

Presence of Phthalates in Vacuum Packaged Kashar Cheeses Sold Retails in Türkiye

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ARTICLE INFO	ABSTRACT				
Research Article Received : 18.04.2023	Phthalate esters (PAEs) are synthetic compounds, commonly used as plasticizers and softeners in plastic material production, and they are recognized as endocrine-disrupting chemicals. This study was focused on monitoring the extent of PAEs migration in vacuum-packaged Kashar cheeses and plastic materials used in their packaging. A total of fifteen cheese samples were tested for PAEs, including benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), diisononyl phthalate (DiNP), di-2-ethylhexyl phthalate (DEHP), and diisodecyl phthalate (DiDP). The quantification (LOQ) and detection (LOD) limits varied between 0.197 to 0.619 µg/mL and 0.059 to 0.185 µg/mL for all				
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Migration Plastic film, Vacuum packaging	analytes, respectively. All phthalate ester concentrations in both of the cheese samples and their packaging materials were below the detectable level LOQ of the analytical method. FTIR spectra also confirmed that the packaging materials which consisted of polypropylene and polyethylene.				
Kashar cheese Phthalate esters					
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Introduction

Plastic materials used in packaging differ in terms of their chemical structures, processing-dependent properties, added additives, and combinations with other polymers (Guerreiro et al., 2018). They are petroleum-based products manufactured by the polymerization of many simple units called monomers. Moreover, plastics are not chemically pure polymers. Therefore, many additives such as lubricants, plasticizers, stabilizers, UV absorbers, antioxidants and antistatic agents, colorants, optical brighteners, etc., are used in the production of plastic materials, that are necessary for improving the quality and the characteristics of the final products (Keleş 2011; Ibarra et al., 2018). These additives and chemicals may migrate from packaging materials to the foods under suitable environmental conditions (Dagdelen, 2016; Korkmaz, 2018).

One of the substances that make the transition from plastic packaging to foods is phthalate esters (PAEs). PAEs, known as di-alkyl or alkyl aryl esters of 1,2-benzene dicarboxylic acid, have been used as plasticizers. Since the 1920s, PAEs have been used as plasticizers to give flexibility, softness, and to increase their durability to plastic materials, especially polyvinyl chloride (PVC) (Ustun et al., 2015; Xu et al., 2020). More than 25 different types of phthalates are used in commercial applications, and each PAE provides a unique quality to the product (Zhang et al., 2021). The most commonly used PAEs in consumer products are dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), di-2-ethylhexyl phthalate (DEHP), diisobutyl phthalate (DiBP), diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), and di-n-octyl phthalate (DnOP). In addition to their use as plasticizers, phthalates also find applications as fragrances in many personal care products such as cologne, perfume, soap, shampoo and other cosmetic products. The extensive production and application of phthalates have resulted in their ubiquitous distribution in all facets of the environment including air, water, and soil.

PAEs are not bonded to plastics by chemical bonds such as covalent (Mondal et al., 2022). Therefore, they can be released directly or indirectly into food during their manufacture, use, processing, and storage (Du et al., 2016; Hou et al., 2022). It has been stated that this situation causes phthalates to be among the most common chemical contaminants (Adenuga et al., 2020).

PAEs are categorized as endocrine-disrupting chemicals all over the world due to their mimicking nature to human estrogens, and they exhibit mutagenic, teratogenic, and carcinogenic effects on humans (Mondal et al., 2022). As a result of increasing epidemiological

studies on health, long-term exposure to PAEs may cause many adverse effects such as delaying of neurodevelopment in infants (Jurewicz and Hanke, 2011; Jones et al., 2018), increasing respiratory diseases and allergic reactions in children (Buckley et al., 2018), and these effects, decreasing of lung function and depression in the elderly (Kim et al., 2018). It has also been observed in studies that it is associated with health problems such as low birth weight, autism, attention deficit and hyperactivity (Otero et al., 2015). The use of certain PAEs such as DBP, BBP, and DEHP have been restricted in foods and plastic packages in most countries due to their adverse effects on health, frequent use as plasticizers, and environmental risks. The current codex in Türkiye complies with the legal regulations of the European Union on the Regulation of the "Use of Plastics in Food Contact Materials" (EU No: 10/2011), and the specific limits for BBP, DHEP, and DBP are determined as 30 mg/kg, 1.5 mg/kg and 0.3 mg/kg, respectively.

In the food industry, packaging techniques with plastic films are widely used because of their positive aspects such as flexibility, versatility and increasing the shelf life of food. The best-known example of these packaging techniques is vacuum packaging. By vacuuming the package, that the air is removed from the package and hermetically sealed it (Patil et al., 2020). Thus, removing the oxygen in the packaging minimizes the development of aerobic microorganisms and oxidation problems (Hecer, 2012). Vacuum packaging is frequently used in the packaging of meat products, semi-hard/hard cheeses, olives, and nuts. In addition to plastic films such as polyamide (PA), polyethylene terephthalate (PET), PVC and ethylene vinyl alcohol (EVOH), polypropylene (PP), polyethylene (PE), and PE-copolymers can also be used in vacuum packaging due to their heat-sealing characteristic (Guerreiro et al., 2018). In this type of packaging, plastic films tightly wrap the food, depending on the contact area and time, some components may pass/migrate from polymers to food. There are few studies involving phthalate transitions from vacuum-packaged or wrapped foods with plastic film. Cao et al. (2014) reported that only DEHP was detected in some cheese samples covered with PVC film at levels from 0.29 to 15 mg/g, with an average of 2.8 mg/g. Guerreiro et al. (2018) investigated a mass spectrometry-based application for the detection of several compounds from plastic, directly from vacuum-packaged meat samples. They reported that this application was capable of identifying contaminants in all pieces of beef that were in contact with the vacuum-plastic packaging.

Although there were many studies on phthalates in packaging materials and food, there is a lack of information dealing with the transmission of PAEs from vacuumpackaged cheeses. Also considering the lipophilic nature of phthalates and the high-fat content of cheeses, it is essential to detect the possible presence of phthalates in cheeses. Therefore, the aim of this study was to determine the migration of PAEs which can be widely found in and whose use is restricted in the legislation, from vacuumpackaged Kashar cheeses. At the same time, some physical and chemical properties of cheeses were analyzed to determine whether the composition of the cheeses had an effect on the possible migration of PAEs through plastic films.

Material and Methods

Material and Reagents

Vacuum-packaged Kashar cheese (a pasta-filata type) samples were used in this study. A total of 15 commercial samples were collected from different companies in Türkiye. Descriptive information about each cheese is listed in Table 1. Kashar cheeses were stored at 4°C before analyses and all analyses were carried out at least in duplicate for each cheese sample.

Standards of PAEs, including BBP, DBP, DiNP, DEHP, and DiDP, were obtained from Sigma-Aldrich (Charleston, USA). The stock solution of the PAEs with the concentration of 100 mg/L was prepared in methanol and stored at -20°C. A series of working solutions of PAEs were prepared by appropriate dilution of the stock solution before use. Phthalate quantitation was performed according to a least four-point calibration curve, which was linear for concentrations. The presence of phthalate compounds was initially identified by observing the m/z 149 ion in the GC–MS spectrum. All standards were also injected onto the GC-MS system and retention times are shown in Figure 1.

Table 1. Descriptive information about analysed Kashar cheeses

Sample No	Type of plastic film Fresh/Ripened Compa		Company	Shape and weight	
C1	PET	Fresh	National	Molded 500 g	
C2	PP	Ripened	National	Sliced 350 g	
C3	LDPE	Fresh	National	Molded 500 g	
C4	Unspecified	Ripened	National	Sliced 436 g	
C5	LDPE	Fresh	National	Molded 500 g	
C6	PE	Ripened	National	Sliced 300 g	
C7	PE	Ripened	National	Sliced 350 g	
C8	Unspecified	Fresh	Local	Molded 500g	
C9	Unspecified	Fresh	National	Molded 600g	
C10	Unspecified	Ripened	Local	Sliced 350 g	
C11	Unspecified	Ripened	National	Sliced 350 g	
C12	Unspecified	Fresh	Local	Molded 1000g	
C13	LDPE	Ripened	National	Sliced 400 g	
C14	Unspecified	Fresh	Local	Molded 500g	
C15	Unspecified	Ripened	Local	Sliced 350 g	

Analytes	-	orrelation coef			Q (µg/mL)	LOD (µg/mL)	
BBP	0.9868				0.619	0.185	
DBP	0.9855				0.351	0.105	
DEHP	0.9958				0.273	0.081	
DINP		0.9980			0.197	0.059	
DIDP		0.9982			0.478	0.143	
(<u>x100,000,000</u> 1.09TIC))					-	
0.75							
0.59		Be	enzyl benzoat DBP	e BBP	DEHP		
0.25					DINP+		
0.00						~~	
7.5	10.0	12.5	15.0	17.5	20.0 2	22.5 25.0	

Table 2. Main validation parameters for phthalates extraction and analysis

Figure 1. The retention times of PAEs and internal standard

The limit of detection (LOD) and limit of quantification (LOQ) for each phthalate were calculated from eight replicated measurements of low-concentration standard solutions according to the literature data (Kafalı, 2008; Kumawat et al., 2022). LOD and LOQ were calculated from the signal/noise ratios which were multiplied with factor 3 and 10, respectively. Details of quality assurance for phthalates analysis, including the LOD, LOQ and R² values are given in Table 2.

Methods

Physicochemical analysis

Cheese samples were analyzed in duplicate by gravimetric method for total dry matter (IDF, 1982), Gerber method for fat, and titration with AgNO₃ for salt (Hayaloglu, 2009). Titratable acidity (as % lactic acid) was determined by titration method with 0.1 N NaOH using phenolphthalein as an indicator (Hayaloglu, 2009). For pH measurement, 10 g cheese was softened in 15 mL distilled water and the pH of the slurry was measured using a pH meter (Mettler-Toledo, Seven Compact TM S210, Schwerzen-bach, Switzerland) with combined electrodes (Hannon et al., 2003). The fat (F) rate in dry matter (DM) (%) and the salt (S) rate in dry matter (%) were calculated using the formulas F/DM x100 and S/DMx100, respectively.

Extraction of phthalate esters from cheeses

Cheeses were cut to pieces 1 cm from the surface at different points and the 15 g of homogenised cheese pieces were weighed into a glass vial. Benzyl benzoate (BB) was added as an internal standard and allowed to infuse into the Then, for 15 cheeses min. 30 mL of acetonitrile:dichloromethane (1:1) mixture was added and shaken in an orbital shaker for 4 hours at room temperature. The samples were centrifuged for 5 min at 3000 rpm (Hettich model 320 R, Tuttlingen, Germany) and the supernatant was frozen (-20 °C) to separate the fat layer. Following evaporation to dryness using a rotary evaporator (Buchi, model RV300, Switzerland), the residue was reconstituted in one mL of acetonitrile and transferred to a glass vial for analysis by GC-MS (Bradley et al., 2013).

Extraction of phthalate esters from packaging material

An area of 0.5 dm^2 was cut from each plastic material used in the vacuum packaging of cheeses, and the weight was recorded. The packaging materials were then cut into small particles of approximately 1×1 cm and transferred to the glass jar. BB was added as an internal standard and allowed to infuse into the packaging for 15 min. Then, 40 mL of acetonitrile:dichloromethane (1:1) mixture was added and shaken in an orbital shaker for 4 hours at room temperature. The extract was poured into a clean glass bottle and evaporated to dryness on a heating block set at 50°C. The resulting residue was dissolved in 1 mL of acetonitrile before analysis for GC-MS (Bradley et al., 2013).

Gas Chromatography-Mass Spectrometry (GC-MS) operating parameters

The determination of the PAEs in vacuum-packaged Kashar cheese samples and plastic material were performed using a gas chromatography coupled to mass spectrometry (GC-MS) system (Shimadzu, Shimadzu QP–2010, Kyoto, Japan). The injection volume was 1 μ L and the split ratio was 10:1. High-purity helium was used as carrier gas and the flow rate of helium was 1 mL/min. The separation of PAEs was achieved using an HP 5-MS (60 m x 0.25 mm x 0.25 μ m) column (Agilent, J&W, Santa Clara, CA, USA). The oven temperature was kept at 80 °C for 2 min and increased to 320 °C with 20 °C/min rate and kept at this temperature for 12 min. All samples were analyzed in duplicate.

Fourier transform infrared spectroscopy (FTIR) analyzes of plastic films

The sections (1 cm²) taken from the plastic materials used in vacuum packaging for FTIR analysis were placed in the reading chamber of the attenuated total reflection (ATR) unit of the Perkin Elmer FTIR (Perkin Elmer Spectrum One FTIR spectrometer, Germany). Absorption or transmittance spectra were taken at a wavelength of 400-4000 cm⁻¹. The type of polymer was determined by comparing the obtained spectra with the electronic certified library (BIO-RAD Sadtler Spectral Databases Library Polymers vol.2) and Polymer Data Handbook (Korkmaz, 2018).

The thickness of the plastic films

The thickness of the plastic films was measured using a digital micrometer (LTF ART 327.13-14).

Results and Discussion

Physicochemical characteristics of cheeses

To determine the effect of chemical composition on the migration of PAEs in vacuum- packaged Kashar cheeses, cheeses were analyzed in terms of some physicochemical properties (Table 3). The dry matter contents of the cheeses ranged from 51.08-63.08%, and it was lower level in fresh

Kashar cheese samples. The fat content of most samples of Kashar cheeses was over 30%, and all cheese samples fall into the full-fat cheese ($45 \le \text{milk fat}$) category according to Turkish Food Codex Cheese Notification (2015/6). The pH and titratable acidity of the cheese samples varied between 4.98 to 5.94 and 0.351% to 1.359%, respectively. Generally, it was determined that the titratable acidity of the cheeses offered for sale as fresh was lower and the pH values were higher. The chemical compositions of cheeses were similar to with previously reported results for fresh and mature Kashar cheeses (Hayaloglu, 2009; Yılmaz and Dagdemir, 2012).

Method Validation

The phthalates in extracts of cheese samples were identified by matching GC retention times against those of standards and by comparing the mass spectra with standards. The GC chromatogram obtained from five phthalate standards is shown in Figure 1. A successful separation of the five analytes was achieved. DBP, BBP and DEHP peaks were sharp and symmetrical. DiNP and DiDP were present as partially co-eluted peaks due to the presence of many isomers. Phthalates were totally separated in less than 25 min. All calibration curves were linear and correlation coefficients (R^2) were in the range of 0.985-0.998. The detection limits (LOQ and LOD) were separately calculated for each phthalate compound from signal/noise. LOQ and LOD values varied between 0.197 to 0.619 μ g/mL and 0.059 to 0.185 μ g/mL for all compounds, respectively (Table 2).

Concentration of phthalates in cheeses and packaging materials

The fifteen homogenized cheese samples were analyzed for PAEs (DEHP, BBP, DBP, DiNP, DiDP), and the data obtained are presented in Table 4. All phthalate ester concentrations in the analyzed samples were below the detectable level (LOQ). In this case, it was possible to state that there was no detectable transition from the plastic films used in vacuum packaging, and these cheeses did not have any significant contact with plastic materials during the production stages.

Table 3. Some physcochemical characteristics of Kashar cheese samples

Samples	Total solid	Fat	Salt	Fat in dry	Salt in dry	Acidty	
	(%)	(%)	(%)	matter (%)	matter (%)	(%)	pН
C1	53.46 ± 0.02	27.75±1.06	2.22 ± 0.16	51.91±1.95	4.15±0.31	0.468 ± 0.05	5.71 ± 0.01
C2	60.99 ± 0.22	35.00 ± 0.00	2.69 ± 0.09	57.39±0.21	4.41 ± 0.15	1.062 ± 0.07	5.28 ± 0.02
C3	58.42 ± 0.02	32.00 ± 0.00	1.61 ± 0.19	54.77±0.01	2.76 ± 0.33	0.540 ± 0.00	5.44 ± 0.00
C4	63.08 ± 0.40	36.00±1.41	2.22 ± 0.16	57.07±1.87	3.52 ± 0.23	0.648 ± 0.05	5.40 ± 0.02
C5	57.56 ± 0.05	33.00 ± 0.70	1.05 ± 0.16	57.33±1.17	1.82 ± 0.28	0.684 ± 0.05	5.52 ± 0.01
C6	62.45 ± 0.07	35.75±1.06	3.39 ± 0.16	57.24±1.63	5.43 ± 0.27	0.693 ± 0.01	5.69 ± 0.01
C7	60.67 ± 0.02	32.25 ± 0.35	3.68 ± 0.08	53.16 ± 0.60	6.06 ± 0.13	1.359 ± 0.03	5.27 ± 0.00
C8	56.70 ± 0.00	31.25±0.35	$2.80{\pm}0.16$	55.11±0.63	4.94 ± 0.29	1.116 ± 0.05	4.98 ± 0.01
C9	51.08 ± 0.18	29.75±0.35	1.57 ± 0.08	58.24 ± 0.90	3.07 ± 0.15	0.540 ± 0.05	5.94 ± 0.03
C10	59.37±0.12	37.50 ± 0.71	3.10 ± 0.08	63.16±1.32	5.22 ± 0.15	0.689 ± 0.03	5.64 ± 0.03
C11	60.03 ± 0.29	39.00 ± 0.00	2.16 ± 0.08	64.97 ± 0.32	3.60 ± 0.15	0.729 ± 0.03	5.50 ± 0.05
C12	55.18 ± 0.08	32.75±0.35	2.74 ± 0.08	59.35±0.73	4.96 ± 0.15	0.351 ± 0.01	5.81 ± 0.00
C13	61.12 ± 0.04	39.50 ± 0.70	2.22 ± 0.16	64.63±1.11	3.63 ± 0.26	0.585 ± 0.01	5.54 ± 0.01
C14	52.44 ± 0.02	26.50 ± 0.70	2.69 ± 0.16	50.53±1.36	5.13 ± 0.31	0.793 ± 0.05	5.59 ± 0.00
C15	57.04±0.21	29.25±0.35	3.15±0.16	51.28±0.43	5.52 ± 0.30	$0.828 {\pm} 0.05$	5.49 ± 0.02

Samples	DEHP	BBP	DBP	DiNP	DiDP	Thickness of plastic films (mm)	FTIR
1	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070±0.01</td><td>PP</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070±0.01</td><td>PP</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070±0.01</td><td>PP</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.070±0.01</td><td>PP</td></loq<></td></loq<>	<loq< td=""><td>0.070±0.01</td><td>PP</td></loq<>	0.070±0.01	PP
2	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070 ± 0.00</td><td>PP</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070 ± 0.00</td><td>PP</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070 ± 0.00</td><td>PP</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.070 ± 0.00</td><td>PP</td></loq<></td></loq<>	<loq< td=""><td>0.070 ± 0.00</td><td>PP</td></loq<>	0.070 ± 0.00	PP
3	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.080 ± 0.00</td><td>LDPE</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.080 ± 0.00</td><td>LDPE</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.080 ± 0.00</td><td>LDPE</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.080 ± 0.00</td><td>LDPE</td></loq<></td></loq<>	<loq< td=""><td>0.080 ± 0.00</td><td>LDPE</td></loq<>	0.080 ± 0.00	LDPE
4	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.080 ± 0.01</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.080 ± 0.01</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.080 ± 0.01</td><td>PE</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.080 ± 0.01</td><td>PE</td></loq<></td></loq<>	<loq< td=""><td>0.080 ± 0.01</td><td>PE</td></loq<>	0.080 ± 0.01	PE
5	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070 ± 0.01</td><td>LDPE</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070 ± 0.01</td><td>LDPE</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070 ± 0.01</td><td>LDPE</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.070 ± 0.01</td><td>LDPE</td></loq<></td></loq<>	<loq< td=""><td>0.070 ± 0.01</td><td>LDPE</td></loq<>	0.070 ± 0.01	LDPE
6	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.060 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.060 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.060 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.060 ± 0.00</td><td>PE</td></loq<></td></loq<>	<loq< td=""><td>0.060 ± 0.00</td><td>PE</td></loq<>	0.060 ± 0.00	PE
7	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.060 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.060 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.060 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.060 ± 0.00</td><td>PE</td></loq<></td></loq<>	<loq< td=""><td>0.060 ± 0.00</td><td>PE</td></loq<>	0.060 ± 0.00	PE
8	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.070 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.070 ± 0.00</td><td>PE</td></loq<></td></loq<>	<loq< td=""><td>0.070 ± 0.00</td><td>PE</td></loq<>	0.070 ± 0.00	PE
9	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.110±0.00</td><td>PP</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.110±0.00</td><td>PP</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.110±0.00</td><td>PP</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.110±0.00</td><td>PP</td></loq<></td></loq<>	<loq< td=""><td>0.110±0.00</td><td>PP</td></loq<>	0.110±0.00	PP
10	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.080 ± 0.00</td><td>PP</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.080 ± 0.00</td><td>PP</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.080 ± 0.00</td><td>PP</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.080 ± 0.00</td><td>PP</td></loq<></td></loq<>	<loq< td=""><td>0.080 ± 0.00</td><td>PP</td></loq<>	0.080 ± 0.00	PP
11	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.085±0.01</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.085±0.01</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.085±0.01</td><td>PE</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.085±0.01</td><td>PE</td></loq<></td></loq<>	<loq< td=""><td>0.085±0.01</td><td>PE</td></loq<>	0.085±0.01	PE
12	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.065 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.065 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.065 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.065 ± 0.00</td><td>PE</td></loq<></td></loq<>	<loq< td=""><td>0.065 ± 0.00</td><td>PE</td></loq<>	0.065 ± 0.00	PE
13	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.075 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.075 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.075 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.075 ± 0.00</td><td>PE</td></loq<></td></loq<>	<loq< td=""><td>0.075 ± 0.00</td><td>PE</td></loq<>	0.075 ± 0.00	PE
14	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.085 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.085 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.085 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.085 ± 0.00</td><td>PE</td></loq<></td></loq<>	<loq< td=""><td>0.085 ± 0.00</td><td>PE</td></loq<>	0.085 ± 0.00	PE
15	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.090 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>0.090 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0.090 ± 0.00</td><td>PE</td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0.090 ± 0.00</td><td>PE</td></loq<></td></loq<>	<loq< td=""><td>0.090 ± 0.00</td><td>PE</td></loq<>	0.090 ± 0.00	PE

Table 4. Migration of PAEs in cheeses and plastic packaging materials

Several studies performed in various countries on the migration of phthalates from different food groups, and the variable results are presented. Yang et al. (2019) reported that DEHP and DBP were detected in 218 of 283 convenience food samples in China, and the highest migration content was found in cakes for DEHP and DBP. In Canada, a total of 118 food samples wrapped with PVC film were analyzed for migration of PAEs, and DEHP was detected in only some of the cheese samples (9 out of 40 samples, at 0.29 to 15 mg/g) and other phthalates were not detected in any of the 118 samples (Cao et al., 2014). Oruc (2020) reported that PAEs were found below the detection limit in samples of flavored carbonated beverages, lemonade and drinking water packaged with plastic material. Beltifa et al. (2018) reported that cheeses repackaged in flexible plastic sold in the Tunisian market contained high quantities of DBP and DEHP with concentrations up to 0.46 and 2.339 mg/kg. Van Holderbeke et al. (2014) tested 591 Belgian foods and 30 packaging materials for phthalate levels. In this study, DEHP was the most detected phthalate compound in milk and dairy products (72 of 79), meat and meat products (35 of 37), fish and fish products (18 of 22), snacks (27 of 29), condiments and sauces (40 of 41) and in packaging materials (30 of 30). Pil-Bala et al. (2019) reported that the concentrations of DBP and DEHP in the cheeses packaged in PE or PP packages ranged from 46 to 62 ng/g and 78 to 96 ng/g, respectively.

Phthalate migration depends on numerous factors, such as temperature and storage period, type and characteristics of the package, contact area and duration with packaging material, and chemical composition of the packaged food. It has been reported that PAEs may accumulate especially in foods with high fat content due to their lipophilic nature (Bogdanovicova and Jarosova, 2015). However, there was no detectable transition despite the fact that the analyzed Kashar cheeses had high-fat ratios (ranging from 26.50% to 39.50%). Similarly, it was stated that the moisture content of the cheeses may have an effect on migration. As seen in Table 3, the moisture contents of the cheese samples were low, this might cause an increase in consistency, preventing the passage of phthalates to some extent. Indeed, Goulas et al. (2000) reported that the moisture content in cheese may have an effect on the migration of phthalates used as plasticizers and that high moisture content may reduce the consistency and increase the diffusion rate of plasticizers such as di-(2-ethylhexyl) adipate (DEHA).

Another factor in migration is the properties of the plastic material, especially the type of plastic material from which the packaging material is made. Therefore, to investigate whether packaging material is the primary source of PAEs all packaging materials were analyzed for phthalates. The transition of PAEs from the plastic films to the solvents was below the detectable level (<LOQ). This result was in agreement with Cao et al. (2014) who reported that phthalates were not detected in any of the extracts of the packaging materials. It is seen that this situation is related to the type of plastic films used in vacuum packaging. The descriptive information on the packaging, showed these were made from PE/LDPE, PET, and PP polymers, and in some samples the type of plastic material used in the packaging was not specified (Table 1). As a matter of fact, it has been reported that plasticizers are mostly used for softening and flexibility of hard materials such as PVC, and theoretically, PP contains fewer plasticizer additives than PVC due to the softness and flexibility coming from polypropylene's molecular structure (Fang et al., 2017). Similarly, it was stated that PE was a naturally flexible polymer and less plasticizer was needed (Cao et al., 2010). The fact that a significant part of the analyzed packaging materials is produced from PE/LDPE and PP polymers can be considered as the reason why PAEs are below the detectable level. De Anda-Flores et al. (2021) reported that only one phthalate was detected in 5 of the 15 commercially obtained PVC cling films used for food packaging in Mexico. Korkmaz (2018) reported that the transitions of PAEs from 10 plastic packages made of PET were below the detectable level.

In addition, the ambient temperature is an essential factor in the transitions, and the migration of PAEs can migrate at a higher level at high temperatures. Vacuumpackaged Kashar cheeses were generally stored at refrigerated temperature. This condition may cause to preventing of possible passage of PAEs. Indeed, Yang et al. (2017) reported that PAEs did not show a significant release from PE plastic films to different food simulants at temperatures of 4 °C and -18 °C, and the transition rates were much lower than the transitions at higher temperatures. The characteristics of plastic films used in food packaging such as type, thickness, and permeability of plastic materials are also considered as an effective factor on migration. The thickness of plastic films ranged from 0.060 to 0.110 mm, and there was not much difference between the thicknesses of the plastic films, except for one sample (sample 9) (Table 4). However, because PAEs could not be determined in both cheese and packaging materials analyzed, a relationship could not be established between the film thickness and the passage of these compounds.

FTIR results

The FTIR spectra of the plastic films used in vacuum packaging were compared with the certified electronic library and literature information. FTIR results verified that sample 2 was comprised of PP, and samples 3, 5, 6, 7, 13 comprised PE/LDPE; however, from the descriptive information on the package, it was stated that sample 1 was produced from PET polymer. But it was more compatible with PP according to the FTIR spectra. Also, some plastic films were not specified which type of plastic film was used on the packaging (Table 1). A part of these samples (4, 8, 11, 12, 14, and 15) gave characteristic absorption bands such as C-H stretching vibrations at 2914 and 2847 cm⁻¹, C-H scissoring vibrations at 1470 and long-chain CH₂ rocking vibration at 718 cm⁻¹, and their spectra matched to a large extent with PE. Considering the specific peaks for PP, it was also concluded that the two samples (9 and 10) could most likely be produced from PP polymer.

Conclusions

In this study, a total of fifteen cheeses and their packaging materials were analyzed for the migration of five PAEs including BBP, DBP, DiNP, DEHP, and DiDP. The present results showed that the concentrations of PAEs in cheeses were under the LOQ, and there was no detectable transition from plastic films used in vacuum packaging to cheese. At the same time, it was determined that the cheese did not come into contact with plastic materials during the production or storage periods. However, it should be kept in mind that although they are present in foods in low concentrations, plastic materials in contact with food constitute the primary source of exposure to phthalates, and considering that this exposure may be lifelong, they may have long-term adverse effects on human health. For this reason, it is thought that it would be beneficial to carry out more detailed studies to determine the transitions of PAEs, taking into account the worst conditions of plastic materials used in packaging, including plastic films used in vacuum packaging.

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