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PHTHALIC ACID ESTERS CONTENT IN YOGHURT WITH CHIA FLOUR AND BAMBOO FIBER DURING STORAGE TIME

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ABSTRACT

Phthalic acid esters are plasticizers, they can migrate freely from plastic to their surroundings. They have negative health effects. European legislation sets specific migration limits for phthalic acid esters. In our study, we deal with two esters of phthalic acid, dibutylphtalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP). It was studied the effect of storage of four types of yoghurt on the concentration of phthalic acid esters. Yoghurts contained 1% chia flour, 5% chia flour, 1% bamboo fiber and 5% bamboo fiber. Yoghurts were stored in plastic cups, which contained both examined phthalic acid esters. Esters of phthalic acid were determined after 1 week of storage then after 2 weeks of storage and in the original raw material. Furthermore, the pH of the yoghurt was determined. The pH values were correlated with phthalate concentrations: the correlation coefficient for DBP with a pH of -0.0265 and for DEHP with a pH of 0.3075. Mean concentrations of DEHP decreased over time, while DBP decreased for yoghurt with 1% chia flour, while in other cases they increased. The mean DBP concentrations in yoghurt were higher than the average concentrations of DEHP. Comparing the mean sample values with t-test for dependent samples for yoghurt of the same type, when comparing the DBP or DEHP concentration in week 1 with the DBP or DEHP concentration at week 2, the mean values were consistent. It can be noted that there was no increase in DEHP concentrations from cups to yoghurts, which was probably due to a lower concentration in cups than the DBP concentration. DBP concentrations increased in 3 of the 4 types of yoghurt. The determined pH in yoghurts did not differ significantly. Apparently a greater effect on the migration of phthalic acid esters will have in our case a different yoghurt consistency than pH. It would be appropriate to examine the effect of food density on the migration of phthalic acid esters. Likewise, it would be appropriate to examine the effect of pH but in the same food with different pH on the migration of phthalic acid esters.

Keywords: yogurt; dietary fiber; contaminant; dibutylphthalate; di-(2-ethylhexyl) phthalate

INTRODUCTION

Phthalic acid esters represents important plasticizers. They are also present in food packaging materials. Yet they are not covalently bonded which means that they can migrate into their surroundings **(Benson, 2014)**. In case of plastic food packaging, which is in contact with food, plasticizers can migrate both in the plastic, and to the surrounding area, respectively into food **(Piotrowska, 2005)**. When components of plastic packaging material – mostly monomers and additional components – migrate, the material is not completely destroyed, it still preserves technologically significant properties. At the same time, migrate the ingredients of the food in the package, which can affect the migration speed of the ingredients from the package to the food **(Velíšek, 2002)**.

Phthalic acid ester migration from packaging materials into fatty food is very important and closely monitored **(Petersen, 2003)**, as phthalic acid esters are strongly lipophilic **(Velíšek and Hajšlová, 2009)**. Being plasticizers, phthalic acid esters are present in a very large amount in softened PVC where they can make up to 50 % of the weight. Food packaging foil is made of softened PVC as well. Phthalates are used abundantly also for non-food purposes, which causes environmental expansion and the possibility of secondary contamination from the environment **(Brimer, 2011)**. Phthalates are also used in nail polish, perfumes, print inks, toys for children, and flooring **(Mikula et al., 2011)**. Phthalates are also found in the room facilities, but there is found a difference in the time. At the time, when the phthalates had not regulated, and today's when is existing phthalate control and regulation. Exposure values dropped compared with measurements fifteen years ago, it was the age without regulation **(Larsson et al., 2017)**. European legislation lays down limits for phthalic acid ester migration **(Brimer, 2011)**. The Commission Regulation (EU) no. 10/2011 defines specific migration limits (SML) which represents the largest permissible amount of released substance that is set free from objects into food. The SML value for DBP is 0.3 mg·kg^{-1} and for DEHP it is 1.5 mg·kg^{-1} food. The overall migration limit represents the highest permitted amount of released non-volatile matter from materials into food. The overall migration limit is defined as 10 mg per 1 dm^2 **(Commission Regulation (EU) No 10/2011)**.

Phthalic acid ester occurrence in food is caused especially by the food getting in touch with the material, be it during processing, handling or transport, but also contamination or fallout on the agricultural crops may occur **(Benson, 2014)**. Yet it has been found out, that even manually milked milk contains phthalic acid esters although it is being presupposed that the cattle digestive tract is to hydrolyse phthalic acid esters to mono-esters and alcohols and excrete them in urine as glucuronide conjugates **(Petersen, 2003)**.

Of course, phthalic acid esters are not sole plasticizers, as plasticizers are used: too adipates, trimellitates, citrates, polymerics, etc. They number about 8 types of commonly used plasticizers **(Andrady and Neal, 2009)**.

Humans are exposed to phthalic acid esters via their skin, they can inhale or swallow it **(Clark et al., 2011)**. Phthalic acid esters have a negative impact especially on the reproductive system; administering phthalates to male rats during the perinatal stadium developed cryptorchism, a low number of sperms and hypospadias **(Martino-Andrade and Chahoud, 2010)**. Hepatotoxicity of phthalates was particularly solved, finally it was found that phthalates are endocrine disruptors **(Latini, 2005)**. In animal studies, in which was the animals exposed of DBP had occurred the development and reproductive changes, decrease fertility, increase the number of fetal malformation, exposure of DBP cause disease of liver, kidney and haematology **(ATSDR, 2001)**. It was found low toxicity in inhalation exposure of DEHP, nevertheless was found mortality in rats, after 2–4 hours of inhalation exposure, lung lesions were observed in neonates with mechanical ventilation where respiratory tubes were made of PVC. Pulmonary mass was increased by rats after inhalation of aerosol with DEHP. By oral exposure, the product of metabolism mono-(2-ethylhexy) phthalate (MEHP) is cardiotoxic. Acute exposure in humans causes diarrhoea and abdominal pain. In mice and rats it causes hyperplasia and hypertrophy of liver cells. Negative effects on the liver were found more in mice and rats than in dogs and monkeys, males have been always more prone than females. It has also been established that administration of DEHP to the rat feed dose resulted in a reduction of body weight. Oral administration of DEHP has a negative effect on the reproductive system - it reduces the weight of the testicles, causes deformities and abnormalities. The effect on the female reproductive system was also found when female mice were unable to have litters. In addition, developmental toxicity was found in mice and rats - malformation or resorption of foetuses, lower fetal and juvenile weight, increased abortion rates. Long-term administration of DEHP led to the formation of liver tumours in mice and rats **(ATSDR, 2002)**.

Animal studies indicate that the developmental and reproductive toxicity of phthalates is most affected by the individual as a fetus. As well as the phthalic acid esters are investigated for suspicion that they cause premature births in humans and impair sperm quality, etc. **(Hauser and Calafat, 2005)**. However, studies of the effects of phthalates on humans are limited by the low number of data and inconsistencies of the data **(Meeker et al., 2009)**. Due to the abundant use of phthalates, they have become ubiquitous **(Velíšek, 2002)**. Phthalic acid esters are easily released, and are found in the environment, e.g. in plants, soil, air, water, sewage, etc. **(Przybylińska and Wyszkowski, 2016)**. In the German study, most of the DEHP was found in surface wastewater and sewage and sludge water, and DBP was found only in low concentrations **(Fromme et al., 2002)**.

Degradation of phthalic acid esters from the environment can be through biodegradation – many organisms can catalyse the hydrolysis of phthalates **(Velíšek, 2002)**. Easy biodegradable are all commonly used phthalic acid esters **(Cadogan, 2002)**. In mammals, up to 90% of phthalates are excreted during metabolism from the organism, but also they are partially deposited in the organism **(Velíšek, 2002)**. Daily estimated phthalate intake of DEHP is around 4.9–18 μg·kg^{-1} and day for humans and daily estimated phthalate intake of DBP is at 0.48 μg·kg^{-1} and day **(Schettler, 2006)**. The tolerated daily intake of DEHP is at 37 μg·kg^{-1} body weight and day **(Koch et al., 2003)**. The tolerated daily intake of DBP is at 10 μg·kg^{-1} body weight and day **(Hines et al., 2011)**. Nevertheless, some studies suggest that the negative effect of phthalic acid esters on the human body is low, yet they are set up legislative limits for articles for human use, especially for items intended for children **(Kamrin, 2009)**.

Yogurt, a fermented milk product, is considered to be a healthy food, both because contained probiotic bacteria and nutrients, vitamins and minerals, especially calcium, which is a good utilisable by the human organism. Nutritional values of yogurt are similar to the milk from which yoghurt is produced, however, it may vary when ingredients are added to yoghurt, such as fruits, cereals, etc. **(McKinley, 2005)**, e.g. fiber. Dietary fiber is important for the prevention of civilization diseases, it is also the prevention of coronary-heart diseases, supports the maintenance of proper body weight, right levels of blood lipids and sugars, promotes the growth of beneficial intestinal microflora, while reducing the number of pathogenic microorganisms. The recommended daily intake of dietary fiber is set at 38 g·day^{-1} for men and 25 g·day^{-1} for women **(Betteridge et al., 2012)**. Yoghurts with fiber, which were found as acceptable for consumers, were with apple, wheat and bamboo fiber, inulin, as well as with date fiber **(Dhingra et al., 2012; Jandlová et al., 2017; Pytel et al., 2018)**. Yogurts are usually wrapped in HDPE (high-density polyethylene) or in PS (polystyrene) plastic cups **(Holm, 2010)**.

Scientific hypothesis

It was studied the effect of storage in four types of yoghurt with different addition of fiber on the concentration of phthalic acid esters. The assumption is that with the storage time, the concentration of phthalic acid esters in yoghurts will increase due to migration from the packaging. Further, the question arises which is the correlation of the concentrations of phthalic acid esters with pH.

MATERIAL AND METHODOLOGY

The yoghurts were produced at the Department of Food Technology at Mendel University. Content of the used milk (Holstein cattle, South-Moravian region, CZ) was determined fat to 3.50%, protein to 3.42% and lactose to 4.50%. The milk was pasteurized for 5 minutes at 85 °C, after that the milk was cooled down to 36 °C, and inoculated with a starter culture (0.5wt%) of original Bulgarian yogurt (GENESIS LABORATORIES, Bulgary). The mixture was fermented at 36 °C for 18 hours. The yoghurt was homogenized for 5 minutes, and divided into groups with additions of chia flour (ADVENI MEDICAL, spol. s.r.o., CZ) or bamboo fiber (J. Rettenmaier & Söhne GmbH & Co. KG, DE). One group was natural yoghurt with chia flour 1%, second yoghurt with chia flour 5%, next yoghurt with bamboo fiber 1% and last group yoghurt with 5% of bamboo fiber. The yoghurts were stored at 4 °C and were analysed in the 1st and 2nd week of storage. They were analysed also mixtures of yoghurts with chia flour/or bamboo fiber before filling into cups. All samples of yoghurts in cups were analysed in six repeats. The samples of yoghurts were analysed according to **Jarošová et al., (1999).** The samples were homogenized, weighed out 25 g into aluminium bowls, in which they were frozen. Afterwards the samples were lyophilized and extracted with hexane (PENTA s.r.o., CZ): acetone (PENTA s.r.o., CZ) (V:V= 1:1). The obtained solvent-free fat was weighed 0.5 grams out, from which the phthalate fraction was separated by gel permeation chromatography (ECOM spol. s.r.o., CZ). The fraction was further purified by sulphuric acid (Lach-Ner s.r.o., CZ): 1 ml of 96% sulphuric acid was added to 1 ml of sample dissolved in hexane, shaken for 10 minutes, 300 rpm, on a shaker (GFL 3005, GFL Gesellschaft für Labortechnik mbH, DE), centrifuged on centrifuge (Hettich-Zentrifugen D-78532 Tuttlingen-universal 32R, DE) (at -4 ° C, 10 min, 3000 rpm). The upper layer was given away as a waste. Then 2 ml of 68% sulfuric acid and 1 ml of hexane were added, all was shaken and centrifuged under the same conditions. The top layer was taken as the sample into 1.5 ml vials. Next, 1 ml of hexane was added, shaken and centrifuged under the same conditions, and taken as the sample into a vial. Subsequently, 1 ml of hexane was added again, shaken and centrifuged under the same conditions, and taken as a sample into a vial. The vials were dried with nitrogen (SIAD Czech spol. s r.o., CZ), and put 1 ml of acetonitrile (SIGMA-ALDRICH spol. s r.o., CZ). Measurement was performed on high-performance liquid chromatography (HPLC) (Agilent Technologies 1100 Series, DE), with UV detection at 224 nm, Zorbax Eclipse C8 column (Agilent Technologies, USA), mobile phase acetonitrile.

The cups from PS (polystyrene) (JEPA Plastics a.s., CZ), which were used, content plasticizer DBP (average concentration 59.45 $\mu g \cdot g^{-1}$) and DEHP (average concentration 8.99 $\mu g \cdot g^{-1}$) measured by **Gajdůšková et al., (1996).** The analyses were carried out in chemical laboratory at the Department of Food Technology at Mendel University. The evaluation was realized by software Agilent Chemstation for LC and LC / MS systems (Agilent Technologies, US).

In every type of yoghurts was determined pH by pH meter Portavo 907 Multi pH (KNICK, DE).

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Statistic analysis

The results were processed by Microsoft Excel 2010 (Microsoft Corporation, USA) and by Statistica 12 (StatSoft, USA).

Normality was tested by Shapiro–Wilk test. It was used Grubbs' test for to detect outliers data.

The t-test for comparison of mean values for dependent samples ($\alpha = 0.05$) was used to compare dibutyl phthalate concentrations and di-(2-ethylhexyl)phthalate concentrations in week 1 and week 2 by the same group of yoghurts.

Furthermore, a correlation coefficient was established between the average DBP concentrations in yoghurts with the measured pH of yoghurts, as well as the correlation coefficient between the average concentrations of DEHP in yoghurts with the measured pH of yoghurts. The correlation coefficient was determined even though the correlation does not imply causality.

RESULTS AND DISCUSSION

The concentrations of phthalic acid esters (DBP and DEHP) in mixtures of yoghurts with chia flour/or bamboo fiber before filling (Table 1) were higher (except for mixture of yogurt with 5% chia flour) than the yoghurts, which were stored.

The concentrations of phthalic acid esters is given in Table 2 and Table 3. Comparing the mean values of samples by t-test for dependent samples in yoghurts of the same type, when the DBP or DEHP concentrations in 1st week were compared with the DBP or DEHP concentrations in 2nd week. It was found no statistic difference ($p > 0.05$).

Table 1 Concentration of DBP and DEHP in mixtures of yoghurts before filling

Mixtures of yoghurts with	DBP [$\mu g \cdot g^{-1}$ of dry matter]	DEHP [$\mu g \cdot g^{-1}$ of dry matter]	DBP [$\mu g \cdot g^{-1}$ of original matter]	DEHP [$\mu g \cdot g^{-1}$ of original matter]
Chia flour 1%	36.43	16.84	6.09	2.82
Chia flour 5%	22.02	9.88	3.71	1.67
Bamboo fiber 1%	6.56	10.66	1.91	3.11
Bamboo fiber 5%	14.26	6.03	2.78	1.18

Table 2 Concentration of DBP and DEHP in yoghurts with chia flour

Yogurts with	1st week DBP [μg·g-1 of original matter]	2nd week DBP [μg·g-1 of original matter]	1st week DEHP [μg·g-1 of original matter]	2nd week DEHP [μg·g-1 of original matter]
Chia flour 1%	1.53	1.03	0.90	0.79
	1.21	1.30	0.88	1.16
	1.91	0.78	0.72	0.48
	1.21	1.34	0.60	0.62
	1.90	1.43	1.01	0.81
	1.55	0.84	0.93	0.69
Chia flour 5%	3.35	4.36	1.63	1.21
	4.07	4.50	5.60	0.85
	5.84	4.18	1.46	1.63
	5.21	5.48	1.94	1.99
	2.65	4.15	0.86	2.56
	3.31	2.81	0.90	1.49

Table 3 Concentration of DBP and DEHP in yoghurts with bamboo fibre

Yogurts with	1st week DBP [μg·g-1 of original matter]	2nd week DBP [μg·g-1 of original matter]	1st week DEHP [μg·g-1 of original matter]	2nd week DEHP [μg·g-1 of original matter]
Bamboo fiber 1%	4.22	5.81	1.80	2.93
	3.51	4.49	1.45	1.14
	3.16	4.29	1.26	1.21
	2.17	3.54	0.86	1.21
	3.72	2.94	1.45	0.76
	3.12	0.16	1.28	0.04
Bamboo fiber 5%	4.11	2.38	1.60	0.53
	2.33	1.93	0.57	0.40
	1.61	2.62	0.72	0.59
	1.43	3.92	0.88	0.89
	3.91	3.57	1.26	0.81
	2.24	3.65	0.78	0.90

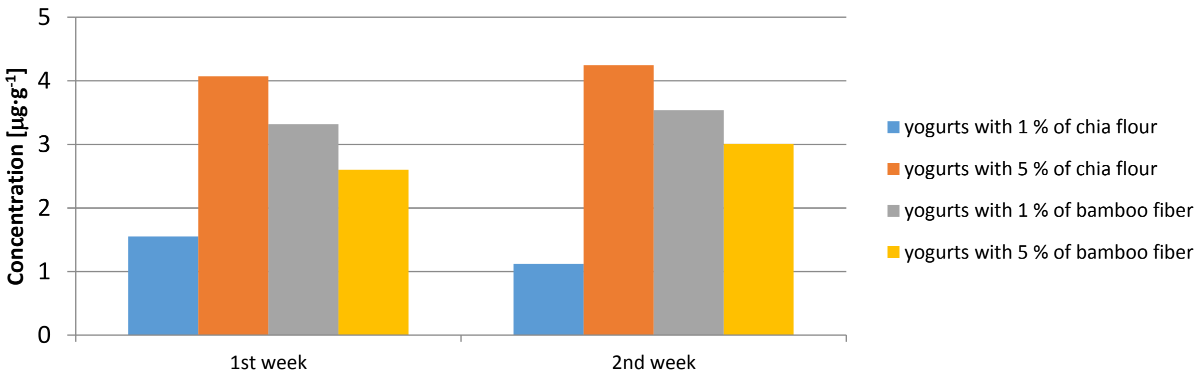

Figure 1 Average concentrations of DBP in yogurts.

Average concentrations of DBP with time of storage increased in yogurts with chia flour 5%, bamboo fiber 1%, and with bamboo fiber 5%. While the concentrations of DBP in yogurt with chia flour 1% decreased (Figure 1). Average concentrations of DEHP in all types of yogurt decreased over time (Figure 2).

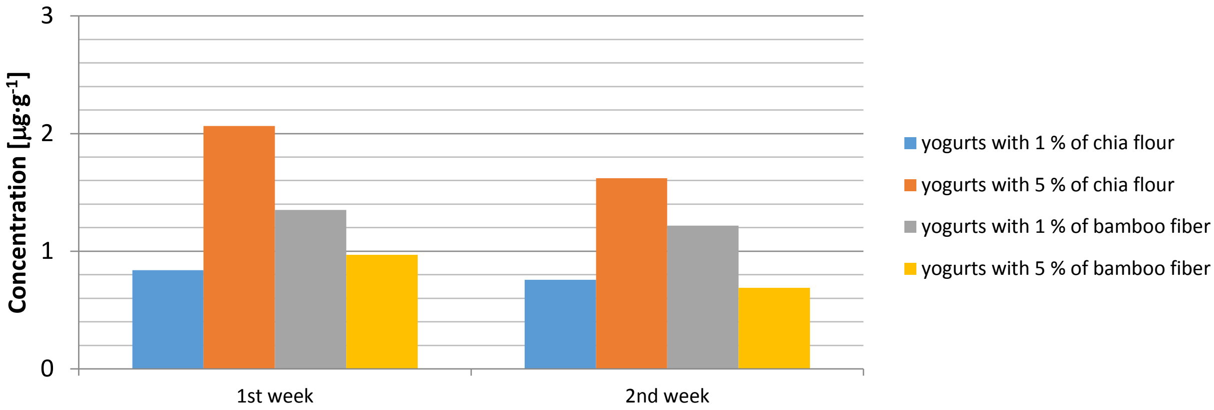

Figure 2 Average concentrations of DEHP in yogurts.

Table 4 pH of yoghurts in first week and in second week of storage.

Yogurts with	pH in 1st week	pH in 2nd week
Chia flour 1%	3.74	3.74
Chia flour 5%	3.78	3.76
Bamboo fiber 1%	3.51	3.62
Bamboo fiber 5%	3.51	3.62

In yoghurts, the average concentrations of DBP have always been found to be higher than the DEHP concentration, which corresponds to the fact that the DBP concentration in the cups was higher than the DEHP concentration.

The pH of each type of yoghurt was not very different (Table 4), but the correlation coefficient was determined. The correlation coefficient for the relationship between average DBP concentrations with pH was -0.0265 and for average DEHP concentrations with pH 0.3075.

In further research, it would be appropriate to test the effect of different pH in the same food on the migration of phthalic acid esters. Or make the study influence of different added concentrations on the migration of phthalic acid esters.

We were looking for some similar research to our research for better comparison, but we did not find any similar research with concentrations of phthalates in yoghurt enriched with flour or fiber. In study by **Rastkari et al., (2017)**, where they examined the transfer of phthalates from PET and HDPE into the acidic fluid during storage (0, 2, 4 and 6 months of measurement), the DEHP concentration increased with time in the liquids, the DBP concentration increased to 2 months, then dropped in the packaged fluid. In **Schecter et al., (2013)** study, the average concentration of DEHP was determined at 144 ng·g^{-1} and the average concentration of DBP was determined at 104.4 ng·g^{-1} in the category "other dairy products" purchased in New York, this category included yoghurt.

In study by **Sørensen, (2006)** was detected in raw milk concentration of DBP less than 9 µg·kg^{-1} and DEHP from 7 to 30 µg·kg^{-1}, and in fruit yoghurt concentration of DBP below 9 µg·kg^{-1} and DEHP from 15 to 37 µg·kg^{-1}.

In other study by **Sharman et al., (1994)** was measured in Norwegian raw milk the total amount of phthalate esters from 0.12 to 0.28 mg·kg^{-1}. Concentration of DEHP in Norwegian cream was determined up to 1.93 mg·kg^{-1} in United Kingdom cream from 0.2 to 2.7 mg·kg^{-1}. And in Spanish dairy products was detected the concentration of DEHP from below 0.01 to 0.55 mg·kg^{-1}.

A study examining the transfer of phthalic acid esters from plastic articles to liquid simulants represented by distilled water, 3% acetic acid and 10% ethanol. The highest phthalate migration values were found in distilled water, followed in the solution of acetic acid and finally in the ethanol solution. However, the established concentrations did not exceed the limits set by legislation **(Bošnir et al., 2003)**.

CONCLUSION

Mean concentrations of di-(2-ethylhexyl) phthalate in yoghurts with time of storage decreased. Mean concentrations of di-(2-ethylhexyl) phthalate was detected in youghurt with 1% of chia flour in the 1st week of 0.84 µg·g^{-1} in the 2nd week of 0.76 µg·g^{-1}, youghurt with 5% of chia flour in the 1st week of 2.06 µg·g^{-1} and in the 2nd week of 1.62 µg·g^{-1}, youghurt with 1% of bamboo fiber in the 1st week of 1.35 µg·g^{-1} and in the 2nd week of 1.22 µg·g^{-1}, youghurt with 5% of bamboo fiber in the 1st week of 0.97 µg·g^{-1} and in the 2nd week of 0.69 µg·g^{-1}. It was compared with t-test mean values of concentrations in the 1st week and 2nd week. It was found no statistic difference between mean values of concentrations (p >0.05).

Average dibutylphthalate concentrations dropped during storage for yoghurt with chia flour at 1% (in the 1st week of 1.55 µg·g^{-1} in the 2nd week of 1.12 µg·g^{-1}) and rose in yoghurts with chia flour at 5% (in the 1st week of 4.07 μg·g^{-1} in the 2nd week of 4.25 μg·g^{-1}) bamboo fiber at 1% (in the 1st week of 3.32 μg·g^{-1} in the 2nd week of 3.54 μg·g^{-1}) and 5% of bamboo fiber (in the 1st week of 2.60 μg·g^{-1} and in the 2nd week of 3.01 μg·g^{-1}). The concentrations were compared with t-test mean values in 1st week and 2nd week. It was found no statistic difference between mean values of concentrations (p >0.05).

The increase of dibutylphthalate concentration in yogurts caused migration from the plastic cups, where the dibutylphthalate concentration was more than 6 times higher than the di-(2-ethylhexyl) phthalate concentration. While the decline of di-(2-ethylhexyl) phthalate has most likely caused either dissociation or, reaction di-(2-ethylhexyl) phthalate with another food ingredient.

It was tested the correlation between the pH and concentrations of phthalic acid, however the pH of the yoghurt was too similar and the yoghurt too different. The correlation coefficient for the relationship between average dibutylphthalate concentrations with pH was -0.03 and for average di-(2-ethylhexyl) phthalate concentrations with pH 0.31. It would be advisable to monitor the migration of phthalic acid esters from the packaging to the same food with different of pH. And it would also be appropriate to determine the effect of migration of phthalates at different fiber concentrations.

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