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Determination of phthalate esters contents in different types of cheese produced and consumed in Saudi Arabia

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ABSTRACT

Background: Phthalates' esters are commonly incorporated into plastic materials to enhance their elasticity, thereby facilitating their migration into food items. **Aims:** To evaluate the levels of phthalate esters in six distinct varieties of locally produced cheese and sold in Saudi Arabia, at the time of purchase (t=0) and after storage for one and two months. **Methods:** Six types of local cheeses (Cheddar, Mozzarella, Feta, Cheeseburger slices, Haloumi and Kashkaval) were purchased and analyzed at three different intervals: at the time of purchase (t=0), after one month, and after two months of storage. The Phthalate ester concentrations were determined using indirect gas chromatography with results expressed in mg/kg of cheese. **Results:** Among the cheese samples analyzed, di(2-ethylhexyl) phthalate (DEHP) was found to be the most prevalent. Halloumi cheese exhibited the highest average concentration of DEHP (4.29 ± 0.51 mg/kg) followed by Mozzarella cheese (3.65 ± 0.46 mg/kg). After two months of storage, more than two-thirds (n=13) of the samples exceeded the permissible limits content of DEHP content while one-third (n=6) and more than half (n=9) exceeded the limits for dibutyl phthalate (DBP) and di-n-octyl phthalate (DNOP), respectively. The levels of phthalates in all cheese types increased with prolonged storage duration with Cheddar cheese exhibiting the highest rate of increase, exceeding fivefold (from 0.34 to 2.29 mg/kg). **Conclusion:** This study confirms the presence of phthalate in cheese samples, with concentrations varying depending on the cheese type, specific phthalate compound, and storage duration. Heightened consumer awareness regarding cheese storage practices is imperative to mitigate potential health risks associated with phthalate consumption.

Keywords: Phthalate, cheese, chemical analysis, content, Saudi Arabia.

ARTICLE INFORMATION

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1 Introduction

Phthalates Softening plastics and polyvinylchloride is ensured using a group of chemical constituents called phthalates [1]. Plastic material become more extensible, elastic, and more workable by adding these polymers [2]. For this reason, phthalates are considered ubiquitous since they are extensively used when producing plastic material intended for human handling and use [3]. As a consequence, the exposure to these chemical compounds seems to be universal, affecting individuals of different ages and sex, as proved by biomonitoring studies [4]. Alarming findings reported that human exposure to phthalates is not without health consequences. Overweight/obesity, type 2 diabetes and insulin resistance, reduced reproductive outcomes, asthma, and allergy are all types of health disorders with significant association with increased exposures to phthalate [5]. Hence, researchers highlighted the global health concern related to phthalates present in consumable plastic [2].

It is well demonstrated that phthalate esters are not bound chemically to the plastic materials leading to their easy release into the environment at any of their production line until destruction. As per this chemical characteristic, phthalates are compounds that can be transferred from plastic to the material adjacent to them including foods and beverages wrapped in plastics. Therefore, food and beverage consumption are featured as considerable routes of human exposure to phthalates [6, 7]. Research studies showed that phthalates can potentially migrate to all types of food and beverages during

any step of the production process. Chemical studies explained that fat content in food is a promoting factor of phthalate migration. For example, a review of food monitoring and epidemiology data revealed that dairy-rich diets resulted in higher phthalate exposure risk [8]. Laboratory analytical studies confirmed this fact by findings indicating the existence of phthalates in most commercial milk products packaged in plastic materials. Thus, these food products strongly represent a possible route of phthalate migration and source of exposure [9, 10]. Similarly, Cruz et al. [11] concluded that cheese wrapped in plastic material and because of their content in fat should be seriously considered a health concern they might represent because of human exposure to phthalates. In the Kingdom Saudi Arabia (KSA), our recently published study conducted among Saudi adults revealed that this population has a poor knowledge of phthalates and poor practices with plastic, indicating a high risk of exposure to phthalates due to cheese consumption [12]. As per dietary habits, cheese consumption one to three times on a daily-basis was reported by more than one-third of Saudis [13]. In line with this fact, the results of the national representative survey carried out in 2016 indicated that the average amount of consumed cheese among men and women all together equaled to 43.7 ± 0.9 g/day [14].

Assuming the raising evidence-based results confirming the association between the human exposure to phthalate through foods and beverages and the negative health consequences, it is crucial to determine the content of phthalates in locally produced cheese in Saudi Arabia. Furthermore, performing this chemical quantification on different times of storage to shed light on how this could impact on this variable. It is important to note that there is currently a lack of research studies available regarding the assessment of phthalates in food products in Arab countries. This underscores the novelty and importance of our study, as it fills a critical gap in the existing literature and provides valuable insights into an area that has been largely unexplored in the region. Therefore, results of the present study would serve of basis to elaborate specific recommendations regarding the cheese handling by Saudi consumers and increasing their awareness regarding these chemical compounds. Emphasis could also be made on opting for healthier alternative wrapping and storing of the cheese food items. Accordingly, the present study aimed to estimate the content of phthalate in six different types of cheese locally produced, sold, and consumed by the Saudi population.

The novelty of our research lies in its pioneering investigation into the presence of phthalates in six varieties of cheese, all locally produced and consumed within Saudi Arabia. This study represents the first of its kind in the region, offering a unique perspective on food safety and environmental health concerns. Furthermore, our research extends beyond mere detection by incorporating an examination of phthalate levels across three distinct storage durations. By simulating home storage conditions, we not only capture real-world scenarios but also provide valuable insights into the potential changes in phthalate concentrations over time. This multi-faceted approach contributes significantly to our understanding of phthalate exposure in a culturally significant food item and underscores the importance of tailored research efforts in addressing national health challenges.

2 Methods

2.1 Cheese samples

Cheddar cheese, Mozzarella cheese, Feta cheese, Cheeseburger slices, Haloumi cheese and Kashkaval were purchased directly from the local market in Jeddah city, Saudi Arabia. Three different were selected for each cheese type to ensure a comprehensive analysis of phthalate esters. All cheeses met the criteria of being produced by Saudi companies, manufactured in Saudi Arabia, and packaged in plastic materials. However, it should be noted that not all brands offered all six types of cheese. Additionally, all samples had a shelf life exceeding two months.

2.2 Storage of the samples

Cheese samples, including Cheddar cheese, Mozzarella cheese, Feta cheese, Cheeseburger slices, Haloumi cheese and Kashk were prepared in dimensions of 8x8 cm with a thickness of 5 mm. Each sample weighed approximately 35 g. and the contact area between the film and cheese was approximately 64 cm^2.

Sampling for phthalates esters analysis was performed at three times: upon purchase (t=0), after one month of storage and after two months of storages. The samples were stored in a refrigerator at 5°C to replicate typical home storage conditions.

2.3 Chemicals

Analytical grade DEHA was purchased from Fluka, Buchs, Switzerland.

2.4 Phthalate analysis

The chemical analysis included 10 phthalate esters with are: Di-2 ethyl hexyly phthalates (DEHP), Di-n-butylphtalates (DBP), Butylbenzylphthalate (BBP), Diisononyl phthalate (DINP), Diisodecyl phthalate (DIDP), Dimethylphthalates (DMP), Diethylphthalated (DEP), Di-n- Octylphthalate (DNOP), Diisobutylphthalates (DIBP), and Di-(2-Ethylhexyl) Adipate (DEHA). The determination of phthalate content was performed according to the method outlined by Aly (2016) [15]. Initially, cheese samples were homogenized in a mortar and subsequently mixed with

anhydrous Na_2SO_4 (40 g). The homogenized samples underwent Soxhlet extraction using 150 mL of hexane for a duration of 6 hours facilitating the transfer of fat and plasticizer into the hexane phase. Hexane was then evaporated using a rotary evaporator, followed by the addition of 85 mL of KOH (2N) in CH_3OH and 35 mL of glycerin to the sample residue. Subsequently, saponification of the samples was achieved via reflux condenser for 3 hours leading to the decomposition of DEHA into adipic acid and 2-ethyl-1-hexanol. After evaporating the CH_3OH, samples were acidified with 25 mL of citric acid (1:1) and subjected to steam distillation after the addition of 35 mL of distilled H_2O until 200 mL of distillate was collected. The distillate was then adjusted to a pH of approximately 9 by adding a 10% aqueous solution of Na_2CO_3, followed by four extractions with 50 mL of diethyl ether. The combined ether extracts were left overnight with 25 g of anhydrous Na_2SO_4. Diethyl ether was then separated from Na_2SO_4 by filtration and evaporated in a roto evaporator. The resulting residue was redissolved in 5 mL of carbon disulfide, and this solution was utilized to determine the 2-ethyl-1-hexanol content via GC using an appropriate standard curve.

To determine the recovery factor of the method, cheese samples were prepared by spiking with known amounts of DEHA. The extraction of plasticizer, saponification, separation of the alcoholic constituent of plasticizer, and GC analysis were conducted using the same procedure as for experimental samples [16].

2.5 GC operational conditions

The determination of the alcoholic component of the DEHA, 2-ethyl-1-hexanol was conducted under specific conditions. The GC unit (Agilent Technologies 7890 A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with an apolar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30m x 0.25mm i.d. and 0.25 µm film thickness). Helium served as the carrier gas with a linear velocity of 1 mL/min.

The identification of components was based on a comparison of their mass spectra and retention time with authentic compounds supplemented by computer matching with NIST and Wiley library. Additionally, the fragmentation pattern of the mass spectral data was compared literature-reported patterns.

2.6 Data analysis

Results are expressed in mg/kg for each sample with the mean content and standard error calculated at three different storage times. The total phthalate content was determined by summing the content of all phthalates in each sample at a specific time of analysis.

3 Results

3.1 Phthalate content at t=0 of storage

Table 1 summarizes the results in the phthalates esters content in 6 types of cheeses locally produced and sold in Saudi Arabia. The analysis was performed at t=0 of storage. In terms of cheese type, six different phthalate esters (DEHP, DBP, BBP, DIDP, DMP, DEHA, and DNOP) were detected in both Mozzarella and Feta cheeses, while three only in Kashkavan cheese (DEHP, DMP and DEHA). Regarding the type of phthalates, DEHP was the most detectable in the cheese samples of the six types of cheese reported. The highest average was obtained for Halloumi cheese (4.29 ± 0.51 mg/kg) followed by Mozzarella cheese (3.65 ± 0.46 mg/kg). Both DBP and BBP were detected in 4 kinds of cheeses, the content in Mozzarella cheese was the highest with 0.18±0.05 mg/kg and 0.543 ± 0.06 mg/kg, respectively. DIDP was found only in two types of cheese (Mozzarella and feta), with Mozzarella showing higher content (0.016 ± 0.001 mg/kg). DMP was detected in four kinds of cheese, and the highest content was obtained in the Feta cheese (0.037 ± 0.03 mg /kg). Halloumi cheese had the highest content of DEHP and DNOP. The three phthalates' esters DINP, DEP, and DIBP were not detected or below the limit of quantification in all the samples. Two thirds (n=12) and 1/4th (n=4) of samples exceeded the permissible limits content of DEHP and DNOP, respectively.

3.2 Phthalate content at t=1 month of storage

After one month of storage, the phthalate ester content showed different patterns across the analyzed cheese samples (Table 2). In general, the content of phthalate esters increased compared to phase I. DEP and DINP were newly detected in several cheese samples, including Cheeseburger slices, Kashkavan cheese, and Feta cheese. The DIBP was the only phthalate ester not detected or below the limit of quantification in the cheese samples. DEHP continued to show the highest contents compared to other phthalate esters in the cheese samples. The highest content in DEHP, DEP, and DNOP were obtained in the Halloumi cheese (4.33±0.39 mg/kg, 0.083±0.009 mg/kg, and 1.02±0.13 mg/kg; respectively. Kashkavan cheese showed the highest content in DMP (0.199±0.018). More than two thirds (n=13) and one third (n=6) of samples exceeded the permissible limits content of DEHP and DBP, respectively. While in 5 samples the content of DNOP exceeded the permissible limits.

Table 1. Phthalates content (mg/kg) at t=0 of storage

Cheese Samples	Phthalates (mg/kg)									
	DEHP	DBP	BBP	DINP	DIDP	DMP	DEP	DIBP	DEHA	DNOP
Permissible limits	<1.5 [17]	<0.3 [17]	<30 [18]	< 9 [17]	< 9 [17]	None	None	None	<18 [19]	< 0.368 [20]
Cheddar cheese										
▪ **Brand A**	0.114	0.22	0.091	ND	LOQ	0.017	ND	ND	ND	0.032
▪ **Brand B**	0.132	LOQ	0.082	ND	LOQ	0.019	ND	ND	ND	ND
▪ **Brand C**	0.11	0.1	0.093	ND	LOQ	LOQ	ND	ND	ND	0.011
Mean ± SE	0.118 ± 0.01	0.106 ± 0.01	0.088 ± 0.001	-------	-----	0.012 ± 0.001	------	------	-----	0.014 ± 0.001
Mozzarella cheese										
▪ **Brand A**	**4.3**	0.27	0.56	ND	0.018	ND	ND	ND	1.91	**1.01**
▪ **Brand D**	**3.55**	0.29	0.66	ND	0.02	ND	ND	ND	2.1	**0.78**
▪ **Brand E**	**3.1**	ND	0.41	<LOQ	0.011	ND	ND	ND	1.5	**0.54**
Mean ± SE	**3.65 ± 0.46**	0.186 ± 0.05	0.543 ± 0.06	-------	0.016 ± 0.001	-------	-------	-------	1.83 ± 0.23	**0.77 ± 0.06**
Cheeseburger Slices										
▪ **Brand A**	1.7	ND	0.24	ND	ND	0.043	ND	ND	0.56	ND
▪ **Brand E**	2.1	ND	0.28	ND	ND	0.03	LOQ	ND	LOQ	0.033
▪ **Brand B**	LOQ	ND	0.3	ND	ND	LOQ	LOQ	<LOQ	ND	<LOQ
Mean ± SE	1.26 ± 0.15	-----	0.273 ± 0.03	-------	----	0.024 ± 0.02	-------	-----	0.18 ± 0.02	0.011 ± 0.01
Halloumi cheese										
▪ **Brand B**	**4.78**	0.25	LOQ	ND	ND	LOQ	LOQ	ND	1.71	1.23
▪ **Brand A**	**4.11**	0.24	LOQ	ND	ND	LOQ	LOQ	ND	1.52	<LOQ

▪ **Brand E**	**3.99**	ND	LOQ	<LOQ	ND	LOQ	LOQ	ND	1.32	1.11
Mean ± SE	**4.29 ± 0.51**	0.16 ± 0.012	-----	-----	-----	-----	------	-----	1.51 ± 0.022	0.78 ± 0.06
Kashkaval cheese										
▪ **Brand A**	1.42	ND	ND	ND	ND	0.022	ND	ND	0.59	ND
▪ **Brand D**	**2.11**	ND	ND	ND	ND	0.035	ND	<LOQ	0.45	<LOQ
▪ **Brand B**	**1.87**	ND	ND	ND	ND	LOQ	ND	ND	0.71	ND
Mean ± SE	**1.8 ± 0.23**	----	-----	-----	------	0.019 ± 0. 001	------	------	0.583 ± 0.065	-----
Feta cheese										
▪ **Brand A Full cream**	LOQ	0.31	0.091	ND	0.022	0.06	ND	ND	LOQ	0.023
▪ **Brand A White**	**2.11**	ND	0.063	<LOQ	0.02	0.051	ND	ND	LOQ	0.013
▪ **Brand E**	**1.56**	0.22	0.076	ND	LOQ	LOQ	LOQ	ND	LOQ	0.01
Mean ± SE	**1.22 ± 0.143**	0.17 ± 0.02	0.076 ± 0.06	----	0.014 ± 0.001	0.037 ± 0.03	----	-----	-----	0.015 ± 0.01

LOQ: Limit of quantification: 0.01 mg/kg, ND: Not detected. In Bold, the permissible limit is exceeded. DEHP: Di-2 ethyl hexyly phthalates, DBP: Di-n-Butyl Phthalate, BBP: Benzyl butyl phthalate, DINP: Diisononyl phthalate, DIDP: Diisodecyl phthalate, DMP: Dimethyl Phthalates, DEP: Diethyl Phthalates, DNOP: Di-n-octylphthalate, DIBP: Diisobutyl phthalates, DEHA: Di-(2-Ethylhexyl) Adipate

Table 2. Phthalates content at t=1 month of storage

Cheese Samples	Phthalates (mg/kg)									
	DEHP	DBP	BBP	DINP	DIDP	DMP	DEP	DIBP	DEHA	DNOP
Permissible limits	<1.5 [17]	<0.3 [17]	<30 [18]	< 9 [17]	< 9 [17]	None	None	None	<18 [19]	< 0.368 [20]
Cheddar cheese										
▪ **Brand A**	0.124	0.42	0.125	LOQ	0.05	0.087	ND	LOQ	ND	**0.741**
▪ **Brand B**	0.152	LOQ	0.23	LOQ	0.11	0.098	0.11	ND	0.66	0.34
▪ **Brand C**	0.121	0.12	0.28	LOQ	0.09	LOQ	ND	LOQ	ND	0.011
Mean ± SE	0.132 ± 0.01	0.18 ± 0.03	0.211 ± 0.04	-----	0.083 ± 0.006	0.061 ± 0.001	0.036 ± 0.002	-----	0.22 ± 0.03	0.364 ± 0.04
Mozzarella cheese										
▪ **Brand A**	**4.47**	**0.3**	0.99	ND	0.072	ND	ND	ND	2.9	**1.44**
▪ **Brand D**	**3.86**	**0.32**	1.2	ND	0.028	LOQ	LOQ	ND	2.6	0.88
▪ **Brand E**	**3.44**	LOQ	0.91	<LOQ	0. 10	LOQ	0.081	ND	2.1	0.62
Mean ± SE	**3.92±0.56**	0.2±0.03	1.03±0.21	------	0.066±0.001	----	0.027 ± 0.008	------	2.53 ± 0.35	0.92 ± 0.078
Cheeseburger Slices										
▪ **Brand A**	**1.8**	ND	0.44	ND	ND	0.11	ND	ND	0.82	ND
▪ **Brand E**	**1.9**	ND	0.71	ND	LOQ	0.09	LOQ	ND	LOQ	**0.92**
▪ **Brand B**	LOQ	ND	0.62	0.04	LOQ	LOQ	LOQ	<LOQ	ND	0.073
Mean ± SE	1.26 ± 0.26	-----	0.59 ± 0.06	0.04	-----	0.066 ± 0.001	------	------	0.27 ± 0.028	0.331 ± 0.065
Halloumi cheese										
▪ **Brand B**	**4.91**	0.29	0.09	0.05	ND	LOQ	0.1	ND	1.8	**1.23**
▪ **Brand A**	**4**	**0.31**	0.11	ND	ND	LOQ	0.086	ND	1.56	0.06

▪ **Brand E**	**4.11**	ND	0.08	<LOQ	ND	LOQ	0.065	ND	1.55	**1.78**
Mean ± SE	**4.33 ± 0.39**	0.200 ± 0.08	0.093 ± 0.003	0.05	----	-----	0.083 ± 0.009	------	1.63 ± 0.18	1.02 ± 0.13
Kashkaval cheese										
▪ **Brand A**	**1.52**	ND	ND	0.02	ND	0.051	ND	ND	1.2	0.1
▪ **Brand D**	**2.41**	ND	ND	0.05	ND	0.039	ND	<LOQ	0.99	0.065
▪ **Brand B**	**2.22**	ND	ND	0.05	ND	0.05	0.12	ND	1.3	ND
Mean ± SE	**2.05 ± 0.28**	----	---	0.04	-----	0.199 ± 0.018	0.04 ± 0.001	-----	1.16 ± 0.17	0.055 ± 0.006
Feta cheese										
▪ **Brand A Full cream**	LOQ	**0.71**	0.86	LOQ	0.052	0.09	ND	ND	LOQ	0.087
▪ **Brand A White**	**2.54**	**0.45**	0.82	<LOQ	0.04	0.088	0.055	ND	LOQ	0.043
▪ **Brand E**	**2.33**	**0.54**	0.76	0.09	0.031	0.06	LOQ	ND	LOQ	0.076
Mean ± SE	**1.62 ± 0.44**	**0.56 ± 0.08**	0.813 ± 0.09	0.03	0.041 ± 0.017	0.079 ± 0.03	0.018 ± 0.001	------	------	0.068 ± 0.003

LOQ: Limit of quantification: 0.01 mg/kg, ND: Not detected. In Bold, permissible limit is exceeded. DEHP: Di-2 ethyl hexyly phthalates, DBP: Di-n-Butyl Phthalate, BBP: Benzyl butyl phthalate, DINP: Diisononyl phthalate, DIDP: Diisodecyl phthalate, DMP: Dimethyl Phthalates, DEP: Diethyl Phthalates, DNOP: Di-n-octylphthalate, DIBP: Diisobutyl phthalates, DEHA: Di-(2-Ethylhexyl) Adipate.

Table 3. Phthalates content at t=2 months of storage

Cheese Samples	Phthalates (mg/kg)									
	DEHP	DBP	BBP	DINP	DIDP	DMP	DEP	DIBP	DEHA	DNOP
Permissible limits	<1.5 [17]	<0.3 [17]	<30 [18]	< 9 [17]	< 9 [17]	None	None	None	<18 [19]	< 0.368 [20]
Cheddar cheese										
▪ **Brand A**	0.126	**0.48**	0.131	ND	0.05	0.087	ND	ND	ND	**0.741**
▪ **Brand B**	0.154	LOQ	0.31	ND	0.25	0.14	0.11	0.055	0.67	**0.56**
▪ **Brand C**	0.125	0.23	0.28	ND	0.16	LOQ	ND	ND	ND	0.081
Mean ± SE	0.135 ± 0.004	**0.237 ± 0.065**	0.240 ± 0.04	----	0.153 ± 0.027	0.076 ± 0.019	0.037 ± 0.017	0.018 ± 0.009	0.223 ± 0.105	0.461 ± 0.093
Mozzarella cheese										
▪ **Brand A**	**4.47**	**0.3**	1.1	ND	0.072	ND	ND	ND	2.8	**1.57**
▪ **Brand D**	**3.88**	**0.32**	1.4	ND	0.032	ND	ND	ND	2.8	0.78
▪ **Brand E**	**3.42**	ND	0.91	<LOQ	0. 15	ND	0.11	ND	2.2	0.77
Mean ± SE	**3.923 ± 0.143**	0.207 ± 0.049	1.137 ± 0.067	0	0.052 ± 0.007	0	0.037 ± 0.017	0.000±	2.600 ± 0.094	1.040 ± 0.125
Cheeseburger Slices										
▪ **Brand A**	1.8	ND	0.74	ND	ND	0.13	ND	ND	1.1	ND
▪ **Brand E**	1.9	ND	0.71	ND	ND	0.11	LOQ	ND	LOQ	**0.92**
▪ **Brand B**	LOQ	ND	0.81	ND	0.05	LOQ	0.07	0.053	ND	0.081
Mean ± SE	1.233 ± 0.291	----	0.753 ± 0.014	----	0.017	0.080 ± 0.019	0.023	0.018	0.367	0.334 ± 0.139
Halloumi cheese										
▪ **Brand B**	**4.78**	0.25	0.14	ND	ND	LOQ	0.15	ND	1.8	**1.53**
▪ **Brand A**	**4.11**	0.24	0.11	ND	ND	LOQ	0.093	ND	1.56	**1.71**

Brand E	**3.99**	ND	0.08	0.056	ND	LOQ	0.067	0.06	1.99	**1.78**
Mean ± SE	**4.293 ± 0.116**	0.163	0.110 ± 0.008	0.019	----	----	0.103 ± 0.012	0.02	1.783 ± 0.059	1.673 ± 0.035
Kashkaval cheese										
Brand A	**1.52**	ND	ND	ND	ND	0.022	ND	ND	1.4	**0.76**
Brand D	**2.41**	ND	ND	ND	ND	0.035	ND	<LOQ	0.99	0.091
Brand B	**2.22**	ND	ND	0.071	ND	LOQ	0.22	ND	1.4	0.03
Mean ± SE	**2.050 ± 0.128**	----	----	0.024	----	0.019 ± 0.005	0.073	----	1.263 ± 0.064	0.294 ± 0.110
Feta cheese										
Brand A Full cream	LOQ	**0.73**	0.88	ND	0.022	0.06	ND	ND	LOQ	**1**
Brand A White cheese	**2.11**	**0.57**	0.83	0.091	0.074	0.083	0.057	ND	LOQ	0.67
Brand E	**1.56**	**0.54**	0.76	ND	LOQ	LOQ	LOQ	ND	LOQ	0.078
Mean ± SE	1.223 ± 0.298	**0.613 ± 0.028**	0.823 ± 0.016	0.03	0.032 ± 0.010	0.048 ± 0.012	0.019 ± 0.009	----	----	0.583 ± 0.127

LOQ: Limit of quantification: 0.01 mg/kg, ND: Not detected. In Bold, the permissible limit is exceeded. DEHP: Di-2 ethyl hexyly phthalates, DBP: Di-n-Butyl Phthalate, BBP: Benzyl butyl phthalate, DINP: Diisononyl phthalate, DIDP: Diisodecyl phthalate, DMP: Dimethyl Phthalates, DEP: Diethyl Phthalates, DNOP: Di-n-octylphthalate, DIBP: Diisobutyl phthalates, DEHA: Di-(2-Ethylhexyl) Adipate.

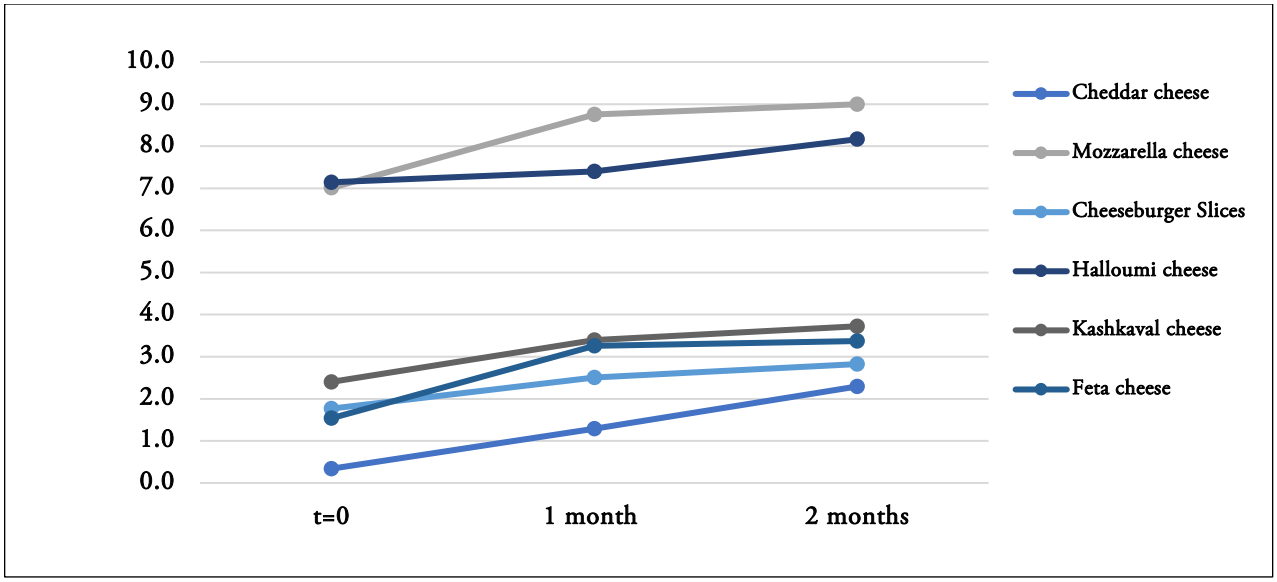

Figure 1. Total content of phthalate esters (mg/kg) in cheese samples according to different times of storage

3.3 Phthalates' content at t=2 months of storage

Table 3 presents the results of the phthalates esters chemical analysis in cheese samples after two months of storage. In general, the detected contents of these compounds revealed that in most cheese samples the phthalates increased compared to the findings after one month of storage. For example, the content has increased including DBP and DEHA in Mozzarella cheese DEHA; and DIDP in and DMP in cheddar cheese and DNOP in Feta cheese. Additionally, the phthalates ester DINP was detected for the first time in 4 cheese types (Cheeseburger, Halloumi, Kashkavan, and Feta). In terms of permissible limits, it was the same as registered in t=1 month of storage for DEHP and DBP while more than half (n=9) of samples revealed content in DNOP higher than the permissible limit.

3.4 Evolution of the total content of phthalate esters in cheese samples according to different times of storage

The evolution of the total content of phthalates esters in cheese samples according to different times of storage is presented in Figure 1. Mozzarella cheese followed by Halloumi cheese contained the highest concentration of phthalates compared to the other kinds of cheese. Furthermore, the content of phthalates in all types of cheese increased with the time of storage. The content ranged between 0.34 - 7.143 mg/kg at=0 versus 2.293 -8.995 mg/kg at t=2 months. The highest increase rate was obtained in Cheddar cheese as the total content increased by more than 5 times (from 0.34 to 2.29 mg/kg). Furthermore, our findings indicate a notable distinction among the various cheese types. Specifically, for Mozzarella and Feta cheese, a consistent level of phthalate esters is observed after one month of storage. In contrast, the opposite trend is noted for Halloumi cheese, where the phthalate ester content exhibits variability over the same duration.

4 Discussion

Various research studies have suggested that for phthalates, the intake of contaminated foods is the most important exposure pathway for the general population. Yet, up to now, findings on phthalate content in different types of food in Saudi Arabia are scarce. Therefore, the present study assessed the content of 10 different phthalate esters in 18 samples belonging to 6 different types of cheese. Of interest, this is the first study of its kind in Saudi Arabia as the samples included in the analysis are locally produced and consumed by the Saudi population. The main findings of our study indicate that the most detectable phthalate ester and most exceeding the permissible limits was DEHP followed by DBP. The cheese types with the most phthalates contents were Cheddar, followed by Mozzarella. While the overall trend of content implies a progressive increase as the duration of storage is lasting. However, the importance of such a rise over the storage time varies among the cheese samples, where the highest rate of increase obtained in Cheddar cheese.

In their studies, Kormak et al. [21] have confirmed that the migration of phthalates to dairy products including cheese might happen from the packaging materials. This is reflected in the cheese samples included in our study, as different phthalate esters were detected. In more detail, a systematic review by da Costa et al. [22] concluded that dairy products were listed among foods with the highest content in DEHP and DBP. These authors also confirmed the legal limits of these phthalates were exceeded, representing a potential risk exposure for both adults and children [22]. Similar results were obtained in our study where the average content of DEHP and DBP was highly detected. When summing up the total content of phthalates, the highest average was 7.143 mg/kg at=0 and reached 8.995 mg/kg at t=2 months of storage (Mozzarella cheese). These values are very elevated compared to the content report in the literature of 3 mg/kg of phthalates in cheese samples[2]. Nevertheless, these differences could be related to the type of cheese and phthalates analyzed.

This evidence from our present study sheds light on potential concerns about the Saudi consumer's safety considering that these molecules are iniquitous, and their intake has been demonstrated to be associated with various health outcomes [23]. Furthermore, we have recently demonstrated a poor level of knowledge of phthalates, plastic use, and handling and storing cheese wrapped in plastic materials among adults in Jeddah (Saudi Arabia) [12]. Indeed, the storage of cheese in plastic material led to a rise in the type and amount of phthalates detected, as shown through the values obtained at different times of storage (t=0, t=1 month of storage and t=2 months of storage). With all samples exceeding a duration of two months in terms of shelf life, this extended storage period could potentially impact the phthalate content in the cheese.

Limitations of this study might be related to the fact that parameters such as temperature of storage, surface content between the cheese, and plastic and difference in packaging plastic were not considered in the analysis. These parameters have been discussed as potential factors influencing in phthalate content in food [21, 24]. However, the authors mimicked the household conditions of storage as real as possible, and in consequence, the obtained values would reflect the real content in phthalate esters.

5 Conclusion

Our study substantiated the presence of phthalates in the cheese samples, with varying concentrations depending on the cheese type, the specific phthalate compound studied, and the duration of storage. Consequently, increasing awareness among Saudi consumers regarding cheese storage practices in crucial to mitigate potential health risks associated with phthalates consumption. Given that individuals may not have the capacity to fully address such risks independently, it is paramount for public health authorities to prioritize measures aimed at minimizing the exposure of Saudi consumers to phthalates. We recommend initiating a call to action directed at relevant authorities in Saudi Arabia to implement proactive measures for enhanced monitoring of phthalate content and migration from plastic packages to food. Given the potential health hazards associated with phthalate exposure, establishing robust monitoring programs to assess phthalate levels in various food products consumed by the population is essential. Furthermore, expanding the scope of investigation to encompass a broader range of frequently consumed foods wrapped in plastic materials in Saudi Arabia is highly advisable to gain a comprehensive understanding of phthalate exposure pathways. By prioritizing these initiatives, authorities can safeguard public health and facilitate evidence-based regulatory measures aimed at reducing phthalate exposure and ensuring the safety of food products in Saudi Arabia. In addition, the new trend to innovate and produce non-phthalate plasticizers should get the appropriate attention and support. This may involve replacing current plastic material packaging with innovative technologies designed to minimize or eliminate plasticizer migration, such as surface sealing of polyethylene (PE) or the use of alternative films containing significantly lower levels of plasticizer. In terms of research, conducting evaluations or risk assessments among the Saudi population based on their typical cheese consumption patterns would complement the findings of our study.

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