Measurement of Phthalate Acid Esters in Non-alcoholic Malt Beverages by MSPE-GC/MS Method in Tehran City: With the Approach of Chemometric

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Abstract

The goal of this research was to assess the phthalic acid esters (DEP, DMP, BBP, DBP, DEHP, DnOP, and total PAE) in non-alcoholic malt beverages bottled by multi-walled carbon nanotubes were magnetized with iron (MWCNT-Fe$_3$O$_4$) using Mass spectrometry is gas chromatography (GC-MS) in Tehran.

The results showed that the mean of DEHP (which has also been found to be carcinogenic) in all samples was lower than the standard levels (5944.73 ng/l). The maximum concentration of DEHP in four samples was upper than the standard levels (8957.87 ng/l). Also, scanning electron microscopy (SEM), scattered energy X-ray (EDX) and X-ray diffraction (XRD) analysis were used to describe the generated MWCNT-Fe$_3$O$_4$. Multivariate techniques and heat-map visualization were used to assess the correlation among the type and levels of PAEs with brand, color, product date, pH, sugar, volume and gas pressure. Therefore, based on heat-map and principal component analysis (PCA) results, the Bis (2-ethyl hexyl) phthalate (DEHP) and total PAEs were the closest accessions, indicating that these variables had similar trends. Based on the results, it can be stated that due to the low average of total phthalate esters in non-alcoholic malt beverages, there is no serious health hazard of these compounds for humans.

1. Introduction

Large amounts of phthalic acid esters (PAEs) are used annually to produce a wide variety of common consumer goods around the world (Arfaeinia et al. 2020, Harunarashid et al. 2017). PAEs are used mainly as plasticizers to increase durability, workability, and flexibility of polymeric materials in polyethylene terephthalate (PET) packaging or other plastic packaging. But, in addition, they can be present in many products such as inks, paints, cosmetics and adhesives. (Rafiei Nazari et al. 2018). These compounds bind to polymers through frail subaltern molecular interactions with the chains of polymer. Also, PAEs have employed in physically attached to the polymer, so easily released from the products and then transported to the environment (with migration way), and finally can cause contaminate air, soil, water, and food products (Dobaradaran et al. 2020, Jarošová 2006, Kouhpayeh et al. 2017). In recent years, food contamination with PAEs has become a problem of public concern. PAEs have been reported in beverages such as alcoholic and non-alcoholic malt beverages, soft drinks, meat and meat products, fruits, vegetables, dairy products, cereals, vegetable edible oils and other food products (Kiani et al. 2018, Liu et al. 2020). PAEs are also widespread pollutants in the environment and appear to be the cause of cancer in humans and animals, as well as endocrine disorders. The USEPA (Environmental Protection Agency of USA) has listed PAEs as the important contaminant (Jarošová 2006, Moazzen et al. 2019, Shen et al. 2019. The most important PAEs include bis(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DnOP), butylbenzyl phthalate (BBP), dimethyl phthalate (DMP), diethyl phthalate (DEP) and dibutyl phthalate (DBP). DEHP is one of the most important these compounds, which causes liver cancer in rodents (Liu et al. 2020, Sinha et al. 2019). DBP, BBP, DEHP and some phthalate esters metabolites in animals have shown teratogenic effects. The phthalates are involved in the pathogenesis of asthma, allergic symptoms, contribute to airway remodeling and the premature puberty in young girls (Harunarashid et al. 2017, Kiani et al. 2018).

Several analytical techniques have been developed to detect PAE in various samples packaged in plastics (especially PET), such as drinking water, beverages, and cosmetics. High-performance liquid chromatography (HPLC), Gas chromatography (GC) and GC combined with mass spectrometry (GC-MS) were commonly used (Dobaradaran et al. 2020, Moazzen et al. 2019, Wu et al. 2014). Numerous preparation methods can be used to measure these compounds in different matrices, including single-drop microextraction (SDME), liquid–liquid extraction (LLE), solvent extraction (SE), liquid-phase microextraction (LPME), LPME method based on the solidification of a floating organic micro drop (LPME-SFO), accelerated solvent extraction (ASE), stir-bar sorptive extraction (SBSE), solid-phase extraction (SPE) and column chromatographic cleanup (CC), but these methods are expensive, insensitive and time-consuming (Kiani et al. 2019, Roudbari et al. 2020, Shariatifar et al. 2020). Recently, a different type for solid-phase extraction (SPE), that called MSPE or magnetic solid-phase extraction has been established (this method based on the using of magnetic adsorbents). Magnetic adsorbents compared with non-magnetic adsorbents, can make separation process faster and easier without any additional procedures such as filtration or centrifugation. The MSPE method can prevent the time-consuming column operations seen in SPE (Kouhpayeh et al. 2017, Moazzen et al. 2018, Shen et al. 2019). Principal component analysis is one of the most important statistical technique used to characterize the interrelationships between variables and visualization of the data patterns (Ghelichkhani et al. 2019, Samiee et al. 2020). Heat-map used to represent the similar or vastly different expression status characteristics values (Arabameri et al. 2019b, Heydarieh et al. 2020). Considering the high consumption of these products in the daily diet, it is necessary to investigate the PAEs contaminants in alcoholic malt beverages, since so far, no research has been performed on PAEs contaminants in non-alcoholic malt beverages in Iran. Therefore, this research was conducted to investigate of PAEs in non-alcoholic malt beverages based on MSPE-GC/MS method by using MWCNT-Fe$_3$O$_4$ as magnetic adsorbent offered in Tehran, Iran. Also the PCA was applied to characterize the interrelationships between variables and visualization of the data patterns and Heat-map was applied to represent similar or vastly different expression characteristics values.
2. Materials And Methods

2.1. Reagents and Chemicals

Phthalate acid esters (DEHP, BBP, DBP, DEP, DMP and DNOP) and other used chemicals including ethanol, methanol, NaCl and n-hexane were obtained from Sigma-Aldrich Company (St. Louis, MO). FeCl3.6H2O, Benzyl benzoate (internal standard (IS)), NaBH4, HCl, NaOH and 3,5-dinitrosalicylic acid (DNSA) were purchased from Merck Company (Germany). The diameter and length of multi walled carbon nanotube (MWCNTs, Panchkula, India) were 30–60 nm and 5.0–30 mm, respectively. The grade of all the other used chemical and solvents were analytical reagent. The stock solution of analyzed contaminant was ready in methanol (100 mg/mL). Afterward, the solutions of phthalate acid esters working standard were ready by consecutive dilutions of the stock solution with distilled water -methanol (50: 50 v/ v). To prepare I.S., benzyl benzoate (10 mg) was dissolved in ethanol (10 mL), and at the end for preparing samples, 100 µL of prepared I.S. was added. On the same day of the study, the QC (quality control) were prepared from diluted solutions of stock standard. The prepared solutions were retained at 4°C and in the dark place, until analysis. In this research, all the laboratory glass dishes (before using) were washed with an Al2O3 solution, and then they were immersed in acetone for 40min, and were washed with n-hexane as well as finally were dried (in the oven) for 5 h at 150°C.

2.2. Determination of sugar

In the first, five samples with different dilutions were prepared from sucrose stock solution (1000 mg/dL) as standard. Afterward, 10mL of non-alcoholic malt beverages samples diluted with distilled water up to the 100 mL as samples. Insert 2 mL of each standard solution into a test tube and insert two mL of distilled water into a separate test tube (blank solution). Then, two mL of HCl hydrochloric acid (six M) solution was added and placed in Bain-marie for 10min and then 8 mL: of 2.5 M NaOH solution was added to neutralize. Afterward, add two mL of 0.05 M 3,5-dinitrosalicylic acid solution and cover the test tube with cover (film) and shake well to mix. Then was placed in Bain-marie for five min followed by so cold water for ten min (The time between 3,5-dinitrosalicylic acid solution addition and measurements should be same for all test solutions.). Finally, the absorbance of the five standards and references at 580 nm was measured using UV/Vis Spectrophotometer( Roig &Thomas 2003).

2.3. Gas measurement

The gas pressure of the non-alcoholic malt beverages samples were determined using a digital gas meter at room temperature set at 25°C.

2.4. The pH measurement

The pH of the non-alcoholic malt beverages samples were determined using a digital pH meter at room temperature and pressure of 1atm(Godwill et al. 2015).

2.5. Preparation of adsorbent

Magnetic adsorbents (MWCNTS-Fe3O4) were prepared according our previous study (Kiani et al. 2018, Kouhpayeh et al. 2017, Moazzen et al. 2018).

2.6. Evaluate the properties of the prepared adsorbent

Phase identification of adsorbent was assessed by XRD (X-ray Diffraction) and characterization of elemental was assessed by EDX (energy dispersive X-ray) and morphological analysis was assessed by SEM (scanning electron microscope) (Moazzen et al. 2019). The models of our devices were SEM: PHILIPS, and S360 Mv2300, EDX: PHILIPS, S360, and Mv2300 and XRD: Philips, X’PertPro 2002.

2.7. Sampling, sample preparation, and instrumentation

In the beginning, 120 non-alcoholic malt beverages samples were bought with five brand of most commonly used from chain store in Tehran, Iran and all samples were degassed (with a bath of ultrasonic) at temperature of room for 20 minute. Afterward, 10mg of prepared adsorbent (MWCNT-Fe3O4) were activated with solutions of methanol and water. To the ten milliliter of each degassed non-alcoholic malt beverage samples, 10 mg the activated adsorbent, 0.5 gr of NaCl, and 100 µL I.S. were added. The mixture was mixed strongly (to extract the contaminant compounds) for four minute, after that adsorbent was gathered (with an exterior magnet) to the side of the laboratory dish (within 90 seconds) and the other compounds in the mixture were discarded. Then, 2 mL of n-hexane was added to the adsorbent and was mixed with blender (2 min) vigorously, to elute contaminant compounds (PAEs) from the adsorbent. After that, by using an external magnet, the adsorbent was collected to the side of the laboratory dish, and the supernatant was moved to a vial. Desorption solvent was
dehydration with a mild stream of \( \text{N}_2 \) gas (at room temperature) and was maintained in the cold place such as refrigerator. Finally, the dehydrated contents of the previous step was dissolved in one mL solvent (n-hexane), and one µL of the mentioned solution was injected into the gas chromatography (Agilent 7890 N) – mass spectrometry (5975) device. The column of chromatographic was DB-5– J & W Scientific (30m, 250µm, 0.5µm). Helium (He) was selected as the gas of carrier at one mL/min (ratio of split of 50:1). Splitless was the mode of injection with an inlet temperature of 290-centigrade degree. The program of GC-MS temperature was: 80-centigrade degree, retained for two minute, 80–285 centigrade degree at seven °C/min, retained for ten minute. For the quantitative determination of PAEs compounds, the mode of selective ion monitoring was used. The device outputs (the retention times (RT), quantitative and qualitative ions) of six phthalate acid esters and I.S. are presented in Table 1.

| Group of Ions | Compounds                        | RT (minute) | Quantitative ions (abundance) (m/z) | Qualitative ions (abundance) (m/z) |
|--------------|----------------------------------|-------------|-------------------------------------|------------------------------------|
| 1            | Di methyl phthalate (DMP)        | 12.8–13.2   | 163                                 | 76, 134, 162, 195                  |
| 2            | Di ethyl phthalate (DEP)         | 14.2–14.8   | 149                                 | 121, 149, 177, 222                 |
| 3            | Di butyl phthalate (DBP)         | 20.1–20.4   | 149                                 | 121, 149, 205, 223                 |
| 4            | Butyl benzyl phthalate (BBP)     | 25.2–25.6   | 149                                 | 91, 149, 206, 238                  |
| 5            | Benzyl benzoate (IS)             | 26.1–26.4   | 105                                 | 212 (40), 194 (35)                 |
| 6            | Bis(2-ethyl hexyl) phthalate (DEHP) | 27.1–27.6   | 149                                 | 113, 149, 167, 279                 |
| 7            | Di-n-octyl phthalate (DNOP)      | 29.3–29.5   | 149                                 | 149, 179, 261, 279                 |

2.8. Optimization of method of the extraction process

The optimization of method was performed according to the 1 factor at a time method. (Kiani et al. 2018, Moazzen et al. 2019).

2.9. Validation of method

The validation of method was performed based on the currently established Guideline of FDA (U.S. Food and Drug Administration) for industries (Kouhpayeh et al. 2017, Moazzen et al. 2018).

2.10. Statistical analysis

The outcomes were statistically analyzed by SPSS version 18 (SPSS Inc, Chicago, IL) for Windows. Data analysis done by test of Kolmogorov–Smirnov and tests of Kruskal-Wallis. Statistical significance was a p-value of <0.05. For a better understanding of the most significant contribution to distribution PAEs levels among the different samples, the PCA was done by the software of SPSS (Arabameri et al. 2019a, Heydarieh et al. 2020). Multivariate techniques were used to assess the correlation between the amount and type of PAEs levels with properties samples. Heat-map analysis was used to analyze the correlation between samples online at https://biit.cs.ut.ee/clustvis/.

3. Results And Discussion

3.1. Quantitative analysis

To draw the calibration curves were prepared five different concentration levels of phthalate acid esters (DEHP, DBP, DEP, DNOP, BBP and DMP) at the range of 10 to 12000 ng/L (including 10, 100, 1000 and 5000 ng/L). The correlation coefficient ranged from 0.9979 to 0.9997 and the LODs (detections limit) and LOQs (quantifications limit) for the target analytes were 13 to 30 ng/L and 39 to 90 ng/L, respectively (Table 2). The recovery values of the 6 PAEs were 94.2–104.3% with the RSDs less than 7.6%. To control the quality three levels (50, 500 and 5000 ng/L) from mix of PAEs were prepared and were analyzed duplicate in several days. The inter- and intra- day precision measured for three consecutive days in triplicate analyzes and they were lower than 7.8% and 8%, respectively (Table 3). The selectivity of method was examined by analyzing 25 non-alcoholic malt beverages.
Table 2
The linear range, LOD, LOQ and coefficient of estimation of the developed MSPE technique for measurement of phthalate acid esters

| Target compound                  | Linear range (ng/L) | Detections limit (LOD) (ng/L) | Quantifications limit (LOQ) (ng/L) | Coefficient of estimation $r^2$ |
|----------------------------------|---------------------|------------------------------|-----------------------------------|-------------------------------|
| Di methyl phthalate (DMP)        | 10–12000            | 23                           | 69                                | 0.9979                        |
| Di ethyl phthalate (DEP)         | 10–12000            | 15                           | 45                                | 0.9984                        |
| Di butyl phthalate (DBP)         | 10–12000            | 13                           | 39                                | 0.9991                        |
| Butyl benzyl phthalate (BBP)     | 10–12000            | 30                           | 90                                | 0.9990                        |
| Di-n-octyl phthalate (DNOP)      | 10–12000            | 18                           | 54                                | 0.9988                        |
| Bis(2-ethyl hexyl) phthalate (DEHP) | 10–12000            | 26                           | 78                                | 0.9997                        |

Table 3
Estimated recoveries, precisions and accuracies for determination of the PAEs compounds at three different concentrations (n = 6) in QC samples.

| Target compound                  | Sample | Nominal concentration (ng/L) | Mean of calculated concentration (ng/L) | RSD(%) of calculated concentration (Intraday) | RSD(%) of calculated concentration (Interday) | RE(%) of calculated concentration | Estimated recoveries (%) | RSD(%) of calculated recovery |
|----------------------------------|--------|------------------------------|----------------------------------------|-----------------------------------------------|-----------------------------------------------|----------------------------------|--------------------------|-------------------------------|
| Di methyl phthalate (DMP)        | QCI    | 50                           | 52                                     | 6.4                                          | 7.3                                          | 4                                | 95.3                     | 7.2                           |
|                                  | QCII   | 500                          | 503                                    | 7.1                                          | 6.6                                          | 0.6                              | 96.0                     | 6.4                           |
|                                  | QCIII  | 5000                         | 5050                                   | 7.8                                          | 7.9                                          | 1                                | 97.3                     | 7.0                           |
| Di ethyl phthalate (DEP)         | QCI    | 50                           | 47                                     | 5.3                                          | 7.1                                          | -6                               | 99.7                     | 6.9                           |
|                                  | QCII   | 500                          | 510                                    | 6.4                                          | 8.0                                          | 2                                | 96.3                     | 7.6                           |
|                                  | QCIII  | 5000                         | 5100                                   | 6.2                                          | 5.8                                          | 2                                | 94.2                     | 5.7                           |
| Di butyl phthalate (DBP)         | QCI    | 50                           | 53                                     | 6.3                                          | 7.2                                          | 6                                | 98.4                     | 7.0                           |
|                                  | QCII   | 500                          | 505                                    | 6.8                                          | 6.7                                          | 1                                | 98.4                     | 5.6                           |
|                                  | QCIII  | 5000                         | 5045                                   | 7.0                                          | 6.9                                          | 0.9                              | 99.8                     | 6.3                           |
| Butyl benzyl phthalate (BBP)     | QCI    | 50                           | 56                                     | 5.6                                          | 5.9                                          | 12                               | 104.3                    | 5.8                           |
|                                  | QCII   | 500                          | 512                                    | 6.4                                          | 7.7                                          | 2.4                              | 98.3                     | 7.2                           |
|                                  | QCIII  | 5000                         | 5039                                   | 6.5                                          | 7.0                                          | 0.78                             | 98.6                     | 6.0                           |
| Di-n-octyl phthalate (DNOP)      | QCI    | 50                           | 56                                     | 5.2                                          | 6.3                                          | 12                               | 98.2                     | 5.7                           |
|                                  | QCII   | 500                          | 504                                    | 7.3                                          | 7.2                                          | 0.8                              | 98.9                     | 6.9                           |
|                                  | QCIII  | 5000                         | 5105                                   | 6.6                                          | 5.9                                          | 2.1                              | 97.1                     | 5.8                           |
| Bis(2-ethyl hexyl) phthalate (DEHP) | QCI    | 50                           | 49                                     | 6.9                                          | 7.8                                          | -2                               | 101.3                    | 7.0                           |
|                                  | QCII   | 500                          | 515                                    | 6.0                                          | 6.7                                          | 3                                | 98.8                     | 5.6                           |
|                                  | QCIII  | 5000                         | 5070                                   | 6.8                                          | 7.3                                          | 1.4                              | 98.4                     | 6.9                           |

3.3. Images of SEM and analysis of EDX

The SEM image and EDX analysis of the magnetic adsorbents are presented in Figs. 1 and 2. The graphs show that the placement of magnetic particle on the surface of multi wallet carbon nanotubes is comparatively uniform and after filling with magnetic particles, the surface of the multi wallet carbon nanotubes became rougher (Fig. 1). Besides, there's no significant modification is detected in the surface of construction of the magnetic adsorbents after extraction procedure. A growth in adsorbent diameter is apparent. In addition, we
concluded that the adsorbent exhibited a chain-like morphology without apparent collection. This conducts to a high ability of adsorption of the adsorbent.

The chemical compounds of the prepared adsorbent were assessed by using EDX analysis. The spectrum of EDX displayed oxygen (O), iron (Fe) and carbon (C). The atomic C, Fe, and O ratio (64.6, 22.7 and 12.7, respectively) as the principal elements in the structure of prepared adsorbent confirmed the quantitative representation of the existence of Fe$_3$O$_4$ nanoparticles on the MWCNT surface (Fig. 2).

3.4. XRD images

By XRD analysis, the construction of multi wallet carbon nanotubes and MWCNT-Fe$_3$O$_4$ composites were more confirmed. The patterns of XRD of adsorbent were exhibited in Fig. 3. The strong diffraction peaks at $2\theta = 31.77^\circ$ and $2\theta = 45.52^\circ$ were shown MWCNTs and Fe$_3$O$_4$, respectively. The get XRD outcomes displayed that the Fe$_3$O$_4$ were efficaciously coated on the surface and texture of multi wallet carbon nanotubes using a co-precipitation technique.

3.5. Evaluation of phthalate esters in non-alcoholic malt beverages bottled in PET bottle

Mean concentrations and other statistical analyses of PAEs in all samples were shown in Table 4. There was a significant difference between the groups in terms of phthalate esters ($P < 0.05$). According to Table 4 from the research mean of all compounds in all the samples were less than the standard defined by the EPA (6000 ng/L) and WHO-EU (8000 ng/L) in drinking water, but maximum of DEHP in samples (4 samples) was upper than the EPA and WHO-EU standards (8957.87 ng/L).

| Target compound | mean  | SD    | min  | max  |
|-----------------|-------|-------|------|------|
| DMP             | 18.31 | 8.75  | 8.6  | 98.1 |
| DEP             | 151.33| 31.73 | 74.83| 268.9|
| DBP             | 496.73| 108.99| 300.76| 803.21|
| BBP             | nd    | --    | nd   | nd   |
| DEHP            | 5944.73| 2518.14| 1897.12| 8957.87|
| DnOP            | 46.16 | 13.24 | 15.43| 97.13|
| Total           | 6657.28| 1600.9| 2412.5| 9483.93|

The mean value and range of pH in samples was 3.3 (3.18–3.53) and sugar (per 100g) was 8.7 (4.4–11.9) and gas pressure was 1140 (830–1398) millimeters of mercury (mmHg). There was a significant difference between the groups in terms of sugar content and gas pressure ($P < 0.05$).

Moazzen, M et al. (2018) showed the highest concentration of PAEs in carbonated soft drinks was DEHP, that it was upper than the standard level (STL) in the 4 samples (14,008, 9301.6, 9201 and 6766.6 ng/L) and other PAEs compounds were lower than the STL, which was somewhat similar to our study (Moazzen et al. 2018).

Xu, X et al. (2019) showed 3 PAEs compounds (DBP, DMP and DEP) were measured in ten common brands of bottled water (Made of PET) in Beijing (China), ranging from 101.97 µg/kg to 709.87 µg/kg (Xu et al. 2020).

Vincenzo Russo, M et al in 2014 with the determination of PAEs in soft drinks and alcoholic drinks (light) showed that DEHP (3.6–101 ng/mL), DBP (1.9–4.4 ng/mL), DiBP (0.2–2.5 ng/mL), DEP (0.1–1.0 ng/mL) and BBP (0.08–0.8 ng/mL), are present in all samples, while iBcEP (0.08 ng/mL) and DMP (1.9 ng/mL) are present only in 1 beer sample, that was higher than compare our study (Russo et al. 2014).

Rafiei Nazari, R et al. (2017) with determine the migration modelling of PAEs from non-alcoholic beer bottles showed that storage duration increased and temperature resulted in an increase in migration level ranging from 0.6 µg/L to 2.9 µg/L, which was lower than our study (Rafiei Nazari et al. 2018).
Carnol, L et al. (2017) identified 6 PAEs of Luxembourgish beer stored in various containers (aluminum, glass and can bottle) and total PAEs were found in all samples at levels of 61.56 µg / L, which was upper than the present study (Carnol et al. 2017).

Victor E. Balderas-Hernández et al. (2020) identified PAEs in tequila beverage and showed that 22% of samples (65 samples) lacked PAE. DINP (1.64–3.43 mg/kg), BBP (0.05–2.91 mg/kg) and DEP (0.13–0.27 mg/kg) were found in 5 (1.69%), 37 (12.54%) and 11 (3.73%) samples, respectively. However, these levels weren’t higher than the maximum standard level of PAEs for alcoholic beverages. DEHP (0.03–4.64 mg/kg) and DBP (0.01–2.20 mg/kg) were found in 224 (75.93%) and 96 (32.54%) samples, from them just 15 (5.08%) and 10 (3.39%) samples, respectively, exceeded the maximum standard levels for alcoholic drinks. Bis(2-ethylhexyl) phthalate was the most repetitious PAEs detected in tequila and detected concentrations of DEHP were two-times higher in ultra-aged tequilas compared to those in white tequilas (Balderas-Hernández et al. 2020).

Yang, JF et al. (2016) measured the PAEs in non-alcoholic drinks bottled in PET from China and showed Bis(2-ethylhexyl) phthalate contained the highest median and mean contents (0.62 ng/g and 1.60 ng/g), followed by DBP (0.27 ng/g and 1.34 ng/g). (Yang et al. 2017).

Rodríguez-Ramos, R et al (2020) by evaluating plastic migrants in non-alcoholic and alcoholic drinks showed that there are 4 PAEs in the range of 0.14–1.1 µg / L in some beers, 1 PAE in the range of 1.2–1.5 µg / L in 3 grape juices and 6 PAE in several cider samples, in the range of 0.3–2.1 µg / L, which was less than the present study (Rodríguez-Ramos et al. 2020).

Wu ,PG et al. (2014) with determination of PAEs in non-alcoholic beverages showed a wide variety of PAEs contents was detected in 48 non-alcoholic beverages. Bis(2-ethylhexyl) phthalate was the most abundant PAEs compound followed by DOP, DPP and DBP. Bis(2-ethylhexyl) phthalate was detected in fruit juice samples (0.022–0.126 mg/L), sport beverages (0.015–0.098 mg/L), coffee (0.028–0.159 mg/L) and tea (0.016–0.123 mg/L), that was higher than our study (Wu et al. 2014).

March, JG et al. (2015) showed low-alcohol beer had DEHP 0.4 ± 0.2 and DBP 2.2 ± 0.4 µg/L, that DEHP was lower than our study and DBP was higher our study (March &Cerdà 2015).

Heinemeyer,G et al. (2013) showed that the DEHP in Beer (alcoholic) was 0.022 and in nonalcoholic Beverages was 0.020 µg/g (Heinemeyer et al. 2013).

Wang, F et al. (2017) showed DEHP in alcoholic beverages (liquor) was ranged from 0.6182 to 1.0890 µg/mL, that was higher than our study (Wang et al. 2017).

Pang, YH et al (2019) showed DEHP in alcoholic carbonated beverage and beer was not found, that was lower than our study (Pang et al. 2020).

The data comparison of this research with other articles shows differences that can be due to reasons such as: alcoholic or non-alcoholic beverage, use of plastic or other containers, contamination of raw materials or secondary contaminants during the production process, carbonated or non-carbonated beverage and also the amount of gas in carbonated beverage (pH), duration Keep in the package as well as the amount of other ingredients in the drink.

### 3.6. Structural relationship of parameters

Multivariate techniques and heat-map visualization were applied to evaluate the correlation between the type and levels of PAEs with brand, color, product date, pH, sugar, volume and gas pressure. Consequently, based on heat-map and PCA results, the Bis(2-ethyl hexyl) phthalate (DEHP) and total PAEs were the closest accessions, indicating that this variables had similar trends. Heat-map clustered the 40 non-alcoholic malt beverages using correlation distance and average linkage, reflecting similarities and relationship among the type and levels of PAEs samples. The heat-map clearly grouped samples into two major clusters and two sub clusters (Fig. 4). First cluster includes DEHP and total PAEs, Second cluster contains two sub-groups with brand, color, product date, pH, sugar, volume, gas pressure, DMP, DEP, DBP, BBP and DNOP.

Quantitative results obtained for the type and levels of PAEs were used to PCA to investigate the most significant contribution among non-alcoholic malt beverages. The compounds included samples brand, color, product date, pH, sugar, volume, gas pressure and levels of PAEs (DEHP, DMP, DNOP, BBP, DBP and DEP).

The dependence relations between different PAEs can be found from Fig. 4, the graph subset illustrates a visual representation about the relations among different PAEs. The nearest neighbor among the properties, the greater significant relationship existed among dependent
As shown in Fig. 5, the first five principal components accounted for 77.86% of the data variance in all samples, and their contribution rates were 26.80%, 15.87%, 14.27%, 11.46% and 9.43%, respectively. The DEHP and total PAEs were the closest accessions, indicating which these variables had similar trends. The Total phthalate, DEP, DBP, BEHP had a high positive correlation with PC1, while had negative correlation with sugar content and gas pressure. The results showed that the sugar, pH, brand had a positive correlation with PC2, while had negative correlation with gas pressure and DOP.

4. Conclusion

In first magnetic adsorbent was ready using a simple, sensitive and cost-effective method for measuring 6 PAEs (DEHP, DMP, BBP, DnOP, DEP and DBP) from non-alcoholic malt beverages. Moreover, SEM, EDX, XRD, VSM and TEM analysis were used to describe the generated MWCNT-Fe3O4. Analysis of non-alcoholic malt beverage samples evaluated in Tehran and showed that the concentration of none of the PAEs released from the bottles was upper than the standard levels (6 µg/L by USEPA). The correlation analysis focuses on the numerical relationship among types and levels of PAEs in all samples. Comparing relationships between PAEs levels from different non-alcoholic malt beverages showed that the Bis (2-ethyl hexyl) phthalate (DEHP) and total PAEs were the closest accessions. Furthermore, the results of heat-map visualization clearly indicated that relationship between PAEs levels under different samples was properly diagnosed. Based on the results, it can be stated that due to the low average of total phthalate esters in non-alcoholic malt beverages, there is no serious health hazard of these compounds for humans.

Declarations

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Competing interests

The authors have no conflicts of interest to declare in this research.

Ethical Approval

“This study does not involve any human or animal testing” or “This study was approved by the School of Public Health of Tehran University of Medical Sciences.

Consent to participate

All authors participated in this work.

Consent to publish

All authors agree to publish.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author’s contributions

Nabi Shariatifar, Gholamreza Jahed: Conceptualization, Supervision, Design of study, Writing- Reviewing and Editing. Hana Rezaei and Mojtaba Moazzen: Data curation, Writing- Original draft preparation. Mohammad Hadi Dehghani: Visualization, Investigation. Mahsa Alikord: Software, Methodology. Majd Arabameri: Software, Validation:

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Figures
Figure 1
SEM of magnetic adsorbent (A: MWCNTs and B: MWCNTs-Fe3O4)

Figure 2
EDX of magnetic MWCNTs/Fe3O4
Figure 3

The XRD image of MWCNTs/Fe3O4
Figure 4

Heat map of PAEs in non-alcoholic malt beverages
Figure 5

Principal component analysis plot of PAEs in non-alcoholic malt beverages samples