Turkish Journal of Agriculture - Food Science and Technology, 12(4): 666-674, 2024
DOI: https://doi.org/10.24925/turjaf.v12i4.666-674.6744

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Investigation and Quantification of Phthalate Esters in Packaged Milk: A Study in Türkiye

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A R T I C L E I N F O

Research Article

Received : 29.02.2024
Accepted : 03.04.2024

Keywords:
Dispersive extraction
Milk
Migration
Phthalate esters
Benzyl butyl phthalate

A B S T R A C T

The aim of this study was to explore the concentrations of PAEs (Phthalate esters) in milk in Türkiye. For this purpose, a methodology was developed to quantify eight different PAEs in milk samples using a combination of dispersive solid-phase extraction (dSPE) and Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS). Employing this methodology, the concentrations of PAEs were evaluated in 34 milk samples. Results indicated the presence of PAEs in the milk samples; however, all tested compounds remained within the specific migration limits established by the EU. Among the analyzed PAEs, BBP (Benzyl butyl phthalate) was not detected in any samples, while DMP (di-methyl phthalate) (ND-5.51 µg/L) and DBP (di-butyl phthalate) (ND-7.91 µg/L) exhibited the lowest concentrations. DEHP (bis(2-ethylhexyl)) was identified as the most prevalent plasticizer with a maximum concentration of 41.31 µg/L. In conclusion, this study successfully investigated PAE concentrations in Turkish milk samples using a developed methodology. The results indicated the presence of PAEs within EU-established limits, with DEHP being the predominant plasticizer. Further research and monitoring efforts are crucial to ensure ongoing safety in packaged milk products.

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Introduction

Adequate and balanced nutrition is essential for the growth and development of the newborn and its protection from both communicable and non-communicable diseases in adult life (Chalupa-Krebzdak, Long, & Bohrer, 2018; Collard & McCormick, 2021). Milk and dairy products are among the most important food groups in a healthy and balanced diet (Pereira, 2014). Milk is a food that provides the physiological needs of mammals in the growing period and contains all the nutrients that play an important role in the nutrition of people of all ages (Silva & Smetana, 2022). These sensitive nutritional products are highly susceptible to chemical and microbial spoilage due to the rich nutritional elements and high water content they contain (Balthazar et al., 2022; Rejeesh & Anto, 2022). Therefore, it is packaged with packaging materials in order to facilitate the storage, transportation process and increase the shelf life (Cadwallader, Gerard, & Drake, 2023; Rejeesh & Anto, 2022). These packaging materials are polymer-based products and are produced by polymerization of many simple units called monomers, as well as additives such as heat and light stabilizers, colorants, lubricants, antioxidants (Arvanitoyannis & Kotsanopoulos, 2013). Additionally, plasticizers such as di-iso decyl phthalate (DIDP), di-n-octyl phthalate (DNOP), DEHP, di-isononyl-phthalate (DINP), DMP, DBP, di-ethyl phthalate (DEP), and BBP are employed to enhance the pliability and suppleness of packaging materials (Cirillo et al., 2015). Since phthalate esters do not have stable chemical bond interactions with the polymers they are included in, they can be separated from the polymer matrix under storage and some unsuitable conditions (vigorous shaking, high temperature, solar radiation etc.)(Yang et al., 2017) and human consumption of these foods may pose a human health concern for PAEs (Arfaeinia et al., 2020; Ebrahimi et al., 2016; Wang et al., 2015). The effects of PAEs on human health are regarded as endocrine disruptors, mainly because they interfere with the endocrine systems of living things (Pang, Skillen, Gunaratne, Rooney, & Robertson, 2021). Increasingly, epidemiological studies have shown that phthalate esters have hepatotoxic, teratogenic and carcinogenic properties, as well as many negative effects (Arbuckle et al., 2014). At the beginning of these effects are seen impairing spermatogenesis and decreasing sperm count in men (Matsumoto, Hirata-Koizumi, & Ema, 2008), allergic reactions with increased respiratory diseases in children (Buckley et al., 2018), prostate development in men, breast cancer formation in women, thyroid gland abnormalities (Arfaeinia et al., 2020). Based on the current literature, it has been reported that many foods contein PAEs such as in milk and dairy product (Dobaradaran et al., 2020; Mirzaei, Ahmadi, Shariatifar, & Ariaii, 2023), bottled water (Abtahi et al., 2019; Mehraie et al., 2022), carbonated soft drinks (Moazzen et al., 2018), infant formula (Isci, 2023) and juices

(Arfaeinia et al., 2020; Isci, 2024; Kargarghomsheh et al., 2023). Milk has the feature of being one of the basic foods for all age groups because it is cheap and easy to produce and contains the energy and nutrients needed by the body. Therefore, monitoring of commercial milk samples for potential contaminants such as PAEs is of great importance for consumer health and food safety (Dobaradaran et al., 2020). This study aims to analyze the packaged milk of all brands available in the Turkish market in terms of PAEs content and determine their compliance with legal regulations.

Materials and Methods

Chemicals And Reagents

The study utilized chemicals and standards of analytical grade. Formic acid (>98%, for analysis EMSURE® ACS, Reag. Ph Eur) and acetonitrile (>99.9%, OmniSolv® LC-MS) were supplied by Merck (Darmstadt, Germany). Di-isodecyl phthalate (DIDP), di-isononyl phthalate (DINP), and methanol (≥99.9%, hypergrade for LC-MS LiChrosolv®) were provided by Sigma-Aldrich (St. Louis, USA). The standard mixture, which included DEHP, di-n-octyl phthalate (DNOP), DMP, di-ethyl phthalate (DEP), DBP, and BBP esters, was sourced from Dr. Ehrenstorfer (Augsburg, Bavaria, Germany). Additionally, ISOLAB (Wertheim, Germany) supplied a 0.45 µm pore size PTFE filter, and RESTEK (Bellefonte, USA) provided the Q-sep ($MgSO_4$ (6000 mg), Sodium Acetate (1500 mg)) extraction salt and Q-sep dSPE tubes ($MgSO_4$ (1200 mg), PSA (400 mg), C18 (400 mg)) for extract cleanup. The selection of these materials was made with care to ensure the accuracy and reliability of the study results.

Sampling Method

A total of 34 milk samples were gathered from 10 international brands accessible in the Turkish market. The research encompassed all brands with products available for retail sale in Türkiye. Each sample was collected in triplicate and stored in a refrigerator until analysis. Milk samples were obtained from supermarkets and retail stores, representing commercially available milk products during the study period. The samples included different types of milk, such as unflavored whole milk (3.0% fat content), unflavored semi-skimmed milk (1.5% fat content), and flavored milk options like strawberry, cocoa, and banana. The analysis covered samples packaged in PET, cartons, and glass containers to assess potential variations in PAEs levels based on different packaging materials.

Sample Extraction Procedure

The determination of PAE contents in the samples was conducted using the methodology described by Isci et al. (2023), employing the dSPE technique. In adherence to this approach, ultrapure water and acetonitrile were introduced to the milk sample, and subsequently, dSPE extraction salt was added, followed by thorough vortexing. After centrifugation (4500 G; Thermo Fisher Scientific Inc.), the extracted supernatant (acetonitrile) was collected. After vacuum drying, cleaning salts were added to the tube, and the mixture underwent another round of centrifugation through vortexing. The resulting supernatant from the final step was then injected into the glass vial.

Instrument

The investigation utilized a triple quadrupole LC/MS system developed by Agilent Technologies based in Loveland, CO, USA. This system incorporated MS/MS capability and additional components, including a Vacuum Degasser, a quaternary pump, an Infinity Autosampler, and a thermostatted column oven. Chromatography procedures were executed using a 120 SB-C18 column (Poroshell, 3.0 mm, 100 mm, 2.7 µm), also procured from Agilent Technologies in Loveland, CO, USA.

LC-MS/MS Conditions and Analysis

The determination of PAEs involved both quantitative and qualitative assessments, employing the multiple reaction monitoring (MRM) mode with precursor-product ion transitions (Table 1 and Figure 1a,b). The LC-MS/MS was employed for this purpose, as depicted in Figure 1, which illustrates the LC-MS/MS MRM chromatogram of eight PAE's fragment ions with m/z ranging from 50 to 550 in milk. The LC-MS/MS system used a mobile phase A consisting of 0.1% formic acid + 5 mM ammonium formate in water, with an injection volume of 5 µl and a flow rate of 0.3 mL/min. Mobile phase B, prepared as 0.1% formic acid in methanol, was also applied. The total run time was 10 min. The LC-MS/MS instrument was configured with specific operational parameters, including a sheath gas flow rate of 10 L/min at a temperature of 400°C. The nebulizing gas flow pressure and temperature were constant at 50 psi and 300°C, respectively. The capillary ion spray voltage was set at 4.0kV, and the SB-C18 column was maintained at a consistent temperature of 40°C. To establish the calibration curve, various concentrations of PAEs (1, 5, 10, 25, 50, 75, 100, 250 µg/L) were injected twice into the LC-MS/MS system.

Table 1. Method verification parameters.

A	RT	SL	Linearly range		Recovery (%)		RSD (%)		Quantification		m/z	Reference ion
			(µg/L)	R^2	SM	WM	SM	WM	LOD (µg/L)	LOQ (µg/L)		
DMP	2.62	100	1.0-100	0.998	93.76	92.19	5.19	4.93	1.882	6.272	195.1>92.1	195.00>77.1
DEP	3.58	100	1.0-100	0.998	95.43	94.19	4.69	5.79	0.350	1.165	223.10>177.0	223.20>121.0
BBP	5.16	100	1.0-100	0.998	94.30	93.17	3.12	4.43	0.517	1.723	313.20>205.0	313.20>91.0
DBP	5.25	100	1.0-100	0.999	92.10	92.73	4.78	5.41	0.567	1.891	279.25>205.0	279.25>57.3
DEHP	7.92	100	1.0-100	0.999	97.24	93.45	6.19	5.73	1.176	3.920	391.30>279.2	391.10>167.0
DNOP	8.02	100	1.0-100	0.999	96.41	95.76	5.19	4.73	1.347	4.490	391.30>261.1	391.10>57.3
DINP	8.24	100	1.0-100	0.996	96.73	92.17	5.64	7.51	1.064	3.546	419.30>148.9	419.30>71.3
DIDP	8.51	100	1.0-100	0.999	95.31	96.15	4.70	5.42	0.474	1.581	447.40>148.9	447.40>85.2

A: Analytes; RT: Retention time (min); SL: Spiking level (µg/L); SM: Skim milk; WM: Whole milk

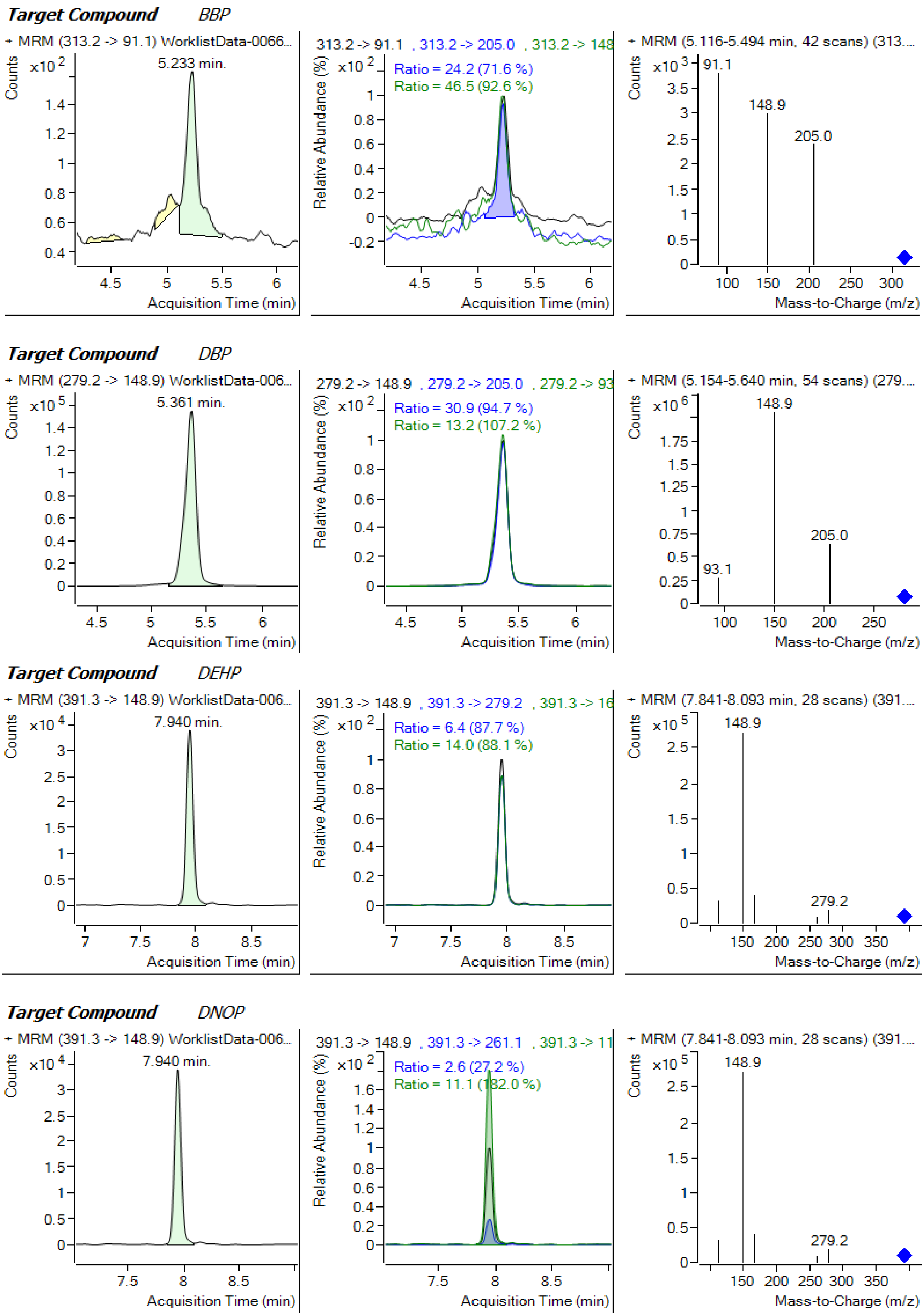

Figure 1a. LC-MS/MS MRM chromatogram of eight PAE's fragment ions m/z 50 and 550 in milk

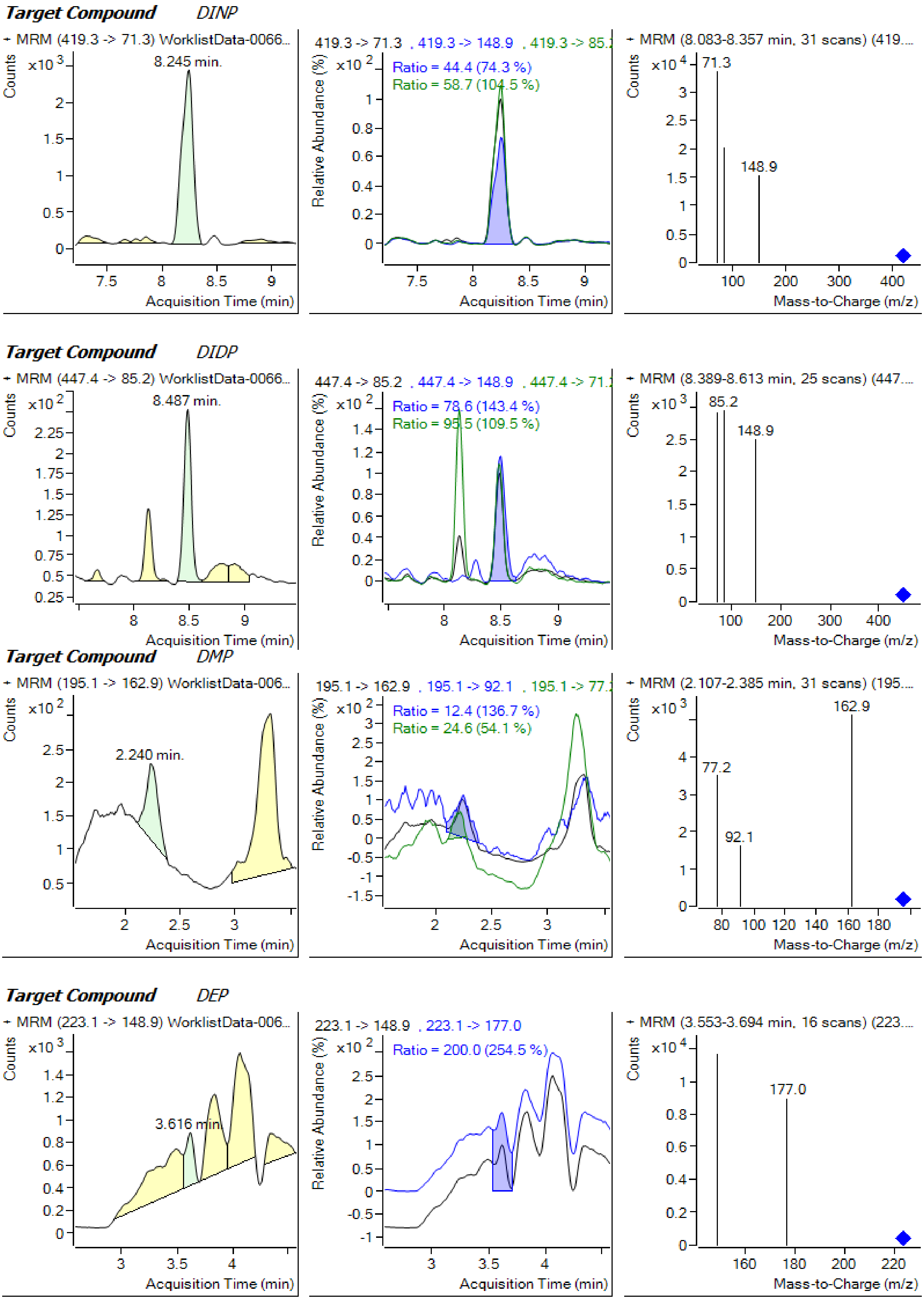

Figure 1b. LC-MS/MS MRM chromatogram of eight PAE's fragment ions m/z 50 and 550 in milk

Statistical Analysis

The statistical analysis of the results included applying PAEs and ANOVA (analysis of variance) to the samples, using SPSS version 26.0 (IBM, Chicago, IL, USA). Particularly for the milk samples, an analysis of variance was performed on the mean PAEs values, with a significance level set at $p < 0.05$. Duncan's multiple tests were employed to identify any statistical differences among the milk samples.

Results and discussion

Quality Control and Quality Assurance

The detected values of PAEs underwent correction by subtracting the average of blank values. To minimize the risk of contamination during PAEs analysis, precautions were taken to prevent contact between reagents, solutions, and plastic materials. Additionally, laboratory glassware was thoroughly cleaned with ultrapure water before utilization.

Table 1 demonstrates that the LOD and LOQ values for all PAEs were below 1.88 µg/L and 6.27 µg/L, respectively. Furthermore, the R^2 exceeded 0.995 for all PAEs. Furthermore, the recovery studies demonstrated results ranging from 92.1% to 97.2%, signifying a favorable recovery, with the RSD values for the target PAEs being under 7.51%. Comparing our results with relevant literature, specifically Kargarghomsheh et al. (2023), our LOD and LOQ values are lower, showcasing the sensitivity of our method. The recovery rates in our study align well with the recovery rates reported by Dobaradaran et al. (2020), affirming the consistency of our findings with established methodologies. In conclusion, our study not only demonstrates a meticulous laboratory protocol but also presents analytical results that are both sensitive and reliable.

PAEs Levels in Milk Samples

The PAE levels of 34 milk samples with different brands and packages which are available in Türkiye are shown in Table 2. All PAE compounds were detected in 100% of the milk samples except for BBP. The DEHP levels of whole milk (unflavored), strawberry flavored milk, cocoa flavored milk, banana flavored milk, semi-skimmed milk (unflavored) was detected to be within the range of <LOD-47.18 µg/L, <LOD-19.84 µg/L, 2.13-8.84 µg/L, 2.75-41.31 µg/L, and <LOD-33.12 µg/L, respectively. DEHP was determined as the most abundant plasticizer in milk samples and its amount in the samples also differed from each other. However, the highest average DEHP amount was found in unflavored milk (47.18 µg/L), while the lowest average value was found in semi-skimmed milk (1.21 µg/L). According to Commission Regulation (EU) No 10/(2011), the specific migration limit (SML) value of 1.5 mg/kg is established for DEHP (EU, 2011). When assessing the samples for compliance with the SML, it was found that the commercial milk samples did not exceed the SML for DEHP. Similar to the current study, DEHP levels in milk samples in plastic bottles were reported to be 198.84-622.37 ng/L by Dobaradaran et al. (2020), 41.3-228.6 ng/L by Mondal et al. (2022), ND-0.154 mg/kg by Kim et al. (2009).There are also some studies that detected higher DEHP levels, 25.0-247.0 µg/L by Selvaraj et al. (2016), 1.0- 936.0 µg/L by Jia et al.(2014), 187.0-201.0 µg/L by Farajzadeh et al. (2012), 13.14-242.39 µg/L by Feng et al.(2005). The PAE level of the same brand of flavored whole milks was statistically higher than the semi-skimmed unflavored milks ($P<0.05$). DEHP (7.73) with higher log partition coefficient (K_{ow}) is more likely to migrate into whole milk than other phthalates (DINP, DEP, DBP and BBP) with lower log K_{ow} (1.0-4.7) (Selvaraj et al., 2016). The DBP levels of whole milk (unflavored), strawberry flavored milk, cocoa flavored milk, banana flavored milk, semi-skimmed milk (unflavored) was detected to be within the range of ND-7.91 µg/L, ND-6.24 µg/L, ND-6.29 µg/L, ND-5.33 µg/L, and ND-4.37 µg/L, respectively. The highest mean DBP level was detected in unflavored milk (7.91µg/L), while the lowest mean concentration was detected in semi-skimmed milk (1.88 µg/L). Commission Regulation No 10/(2011), value of 0.3 mg/kg is reported for DBP (EU, 2011). When samples were evaluated for specific migration limits, commercial milk samples did not exceed the SML for DBP. When current studies are evaluated, DBP levels in plastic bottled milk samples were reported to be 105.0-498.0 ng/L by Dobaradaran et al. (2020), 3.30-150.2 ng/L by Mondal et al. (2022), 0.43-54.3 µg/L by Selvaraj et al. (2016), 99.0 µg/L by Kim et al. (2009), 4.0-10.0 µg/L by Feng et al. (2005). The levels of DINP in the different milk samples varied. For whole milk (unflavored), the detected levels ranged from ND to 28.82 µg/L. Similarly, strawberry flavored milk showed levels ranging from ND to 3.37 µg/L, while cocoa flavored milk exhibited levels between ND and 9.29 µg/L. Banana flavored milk had levels ranging from ND to 12.31 µg/L. Among the samples, semi-skimmed milk (unflavored) showed levels varying from ND to 12.42 µg/L. In the literature research, DINP has been studied in very few studies in packaged milk samples. The mean DINP level in this study is consistent with the mean level determined by Sørensen (2006) (5.0-12.0 µg/L). The DIDP levels of whole milk (unflavored), strawberry flavored milk, cocoa flavored milk, banana flavored milk, semi-skimmed milk (unflavored) was detected to be within the range of ND-8.43 µg/L, ND-9.84 µg/L, ND-10.77 µg/L, ND-2.22 µg/L, and ND-1.95 µg/L, respectively. The DIDP level of whole milk flavored are different from semi-skimmed milks. DIDP has not been investigated in milk and dairy products in the literature. However, according to Commission Regulation No 10/(2011), the SML for both DINP and DIDP is reported to be 9 mg/kg (EU, 2011). In this study, when the milk samples were evaluated for compliance with these SML values, it was found that the concentrations of DINP and DIDP in the commercial milk samples did not exceed the established limits. This suggests that the milk products examined in this study meet the regulatory requirements regarding the presence of DINP and DIDP. The BBP compound is very difficult to decompose under natural environmental conditions and is considered a carcinogen by the Integrated Risk Information System (IRIS) (1988), was not detected in any of the milk samples analyzed in this study. Similar to reported findings of Lin et al. (2015) reported that the BBP levels in three milk brands in plastic bottles ranged from ND-0.00 ng/L, <LOD (4.0 µg/L) by Sørensen (2006). There are also some studies that detected higher BBP level in milk samples

19.0- 85.0 µg/L by Jia et al. (2014), ND- 21.0 µg/L by Selvaraj et al. (2016), and ND-46.1 by Mondal et al. (2022). The DEP levels of whole milk (unflavored), strawberry flavored milk, cocoa flavored milk, banana flavored milk, semi-skimmed milk (unflavored) was detected to be within the range of ND-54.67 µg/L, ND-4.55 µg/L, ND-4.37 µg/L, ND-11.22 µg/L, and ND-9.51 µg/L, respectively. DEP concentrations of whole milk are different from each other, and DEP level of whole fat milks is higher than that of semi-skimmed milks. The highest DEP concentration was found in brand 1(54.67 µg/L) of the whole milk samples. When comparing our findings to previous studies, it is important to consider the range of results reported in the literature. Some studies, such as Farajzadeh et al. (2012), Lin et al. (2015), and Sajid et al. (2016), have reported similar values of (ND), indicating that the compound was below the limit of detection in their samples as well. On the other hand, studies conducted by Selvaraj et al. (2016) and Jia et al. (2014) have reported concentrations ranging from 0.43 µg/L to 54.3 µg/L and 13.0 µg/L, respectively, indicating the presence of the compound in those samples. Additionally, studies by Dobaradaran et al. (2020) and Mondal et al. (2022) have reported higher values ranging from ND to 16.24 ng/L and ND to 33.3 ng/L, respectively. These variations in results highlight the importance of considering different factors such as sample collection methods, analytical techniques, and geographical variations when comparing findings across studies. The DNOP levels of whole milk (unflavored), strawberry flavored milk, cocoa flavored milk, banana flavored milk, semi-skimmed milk (unflavored) was detected to be within the range of ND-5.56 µg/L, ND-4.17 µg/L, ND-5.31 µg/L, ND-4.13 µg/L, and ND-8.13 µg/L, respectively. The DNOP level of flavored whole milks is not different from semi-skimmed milk. The DNOP level was determined to be the highest in whole milk (8.13 µg/L). Most of the studies in the literature reported DNOP levels in commercial milk samples as below the detectable limit (Lin et al., 2015; Sajid et al., 2016). However, there are studies that found higher values ND-1795.00 ng/L (Dobaradaran et al., 2020). The DMP levels of whole milk (unflavored), strawberry flavored milk, cocoa flavored milk, banana flavored milk, semi-skimmed milk (unflavored) was detected to be within the range of <LOD, ND-4.18 µg/L, ND-5.51 µg/L, <LOD, and ND-2.22 µg/L, respectively. DMP level was determined to be the highest in cocoa flavored milk (5.51 µg/L). However, Herrero et al. (2021) reported as 0.43 µg/L. In another study Dobaradaran et al. (2020) reported as ND-16.24 ng/L. PAEs concentrations of different packaged milk samples examined in this study show differences. These differences may be caused by the flavoring and coloring agents used during production and the production method. In addition, it is seen that the machine milking and equipment used in the production processes of the products in the supply of raw materials and the packaging used in their sales can show great differences. The main sources of contamination of PAEs in packaged milk can be feeding of lactating animals with contaminated water and food, milking by machinery with plastic parts, containers used for the transport of milk and plastic packaging bottles (Fierens, Van Holderbeke, Willems, De Henauw, & Sioen, 2013).

Effect of Packaging Type on Paes Migration

The PAE concentrations determined in different packaging types are given in the Table 2. As can be seen, all level of PAEs expressed in µg/L of the 34 packaged milk samples analysed. Statistics analyses were conducted considering 34 milk samples in 10 brands and considering glass, PET, and cartons packaging type separately. The qualitative and quantitative levels of PAE found were different for PAEs and packaging (Figure 2). Total PAE concentration was determined in carton> PET> glass. The highest Total PAEs level were found for carton packaging (Brand1 in whole milk;153.53 µg/L). Carton food packaging includes on their inside PE, which protect the food content from the external factors, such as moisture, spoilage microorganisms (Bekhta, Lyutyy, Hiziroglu, & Ortynska, 2016). In this situation, these interior PE linings can release plasticizer, such as PAEs. In addition to food packaging material, different food additives (flavored, sweeteners, color additives), migration of PAEs from milk plastic transporting tank, automated milking machines (with PVC tubing) and processing apparatus (Fierens et al., 2012) may be the possible sources for higher PAEs observed in packed milk. Similar to the results of this research, Herrero et al. (2021) reported that highest total PAE level were found for metal pail (9094 pg/g f.w.) and carton packaging materials (8193 pg/g f.w.). The lowest Total PAE level were found for glass packaging. Glass materials of packaging does not contain polymer-based layers in their composition. Therefore, PAEs are not expected to be found in dairy products sold in glass packaging.

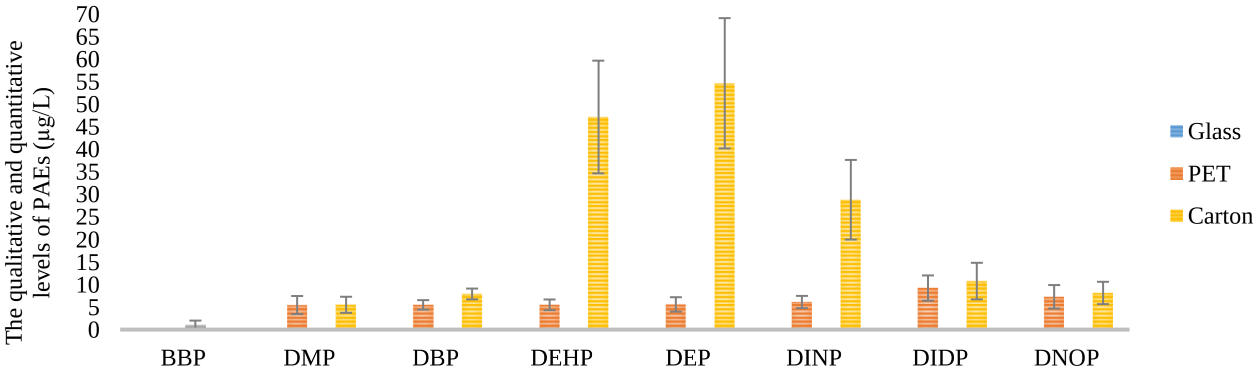

Figure 2. Concentration of PAEs in milk in different types of packing

Table 2. The PAE level of different milk samples (µg/L)

	Whole Milk Fat content (% 3.00)								
	BBP	DMP	DBP	DEHP	DEP	DINP	DIDP	DNOP	P
				Unflavored					
Brand 1	ND	<LOD	7.91±1.16	47.18±9.11	54.67±33.27	28.82±2.21	8.35±8.28	5.34±5.97	C
Brand 2	ND	<LOD	4.95±1.31	8.30±3.78	2.75±1.56	4.63±1.33	6.86±7.74	4.68±5.23	C
Brand 3	ND	<LOD	4.83±2.35	2.54±1.20	2.45±1.68	4.42±1.65	8.43±8.83	5.56±6.17	C
Brand 4	ND	<LOD	4.35±2.56	2.37±1.12	2.72±1.15	3.16±1.30	7.76±8.18	5.40±5.93	PT
Brand 5	ND	<LOD	5.49±2.69	2.18±1.12	2.21±2.32	3.17±1.36	7.21±7.96	5.14±5.64	PT
Brand 6	ND	ND	ND	<LOD	ND	ND	ND	ND	G
Mean± SD	ND	<LOD	5.50±2.01	12.51±3.27	13.03±7.99	8.84±1.56	7.72±7.19	5.22±5.79	
(min. -max.)			(ND-7.91)	(<LOD-47.18)	(ND-54.67)	(ND-28.82)	(ND-8.35)	(ND-5.56)	
				Strawberry flavored					
	BBP	DMP	DBP	DEHP	DEP	DINP	DIDP	DNOP	P
Brand 1	ND	2.51±2.79	3.36±2.38	2.13±1.45	2.62±2.52	2.19±1.14	8.85±10.52	2.63±0.28	C
Brand 2	ND	4.18±2.60	4.71±3.33	2.12±1.50	3.65±2.75	2.89±1.63	7.48±8.60	3.62±3.46	C
Brand 3	ND	3.10±2.96	3.93±2.78	5.55±2.56	4.55±2.44	3.37±1.97	9.84±12.60	3.85±2.98	C
Brand 4	ND	3.00±2.77	6.24±4.26	19.84±11.13	2.66±2.53	2.84±1.45	9.23±12.40	3.99±4.07	PT
Brand 5	ND	3.12±2.84	4.72±4.11	2.22±1.51	2.12±1.84	2.26±3.16	9.23±11.46	4.17±4.04	PT
Brand 6	ND	ND	ND	<LOD	ND	ND	ND	ND	G
Mean± SD	ND	3.18±2.79	4.59±3.37	6.39±3.63	3.12±2.42	2.71±1.58	8.93±11.12	3.65±2.97	
(min. -max.)		(ND-4.18)	(ND-6.24)	(<LOD-19.84)	(ND-4.55)	(ND-3.37)	(ND-9.84)	(ND-4.17)	
				Cocoa flavored					
	BBP	DMP	DBP	DEHP	DEP	DINP	DIDP	DNOP	P
Brand 1	ND	5.51±9.63	4.77±1.78	8.34±3.94	2.53±1.59	3.25±1.71	8.60±9.48	ND	C
Brand 2	ND	4.12±9.09	3.25±1.52	2.70±1.13	3.17±2.13	2.53±1.4	9.69±8.99	3.55±8.71	C
Brand 3	ND	4.58±10.41	6.29±1.68	2.13±1.60	4.37±1.35	3.35±2.81	10.77±9.70	4.59±11.25	C
Brand 4	ND	3.93±9.26	5.83±1.80	7.93±2.90	3.91±1.36	9.29±2.83	8.79±10.15	5.12±12.53	PT
Brand 5	ND	5.45±8.73	4.14±2.93	2.60±1.33	3.90±2.17	6.12±2.84	8.66±10.24	5.31±13.02	PT
Brand 6	ND	ND	ND	2.54±1.70	ND	ND	ND	ND	G
Mean± SD	ND	4.73±9.60	4.86±1.94	4.74±2.17	3.52±1.72	4.91±2.32	9.72±9.71	4.64±11.36	
(min. -max.)		(ND-5.51)	(ND-6.29)	(2.13-8.84)	(ND-4.37)	(ND-9.29)	(ND-10.77)	(ND-5.31)	
				Banana flavored					
	BBP	DMP	DBP	DEHP	DEP	DINP	DIDP	DNOP	P
Brand 1	ND	<LOD	3.16±2.23	2.75±1.13	6.51±2.37	8.65±4.70	1.93±1.72	4.13±1.28	C
Brand 2	ND	<LOD	3.16±1.96	24.13±6.24	6.60±2.43	11.19±4.39	1.98±1.85	3.87±1.94	C
Brand 3	ND	<LOD	3.47±2.45	41.31±28.17	11.22±5.48	12.31±7.29	2.22±2.02	2.86±1.09	C
Brand 4	ND	<LOD	5.33±3.20	15.17±8.37	6.20±2.61	5.30±1.76	2.00±1.79	2.81±0.74	PT
Brand 5	ND	<LOD	3.14±1.99	4.34±2.34	2.81±2.16	5.11±2.15	1.95±1.72	3.92±1.72	PT
Brand 6	ND	ND	ND	3.14±1.10	ND	ND	ND	ND	G
Mean± SD	ND	<LOD	3.65±2.39	17.52±9.26	6.66±3.00	8.51±4.06	2.01±1.82	3.52±1.35	
(min. -max.)			(ND-5.33)	(2.75-41.31)	(ND-11.22)	(ND-12.31)	(ND-2.22)	(ND-4.13)	
				Semi-skimmed milk Fat content (% 1.50					
	BBP	DMP	DBP	DEHP	DEP	DINP	DIDP	DNOP	P
Brand 1	ND	<LOD	4.37±1.13	<LOD	2.20±1.54	1.76±1.88	1.79±1.63	8.13±0.06	C
Brand 2	ND	<LOD	1.88±1.56	1.93±1.67	1.53±2.95	2.45±1.96	1.81±1.62	2.80±0.09	C
Brand 3	ND	<LOD	2.73±1.85	1.21±1.93	2.26±2.33	1.29±1.13	1.74±1.53	2.51±0.041	C
Brand 4	ND	<LOD	1.94±1.37	19.93±12.72	4.36±1.71	4.87±3.45	1.90±1.65	2.80±0.123	PT
Brand 5	ND	<LOD	2.13±1.41	1.90±1.17	1.97±2.22	1.13±1.80	1.95±1.47	2.45±0.071	PT
Brand 6	ND	ND	ND	<LOD	ND	ND	ND	ND	G
Brand7	ND	<LOD	4.12±1.42	33.12±21.51	5.93±1.28	11.75±6.11	1.65±1.51	3.53±0.103	C
Brand8	ND	1.93±1.72	3.13±1.55	<LOD	6.33±2.27	3.59±1.87	1.48±1.31	3.50±0.05	PT
Brand9	ND	1.98±1.85	3.72±1.10	23.34±13.87	9.51±4.10	12.42±7.24	1.33±1.93	6.83±0.02	PT
Brand 10	ND	2.22±2.02	2.52±1.55	5.50±2.11	5.59±2.18	1.22±1.63	1.41±1.50	7.29±0.05	PT
Mean± SD	ND	2.04±4.24	2.93±1.53	9.60±6.31	4.38±2.18	4.34±2.93	1.74±1.60	4.36±0.03	
(min. -max.)		(ND-2.22)	(ND-4.37)	(<LOD-33.12)	(ND-9.51)	(ND-12.42)	(ND-2.37)	(ND-8.13)	

SD: Standart Deviation, ND, Not Detected; P: Packaging; C: Carton; PT; PET; G: Glass

However, It may be contaminated by lacquer on the cap, or from milking machinery and equipment used during production (Fierens et al., 2012). Average PAE levels of PET packaging materials lower than carton packaging materials and higher than glass packaging materials. The contributions of each PAEs to the total PAE level are shown in Table 2 and Figure 2. DEHP contributed the highest to the total PAE concentration, while DMP contributed the lowest. BBP, which has a carcinogenic effect, has not been detected in any packaging material. As a result, this study found that 34 commercial milk samples did not exceed the SML (specific migration limit) reported by the Commission Regulation (EU) No 10/2011. This monitoring is particularly important for plastic materials and articles intended for food contact to maintain consumer safety and prevent any potential risks associated with PAE exposure.

Conclusions

This research contributes novel insights into the occurrence of PAEs by examining milk samples in Türkiye. The results consistently demonstrate PAE levels in the milk samples that are below the standards established by the EU Regulation for all tested compounds, specifically adhering to the SML. DEHP and DEP emerged as the primary plasticizers among the analyzed PAEs. These findings underscore the significance of continuous monitoring and regulatory measures to safeguard the safety of packaged milk and address potential long-term exposure risks linked to PAEs. Persistent research and collaborative efforts are essential to reduce PAE concentrations in packaged milk, ultimately enhancing consumer safety in the long term.

Acknowledgements

There is no conflict of interest.

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