Diminution of Migration of Phthalic Acid Esters in Tequila Beverage by the Year of Production

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ABSTRACT

The presence of diethyl-phthalate (DEP), dibutyl-phthalate (DBP), butylbenzyl-phthalate (BBP), diethylhexyl-phthalate (DEHP) and diisononyl- phthalate (DINP) was determined in 295 tequila samples. They were grouped by age of maturation and year of production. Gas Chromatography coupled with Mass Spectrometry was used for identification and quantification. The results showed 65 (22%) samples were phthalate free. DEP (0.13-0.27 mg/kg), BBP (0.05-2.91 mg/kg) and DINP (1.64-3.43 mg/kg) were detected in 11 (3.73%), 37 (12.54%) and 5 (1.69%) samples, respectively, but these concentrations did not exceed the limits admitted for alcoholic beverages. DBP (0.01- 2.20 mg/kg) and DEHP (0.03-4.64 mg/kg) were detected in 96 (32.54%) and 224 (75.93%) samples, from them only 10 (3.39%) and 15 (5.08%) samples exceed the limits admitted for alcoholic beverages and they were few tequilas produced in the year 2014 or before. All tequilas produced in 2015 and after, satisfied the international standards for these compounds. We concluded that a moderate consumption of tequila does not pose a health risk regarding the tolerable daily intake of phthalates according to the international standards for no fat meals and distilled alcoholic beverages.

Keywords: Alcoholic beverages, endocrine disruptor, phthalate, tequila, spirits.

1. INTRODUCTION

Phthalic acid esters or phthalates (Fig. 1) are plasticizer substances widely used in several industrial processes and they have been detected in toys, food packaging, medical materials and other articles [1–3]. Phthalates are not chemically bonded the plastic and they can migrate and contaminate food and beverages during manufacturing or storage [4–7]. Special attention has been paid to the presence of phthalates in food and beverages as they have been referred to as substances with endocrine disruptor and cytotoxicity activity [1,8]. Experimental phthalate exposition using rats has been shown to cause anti-androgenic effects such as cryptorchidism, diminution of testosterone levels and infertility [9–11]. Studies on human exposition to phthalates and their metabolites have been correlated with DNA damage in adult sperm as well as genital malformation among male infants with prenatal exposition [12,13]. Phthalates has been related to cancer development; for instance, dibutyl phthalate and benzyl butyl phthalate increase the in vitro and *in vivo* proliferation of human breast cancer cell-lines [14]. International safety authorities have regulated the concentration limits of certain phthalates in consumer products [1]. In the case of foods and beverages, international regulations [15,16] have established the permissible limits for some phthalates as shown in Table 1.

Tequila is the main alcoholic beverage produced in Mexico from the distillation and fermentation of sugars from *Agave tequilana* Weber var. *azul*. Tequila has the denomination of origin and exclusive trademarks with worldwide recognition. Tequila is produced in factories with steel fermenters and distilling devices, and it is classified as white or silver tequila when it is distilled and bottled after the alcoholic grade is fixed to 38ºalc. vol.; aged, extra aged or ultra aged, for tequila maturated in oak casks for at least 3, 12, and 36 months, respectively in agreement with the Mexican Official norm NOM-006-SCFI-2012 [17,18].

The aim of this work was to determine the phthalate content in a tequila collection of 295 samples produced from 2013-2018 by gas chromatography coupled to mass spectrometry. This analytical technique has been adopted for the routine analysis of phthalates in alcoholic beverages and biological samples such as serum and urine [19–21].

2. MATERIALS AND METHODS

2.1. Chemicals

Diethyl-phthalate (DEP), dibutyl-phthalate (DBP), butylbenzyl-phthalate (BBP), diethylhexyl-phthalate (DEHP), diisononyl- phthalate (DINP) and other chemicals were purchased from Sigma-Aldrich with purity up of 99.9%. Methanol (HPLC grade) was used to wash all glass material to eliminate lab phthalate contamination, which was also confirmed by analysis as described below.

2.2. Samples Collections

295 tequila samples (glass bottled) produced from 2013 to 2018 were analyzed, from them 185 were white, 48 aged, 36 extra aged and 26 ultra aged tequilas (Table 2). Each sample was processed as described previously by Gonzalez-Castro et al.[2] and filtrated using 0.22 µm filters (Millipore).

2.3. Instrumental Analysis

Samples were analyzed by a Gas Chromatograph coupled to a Mass Spectrometry detector 7820A/5977E System (Agilent Technologies) with an automatic injector 7683 Series (Agilent Technologies) using a capillary column HP5-MS (Agilent Technologies) 30 m long, 250 µm inner diameter and 0.25 µm-film thickness. The injector temperature was 250°C. The temperature program started at 60°C for 1 min, and it was increased at a rate of 20°C/min until 220°C. The temperature was held at 220°C for 1 min, then increased at a rate of 5°C/min to 280°C and held at 280°C for 4 min. The ionization potential was 70 eV and a scan function with 45-300 *m/z* for identification and SIM mode for quantification [2]. Compounds were identified by comparing their mass spectra with those obtained in the NIST 14 library (Gaithersburg, MD, USA) from the MS database, and by using commercial standards (Table 3).

2.4. Method Validation

Detection and quantification limits were measured by the signal/noise method and by using the standard deviation of the response and the slope [22]. The linear response of detectors was determined by straight-line calibration curves from the analysis of five standards with increasing the phthalate concentrations (Table 4) [23].

3. RESULTS AND DISCUSSION

A summary of phthalates detected in the tequila samples is shown in Table 5 and Fig. 2. As noted 65 samples (22%) were phthalate free, 112 samples (38%) contained one phthalate (DEHP), 99 samples (33.6%) presented two phthalates (DEHP and DBP), 13 tequila samples (4.4%) contained three phthalates (DEHP, DBP and BBP), and six samples (2%) presented four phthalates (DBP, BBP, DEHP and DEP or DINP). None tequila samples showed five phthalate simultaneously.

Tequila were grouped with respect to the age of maturation as White (n=185), Aged (n=48), Extra aged (n=36), and Ultra aged (n=26) (Table 2). From them, DEP (0.13-0.27 mg/kg), BBP (0.05-2.91 mg/kg) and DINP (1.64-3.43 mg/kg) were detected in 11, 37 and 5 samples, respectively. In all cases, phthalate concentration did not exceed the admitted limit for alcoholic beverages. DBP was detected in 96 tequilas from the total samples representing the 32.5%, but only 3.39% exceeded the limit of 0.3 mg/kg. Meanwhile 224 samples (75.9%) contained DEHP and 5.08 % exceeded the permissible limit of 1.5 mg/kg (Table 6).

Since DEHP was the most frequent phthalate detected, special attention was paid to this phthalate. Fig. 3 shows DEHP content in tequila samples grouped by age of maturation and year of production. As noted White tequilas presented DEHP below 0.47±0.35 mg/kg or less. Only aged tequila produced in 2013 exceeded the permitted limit with an average concentration of 2.2±1.86 mg/kg, whereas the subsequent years the DEHP concentration dropped to 0.08±0.02 mg/kg in 2018 samples. Extra aged tequilas from 2013 and 2014 showed DEHP of 0.97±0.81 and 1.1±1.4 mg/kg, respectively, indicating that some samples were out of the permitted limit, but in the subsequent years, the DEHP concentration was reduced until 0.17±0.04 mg/kg. Ultra aged tequilas produced from years 2014 to 2018 showed DEHP from 1.2±0.41 to 0.19±0.04 mg/kg. The important reduction of DEHP content in tequila is evidenced in the drop from 1.49±1.58 to 0.14±0.06 mg/kg from 2013 to 2018 total production, as a result of the improvement on the factories, quality control enhancement and reduction in the use of synthetic materials during the beverage elaboration.

DEHP is still the most used plasticizer and also the most studied phthalate; it has been detected in quotidian materials including food and alcoholic and non-alcoholic beverages. Some authors refer to it as an "omnipresent contaminant" [24]. Ye et al. reported that DBP and DEHP were the main phthalates found in three bottles of Chinese beer, and the total concentrations were between 6.22 and 7.76 μg/L [25]. Migration tests revealed that the high content of DEHP incorporated in polyvinyl chloride gaskets in the lids could be a potential source of phthalate contamination in bottled beers during transportation and storage. Studies on DEHP metabolism in humans showed that the major metabolite in serum was mono 2-ethylhexyl phthalate (MEHP), whereas the major metabolite in urine was 5OH-MEHP, 5oxo-MEHP and MEHP [24]. These metabolites are shown after short-term oral-exposition to DEHP, whereas mono 2-ethyl-5-carboxypentyl phthalate (5cx-MEPP) and mono [2-(carboxymethyl)hexyl] phthalate (2cx-MMHP) are also found after long-term oral exposure [26].

Other phthalates have been detected in alcoholic beverages, for instance Russo et al. [27] analyzed 6 commercial samples of Italian wine; they found 7.3 to 23 µg/L DBP, 0.1 to 7 µg/L BBP, and 3.1 to 16 µg/L DEHP. Cinelli et al. [21] found 0.0333 to 0.3124 mg/L of DBP, 0.046 to 0.135 mg/L BBP and 0.0115 to 0.0266 mg/L DEHP in 11 samples of Italian wine. Duca et al. [28] reported that 8% of bottled wine from a total of 2000 samples produced in the Republic of Moldova had DBP >0.3 mg/kg, which exceeds the limit established by the international regulation. Montevecchi et al. [29] studied the repartition of phthalates during distilling of wine for spirit production such as brandy; they reported values up 0.62 mg±0.05 and 0.47 ±0.04 for DBP and DEHP, respectively. They concluded it is very difficult to suggest modification of the distillation process without impairing the quality of brandies. Montevecchi et al. [30] determined phthalates by GC/MS in brandy samples from 1987 to 2014. They reported values up to 0.4 mg/kg DBP and 4.18 mg/kg DEHP, for samples produced in 1987 and 1989, respectively, whereas brandies produced in 2014, values were reduced to 0.03 mg/kg DBP and 0.3 mg/kg DEHP, respectively. Jurica et al. analyzed seven phthalates in samples during the plum spirit production and in the final product (plum is a spirit manufactured by registered producers from five European countries), they reported values of 0.822 mg/kg DBP and 1.638 mg/kg DEHP, which exceeded the limits established by the international regulations, but they concluded that a moderate daily consumption of plum spirit does not pose a health risk regarding the Tolerable Daily Intake of BBP, DEHP, and DBP [31].

4. CONCLUSIONS

The great majority of samples analyzed here did not exceed the limit accepted by the international regulations, but some samples of tequila produced in 2014 or previous years exceeded the allowable limits for DBP or DEHP, but beverages produced from 2015 to 2018 do satisfied fully the permissible limits of the international standards authorized for beverages. Our results suggest the risk of human exposure to phthalate by consuming tequila is really low and insignificant. Whit this, we concluded that tequila is a safety alcoholic beverage, however, routine monitoring in each lot of beverages produced and commercialized must be mandatory to guarantee that the beverages are safe for human consumption. On the other hand, monitoring and analysis at every stage of the production process including raw materials, fermentation and packing is strongly recommended in order to detect and discard possible phthalate contamination points.

5. ACKNOWLEDGMENTS

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7. FIGURE CAPTIONS

Fig. 1. Structure of common phthalates. The structures of the phthalates: a) Diethyl phthalate (DEP), b) Dibutyl phthalate (DBP) structure, c) Benzyl butyl phthalate (BBP) structure, d) Bis (2-ethylhexyl) phthalate (DEHP) structure, and e) diisononyl phthalate.

Fig. 2. Percentage of beverages containing no phthalate, one or more phthalate simultaneously. 0: Phthalate free; 1: DEHP; 2: DEHP and DBP; 3: DEHP, DBP and BBP; 4: DEHP, DNP, BBP and DEP or DINP; 5: DEHP, DNP, BBP, DEP and DINP.

Fig. 3. DEPH content in tequila samples grouped by age of maturation and year of production.

8. FIGURES (DIAGRAMS) AND TABLES

Table 1 Phthalates concentrations permitted in foods according to international standards for no fat meals including distilled alcoholic beverages.

Abbreviation	**Name**	**Limit premised (mg/kg)**
DEP	Diethyl phthalate	Not reported
DBP	Dibutyl phthalate	0.3
BBP	Benzyl butyl phthalate	30.0
DEHP	Di-2-ethyl-hexyl phthalate	1.5
DINP	Diisononyl phthalate	9.0

Table 2. Summary of tequila samples (n) analyzed in this work.

Year	White	Aged	Extra aged	Ultra aged	Total
2013	4	8	3	0	15
2014	86	22	10	6	124
2015	49	7	5	3	64
2016	18	5	6	7	36
2017	24	3	8	7	42
2018	4	3	4	3	14
Total	185	48	36	26	295

Table 3 Summary of chemical characteristics of the phthalate analyzed.

Chemical	**CAS number**	**Retention time (min)**	**Ions for identification**	**Ion for quantification**	**Molecular weight (g/mol)**
DEP	84-66-2	8.72	149, 177, 76, 105	149	222,24
DBP	84-74-2	11.29	149, 104, 76, 223	149	278,34
BBP	85-68-7	15.41	149, 91, 206, 65	149	312.36
DEHP	117-81-7	17.79	149, 167, 57, 71	149	390.56
DINP	28553-12-0	20.0-21.3	149, 71, 57, 293	149	418.61

Table 4. Typical standard curves used for phthalate determination. Evaluation of linearity (R^2), limit of detection (LOD) and limit of quantification (LOQ).

Chemical	Minimum level (mg/L)	Maximum level (mg/L)	Slope	Intercept origin	R^2	LOD (mg/L)	LOQ (mg/L)
DEP	0.053	1.70	182525	-2775.8	0.99771	0.005	0.013
DBP	0.058	1.84	365291	-40707	0.99448	0.004	0.01
BBP	0.056	1.80	96154	-4398.4	0.99512	0.01	0.05
DEHP	0.055	1.76	183410	542.73	0.99390	0.01	0.03
DINP	0.631	20.2	8959.6	496.43	0.99775	0.4	0.99

Table 5. Number of phthalates detected in the tequila samples.

Year	0	1	2	3	4	5	Total
2013	0	2	13	0	0	0	15
2014	31	37	39	11	6	0	124
2015	20	37	5	2	0	0	64
2016	4	14	18	0	0	0	36
2017	10	20	12	0	0	0	42
2018	0	2	12	0	0	0	14
Total	65	112	99	13	6	0	295

Table 6. Summary of tequila samples contaminated with phthalates.

Chemical	Samples contained phthalate	Min (mg/kg)	Max (mg/kg)	Samples out of norm	% of samples out of norm
DEP	11	0.13	0.27	0	0
DBP	96	0.01	2.20	10	3.39
BBP	37	0.05	2.91	0	0
DEHP	224	0.03	4.64	15	5.08
DINP	5	1.64	3.43	0	0

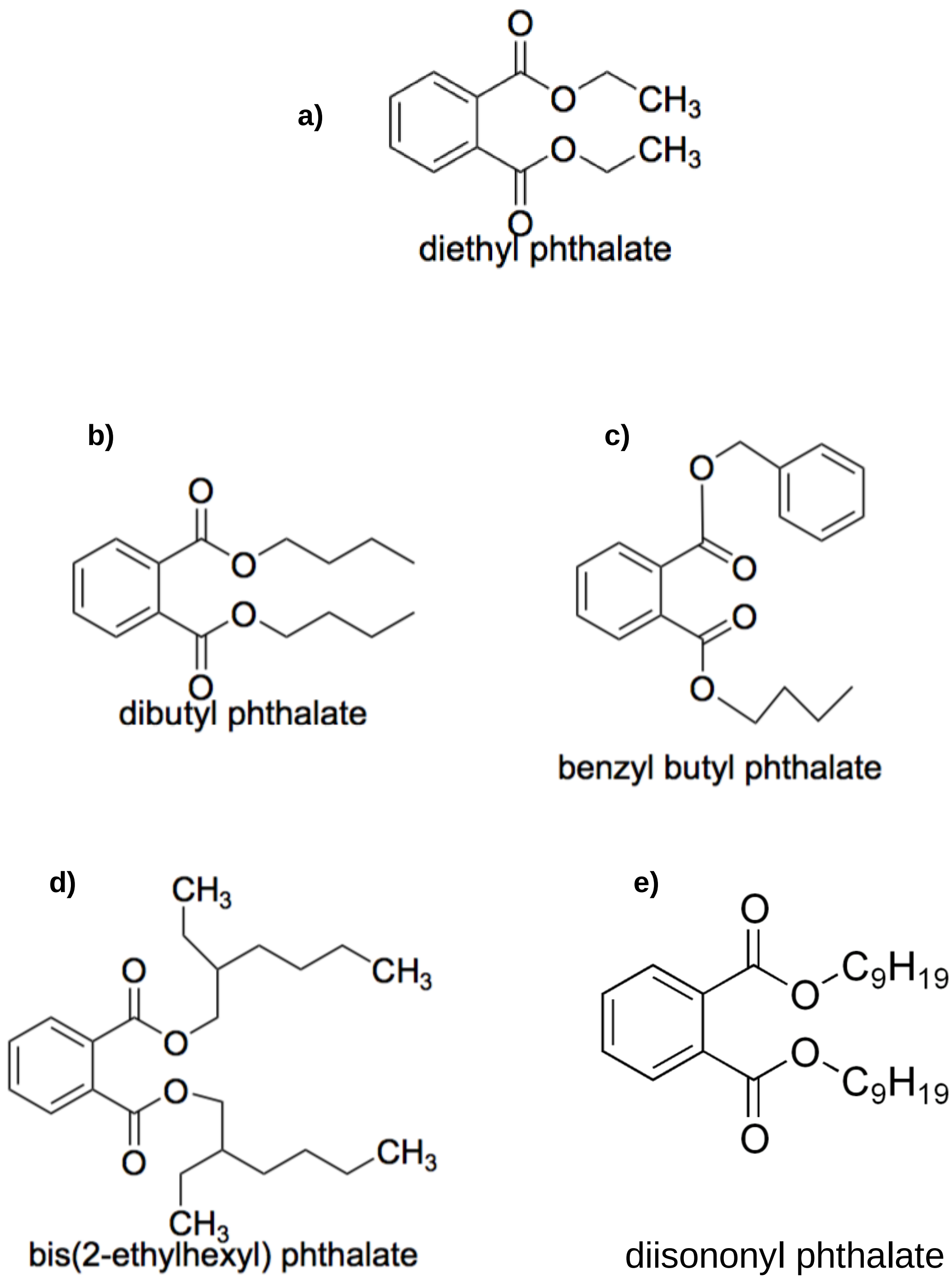
a)
O
O
CH3
O
CH3
O
diethyl phthalate
b)
dibutyl phthalate
c)
benzyl butyl phthalate
d)
CH3
bis(2-ethylhexyl) phthalate
e)
O
C9H19
diisononyl phthalate

Fig.1

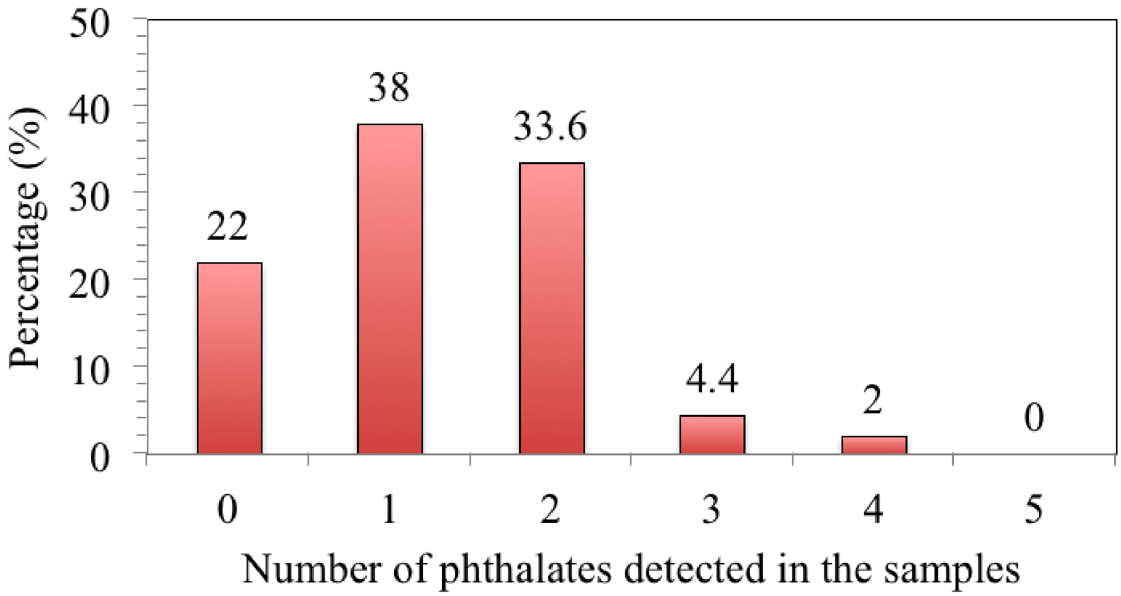
50
40
30
20
10
0
Percentage (%)
22
38
33.6
4.4
2
0
0
1
2
3
4
5
Number of phthalates detected in the samples

Fig. 2.

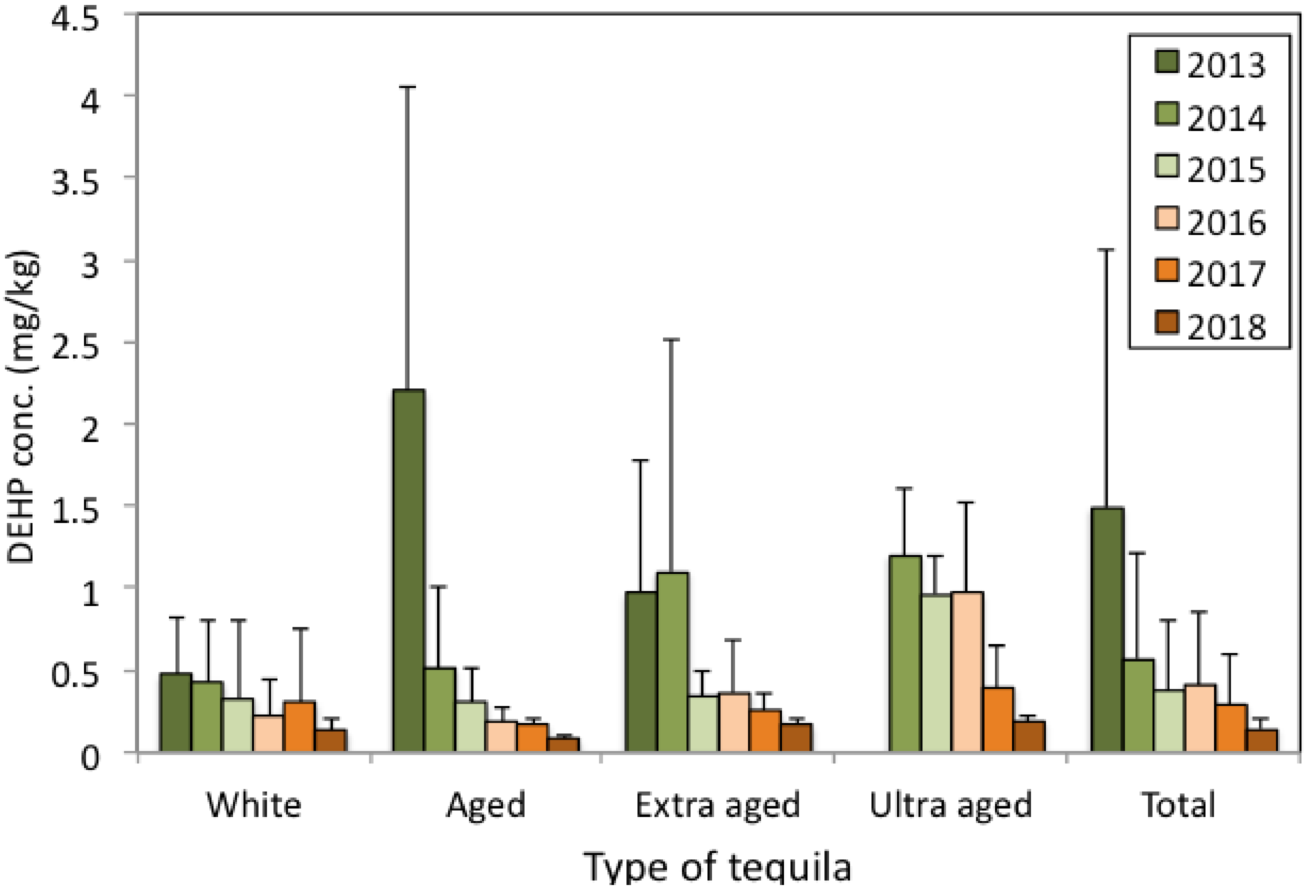
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
DEHP conc. (mg/kg)
2013
2014
2015
2016
2017
2018
White
Aged
Extra aged
Ultra aged
Total
Type of tequila

Fig. 3.