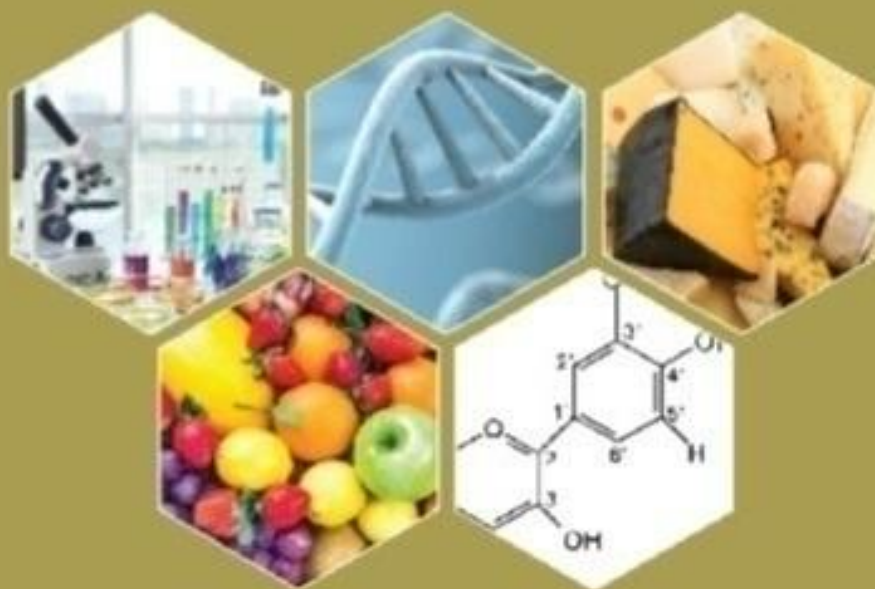


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The effect of ginger use in chicken meatball production on lipid oxidation, sensory properties and other quality criteria

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ABSTRACT

In this study, ginger was used in different proportions (control, 0.25%, 0.50%, 0.75% and 1%) in the production of chicken meatball and the samples were cooked at different temperatures (175 °C and 200 °C). Total aerobic mesophilic bacteria (TAMB) count, pH, moisture, thiobarbituric acid reactive substances (TBARS), ash, cooking yield and sensory analyses were applied on the samples. Ginger ratio (GR) in the meatballs caused statistically significant differences in TAMB, moisture, TBARS, ash, cooking yield and taste values ($P < 0.01$). On the other hand, pH value was affected by this ratio at the level of $P < 0.01$. Especially, with the increase in ginger ratio, lipid oxidation decreased while cooking yield increased. Cooking temperature (CT) caused changes in TAMB, TBARS and ash values. High cooking temperature caused a decrease in TAMB count and TBARS values. GR-CT interaction was statistically effective on TAMB, moisture, ash, cooking efficiency and general acceptability from sensory parameters. Considering the sensory analysis results, it was concluded that up to 0.75% ginger could be used in meatballs to increase functional properties and provide antioxidant and antimicrobial effects.

1. Introduction

Meatball is a meat product that is consumed worldwide and is usually prepared with minced meat, spices, binding agents and various additives (Kirkyol & Akköse, 2023). Despite the fact that they are prepared using a variety of recipes in different countries and cultures, its basic ingredients and production process are similar to each other. Meatball stands out as a healthy food option in nutrition, especially with their high protein, vitamin and mineral content. In addition, due to the ease of preparation and sensory diversity, meatball is gaining importance as an indispensable food in both homemade meals and industrial production, making it commercially attractive. Especially in the ready-to-eat food sector, meatball is prepared with different types of meat and appeal to a wide range of consumer groups (Meng et al., 2022).

The types of meat used in making meatball have a direct effect on the flavor, texture, nutritional value and cost of the product. While red meats such as goat, lamb (Silva et al., 2023), pork (Madsen et al., 1996; He et al., 2022) and beef (Kirkyol & Akköse, 2023) are generally preferred in traditional recipes; other alternative protein sources (white meats) such as chicken (Racan Ricci et al., 2004; Kesemen &

Akköse 2024), turkey (Karpińska-Tymoszczyk & Draszanowska, 2019) and even fish (Guan et al., 2019) are used in the modern food sector. White meats are preferred especially for lower-cost and low-fat products (Karpińska-Tymoszczyk & Draszanowska, 2019).

White meats stand out as a healthy alternative with their low cost, low saturated fat and high protein content (De Lima et al., 2015; Al-Juhaimi et al., 2018; Karpińska-Tymoszczyk, and Draszanowska, 2019). Chicken and turkey meats have more advantages compared to red meats in terms of polyunsaturated fatty acids (Racan Ricci et al., 2011; Petcu et al., 2023). This situation makes meatballs containing white meat more attractive, especially for health-conscious consumers. What is more, its low cholesterol level makes chicken meatballs preferable in terms of cardiovascular health (Lima et al., 2016). The neutral taste of chicken meat can be easily blended with spices and herbal extracts. This allows the meatball to be adapted to different palates (Jaworska et al., 2021). The sensory properties of chicken meatballs can be enriched, especially when produced using ginger (Rongsensusang et al., 2005), rosemary (Karpińska-Tymoszczyk & Draszanowska, 2019) and other herbal additives (Racan Ricci et al., 2004).

As a processed meat product, meatball is susceptible to the problems such as lipid oxidation and microbial spoilage. This

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situation may shorten shelf life, deteriorate sensory properties and increase food safety risks (Lima et al., 2016; Jaworska et al., 2021; Petcu et al., 2023). Antioxidants and antimicrobial agents play a critical role in preventing these problems (Guan et al., 2019; Petcu et al., 2023). Lipid oxidation causes loss of flavor, color change and decrease in nutritional value in the products (Jayasena and Jo, 2014; Karpińska-Tymoszczyk & Draszanowska, 2019). Antioxidants slow down oxidation by neutralizing free radicals. Synthetic antioxidants (e.g. BHA, BHT) have been used as an effective solution for many years, however the demand for natural antioxidants is increasing due to the toxic effects of synthetic ones (Jaworska et al., 2021; Petcu et al., 2023). Microbial spoilage in meatballs increases the risk of foodborne illness (Morsy et al., 2023). Natural antimicrobial agents provide a safe product by preventing the growth of pathogenic microorganisms (Jaworska et al., 2021; He et al., 2022; Petcu et al., 2023). Herbal extracts and natural-sourced components such as ginger and rosemary stand out with both antioxidant and antimicrobial properties (He et al., 2022). In addition, natural components are preferred since they are more suitable for environmentally friendly production processes (Shaukat et al., 2023).

Ginger (*Zingiber officinale*) is one of the most important herbal extracts used in meatball production. It contains phenolic compounds such as gingerol and shogaol which protect the flavor and nutritional value of meatballs by slowing down lipid oxidation (Laelago Erasedo et al., 2023; Shaukat et al., 2023). In addition, ginger increases food safety by preventing the growth of pathogenic microorganisms, which is also crucial in extending shelf life (Beristain-Bauza et al. 2019; Laelago Erasedo et al., 2023; Shaukat et al., 2023). Ginger supports the immune system with its anti-inflammatory and antioxidant properties and turns meatball into a functional food (Laelago Erasedo et al., 2023; Shaukat et al., 2023).

In chicken meatball production, dittany and rosemary herb (Racanucci et al., 2004), mate (Racanucci et al., 2008), argel leaf extract (Al-Juhaimi et al., 2018), moringa leaf powder (Nisar et al., 2020) and ginger (Rongsensusang et al., 2005) were used as antioxidants. In the study conducted by Rongsensusang et al., (2005); 2, 4 and 6% ginger was added to meatballs made from chicken meat and the product was examined in terms of pH, penetrometer values, TBA, microbial load and sensory properties during storage. However, the effects of different cooking temperatures on meatballs with ginger added have not been investigated in the literature. In addition, cooking efficiency, moisture and ash analyzes have not been performed on chicken meatballs with ginger added. In this study, different amounts of ginger (control, 0.25%, 0.50%, 0.75% and 1%) were used in meatballs produced using chicken meat and they were cooked at different temperatures (175 °C and 200 °C). At the end of production, samples were analyzed in terms of total aerobic mesophilic bacteria (TAMB), pH, moisture, thiobarbituric acid reactive substances (TBARS), ash, cooking efficiency and sensory ways (color, appearance, odor, texture, taste and general acceptability).

2. Materials and Methods

2.1. Materials

The chicken meat, beef fat, salt, onion, breadcrumbs, eggs and spices (hot pepper powder, sweet pepper powder, black pepper, cumin and ginger) used in this study were supplied

from the Kastamonu market.

2.2. Methods

Production of meatball

To start with the ingredients, 71% chicken meat, 12% beef fat, 1% salt, 6% onion, 6% breadcrumbs, 2.5% egg, 0.3% hot pepper powder, 0.6% sweet pepper powder, 0.3% black pepper and 0.3% cumin were used for meatball production. The first group prepared with this formulation was considered as control and ginger was not used in production. In the other groups, ginger was added in addition to this formulation at different rates (0.25%, 0.5%, 0.75% and 1%). 40 g of meatballs were taken and shaped using a metal mold (6.5 cm diameter and 1 cm thickness). The produced meatballs were cooked on a hot plate (Elektromag M4060, Türkiye) at different temperatures (175 °C and 200 °C) and then analyzed for total aerobic mesophilic bacteria (TAMB), pH, moisture, thiobarbituric acid reactive substances (TBARS), ash, and cooking efficiency. Sensory analysis was also applied to the samples.

TAMB

PCA (plate count agar) was used for TAMB count. 25 g of meatball samples were weighed; 225 mL of dilution fluid was added and homogenized using the Stomacher device. After that, the homogenate was diluted with the rate of 1:10. Appropriate dilutions were cultured by the spreading method and the petri plates were incubated at 30 °C for 2 days. At the end of the period, the TAMB count was determined as log cfu/g.

pH

Before determining the pH values of the samples, the pH meter was calibrated using buffer solutions (pH 4.00 and pH 7.00). Then, 100 mL of pure water was added onto 10 g of sample and homogenized using an ultra-turrax device (Velp Scientific, Italy). The pH value of the homogenate was determined using a calibrated pH meter (Isolab, Germany) (Gökalp et al., 2010).

Moisture

The nickel containers to be used in the analysis were dried and tared. Then, 10 g of meatball samples were weighed on those containers and dried at 105 °C until a constant weight was reached. The moisture content was stated as % at the end of drying (Gökalp et al., 2010).

TBARS

To determine lipid oxidation in meatballs, 12 mL of 7.5% TCA solution was added on 2 g of weighed sample; the mixture was homogenized using ultra-turrax (Velp Scientific, Italy) and filtered by Whatman No.1 filter paper. 3 mL of this filtrate and 3 mL of 0.02 M TBA solution were mixed together, kept in a boiling water bath for 40 minutes and cooled. After the mixture was cooled, then it was centrifuged for 5 minutes at 2000 G and the absorbance was determined at 530 nm in a spectrophotometer. The result was expressed as mg malondialdehyde/kg (Lemon, 1975).

$$TBARS (\mu\text{molMDA/kg}) = \frac{\left(\frac{\text{Absorbance}}{\text{Standard}} \times 2\right)}{\text{Sample Weight}} \times 6.8 \quad (1)$$

Ash

Ash crucibles were dried and tared before use, 5 g of

meatball sample was weighed on these crucibles and placed in a muffle furnace; and the temperature was gradually increased and brought to 525 °C. The burning process was terminated when the color of the samples became gray-white and the results were calculated as ash% on dry matter (Gökalp et al., 2010).

Cooking yield

The 40 g meatball samples were weighed before and after cooking. Then, the cooking yield was determined using these results (Pinero et al., 2008).

$$CY = \frac{A}{B} \times 100 \quad (2)$$

where CY, cooking yield (%); A, cooked meatball weight (g); B, Raw meatball weight (g)

Sensory analysis

In the sensory analysis, 10 semi-trained panelists evaluated the cooked meatball samples in terms of color, appearance, odor, texture, taste and general acceptability using the hedonic type scale (1-9). In the scoring, 1 point was accepted as “undesirable” and 9 points as “typically desirable”.

2.4. Statistical analysis

Ginger ratio and cooking temperature were selected as factors in the study. The trial was carried out with 2 replications and completely random. TAMB, pH, moisture, ash and cooking efficiency were carried out with two replications. TBARS and sensory analysis were carried out with 3 and 10 replications, respectively. Variance analysis was applied to the data obtained from the analyses and statistically significant results were compared with the Duncan multiple comparison test (IBM SPSS Statistics 2).

3. Results and Discussion

Total aerobic mesophilic bacteria (TAMB) counts of meatballs cooked at different temperatures using different amounts of ginger are shown in Table 1. As seen from the table, the TAMB counts of the groups using 1% ginger were

found to be higher than the other groups. This result is thought to be due to contamination that occurs especially during the drying of ginger. However it has been stated that the TAMB count decreased with the use of argel leaf extract in chicken meatballs (Al-Juhaimi et al., 2018). At high cooking temperatures, there was a statistical increase in the number of these bacteria and a lower average value was detected in meatballs cooked at 200 °C. In addition, a significant effect of GR-CT interaction on TAMB was determined and while a lower TAMB count was determined in meatballs cooked at 175 °C in the control group, cooking at 200 °C caused higher TAMB counts in the groups using 0.75% and 1% ginger (Figure 1).

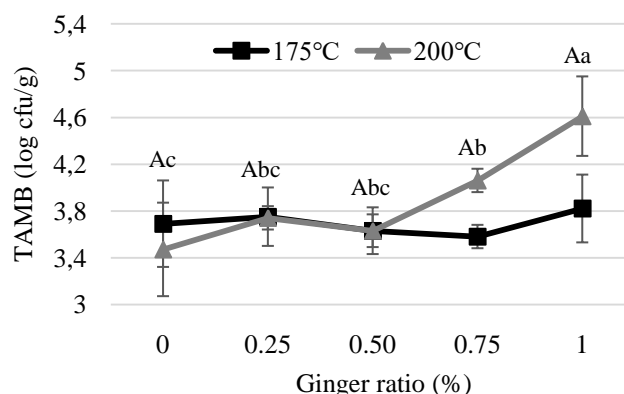


Figure 1. The effect of ginger level x cooking temperature interaction on TAMB number (A: Same letters indicate statistical no differences ($P>0.05$) for meatball cooking at 175 °C; a-c: Different letters indicate statistical differences ($P<0.05$) for meatball cooking at 200 °C)

The ginger ratio, cooking temperature and the interaction of these factors did not have a significant effect on the pH value of the meatballs ($P>0.05$) (Table 1). The use of ginger in meatballs produced from pork did not show a significant effect on the pH value as well (He et al., 2022). On the other hand, in the study conducted by Al-Juhaimi et al. (2018) on chicken meatballs, argel leaf extract caused a decrease in the pH value.

Table 1. The effect of ginger level and cooking temperature on TAMB, pH, moisture, TBARS, ash and cooking yield of meatball

	TAMB (log cfu/g)	pH	Moisture (%)	TBARS (mg MDA/kg)	Ash (%)	Cooking Yield
Ginger ratio (GR) (%)						
0-Control	3.58±0.37 ^b	6.05±0.05	58.81±0.73 ^b	0.841±0.013 ^a	2.61±0.10 ^a	85.06±3.43 ^c
0.25	3.75±0.17 ^b	6.10±0.01	60.27±0.51 ^a	0.778±0.014 ^b	2.55±0.08 ^b	85.85±1.98 ^{bc}
0.50	3.63±0.16 ^b	6.08±0.02	59.40±2.01 ^{ab}	0.766±0.011 ^c	2.56±0.03 ^b	85.14±1.80 ^c
0.75	3.82±0.28 ^b	6.10±0.05	58.47±0.33 ^b	0.711±0.009 ^d	2.53±0.11 ^b	86.76±2.85 ^b
1	4.22±0.52 ^a	6.14±0.02	58.74±0.66 ^b	0.661±0.012 ^e	2.36±0.07 ^c	89.62±1.47 ^a
Sig.	**	NS	*	**	**	**
Cooking temperature (CT) (°C)						
175	3.69±0.23 ^b	6.09±0.04	59.39±1.15	0.760±0.065 ^a	2.56±0.14 ^a	86.19±3.31
200	3.90±0.47 ^a	6.10±0.04	58.88±1.12	0.743±0.062 ^b	2.48±0.06 ^b	86.78±2.38
Sig.	*	NS	NS	**	**	NS
GRxCT	*	NS	**	NS	**	**

^{a-c}: Different letters indicate statistical difference ($P<0.05$) in each column; NS: Not significant; * $P<0.05$; ** $P<0.01$

The ginger ratio and GR-CT interaction caused a statistical change in the moisture value. The moisture value became the highest in the group containing 0.25% ginger (Table 1). On the other hand, He et al. (2022) did not determine a significant effect of ginger use on the moisture value in pork meatballs. According to the GR-CT interaction, cooking at 200 °C caused a higher moisture value in the control and 0.25% ginger groups, while the opposite was observed in the other groups (Figure 2).

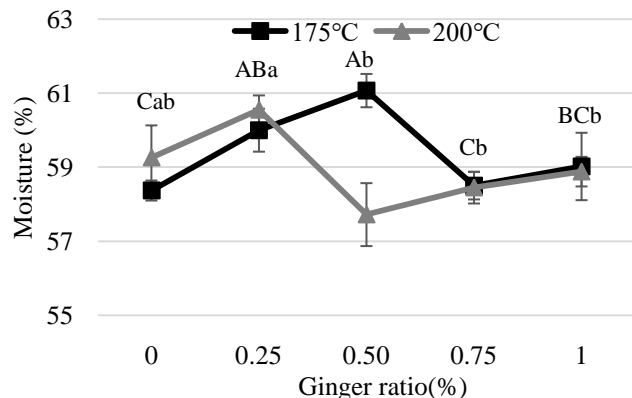


Figure 2. The effect of ginger level x cooking temperature interaction on moisture (A-C: Different letters indicate statistical differences ($P<0.05$) for meatball cooking at 175 °C; a-b: Different letters indicate statistical differences ($P<0.05$) for meatball cooking at 200 °C)

The TBARS value, which is an indicator of lipid oxidation, was significantly affected by all main sources of variation and the interaction of those sources. According to the results, the use of ginger and the application of high cooking temperature caused a decrease in lipid oxidation (Table 1). This result is thought to be due to the antioxidant effect of ginger, since it contains the related phenolic compounds (Shaukat et al., 2023). In a study conducted on chicken meatballs, the use of dittany herb and rosemary caused a decrease in the TBARS value (Racanincci et al., 2004). Bulan & Öz (2022) found that the TBARS value of beef meatballs decreased with the use of tarragon. In addition, this study also concluded that since the internal temperature of the sample group that were applied high-temperature cooking process reached the desired level in a shorter time, it caused a lower TBARS value in these groups (Table 1).

The ginger ratio, cooking temperature and interaction of these two factors had a significant effect on the ash content of the meatball samples. As seen in Table 1, the control group showed a higher ash content than the other samples. Contrary to this study, the use of argel leaf extract in chicken meatballs did not cause a statistical change in the ash value (Al-Juhaimi et al., 2018). On the other hand, this study also provided that the ash content in meatballs cooked at 175 °C was found to be higher than other groups. The effect of GR-CT interaction on the ash content is given in Figure 3. As seen in the figure, while the samples cooked at 200 °C in the control, 0.25% and 0.75% ginger groups showed lower ash content, the meatballs cooked at 200 °C in the 1% ginger group showed higher values (Figure 3).

The cooking yield results of chicken meatballs produced using different ginger ratios and cooking temperatures are shown in Table 1. As it can be understood from the results, the use of 0.75% and 1% ginger had positive effects on cooking yield. The highest average cooking yield value (89.62) was determined in the group containing 1% ginger. In a study conducted on pork meatballs, the use of ginger reduced

cooking loss and the lowest value was found in the group using 1.25% ginger (He et al., 2022). In this study, cooking temperature had no significant effect on cooking yield ($P>0.05$). However, the GR-CT interaction affected cooking yield very significantly. While cooking at 200 °C increased cooking yield in the control and 0.25% ginger samples, cooking at 200 °C caused lower values in the samples containing 0.75% ginger (Figure 4).

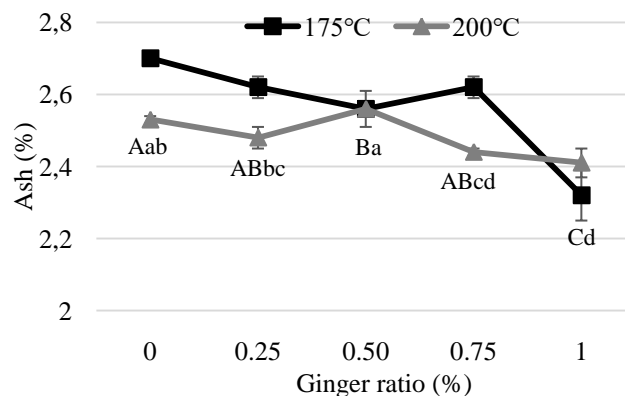


Figure 3. The effect of ginger level x cooking temperature interaction on ash (A-C: Different letters indicate statistical differences ($P<0.05$) for meatball cooking at 175 °C; a-d: Different letters indicate statistical differences ($P<0.05$) for meatball cooking at 200 °C)

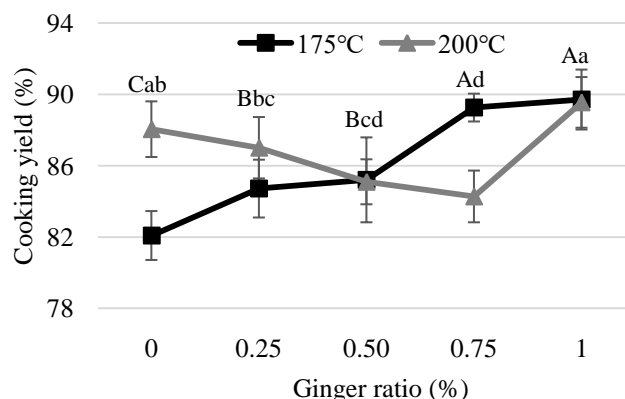


Figure 4. The effect of ginger level x cooking temperature interaction on cooking yield (A-C: Different letters indicate statistical differences ($P<0.05$) for meatball cooking at 175 °C; a-d: Different letters indicate statistical differences ($P<0.05$) for meatball cooking at 200 °C)

The sensory analysis results of meatballs produced with different ginger ratios and cooking temperatures are presented in Table 2. While the ginger ratio statistically affected only the taste of the product, the cooking temperature did not have a significant effect on the average sensory analysis values. In terms of taste, especially the use of 1% ginger received lower scores from the panelists. In the study conducted by Nisar et al., (2020), it was determined that moringa leaf powder application on chicken meatball did not affect the sensory properties. The interaction of these two values had a statistical effect on general acceptability at $P<0.05$ level. As seen from Figure 5, while cooking at 200 °C caused lower general acceptability scores in the control and 0.25% ginger groups, it increased general acceptability in the other groups.

Table 2. The effect of ginger level and cooking temperature on sensory parameters of meatball

	Color	Appearance	Odor	Texture	Taste	General acceptability
Ginger ratio (GR) (%)						
0-Control	7.79±1.32	7.67±1.34	7.86±1.03	7.92±1.41	7.88±1.26 ^a	7.83±1.13
0.25	7.75±1.07	7.58±1.14	7.79±1.28	7.58±1.10	7.75±1.11 ^a	7.79±0.93
0.50	8.00±1.02	7.96±1.12	7.96±1.20	7.29±1.60	7.38±1.44 ^{ab}	7.75±1.03
0.75	7.63±1.06	8.04±1.08	8.17±0.76	7.58±0.97	6.92±1.84 ^{ab}	7.38±1.31
1	7.96±1.08	8.13±0.95	7.96±1.27	7.96±1.08	6.42±2.19 ^b	7.00±1.79
Sig.	NS	NS	NS	NS	*	NS
Cooking temperature (CT) (°C)						
175	7.82±1.16	7.88±1.12	7.80±1.22	7.57±1.37	7.15±1.82	7.48±1.42
200	7.83±1.06	7.87±1.16	8.10±0.99	7.77±1.14	7.38±1.53	7.62±1.17
Sig.	NS	NS	NS	NS	NS	NS
GRxCT	NS	NS	NS	NS	NS	*

^{a-b}: Different letters indicate statistical difference (P <0.05) in each column; NS: Not significant; *P < 0.05

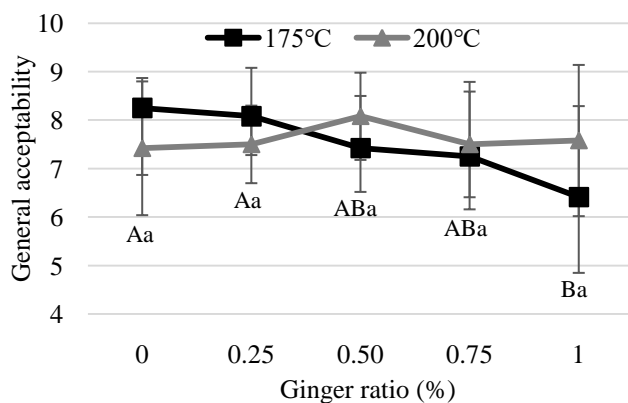


Figure 5. The effect of ginger level x cooking temperature interaction on general acceptability (A-B: Different letters indicate statistical differences (P<0.05) for meatball cooking at 175 °C; a-c: Same letters indicate statistical no differences (P>0.05) for meatball cooking at 200 °C)

4. Conclusions

The use of ginger in meatballs increased cooking efficiency while limiting lipid oxidation. On the other hand, while the use of 1% ginger caused an increase in TAMB numbers, it decreased the ash value and received lower scores from the panelists in terms of taste. Considering all these outcomes, it is thought that the use of ginger up to 0.75% in meatball production is appropriate. In addition, when the TBARS values were considered in terms of cooking temperature, it was observed that cooking at 200 °C gave more positive results.

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Conflicts of Interest

The authors state that they have no conflicts of interest.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Carcinogenic potential of food additives: A review

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ABSTRACT

Food additives are substances intentionally added to food to achieve specific technical or physical effects, such as reducing spoilage, enhancing colour, or improving flavour. This review focused on food additives with carcinogenic potential in humans or animals, as identified by the Food and Drug Administration (FDA) and the International Agency for Research on Cancer (IARC). Food additives benefit manufacturers, processors, and consumers, including acids that inhibit microbial growth and prevent foodborne diseases. Some commonly consumed foods and beverages contain compounds with carcinogenic potential in animal models. Furthermore, human exposure to several of these compounds has been associated with increased cancer risk. This review examined the role of food additives in dietary consumption, their classification as direct or indirect additives, and their potential ecological impacts. It highlights the importance of rigorous safety assessment and regulation of food additives to protect public health, considering both the benefits and potential risks associated with their use in the food industry.

1. Introduction

Food risk components (FRCs) provide worldwide challenges. Skill impediments threaten community well-being and the pliability of the food system (Jin et al., 2020, Zhang et al., 2020). FRCs comprise anthropogenic and biogenic substances, including pesticide residues, environmental contaminants, illicit or hazardous additions, and biogenic food toxins (Shi et al., 2024). The World Health Organisation (WHO) estimates that food contaminated with FRC leads to disease in 1 in 10 individuals, leading to 420,000 fatalities per year (Wicker et al., 2016). Global industrialisation and resultant environmental pollution, including industrial wastewater discharge, metal mining and smelting, pesticide application, and solid waste accumulation, have controlled an increase in the detection of anthropogenic pollutants in food, particularly with the introduction of FRCs (Wang et al., 2020).

A principal pathway for anthropogenic contaminants to infiltrate food crops and livestock occurs when agricultural activities are conducted near pollution sources, such as contaminated soil from adjacent industrial operations or irrigation utilising polluted water (Adnan et al., 2022). Extensive education has established the detrimental effects of pesticide remains on soil, worldly, and water ecosystems, as well as optimistic associations with the danger of neurological

and generative disorders and cancer. Additionally, veterinarian medication remains have been shown to induce hearing loss, liver and kidney harm, disrupt microbial balance, and have carcinogenic properties (Carvalho, 2017, Thompson et al., 2017, Bacanlı & Başaran, 2019). Infants and toddlers are particularly vulnerable to these foodborne pathogens. Extended exposure to these chemicals may lead to detrimental health consequences, including developmental problems (Mielech et al., 2021). Adults and immunocompromised individuals constitute a height-risk demographic for foodborne infections because of various variables, including reduced resistant classifications, long-lasting conditions, and the consequent elevated risk of problems (Thaivalappil et al., 2020).

In addition to anthropogenic contaminants, biogenic poisons in flora and fauna and those produced by fungi and bacteria pose significant risks to human health. Mycotoxins, which are minor deadly metabolites synthesised primarily by fungi of the genera *Aspergillus*, *Alternaria*, *Fusarium*, and *Penicillium*, lead to the adulteration of nutrition, vegetables, and fruits (Tian et al., 2022). Bacterial toxins consist of poisonous particles, polypeptides, and proteins generated by bacteria that can disrupt cell membranes and the extracellular matrix (Kuzmenkov et al., 2016). Edible plants may also harbour specific poisonous metabolites, for example, glycoalkaloids in potatoes and aristolochic acid in fish mint,

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which can result in liver and kidney failure along with additional well-being hazards (Kemprai et al., 2023). Furthermore, specific peptides and proteins found in animal crops can delay the functions of essential enzymes, ion channels, and receptors in human digestion, compromising homeostasis and adversely affecting the nervous and cardiovascular systems (Akbarian et al., 2022). This review focuses on food additives with carcinogenic potential in humans or animals, as identified by the Food and Drug Administration (FDA) and the International Agency for Research on Cancer (IARC).

2. Food and Beverages

Foods and beverages are fundamentally intricate combinations of substances ingested for nourishment or enjoyment. The diversity of compounds included in food is extensive, as are their differing qualities (Kobets et al., 2022). Substances exhibiting carcinogenic activity in rat models have long been identified in numerous regularly consumed foods. The complexity of food safety and quality arises from diverse sources such as flora, microbes, contaminants, additive applications, and reactions that transpire during processing, cooking, and storage (Jackson, 2000, Blandino et al., 2003).

Additionally, carcinogens may be generated endogenously from dietary constituents (Tricker & Preussmann, 1991, Rietjens et al., 2022). It fails to consider that drinking water toxins like arsenic, the effects of caloric content and macronutrients, for instance, overweight, or the overconsumption of intoxicating drinks, altogether of which have been related to heightened cancer dangers in people (Pflaum et al., 2016, Key et al., 2020).

The carcinogenic mechanism of action (MoA) is strongminded by chemical construction. DNA-reactive carcinogens possess structures that produce reactive electrophiles, either directly or after undergoing bioactivation. In contrast, epigenetic carcinogens do not have these properties but feature structures that interact with additional molecular and cellular mechanisms that are critical to cancer development (Williams et al., 2014, Kobets et al., 2019). These disparities in MoA highlight the hazards of human cancer due to exposure (Williams, 1997, Williams, 2008).

3. Food and Cancer

Food is ingested for its nutritional benefits and sensory gratification, including flavour and fragrance. The United States (US) food system guidelines rely on future applications within specific food categories, demographic consumption groups, and projected health claims associated with those items. Direct or indirect food substances may only be lawfully introduced if the manufacturer has proven free from harmful qualities under designated use circumstances (Rolls, 2015). A novel product or component might be classified as a direct food preservative or a generally recognised safe (GRAS) component; however, its purpose may be uncertain regarding its incorporation into food or its influence by hereditary and ecological issues. A substance designed to provide colour upon addition or application to food is controlled independently as a colour additive (Pressman et al., 2017).

Cancer is a multifactorial disease influenced by genetic

and environmental factors. Nutrition contributes to the elevated incidence of cancer. The characteristics, ingested quantity, and additive composition of dietary products may be significant in the potential incidence of cancer. Consuming refined carbohydrates may help the growth of colon cancer (Chan & Giovannucci, 2010). Excessive eating of red meat might raise the risk of colon cancer (McAfee et al., 2010). Additional variables contributing to elevated cancer risk stem from the chemicals incorporated into processed foods (Belpoggi et al., 2006, Mueller et al., 2010, Bastide et al., 2011).

4. Food Additives

Food additives are substances intentionally added to food products to achieve specific desirable effects, for example, enhancing flavour, appearance, and shelf life (Ukwo et al., 2022). They are primarily used in processed foods and are not typically consumed on their own (Sadler et al., 2021).

Food additives are essential in the food industry, encompassing direct additives deliberately added to food for specific effects and indirect additives that may migrate from packaging materials. With thousands of additives developed to enhance food safety, convenience, and nutrition, their regulation and safety assessment are paramount for public health protection (Zang & Kabadi, 2001). These additives serve various functions, including sensory enhancement, nutrient fortification, processing aid, and preservation, with different categories like colourants, sweeteners, vitamins, and antioxidants (Aleksieva et al., 2022, Owusu-Apenten & Vieira, 2022). Additionally, food hydrocolloids, including polysaccharides and proteins, are widely employed as thickening agents, stabilisers, and delivery transporters for bioactive compounds, offering health benefits and varied requests in food and biomedicine productions (Kutlu et al., 2020, Kutlu et al., 2021, Lu et al., 2021). The emergence of nutraceuticals, functional foods, and supplements further highlights the evolving landscape of food research to address health concerns and develop alternative therapies (Ditu et al., 2018). Food additives can be categorised into many classifications according to their functionality (Table 1).

4.1. Role of food additives in dietary consumption

All individuals must consume food! The human diet consists of different biochemical compounds, primarily of natural origin, and intentionally added components such as nutrients, colourants, and flavour enhancers. During processing and food preparation, additional chemicals may be incorporated into food, resulting in chemical alterations and the introduction of molecules not typically present in raw agricultural items (Pressman et al., 2017). Chemicals are additionally used to attain positive practical effects, including colouration, preservation, constancy (e.g., emulsification), flavour enhancement, sweetener, and other physical alterations. Supplementary compounds are frequently incorporated, typically in minimal quantities, primarily as byproducts of cultivation and packaging. These may include pesticides, pharmaceuticals utilised in livestock organisations, and chemicals that leach from food interaction of packaging surfaces (FCSs) (Marone et al., 2016, Pressman et al., 2017).

Table 1. Main types of food additives and their uses with examples

Food additives	Examples	Uses
Nutritional additives	Examples include vitamins A, D, and B vitamins, vitamin C, essential fatty acids, minerals like calcium and iron, and dietary fiber (Pogorzelska-Nowicka et al., 2018)	Used to restore nutrients lost during processing or enrich foods to correct dietary deficiencies (Augustin et al., 2016)
Processing agents	Examples include anticaking, bleaching, clarifying, emulsifiers, leavening, humectants, PH control, and stabilisers/thickeners (Abdulmumeen et al., 2012)	Used to aid in processing or maintaining desired product consistency (Chandan, 2015)
Preservatives	Examples include salt, spices, sulfites, benzoates, sorbates, propionates, and nitrites (Silva & Lidon, 2016)	Used to prevent spoilage and extend shelf life (Mei et al., 2019)
Sensory agents	Examples include flavouring agents, flavour enhancers, and colourants (Ramesh & Muthuraman, 2018)	Used to enhance taste, aroma, or appearance (Lesme et al., 2020)

The human diet also includes several undesirable pollutants from natural sources, including bacteria and their metabolites and chemicals inherent to plants (Kruger et al., 2014, Pressman et al., 2017). The Food Additive Alteration near the Federal Food, Drug, and Cosmetic Act (FDCA) mandated regulatory oversight of food additives. It empowered the FDA to request evidence from manufacturers showing that the additive posed minimal risk before its incorporation into the food source. The Food and Drug Administration (FDA) defines the kinds of poisonousness and chemical assessments necessary to evaluate the care of food additives and Generally Recognized as Safe (GRAS) substances. The safety evaluation standards for food additives and compounds undergoing (GRAS) appraisal match. The primary distinction is in the period to market and then the qualifications of professionals assessing the readily accessible protection information (Roberts, 1981).

4.2. Direct and indirect food additives

A substance anticipated to be incorporated into food qualifies as a food additive. It requires premarket approval from the FDA without being deemed Generally Recognised as Safe (GRAS) through competent specialists or falls under other exclusions specified by the FDCA. Since 1958, additives have been the focus of food additive petitions that succumbed to the FDA (Noah & Merrill, 1998, Mosley, 2014). Petitions must include suitable protection data to enable the agency to fulfil its approval standards. Substances incorporated into food intended for a detailed function are recognised by way of "direct additives" and are listed and arranged as the component marker of the respective food product. Aspartame, a low-calorie sweetener, is an intentional ingredient in puddings, yoghurt, soft drinks, and other food products. An indirect additive integrates into the food in minimal quantities during processing, storage, or packaging (Adhikari, 2021).

Additives fulfil important practical roles, including preserving the nutritional integrity of food (Kruger et al., 2014), improving stability or quality, hence decreasing food waste (Roberts, 1981), enhancing consumer appeal (FDA, 2014) and offering necessary assistance during processing. Currently, many compounds occur in the food consumed in the US, most of which are manufactured by manufacturers. Indirect additives are required by law to disclose their concentrations in food when they approach levels that may lead to discernible negative effects. Despite direct and indirect food additives, dispensation assistances are not obligatory to

be listed on the element declaration (Kwon et al., 2023).

4.3. Ecological impacts of food additives

A food additive may remain interested in the released surroundings through its production and application. Taste sensations experienced through eating or drinking can spread through the environment through manure systems. Chemicals utilised to produce food additives might also be incorporated into wastewater treatment, industrial, or processing facilities (Pressman et al., 2017). Alternative pathways for introducing food additives encompass landfill solid waste disposal, food processing, and solid waste combustion. The National Environmental Policy Act (NEPA) mandates that the FDA evaluate its regulatory actions' environmental consequences (1985). Petitioners must conduct an environmental evaluation before FDA approval of a FAP (Gibbs & Kahan, 1986). Addressed issues encompass the proposed application of physical and chemical properties. Metabolic grade about ecological fate in air, utilisation, soil, and water; anticipated ecological attentions; potential toxicological impacts on water and global organisms; and ecological ramifications of production and final disposal. Introduction levels and environmental fate absorption rates and soil are assessed to forecast the maximum attentiveness of the additive in related ecological media (Cousins et al., 2002).

After feasible, developments manipulating the transportation and alteration of food additives are utilised for predicting ecological concentration. Relevant data encompass chemical constancy, biodegradability, and movement in waste media (oil sorption, water volatility, and solubility) (Kahrilas et al., 2015). Following the estimation of the material released into the atmosphere, the ecological assessment entails a review of existing data regarding toxicity to fauna, flora, and other organisms within the environmental section (terrestrial ecosystems, air, estuarine, lake, and maritime) at the ecosystem level. The toxicity database is thereafter analysed alongside the degree of ecological contact to evaluate potential threat (Lytle & Lytle, 2001).

5. Food Additives and Carcinogenesis

Food additives undergo toxicologic assessments meant for safety assessment. Additives scientifically and officially validated as safe are permitted for usage in the food industry. However, using about processed meals comprising specific

food additives hawthorn increases the hazard of human cancer (Table 2), despite adherence to the regulatory limitations for these compounds in such meals. Recent research reveals that processed meat containing preservatives like nitrite and nitrate elevates the risks of colorectal and pancreatic cancer (Bastide et al., 2011, Larsson & Wolk, 2012). Consumption of soft drinks like Coke may elevate the risk of some cancer types, as demonstrated by the research (Belpoggi et al., 2006). This study had rats maintain a typical diet during their lifespan, with one group receiving regular tap water and the other group receiving Coke as their drinking water source. The prevalence of breast cancer in women and pancreatic cancer in females and males was elevated in the collection consuming expel compared to the group receiving typical water discharge (Qadir et al., 2024). A comparable study indicated that consuming beverages with food additives may elevate tumour danger. The study shows that 600 Singaporeans who consumed two or more glasses of non-alcoholic beverages weekly for 14 years exhibited an increased incidence of pancreatic cancer, while no similar evidence was found among those who drank fruit juices (Mueller et al., 2010).

Food additives are incorporated into food to enhance or preserve specific attributes, including texture, flavour, appearance, or safety. Several substances are directly incorporated into foods, whereas others infiltrate foods in minimal quantities during storage, handling, or packaging (FDA, 2010). FDA rules from the late 1950s prohibit the addition of any direct food additive with carcinogenic possible to food. However, contemporary understandings of the various mechanisms of biochemical carcinogenesis call the validity of these regulations into question (Williams et al., 1996; Krishan et al., 2021).

In Japan, madder colour produced from the roots of *Rubia tinctorum* (madder root) is used in food colouring. In a carcinogenicity investigation, F344 rats nursed a regime covering *Rubia tinctorum* colour excerpt for 104 weeks and significantly higher renal and liver cell carcinoma rates in mutual sex activity. The *Rubia tinctorum* colour excerpt was banned from usage in 2004 (Inoue et al., 2009) because of carcinogenic findings, combined through its high genotoxicity, yet its present IARC categorisation remnants Cluster 3. In systematic research, a metabolite of a *Rubia tinctorum* colour constituent has been revealed to produce a significant character in carcinogenicity (Inoue et al., 2009). Potassium bromate is an oxidising substance commonly used as a food ingredient, particularly in bread-making. Potassium bromate causes renal cell cancer in pests (Kurokawa et al., 1983, DeAngelo et al., 1998). Potassium bromate has both initiating and promoting properties in swine kidney carcinogenesis. Its potency in pests appears lower than that of rats with hamsters. In contrast to its modest genotoxicity in microbiological studies, potassium bromate had a relatively high ability to cause chromosomal abnormalities. The consequences of 8-hydroxydeoxyguanosine synthesis in the rat kidney suggested that reactive oxygen species produced by potassium bromate were responsible for its hazardous and carcinogenic impacts (Sai et al., 1992).

These cases illustrate that certain processed foods with approved ingredients may elevate the risk of carcinogenicity despite the absence of any publicly proclaimed safety concerns regarding these compounds. Consequently, one may hypothesise the following causes for the potential carcinogenicity of certain chemicals in food crops. No carcinogenic concerns were established in investigational trials examining alterations in food structure, potential adverse synergistic effects from additional carcinogenic compounds in

flavourings, prolonged exposure due to poor storage conditions, or the possibility of exceeding acceptable limits (Felter et al., 2021).

Table 2. Food additives promote carcinogenicity at high exposure doses

Food additives	Cancer types
Cyclamic acid and its Na and Ca Salts	Colon and hepatocellular tumours, Prostate adenocarcinoma, Thyroid and Uterus adenomas (Takayama et al., 2000)
Allura Red AC	Colon tumor (Tsuda et al., 2001)
Acesulfame potassium	Urinary tract tumour (Andreatta et al., 2008)
Aspartame	Urinary tract tumours (Soffritti et al., 2007), Lymphoma, leukaemia, and breast tumours (Ter Veld et al., 2006)
BHA(Butylated hydroxyanisole)	Breast tumour (Lu et al., 2002, Qadir et al., 2024)
BHT(Butylated hydroxytoluene)	Bladder tumour (Saito et al., 2003), Lung tumour (Plesner & Hansen, 1983)
Hexamethylenetetramine	Adrenal gland pheochromocytoma and Harderian gland tumour (Mahapatra & Parija, 2018)
Carboxymethyl cellulose, Sodium carboxymethyl cellulose	Fibrosarcoma at the side of subcutaneous injection (Uittamo et al., 2011)
Xylitol	Adrenal medulla tumour (Cross et al., 2010)
Nitrates, Nitrites	Colorectal cancer and Bladder tumour (Ferrucci et al., 2010), non-Hodgkin lymphoma (Kilfoy et al., 2010), Thyroid tumour (Kilfoy et al., 2011), Brain (Preston-Martin et al., 1982), Hepatocellular tumour (Sayed-Ahmed et al., 2010), Advanced prostate cancer (Sinha et al., 2009)
Propionic acid and salts	Fore stomach tumour (Harrison, 1992)
Saccharin and its salts	Bladder tumour (Tisdell et al., 1974), Thyroid tumour (Prasad & Rai, 1986)
Talc	Adrenal gland and lung adenoma/carcinoma (NTP, 1993) Endometrial cancer (in genital usage of women as talcum powder) (Karageorgi et al., 2010)
Polyoxyethylene stearate	Bladder papilloma (Shubik, 1975)
4-Hexylresorcinol	Adrenal gland pheochromocytoma and Harderian gland tumor (Chhabra et al., 1988)

6. Potential Factors Contributing to the Development of Additive Carcinogenicity

6.1. Structural changes

When food additives interact with other food components, their chemical structure may alter due to physical, chemical, or enzymatic activities. Nitrites and nitrates are transformed into nitrosamines in meat crops (SIDS, 2005). The primary nitrosamines current in meat and dairy foodstuffs of crops are

N-nitrosodimethylamine and N-nitrosopyrrolidine. In Belgique, 101 thirsty agitated sausages were analysed for remaining sodium nitrate and nitrite concentrations, biogenic amines, and volatile N-nitrosamine levels. The findings revealed that N-nitroso morpholine and N-nitroso piperidine were present in a notable percentage of samples (22% and 28%, respectively) (Catsburg et al., 2014, De Mey et al., 2014), and observed the part of dietetic bases of N-nitroso compounds (NOCs) and NOC precursors as potential risk factors for bladder cancer in a situation-regulator training performed in Los Angeles. The intake of treated meats, such as pastrami, corned beef, salami, and liver, which contain amines and nitrosamines, was meaningfully related to a heightened risk of bladder cancer.

6.2. Adverse synergistic effects

The influence of the interaction among various food additives on carcinogenicity may have been neglected in assessments of their individual carcinogenic risks. The risk may have been exacerbated by a food ingredient that elicited an adverse reaction. Scientific evidence exists to substantiate this hypothesis. The mixture of potassium sorbate, ascorbic acid, and ferric or ferrous salts has demonstrated mutagenicity and DNA-damaging effects, but their individual use does not exhibit such activity (Kitano et al., 2002). A different study investigated the synergistic impact of a combination of six prevalent reproduction food colourants (allura red, brilliant blue, new cocaine, erythrosine, tartrazine, and fast green) on the poisonousness of the carcinogen 3-amino-1,4-dimethyl-5H-pyrido[4,3-b] indole (Trp-P-1) utilising primary refined swine hepatocytes, demonstrating that the food colourant combination heightened the cytotoxicity of Trp-P-1 (Ashida et al., 2000).

7. Various Safe Chemicals may be Associated with Cancer

As per the International Agency for Research on Cancer (IARC), approximately the additives are deemed safe because they present no significant risk level (NSRL) despite a minimal cancer risk. Only permissible quantities are sanctioned for human ingesting. "All additives" referenced in this document are deemed harmless after ingested within permissible amounts. Consequently, the factors above may have compromised the safety of certain chemicals, potentially elevating their carcinogenic effects or hazards, particularly in processed food (Cohen & Ito, 2002, Gultekin et al., 2015).

7.1. Mechanisms of carcinogenicity of DNA-reactive carcinogens

DNA-reactive carcinogens possess characteristics that facilitate the development of electrophilic reactants, which can covalently attach (adduct) to nucleophilic sites in nuclear DNA and other macromolecules, such as RNA and proteins, within the board tissues of carcinogenicity (Miller & Miller, 1981, Hartwig et al., 2020). Inboard tissues, only DNA reactant may produce multiple DNA adducts at distinct nucleophilic places on the same dishonourable before across bases. The pace of repair for each adduct may differ depending on its chromosomal position. The global reparation scheme controls adducts in cutting-edge transcriptionally inactive regions, whereas the transcription-coupled repair mechanism deals with adducts in transcriptionally lively areas

(Hanawalt et al., 2003). The concentration of DNA adducts from exposures is determined by various parameters, including contact incidence, dosage, and the effectiveness of DNA overhaul used for exact adducts. Respectively, adduct has a unique ability to induce mutations, especially at base-pairing sites, which are extra mutagenic. "Pro-mutagenic DNA alterations" develop into changes through cellular repetition (Fuchs, 2002). Changes in key growing regulatory genetic factors cause neoplastic transformation and progression (Vogelstein et al., 2013).

"DNA-reactive carcinogens" may also induce other biological effects, including cytotoxicity, which can promote increased cell proliferation and donate to their carcinogenic potential (Poirier, 2012). DNA-reactive carcinogens may exhibit cumulative effects within their board structures. Specific DNA adducts do not result in carcinogenicity, as adducts are present where cancer formation does not occur after food administration (Poirier & Beland, 1994, Poirier, 2012). Acrylamide, as mentioned below, generates adducts in both non-target and target tissues (Doerge et al., 2005). Epigenetic changes may be necessary for neoplastic conversion induced by specific adducts (Lafferty et al., 2004, Pavanello et al., 2009).

DNA-reactive carcinogens are generally genotoxic in test techniques that accurately depict the necessary bioactivation due to DNA interactions (Williams et al., 1996, Preston & Williams, 2005, Phillips & Arlt, 2009). Furthermore, DNA-reactive carcinogens frequently induce cancer at many locations and with brief exposure periods, even following the administration of a single dosage in some examples. This feature underpins their engagement in restricted "short-term bioassays" (Williams et al., 2014). Several DNA-reactive carcinogenic agents remained identified as lacking observable detrimental impact levels (NOAELs) for carcinogenic belongings popular visceral replicas (Neumann, 2009, Williams et al., 2012), despite the existence of contradictory data. It is clear that biological verges potentially affect the probability of cancer growth for genotoxic carcinogens, according to the phases of carcinogenesis. Currently, criteria for DNA-reactive carcinogens are not widely accepted from a risk assessment and organisation position (Adeyeye, 2020).

7.2. Carcinogenicity mechanisms of epigenetic carcinogens

Epigenetic carcinogens do not engage in chemical reactions with DNA (Williams, 1992, Pogribny & Rusyn, 2012). In the board matters of carcinogenicity, the mechanisms of the act of these carcinogens include molecular or cellular changes that may indirectly principal to alterations in DNA function or cellular behaviour through secondary pathways (Kobets et al., 2019). "Epigenetic carcinogens" may persuade oxidative pressure, subsequent trendy oxidative DNA injury (Klaunig & Kamendulis, 2004, Pogribny & Rusyn, 2012), which may cause neoplastic alterations or increased cell propagation, easing neoplastic growth, frequently arising after cryptogenic pre-neoplastic cells. Epigenetic hazards can touch genetic factors, foremost toward neoplastic transformation. Epigenetic carcinogens may touch genetic factor appearance, foremost toward neoplastic transformation (Jones & Baylin, 2007, Baylin & Jones, 2016), like belongings, which are frequently particular to rodents.

Epigenetic carcinogens can augment the carcinogenic potential of "DNA-reactive carcinogens" via interacting mechanisms, such as neoplasm advancement. Epigenetic carcinogens generally yield negative results in genotoxicity studies due to their absence of direct DNA responsiveness,

unlike DNA-reactive chemicals, even when bioactivation occurs, unless influenced by an artefact such as severe cytotoxicity that induces mutagenicity. Epigenetic drugs typically necessitate extended high-level exposures to manifest their carcinogenicity. Their mechanism of action indicates that in restricted bio-assays, they exhibit harmful results for starting action. However, they may demonstrate promising results for endorsing action (Williams et al., 2014). Epigenetic carcinogen agents stay recognised toward demonstrating NOAELs intended for the cellular effects that contribute to the carcinogenicity from bodily replicas (Williams, 2001, Kobets et al., 2019) by way of examining some foodborne carcinogens addressed in this article. Thresholds for DNA-sensitive hazards are typically acknowledged from a hazard valuation viewpoint (Adeyeye, 2020).

8. Establishing Interaction with Additional Carcinogenic By-products in Profitable Additives

Unknown substances may be present in certain foods, potentially posing a consumer risk. For example, after ammonium is utilised, unwanted by-products like 4-methylimidazole may be generated during caramel synthesis. The derivative of (4-methylimidazole) induces lung cancer in mutual females with male pests by elevated dosages and precipitates leukaemia in women (National Toxicology Program, 2007, Chan et al., 2008). Certain Coke crops were discovered to have raised stages of 4-methylimidazole in their honey ingredients, exceeding the no significant risk level (NSRL). On February 16, 2011, the Centre for Knowledge in the Community Interest initiated a public request urging the United States FDA to prohibit the use of these caramels in such products (Gultekin et al., 2015).

8.1. Insufficient and extended packing conditions

Inappropriate surroundings might cause an alteration in chemical construction. Benzoates, such as sodium, potassium, and calcium) benzoate and benzoic acid exemplify common cases. They may undergo decarboxylation reactions to produce a carcinogenic compound, benzene, in the presence of erythorbic acid and ascorbic acid under appropriate UV light, pH, or temperature conditions (Gardner & Lawrence, 1993).

8.2. Surpassing the permissible thresholds

Food additives are marketed only once their Acceptable Daily Intake (ADI) levels are officially determined. The permissible maximum quantity that may be incorporated in foods is established to meet these ADI limits. Nevertheless, foods covering chemicals are expended typically, resulting in sustainable production to satisfy market requests. Consequently, this legal stipulation for maximum limits may have been surpassed using standard production processes (Stanković & Ćirić, 2021).

Research examining the effect of micro-particles in Crohn's disease indicated that titanium dioxide microparticles were consumed in amounts surpassing the acceptable daily intake (ADI) (Lomer et al., 2002). A comparable study in Italy showed that the antioxidant BHT was ingested in quantities exceeding the ADI (Leclercq et al., 2000). Phosphorus consumption in the US has been shown to exceed the ADI standard (Calvo & Park, 1996). The nutritional intake of

nitrite and nitrate from natural foods was evaluated in France. The study revealed that dietary nitrite intake exceeded the ADI threshold in 0.7%-16.4% of adults and 10.5%-66.2% of kids, correspondingly (Menard et al., 2008). In Estonia, the concentration of nitrite and/or nitrate in meat crops was evaluated, revealing that nitrite consumption surpassed the ADI standard by as much as 140% for kids old 1 to 6 years (Reinik et al., 2005). Research investigating the use of reproduction nutrition colours amongst 3,141 broods in Kuwait revealed that sundown yellow, tartrazine, carmoisine, and Allura red were ingested in quantities exceeding the acceptable daily intake (ADI) (Toledo et al., 1992, Husain et al., 2006).

8.3. Epigenetic carcinogen risk assessments

The role and significance of epigenetic mechanisms induced by dietary variables in human cancer development are uncertain (Herceg, 2007), and the optimal method for assessing the risk of such carcinogens continues to be a subject of contention (Braakhuis et al., 2018). However, at low intermittent dosages (below 1 mg per day), epigenetic Carcinogens be situated non carefully for tumour dangers on the way to persons (Williams, 2008). This might indicate the lack of analogous mechanisms in humans compared to rodents, such as (d-limonene α 2 μ (α 2 μ)-globulin) nephropathy in male pests resulting in kidney cancer (Swenberg & Lehman-McKeeman, 1999), or the significantly lesser human contacts, demonstrated by forestomach prevention in rats induced by Butylated Hydroxyanisole (BHA) critical toward squamous prison cell carcinoma (Williams & Whysner, 1996). Furthermore, the reversibility of epigenetic alterations may mitigate potential human harm. Consequently, epigenetic Carcinogens (NOAELs) stay employed toward established care standards, including tolerated daily intake TDI (Williams, 2008).

8.4. Food-derived carcinogens risk assessments

Application of carcinogenicity data to human risk

Risk assessment utilises dual categories of carcinogenicity information, human epidemiological data, and cancer data derived from rodent model tests (IARC, 2009). The former is deemed more pertinent for several reasons (Barlow & Schlatter, 2010). However, such data frequently lack comprehensive human revelation information and may be inadequately regulated (Raffaele et al., 2011). Animal data are often more reliable; however, they often contain results with dubious relevance to humans (Raffaele et al., 2011, Edler et al., 2014). The tumorigenic effects entail mechanisms of action that operate solely in rats. Furthermore, rodent studies fail to replicate actual human exposure in terms of both attentiveness and incidence. The social diet has a combination of elements that promote and impede carcinogenicity. Therefore, by evaluating human hazards, binary factors be considered paramount: the mode of action of carcinogenicity and the human exposure dosage (Hartwig et al., 2020).

After identifying a chemical in a nutrition product and determining its structure, an in silico study can be conducted to assess the possibility of DNA reactivity based on structure-activity connections (Rosenkranz, 2004). Although this method is effective for comparatively simple chemicals, the intricacies of several accepted crops render the nuances of metabolic initiation progressively challenging to anticipate.

Table 3. Description of the risk assessment of chemical agents

Risk assessment of chemical agents	Description
Hazard identification	The determination of potential adverse health effects from exposure to a substance. This determination is based upon a review of the toxicity data, which includes toxicity testing results in experimental animals and any knowledge of effects on human health and the mechanism/mode of carcinogenesis (Chartres et al., 2019).
Hazard characterisation	Determining the dose-response relationship and relevance to humans, incorporating factors such as interspecies variation in susceptibility and the relevance of mode/mechanism of action to humans (Cohen Hubal et al., 2010).
Exposure assessment	The amount of human exposure to a substance is determined. This determination uses data collected on the contaminant levels in food and specific food intake (consumption) information to calculate probable human exposure (Moretto, 2015).
Risk characterisation	Exposure to a hazardous chemical poses an estimated risk to human health. This procedure can be used to conclude, for example, what level of exposure to the hazardous chemical is associated with an increase in carcinogenic risk, even if very small. In addition, the risk characterisation is used to inform risk managers what level of risk may be acceptable or tolerable. To arrive at these estimates, consideration is given to the toxicity profile of the chemical in question, mechanisms of action, relevance to humans, dose response, and potential human exposure (Tice et al., 2013).

When adequate physical remains obtainable, a straight challenge used for DNA responsiveness stands as the preferable method (IARC, 2009, Turner et al., 2023). Table 3 presents information on the organization of Carcinogens through administration activities and their carcinogenic strengths (TD50) derived from rodent tumorigenicity studies.

In contrast to pharmaceuticals, the FDCA does not mandate the acquisition of scientific protection information for food additives. The protection valuation method for food additives may rely exclusively on the outcomes of tentative investigations. When human data are accessible, they should be integrated into the protection outline of the food additive (Pressman et al., 2017). When substantial human consumption is anticipated, petitioners may want to perform human research following a comprehensive nonclinical examination (NRC, 2004). Clinical studies on specific macro ingredient food additives might be necessary, as excessive consumption of macro ingredients in rodents has demonstrated changes in normal physiology, resulting in misleading toxicological effects irrelevant to humans. Moreover, inquiries concerning the impact of elevated amounts of these additives on nutritional caloric gratified with the alteration of micronutrient homeostasis are most effectively addressed in humanoid subjects (Pressman et al., 2017, Reddy & Hayes, 2018).

Assessment of risks associated with DNA-reactive rodent carcinogens

On the way to assess potential care anxieties associated with the attendance of carcinogens that operate through DNA-reactive mechanisms in food, various controlling and optional organisations, including the European Food Safety Authority Panel on Contaminants in the Food Chain (EFSA CONTAM) and the Combined Food and Cultivation Organisation of the United Nations (FAO), WHO, JECFA, and MoE, employ specific methodologies (O'Brien et al., 2006, Benford et al., 2010). The Margin of Exposure (MoE) is intended using the relation amid a pertinent Fact of Parting for cancer reply; for instance, No Observed Adverse Effect Levels (NOAELs) found in beast studies and a projected or expected human revelation level (Edler et al., 2014, Paustenbach & Cox Jr, 2024).

9. Conclusion

This review underscores the dual nature of food additives, acknowledging their benefits in enhancing food preservation, sensory appeal, and processing efficiency while highlighting potential carcinogenic risks. While food additives play a crucial role in the modern food industry, rigorous safety assessment and continuous monitoring are paramount to safeguarding public health. The carcinogenic potential of certain additives, as identified by the FDA and IARC, necessitates a balanced approach that considers both the advantages and potential hazards associated with their use. Future research should focus on elucidating the long-term effects of food additive exposure, refining risk assessment methodologies, and exploring safer alternatives to ensure a secure and sustainable food supply.

Author's Contributions

The authors contributed equally. Amjad Mahmood Qadir: Conceptualization, Software and Resources, Tables, Revision and Supervision; Dastan Jamal Salih, Software and Resources, Tables. The authors have reviewed and consented to the published version of the manuscript.

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Conflicts of Interest

The authors disclose no conflicts of interest.

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

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ABSTRACT

Red beetroot juice (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).

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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 \pm 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 $\geq 7.5\%$ 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 \pm 13 ^e	217 \pm 1 ^b	14.88 \pm 0.12 ^c	11.64 \pm 0.05 ^c	19.79 \pm 0.17 ^e	0.17	1.33	6.27
RBJ-2	1273 \pm 5 ^{de}	62.83 \pm 0.78 ^g	9.98 \pm 0.14 ^f	6.18 \pm 0.08 ^e	17.30 \pm 0.29 ^f	0.05	1.73	2.30
RBJ-3	1224 \pm 11 ^f	87.84 \pm 0.27 ^e	12.66 \pm 0.02 ^e	9.04 \pm 0.16 ^d	26.06 \pm 0.14 ^b	0.07	2.06	2.27
RBJ-4	796 \pm 3 ^h	93.10 \pm 0.46 ^d	13.72 \pm 0.15 ^d	8.82 \pm 0.51 ^d	14.81 \pm 0.07 ^h	0.12	1.08	3.26
RBJ-5	579 \pm 7 ⁱ	179 \pm 2.84 ^c	20.20 \pm 0.20 ^b	9.21 \pm 0.16 ^d	15.17 \pm 0.4 ^h	0.31	0.75	5.06
RBJ-6	1405 \pm 12 ^b	175 \pm 0.65 ^c	20.30 \pm 0.13 ^b	20.91 \pm 0.88 ^b	42.94 \pm 0.12 ^a	0.12	2.12	2.77
RBJ-7	1110 \pm 6 ^g	29.78 \pm 0.47 ⁱ	4.32 \pm 0.06 ⁱ	6.75 \pm 0.30 ^e	20.90 \pm 0.15 ^d	0.03	4.84	1.18
RBJ-8	1333 \pm 8 ^c	54.57 \pm 0.21 ^h	8.25 \pm 0.02 ^g	9.01 \pm 0.10 ^d	12.97 \pm 12.87 ⁱ	0.04	1.57	2.57
RBJ-9	1303 \pm 18 ^{cd}	72.59 \pm 0.53 ^f	5.27 \pm 0.06 ^h	11.21 \pm 0.20 ^c	16.51 \pm 0.27 ^g	0.06	3.13	3.33
RBJ-10	1503 \pm 25 ^a	266 \pm 4.05 ^a	24.09 \pm 0.49 ^a	39.36 \pm 0.71 ^a	25.31 \pm 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 & Kłasa 2009). The Ca/Mg ratios ranged from 0.75 to 4.84. According to Francke & Kłasa (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.

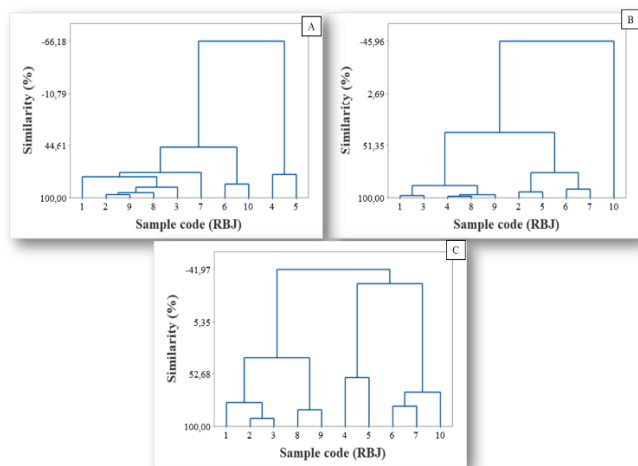


Figure 1. Dendrographic classification of RBJ samples according to their elements. (A. macroelements, B: essential microelements, C: toxic microelements)

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 $\mu\text{g } 100 \text{ mL}^{-1}$; Mn, 85.8 $\mu\text{g } 100 \text{ mL}^{-1}$; Zn, 25.71 $\mu\text{g } 100 \text{ mL}^{-1}$; Cu, 9.68 $\mu\text{g } 100 \text{ mL}^{-1}$; Co, 0.345 $\mu\text{g } 100 \text{ mL}^{-1}$; Se, 9.05 $\mu\text{g } 100 \text{ mL}^{-1}$; and Mo, 1.197 $\mu\text{g } 100 \text{ mL}^{-1}$. 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 $\mu\text{g L}^{-1}$, which significantly exceeded the maximum allowable concentration of 200 $\mu\text{g L}^{-1}$ set by EU, WHO, and Turkish regulations.

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

Sample code	Fe	Mn	Zn	Cu	Co	Se	Mo
RBJ-1	101 \pm 8 ^h	76.76 \pm 0.03 ^{cd}	85.80 \pm 1.15 ^b	20.19 \pm 0.46 ^d	0.291 \pm 0.020 ^c	4.195 \pm 1.661	0.545 \pm 0.066
RBJ-2	92.69 \pm 2.25 ^h	35.09 \pm 0.06 ^f	44.99 \pm 0.38 ^e	12.16 \pm 0.20 ^f	0.067 \pm 0.010 ^d	5.378 \pm 0.56	0.480 \pm 0.052
RBJ-3	253 \pm 1 ^{fg}	31.96 \pm 0.04 ^f	44.08 \pm 0.63 ^e	21.98 \pm 0.60 ^{cd}	0.595 \pm 0.030 ^b	8.38 \pm 2.24	0.576 \pm 0.108
RBJ-4	448 \pm 30 ^e	83.67 \pm 5.40 ^c	79.94 \pm 0.87 ^c	24.12 \pm 1.74 ^c	0.084 \pm 0.017 ^d	6.531 \pm 1.558	0.511 \pm 0.088
RBJ-5	776 \pm 1 ^c	262 \pm 5 ^a	49.54 \pm 0.55 ^d	19.07 \pm 0.21 ^{de}	0.139 \pm 0.009 ^d	3.647 \pm 1.101	0.625 \pm 0.041
RBJ-6	958 \pm 37 ^b	165 \pm 8 ^b	107 \pm 2 ^a	42.58 \pm 1.66 ^b	0.976 \pm 0.103 ^a	6.32 \pm 1.77	1.123 \pm 0.110
RBJ-7	289 \pm 25 ^f	39.73 \pm 1.15 ^f	26.02 \pm 0.46 ^g	10.28 \pm 0.91 ^f	0.162 \pm 0.035 ^d	5.005 \pm 1.038	0.494 \pm 0.0803
RBJ-8	202 \pm 10 ^g	37.32 \pm 0.70 ^f	31.35 \pm 0.34 ^f	16.66 \pm 0.03 ^e	0.117 \pm 0.030 ^d	8.95 \pm 1.85	1.401 \pm 0.060
RBJ-9	2647 \pm 16 ^a	56.92 \pm 0.34 ^e	49.83 \pm 0.03 ^d	23.47 \pm 0.16 ^c	0.644 \pm 0.024 ^b	8.14 \pm 2.39	3.556 \pm 0.178
RBJ-10	577 \pm 13 ^d	69.94 \pm 1.05 ^d	46.47 \pm 1.59 ^e	77.16 \pm 2.23 ^a	0.379 \pm 0.057 ^c	34 \pm 2.78	2.663 \pm 0.084
Average	634 \pm 737	85.8 \pm 70.8	56.46 \pm 24.85	26.77 \pm 19.11	0.345 \pm 0.295	9.05 \pm 8.76	1.197 \pm 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 \pm 68 ^{de}	1762 \pm 21 ^d	49.88 \pm 2.28 ^e	17.41 \pm 1.18 ^d	-	0.29 \pm 0.17 ^{cd}	-
RBJ-2	6023 \pm 160 ^{de}	2081 \pm 38 ^{bc}	49.81 \pm 0.64 ^e	20.72 \pm 2.27 ^{cd}	-	0.35 \pm 0.26 ^{bcd}	-
RBJ-3	5884 \pm 47 ^e	2151 \pm 33 ^b	70.57 \pm 2.12 ^{de}	28.47 \pm 2.6 ^b	-	0.39 \pm 0.50 ^{bcd}	8.37 \pm 0.60 ^d
RBJ-4	7273 \pm 73 ^a	582 \pm 32 ^g	87.42 \pm 6.28 ^d	25.51 \pm 3.51 ^{bc}	-	1.18 \pm 0.57 ^{bc}	24.79 \pm 2.7 ^a
RBJ-5	6389 \pm 92 ^{cd}	875 \pm 4 ^f	84.07 \pm 3.03 ^d	25.69 \pm 3.02 ^{bc}	-	0.79 \pm 0.13 ^{bcd}	-
RBJ-6	6810 \pm 364 ^{bc}	2073 \pm 91 ^{bc}	437 \pm 22 ^a	23.94 \pm 1.10 ^{bcd}	64.91 \pm 6.36 ^a	4.91 \pm 0.32 ^a	13.85 \pm 1.25 ^b
RBJ-7	6821 \pm 58 ^b	1944 \pm 77 ^c	76.98 \pm 4.28 ^d	20.42 \pm 1.25 ^{cd}	-	0.14 \pm 0.10 ^d	-
RBJ-8	5670 \pm 23 ^e	1462 \pm 24 ^e	82.77 \pm 2.72 ^d	22.89 \pm 1.82 ^{bcd}	-	0.96 \pm 0.37 ^{bcd}	-
RBJ-9	5713 \pm 28 ^e	1335 \pm 7 ^e	333.85 \pm 7.75 ^b	25.6 \pm 0.9 ^{bc}	142.7 \pm 2.51 ^b	0.41 \pm 0.14 ^{bcd}	9.39 \pm 0.50 ^c
RBJ-10	7022 \pm 169 ^{ab}	2508 \pm 69 ^a	151.65 \pm 6.21 ^c	75.94 \pm 3.94 ^a	-	1.34 \pm 0.52 ^b	2.05 \pm 0.24 ^d
Average	6369 \pm 570	1677 \pm 587	142.4 \pm 128.9	28.66 \pm 1.65	20.76 \pm 45.82	1.08 \pm 0.25	5.84 \pm 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 Türkiye 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 $\mu\text{g L}^{-1}$, which was below the EU’s maximum permissible limit of 7000 $\mu\text{g L}^{-1}$. However, the average Cr content was 142 $\mu\text{g L}^{-1}$, exceeding the recommended limit of 50 $\mu\text{g L}^{-1}$. 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 $\mu\text{g L}^{-1}$ and 1.076 $\mu\text{g L}^{-1}$, respectively. The maximum permissible level of As in drinking water is 10 $\mu\text{g L}^{-1}$. 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 $2 \mu\text{g L}^{-1}$. According to the Regulation on Water Intended for Human Consumption, the maximum permissible concentration of cadmium (Cd) in drinking water is $5 \mu\text{g/L}$. 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 $5 \mu\text{g/L}$. 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 $20.76 \mu\text{g L}^{-1}$. 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 $20 \mu\text{g/L}$, whereas the World Health Organization (WHO) allows a higher threshold of $70 \mu\text{g L}^{-1}$. 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 $5.84 \mu\text{g L}^{-1}$. 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 $10 \mu\text{g/L}$. 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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