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Measurement of bisphenol A in non-alcoholic malt beverages by the MSPE/GC-MS technique: A risk assessment study

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ABSTRACT

The goal of this research was to assess the level of Bisphenol A (BPA) in samples of non-alcoholic malt beverage using the technique of MSPE/GC-MS (magnetic graphene solid-phase extraction/gas chromatography-mass spectrometry). Results revealed that the mean ± standard deviation (min-max) of BPA in all samples was 2.41 ± 0.60 µg/L (1.08 - 4.25 µg/L). As the data show, the BPA content in non-alcoholic malt beverages was upper than the standard level of EU (European Union) (the new EU standard level, no amount of bisphenol A is allowed in food). The maximum average of BPA was in the 330 mL (Can) volume (2.53 ± 0.53 µg/L) and the minimum average was in the 1000 mL (PET) volume (2.30 ± 0.47 µg/L). The maximum average of BPA was in samples stored at 27°C (2.47 ± 0.53 µg/L) and the minimum average was in samples stored at 7°C (2.35 ± 0.57 µg/L). The contamination level increased slightly over time, with the highest mean BPA observed in samples close to the expiration date (2.53 ± 0.63 µg/L) and the lowest mean in samples close to the production date (2.15 ± 0.47 µg/L). The 95th percentile chronic daily intake (CDI) values of BPA in non-alcoholic malt beverages for children, adolescents, and adults were 3.64E-6, 1.61E-6, and 1.05E-6 mg/kg bw/day, respectively. The 95th percentile target hazard quotient (THQ) values for children, adolescents, and adults were 1.92E+1, 8.63E+0, and 5.48E+0, respectively, all of which exceeded the acceptable threshold of 1, demonstrating potential non-carcinogenic risks of human health. Based on the study's findings, it can be concluded that—given the risks associated with this compound in Iranian beverages—further investigation and enhanced monitoring are essential

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1. Introduction

The production of epoxy resins and polycarbonate plastics commonly relies on a synthetic organic material known as Bisphenol A (BPA) (1-3). Owing to its pervasive presence in various consumer products, including containers of foodstuff, bottles of water, and coatings, BPA has raised significant health worries. Research has linked BPA exposure to various adverse health effects, including reproductive issues,

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hormonal disruptions, and an enhanced hazard of chronic diseases like obesity and diabetes (4-6).

The compound can leach into foodstuffs and beverages, especially when containers are subjected to heat or wear, making its presence in food products a critical public health issue (7-9). Under the latest regulations set by the European Union, bisphenol A is completely prohibited from being present in food (10). In 2023, the European Food Safety Authority (EFSA) revised the Tolerable Daily Intake (TDI) downward, establishing a more stringent toxicological benchmark of 0.0002 µg/kg body weight per day for assessing long-term risk (11).

Malt beverages, which include a variety of drinks like malt beer, malted milk, and non-alcoholic malt beverages, are increasingly popular owing to their unique flavors and nutritional benefits. These beverages often comprise essential minerals and vitamins, making them appealing to consumers seeking healthier alternatives. The ingredients of non-alcoholic and unflavored malt beverages (classic) usually include barley malt, CO_2, lactic acid, citric acid, hops extract, ascorbic acid, and water (12, 13). Nonetheless, the production process and packaging materials applied for malt beverages can introduce contaminants like BPA. The use of BPA-containing materials in the manufacturing and storage of these beverages raises concerns about potential exposure to this harmful chemical, especially among vulnerable populations like children and pregnant women (12, 14, 15).

Several techniques exist for measuring BPA levels in foodstuffs, each technique has its own advantages and limitations. Traditional analytical techniques often lack the sensitivity required for trace detection of BPA in complex matrices. MSPE combined with GC-MS offers a robust solution for this challenge. The MSPE technique enhances the extraction efficiency of BPA from food matrices, allowing for lower LODs (detection limits) and improved accuracy. GC-MS further enables precise identification and quantification of BPA, making it an ideal choice for comprehensive analysis in studies of food safety (2, 6, 16, 17).

Risk assessment is crucial in understanding the potential health implications of BPA exposure from malt beverages. Monte Carlo simulation (MCS) provides a powerful statistical approach for evaluating risk by incorporating variability and uncertainty in exposure data. This technique allows researchers to model different scenarios and evaluate the likelihood of adverse health outcomes associated with BPA consumption. By employing MCS, researchers can better recognize the risks posed by BPA in malt beverages and inform regulatory decisions aimed at protecting public health (2, 16).

Despite the growing consumption of non-alcoholic malt beverages in Iran and worldwide, scientific data regarding BPA contamination in these products remain scarce. Most previous studies have focused on carbonated soft drinks, fruit juices, and canned foods. To the best of our knowledge, no comprehensive study has simultaneously investigated BPA levels in malt beverages alongside critical factors influencing migration, such as packaging size, brand variation, storage temperature, and storage time. This research gap is particularly concerning given the high consumption of malt beverages among children and adolescents populations, which exhibit greater

susceptibility to the hormone-disrupting properties of BPA (2, 6, 16).

This study aimed to measure BPA concentrations in non-alcoholic malt beverages using magnetic solid-phase extraction coupled with gas chromatography-mass spectrometry (MSPE/GC-MS), and to assess associated health risks via Monte Carlo simulation. The novelty lies in simultaneously investigating four factors affecting BPA migration (brand, packaging volume, temperature, and storage time) and applying advanced probabilistic risk assessment to better estimate actual consumer exposure. The findings can inform national standards and regulatory guidelines for the malt beverage industry and raise awareness among consumers and policymakers regarding product safety.

2. Materials and Methods

2.1. Reagents

The MWCNTs or Multi-Walled Carbon Nanotubes (5-30 µm length and 30–60 nm diameter) were bought from Nanoshel Co. (Panchkula, India). The Sigma-Aldrich company (West Chester, PA, USA) supplied the BPA standard solution used in this study. Other chemicals and solvents were ordered from Merck Co. (Darmstadt, Germany) with analytical grade.

2.2. The process of fabricating an adsorbent with magnetic properties

The MWCNTs-Fe_3O_4 was prepared as described in previous articles (2, 18, 19).

2.3. Sample collection

A total of 48 samples (in duplicated) were collected. Sampling was performed from 3 brands in 2 different volumes [330 (Can) and 1000 (PET) mL]. The samples were kept under 2 conditions of time including at the sixth month (i.e., expiration date) and at the first week after production (i.e., production date) and also at 2 temperatures: 27°C (ambient temperature) and 7°C (refrigeration temperature). Brands of non-alcoholic malt beverages were selected from among the most widely consumed brands using sampling method of simple random; sampling was conducted at supermarkets in Tehran, Iran, Between March and May 2026. After transfer to the laboratory, samples were kept at room temperature (25 ± 2 °C) or under refrigeration (4 ± 1 °C), depending on the study design, until analysis.

2.4. BPA measurement

2.4.1. Preparation of samples

At the beginning of the experiment, the degassed samples (10 mL) were mixed with NaCl (0.5 g), MWCNT-Fe_3O_4 composite (0.1 g), and phenol (100 µL, as internal standard). The mixture was stirred using a magnetic stirrer for 5 min and allowed to stand for 10 min. Subsequently, the supernatant was removed (gently) and the magnetic adsorbents were then collected using an external magnet. Subsequently, the magnetic adsorbents were washed with 2 mL of dichloromethane. Next, the magnet (external) was used to separate the magnetic adsorbents from the dichloromethane including the compound of BPA. Next step, the solution containing the BPA compound was filtered using syringe filters into a GC vial and stored at 4 °C (refrigerator). Finally, to the prepared solution, MSTFA (N-Methyl-N-trimethylsilyltrifluoroacetamide) as derivatizer (50 µL) was added and incubated for 1 h at 50 °C. Finally, 1 µL of the prepared solution was injected into the GC–MS technology (2, 17).

2.4.2. The settings and specifications applied during GC-MS analysis

The analyses conducted in this study were performed on a GC-MS system—specifically, an Agilent 7890 gas chromatograph coupled with an Agilent 5975 mass spectrometer (Palo Alto, CA, USA). The instrument was equipped with an HP-5MS capillary column (with dimensions of 30 m in length, an internal diameter of 0.25 mm, and a film thickness of 0.25 μm). All GC-MS operational parameter, including carrier gas flow rate, injection and ion source temperatures, oven temperature program, and retention times, were optimized based on established protocols (2, 6). The GC–MS was operated under the following parameters: helium served as the carrier gas at a purity of 99.999% and a flow rate of 1 mL/min. Samples were introduced in splitless mode with a 1 μL injection volume. The oven temperature program was set as follows: the injector was maintained at 280 °C, while the initial oven temperature was held at 100 °C for 1 min. The temperature was then raised to 225 °C at a rate of 20 °C/min, held for 5 min, and subsequently increased to 325 °C at 35 °C/min, where it was held for another 5 min. The monitored m/z ions for the internal standard (IS) were 94, 187, and 188, while for BPA, the quantification ion was m/z 285 and the confirmation ions were m/z 357 and 285.

2.5. Method validation

A six-point calibration curve was constructed by analyzing standard BPA solutions in methanol at concentrations of 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 μg/L. The curve was plotted as peak area versus concentration, and the calibration curve equation y = ax + b (where y is the peak area and x is the concentration) was calculated, along with the coefficient of determination (R^2).

Values for the detection limit (LOD) and the quantification limit (LOQ) were calculated based on the following equations:

Equation 1: $LOD = 3.3 \times \sigma/S$

Equation 2: $LOQ = 10 \times \sigma/S$

Where σ = the standard deviation of the response and S = slope of the calibration curve. These formulas are consistent with IUPAC and ICH guidelines.

For intra-day precision (repeatability), the relative standard deviation (RSD%) was determined by analyzing six independently prepared standard solutions at three concentration levels (1.0, 5.0, and 9.0 μg/L) on the same day under identical conditions. Inter-day precision was assessed by analyzing the same concentrations on three consecutive days.

Recovery (accuracy) studies were performed by spiking a real malt beverage with BPA at three concentration levels (low, medium, and high: 1.0, 5.0, and 9.0 μg/L). Each level was prepared and analyzed in triplicate (n=3). The spiked samples were extracted and analyzed using the same MSPE/GC-MS procedure. Recovery percentage was calculated as:

Equation 3: $\text{Recovery } (\%) = [(C_{spiked} - C_0) / C_{added}] \times 100$

Where C spiked is the concentration measured in the spiked sample, C_0 is the concentration measured in the unspiked sample (native concentration), and C added is the theoretical spiked concentration.

Matrix effect evaluation: To assess the potential influence of the beverage matrix on the GC-MS analysis, the matrix effect was evaluated using the post-extraction spiking method. Calibration curves were

prepared in both neat solvent (methanol) and blank matrix extract (post-extraction spiked). The matrix effect percentage (%ME) was calculated as:

Equation 4: %ME = [(slope_matrix − slope_solvent) / slope_solvent] × 100

Where slope_matrix is the slope of the calibration curve prepared in the blank matrix extract and slope_solvent is the slope of the calibration curve prepared in neat solvent. Values of %ME close to zero indicate negligible matrix effects; positive values indicate signal enhancement and negative values indicate signal suppression.

2.6. Estimate of dietary exposure

To quantify the oral exposure dose of hazardous compounds, the chronic daily intake (CDI) (mg/kg bw/day) and target hazard quotient (THQ) were employed as metrics for characterizing the health risk posed by BPA and assessing the magnitude of its potential adverse effects. These parameters were derived using the following equations:

$$CDI = \frac{C \times IR}{BW} \quad \text{(Equation 5)}$$

$$THQ = CDI \,/\, TDI \quad \text{(Equation 6)}$$

Where C is the BPA level in non-alcoholic malt beverages (mg/kg) (20), IR is the daily ingestion rate in Iran (0.23 L/day) based on manufacturer recommendations (21), BW is the average human body weight (Adults = 70 kg, Children = 25 kg, adolescents=50 kg) (22), and the TDI is tolerable daily intake for non-carcinogenic risk. The EFSA (April 2023) established TDI of 0.0002 µg/kg/day as the threshold for BPA (23). To manage uncertainties associated with BPA-related health risks, a Monte Carlo simulation approach was applied in the present investigation. In contrast to conventional deterministic models with fixed estimates which can lead to either under- or overestimation of risk, Monte Carlo simulation accounts for variability across multiple parameters (24). This method provides a more robust, probability-based risk characterization by incorporating uncertainties from environmental sources, population differences, and analytical measurements. BPA concentration, ingestion rate, and body weight were modeled with log-normal, triangular, and normal distributions, respectively, for a conservative, worst-case screening-level probabilistic exposure assessment. A total of 10,000 iterations per scenario were conducted using Crystal Ball (Oracle® Crystal Ball, Oracle Corporation, USA) based on Monte Carlo techniques. Probability distributions were assigned to the input variables, which included BPA concentration levels and exposure-related factors such as ingestion rate and body weight (25). Through the probability distributions obtained from the simulations, a more detailed risk evaluation was made possible concerning the health effects of non-alcoholic malt beverage consumption.

2.7. Data analysis

Statistical evaluation was carried out with SPSS software for Windows (version 22; SPSS Inc., Chicago, IL, USA). Data distribution was checked for normality through the Kolmogorov–Smirnov test. Differences between sample groups were identified using the Kruskal–Wallis test, and a p-value below 0.05 was regarded as statistically significant. To assess the main effects along with all interaction terms among time, temperature, brand, and size simultaneously, a generalized linear model (GLM) employing a Gamma distribution and a log-link function was applied.

3. Results

3.1. Analytical method validation

According to the validation outcomes in the present article, relative standard deviation (RSD), R^2 (determination coefficient), LOD, and LOQ were 10.8 %, 0.993, 0.0030 µg/L, and 0.0090 µg/L, respectively. Also, the recoveries obtained at the three spiking levels (1.0, 5.0, and 9.0 µg/L) were 94.2% ± 2.1%, 96.8% ± 1.8%, and 95.8% ± 1.5%, respectively (n=3). The overall mean recovery across all levels was 95.6%.

3.2. BPA concentrations in all samples

The results of BPA measurement in all non-alcoholic malt beverage samples can be seen in Table 1. Referring to the information in mentioned table, the mean±SD (min-max) of BPA in all non-alcoholic malt beverage samples was 2.41 ± 0.60 µg/L (1.08 - 4.25 µg/L). As the data show, the BPA content in non-alcoholic malt beverages was upper than the standard level of EU (10).

3.3. BPA level in three brands of non-alcoholic malt beverages

Table 2 presents the BPA levels in different brands of non-alcoholic malt beverages. Drawing on the results obtained, the maximum average level of BPA was detected in brand C (2.41±0.40 µg/L) and the minimum average level of BPA was detected in brand A (2.39±0.41 µg/L). Among the various brands of non-alcoholic malt beverages tested, the average BPA contamination levels followed the sequence A < B < C. Based on the very close mean and median values across brands (A: 2.39, B: 2.40 and C: 2.41) and the p-value of 0.99, which is much larger than 0.05, there is no significant difference in BPA levels between the brands.

3.4. BPA level in two packaging of malt (non-alcoholic) beverages

Table 3 presents the level of BPA contamination in different volumes (1000 (PET) and 330 (Can) mL) of samples, with the maximum average in the 330 mL volume (2.53±0.53 µg/L) and the minimum average in the 1000 mL volume (2.30±0.47 µg/L). Among the various volumes of non-alcoholic malt beverages tested, the average BPA contamination levels followed the sequence 330 mL (Can) > 1000 mL (PET). Based on the p-value of 0.79, which is considerably larger than 0.05, there is no significant difference in levels of BPA between the different packaging sizes (1000 mL vs. 330 mL). Although the mean BPA level for the 330 mL size (2.53 µg/L) is slightly higher than that of the 1000 mL size (2.30 µg/L), this variation lacks statistical significance and can most likely be attributed to random chance.

3.5. BPA level at two temperatures of storage of non-alcoholic malt beverages

Table 4 presents the BPA measurement outcomes for non-alcoholic malt beverage samples kept at various temperatures of storage. As can be seen from the table's results, the maximum average contamination was in samples kept at 27°C (2.47±0.53 µg/L) and the minimum average was in samples kept at 7°C (2.35±0.57 µg/L). Among the various storage temperatures tested for non-alcoholic malt beverages, the average BPA contamination levels followed the sequence 27°C > 7°C. The p-value is 0.219, which is greater than the significance level of 0.05. Therefore, there is no significant difference in BPA levels between non-alcoholic malt beverages stored at 7°C (refrigerator

temperature) and 27°C (room temperature). Although the mean BPA level at 27°C (2.47 µg/L) is slightly higher than that at 7°C (2.35 µg/L), this small difference is not statistically significant.

3.6. BPA levels at two times of storage in non-alcoholic malt beverages

Table 5 presents the levels of BPA in non-alcoholic malt beverages at different storage times. The results show a slight increase in contamination over time, with the maximum average concentration observed in samples near the expiration date (2.53 ± 0.63 µg/L) and the minimum average in samples near the production date (2.15 ± 0.47 µg/L). Accordingly, the mean BPA levels followed the order: expiration date > production date. The p-value is 0.038, which is less than the significance level of 0.05. Therefore, there is a significant difference in BPA levels between the first week (production date) and the sixth month (expiration date). The mean BPA level at the sixth month (2.53 µg/L) is significantly higher than that at the first week (2.15 µg/L). Even a modest 17.7% increase over the storage period could be relevant for extended shelf life. However, the consistent upward trend reinforces the recommendation to minimize storage duration as a food safety precaution, especially for vulnerable populations.

3.7. Multifactorial analysis of BPA using generalized linear model

Table 6 presents the Wald chi-square tests for the generalized linear model (GLM) .The model was fitted with a Gamma distribution and log-link function to account for the positive skewness and heteroscedasticity of the BPA concentration data. The analysis revealed that, among the main effects, only storage time had a statistically significant influence on BPA migration (χ^2 = 5.54, df = 1, p = 0.038). All interaction terms, including time×size (χ^2 = 0.88, df = 1, p = 0.471), time×brand (χ^2 = 1.00, df = 2, p = 0.606), temperature×time (χ^2 = 0.99, df = 1, p = 0.320), the three-way interaction of time×brand×temperature (χ^2 = 1.08, df = 2, p = 0.584), and the four-way interaction of time×brand×temperature×size (χ^2 = 1.87, df = 1, p = 0.171), were not statistically significant (all p > 0.05).

3.8. Chronic daily intake (CDI) of BPA

The CDI was calculated for three population groups: adults, adolescents, and children. The 50th (median) and 95th percentile concentrations were used to represent average and high-end exposure scenarios, respectively. Figure 1 presents the CDIs of BPA in all non-alcoholic malt beverage samples. According to this figure, the 95th percentile CDI of BPA in all non-alcoholic malt beverage samples for children, adolescents, and adults were 3.64E-6, 1.61E-6, and 1.05E-6 mg/kg bw/day, respectively.

Figure 1 illustrates the findings regarding the non-carcinogenic health risk associated with BPA intake through the consumption of non-alcoholic malt beverages. Results demonstrated that the 95th percentile THQ values of non-alcoholic malt beverage samples for children, adolescents, and adults were 1.92E+1, 8.63E+0, and 5.48E+0, respectively.

Table 1. BPA concentrations in all non-alcoholic beverage samples (µg/L)

	Mean	Maximum	Minimum	Median	SD
Total samples	2.41	4.25	1.08	2.30	0.60

Table 2. BPA concentrations by brand in non-alcoholic malt beverages (µg/L)

Brand	Mean	Maximum	Minimum	Median	SD	p-value
A	2.39	4.24	1.15	2.39	0.41	
B	2.40	4.25	1.08	2.36	0.51	0.99
C	2.41	4.23	1.22	2.38	0.40	

Table 3. BPA concentrations by packaging size in non-alcoholic malt beverages (µg/L)

Volume	Mean	Maximum	Minimum	Median	SD	p-value
1000 mL (PET)	2.30	4.02	1.08	2.19	0.47	0.79
330 mL (Can)	2.53	4.25	1.26	2.36	0.53	

Table 4. BPA concentrations at two temperatures of storage in non-alcoholic malt beverages (µg/L)

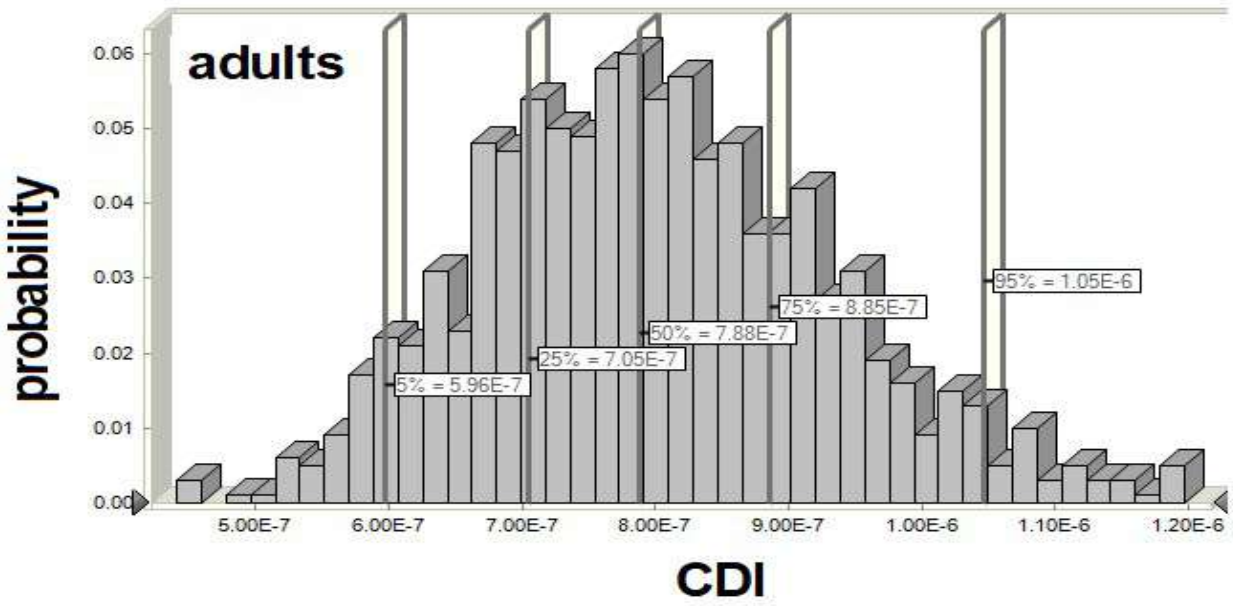
Temp (ºC)	Mean	Maximum	Minimum	Median	SD	p-value
7.0	2.35	4.25	1.15	2.32	0.57	0.219
27.0	2.47	4.24	1.08	2.29	0.53	

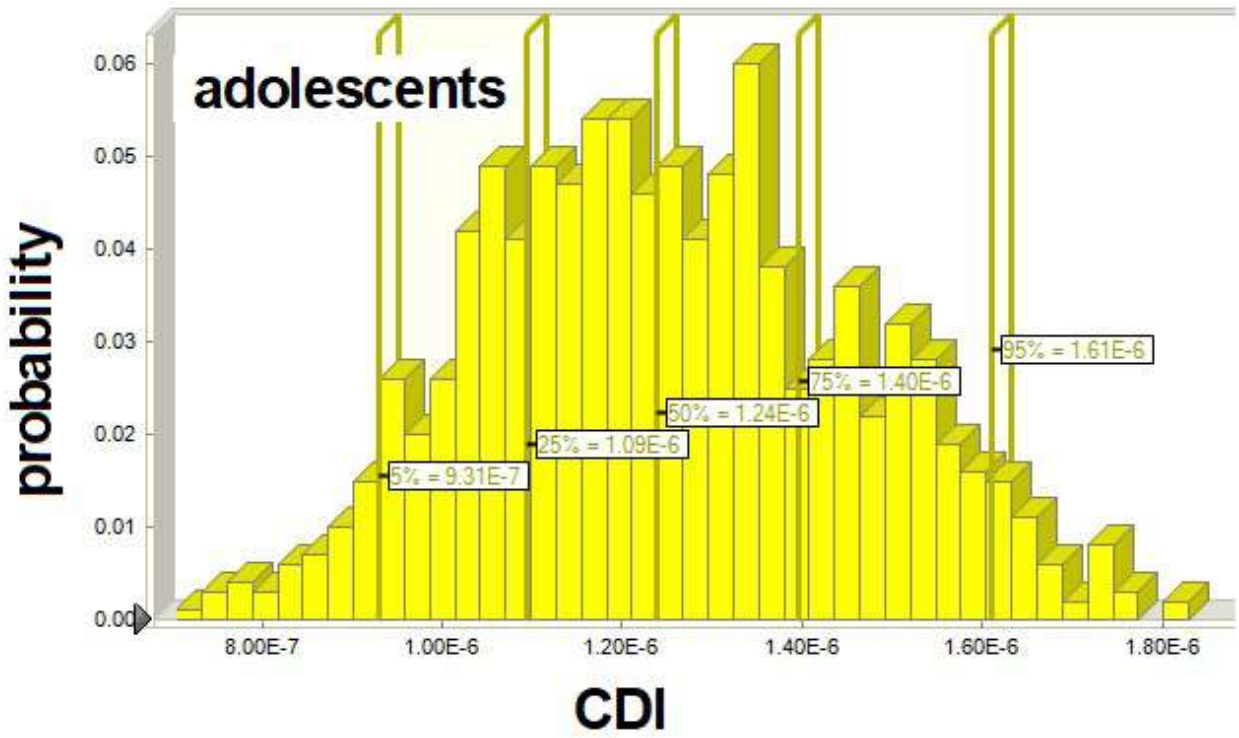
Table 5. BPA concentrations at different storage times in non-alcoholic malt beverages (µg/L)

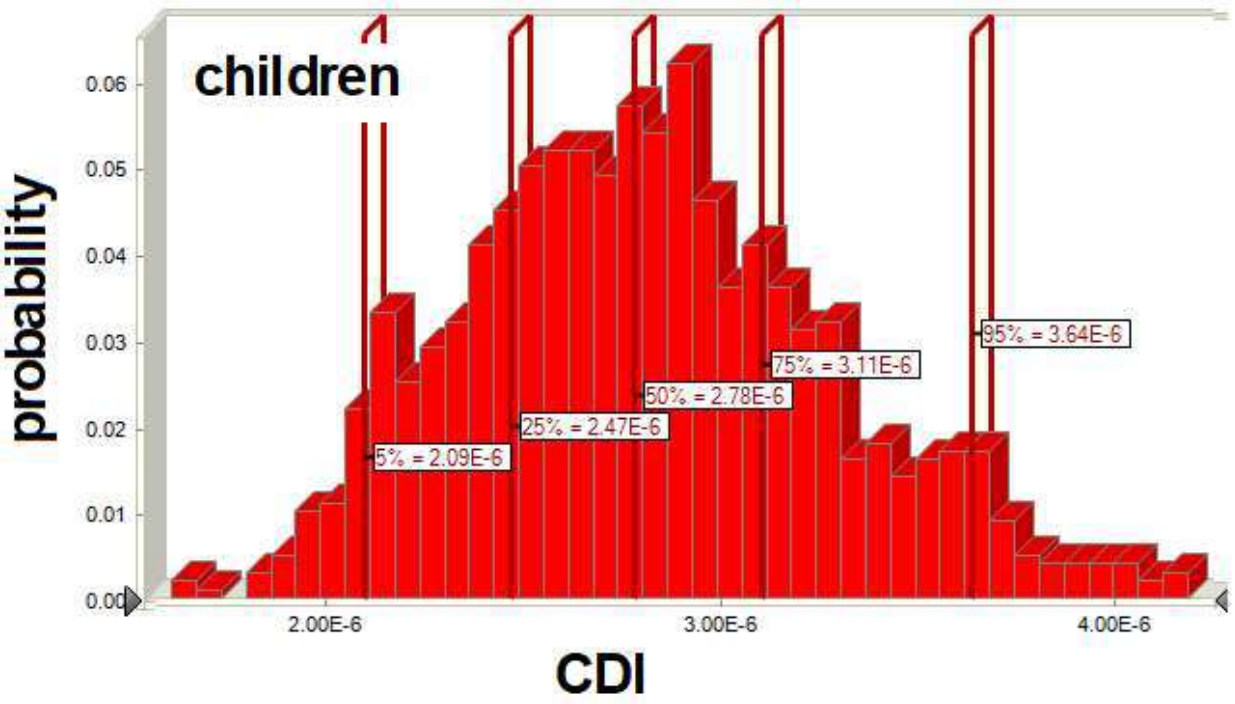
Time	Mean	Maximum	Minimum	Median	SD	p-value
First week (production date)	2.15	4.02	1.08	2.19	0.47	0.038
Sixth month (expiration date)	2.53	4.25	1.05	2.36	0.63	

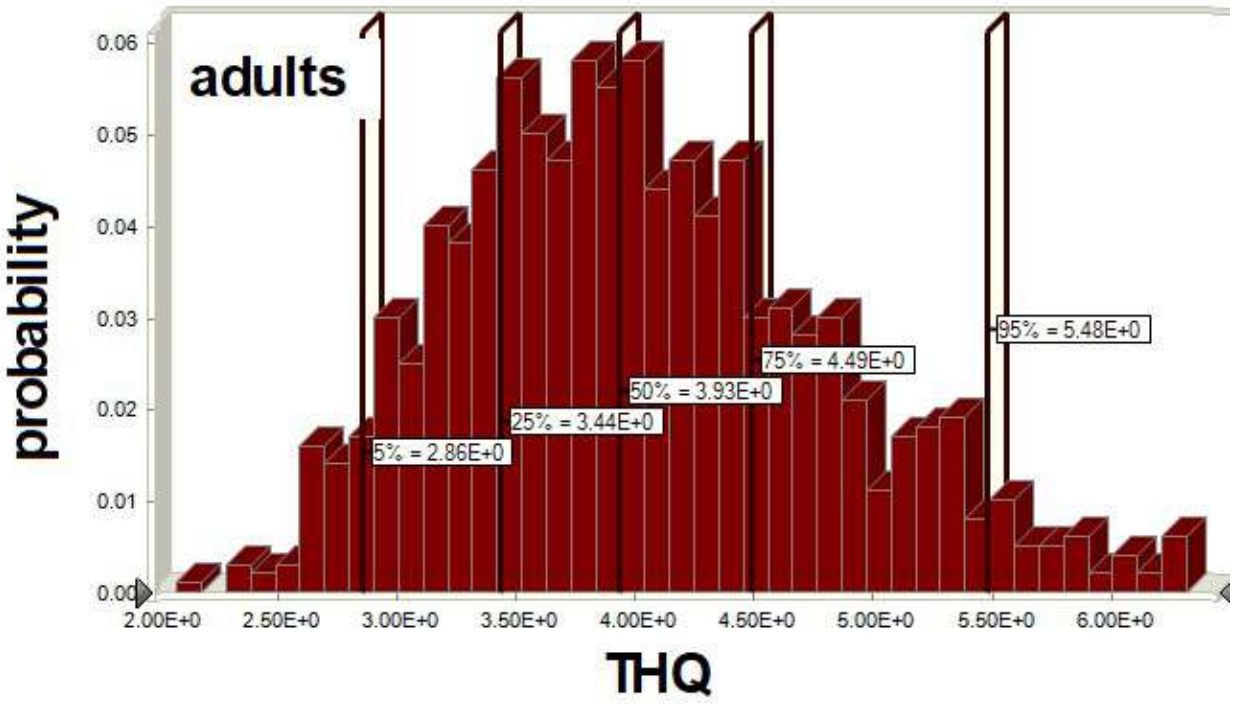
Table 6. Generalized linear model (GLM) for main effects and interactions in non-alcoholic malt beverages.

Source	Wald χ^2	df	p-value
Time	5.54	1	0.038
Brand	0.91	1	0.990
Size	1.08	1	0.790
Temperature	0.78	1	0.219
Time × Size	0.88	1	0.471
Time × Brand	1.00	1	0.606
Temperature × Time	0.99	1	0.320
Time × Brand × Temperature	1.08	2	0.584
Time × Brand × Temp × Size	1.87	2	0.171


adults
probability
0.06
0.05
0.04
0.03
0.02
0.01
0.00
5% = 5.96E-7
25% = 7.05E-7
50% = 7.88E-7
75% = 8.85E-7
95% = 1.05E-6
5.00E-7
6.00E-7
7.00E-7
8.00E-7
9.00E-7
1.00E-6
1.10E-6
1.20E-6
CDI


adolescents
probability
0.06
0.05
0.04
0.03
0.02
0.01
0.00
5% = 9.31E-7
25% = 1.09E-6
50% = 1.24E-6
75% = 1.40E-6
95% = 1.61E-6
8.00E-7
1.00E-6
1.20E-6
1.40E-6
1.60E-6
1.80E-6
CDI


children
probability
0.06
0.05
0.04
0.03
0.02
0.01
0.00
5% = 2.09E-6
25% = 2.47E-6
50% = 2.78E-6
75% = 3.11E-6
95% = 3.64E-6
2.00E-6
3.00E-6
4.00E-6
CDI


adults
probability
0.06
0.05
0.04
0.03
0.02
0.01
0.00
5% = 2.86E+0
25% = 3.44E+0
50% = 3.93E+0
75% = 4.49E+0
95% = 5.48E+0
2.00E+0
2.50E+0
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5.00E+0
5.50E+0
6.00E+0
THQ

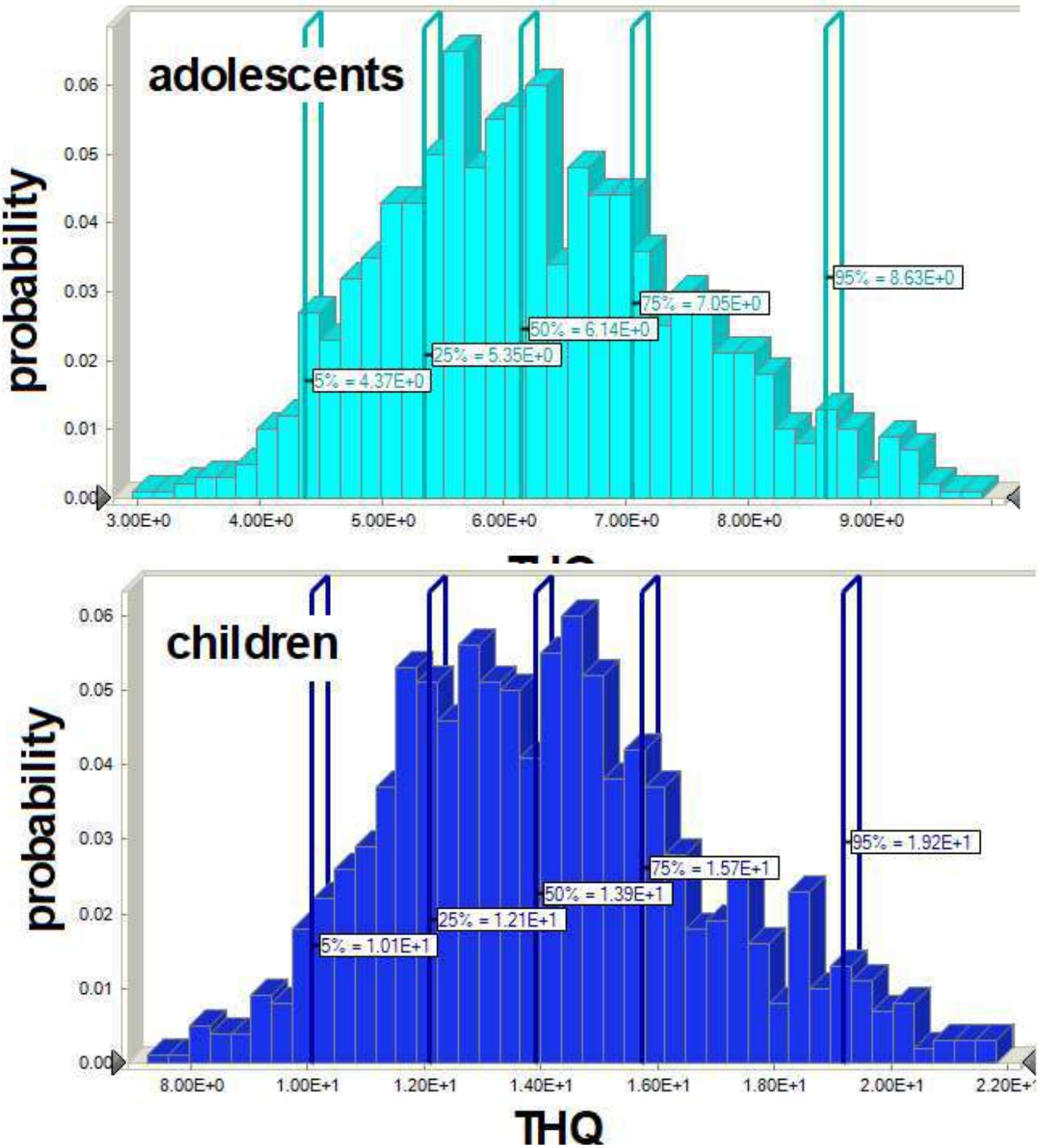


Figure 1. Values of chronic daily intake (CDI) and target hazard quotient (THQ) associated with BPA exposure through the consumption of non-alcoholic malt beverages

4. Discussion

4.1. Analytical method validation

The findings of the current research are consistent with and confirm those of previous studies. In a similar study, Zang et al. used procedure of MSPE (by graphene grafted magnetic microspheres) and a LOD (limit of detection) of 0.01 µg/L (26). In another study, Jiao et al. used adsorbent of MWCNT-MNP with GC-MS/MS and reported the LOD, RSD (%), recovery (%) and R^2 were 0.001 µg/L, lower than 10, 90.3–103.7, and 0.9988, respectively (17). In addition, Hazrati-Raziabad et al. used procedure of derivatization with GC-MS system and stated the recovery, determination coefficient (R^2), RSD, LOD, and LOQ were 99.8%, 0.991, 8.9%, 0.1 µg/kg, and 0.35 µg/kg, respectively (6).

4.2. BPA concentrations in all samples

The migration of BPA from can coatings is influenced by multiple reasons, coating thickness and uniformity, raw material quality, and even can washing during recycling cycles all influence the migration rate. Storage temperature is one of the most critical factors, significantly accelerating BPA migration. Research has shown that refrigerated storage can substantially reduce migration rates. Furthermore, the glass transition temperature (Tg) of epoxy resin (approximately 105 °C) represents a critical threshold for BPA migration. Above this temperature, the polymer structure undergoes a phase transition and the migration rate increases dramatically. Exposure to sunlight and prolonged storage duration are other factors that accelerate migration. The composition of the beverage matrix, particularly acidity (low pH) and ethanol content, also plays an important role in migration, with higher migration reported in acidic and ethanol-containing media. Physical damage such as denting or impact to cans can compromise coating integrity and create new pathways for migration. In the present study, samples were collected under controlled laboratory conditions following standard sampling procedures. However, variations across different brands, differences in internal coating quality, and transportation conditions prior to sampling are among the factors that may have influenced the final BPA concentrations. The lack of precise control over these variables should be considered a limitation of this study. Overall, the findings of this study are consistent with previous research showing that BPA migration is a function of temperature, time, pH, and coating quality (2, 14-16).

The migration of Bisphenol A from food packaging into beverages is significantly influenced by the physicochemical properties of the beverage matrix, and understanding these factors is essential for accurate interpretation of results and risk assessment. Among the most critical factors is the pH of the beverage; studies have demonstrated that acidic environments (particularly pH < 3) dramatically increase BPA migration, as hydrogen ions promote hydrolysis of ester bonds in the epoxy coating, accelerating polymer degradation . In the present study, non-alcoholic malt beverages typically had a pH in the range of 4–5, which is less aggressive toward the coating compared to highly acidic beverages such as cola or fruit juices (pH ~ 2.5–3); nevertheless, measurable BPA migration still occurs even at this pH range, and the measured concentrations are consistent with previous findings in similar beverage matrices. Ionic strength and the presence of soluble salts also play a significant role in BPA migration. Research has shown that increasing sodium chloride (NaCl) concentration in beverages

directly enhances BPA migration, and this effect is even more pronounced for divalent salts (such as $CaCl_2$ and $MgCl_2$) at very low concentrations (0.01%). This phenomenon can be attributed to the salting-out effect, which increases the thermodynamic activity of the analyte in the aqueous phase, driving BPA toward the liquid phase. In malt beverages, compounds such as sugars and malt extract may also influence migration. Studies have shown that increasing concentrations of simple sugars like glucose and sucrose enhance BPA migration, with significant differences observed at concentrations of 1–2% compared with distilled water. This is particularly relevant for malt beverages, which contain substantial amounts of carbohydrates. Other matrix components, including organic acids (such as citric acid and lactic acid, which are present during fermentation and malt production), proteins, and lipids, may also affect both the migration rate and the extraction efficiency of the MSPE/GC-MS method through molecular interactions with BPA. The presence of these compounds can cause matrix effects in chromatographic analysis, potentially influencing detector response; therefore, matrix-matched calibration or the use of an internal standard were employed in this study to compensate for these effects (2, 14-16).

It is important to note that the presence of BPA in non-alcoholic malt beverages is not exclusively attributable to migration from packaging materials. BPA is a ubiquitous environmental contaminant, and its occurrence has been documented in various water sources, including surface water and groundwater, at concentrations ranging from below detection limits to several micrograms per liter, depending on the region. Furthermore, BPA can enter the production chain through raw materials, as it has been detected in agricultural products and food ingredients. A comprehensive review on endocrine-disrupting chemicals in beverages confirmed that contamination can originate from raw materials, processing equipment, and environmental sources, in addition to food contact materials. Therefore, the BPA levels measured in our study reflect not only potential migration from PET and can packaging but also the cumulative contribution from water used in production and the malt-based ingredients themselves. This multi-source origin of BPA contamination has been similarly reported in other beverage matrices, including dairy products and soft drinks (2, 14-16).

The presence of bisphenol A (BPA) in non-alcoholic malt beverages at concentrations exceeding the EU limit can be attributed to several contributing factors. The main source is the migration of BPA from epoxy resin coatings applied to the inner surface of metal cans, which act as a protective barrier between the beverage and the metal container. Studies have shown that storage conditions significantly affect the migration rate; high temperatures, exposure to sunlight and long storage times significantly accelerate the release of BPA into the beverage. Other factors, including the low pH of the beverage, the quality and thickness of the can inner coating, the manufacturing processes and even the washing of the can during recycling cycles, can affect the migration rate of BPA. Given that the EU specific migration limit for BPA in food is very low, even relatively small migrations can lead to concentrations exceeding the regulatory threshold. This emphasizes the critical importance of strict quality

control during production and storage processes to ensure consumer safety and regulatory compliance (2, 14-16).

The findings from earlier studies on BPA concentrations are shown in Table 7. Variations among the reported values can likely be attributed to differences in the materials (raw) used, the types of packaging, the preparation methods, and the measurement procedures employed for this compound across various beverages (27-29).

4.3. BPA level in three brands of non-alcoholic malt beverages

This finding regarding different brands is noteworthy for several reasons:

First, the remarkable similarity in BPA concentrations across different brands suggests that industry-wide common factors—such as the standard type of epoxy coatings used, general production conditions, and common filling and packaging processes—exert a more dominant influence on BPA migration than brand-specific differences. In other words, despite brand diversity, they all employ similar technologies for internal coatings, resulting in comparable migration behavior. Second, this finding is consistent with previous studies that have demonstrated that the primary source of BPA in beverages is the internal coating, not the beverage's raw materials. Therefore, the similarity in BPA concentrations across brands can be attributed to the widespread use of epoxy coatings of comparable quality throughout the non-alcoholic malt beverage industry. Third, the absence of significant differences between brands weakens the hypothesis of background BPA contamination in raw materials (water, malt, additives); if raw materials were the primary source, greater variability in BPA concentrations across brands with different raw material suppliers would be expected. Fourth, from a regulatory and consumer health perspective, this finding is particularly important because it indicates that BPA contamination is not limited to a specific brand but is a widespread challenge across the entire industry. Therefore, regulatory and corrective solutions should not target any specific brand but should be pursued at the level of overarching industry policy and the mandatory adoption of BPA-free coatings across all brands. Fifth, the lack of significant difference between brands represents a methodological advantage for the present study, indicating that sampling from three different brands did not introduce considerable variability into the data, and the results are more generalizable. In other words, the measured BPA concentrations in this study are representative of the overall status of the non-alcoholic malt beverage industry in the studied region and do not merely reflect the conditions of a specific brand. In conclusion, while the absence of brand-to-brand variation may initially appear to contradict expectations, from both scientific and policy-making perspectives, this finding conveys a clear message: all brands operate under similar conditions, and integrated actions at the industry-wide level are urgently needed to reduce consumer exposure to BPA (1, 2, 6).

Table 7. Comparison of BPA levels with previous studies

Author	Year	Country	Food matrix	Analysis method	Outcome	Ref
Mastanjević et al.	2025	Croatia	Beer samples	QuEChERS/GC-MS	From 0.020 ± 0.003 to 0.087 ± 0.015 µg/L	(14)
Tsalbouris et al.	2021	Greece	Alcoholic and non-alcoholic beverages	Molecularly imprinted solid phase extraction (MISPE)-HPLC-DAD	From 0.011 to 0.041 µg/L.	(30)
Cao et al.	2010	Canada	Beer products and canned soft drink samples	SPE-Acetylation Derivatization-LLE followed by GC-MS	In beer products= ND to 0.54 µg/L and in soft drink samples= ND to 0.21 µg/L	(31)
Gallart-Ayala et al.	2011	Spain	Canned soft drink samples	On-line SPE/ LC-MS-MS	From ND to 0.607 µg/L	(32)
Li et al.	2013	China	Soft drink samples	SPE/GC-MS	From ND to 0.86 µg/L	(33)
Yazdinezhad et al.	2013	Spain	Soft drink samples	MSPE- LC-MS/MS	From 0.066 to 1.08 µg/L	(34)
Koti et al.	2025	Iran	Samples of soft drink	MSPE/GC-MS	Mean=1.83±1.16 (ranging from 0.45 to 5.10 µg/L).	(2)
Rezapour et al.	2025	Iran	Samples of doogh (Iranian yogurt-based drink)	MSPE/GC-MS	Mean=3.50 (ranging from 0.63 to 6.75 µg/L).	(16)
Tzatzarakis et al.	2017	Greece	Soft drink samples	SPE/ LC-MS	Mean=2.30±0.18 (0.40–10.2 µg/L)	(29)
Fasano et al.	2015	Italy	Beverages including non-carbonated and sugary carbonated samples	SPE/ HPLC-FLD and LC-MS-MS	Median=1.24 (< LOD–4.98 µg/L) in canned carbonated beverages and median= 0.18 (< LOD–1.78 µg/L) in non-canned carbonated beverages	(28)
Kumar et al.	2023	India	Canned soft drink and plastic-bottled water samples	Microextraction in packed syringe (MEPS)-GC-MS	BPA levels= 0.0082 -0.01401 µg/L in canned soft drink samples and BPA levels= 0.06-0.09 µg/L in water samples	(35)

4.4. BPA level in two packaging of malt (non-alcoholic) beverages

The variation in BPA contamination levels between different packaging of malt (non-alcoholic) beverages could be attributed to several reasons. One possible reason is that smaller packages, such as the 330 mL size, may have a higher surface area-to-volume ratio, leading to increased leaching of BPA from the packaging materials into the beverage. Additionally, the production and storage conditions for smaller packages might differ from those of larger packages, potentially affecting the stability of the packaging and its interaction with the contents. Furthermore, the type of materials used in different sizes could vary, with smaller packages possibly utilizing different coatings or plastics that may contain higher levels of BPA (6, 15, 16, 36).

4.5. BPA level at two temperatures of storage of non-alcoholic malt beverages

Although the difference in BPA levels between the two storage temperatures (7 °C and 27 °C) did not reach statistical significance ($p = 0.219$), a consistent upward trend was observed at the higher temperature. This suggests that storage temperature may influence the extent of BPA contamination in malt beverages, though the effect was not statistically significant in the present study. The lack of significance could be attributed to the limited sample size and the relatively short storage duration, and further studies with larger sample sizes are needed to confirm this trend (2, 6, 14, 15).

4.6. BPA levels at two times of storage in non-alcoholic malt beverages

The observed increase in BPA contamination levels in malt (non-alcoholic) beverage samples measured at the expiration date compared to those measured at the production date can be attributed to numerous reasons. Over storage time, the integrity of packaging materials may deteriorate due to exposure to light, heat, and other environmental conditions, leading to enhanced leaching of BPA into the beverage. Furthermore, chemical interactions between the beverage and the packaging may intensify as the product ages, further enhancing BPA migration. Furthermore, the storage conditions and duration can influence the stability of both the beverage and the packaging, potentially resulting in higher BPA levels as the product approaches its expiration date (6, 14-16).

4.7. Multifactorial analysis of BPA using generalized linear model

These findings indicate that while storage time alone significantly affects BPA migration, its effect is independent of temperature, brand, and package size. The absence of significant interactions suggests that the rate of BPA release over time remains consistent across different experimental conditions, and no synergistic or antagonistic effects exist among the studied factors under the tested conditions. Therefore, storage duration emerges as the primary and most reliable predictor of BPA migration, regardless of the packaging type or storage temperature.

4.8. Chronic daily intake (CDI) of BPA

Based on the findings of our article, the risk posed by Bisphenol A through the intake of malt beverages was found to be above the established safety thresholds (standard level of EU for TDI value of BPA= 2E-7 mg /kg bw/day). This suggests that daily exposure from malt beverage consumption exceeds the recommended safety limit. A comparison of the present findings with

existing literature reveals both consistency and differences in health risk. For example, a study on BPA in the sixth total diet study in China reported that the estimated dietary exposure of BPA for adults was 0.0181 mg/kg body weight per day (37).

The probabilistic risk assessment conducted in this study demonstrated substantial variability in population exposure levels, using Monte Carlo simulation to identify high-risk subgroups and support evidence-based regulatory decision-making. In 2023, the EFSA CEP Panel established a new TDI of 0.0002 µg/kg bw/day—approximately 20,000 times lower than the previous value. This revision was based on observed reductions in peripheral blood lymphocyte counts in a 90-day toxicity study, with an additional uncertainty factor applied to account for potential immunotoxicity and developmental neurotoxicity.

Our findings suggests that THQ values from malt beverage consumption exceed the recommended safety limit (THQ < 1), which points to a possible health concern in light of the EFSA's recommended limits.

A comprehensive study by Koti et al. (2) analyzing different soft drink samples in Iran reported that the non-carcinogenic effects pose a significant risk to consumers, which is directly correlated with our THQ findings (2).

A study conducted on various soft drink categories in Iran applied Monte Carlo Simulation (MCS) for non-carcinogenic risk assessment and concluded that BPA exposure presents a considerable threat to consumers, which aligns closely with our own THQ results. A distinct split in risk outcomes emerges depending on which reference value is applied. Investigations that rely on older, less stringent tolerance thresholds tend to conclude that exposure is safe (THQ < 1). Risk conclusions vary significantly based on the selected reference value. Research that uses older, higher permissible limits generally finds the exposure to be safe (THQ < 1).

These represent a significant public health concern, particularly given that BPA is an endocrine-disrupting chemical (EDC) with documented effects on reproductive, metabolic, immunological, and neurodevelopmental systems at very low doses.

5. Conclusion

The main goal of this study was to measure BPA levels in non-alcoholic malt beverages from Iran (Tehran), by the procedure of MSPE/GC-MS. Our results revealed that the levels of BPA detected in non-alcoholic malt beverages exceeded the permissible limit. Among the various brands of non-alcoholic malt beverages tested, the average BPA contamination levels followed the sequence A < B < C. Among the various volumes of non-alcoholic malt beverages tested, the average BPA contamination levels followed the sequence 330 mL (Can) > 1000 mL (PET). Among the various storage temperatures tested for non-alcoholic malt beverages, the average BPA contamination levels followed the sequence 27°C > 7°C. By storage time, the mean order was expiration date > production date. Our research shows that consumption of malt (non-alcoholic) beverages carries a significant health risk. The long-term health effects of ongoing BPA exposure through regular dietary intake also remain a concern. Therefore, these findings emphasize the importance of: (1) regular monitoring of BPA levels in food products, (2) considering combined exposure from multiple dietary and environmental sources, (3) strengthening

regulations, and (4) developing BPA-free packaging alternatives. A key limitation of this study was the restricted geographical scope of sampling, which may affect the generalizability of the findings. Subsequent investigations ought to broaden the scope of sampling, integrate exposure evaluations across multiple pathways, and include human biomonitoring in order to more effectively capture cumulative risks within this susceptible group.

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Author contributions

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Declaration of competing interest

The authors declare that they have no competing interests.

Date Availability

Data is provided within the manuscript.

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