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Research Article

Migration Of Phthalates From Plastic Packages Into Dairy Products

Seda Dicle KORKMAZ [1 (*)] Özlem KÜPLÜLÜ [2] Güzin İPLİKÇİOĞLU ARAL [2] Mehmet Emin ŞEKER [3]

[1] Giresun University, Espiye Vocational School, Department of Food Production, TR-28600 Giresun - TÜRKİYE
[2] Ankara University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, TR-06110 Ankara - TÜRKİYE
[3] Giresun University, Espiye Vocational School, Department of Crop and Animal Production, TR-28600 Giresun - TÜRKİYE

(*) **Corresponding author:** Seda Dicle KORKMAZ
Phone: +90 454 310 1430
Cellular phone: +90 555 631 6070
E-mail: diclekahraman@gmail.com

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ABSTRACT

In this study, it was aimed to determine the possible migration from the packaging material by determining the phthalates during the storage period in dairy products. For this purpose, selected phthalate esters were determined by LC-MS Q-TOF from the dairy product samples taken on different times of storage, following the raw milk stage and packaged with plastic. Accordingly, only DEHP was detected from phthalate esters in all samples. In white cheeses, DEHP values was 226 µg/kg in glass packaging and 244 µg/kg in plastic packaging on the 1st day, it increased to 259 µg/kg in glass packaging and 420 µg/kg in plastic packaging in the 9th month. Likewise, the DEHP values of Kashar cheese, which were 236 µg/kg and 262 µg/kg in glass and plastic packaging samples on the 1st day, reached 241 µg/kg and 346 µg/kg, respectively, in the 9th month. In yogurt samples, on the 1st day, there was 253 µg/kg DEHP in the glass package and 255 µg/kg in the plastic package, while on the 30th day, 262 µg/kg in the glass package and 288 µg/kg in the plastic package. Although the determined values are below the legal limits, in case of high phthalate in the packaging material, the potential risk that may occur in foods has been revealed.

Keywords: Cheese, Dairy Products, LC-MS Q-TOF, Phthalates, Yogurt

Introduction

Phthalates are organic compounds defined as plasticizers, especially used to make plastic materials more elastic, lightweight, durable and soft [1,2]. Phthalates, which are not chemically bonded to plastic, have a lipophilic character and tend to evaporate easily. All these properties play an important role in the contamination of these substances to the environment and food [3-5]. Due to they are heavily used in food contact materials to be present in the environment and especially Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Dipropyl phthalate (DPP), Di-isobutyl phthalate (DIBP), Di-n-butyl phthalate (DBP), Butyl benzyl phthalate (BBP), Dicyclohexyl phthalate (DCHP), Di-n-hexyl phthalate (DHP), Di-2-ethylhexyl phthalate (DEHP), Di-n-octyl phthalate (DOP), Di-isononyl phthalate (DINP) and Di-isodecyl phthalate (DIDP) are (DIDP) are common phthalate residues detected in foods [6,7]. The higher molecular weight PAEs, such as DEHP, DINP, and DIDP are used as plasticizing agents to soften poly (vinyl chloride) products. DBP, DEP and BBP as the lower molecular weight PAEs, usually act as thickeners and flavor enhancers in a variety of personal care products [8,9]. Packaging materials, which are also often used in the packaging of milk and dairy products, are the main source of contamination, especially for phthalates such as DEHP, DBP and DIBP. Phthalate contamination of food could occur not only from packaging material, but also from soil, water, air, as well as during transport, production, storage, or even cooking in houses [10-12]. Owing to the lipophilic property of PAEs, milk products with high protein and lipid content are prone to accelerated the migration of PAEs from contacted plastic materials during processing, transport and storage [8,13]. Therefore, the amount of phthalate in packed food depends on both the phthalate concentration in the packaging material, the storage time and temperature, the fat content of the food and the contact surface [14-17]. For this reason, milk and dairy products can be easily exposed to phthalate migration due to their fat content and the use of plastic for their packaging. As

with all raw foods, the amount of phthalates in raw milk is low. It is reported that the amount of phthalates in raw and unprocessed milk does not exceed 120-180 µg/kg. In most cases, phthalates pass into the final product at the processing and packaging stages in milk products [10,15,18]. For example, applications during production in cream and cheese increase the amount of DEHP in the final product by 5-100 times [5]. In one of the studies, total phthalate and DEHP levels in raw milk and dairy products taken during the collection, transportation and packaging were found to average 20 and 280 µg/kg, <10-70 µg/kg in low-fat milk and 1930 µg/kg in cream. In another study, they determined the amount of DEHP in retail dairy products as <10-50 µg/kg and the total phthalate level in dairy products as 3000 µg/kg, the highest in creams [19,20]. Also, Yang et al.[8] investigated ten well known dairy brands of China, and found that the BBP, DEHP and DOP in plastic packages were higher than those in other type of packages.

Phthalates used as softening agent in packaging material and particularly DEHP are specified as "environmental hormones" which have effect of disrupting the endocrine system of animals and humans. The phthalates pose a serious threat to food safety and public health [21]. People can be exposed to phthalates through skin, respiration and digestion. Digestion, in particular, is an important pathway in phthalate exposure. Phthalates are taken into the body by food, supplements and drugs, even young children are exposed to phthalates as a result of swallowing toys or taking them to the mouth. Phthalate contamination of food occurs not in primary parts of production, such as farm and field, but mostly in final stages, such as processing and packaging. Phthalate migration occurs especially from plastic packaging materials to foods. Lipophilic foods such as milk, butter and meat are reported to be the main source of exposure for some phthalates such as DEHP, DBP and DIBP [13,22,23].

Animal experiments conducted in recent years showed that some PAEs, their main metabolites and degradation substances, have toxic effects on various organs, including the liver, reproductive system, kidneys, lungs and heart. DEHP and DBP, the most commonly used phthalate esters during the production, processing and preservation of foods, are evaluated in the 'endocrine disrupting chemical' group since they are estrogen and testosterone antagonists. Among phthalate esters, DBP and DEHP are reported to have negative effects on germ cell development, BBP on epididymal spermatozoa concentration, DINP and DIDP on liver cells. It has been found to cause low sperm count in men, decreased fertility and testicular changes, and low birth weight and malformation in females, especially during pregnancy [24-26]. The effects of phthalates in the organism can vary depending on age, duration of exposure and amount of exposure. In this respect, the most sensitive periods are pregnancy, infancy and puberty [25,27,28]. Col´on et al.[29] suggested a possible association between PAEs with known estrogenic and antiandrogenic activity and the cause of premature breast development in young Puerto Rican girls. In addition, the carcinogenic, teratogenic and mutagenic effect of phthalates revealed by experimental studies is also an important threat to human health [30-32]. The presence of phthalates in foods is legally regulated by some authorities. Because of the potential risks to human health DMP, DEP, BBP, DBP and DEHP phthalate esters, are listed as "Priority toxic pollutants" by United States Environmental Protection Agency (USEPA) in 1976 and reference values for DEHP, BBP and DBP were determined 20, 200, and 100 g/kg body weight (bw)/day, respectively [33,34]. The tolerable daily dose for DBP, BBP, DEHP, DINP and DIDP was determined by EFSA (European Food Safety Authority) as 0.01, 0.5, 0.05, 0.15 and 0.015 mg/kg/day respectively [35-37]. European Union directives set the legal limits for BBP, DEHP, DBP, DINP and DIDP that may generate migration when in contact with food 30, 1.5, 0.3, 9, and 9 mg/kg respectively while total phthalate levels from plastic into food has been limited to 60 mg/kg [38]. Similar practices and limits have also been adapted to Turkey by legal legislation [39].

Yogurt and cheese, which are offered for sale in plastic packaging in Turkey, are dairy products with high nutritional value that are widely consumed by people of all ages. The purpose of this study was, determination of possible potential food safety/public health risks arising from phthalate migration depending on storage period. Yogurt, white cheese and kashar samples were analyzed for six phthalates esters during the shelf life, all samples were produced in an integrated dairy plant using machinery milking.

Material and Methods

Material

In the study, samples were taken before production (raw material), at the beginning of production (pasteurization) and at the end of production (from the final product). Raw milk, pasteurized milk, and white cheese, kashar cheese and yogurt samples produced from this milk, including feed, from an integrated dairy farm with machine milking were used as material in order to determine the presence and amount of phthalates during the storage of the final products. For this purpose, before production 6 samples were taken from each ration of feed and finger milking to evaluate possible phthalate contamination. 18 samples, 6 of each, from raw milk used in the production of yogurt, white cheese and kashar cheese simultaneously after milking with the machine, 18 samples, 6 of each, from milk used in the production of yogurt, white cheese and kashar cheese after pasteurization, and the last stage of

production, a total of 84 samples were taken, 6 of each from yogurt, white cheese and kashar cheese packaged with plastic materials, and 6 of each from yogurt, white cheese and kashar cheese placed in glass jars. All dairy products taken in glass jars were used as the control group. Therefore, to prevent possible phthalate contamination in the glass jars used, the jars, cleaned with ultrapure water, methanol, hexane and acetonitrile respectively and dried. The samples were brought to the laboratory under the cold chain and stored in the refrigerator (4°C) throughout their shelf life. Additionally, a total of nine packaging material samples, three of each used in the packaging of yogurt, white cheese and kashar cheese, were subjected to migration testing and analyzed for phthalate esters.

The presence and amount of DBP, DEHP, BBP, DINP, DNOP, DIDP phthalate esters in all samples were determined by LC/MS Q-TOF. All samples taken from the factory were immediately analyzed in terms of phthalate, and the initial phthalate values of the production date (1st day) were determined. Phthalate analysis was performed on the 1st, 3rd, 6th and 9th months of the shelf life of white cheese and kashar cheese samples while it was performed on the 15th and 30th days of shelf life of yogurt samples in order to determine the effect of storage time on phthalate migration.

Chemicals

Formic acid (98-100%), Acetic acid (100%), Acetonitrile, and Methanol (Hypergrade for LC-MS) from Merck (Darmstad-Almanya). Ultra pure water (18.2MΩ) for mobile phase was from Millipore Simplicity 185 (Millipore SAS, Molsheim, Fransa) water purification system. Analytes DBP, DEHP, BBP, DINP, DNOP, and DIDP referance standards from Dr. Ehrenstorfer (Augsburg -Almanya). Stock solutions were prepared in methanol with concentration of 1 mg/mL, Each of them were weighted 10.0±0.1 and dissolved in 10 mL methanol. Stock solutions were stored in -18°C. Working solutions of 6 compounds were mixed and diluted to 10 µg/ml with methanol. Calibration solutions were prepared diluting 10 µg/mL solution.

Instrumentation

Agilent 1260 series LC coupled with Agilent 6550 LC-MS Q-TOF (Agilent, Santa Clara, USA) was used to determinate DBP, DEHP, BBP, DINP, DNOP, and DIDP. Agilent Zorbax SB-C18 (2.1 mm x 50 mm x 1.8 µm) (Agilent, Santa Clara, ABD) column was used for chromatographic seperation. Precursor ions (M+H) for DBP, DEHP, BBP, DINP, DNOP, and DIDP used or measurement were 279.1595, 391.2847, 313.1436, 419.3156, 391.2847 ve 447.3469 respectively. Referance ions of 121.0508 and 922.0098 were used to correct if mass shift occurs during the run. First 2 min of run was diverted to waste to deliver unretained and matrix components without entering the ion source. All analysis were run in MS and MSMS mode. Injection volume was 1 µL. Parameters for the LC-MS system are shown in *Table 1*.

Glassware

Sample contamination can occur in any steps of sample prep and instrumental part [40,41]. For this reason, only glass materials were used. All the glassware were rinsed with ultra pure water, methanol, hexan and asetonitrile respectively. They left in an oven at 400°C for 12 h. Before use they were rinsed with methanol.

Sample Preperation

Samples were prepared according to FDA [42] method with a slight modification. 5.0±0.1 g of sample weighted and put in a 100 mL beaker and 45 mL methanol was added. It was mixed 30 min in an ultrasonic bath at room temperature. After cooling the volume was filled up 50 mL with methanol. Ten mL of this solution was taken and evaporated under nitrogen at 40°C. The process

Table 1. *LC-MS parameters*

Parameter	Value	Parameter	Value
Mobil phase A	0.1% Formic acid	Column	Agilent Zorbax SB-C18 2.1 mm x 50 mm x 1.8 µm
Mobil phase B	Methanol	Column oven	35°C
Flow rate	0.3 mL/min	Ionisation mode	Positive electrospray
Gradient	0 min - 20% B	Drying gas temp	250°C
	0.5 min - 20% B	Drying gas flow	14 L/min
	3 min - 95% B	Nebuliser	35 psi
	6 min - 95% B	Sheath gas temp	350°C
	6.1 min - 20% B	Sheath gas flow	11 L/min
Analysis time	12 min	Capillary voltage	3000 V
Injection	1 µL	Nozzle voltage	0 V

was stopped when solution volume was left around 1ml. Remaining part dissolved and diluted to 2 mL with methanol. The solution was centrifuged 3500 rpm for 10 min, and 1 mL of supernatant part was transferred to vial and injected to LC-MS.

Migration Test

Migration test was performed acoording to European Directive (EU 10/2011). In this study, 3% acetic acid solution were used for extraction media. 100 mL 3% acetic acid solution put in container and waited in an oven for 10 days at 40°C. Final solution was diluted with methanol (1:1 ratio) in a vial and injected to LC-MS system [36].

Validation

To validate the method, parameters such as selectivity, lineerity, trueness, precision, limit of detection (LOD) and limit of quantitation (LOQ) were determined using the spiked samples according to Eurochem Guide [43]. Standart addition and matrix matched calibration approaches were also used for quantification purposes. Cheese samples were prepared at 50, 100 and 400 µg/kg spiked levels. 20-1000 ng/mL lineer range was used for calibration and first level was checked for signal to ratio to avaoid any contamination. Calibration solutions were prepared in pure solvent and matrix extract. Slope ratio of each compounds calibration were compared to to check matrix effects on each phthalate. LOD and LOQ values were calculated using spiked samples. S/N ratio was taken 3 as LOD and ratio 10 was taken as LOQ. Precision was evaluated at three levels as repeatability and intermediate precision. Trueness was evaluated at three concentration levels (50, 100 and 400 µg/kg) using blank samples spiked with a standard solution. To validate the method used for migration test, blank samples (ultra pure water) were spiked with 50-100-400 µg/L and extracted as the same with migration procedure.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 20.0 was used for analysis of the data obtained. Normality assumption was evaluated using the repeated measure variance analysis was used to examine the milk and dairy products change over time. And independent sample t test was used to compare the mean of samples in glass jar, samples in plastic packaging and the finger milking, machine milking groups, mean and standard deviation data were presented. $P<0.05$ was considered to indicate a statistically significant difference among groups.

Results

Within the scope of the study, 93 samples were taken in total, including 84 samples from raw milk, pasteurized milk and the final products (yogurt, kashar cheese, white cheese) and 9 packaging materials of packaged products, and 6 phthalate esters (DBP, DEHP, BBP, DINP, DNOP, DIDP) were analyzed by LC-MS Q-TOF instrument. Chromatographic separations of DEHP and DNOP were achieved by appyling the gradient program due to the fact that DEHP and DNOP of phthalate esters having the same m/z (391.2847) value. DEHP fragmentation products were obtained within certain ion ratios as the major ion 391.2847, and were verified. The fragmentation ions were selected as 279.1591 and 167.0339 ions. *Fig. 1* shows the ion ratios.

Quantification of the positive results, which were obtained, was calculated from the injected 20-1000 ppb series (8 levels). Blank vials were considered before starting the batch run. Blank reading was done at least 3 times before analysis, and the contamination, which may come from the system and solvents, was checked. Due to the fact that the matrix separation cannot be removed efficiently during extraction, recovery value was determined between 65-86% while the RSD value in repeatability studies was obtained in the range of 3-20%. It is a disadvantage that the recovery and repeatability values are at LOQ levels, and it was resulted from the possible matrix components. Particularly, the fat content of the samples and the insufficient removal of it during the extraction could lead the low recovery values [18].

Among the phthalate esters, only DEHP was detected within the performed test for the plastic packages used in the samples *(Fig. 2)*. Other PAEs (DBP, BBP, DINP, DNOP, DIDP) remained below the detection limit ≤50 µg/L).

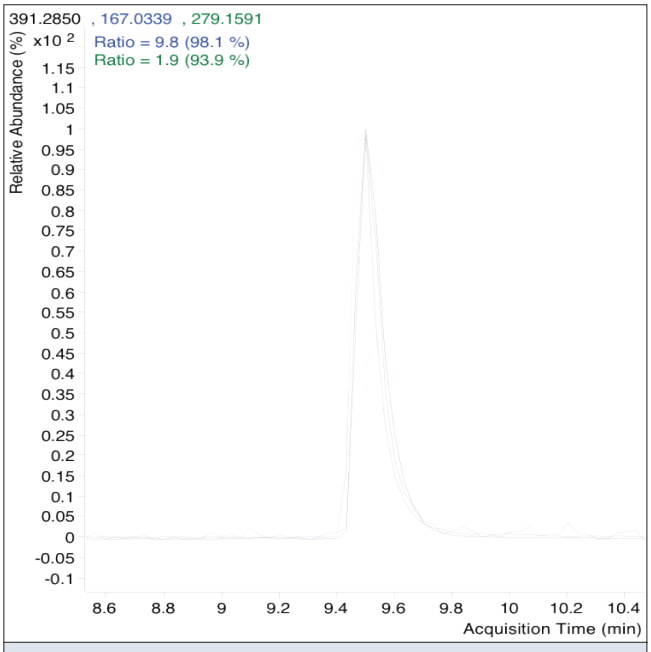

Fig 1. Chromatogram showing the ratios of major ion (391.2847) and fragmentation ions (167.0339 and 279.1591) for DEHP (Ion ratios 9.8% for 167 and 1.9% for 279 ions)

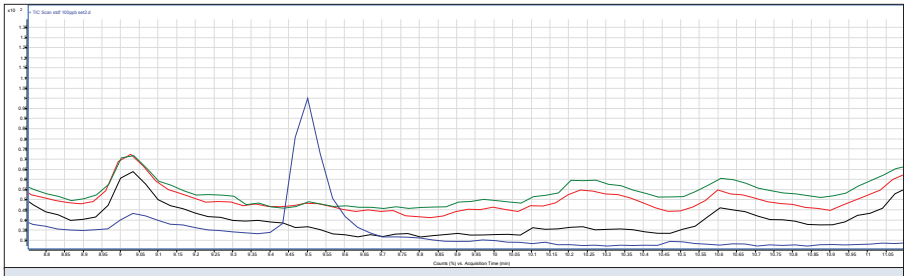

Fig 2. For DEHP, signal at LOQ level (Blue), sample below LOQ level (Green/Red), and solvent injection (Black)

Table 2. *DEHP values of packaging material according to migration test results*

Sample (n=3)	**DEHP Amount* (µg/lt; mean±SD)**
Yogurt Packaging	92±6
White Cheese Packaging	272±7
Kashar Cheese Packaging	118±8

Table 3. *DEHP values of the samples taken from the factory (µg/kg)*

Sample (n=6)		**DEHP (µg/kg; mean±SD)**					
		1st Day	**15th Day**	**1st Month**	**3rd Month**	**6th Month**	**9th Month**
Feed	**Feed**	**<**LOD**	-	-	-	-	-
Milk	Finger milking	161±7	-	-	-	-	-
	Raw milk tank[a]	239±9	-	-	-	-	-
	Raw milk tank[b]	234±9	-	-	-	-	-
	Raw milk tank[c]	226±9	-	-	-	-	-
Yogurt	Pasteurized milk	246±8	-	-	-	-	-
	Glass	253±8	259±9	262±10			
	Plastic	255±9	286±10	288±10			
White Cheese	Pasteurized milk	251±7					
	Glass	246±8		260±8	251±10	253±10	259±8
	Plastic	244±8		268±7	299±10	339±9*	420±8*
Kashar Cheese	Pasteurized milk	256±7	-	-	-	-	-
	Glass	240±10	-	236±10	232±10	244±11	241±11
	Plastic	262±10	-	318±10*	330±10	328±11	346±11

[a]*Value for yogurt milk, Statiscally significant * P=0,017 ** LOD: Limit Of Detection (<50 µg/kg);* [b]*Value for white cheese;* [c]*Value for kashar cheese*

Analyzed amount of DEHP in yogurt, white cheese and kashar cheese packaging samples were determined as 92 µg/L, 272 µg/L and 118 µg/L, respectively *(Table 2)*.

Among the 6 phthalate esters (DBP, DEHP, BBP, DINP, DNOP, DIDP), only DEHP was detected in the samples analyzed for milk and dairy products in the factory, and the obtained values were determined in the range of 161 µg/kg - 420 µg/kg. Other PAEs were detected below detection limit (≤50 µg/kg). The highest DEHP value (239 µg/kg) of the raw milk samples (n=24) taken before production was found in the machine milking to be used for yogurt production, while the lowest value (161 µg/kg) was found in the finger milking sample. The DEHP values in pasteurized milk samples (n=18) were 246 µg/kg, 251 µg/kg and 256 µg/kg for milk used in the production of yogurt, white cheese and kashar cheese, respectively.

The 1st day DEHP values for white cheese samples were determined as (n=6) 246 µg/kg for the white cheese samples in glass jar, and (n=6) 244 µg/kg for the ones in plastic-packaged, respectively. These values at the end of the 9th month were detected as 259 µg/kg for white cheese in glass jar and 420 µg/kg for plastic-packaged cheese. The 1st day DEHP value for kashar cheese were determined as (n=6) 240 µg/kg for the kashar cheese samples in glass jar

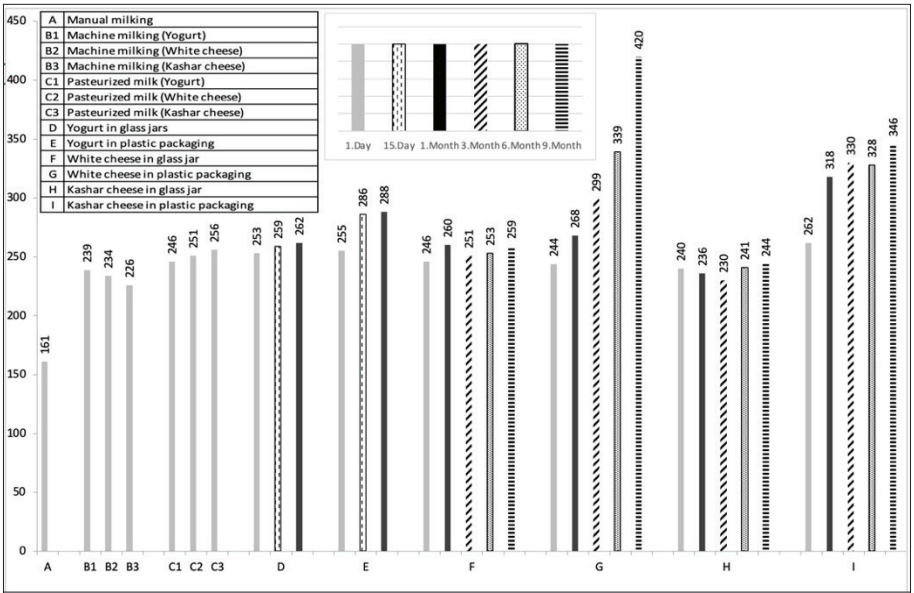

Fig 3. Initial DEHP values of the samples taken from the factory and DEHP change during storage in dairy products (µg/kg)

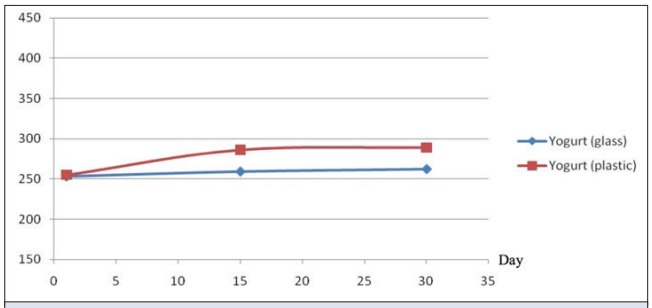

Fig 4. DEHP change of yogurt samples in glass jar and plastic packaged during storage (1st, 15th, 30th day) (µg/kg)

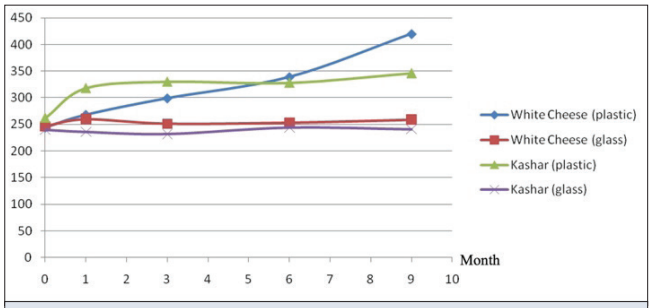

Fig 5. DEHP change in white cheese and kashar cheese samples in glass jar and plastic packaged during storage (1st, 3th, 6th, 9th month) (µg/kg)

and (n=6) 262 µg/kg for the ones in plastic-packaged. In the course of the storage period, the DEHP values of the samples in the glass jar at the 1st, 3rd, 6th and 9th months were found to be as 236, 232, 244, 241, respectively while the ones in plastic-packaged samples were determined as 318, 330, 328, 346 µg/kg, respectively. According to statistical analysis, differences between groups were found to be significant (P<0.05).

The 1st day DEHP value for yogurt samples was determined as (n=6) 253 µg/kg in glass jar yogurt and (n=6) 255 µg/kg in plastic-packaged yogurt samples, respectively. In the analyzes done during storage period, the values for glass jar yogurt samples were found to be as 259 µg/kg at the end of the 15th day and 262 µg/kg at the end of the 30th day, while the values for plastic-packaged yogurt samples were determined as 286 µg/kg at the end of the 15th day and 288 µg/kg at the end of the 30th day. Initial DEHP values of the samples taken from the factory and DEHP change in dairy products during storage period are shown in *Table 3* and *Fig. 3*. The 1st, 15th and 30th day DEHP changes of yogurt samples are demonstrated in *Fig. 4*, and the DEHP changes (1st, 3rd, 6th, and 9th months) of white cheese and kashar cheese samples during the storage period are shown in *Fig. 5*. According to statistical analysis, differences between groups were found to be significant (P<0.05).

Discussion

To know the levels of phthalate contamination before packaging (feed, raw milk and pasteurized milk) are important to clearly demonstrate packaging-induced phthalate migration in dairy products. In this context, analyzes were made in feedi raw milk and pasteurized milk samples in order to understand whether there is phthalate contamination in the pre-packaging stages. Accordingly, DEHP in feed was found below the LOD value. In the study, DEHP values in raw milk samples obtained by machine milking used in the production of yogurt, white cheese and kashar cheese were determined as 239 µg/kg, 234 µg/kg and 226 µg/kg, respectively, and these values were significantly higher than the DEHP value (161 µg/kg) in finger-milked raw milk samples (P=0.05). This difference has been associated with the potential migration of DEHP which will possibly result from the pvc milking pipes in

the milking machine and the milk tank [8,44].

When the 1st day DEHP values of the pasteurized milk used in production and the final products are compared in general, it is seen that there was no remarkable phthalate contamination during the production. Feng et al.[45] examined the PAEs in raw milk samples obtained by finger milking and machine milking from six cows in a factory. In the study, DEHP values of raw milk obtained by machine milking (111.67-283.90 ng/g) were 10-20 times higher than DEHP values of raw milk done by finger milking (8.40-23.72 ng/g), and it was concluded that this difference resulted from the migration of DEHP in the pipes of the milking machines into the milk. In another study which phthalate esters were investigated by GC/TOF-MS in 30 raw milk samples, DEHP values were detected in the range of 0-154 µg/kg in 15 samples, while DEP, BBP and DNOP were not found in any sample [18]. The values of the samples whose milking techniques, not specified, are similar to the values of the samples obtained by finger milking in our study, and these are considerably lower than the values of the machine milking samples.

In white cheese production, the DEHP values of glass jar and plastic packaged white cheese samples on 1st day are 246 and 244 µg/kg, respectively. While no noticeable increase in DEHP was observed in the white cheese samples in glass jars during the storage period (P>0.05), there was a significant increase especially in the 6th and 9th months in packaged samples (P=0.017). It was concluded that the increase possibly resulted from the white cheese packaging material, having the highest DEHP value of 272 µg/kg in the packaging material migration test. In addition, a significant increase in DEHP level towards the end of the storage period, in other words, acceleration of migration, was associated with increased acidity in white cheeses as a result of increased starter culture activity due to ripening [46]. The 1st day DEHP values of the kashar cheese samples in glass jars and plastic packaging for the production of kashar cheese were determined as 240 µg/kg and 262 µg/kg, respectively. Detecting these values similar to the amount of DEHP (251 µg/kg for white cheese, 256 µg/kg for kashar cheese) in pasteurized milk samples (n=18) used in production shows that any significant phthalate contamination is not formed at any stage of production after pasteurization. Any increase in the amount of DEHP was observed during the storage period (9 months) in kashar cheese samples conservation in glass jars. However, the amount of DEHP was 262 µg/kg on the 1st day in packaged samples (P>0.05) while it increased significantly in parallel due to the increasing acidity and became 318 µg/kg (P=0.017) at the end of 30th day. It was determined that this situation is caused by phthalate migration from packaging, as indicated in white cheese.

In yogurt production, regarding the 1st day of sampling DEHP values of the yogurt samples in glass jars and plastic packaging were 253 and 255 µg/kg, respectively. No significant change was observed in DEHP in the yogurt samples in both type packages during storage period (P>0.05). However, DEHP amount in the samples with plastic packaged were detected as 286 µg/kg and 288 µg/kg on 30th day. This increase is quite remarkable when short time of storage (30 days) is considered in terms of migration.

The results of the study could not be compared in this aspect due to the fact that there was no similar study on migration from pack to food. However, there are studies on the presence of phthalates in raw milk and dairy products (milk, yogurt and cheese). Within this scope, a study in which only DBP, DEHP and BBP detected from 6 PAEs by LC-MS/MS in plain yogurt and ayran samples DEHP values were stated as 24-122 µg/kg [47]. In a study carried by Ren et al.[48], 17 PAEs in yogurt and drinking milk by GC/MS were examined, the values of DEHP were determined as ND-144.5 µg/kg. In another study analyzed 16 PAEs in yogurt and drinking milk samples by GC/MS, the average DEHP values were reported as 52.4 µg/kg [49]. In Denmark, a study in which PAEs in raw milk, pasteurized milk, fruit yogurt and baby foods were examined, DEHP determined in all samples was found in the range of 7-138 ng/g [19]. All of these results are quite lower than the findings in our study. Raw milk, milking machine/technique, packaging material, etc. are thought to be the reasons of this situation. In a study the levels of DMP, DEP, DBP, BBP, DEHP, DNOP in fruit yogurt sold in the supermarkets and the migration levels of packaging materials analyzed, and it was stated that DBP, DEHP, DMP, DEP, DNOP and BBP were 76%, 70%, 70%, 54%, 20% and 8%, respectively, and that they were at levels from <LOQ to 1640 µg/kg. In general, it is seen that the values are quite higher than those detected in this study, and it is thought that this may result from fruits that come into contact with various sources of contamination (such as plastic irrigation hoses, plastic boots, plastic transport containers) during production [41]. DEP and DEHP were reported as PAEs determined in all samples from the packaging materials. As known, migration is affected by many factors such as raw materials, production equipment, packaging material, storage conditions. Therefore, it is an expected result that different PAEs are detected at various levels in the studies done on the subject.

The study, in which 6 PAEs in 11 different food groups obtained from Norway market were analysed by Sakhi et al.[50] using gas and liquid chromatography coupled with mass spectrometry, the highest DEHP value in cheese samples found was 173 µg/kg, while this in milk samples was 19 µg/kg. It was observed that the values were

considerably lower than the results of the study. In another study cheese, butter and fatty foods samples in England were also examined. It was observed that the highest DEHP value belonged to cheese sample with 17 mg/kg, and the total phthalate value was 114 mg/kg. In addition, the average DEHP value of the samples was 0.6-3.0 mg/kg. It was stated that the amount of phthalate, detected at a very high level in the samples, resulted from an effective source of contamination such as milking, production, packaging, etc. other than milk-raw material [20].

In conclusion, in this study, the legal limit value of the European Union that can cause migration when in contact with food was taken into account and DEHP values in packaged yogurt, white and kashar cheese samples at the end of the storage period were found below the legal limit (1.5 mg/kg) [19]. Although the phthalate values of the migration test results of the packages are low, the amount of DEHP that migrates to the packaged dairy products is at a level that cannot be ignored, and the risk, which may occur if the amount in the package is high, were showed. In other words, when the migration and the amount of DEHP in the packaged samples and the amount of DEHP detected in plastic packages were evaluated together, how important the material selection used in the food packaging is emphasized. In this respect, the study reveals that the migration test of the packaging material to be used in production and the determination of whether the packaging material is safe have great importance for the protection of public health. In this direction, it is thought that it is necessary to make legal regulations on the subject by taking into account the packaging safety in dairy industry.

Availability of Data and Materials

Data that support the findings of this study are available on reasonable request from the corresponding author (S. D. Korkmaz).

Ethical Approval

The study does not require any ethical approval.

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Competing Interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication

Author Contributions

ÖK and SDK planned and designed the study. SDK and MEŞ performed the experiments; SDK, GİA, and ÖK contributed to the analysis and interpretation of data. SDK, ÖK and GİA drafted the manuscript. All authors read and approved the final manuscript.

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