a price range of 1.3 - 6.36 dollars (35 - 180 Turkish Liras) at that time scale.

2.2. The pH analysis

The pH values of the samples were determined using a pH meter (Hanna HI 1221, Czech Republic).

2.3. Elemental analysis

Elemental analysis of the samples was conducted using the Thermo Scientific iCAP-Q inductively ICP-MS (Thermo Scientific, Bremen, Germany), which is a highly sensitive and precise analytical technique widely used for multi-element detection in complex matrices. ICP-MS enables the rapid quantification of both macroelements (e.g., Ca, K, Mg) and microelements (e.g., Fe, Zn, Cu), and potentially toxic trace metals such as Pb, Cd, and As, in a wide range of biological and environmental samples including fruits, vegetables, and beverages (Bueno et al., 2021).

Before ICP-MS analysis, all samples were subjected to wet digestion to ensure complete breakdown of organic matter and effective release of bound elements. Digestion was performed using an Automatic Microwave Digestion System (Milestone, Sorisole, Italy), which allows for controlled temperature and pressure conditions to enhance digestion efficiency and reproducibility. For each sample, a 1 mL aliquot was transferred into a high-purity Teflon digestion vessel. Subsequently, 4 mL of concentrated nitric acid (HNO_3), 2 mL of 30% hydrogen peroxide (H_2O_2), and 2 mL of ultrapure deionized water were added to each vessel. The mixture was subjected to a microwave digestion program optimized for plant-based matrices.

Following digestion, the resulting solution was diluted to a final volume of 50 mL using a matrix-matching diluent containing 3% HNO_3 and 0.5% hydrochloric acid (HCl). This step ensured the stability of the analytes and minimized potential matrix interference during ICP-MS analysis. All samples were prepared and diluted using the same standardized protocol to ensure consistency and comparability of results. Calibration of the ICP-MS instrument was performed using certified multi-element standard solutions, and internal standards were employed to correct for potential instrumental drift and signal suppression or enhancement. Quality control measures, including analysis of procedural blanks and standard reference materials, were applied throughout the procedure to validate the accuracy and precision of the results.

Quality control procedures were implemented throughout the analysis to ensure data accuracy and reliability, as reported by (Tanase et al., 2015). Procedural blanks were included in each batch to detect possible contamination. Duplicate sample measurements were performed to assess the analytical precision. Instrument calibration was performed using certified multi-element standard solutions, and internal standards were used to correct for matrix effects, instrumental drift, and potential signal suppression or enhancement. Additionally, certified reference materials (CRMs) were analyzed along with the samples to validate the accuracy of the method. The recovery values for the CRMs fell within acceptable limits (90-

110%), confirming the robustness of the analytical process.

2.4. Statistical analysis

All measurements were performed in triplicate, and the results are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to identify significant differences in mean values. The Tukey test revealed the differences between each element of the samples. A significance level of $P<0.05$ was considered statistically significant.

To determine the relationship between the examined samples and the data, multivariate analysis was performed using a pattern recognition method called hierarchical grouping of samples (or cluster analysis). The brands were assessed based on their similarity or dissimilarity and represented using dendrogram-type graphics using the Ward method (Asare et al., 2011). The statistical analysis was conducted using MINITAB 20 software (State College, PA, USA).

3. Results and Discussion

3.1. The pH values of the samples

The pH value, a crucial factor influencing fermentation, is closely linked to changes in both microbiota and phytochemicals composition during and after the fermentation process. The activities of LAB during RBJ fermentation results in the production of organic acids, primarily lactic acid, which leads to a decrease in pH (Bueno et al., 2021). As a result of lactic acid fermentation, fermented RBJ contains the highest concentration of lactic acid, followed by acetic acid (Duyar et al., 2024).

In this study, the pH values of RBJ samples ranged from 2.40 to 3.90. These values fall within the expected range for fermented beverages and are consistent with previous studies on fermented vegetable juices (Yoon et al., 2004; 2006; Kazimierczak et al., 2014; Panghal et al., 2018; Duyar et al., 2024).

3.2. Levels of macro and micro-elements

Macroelements

The concentration of Na, K, Ca, P, and Mg in the RBJ samples are presented in Table 1. Among these macroelements, Na exhibited the highest concentration, with an average of 1179 mg 100 mL^{-1}. The recommended daily intake of Na for healthy adults is 500 mg (Jayedi et al., 2019). According to the UK Food Standards Agency, beverages containing more than 600 mg of Na per 100 mL are classified as high in sodium, those with 120-600 mg as medium, and ≤120 mg as low (Kraemer et al., 2016). All samples, except for RBJ-5, exceeded the high-sodium threshold and therefore may pose health concerns if consumed in large quantities.

The average concentrations of K, Ca, Mg, and P across all RBJ samples were 124, 21.18, 13.37, and 13.21 mg 100 mL^{-1}, respectively. According to the Turkish Food Codex Nutrition Declarations Regulation (TMAF, 20.04.2023), the daily reference intake values for individuals aged 4 years and older are 2000 mg for K, 800 mg for Ca, 375 mg for Mg, and 700 mg for P (Turkish Food Codex, 2023). Consumption of 100 mL of RBJ contributed 2.73-13.29% of the daily K requirement, 1.61-5.37% of Ca, 1.15-6.42% of Mg, and 0.88-5.62% of P. According to the same regulation, a product can be considered a significant source of a nutrient if it provides ≥7.5% of the daily reference intake per 100 mL. Based on this criterion, samples RBJ-1, RBJ-5, RBJ-6, and RBJ-10, which contributed 10.77%, 8.95%, 8.76%, and 13.28% of the daily K intake, respectively, may be regarded as excellent sources of K. However, none of the samples met the threshold to be considered significant sources of Ca, Mg, or P. It is important to note that the daily requirements for these minerals are generally met through a balanced diet rich in legumes, meat, eggs, and dairy products (Leterme et al., 2006). Nevertheless, excessive sodium intake should be avoided, as it is prevalent in a wide range of food sources.

Beyond measuring the elemental concentrations, evaluating elemental ratios is essential due to potential interactions among minerals, which can influence bioavailability and nutritional quality. The K/Na ratios of the samples ranged from 0.03 to 0.31 (Table 1).

Table 1. Macroelement contents and recommended daily reference intake values for RBJ samples

Sample code	Na	K	Mg	P	Ca	K/Na	Ca/Mg	K/[Ca+Mg]
	mg 100 mL^{-1}							
RBJ-1	1263±13^{e}	217±1^{b}	14.88±0.12^{c}	11.64±0.05^{c}	19.79±0.17^{e}	0.17	1.33	6.27
RBJ-2	1273±5de	62.83±0.78^{g}	9.98±0.14^{f}	6.18±0.08^{e}	17.30±0.29^{f}	0.05	1.73	2.30
RBJ-3	1224±11^{f}	87.84±0.27^{e}	12.66±0.02^{e}	9.04±0.16^{d}	26.06±0.14^{b}	0.07	2.06	2.27
RBJ-4	796±3^{h}	93.10±0.46^{d}	13.72±0.15^{d}	8.82±0.51^{d}	14.81±0.07^{h}	0.12	1.08	3.26
RBJ-5	579±7^{ı}	179±2.84^{c}	20.20±0.20^{b}	9.21±0.16^{d}	15.17±0.4^{h}	0.31	0.75	5.06
RBJ-6	1405±12^{b}	175±0.65^{c}	20.30±0.13^{b}	20.91±0.88^{b}	42.94±0.12^{a}	0.12	2.12	2.77
RBJ-7	1110±6^{g}	29.78±0.47^{ı}	4.32±0.06^{ı}	6.75±0.30^{e}	20.90±0.15^{d}	0.03	4.84	1.18
RBJ-8	1333±8^{c}	54.57±0.21^{h}	8.25±0.02^{g}	9.01±0.10^{d}	12.97±12.87^{ı}	0.04	1.57	2.57
RBJ-9	1303±18^{cd}	72.59±0.53^{f}	5.27±0.06^{h}	11.21±0.20^{c}	16.51±0.27^{g}	0.06	3.13	3.33
RBJ-10	1503±25^{a}	266±4.05^{a}	24.09±0.49^{a}	39.36±0.71^{a}	25.31±0.28^{c}	0.18	1.05	5.38
Average	1179	124	13.37	13.21	21.18			
Minimum	571	29.5	4.29	6.09	12.87			
Maximum	1528	270	24.61	40.16	43.07			
CV, %	23.26	61.93	47.99	73.52	40.12			
GRAD*	-	2000	375	700	800			

*Recommended daily reference intake value for healthy individuals aged 4 years and above (mg) (Turkish Food Codex 2023)
Different letters within the same column indicate statistically significant differences (P<0.05).

These relatively low ratios are primarily attributed to elevated Na levels. Such low K/Na ratios are not ideal, especially for individuals with cardiovascular diseases or type II diabetes, who are advised to moderate their sodium intake (Kong et al., 2016). In contrast, a study on commercially available RBJ in Poland reported K/Na ratios ranging from 5.8 to 10.0, with an average of 7.5, suggesting potential cardiovascular benefits associated with higher potassium relative to sodium (Pohl et al., 2019). These differences highlight the influence of geographical location and soil characteristics on the elemental composition of vegetable-based products (Peralta-Videa et al., 2009; Han et al., 2011; Stagnari et al., 2014).

The K/(Ca + Mg) ratios in the RBJ samples ranged from 1.18 to 6.27. While some studies consider these ratios acceptable for human nutrition (Pohl et al., 2019), others suggest an optimal range of 1.6-2.2, with higher values potentially indicating inadequate intake of Ca or Mg (Francke & Klasa 2009). The Ca/Mg ratios ranged from 0.75 to 4.84. According to Francke & Klasa (2009), this ratio should not exceed 3.0 to maintain a balanced mineral profile. With the exception for RBJ-7, all samples remained within or near the recommended range. However, the presence of oxalic acid and phenolic compounds in RBJ may bind with Ca and Mg, forming complexes that reduce their bioaccessibility (Pohl et al., 2019).

Statistical analysis using ANOVA revealed significant differences ($P<0.001$) in the macroelement concentrations among the RBJ samples. The coefficients of variation (CVs) indicated a high degree of variability for K (61.93%) and P (73.52%), whereas Na (23.26%), Mg (47.99%), and Ca (0.12%) showed lower variability, suggesting more uniform distribution of the latter elements across the samples. These variations can be attributed to differences in red beet cultivars, growing conditions, soil characteristics, fertilization practices, and juice production methods (Stagnari et al., 2014).

Hierarchical cluster analysis was performed based on the macroelement composition to identify similarities among the RBJ samples (Dippong et al., 2024). The resulting dendrogram (Figure 1a) revealed two main clusters: RBJ-4 and RBJ-5 formed the first cluster, while the remaining eight samples comprised the second. Within this second cluster, RBJ-2 and RBJ-9 showed the highest similarity (96.33%), followed by RBJ-8 (94.22%), RBJ-3 (88.46%), and the RBJ-10/RBJ-6 pair (85.14%). RBJ-4 and RBJ-5 exhibited a similarity of 75.86%. These groupings likely reflect differences in raw material sources and processing methods.

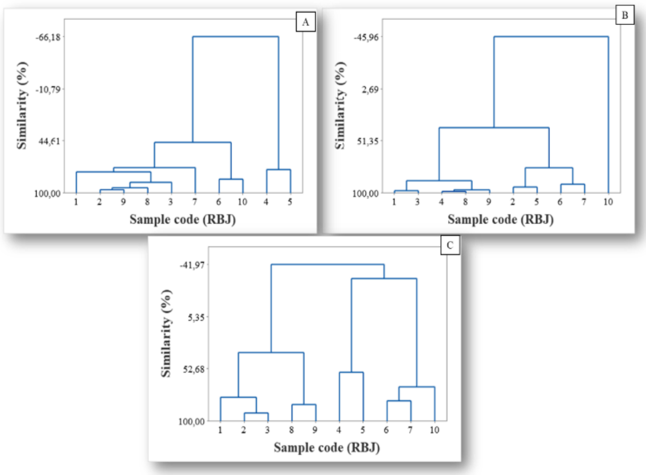

Figure 1. Dendographic classification of RBJ samples according to their elements. (A. macroelements, B: essential microelements, C: toxic microelements)

Microelements

Table 2 presents the concentrations of the essential microelements Fe, Mn, Zn, Cu, Co, Se, and Mo in the RBJ samples analyzed in this study. The mean concentrations were as follows: Fe, 634 µg 100 $mL^{-1;}$ Mn, 85.8 µg 100 mL^{-1}; Zn, 25.71 µg 100 mL^{-1}; Cu, 9.68 µg 100 mL^{-1}; Co, 0.345 µg 100 mL^{-1}; Se, 9.05 µg 100 mL^{-1}; and Mo, 1.197 µg 100 mL^{-1}. statistically significant differences ($P<0.05$) among the samples for all microelements. As shown in Table 2, most elements exhibited high variability, with coefficients of variation (CVs) ranging from 71.38% to 116.18%, except for Zn, which had a relatively lower CV of 44%.

According to the Turkish Food Codex Nutrition Declarations Regulation (TMAF, 2023), the recommended daily intake values for individuals aged 4 years and older are as follows: Fe - 14 mg, Mn - 2 mg, Zn - 10 mg, Cu - 1 mg, Se - 55 µg, and Mo - 50 µg. Based on these reference values, the consumption of 100 mL of the RBJ samples did not provide a significant contribution to the daily recommended intake levels of Zn, Cu, or Mo. Among the samples, only RBJ-9 met 18.91% of the daily Fe requirement, while RBJ-5 and RBJ-6 contributed 13.10% and 8.24% of the Mn, respectively. For Se, all samples, except for RBJ-5, met the recommended daily intake, contributing between 7.6% and 61.82%. Similar findings were reported by Wruss et al. (2015), who analyzed the mineral content of RBJ prepared from different red beet varieties. Similarly, the Cu, Fe, Zn, and Mn levels were consistent with those found in commercially available RBJ in Poland (Sentkowska & Pyrzynska 2020). Se is particularly important in the human diet because of its role as a key component of antioxidant enzymes that protect cells against oxidative stress (Schomburg, 2017).

The study applied hierarchical cluster analysis to assess similarities between RBJ samples based on essential microelement levels. Figure 1b shows two distinct primary clusters. While a cluster contained the sample coded RBJ-2, the other cluster contained all the samples except the sample coded RBJ-2. RBJ-4 and RBJ-8 (98.33%), RBJ 1 and RBJ-3 (97.67%), RBJ-4 and RBJ-9 (96.88%), RBJ-5 and RBJ-10 (94.24%), RBJ-6, and high similarities were detected between RBJ-7 (91%). A similarity of 88.38% was determined between the samples coded RBJ-1, RBJ-3, RBJ-4, RBJ-8, and RBJ-9.

The concentrations of toxic microelements in the RBJ samples are listed in Table 3. These values were compared against drinking water quality standards provided by the International Council Directive 98/83/EC, the World Health Organization (WHO) guidelines, and the Regulation on Water Intended for Human Consumption No. 25730, published by the TMH, ANNEX-1 (as amended by OG-7/3/2013-28580). Although these are water-based regulatory values, they are used here for comparison due to the lack of specific juice-based thresholds. The toxic elements were ranked in descending order of concentration as follows: Al, Sr, Cr, Tb, As, Ni, Cd, and Pb.

Among these elements, Ni was detected in only two samples (RBJ-6 and RBJ-9), while Pb was found in five samples (RBJ-3, RBJ-4, RBJ-6, RBJ-9, and RBJ-10). However, all samples contained measurable levels of the other toxic elements examined. Although the concentrations observed may not pose immediate health risks, long-term exposure to elevated levels of these elements can have serious adverse health effects and may lead to toxicity or chronic poisoning.

The average Al concentration across all samples was 6369 µg L^{-1}, which significantly exceeded the maximum allowable concentration of 200 µg L^{-1} set by EU, WHO, and Turkish regulations.

Table 2. Essential microelement contents of the RBJ samples (µg 100 mL^{-1}) and their maximum limits defined by the standards

Sample code	Fe	Mn	Zn	Cu	Co	Se	Mo
RBJ-1	101±8^{h}	76.76±0.03^{cd}	85.80±1.15^{b}	20.19±0.46^{d}	0.291±0.020^{c}	4.195±1.661	0.545±0.066
RBJ-2	92.69±2.25^{h}	35.09±0.06^{f}	44.99±0.38^{e}	12.16±0.20^{f}	0.067±0.010^{d}	5.378±0.56	0.480±0.052
RBJ-3	253±1^{fg}	31.96±0.04^{f}	44.08±0.63^{e}	21.98±0.60^{cd}	0.595±0.030^{b}	8.38±2.24	0.576±0.108
RBJ-4	448±30^{e}	83.67±5.40^{c}	79.94±0.87^{c}	24.12±1.74^{c}	0.084±0.017^{d}	6.531±1.558	0.511±0.088
RBJ-5	776±1^{c}	262±5^{a}	49.54±0.55^{d}	19.07±0.21^{de}	0.139±0.009^{d}	3.647±1.101	0.625±0.041
RBJ-6	958±37^{b}	165±8^{b}	107±2^{a}	42.58±1.66^{b}	0.976±0.103^{a}	6.32±1.77	1.123±0.110
RBJ-7	289±25^{f}	39.73±1.15^{f}	26.02±0.46^{g}	10.28±0.91^{f}	0.162±0.035^{d}	5.005±1.038	0.494±0.0803
RBJ-8	202±10^{g}	37.32±0.70^{f}	31.35±0.34^{f}	16.66±0.03^{e}	0.117±0.030^{d}	8.95±1.85	1.401±0.060
RBJ-9	2647±16^{a}	56.92±0.34^{e}	49.83±0.03^{d}	23.47±0.16^{c}	0.644±0.024^{b}	8.14±2.39	3.556±0.178
RBJ-10	577±13^{d}	69.94±1.05^{d}	46.47±1.59^{e}	77.16±2.23^{a}	0.379±0.057^{c}	34±2.78	2.663±0.084
Average	634±737	85.8±70.8	56.46±24.85	26.77±19.11	0.345±0.295	9.05±8.76	1.197±1.039
Minimum	90	31.6	25.71	9.68	0.058	2.46	0.402
Maximum	2660	267	109	79.59	1.057	37.17	3.671
CV, %	116.18	82.47	44.01	71.38	85.32	96.76	86.77
TMAF^{a}	14000	2000	10000	1000	-	55	50
EU directive ^{b}	200	50	5000	100			-
WHO ^{c,d}	300	80	-	2000	-	10	

Different letters within the same column indicate statistically significant differences (P<0.05).
TMAF, Turkish Ministry of Agriculture and Forestry; EU Directive, European Union Drinking Water Directive; WHO, World Health Organization
a, Turkish Food Codex (2023); b, Council of the European Union (2010); c, World Health Organization (2003); d, Karami et al. (2023)

Table 3. Toxic elementalelement contents of RBJ samples and their maximum limits defined by standards

Sample code	Al	Sr	Cr	As	Ni	Cd	Pb
RBJ-1	6083±68de	1762±21^{d}	49.88±2.28^{e}	17.41±1.18^{d}	-	0.29±0.17^{cd}	-
RBJ-2	6023±160de	2081±38^{bc}	49.81±0.64^{e}	20.72±2.27^{cd}	-	0.35±0.26^{bcd}	-
RBJ-3	5884±47^{e}	2151±33^{b}	70.57±2.12^{de}	28.47±2.6^{b}	-	0.39±0.50^{bcd}	8.37±0.60^{d}
RBJ-4	7273±73^{a}	582±32^{g}	87.42±6.28^{d}	25.51±3.51^{bc}	-	1.18±0.57^{bc}	24.79±2.7^{a}
RBJ-5	6389±92^{cd}	875±4^{f}	84.07±3.03^{d}	25.69±3.02^{bc}	-	0.79±0.13^{bcd}	-
RBJ-6	6810±364^{bc}	2073±91^{bc}	437±22^{a}	23.94±1.10^{bcd}	64.91±6.36^{a}	4.91±0.32^{a}	13.85±1.25^{b}
RBJ-7	6821±58^{b}	1944±77^{c}	76.98±4.28^{d}	20.42±1.25^{cd}	-	0.14±0.10^{d}	-
RBJ-8	5670±23^{e}	1462±24^{e}	82.77±2.72^{d}	22.89±1.82^{bcd}	-	0.96±0.37^{bcd}	-
RBJ-9	5713±28^{e}	1335±7^{e}	333.85±7.75^{b}	25.6±0.9^{bc}	142.7±2.51^{b}	0.41±0.14^{bcd}	9.39±0.50^{c}
RBJ-10	7022±169^{ab}	2508±69^{a}	151.65±6.21^{c}	75.94±3.94^{a}	-	1.34±0.52^{b}	2.05±0.24^{d}
Average	6369±570	1677±587	142.4±128.9	28.66±1.65	20.76±45.82	1.08±0.25	5.84±8.09
Minimum	5644	555	47.7	16.55	-	0.055	-
Maximum	7351	2587	456	80.43		5.198	27.88
CV, %	8.95	35.02	90.52	57.39	219.88	129.05	138.50
TMH*^{a}	200	7000	50	10	20		10
EU directive ^{b}	200	-	50	10	70		
WHO ^{c}	200		50	10	20	5	10

* Regulation on Water Intended for Human Consumption No. 25730 published in the Republic of Turkiye Ministry of Health (TMH), ANNEX - 1 (Amended: Compared with OG-7/3/2013-28580).
Different letters within the same column indicate statistically significant differences (P<0.05)
"-" indicates values below the detection limit.
TMH, Turkish Ministry of Health; EU Directive, European Union Drinking Water Directive; WHO, World Health Organization.
a, Turkish Food Codex (2023); b, Council of the European Union (2010); c, WHO (2004)

Chronic exposure to elevated Al levels has been associated with toxic effects on the musculoskeletal, renal, hepatic, respiratory, and nervous systems (Basha et al., 2024). Furthermore, Al can interfere with the absorption of essential nutrients such as Ca, Mg, Fe, vitamin B6, vitamin C, and sulfur-containing amino acids (Unar et al., 2024), and it has been implicated in the progression of neurodegenerative disorders, including Alzheimer's disease (Flaten, 2001).

The RBJ samples had an average Sr concentration of 1677 µg L^{-1}, which was below the EU's maximum permissible limit of 7000 µg L^{-1}. However, the average Cr content was 142 µg L^{-1}, exceeding the recommended limit of 50 µg L^{-1}. Chromium contamination in the environment is commonly attributed to industrial applications in metallurgy, refractory materials, and chemical manufacturing (Muneer et al., 2022). Chronic Cr exposure can lead to a range of health issues, including developmental abnormalities, infertility, cardiovascular disease, and various types of cancer (Dippong et al., 2024).

All samples also contained As and Cd at mean concentrations of 28.66 µg L^{-1} and 1.076 µg L^{-1}, respectively. The maximum permissible level of As in drinking water is 10 µg L^{-1}. All samples exceeded this threshold. Contamination, often resulting from mining, industrial activities, or natural geological sources, is associated with various serious health effects. These include cancers of the lung, skin, and bladder, vascular disorders, dermatological conditions, and other acute and chronic toxic effects (Dippong et al., 2024).

All samples exhibited Cd concentrations below 2 µg L^{-1}. According to the Regulation on Water Intended for Human Consumption, the maximum permissible concentration of cadmium (Cd) in drinking water is 5 µg/L. All RBJ samples analyzed in this study complied with this standard. Although Cd occurs naturally in the environment, its levels are significantly elevated due to anthropogenic activities such as non-ferrous metal smelting and refining, fossil fuel combustion, phosphate fertilizer production, electronic and metal waste recycling, and municipal waste incineration (Turner, 2019). Cd is a highly toxic metal, with particularly harmful effects on the kidneys and skeletal system (Muneer et al., 2022). A study conducted in Serbia revealed Al and Cd in commercial fruit juices, with levels exceeding the standard limits (Velimirović et al., 2013). The EU directive defines the maximum allowable concentration of Co in drinking water at 5 µg/L. According to the data presented in Table 3, it is obvious that three samples (RBJ-3, RBJ-6, RBJ-9) above the previously established limits. Ni was found in two of the samples analyzed (RBJ-6, RBJ-9), with an average value of 20.76 µg L^{-1}. The maximum permissible level of Ni varies depending on the regulatory authority: the EU directive and Turkish drinking water regulations both set the limit at 20 µg/L, whereas the World Health Organization (WHO) allows a higher threshold of 70 µg L^{-1}. Accordingly, the Ni concentration of RBJ-9 slightly exceeded the EU and Turkish limits, whereas that of RBJ-6 remained below the WHO threshold but close to the regulatory maximum. Ni is widely used in industrial applications, including stainless steel alloys, nickel-cadmium (Ni-Cd) batteries, electroplating, ceramic and glass mold production, pigments, and various electronic and medical devices. However, excessive Ni exposure is associated with carcinogenic outcomes and poses significant health risks (Muneer et al., 2022).

Five of the samples (RBJ-3, RBJ-4, RBJ-6, RBJ-9, RBJ-10) contained Pb. The mean Pb concentration in the samples were 5.84 µg L^{-1}. According to EU directive, World Health Organization (WHO) standards, and the Regulation on Water for Human Consumption, the maximum allowable concentration of Pb in drinking water is set at 10 µg/L. Out of the samples, RBJ-4 and RBJ-6 exceeded these limits, whereas KP-9 almost reached this limit. The toxicity of Pb is enhanced because of its accumulation in tissues. In addition, it exerts an influence on the brain and cognitive development of early children. Prolonged exposure in both children and adults can result in harm to the kidneys, reproductive, and immunological systems, as well as harmful effects on the brain system (Ackah et al., 2014). In another study, fruit juices were analyzed for the presence of trace and toxic substances, and the findings revealed that metals could potentially contaminate the juices during the fruit processing stage, either through the use of water or additives employed by the manufacturer. They also reported that the utilization of piping and containers in the factory for the purpose of processing and storage can also result in an elevation of the metal concentration in the juice (Tormen et al., 2011).

Hierarchical cluster analysis was used to evaluate similarities and differences in toxic microelement levels among the RBJ samples. As illustrated in Figure 1c, the samples were grouped into three main clusters. In the first cluster, RBJ-2 and RBJ-3 showed a high degree of similarity (92.48%), while RBJ-1 shared a similarity of 78.22% with this pair. In a separate cluster, RBJ-8 and RBJ-9 exhibited similarity of 84.79%. Another cluster included RBJ-6 and RBJ-7, which showed a similarity of 81.46%, with RBJ-10 displaying 68.81% similarity to this group. Lastly, RBJ-4 and RBJ-5 formed a distinct cluster with a lower similarity of 55.57%. These clustering patterns reflect variations in toxic element composition, which may be influenced by factors such as raw material origin, processing conditions, and environmental exposure.

Vegetable juices may have elevated levels of toxic substances as a result of the raw materials, water consumed during production, and the pipes and containers used in the industry (Tormen et al., 2011; Ličina et al., 2014). Soil-based vegetables have the capacity to accumulate toxic elements through a complex absorption process influenced by multiple interconnected factors. The degree of accumulation can vary to some extent based on individual features and genetics. The overall concentration of heavy metals in different soils is influenced by several factors, such as pH, organic matter, clay content, and other variables. The outcome is predominantly influenced by the characteristics of the soil (Kabata-Pendias & Mukherjee 2007).

4. Conclusions

This study provides a comprehensive evaluation of the macro, essential micro, and toxic elements in commercially available RBJ samples. The results indicate that RBJ can serve as a moderate dietary source of essential elements such as K, Mn, and Se. However, it contributes only marginally to the recommended daily intake levels of other nutrients, including Ca, Mg, and Zn.

A notable finding was the presence of several toxic elements - particularly Al, As, and Cr - at concentrations exceeding the maximum allowable limits established by the EU Directive, WHO guidelines, and national drinking water regulations. Although Cd, Co, Pb, and Ni were detected at lower levels, most remained within or near the safety thresholds. Nonetheless, chronic exposure to trace levels of these toxic elements can pose health risks over time.

Hierarchical cluster analysis based on macro, essential micro, and toxic element content revealed clear groupings among the RBJ samples, indicating variability likely attributable to differences in raw material origin, cultivation soil composition, environmental exposure, and production processes.

Environmental pollution, a growing global concern, has indirect implications for the safety and quality of vegetable juices, potentially increasing the risks to human health. Therefore, future studies should compare the elemental profiles of vegetable juices produced from raw materials of different geographical origins. Such research could help identify sources with optimal levels of essential elements and minimal concentrations of toxic contaminants. Additionally, the use of high-quality raw materials and purified water is strongly recommended during the production of vegetable juices to ensure product safety.

In conclusion, while RBJ offers nutritional value due to its content of beneficial bioactive compounds and essential minerals, the detection of potentially harmful levels of toxic elements underscores the need for routine monitoring and quality control. Regional studies and stricter regulatory oversight are recommended to ensure consumer safety and to maintain product quality over time.

Contribution Rate Statement Summary of Researchers

H.A.K. conceptualized the study, performed the experiments, and contributed to data analysis and manuscript writing. C.T. contributed to the methodology and data interpretation. Both authors have reviewed and approved the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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