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Analytical Methods

Determination of phthalate esters in wine using solid-phase extraction and gas chromatography–mass spectrometry

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ABSTRACT

A method for the determination of six phthalate esters in wine samples has been developed. The phthalates were extracted from wine samples with an optimised solid-phase extraction method on C18 column and quantification was achieved via gas chromatography coupled with a mass spectrometer. The method was linear between 0.015 and 5.000 μg mL^{-1} for DMP, DEP and DEHP and between 0.018 and 5.000 μg mL^{-1} for iBP, DBP and BBP. The LOQs of DMP, DEP and DEH were 0.024 μg mL^{-1} while those of iBP, DBP and BBP were 0.029 μg mL^{-1}. The intra-day method repeatability was between 10% and 15% RSD, whereas the inter-day method repeatability was between 13% and 21% RSD. A survey was performed on white and red wines (n = 62) from the market, winemakers and an experimental pilot plant. All the analysed samples were phthalate contaminated. Commercial wine showed higher detection frequency and level of total phthalate, DBP and BBP than those produced in a pilot plant. iBP and DEHP concentrations were similar in all the groups of samples. iBP concentration was higher in red wines than in white ones.

1. Introduction

Phthalates (PAEs) constitute a group of chemical compounds that are mainly used as plasticizers in plastics industries. Manufacturers produce about 400,000 tons of PAEs per year (Stanley, Robillard, & Staples, 2003) and these represent an important group of contaminants due to their environmental persistence (Castello, Barcelo, Pereira, & Aquino Neto, 1999; Holadovà & Hajslovà, 1995). Penetration of PAEs in environment and food may occur because they are not covalently bound to plastics (Balafas, Shaw, & Whitfield, 1999; Castle, Mercer, Startin, & Gilbert, 1988; Page & Lacroix, 1992), therefore they can leak into food and beverages from packaging material (Holadova, Prokupkova, Hajslova, & Poustka, 2007; Lau & Wong, 1996) and also in the environment from plastic waste (Yin & Su, 1996). An endocrine disrupting activity of PAEs (Petrovic, Eljarrat, Lòpez de Alda, & Barcelò, 2001), linked to estrogenic properties, has been described (Gray, Ostby, Furr, Veeramachaneni, & Parks, 2000); moreover their mutagenic and carcinogenic activity has also been reported (Harrison, Holmes, & Humfrey, 1997).

Due to their widespread use, environmental persistence, abundant presence in many plastic materials (including packaging, pumps, tubing) there exist a potential risk of PAEs contamination during winemaking. This may arise both from the grapes and the use of plastics during processing; moreover additives and technological co-adjuvant may contribute to increase the potential impact of PAEs. Even though PAE contamination is likely to occur in wines, there is not any report, to the authors knowledge, on their detection in grape wines. Determination of PAEs is not an easy task, in fact the widespread presence of PAEs in the laboratory environment, including air, glassware and reagents can produce false positive outputs (Fankhauser-Noti & Grob, 2007; Prokupkovà, Holadovà, Poustka, & Hajslova, 2002). In order to detect PAEs at sub ppm levels a clean up/preconcentration step is necessary before instrumental analysis. Various liquid–liquid extraction (LLE) approaches have been used for isolation of PAEs from aqueous samples (Giam & Wong, 1987; Yasuhara et al., 1997; Zhu, 2006). More recently, solid-phase microextraction (SPME) has gained importance in the determination of semivolatile compounds (Alpendurada, 2000; Negrao & Alpendurada, 1999; Zygmunt, Jastrzebska, & Namiesnik, 2001) including PAEs (Cai, Jiasng, Liu, & Zhou, 2003; Cortazar et al., 2002; Kataoka, Ise, & Narimatsu, 2002; Kotowska & Garbowska, 2006; Luks-Betlej, Popp, Janoszka, & Paschke, 2001; Peñalver, Pocurull, Borrull, & Marce, 2000, 2001; Valor, Moltò, Apraiz, & Font, 1997). This technique is an interesting alternative for the determination of PAEs in liquid samples, because the risk of contamination during sample handling can be significantly reduced, but it appears not applicable to wine analysis because in this matrix the PAEs partition in the liquid phase is enhanced by the high

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percentage of ethanol. Other authors used solid-phase extraction (SPE) for PAEs recovery form different matrices, including water and sludge (Davi, Liboni, & Malfatto, 1999; Holadovà & Hajslovà, 1995; Jara, Lysebo, Greinbrokk, & Lundanes, 2000). SPE appears a more suitable technique with respect to LLE as it requires a minimal use of organic solvents, thus reducing health risk and sample contamination, and it could permit the simultaneous extraction of multiple samples. As far as it concerns the instrumental analysis, gas chromatography (GC) methods with flame ionization detection (Batlle & Nerìn, 2004; Polo, Llompart, Garcia-Jares, & Cela, 2005) or with mass spectrometry detection, operating both in full scan mode (Kotowska & Garbowska, 2006; Sablayrolles, Montrèjaud-Vignoles, Benanou, Patria, & Treilhou, 2005), and single ion monitoring (Feng, Zhu, & Sensenstein, 2005; Jonsson & Boren, 2002; Shen, 2005) have been reported for PAEs determination, but other techniques, including reversed-phase liquid chromatography, have been also used (Jara, Lysebo, Greinbrokk, & Lundanes, 2000).

The purpose of the present study was the development and optimisation of an analytical procedure able to detect PAEs in wines at sub ppm level. The method developed was based on a SPE procedure followed by GC–MS analysis. PAEs contamination in commercial ($n = 36$), private wine producers (winemakers) ($n = 18$), and pilot plant ($n = 8$) wines was successfully determined.

2. Materials and methods

2.1. Reagents and samples

Acetone, anhydrous sodium sulphate, dichloromethane, hexane, methanol, dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzylbutyl phthalate (BBP), isobutyl phthalate (iBP), diethylexyl phthalate (DEHP) standards were of analytical grade, water was HPLC grade, all the reagents were from Sigma–Aldrich (Milan, Italy); 6 mL polyethylene SPE cartridges and C18 sorbent (particle size 40–70 μm) were purchased from StepBio (Bologna, Italy).

Individual stock solutions of each phthalate ester (10,000.0 μg mL^{-1}) were prepared in hexane. A standard mixture of the six target analytes (100.0 μg mL^{-1}) in hexane was used for daily preparation of the calibrating solutions. For the standard addition measurement PAEs mix at different concentrations were prepared in methanol.

Commercial red and white wines (36 samples) were purchased in local markets, 10 were packed in polyethylene coupled film brick (PEC) and 26 in glass bottles (GB). Eighteen glass bottled winemakers wines were obtained from local producers (WM) and eight glass bottled sample of wines from an experimental pilot plant (PP). Pilot plant wines were produced in stainless steel tanks, with no use of process adjuvants.

2.2. Glassware and reagent control

To avoid PAE contamination, all glassware used in the study were soaked in acetone for at least 30 min, then washed with acetone, rinsed with hexane, and dried at 120 °C for at least 4 h. All the glassware and reagents were checked for potentially occurring phthalate contamination. Hexane and dichloromethane were checked by GC–MS analysis; moreover the contamination level determined from the SPE procedure was also checked daily.

2.3. Chromatographic analysis by GC–MS

An Autosystem XL gas chromatograph coupled with a Turbomass quadrupole mass spectrometer (Perkin Elmer, Monza Italy) was used for PAEs determination. The chromatograph was equipped with a Restek RTX-5MS capillary column (5% diphenyl; 95% dimethylpolysiloxane) 30 m long, 0.25 mm internal diameter, 0.25 μm film thickness (Restek, Superchrom Italy). Helium (99.998%, Rivoira Milan, Italy) was used as carrier gas at flow rate of 1.0 mL min^{-1}.

A 1 μL sample was injected into the split/splitless inlet in splitless mode (splitless for 1 min, with split flow 50 mL min^{-1}) at 280 °C. The temperature of the GC–MS interface was 280 °C. The oven temperature program started at 70 °C for 1 min, was increased of 20 °C min^{-1} to 160 °C, and then of 10 °C min^{-1} to 280 °C which was maintained for 2 min. Full scan mode (33–550 amu) was used for data acquisition. Selected ion mass monitoring (SIM) was used for quantification (m/z 163 for DMP and m/z 149 for DEP, iBP, DPB, BBP, DEHP) and full scan acquisition was used for analytes identification. The peak areas was reported as a function of the injected concentration and the calibration curves of the six PAEs were obtained by linear regression. Calibration solutions for the GC–MS method were prepared in hexane at 0.100, 0.250, 0.500, 1.000, 2.500, and 5.000 μg mL^{-1} before use. Intra-day repeatability was calculated using values from five injections of each standard solution, and inter-day repeatability was calculated using the value of one measurement, randomly chosen among five, per day over a total 5 days trial. The limit of detection (LOD) was calculated from the apparent measured value of blank injections (mean + 3 × standard deviation), the limit of quantification (LOQ) was calculated using the apparent measured value of blank injections (mean + 10 × standard deviation).

2.4. SPE procedure

SPE procedure was modified from EPA method 506 (Kawahara & Hodgeson, 1995), which reports PAEs determination in drinking water. SPE procedure was optimised with respect to: (1) C18 phase amount, (2) phase conditioning, (3) sample treatment, and (4) sample size. All the cited parameters were studied by recovery and repeatability studies in red and white wines fortified at 0.500 μg mL^{-1}. The C18 phase amount was evaluated by using 0.5 g increase steps in the interval 1–3 g. The effect of water during phase conditioning, sample dilution in water, as well as the addition of salt in diluted samples (NaCl at 0.0, 0.5 and 2.0 g mL^{-1}) was also optimised. Finally, the SPE procedure used for wine samples analysis was the following: 2.5 g C18 phase conditioning with 10 mL dichloromethane (2 × 5 mL), plus 2.5 mL methanol; then 5 mL of sample, diluted to 50 mL with water plus 2 g mL^{-1} of NaCl, were loaded at 1 mL min^{-1} flow rate. The sample vial was further washed with 5 mL of water that were loaded onto SPE column as well. The elution was carried out with 5.0 mL of dichloromethane (2 × 2.5 mL aliquots). The two aliquots were mixed and filtered on anhydrous Na_2SO_4; the filter was then washed with 10 mL of dichloromethane (2 × 5 mL aliquots). All the portions (15 mL) were dried under nitrogen at 28 °C. The dried sample was re-dissolved in 2 mL of hexane and thus concentration factor of 2.5 was introduced.

The recovery of the optimised SPE procedure was evaluated for the six chosen PAEs at 0.100, 0.250 and 0.500 μg mL^{-1}; for each concentration level the repeatability has been evaluated on 10 different red wine samples and 5 white wine samples. The linearity of the method was studied via fortification of pooled wine samples ($n = 6$) in the interval 0.010–5.000 μg mL^{-1} for all the investigated PAEs. The limit of detection (LOD) was calculated from the apparent measured value of the pooled blank sample (mean + 3 × standard deviation), and the limit of quantification (LOQ) was calculated using the apparent measured value of the pooled blank sample (mean + 10 × standard deviation).

2.5. Sample analysis

PAEs were extracted from wine samples using the optimised SPE procedure and analysed with the described GC–MS protocol. PAEs quantification was performed using the standard single addition method. Each sample extract was firstly analysed; then a mix of 20 μL standard solution was added to 1 mL of the sample extract for the second run. The standard solution consisted in PAEs mix at circa the same level of concentration found in the first run calculated on the calibration curve obtained in the matrix extract. The sample was equilibrated for 15 min before injection. No addition was done for PAEs under the detectable level in the first run.

2.6. Statistical analysis

The statistical significance of differences between PAEs level of different sample groups was determined by non-parametric procedures (ANOVA of Kruskall–Wallis and median test). Box and whiskers plots were used to visualize data distribution and in the construction of the graphs the outliers were selected adopting a coefficient of 1.5. Data were processed using the Statistica for Windows (Statsoft, Tulsa, OK) package.

3. Results and Discussion

3.1. GC–MS PAEs analysis in standard solution

GC–MS was used for the identification (full scan mode) and quantification (SIM mode) of PAEs. Linear calibration curves for the PAEs dissolved in hexane were obtained in the range 0.100–5.000 μg mL^{-1} for DMP, DEP, DEHP and 0.150–5.000 μg mL^{-1} for iBP, DBP and BBP. The calculated LOD was 0.100 μg mL^{-1} for DMP, DEP and DEHP and 0.150 μg mL^{-1} for iBP, DBP and BBP. The calculated LOQ was 0.166 μg mL^{-1} for DMP, DEP and DEHP and 0.250 μg mL^{-1} for iBP, DBP and BBP. The relative standard deviation of the instrumental analysis was between 7% and 20%.

3.2. GC–MS PAEs analysis in matrix extract

Preliminary experiments demonstrated an increase of the sensitivity for analysis carried out in matrix extracts up to 300%. For this reason calibration curves of the six PAEs were constructed using a spiked matrix extract obtained from a pool of samples ($n = 6$) and performing a multiple standard addition in the concentration interval 0.100–5.000 μg mL^{-1} and 0.150–5.000 μg mL^{-1} for DMP, DEP, DEHP and iBP, DBP, BBP, respectively. The PAEs concentration of the pooled extract was <0.042 μg mL^{-1} for DMP and DEP; <0.058 μg mL^{-1} for iBP; 0.060 μg mL^{-1} for DEHP; 0.065 μg mL^{-1} for DBP and 0.068 μg mL^{-1} for BBP.

The increased instrumental response was confirmed and resulted in a decrease of the calculated LODs to 0.025 μg mL^{-1} for DMP, DEP and DEHP, and 0.035 μg mL^{-1} for iBP, DBP and BBP. The calculated LOQs were 0.042 μg mL^{-1} for DMP, DEP and DEHP, and 0.058 μg mL^{-1} for iBP, DBP and BBP. This described response enhancement did not affect the intra-day and inter-day repeatability. In Table 1 a summary of the principal analytical parameters for the calibrations obtained both in hexane and matrix extract are reported.

This matrix induced response enhancement is frequently reported in the literature for semi-polar compound determination (i.e organophosphate pesticides) in food matrices (Kirchner, Matisova, Otrekal, Hercegova, & de Zeeuw, 2005). No description of such occurrence was reported for PAEs determination, possibly because the literature is mainly focused on their determination in water samples. The phenomenon is commonly attributed to a protection effect of the matrix in the injection and/or detection of semi-polar compounds (Anastassiades, Maštovská, & Lehotay, 2003). In order to avoid false positive results, the matrix induced response enhancement needs to be addressed. To this purpose an external matrix-matched calibration could be used (Anastassiades et al., 2003) but this might lead to erroneous quantification in wine analysis due to the unpredictable variability from sample to sample. Moreover, the use of a single internal standard (I.S.) was not feasible due to the variability also on each single PAE and, finally, the use of multiple I.S. was limited by costs (Hajslova & Zrostlıkova, 2003). Therefore the external standard single addition method can be used for sample quantification as explained below (Section 3.4).

3.3. SPE–GC–MS method development

Despite materials such as polystyrene have been successfully used for PAEs extraction from water samples (Jara et al., 2000), C18 was selected since it is applied in the official EPA method for phthalate analysis in water samples (Kawahara & Hodgeson, 1995). The main parameters that could affect the SPE process were optimised. Initially, the amount of C18 phase to be used was evaluated. In the experimental conditions, the target analytes as well as the phenolic compounds of wines (anthocyanins, catechins and other phenolics) are retained by the conditioned phase; as a result a phase saturation may occur if an inadequate amount of phase is used. As an example, using 1 and 1.5 g of C18 resin it was observed a visible saturation by anthocyanins, which leaked during the sample loading. This phenomenon may affect the PAEs retention due to column saturation; therefore a higher amount of phase was loaded into the cartridge; hence 2.5 g of C18 were found to be sufficient for optimal recovery.

The high percentage of alcohol of wines may affect the phase adsorption ability, therefore both undiluted and diluted samples were examined. As expected, a higher PAEs recovery was obtained by diluting the sample 1:10 in HPLC water before SPE loading and this improved the recovery efficiency of an average 30%.

The effect of salt addition to the diluted samples in a 0–2 g mL^{-1} range was also evaluated. Results showed that the addition of NaCl to the diluted (1:10) sample had a positive effect on the recovery of all the investigated PAEs except DMP and DEP. A higher recovery of all PAEs except DMP and DEP, was obtained when 2 g mL^{-1} of NaCl was added. Therefore this salt concentration was used.

3.4. SPE–GC–MS method validation

The recovery of the investigated PAEs in the optimised conditions was finally evaluated in a 0.100–0.500 μg mL^{-1} range for red wine and at 0.500 μg mL^{-1} for white wines. In Table 2 the recoveries obtained from red ($n = 10$) and white wines ($n = 5$) are reported for each investigated compounds. A higher recovery was obtained from white wines, particularly for DMP and DEP. The difference between red and white wines in terms of recovery was not significant for the other PAEs (iBP, DBP, BBP, and DEHP) which exhibited a recovery between 70% and 92% from red wines and 68% and 109% from white wines. Three spiking levels were tested for red wines. The recovery of DMP, the most polar of the investigated series, was improved by decreasing the spiking level, but the recoveries of less polar compounds such as DBP and BBP were decreased due to a possible competition with the phenolic fraction for the C18 phase. iBP recovery was not affected by the spiking level. The average recovery value was around 70% for all the analytes of interest and, therefore, the extraction method was considered useful for the purpose of the analysis. In fact this recovery was similar to those reported in the EPA method 506 where an extraction protocol was validated on a less challenging matrix as drinking water (Kawahara & Hodgeson, 1995). To our knowledge no data

Table 1
Linear interval, determination coefficient, LODs, LOQs and repeatability (RSD) obtained for the six investigated compounds in hexane and spiked matrix extract solutions

	Linear interval (μg mL^{-1})	R^2	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	Inter-day repeatability (%)	Intra-day repeatability (%)
Hexane						
DMP	0.100–5.000	0.999	0.100	0.166	10	7
DEP	0.100–5.000	0.998	0.100	0.166	15	15
iBP	0.150–5.000	0.998	0.150	0.250	20	14
DBP	0.150–5.000	0.998	0.150	0.250	14	12
BBP	0.150–5.000	0.997	0.150	0.250	10	8
DEHP	0.100–5.000	0.999	0.100	0.166	16	9
Matrix extract						
DMP	0.025–5.000	0.998	0.025	0.042	10	8
DEP	0.025–5.000	0.998	0.025	0.042	11	11
iBP	0.035–5.000	0.999	0.035	0.058	13	12
DBP	0.035–5.000	0.998	0.035	0.058	15	13
BBP	0.035–5.000	0.997	0.035	0.058	15	9
DEHP	0.025–5.000	0.998	0.025	0.042	12	12

Table 2
Recovery values, mean and standard deviation, for red (n = 10) and white (n = 5) wines

Spiked level (μg mL^{-1})	% Recovery					
	DMP	DEP	iBP	DBP	BBP	DEHP
Red wines						
0.500	33 ± 8	50 ± 5	68 ± 15	109 ± 12	100 ± 10	90 ± 12
0.250	57 ± 20	38 ± 15	65 ± 8	65 ± 10	75 ± 18	72 ± 17
0.100	58 ± 17	73 ± 15	68 ± 14	67 ± 15	71 ± 20	69 ± 20
White wines						
0.500	77 ± 15	65 ± 15	70 ± 14	87 ± 15	92 ± 18	75 ± 20

on PAEs extraction from wines are available for comparison. The linearity of the method was studied in the interval 0.010–5.000 μg mL^{-1} for all the investigated PAEs via fortification of the pooled wine sample (n = 6). In Table 3 a summary of the principal analytical parameters of the calibrations are reported. The intra-day method repeatability was between 10% and 15% RSD, whereas the inter-day method repeatability was between 13% and 21% RSD. The LODs were 0.015 μg mL^{-1} for DMP, DEP and DEHP and 0.018 μg mL^{-1} for iBP, DBP and BBP. The LOQs were 0.024 μg mL^{-1} for DMP, DEP and DEH and 0.029 μg mL^{-1} for iBP, DBP and BBP. The standard addition method was further used for sample quantification and the data were corrected for recovery.

3.5. Sample analysis

The optimised analytical procedure was applied to 62 wine samples of different origin and type: 10 commercial samples packed in polyethylene coupled film brick (PEC), 26 commercial samples in glass bottle (GB), 18 winemakers samples glass bottled from local producers (WM) and eight glass bottled samples from an experimental pilot plant (PP). The mass spectra and the retention times were used for compound identification. The peak area in the SIM mode was used for peak quantification using the standard single addition method.

A possible source of false positive results in real samples is the PAEs laboratory contamination. This was strictly controlled by appropriate glassware and reagents cleaning and by direct GC–MS analysis of hexane and dichloromethane. The concentration of PAEs in hexane and dichloromethane was below the detectable level. Contamination from other sources was estimated using 45 mL of HPLC water recovered in 2 mL of hexane and found to be 0.031 ± 0.005 μg mL^{-1}, 0.009 ± 0.002 μg mL^{-1} and 0.049 ± 0.007 μg mL^{-1} for iBP, DBP and DEHP, respectively (n = 10). The blank mean concentration of each PAE was subtracted from the concentration found in the sample.

The minimum, the maximum and the median level as well as the detection frequency of each target compound for all of the investigated sample groups are reported in Table 4. The PAEs detection frequency was dependent on the type of sample (GB, PEC, WM, PP). DMP and DEP were not found in any of the analysed samples. On the contrary, iBP and DEHP were found in the majority of the analysed samples, with a mean detection frequency of 97% and 92%, respectively. The high detection frequency of iBP and DEHP contamination in all the groups of sample suggested an environmental origin of these contaminants. On the other hand a variable detection frequency and level of DBP and BBP contamination was observed in all the analysed groups of samples (Table 4). These two PAEs were not found in PP samples.

Table 3
Linear interval, determination coefficient, LODs, LOQs and repeatability data obtained for a spiked pooled wine sample

	Linear interval (μg mL^{-1})	R^2	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	Inter-day repeatability (%)	Intra-day repeatability (%)
DMP	0.015–5.000	0.990	0.015	0.024	13	10
DEP	0.015–5.000	0.991	0.015	0.024	18	15
iBP	0.018–5.000	0.995	0.018	0.029	21	14
DBP	0.018–5.000	0.995	0.018	0.029	16	15
BBP	0.018–5.000	0.996	0.018	0.029	18	11
DEHP	0.015–5.000	0.997	0.015	0.024	16	13

Table 4
Minimum, maximum, mean value and detection frequency for each detected contaminant

Name	Min ($\mu g\ mL^{-1}$)	Max ($\mu g\ mL^{-1}$)	Median ($\mu g\ mL^{-1}$)	Detection frequency (%)
GB				
DMP	<LOQ	<LOQ	<LOQ	0
DEP	<LOQ	<LOQ	<LOQ	0
iBP	0.047	0.260	0.099	100
DBP	<LOQ	0.244	0.053	90
BBP	<LOQ	0.269	0.040	40
DEHP	<LOQ	0.242	0.076	100
PEC				
DMP	<LOQ	<LOQ	<LOQ	0
DEP	<LOQ	<LOQ	<LOQ	0
iBP	<LOQ	0.173	0.076	100
DBP	<LOQ	0.240	0.115	85
BBP	<LOQ	0.252	<LOQ	69
DEHP	0.0250	0.276	0.078	96
WM				
DMP	<LOQ	<LOQ	<LOQ	0
DEP	<LOQ	<LOQ	<LOQ	0
iBP	<LOQ	0.254	0.119	89
DBP	<LOQ	0.125	<LOQ	56
BBP	<LOQ	0.237	<LOQ	22
DEHP	<LOQ	0.133	0.057	72
PP				
DMP	<LOQ	<LOQ	<LOQ	0
DEP	<LOQ	<LOQ	<LOQ	0
iBP	0.062	0.197	0.081	100
DBP	<LOQ	<LOQ	<LOQ	0
BBP	<LOQ	<LOQ	<LOQ	0
DEHP	<LOQ	0.061	0.057	100

(GB) wines in glass bottle, (PEC) wines in polyethylene coupled film brick, (WM) wines from winemakers producer, (PP) wines from experimental pilot plant.

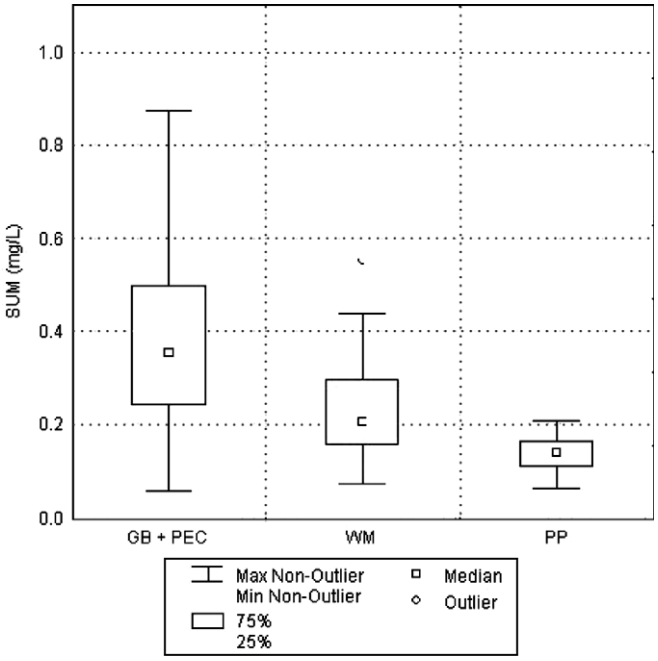

Fig. 1. Box and whiskers plot showing the distribution of total phthalate content in three groups of samples: commercial wines ($n = 36$), winemakers wines ($n = 18$) and pilot plant wines ($n = 8$). (GB) wines in glass bottle, (PEC) wines in polyethylene coupled film brick, (WM) wines from winemakers producer, (PP) wines from experimental pilot plant.

The statistical significance of differences between PAEs content in different groups of samples was determined by non-parametric tests due to the heterogeneous distribution and heteroschedasticity of the data (Figs. 1 and 2). No influence of the packaging material (either glass or polyethylene coupled film) on total and single PAEs content was found in the commercial samples (data not shown). Hence all the commercial wines were treated as a single group (GB + PEC). The total PAE concentration depended on different sample groups as reported in Fig. 1. The median value ($0.385\ \mu g\ mL^{-1}$) of commercial wines (GB + PEC) was significantly higher than that of the winemakers wines ($0.204\ \mu g\ mL^{-1}$), which, in turn, was higher than that of the wines from the pilot plant ($0.138\ \mu g\ mL^{-1}$). All the three groups were significantly different among them ($p < 0.005$).

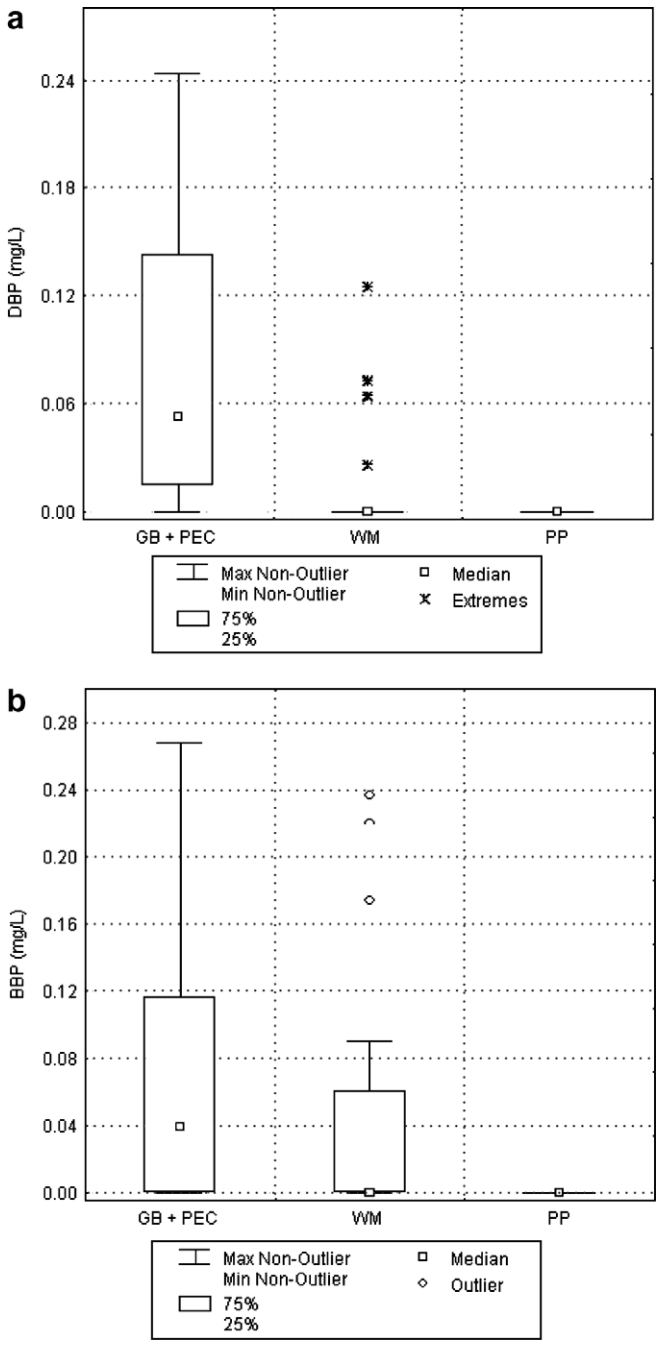

Fig. 2. Box and whiskers plot showing the distribution of DBP (a) and BBP (b) content in three groups of samples: commercial wines ($n = 36$), winemakers wines ($n = 18$) and pilot plant wines ($n = 8$). (GB) wines in glass bottle, (PEC) wines in polyethylene coupled film brick, (WM) wines from winemakers producer, (PP) wines from experimental pilot plant.

By considering the data obtained for each PAE, no difference in iBP and DEHP content was found in samples from different groups. This result supports the above mentioned hypothesis of a possible environmental origin of these contaminants.

On the other hand, DBP and BBP concentration differed among the considered sample groups (Fig. 2a and b). Even though a decreasing trend of average values could be observed from commercial to winemakers and pilot plant wines, a significant difference ($p < 0.005$) was only found between commercial and pilot plant wines. It is worth to notice that the pilot plant wines were produced with no use of process adjuvants and only stainless steel tanks and tubing were employed during winemaking. Thus their lower DBP and BBP contamination may support the hypothesis of an influence of the wine making process on the level and frequency of these compounds.

A further statistical evaluation of total and single phthalate concentration in white and red wines showed that the total PAEs level was not affected by the vinification process, as well as the DPB, BBP and DEHP content. On the contrary, red wines showed a higher iBP content than white wines ($p < 0.005$). The contamination from iBP, which was previously hypothesized to be an environmental contaminant, may arise from the vinification process due to the prolonged contact between grape skins and must.

This preliminary survey performed on 62 samples showed a high detection frequency of PAEs in wines. In particular, an environmental origin of iBP and DEHP contamination may be suggested on the basis of the contamination frequencies and levels in differently processed wines. No DBP and BBP contamination was detected in samples produced in stainless steel tanks, with no use of process adjuvants. On the contrary in commercial wines, where extended adjuvants use is expected, the detection frequency was 88% and 55%, respectively.

On the basis of experimental results obtained from commercial wines packed either in glass bottles or polyethylene coupled film bricks, no leakage of PAEs from the packaging material could be hypothesized. Finally, multiple sources of PAEs contamination may be considered for wines, including agricultural tools (such as plastic foils and laces), oenological adjuvants, materials getting in contact during winemaking (such as tubes and pumps). In this respect our study should be considered a pilot study and dedicated experimentations are needed to better understand the contribution of each possible source to PAEs contamination.

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