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Keywords: Phthalate Esters; Dispersive Liquid-Liquid Micro-Extraction; Gas Chromatography-Mass Spectrometry; Distillation; Distilled Alcoholic beverages.

Corresponding Author: Dr. Giuseppe Montecvecchi, Ph.D.

Corresponding Author's Institution: University of Modena and Reggio Emilia

First Author: Giuseppe Montecvecchi, Ph.D.

Order of Authors: Giuseppe Montecvecchi, Ph.D.; Francesca Masino, Researcher; Nicolas Di Pascale; Giuseppe Vasile Simone, Ph.D.; Andrea Antonelli, Full Professor

Abstract: Due to health concerns and legal matters, an investigation to limit phthalates esters (PEAs) in spirits is necessary. A lab still was used to perform pilot distillations according to the official method for brandy production in order to explore the repartition into the distilled fractions of each PAE. The process was divided in two steps: a première chauffe and a bonne chauffe. The former step included the cut into heads, heart and tails, while the latter into heads, brandy, secondes, and tails. The behaviour of each PAE during distillation was affected by its own chemical nature. Dibutyl phthalate (DBP) was entirely carried over into the distillate, while bis(2-ethylhexyl) phthalate (DEHP) only partially, and diisononyl phthalate (DINP) accumulated in the stillage. During the bonne chauffe, DBP and DEHP accumulated in the secondes more than in the brandy. A rectification step of the secondes was demonstrated to considerably reduce PAEs concentration.



**Centro Interdipartimentale per il Miglioramento e la
Valorizzazione delle Risorse Biologiche Agro-Alimentari**
Piazzale Europa, 1 - 42124 Reggio Emilia (Italia)

May 4th, 2017

Dear Editor,

I am sending you a copy of the revised Ms. entitled: "Study of the repartition of phthalate esters during distillation of wine for spirit production".

All reviewers' comments were carefully considered and accepted in large part.

Short title: Repartition of phthalates during distillation for spirit production

Corresponding author's name; address; telephone and fax numbers; e-mail address:

Giuseppe Montecvecchi; Centro di Ricerca Interdipartimentale per il Miglioramento e la Valorizzazione delle Risorse Biologiche Agro-Alimentari BIOGEST-SITEIA, Università degli Studi di Modena e Reggio Emilia, Tecnopolo di Reggio Emilia, Piazzale Europa 1, 42124 Reggio Emilia, Italy; Tel: +39-0522-523541, Fax: +39-0522-522027; e-mail: giuseppe.montecvecchi@unimore.it

Coauthors' name, e-mail address, and address:

Francesca Masino (francesca.masino@unimore.it)

Nicolas Di Pascale (182647@studenti.unimore.it)

Giuseppe Vasile Simone (giuseppe.vasilesimone@unimore.it)

Andrea Antonelli (andrea.antonelli@unimore.it)

Dipartimento di Scienze della Vita, Università degli Studi di Modena e Reggio Emilia, Via G. Amendola 2, 42122 Reggio Emilia, Italy.

Best regards,

Giuseppe Montecvecchi

Response to reviewers' comments:

Reviewer #1: The work "Study of the repartition of phthalate esters during distillation of wine for spirits production" overall is good science. My opinion is that it can be considered for publication in Food Chemistry, after a revision of English.

The authors recommend taking into account the recent publication:

1) Extraction and GC-MS analysis of phthalate esters in food matrices: a review. MV Russo, Avino P, L Perugini, I Notardonato. RSC Advances 5 (46), 37023-37043 (2015)

The English was accurately revised. We included the suggested reference in the text.

Reviewer #2: PAEs are used as surfactants, emulsifying agents, stabilizers, dispersants, and lubricants. As a consequence of their diffusion, PAEs become process contaminants for food. These substances are cause of great concern as endocrine disrupting chemicals, mainly related to human reproduction. Though, the content of PAEs in the samples was influenced by the level of PAEs contamination in the raw material and by the effect of the concentration that occurred during ageing, the repartition into heads, heart, and tails of each PAE during distillation of the base wine is unknown. This paper studied for that in order to provide a tool to improve brandy purification. The work was well-done. I recommend it to be accepted after minor revision.

1. HPLC chromatogram and GC/MS chromatogram should be given.

HPLC chromatogram has just one peak at 24 min (line 185), while GC/MS chromatogram is now provided as a figure and more details about peaks identification and quantification are now included (lines 171-175).

2. The language needs polish and the table should be changed as three-line table.

English was accurately revised and Table 2 was converted into a three-line table.

EDITORIAL COMMENTS

Abstract: Put all sentences into a single paragraph.

Done.

Line 23-24: Provide DBP, DEHP and DINP in full rather than abbreviations.

Done.

Line 75: are the concentrations given for DBP, DEHP and DINP acceptable limits? Indicate what these numbers refer to.

The values (now line 77) are the maximum concentrations imposed for each substance by the Chinese law. The sentence was rewritten to explain it in a clearer way.

Table 2: Do not use more than two significant digits in the uncertainty value and match the level of precision in the number, e.g. 19.4 ± 1.3 rather than 19.440 ± 1.330 ; 2.7 ± 0.2 rather than 2.694 ± 0.167 etc.

Done.

Study of the repartition of phthalate esters during distillation of wine for spirit production

Giuseppe Montevicchi, Francesca Masino, Nicolas Di Pascale, Giuseppe Vasile Simone, Andrea Antonelli

Highlights: > It is necessary to limit contaminants such as phthalates esters (PAEs) > The behaviour of PAEs during distillation was investigated using a lab still > DBP and DEHP were carried over into the distillate, DINP accumulated in the stillage > Rectification of the secondes is effective to considerably reduce PAEs concentration

1 **Study of the repartition of phthalate esters during distillation of wine for spirit production**

2

3 Giuseppe Montevecchi^{1,*}, Francesca Masino^{1,2}, Nicolas Di Pascale², Giuseppe Vasile Simone¹,

4 Andrea Antonelli^{1,2}

5

6 ¹ Centro di Ricerca Interdipartimentale per il Miglioramento e la Valorizzazione delle Risorse

7 Biologiche Agro-Alimentari BIOGEST – SITEIA, Università degli Studi di Modena e Reggio

8 Emilia, Tecnopolo di Reggio Emilia, Piazzale Europa 1, 42124 Reggio Emilia, Italy

9 ² Dipartimento di Scienze della Vita (Area Scienze Agro-Alimentari), Università degli Studi di

10 Modena e Reggio Emilia, Via G. Amendola 2, 42122 Reggio Emilia, Italy.

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12 * Corresponding author. Tel.: +39 0522523541; fax: +39 0522 522027.

13 E-mail address: giuseppe.montevecchi@unimore.it (G. Montevecchi).

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Abstract

Due to health concerns and legal matters, an investigation to limit phthalates esters (PEAs) in spirits is necessary. A lab still was used to perform pilot distillations according to the official method for brandy production in order to explore the repartition into the distilled fractions of each PAE. The process was divided in two steps: a *première chauffe* and a *bonne chauffe*. The former step included the cut into heads, heart and tails, while the latter into heads, brandy, secondes, and tails. The behaviour of each PAE during distillation was affected by its own chemical nature. Dibutyl phthalate (DBP) was entirely carried over into the distillate, while bis(2-ethylhexyl) phthalate (DEHP) only partially, and diisononyl phthalate (DINP) accumulated in the stillage. During the *bonne chauffe*, DBP and DEHP accumulated in the secondes more than in the brandy. A rectification step of the secondes was demonstrated to considerably reduce PAEs concentration.

Keywords: Phthalate Esters; Dispersive Liquid-Liquid Micro-Extraction; Gas Chromatography-Mass Spectrometry; Distillation; Distilled Alcoholic beverages.

Chemical compounds studied in this article:

Dibutyl phthalate, DBP (PubChem CID: 3026)

Bis(2-ethylhexyl) phthalate, DEHP (PubChem CID: 8343)

Diisononyl phthalate, DINP (PubChem CID: 590836)

Benzyl butyl phthalate, BBP (PubChem CID: 2347)

39 **1. Introduction**

40

41 Phthalate esters (dialkyl or alkylarylesters of 1,2-benzenedicarboxylic acid, also known as PAEs)
42 are a family of chemicals abundantly used since 1930 for a wide spectrum of different industrial
43 applications. PAEs are chemically produced by the reaction of phthalic anhydride with straight-
44 chain or branched alcohols. Therefore, a large variety of PAEs can be synthesized with a wide range
45 of different physical and chemical properties according to their specific use (Moret, Marega, Conte,
46 & Purcaro, 2012; Russo, Avino, Perugini, & Notardonato, 2015; Staples, 2003). High-molecular
47 weight species, such as bis(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DIMP), are
48 applied as plasticizers for PVC, while low-molecular-weight PAEs, such as dibutyl phthalate (DBP)
49 and diisobutyl phthalate (not considered in this study) are used for cellulose acetate, or as
50 surfactants, emulsifying agents, stabilizers, dispersants, and lubricants (Moret et al., 2012; Russo et
51 al., 2015).

52 As a consequence of their diffusion, PAEs become process contaminants for food (Cariou et al.,
53 2016; Chou & Wright, 2006; Wormuth, Scheringer, Vollenweider, & Hungerbühler, 2006),
54 including baby foods (Fierens et al., 2012).

55 Recently, fast food was indicated as an important source of exposure to DEHP and DINP (Zota,
56 Phillips, & Mitro, 2016). Although migration from packaging materials is probably the main source
57 of PAEs contamination in food, PAEs can arise from any step of the productive chain, from the
58 field to the supply chain (Triantafyllou, Akrida-Demertzi, & Demertzis, 2007; Moret et al., 2012).

59 For the same reason, particular attention must be given to their analysis, as environmental PAEs can
60 give false positives or enhance sample content (Fankhauser-Noti & Grob, 2007; Russo et al., 2015).

61 PAEs are not chemically bound to the polymeric matrix, but they can be described as a freely
62 mobile phase. The release into the environment and the migration into products through the contact
63 materials are the main ways of their diffusion (Moret et al., 2012; Russo et al., 2015).

64 Various government agencies, including the EFSA in Europe, and the FDA in the United States, are
65 still assessing the effects of PAEs on human health. These substances are cause of great concern as
66 endocrine disrupting chemicals, mainly related to human reproduction (Committee on the Health
67 Risks of Phthalates, 2008; Owens, 2015). The strongest and most consistent evidence was described
68 for dibutyl phthalate and bis(2-ethylhexyl) phthalate and associated with lower semen quality
69 (Mariana, Feiteiro, Verde, & Cairrao, 2016), although other endocrine functions and different
70 physiological systems are suspected to be involved (Johns, Ferguson, & Meeker, 2016; Mariana et
71 al., 2016).

72 In order to export distillates produced in other countries to China, the Chinese authority responsible
73 for imports and exports (AQSIQ) has made an analytical report mandatory (circular from the
74 Ministry of Health of 22 January 2013). The report must state the concentrations of three PAEs in
75 accordance with the specification of the law that imposes maximum tolerated concentrations of 0.30
76 mg/kg for DBP, 1.50 mg/kg for DEHP, and 9.00 mg/kg for DINP. This brings about a severe
77 hindrance to the export of spirits to China, but it is not excluded that other countries might follow
78 the China action imposing a similar trade barrier.

79 Hence, it is crucial to explore the source of contamination in distilled beverages, as the high
80 percentage of ethanol acts as a solvent for PAEs extraction. Moreover, the fate of different PAEs
81 during the distillation process is so far unknown. Finally, the long ageing (even for decades) in oak
82 casks makes distillates ideal candidates to concentrate PAEs.

83 In a preliminary study, a robust protocol of analysis for the determination of PAEs in brandies was
84 set up (Montevecchi, Masino, Zanasi, & Antonelli, 2017). The procedure is based on an ultrasound-
85 vortex-assisted dispersive liquid-liquid microextraction (USVADLLME) (Cinelli, Avino,
86 Notardonato, Centola, & Russo, 2013; Russo, Notardonato, Avino, & Cinelli, 2014) originally set
87 up for wine and now optimized for high strength beverages. The method is environmentally friendly
88 because of the limited volume of solvent required, particularly chlorinated (Yan, Cheng, & Liu,

89 2011). This is also useful because it minimizes the risk of false positive cases due to the presence of
90 phthalates in the solvent.

91 The results of a screening on PAEs content performed on a historical brandy series (from 1987 to
92 2014) showed that PAEs concentrations exceeded the limits imposed by the Chinese law
93 (Montevecchi et al., 2017) only in a few of the most aged samples. The content of PAEs in the
94 samples was influenced by the level of PAEs contamination in the raw material (base wines) and by
95 the effect of the concentration that occurred during ageing. However, the repartition into heads,
96 heart, and tails of each PAE during distillation of the base wine is still not investigated. The
97 objective of this paper is to fill this gap, thus providing a tool to improve brandy purification
98 without altering the unique quality of many distillates all over the world.

99

100 **2. Materials and methods**

101 *2.1. Sampling*

102 The base wine (Trebiano without sulphur dioxide, 10 L) used for distillations was collected by a
103 local winemaker. It was part of a lot used for the process on an industrial scale.

104

105 *2.2. Chemicals and instruments*

106 All solvents and reagents were of analytical grade. Acetone, dichloromethane, and absolute ethanol
107 were obtained from WVR Srl (Milan, Italy). The pure standards diisobutyl phthalate, bis(2-
108 ethylhexyl) phthalate, diisononyl phthalate, and benzyl butyl phthalate, as well as carborundum,
109 sodium chloride, and sodium hydroxide were purchased from Fluka Sigma-Aldrich[®] Srl (Milan,
110 Italy). Deionised water was obtained by a Milli-Q purification system (Millipore, Milan, Italy).

111

112 *2.3. Preparation of materials: solvents, chemicals, and glassware PAE free (PF)*

113 All the solvents used (acetone, dichloromethane, absolute ethanol, and water) were distilled to
114 eliminate the presence of PAEs as described by Montevecchi and coll. (2017). In a few words, all

115 solvents were distilled before use and water, in particular, was distilled on NaOH to prevent steam
116 distillation of PAEs. Then the presence of PAEs was excluded by gas chromatography-mass
117 spectrometry (GC/MS) on concentrated (100:1) solvent samples. Glassware and solid reagents were
118 previously rinsed with acetone PF and were then heated at 400 °C for 4 h. Finally, reagents were
119 stored in PF containers with ground glass stoppers, while glassware was kept separate from dust.

120

121 2.4. Pilot distillations

122

123 2.4.1. Distillation of base wine spiked with PAEs by a lab still according to the procedure of brandy 124 production

125 Except for the number of the first distillations (*première chauffe*; only 3 instead of 4), distillation
126 was carried out in accordance with Léauté (1990). A lot of 5.0 L of white Trebbiano base wine was
127 homogenized to suspend yeast lees and divided into three batches (1 L each) to be subjected to
128 distillation. Each batch was spiked with DBP (1 mg in the sample), DEHP (5 mg in the sample),
129 and DINP (20 mg in the sample) and subjected to first distillation (*première chauffe*) in a lab still
130 equipped with a distillation flask (2 L) heated by an electric heating mantle, a column, and a
131 condenser cooled down by cold tap water (Fig. 1).

132 The distillate was collected in different flasks in accordance with the brandy production method and
133 cut in heads, heart (or *brouillis*), and tails (Table 1). After sampling, heads and tails were re-distilled
134 with the succeeding batch of fresh base wine. At the end of the distillation of all the batches of
135 wine, the three *brouillis* were joined and subjected to a second distillation (*bonne chauffe*). During
136 the *bonne chauffe* four fractions were collected (Table 1), heads, heart 1 (or brandy), heart 2 (or
137 secondes), and tails.

138

139 2.4.2. Distillation of base wine spiked with PAEs by a lab still and collection of 8 mL fractions

140 A lot of white Trebbiano base wine measuring 2.5 L was homogenized and divided into two batches
141 (1 L each) to be subjected to distillation. Each batch was spiked with DBP (1 mg in the sample),
142 DEHP (5 mg in the sample), and DINP (20 mg in the sample) and subjected to distillation with the
143 lab equipment described above.

144 The distilled fractions (8 mL each) were collected in different tubes. The distillation was stopped
145 after collecting 27 fractions (216 mL in total).

146

147 *2.4.3. Distillation of base wine spiked with PAEs by a lab still with the column filled with Raschig*
148 *rings and collection of 8 mL fractions*

149 Another lot of wine (2.5 L) was distilled with the same protocol described in section 2.4.2., but the
150 column was filled with Raschig rings in order to improve rectification during distillation.

151

152 *2.5. Extraction method (Montevecchi et al., 2017)*

153 Samples (8.000 g for wine, stillage, and tails; 1.500 g for heads, heart, brandy, and secondes) were
154 accurately weighed and transferred into a conical tube with ground glass stoppers. Internal standard
155 (benzyl butyl phthalate), up to 8 mL of water (if necessary), and 1.50 g of NaCl were added to each
156 tube. Each sample was vortexed to dissolve the salt. Then dichloromethane PF (160 μ L) was added
157 and the mixture was vortexed again for 30 sec. Finally, each tube was sonicated for 10 min
158 (Ultrasonic frequency 37 kHz, Elmasonic S30, Elma Schmidbauer GmbH, Singen, Germany),
159 cooled to 4 °C for 5 min and then centrifuged for 2 min at 5000 rpm. The ready-for-analysis extract
160 was collected from the organic phase at the bottom of the tube.

161

162 *2.6. GC-MS determination of PAEs*

163 GC-MS analysis was previously described in detail (Montevecchi et al., 2017). In a few words, the
164 extract was injected (splitless mode, 335 °C, splitless time: 30 sec) onto SE52 crossbond capillary
165 column (5% phenyl, 95% methyl polysiloxane; 25 m length, 0.25 mm i.d., 0.25 μ m f.t., carrier gas

166 He at constant flow rate 1 mL/min). Oven temperature: 150 °C for 1 min then up to 330 °C for
167 2 min, rate 15 °C/min. The injection was performed in splitless mode at 335 °C (splitless time
168 30 sec), the temperature of the transfer line was set at 330 °C.

169 The mass spectrometer detector was operated in electron ionization (EI) mode at 70 eV (33-500
170 m/z). Identification was carried out by comparing the retention time of the analytes to that of the
171 pure standards (Fig. 2) and confirmed by the qualifying ions (76 and 104 m/z , 167 and 279 m/z , 167
172 and 293 m/z , for DBP, DEHP, and DINP, respectively). Quantification was carried out by
173 measuring the quantifying ion's (149 m/z) relative peak area in relationship to that of the internal
174 standard. Each analysis was duplicated.

175

176 *2.7. HPLC determination of alcohol by volume in fractions collected during distillation*

177 Alcohol by volume (ABV) was determined by a Perkin Elmer HPLC system (Series 200 LCP,
178 Norwalk, U.S.A) equipped with a refractive index detector (RI detector, Series 200). Diluted
179 samples were filtered through 0.45- μ m nylon membrane and injected with a 20- μ L loop using an
180 injection valve (Rheodyne Inc., Cotati, CA, U.S.A.) onto a Bio-Rad Aminex HPX-87H (Hercules,
181 CA, U.S.A.) hydrogen-form cation exchange resin-based column (300 mm \times 7.8-mm i.d.). The
182 column was thermostated at 50 °C into a column oven (Perkin Elmer, Series 200). The solvent
183 system was composed by aqueous H₂SO₄ (pH 2.70), added of CH₃CN (10%). The isocratic elution
184 was carried out with 0.5 mL/min flow. The ethanol retention time was 24 min.

185 Quantification was carried out by means external of standard method and assessing the linearity of
186 the response. The chromatograms were acquired and processed using the TotalChrom Workstation
187 version 6.2.1 software (Perkin Elmer, Inc.).

188

189 **3. Results and discussion**

190 *3.1. Behaviour of PAEs during distillation of base wine by a lab still*

191 Première chauffe

192 Concentration of DBP and DEHP in the base wine ranged from 0.012-0.079 mg/kg and 0.005-0.041
193 mg/kg, respectively. Their concentration was largely below the limits of the Chinese law. DINP was
194 not detected in any sample.

195 Samples of base wines spiked with standard solutions of PAEs showed concentrations consistent
196 with the amount added (table 2), thus confirming the robustness of the method.

197 In the stillage, DBP was not detected in any of the cases. On the contrary, about 84 % (average of
198 the 3 distillations) of DEHP remained in the stillage, while DINP did not distil at all, showing very
199 similar figures to the original amount used in spiked samples.

200 During the first distillation, DBP tended to accumulate in the heart, while only about the 15% of the
201 original DEHP accumulated in the heart (average of the 3 distillations). According to their
202 volatility, DBP behaved as head compound more than DEHP did, and both resulted in the heart
203 with average amounts of 1.1 and 0.81 mg, respectively, although DEHP was 5-times more
204 concentrated in the spiked wine. Finally, they were still present in the tails at an average amount
205 below 0.02 mg. DINP was not detected in any of the distilled fractions (table 2).

206 Bonne chauffe

207 In the joint *bruillis*, DBP concentration enhanced 3.5 folds, while DEHP was only about 16 % of
208 the original content (table 2). In the stillage of the *bonne chauffe*, DBP was not detected, while
209 DEHP was as low as 6% of the original amount. As expected, DINP was not detected in any
210 sample.

211 ABV of *brouillis* (about 40 % v/v) in the *bonne chauffe* is by far higher than in the *première*
212 *chauffe*. For this reason, in the *bonne chauffe* the distillation was cut in four fractions (instead of
213 three), as for industrial scale process.

214 DBP and DEHP amounts in the heads (about 79 % ABV) and in the tails (about 0.7 % ABV) were
215 very low. The highest amount of the two substances was concentrated in the two hearts. In the
216 brandy or heart 1 (about 78 % ABV), DBP and DEHP amounts were 0.62 mg and 0.47 mg,

217 respectively, while in the secondes or heart 2 (about 19 % ABV) their amounts were markedly
218 higher. In fact, DBP and DEHP showed values as high as 2.7 mg and 1.0 mg, respectively.

219

220 The general analysis of the data clearly showed that DBP, which has the lowest molecular weight
221 (278.35 g/mol) and the lowest boiling point (340 °C), was entirely carried over into the distillate
222 during the *première chauffe*. The phenomenon was even clearer during the *bonne chauffe*, whereas
223 the carrying over of DBP into the distillate occurred mainly during the distillation of the secondes.
224 In this cut the water content was higher to allow a hydrodistillation of DBP. DBP behaviour was
225 similar to those components that are carried over by the water vapour, as most of the volatile
226 compounds during the hydrodistillation process for essential oil production (Léauté, 1990). On the
227 other hand, PAEs were already found as contaminants in essential oils obtained through
228 hydrodistillation, thus confirming that this process is able to carry over and concentrate them during
229 distillation (Di Bella, Saitta, Lo Curto, Salvo, Licandro, & Dugo, 2001; Ricking, Schwarzbauer, &
230 Franke, 2003; Song et al., 2007; Maltese, van der Kooy, & Verpoorte, 2009; Radulović &
231 Blagojević, 2012; Firouzi, Gohari, Rustaiyan, Larijani, & Saeidnia, 2013; Manayi, Saeidnia,
232 Shekarchi, Hadjiakhoondi, Shams Ardekani, & Khanavi, 2014a; Manayi, Kurepaz-mahmoodabadi,
233 Gohari, Ajani, & Saeidnia, 2014b; Wu, Wang, Liu, Zou, & Chen, 2015).

234 Due to its higher molar mass (418.61 g/mol) and boiling point (estimated at about 426-437 °C),
235 DINP was not carried over at all into the distillate, so it did not represent a concern during the
236 distillation process. Finally, DEHP was in an intermediate position (molar mass 390.56 g/mol and
237 boiling point 385 °C). In fact, it was found in an appreciable amount in the stillage, but it was also
238 present in the secondes, although its solubility in water was as low as 0.00003% (23.8 °C).

239

240 *3.2. Behaviour of PAEs during distillation of base wine by a lab still (8-mL fractions)*

241 Consistently with the previous experiment, PAEs amounts were very low in the base wine. At the
242 end of distillation, DBP was not detected in the stillage, DEHP amount was reduced and, finally,
243 DINP amount was constant.

244 The analysis of the 27 fractions collected during distillation showed that DBP (Fig. 3A) was even
245 carried over at the early stage. Its maximum amount was reached in the eleventh fraction (about
246 0.27 mg) and, after this, its amount was reduced until it disappeared completely in the twentieth
247 fraction.

248 DEHP (Fig. 3B) achieved its highest amount in the eleventh fraction (about 0.09 mg), and thereafter
249 showed fluctuating values up to the end of the process. DINP was not detected in any fraction.

250 These data suggested that DBP achieved its highest amount at the end of the ethanol carry-over and
251 during the shift of the boiling point of the sample (dotted line in Fig. 3A). The greatest part of the
252 DBP was carried over into the distillate when full grade was reached and was sharply reduced when
253 the ethanol disappeared.

254 Even if the DEHP profile is less regular, it behaved in a quite similar way, showing a clear peak at
255 almost the same point of the distillation process as for DBP. From then onwards, DEHP
256 concentration was less regular. However, two observations must be pointed out: i) when volumes
257 are so tiny a little spurt or a single drop can make the difference; ii) DEHP is a very high boiling
258 substance and this could explain some fluctuation in its distillation.

259

260 *3.3. Behaviour of PAEs during distillation of base wine by a lab still (8-mL fractions) with*
261 *rectification (column filled with Raschig rings)*

262 The previous experiment was repeated using the same conditions, but the column was filled with
263 Raschig rings in order to evaluate the effect of the rectification on PAEs behaviour. In the stillage,
264 DBP was not detected, while DINP showed values similar to the previous experiment. Conversely,
265 DEHP showed higher amounts in the stillage in comparison with what was detected in the previous
266 test.

267 The DBP trend showed that it was present starting only from the eighth fraction (Fig. 4A), then it
268 achieved its maximum amount in the tenth fraction (about 0.37 mg) and, finally, it had already
269 almost zeroed in the eighteenth fraction.

270 For DEHP (Fig. 4B), its presence started from the eighth fraction, as well, and reached the
271 maximum amount in the eleventh fraction (about 0.18 mg), and then it dropped down without
272 zeroing at all. DINP was never detected in any fraction.

273 As a matter of fact, rectification allowed the modification of the trend of DBP and DEHP carry-
274 over. For both of them there was a retarded carry-over, but it was concentrated in a smaller volume,
275 especially for DBP. This effect can be exploited to reduce PAEs contamination in the secondes, the
276 fraction where DBP and DEHP accumulated during the *bonne chauffe*. Secondes are considered a
277 valuable fraction for ethanol concentration (about 19 % ABV) and aroma compounds, and for this
278 reason they are kept to be reintroduced in the pot still during the subsequent *bonne chauffe*. As a
279 consequence of this, manufacturers can take advantage of the rectification step on the secondes in
280 order to eliminate only the most contaminated part of them and save the rest.

281

282 **4. Conclusions**

283

284 The results highlighted how the chemical nature of the individual PAE influenced its own behaviour
285 during the distillation process. DBP was entirely found in the distillate, while DINP accumulated in
286 the stillage. DEHP showed an intermediate behaviour, since it was only partially carried over into
287 the distillate.

288 The most impressive phenomenon was highlighted during the *bonne chauffe*, whereas DBP and
289 DEHP accumulated in the secondes more than in the heart 1. A rectification step of the secondes
290 can allow a considerable reduction of PAEs concentration in order to reintroduce this valuable
291 fraction cleaner in the distillation process, thus improving the quality of brandies.

292 Considering the behaviour of DBP and DEHP, it is very difficult to suggest modification of the
293 distillation process without impairing the quality of brandies. The prevention of PAEs wine
294 contamination seems to be the only way to have a low content of PAEs in the brandy. Pipelines
295 used for the pouring of brandy can be a source of PAEs: plastic hosepipes and other plastic objects
296 should be kept separate and the contact with distillates avoided.

297

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418 **Figure captions**

419

420 **Fig. 1.** Lab still equipped with a distillation flask (2 L) heated by an electric heating mantle, a
421 column, and a condenser cooled down by cold tap water.

422

423 **Fig. 2.** GC trace of a wine sample spiked with standard solutions of PAEs.

424 DBP = dibutyl phthalate; DEHP = bis(2-ethylhexyl) phthalate; DINP = diisononyl phthalate. IS =
425 internal standard (BBP, benzyl butyl phthalate).

426

427 **Fig. 3 A.** Trends of amounts (expressed in mg) of DBP in the 27 fractions (8 mL each) collected in
428 the base wine pilot distillation. **B.** Trends of amounts (expressed in mg) of DEHP in the 27 fractions
429 (8 mL each) collected in the base wine pilot distillation.

430 Dotted lines indicate the shift of the boiling point of the sample from 78 °C toward the boiling point
431 of water.

432

433 **Fig. 4 A.** Trends of amounts (expressed in mg) of DBP in the 27 fractions (8 mL each) collected in
434 the base wine pilot distillation with Raschig rings. **B.** Trends of amounts (expressed in mg) of
435 DEHP in the 27 fractions (8 mL each) collected in the base wine pilot distillation with Raschig
436 rings.

437 Dotted lines indicate the shift of the boiling point of the sample from 78°C toward the boiling point
438 of water.

Table 1 – Schematization of fractionation during distillation

| <i>Première chauffe</i> | | | | |
|-------------------------|------------|-----------------------------------|-----------------------------|------------|
| Wine (mL) | Heads (mL) | Heart or <i>Brouillis</i> (mL) | | Tails (mL) |
| 1000 | 4 | 280 | | 60 |
| 1064* | 4 | 280 | | 60 |
| 1064* | 4 | 280 | | 60 |
| <i>Bonne chauffe</i> | | | | |
| <i>Brouillis</i> (mL) | Heads (mL) | Heart 1 or Brandy (mL) | Heart 2 or Secondes (mL) | Tails (mL) |
| 840 | 10 | 280 | 240 | 60 |

* Comprehensive of 1000 mL of wine, 4 mL of heads and 60 mL of tails of the previous distillation.

Table 2

Amount of PAEs (DBP, DEHP, and DINP) expressed in mg and alcohol by volume recorded during the distillation tests.

| | | DBP (mg ± st. dev.) | DEHP (mg ± st. dev.) | DINP (mg ± st. dev.) | ABV (% v/v ± st. dev.) |
|-------------------------|------------------------|---------------------------|----------------------------|----------------------------|------------------------------|
| <i>Première chauffe</i> | | | | | |
| 1 | Wine | 0.015 (±0.004) | 0.008 (±0.002) | N.D. | 9.11 (±0.02) |
| | Wine + PAEs | 0.95 (±0.13) | 4.74 (±0.28) | 19.4 (±1.3) | 9.13 (±0.04) |
| | Stillage | N.D. | 3.94 (±0.17) | 19.2 (±1.3) | N.D. |
| | Heads | 0.001 (±0.000) | 0.009 (±0.000) | N.D. | 66.93 (±0.61) |
| | Heart | 0.94 (±0.07) | 0.78 (±0.05) | N.D. | 36.96 (±0.45) |
| | Tails | 0.009 (±0.000) | 0.017 (±0.000) | N.D. | 0.69 (±0.01) |
| | 2 | Wine | 0.012 (±0.003) | 0.005 (±0.002) | N.D. |
| Wine + PAEs | | 1.11 (±0.08) | 5.19 (±0.70) | 19.2 (±0.5) | 9.57 (±0.21) |
| Stillage | | N.D. | 4.32 (±0.20) | 19.8 (±1.7) | N.D. |
| Heads | | 0.002 (±0.000) | 0.009 (±0.000) | N.D. | 72.39 (±0.37) |
| Heart | | 1.10 (±0.08) | 0.85 (±0.07) | N.D. | 38.70 (±0.07) |
| Tails | | 0.008 (±0.000) | 0.013 (±0.001) | N.D. | 0.70 (±0.03) |
| 3 | | Wine | 0.079 (±0.004) | 0.041 (±0.010) | N.D. |
| | Wine + PAEs | 1.19 (±0.05) | 5.31 (±0.21) | 19.9 (±1.2) | 9.48 (±0.33) |
| | Stillage | N.D. | 4.49 (±0.28) | 19.8 (±1.6) | N.D. |
| | Heads | 0.004 (±0.000) | 0.008 (±0.000) | N.D. | 62.22 (±0.32) |
| | Heart | 1.18 (±0.06) | 0.79 (±0.05) | N.D. | 38.87 (±0.52) |
| | Tails | 0.006 (±0.000) | 0.010 (±0.000) | N.D. | 0.67 (±0.01) |
| | <i>Bonne chauffe</i> | | | | |
| | <i>Joint Brouillis</i> | 3.32 (±0.05) | 2.44 (±0.33) | N.D. | 38.29 (±0.38) |

| | | | | |
|----------|-------------------|-------------------|------|------------------|
| Stillage | N.D. | 0.95 (±0.68) | N.D. | N.D. |
| Heads | 0.001 (±0.000) | 0.005 (±0.000) | N.D. | 78.64 (±0.02) |
| Brandy | 0.62 (±0.05) | 0.47 (±0.04) | N.D. | 77.64 (±0.01) |
| Secondes | 2.69 (±0.17) | 1.01 (±0.06) | N.D. | 18.66 (±0.20) |
| Tails | 0.008 (±0.000) | 0.016 (±0.002) | N.D. | 0.66 (±0.03) |

st. dev. = standard deviation; ABV = alcohol by volume; N.D. = not detected.

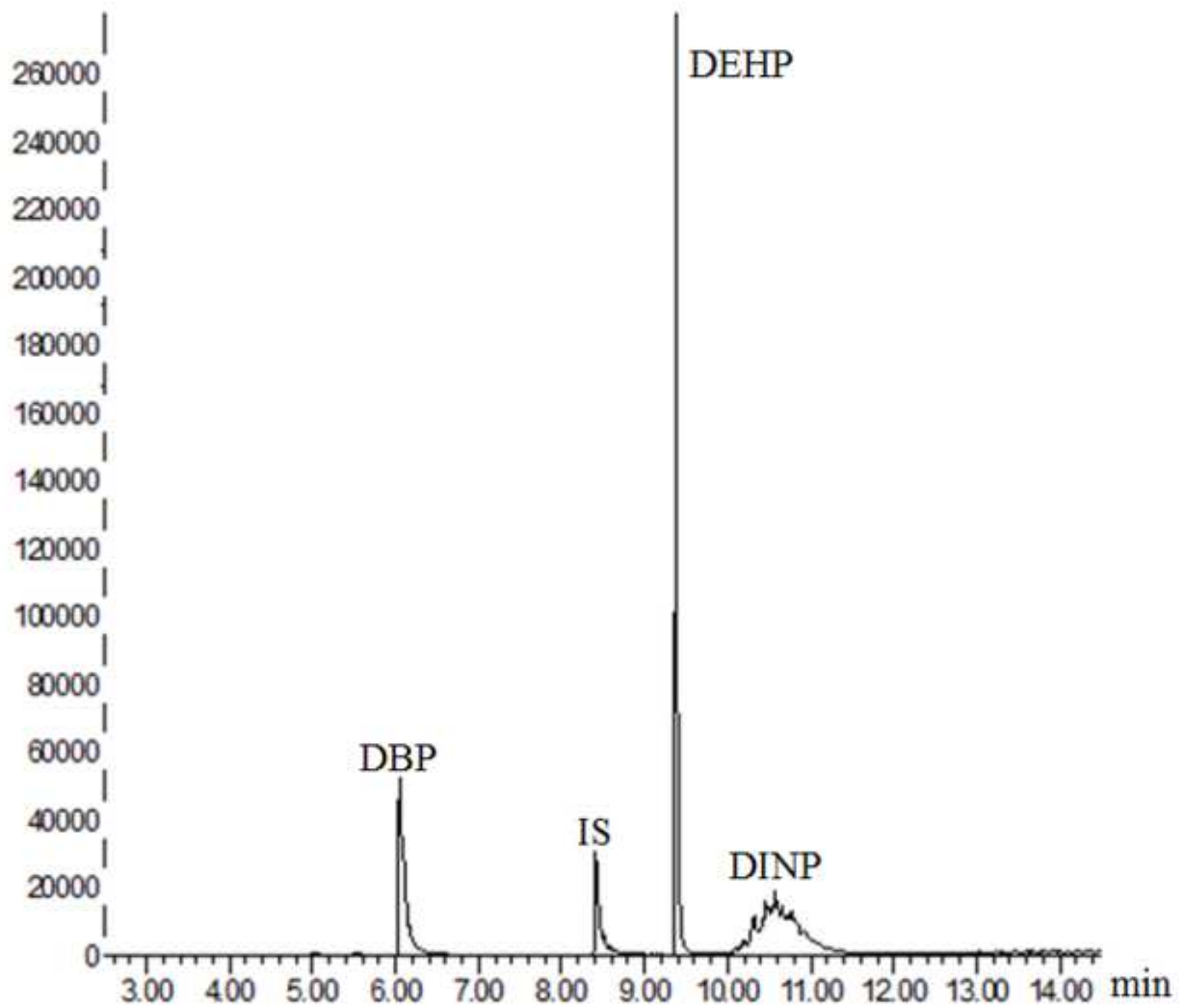
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