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PHTHALATE RESIDUES IN PLASTIC PACKAGED MILK AND DAIRY PRODUCTS

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ABSTRACT

This study aims to search for the organic lipophilic of phthalates in plastic packaged pasteurized milk and the dairy products of fermented milk "Rayeb" and Domuatti cheese, locally produced and marketed in Cairo of Egypt. A pre-validated method using GC-MS was applied to detect the residues of the 6 phthalate congeners commonly used as plasticizers in bottles and food containers manufacturing. Such plasticizers are intentionally added to enhance the technical and industrial properties of the plastic containers used in food industry. Data of spiked samples obtained from the applied method showed average recovery percentages of 90.8 ± 5.3 and a percentage of coefficients of variation equivalent to 4.41 ± 0.6, depending upon both phthalate congener and the concentration of spiked samples. The corresponding percentages of recovery average of Rayeb and Domuatti cheese were 91.0 ± 2.1 and 88.3 ± 1.8, respectively, while the percentages of coefficient of variation were 3.31 ± 0.1 and 3.85 ± 0.9, respectively. However, data revealed that none of the 6 phthalates of DMP, DEP, DBP, BBP, DEHP and DOP were observed in any sample of milk, Rayeb and Domuatti cheese samples examined during the first month of production in both bottle and container sizes. As well, none of the 4 phthalates of DMP, DEP, DBP and/ or BBP were found in the 3 studied products up to the last month of expiry. Only 2 and 3, each out of 24 and 2 out of 18 samples of milk, Rayeb and Domuatti cheese, respectively showed low levels of contamination with DEHP or DOP. The determined residues of DEHP or DOP phthalates were ranged between 30-88/ng/ml. Moreover, the effect of the variables of 6 phthalate congeners, different brands, different container sizes and the storage time were discussed in this work.

KEYWORDS: Phthalate congeners – lipophilic – fermented milk "Rayeb" – Domuatti cheese – coefficient of variation

INTRODUCTION

All food packaging materials contains substances that can migrate into the food they contact, including the plasticizers which commonly used to enhance the technical and industrial properties of plastic containers. Phthalates are a group of organic lipophilic chemicals primarily used as plasticizers (Amrit et al., 2014). Phthalate plasticizers (PAE's) are defined as benzene-di-carboxylic acid esters with different degrees of toxic effects especially endocrine disrupting changes (Saad 2014). Humans are exposed to phthalates through the 3 known routes of ingestion, inhalation and dermal exposure. Both dermal exposure and inhalation are common to short chain phthalates like dimethyl and diethyl phthalates (DMP & DEP) due to the daily usage of soap, shampoo, conditioner and other personal care products. While, oral exposure is mainly due to ingestion of phthalate-contaminated food with the long chain phthalates like di-ethylhexyl and di-n-octyl phthalates "DEHP, DOP" (Latini et al.,(2005). Under certain circumstances, higher exposure could occur based on occupational workplace and/ or the medical settings when using plastic syringes, tubing and bags (Douell et al., 1999). So exposure to different phthalate congeners should be discussed within the different cultures, habits, medical and nutritional status of population (Zhang et al., 2013). Recent studies observed that phthalates were found in all environmental media (air, water, soil and sediment) at different concentrations in many countries which reflect the widespread usage of plastic products (Saad, 2014). However, human exposure to phthalate esters mainly occur through dietary intake, especially plastic packaged foods due to the weak non covalent bond between phthalates and their parent materials which leads to release and bioaccumulation of phthalate esters into the packaged consumed food (Koch et al., 2003 and Wittassek et al., 2011). The long-side chain high molecular weight phthalate of DEHP is commonly used in processing and manufacturing the food packaging containers (Latini, 2005). Phthalate plasticizers have been shown to release at constant rate from plastic containers to the surrounded media and/ or food (Zhang et al., 2013). As well, Marja et al., (2014) reported that processed and packaged foods are the major sources of human exposure to phthalates and DEHP was the most detected phthalate congener contaminated milk and dairy products. So, this study was designed and conducted to search for the most common phthalates of di-methyl phthalate (DMP), di-ethyl phthalate (DEP), di-brobyl phthalate (DBP), benzylbrobyl phthalate (BBP), di-ethylhexyl phthalate (DEHP) and dsi-n-octyl

phthalate (DOP) in random samples of pasteurized milk, fermented milk "Rayeb" and Domuatti cheese packaged in plastic bottles and containers and sold in local markets of Cairo.

MATERIALS AND METHODS

Twenty four samples, each of pasteurized milk and fermented milk "Rayeb", packaged in plastic bottles and 18 samples of Domuatti white cheese - kept in plastic containers too - were collected randomly from different markets of Cairo. The collected samples, representing different commercial brands, 2 size of containers and storage time, were analyzed for the residues of the studied 6 phthalates of DMP, DEP, DBP, BBP, DEHP and DOP. All samples were analyzed at 2 dates of intervals, the first month of production and at the 9th month before the expiration date as recommended by the Egyptian Organization of Standards (EOS). The samples which analyzed at the second interval were kept sealed well at a refrigerator (4 - 5 C) as recommended by the manufacturer instructions on the label, till the time of assay after 9 months. An individual phthalate ester standard (s) and the mixture of the studied 6 phthalates were obtained from Restek Co, UK, each was dissolved in methanol at concentration of 2 mg/ ml. An individual standard(s) of phthalates were used to confirm and validate the data of retention time, peak shape and peak area corresponding to those obtained from the mixture of the 6 standards and the attached typical chromatogram, as well. The adopted solvents in this method were of chromatographic grade (PA-ACS-Areca-EU) and other reagents were obtained from Sigma-Aldrich Co.USA. Gas Chromatography with Mass Spectrum detection (GC-MS) was the instrument applied in this current research as recommended by many previous studies (Mahmoud & Amamar, 2008, Mohammed et al., 2013 and Saad et al., 2015). The dimensions of 30 m x 0.25 mm. x 0.1 m- film thickness. Helium gas was the carrier flowed at a constant rate of 1 ml/ min. The program of temperature was initiated at 40 C (hold 3 min.) up to 280 C, as a final temperature 5 C (hold 5 min.) and the injection temperature was 280 C and the electron ionization energy of 70 eV was used for GC-MS detection as recommended by Saad et al., (2015).

200 ml. of bottled pasteurized milk and/ or fermented milk "Rayeb" or 200 gm of Domuatti white cheese, each was homogenized well with 250 ml. of hexane-acetone (80:20 v/v) for approximately 10 min. Filtration of the homogenized sample was obtained using filter paper Whatman No.4 and transferred to solid phase cartridge then washed with 20 ml diethyl ether before extracting the sample with 20 ml methanol. The final extract of methanol was

completely dried under stream of nitrogen at 50 C, and then the dry film was reconstituted for injection. Three sets of spiked samples of each of the different 3 products were prepared at concentrations of 25, 50 & 100 ng/ ml. of each of the 6 studied phthalates. The applied method was validated by checking the percentages of the recovery and the percentages of coefficient of variation of the injected spiked samples as recommended by (ICH, 2005). The percentages of recovery were evaluated by comparing the peak heights of spiking blank samples of each product with the relative chromatogram of the 6 studied phthalate congeners. Only cheese samples showed the need for further clean-up steps to avoid the interfering layers. So, clean-up was conducted using florisil columns and washed with a system of 20 ml. each of ethyl acetate and dichloroethane, respectively before eluting with methanol.

RESULTS AND DISCUSSION

Tables 1-3 show the calibration data of spiked blank samples of pasteurized milk, fermented milk "Rayeb" and Domuatti cheese with 25, 50 and 100 ng/ ml. of each of the 6 studied phthalate congeners. The highest percentages of recovery were obtained with the plasticizer of BBP I n spiked milk samples at concentrations of 100 ng/ ml., being 94.6%. While, the lowest corresponding values of percentages of recovery were obtained from Domuatti cheese samples spiked with 25 ng/ ml. of the same congener BBP being 82.9%. However, the averages of recovery % of both milk and Rayeb at the 3 different levels of spiking were 90.8 ± 5.3 and 91.0 ± 2.1, respectively. As well, the average coefficient of variation % was 4.41 ± 0.6 and 3.31 ± 0.1, respectively for milk and Rayeb spiked samples at the 3 different levels of spiking.

Table(1). The percentages of average of recovery and average of coefficient of variation of spiked blank pasteurized milk samples at the 3 levels of the 6 studied phthalates

PHTHALATE(s)	25 (ng/ml)	50 (ng/ml)	100 (ng/ml)	Average of Recovery%	Av.Coeff. of Var.%
DMP	86.6	92.1	94.3	91.0	4.8
DEP	85.7	88.4	91.6	88.6	5.2
DBP	93.8	89.9	93.4	92.4	3.9
BBP	94.6	91.4	92.5	92.8	4.1
DEHP	88.4	87.8	94.3	90.2	4.7
DOP	91.3	90.1	88.4	89.9	3.8
Av. Mean ± SD				90.8± 5.3	4.41± 0.6

Table (2).The percentages of average of recovery and average of coefficient of variation of spiked blank Rayeb samples at the 3 levels of the 6 studied phthalates.

PHTHALATE(s)	25 (ng/ml)	50 (ng/ml)	100 (ng/ml)	Average of Recovery%	Av.Coeff. of Var.%
DMP	88.6	91.5	94.1	91.4	3.9
DEP	87.7	92.0	93.2	91.0	2.8
DBP	91.3	90.4	94.3	92.0	2.6
BBP	90.8	89.9	91.1	90.6	3.6
DEHP	89.6	88.8	93.7	90.7	3.7
DOP	87.4	90.3	92.1	90.0	3.3
Av. Mean ± SD				90.95± 2.1	3.31± 0.1

The corresponding averages of recovery % obtained from spiked samples of Domuatti cheese showed less percentage values than both milk and Rayeb being 88.3 ± 1.8 and the average coefficient of variation % was 3.85 ± 0.9. The less obtained values for the average recovery % might be due to the extra step of clean-up the procedure applied only with the samples of Domuatti cheese.

Table (3). The percentages of average of recovery and average coefficient of variation of blank spiked samples of Domuatti cheese with 3 levels of the 6 studied phthalates.

PHTHALATE(s)	25 (ng/ml)	50 (ng/ml)	100 (ng/ml)	Average of Recovery%	Av.Coeff. of Var.%
DMP	85.3	88.7	94.6	89.5	5.4
DEP	84.7	87.9	91.3	88.0	3.1
DBP	83.8	86.5	90.4	86.9	4.1
BBP	82.9	91.4	91.0	88.4	3.7
DEHP	84.1	90.7	89.7	88.2	2.9
DOP	83.3	91.3	91.3	88.6	3.9
Av. Mean ± SD				88.3± 1.8	3.85± 0.9

Wendi et al. (2009) reported that on processing, the DEHP contaminated-milk and dairy products lead to high concentrations of DEHP in the yielded cream, whereas low fat milk contained the less concentrations of phthalate plasticizers. Also, Yong et al. (2005) exhibited that the detection limit for the phthalate DEHP was from 0.31 to 3.3 ng/ g for samples containing up to 10.8% fat.

Tables (4 & 5) show phthalate residues of the 6 studied congeners in pasteurized milk and the fermented milk "Rayeb" in random samples collected from markets of Cairo. The collected samples representing, two manufacturer brands, 2 plastic container/ bottle sizes and 2 dates of

intervals (the date of month of production and the date of the last month before expiry). Data show that none of the 4 phthalates of DMP, DEP, DBP and BBP were observed in all examined samples of milk and/ or Rayeb packaged in either small or large bottles. Data revealed that out of 24 samples, each of pasteurized milk and Rayeb only 3 and 2, respectively showed positively phthalates-contaminated samples.

Table(4). Phthalate residues of the 6 studied congeners in pasteurized milk sold in Cairo markets.

PHTHALATES	BRAND 1				BRAND 2			
	LARGE		SMALL		LARGE		SMALL	
	1	9	1	9	1	9	1	9
DMP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	3 N
DEP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	3 N
DBP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	3 N
BBP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	3 N
DEHP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	(2 P)
DOP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	(1 P)

1 – Data obtained during the first month of production

9 – Data within the last month before expiration date as recommended by labeling.

3 N – all the 3 tested samples were negative for the 6 phthalates.

1 & 2 P – Number of positive contaminated samples (out of 3).

Large – 1 liter plastic bottles of commercial pasteurized milk.

Small - 500 ml. plastic bottles of pasteurized milk.

Table (5). Phthalate residues of the 6 studied congeners in fermented milk "Rayeb".

PHTHALATES	BRAND 1				BRAND 2			
	LARGE		SMALL		LARGE		SMALL	
	1	9	1	9	1	9	1	9
DMP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	3 N
DEP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	3 N
DBP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	3 N
BBP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	3 N
DEHP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	(1 P)
DOP	3 N	3 N	3 N	3 N	3 N	3 N	3 N	(1 P)

Quantitative assay of the 5 positive samples show levels of contamination ranged between 30 – 50 ng/ ml. of either DEHP or DOP, only. Latini et al. (2005) reported that DEHP is the most abundant phthalate in both industry and environment. Samantha et al. (2014) revealed that the short-chain low molecular weight phthalates like DMP and DEP were mainly used in

cosmetics and personal care products, while the long-chain high molecular weight phthalates like DEHP and DOP were mainly used in toys, bottles and food products packaging. Wendi et al. (2009) found residues of DEHP in the raw milk of Norway obtained during different stages of collection, transportation and packaging. The same authors added that the total levels of contamination (expressed as DEHP equivalent) in the raw milk were between 0.12 – 0.28 mg/ kg. They suggested that on processing the DEHP phthalate is concentrated in the cream at levels up to 1.93 mg/ kg, whereas low fat milk contained from 0.01 to 0.07 mg/ kg. As well, In Spain, retail dairy products showed contamination level of 0.01 – 0.55 mg/kg. and total phthalates of 0 – 3 mg/ kg. in cream samples. In UK pooled milk samples from doorstep delivery obtained from different regions of the country showed low levels of contamination with DEHP being 0.01 – 0.09 mg/ kg and total phthalates ranged between 0.06 – 0.32 mg/ kg.(Wendi et al., 2009).

Table(6). Phthalate residues of the 6 studied congeners in Domuatti white cheese sold in markets of Cairo.

PHTHALATE Congener(s)	Brand (1)		Brand (2)		Brand (3)	
	1	9	1	9	1	9
DMP	3 N	3 N	3 N	3 N	3 N	3 N
DEP	3 N	3 N	3 N	3 N	3 N	3 N
DBP	3 N	3 N	3 N	3 N	3 N	3 N
BBP	3 N	3 N	3 N	3 N	3 N	3 N
DEHP	3 N	3 N	3 N	(2 P)	3 N	3 N
DOP	3 N	3 N	3 N	3 N	3 N	3 N

1 – Data obtained during the first month of production.

3 N – all the 3 tested samples were negative for the 6 phthalates.

2 P – Number of positive contaminated samples (out of 3).

All samples tested were packaged in plastic box containers of 1 kg Domuatti cheese.

Table 6 shows the phthalate residues of Domuatti cheese samples intervals (the 1 st month of production and the 9th month before expiry). Data revealed that none of the 6 studied phthalates were observed in any of the tested samples of the 3 commercial brands during the first month of production. As well, all samples of brands 1 and 3 were remained phthalate(s)-free up to the last month of expiration. Only 2 out of 3 samples of brand 2 showed low level of contamination with only the DEHP plasticizer when examined during the last month before expiry. Phthalate plasticizers have been shown to release at constant rate from plastic containers to the surrounding media (Zhang et al., 2013). The determined concentrations of

DEHP in the 2 contaminated samples were o.45 and 0.88 mg/ kg. In a comprehensive study conducted in 3 European countries (UK – Norway & Spain), DEHP and total phthalate esters were determined in milk, cream, butter and cheese samples. The lowest levels of contamination were observed in Norway being 0.12 – 29 mg// kg. in the raw milk, while the highest level of contamination, being 17 – 114 mg/ kg. of total phthalates were obtained from cheese samples collected from UK (Wendi et al., 2009). Regarding our best of knowledge, no literature is available dealing with the contamination levels of dairy products or other food products packaged in plastic containers and sold in Egyptian markets with phthalate plasticizers. Only, Mahmoud and Ammar (2008) who reported high levels of contamination with the 6 phthalates of DMP, DEP,DBP, BBP, DEHP & DEHA when they working on the traditional Egyptian food (Koushry and Medams), black tea and constant coffee. But, they reported that all determined levels were below the restricting limits of the European Union Council (2000). Also, Saad et al. (2015) found that only 2 out of 81 samples had traces of DEHP and/ or DOP when analyzing water bottled samples for the 6 common phthalate congeners. They added that all tested samples were proved to be phthalate(s)-free analyzed at the interval of 6 month of storage which show low level of contamination (22 ng/ ml.).

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