1   **Ambient mass spectrometry as a tool for a rapid and simultaneous determination of**

2   **migrants coming from a bamboo-based biopolymer packaging.**

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17   **Abstract**

18   New bamboo-based biopolymers are used as food packaging materials, but it must be

19   evaluated to ensure consumers safety. In this study, migration from a commercial bamboo-

20   based biopolymer to ethanol 10% (v/v), acetic acid 3% (w/v) and ethanol 95% (v/v) was

21   studied. The migrants were determined from three different perspectives. Volatile and semi-

22   volatile compounds were analyzed by gas chromatography-mass spectrometry (GC-MS).

23   Twenty-five compounds were detected. In addition, a number of phytosterols were detected

24   in ethanol 95%. Non-volatile compounds were identified and quantified by ultra-performance

liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-Q/ToF). Twelve non-volatile compounds were detected in migration solutions, mainly melamine and its derivatives, coming from polymer resins present in the biopolymer. Melamine migration was higher than 50 mg/Kg in the third sequential migration test. Finally, the migration samples were analyzed by DART-SVP (direct analysis in real time coupled to standardized voltage and pressure). This methodology was able to detect simultaneously the main volatile and non-volatile migrants and their adducts in a very rapid and effective way and is shown as a promising tool to test the safety and legal compliance of food packaging materials.

1. Introduction

There is a great demand for biopolymers in the sector of food contact materials manufacturing. This is because biopolymers are a green alternative to the traditional packaging materials used in the food industry, especially those which are abundant, renewable, biodegradable, and biocompatible (Khlestkin, Peltek, & Kolchanov, 2018). Biopolymers can be classified into two groups, polymers produced from biological products that can be extracted from biomass or produced in fermentation processes, such as carbohydrates, proteins or lipids; and biodegradable and/or compostable polymers (Aznar, Ubeda, Dreolin, & Nerín, 2019; Geueke, 2014; Maisanaba et al., 2018). The addition of additives to the biopolymers is necessary in order to improve their physico-chemical properties. These additives can be for example: polyols, used as non-volatile plasticizers (e.g., glycerol, glycol, and sorbitol); and resins, that mold the material (e.g., urea, melamine, melamine formaldehyde, and amines) (Eckardt, Kubicova, & Simat, 2018; Geueke, 2013). Surprisingly, melamine and other resins are neither biodegradable or come from natural sources (Chien et al., 2011; Geueke, 2013).

49   In recent years, food packaging industries have been highly innovative in the production of

50   new biopolymers derived from various natural resources, such as starch, cellulose, bamboo,

51   and chitosan. Among them, bamboo-base biopolymers are of enormous importance. This

52   fibre is attractive as a natural-based raw material, since it is economical, eco-friendly and

53   biodegradable (Jena, 2018; Xie, Zhou, & Yan, 2019). Bamboo plants are easily available due

54   to its very short life cycle and because bamboo timber can be harvested multiple times from a

55   single planting, which makes its use very sustainable. Bamboo-based biopolymers have better

56   mechanical properties than other biomaterials, such as a good impact resistance, high

57   durability and crack resistance, and good flexural properties (Xie et al., 2019). As any food

58   packaging material or food contact article, the biopolymers must be evaluated to confirm

59   their acceptability and safety. The bamboo-base biomaterials can contain other resins and

60   also non intentionally added substances (NIAS), such as impurities from raw materials,

61   degradation compounds or novel substances coming from the reaction between different

62   reagents (Xu, Xiong, Zhao, Xiang, & Wu, 2020). These compounds, as well as those

63   additives intentionally added, are present in the packaging and could migrate to the food.

64   Migration tests are required in order to guarantee the consumers safety, food preservation and

65   organoleptic properties (Bradley, 2010). These tests require specific conditions of

66   temperature, time and simulants, which should be selected according to the intended use of

67   the material. In the specific migration tests, compounds previously identified in the material

68   as well as new formed compounds can be detected (Nerin, Alfaro, Aznar, & Domeño, 2013).

69   Pure biopolymers are not specifically included in the European plastics regulation and only

70   the frame regulation 1935/2004/CEE applies. However, if they contain some resin or

71   conventional plastic, the regulation on plastics should be applied. European legislation on

72   food contact plastics (Regulation No 10/2011/EU) establishes a positive list with substances

73 that can be used in the manufacturing process and also their specific migration limits (Osorio,

74 Dreolin, Aznar, Nerín, & Hancock, 2019).

75 Since the migrants can be volatile as well as non-volatile compounds, different technologies

76 should be applied in order to have a global view of the safety of the material. Gas

77 chromatography can be applied to the separation of the most volatile and semi-volatile

78 compounds (Biedermann & Grob, 2019) and liquid chromatography to the less volatile ones.

79 Both of them coupled to mass spectrometry detection will allow the identification of the

80 NIAS present in the packaging material. Ambient mass spectrometry techniques can be also

81 used to get a quick confirmation of the presence/absence of the identified compounds.

82 Ambient mass spectrometry (AMS) or ambient ionization techniques (AMI) are ionization

83 sources used in mass spectrometry for direct and rapid analysis of compounds present in solid

84 or liquid samples (Black, Chevallier, & Elliott, 2016; Lu et al., 2018). Additionally, AMS has

85 been utilized as screening method in food safety analysis, criminal investigation,

86 environmental test and other different analytical objectives (Gross, 2014; Lennert & Bridge,

87 2019; Zhang et al., 2018). DESI-MS (Desorption ElectroSpray Ionization Mass

88 Spectrometry) was the first AMS technique developed. Since then, other techniques such as

89 direct analysis in real time (DART), extractive electrospray ionization (EESI), desorption

90 atmospheric pressure chemical ionization (DAPCI), dielectric-barrier-discharge ionization

91 (DBDI) or low-temperature plasma (LTP) have been designed (Barnett & Zhang, 2018).

92 AMS techniques had been previously used in the study of food packaging materials by other

93 groups. For example, Bentayeb et al. determined the compounds present due to print set-off

94 process (Bentayeb et al., 2012; ten Dam et al., 2016) while Aznar et al. analyzed the

95 distribution of ink components in a packaging material and Rothenbacher et al. studied the

96 plasticizers in poly(vinyl chloride) (PVC) (Aznar, Alfaro, Nerín, Jones, & Riches, 2016).

Direct analysis in real time (DART) is one of the most popular ambient pressure ionization methods. In this technique, the molecules are ionized by excited helium molecules and adducts, mainly protonated/deprotonated molecular ions, are commonly formed (Black et al., 2016). Some of its main advantages are: the possibility of direct sampling at ambient conditions; to generate ions from liquid or solid samples; to be simple to operate and to have a rapid analysis speed (acquisition time < 60 s) compared to UPLC and GC-MS methods (Nerin et al., 2013). DART-SVP technique has diverse applications such as food quality and safety control, drug screening, contamination analysis and environmental monitoring (Barnett & Zhang, 2018; Djelal, Cornée, Tartivel, Lavastre, & Abdeltif, 2017; Lennert & Bridge, 2018).

In the present study, the migration from a bamboo-based biopolymer intended for food contact to different food simulants was evaluated. Volatile, semi-volatile and non-volatile compounds were firstly identified by GC-MS and UPLC-Q/ToF. Additionally, DART-SVP was used as a tool to assess the presence of all potential migrants in a very short analysis time.

2. Materials and methods

2.1. Chemicals and Reagents

Melamine 99% [108-78-1] (IUPAC name: 1,3,5-triazine-2,4,6-triamine) was from Sigma–Aldrich (Madrid, Spain). Ethanol absolute (HPLC grade), methanol (UHPLC-MS grade), acetic acid (HPLC grade) were supplied by Scharlau (Setmenat, Spain) for the analysis in GC-MS and UPLC-Q/ToF. Ethanol absolute (HPLC grade) supplied by Merck (Darmstadt, Germany) for the analysis in DART-SVP. Ultra-pure water was obtained from Millipore Milli-Q system (Billerica, MA, USA). The nitrogen evaporator was a TECHNE sample

120 concentrator (Cole-Parmer Ltd., UK). SPME fibers were purchased at Supelco (Bellefonte,

121 PA, USA).

122 2.2. Samples

123 Bamboo-based biopolymer samples were purchased at a local supermarket in the form of

124 cups, dishes and jugs. All of these were monolayer materials.

125 2.3. Migration assays

126 Migration assays were carried out by total immersion of the sample (5 cm x 1 cm) into 20 mL

127 of the simulant. The process was established in accordance with the European legislation on

128 food contact materials (Regulation No 10/2011/EU) (European Commission, 2011a). Three

129 simulants were evaluated: ethanol 10% (v/v) (EtOH 10%) (simulant A), acetic acid 3% (w/v)

130 (AcH 3%) (simulant B) and ethanol 95% (v/v) (EtOH 95%) (simulant D2 substitute). Overall

131 migration studies were performed during 2 hours at 70 ºC. The overall simulant solutions

132 were evaporated to dryness at 110 ºC. Overall migration was calculated by weighting the dry

133 residue. Specific migration was performed during 6 hours at 70 ºC. Since the food contact

134 materials of this study were intended for repeated use, the migration tests were performed

135 three consecutive times. The assays were carried out in triplicate for each sample and a blank.

136 2.4. Analysis by GC-MS

137 The analyses of volatile and semi-volatile compounds were carried out using a gas

138 chromatograph 6890N equipped with an electron ionization ion source operating at 70 eV,

139 and coupled to a quadrupole mass spectrometry detector 5975D (Agilent Technologies. Santa

140 Clara, CA). It was set in SCAN acquisition mode (50 - 450 m/z). The transfer line heater was

141 set at 280 ºC. The autosampler was a Combi PAL (CTC Analytics, Zwingen, Switzerland).

142 The injector was used in two modes. Liquid injection for migration samples in EtOH 95%, 1

143 µL of liquid samples was injected at 250 ºC in splitless mode. SPME injection was used for

144 aqueous migration samples (AcH 3% and EtOH 10%). The stationary phase of

145 polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR 50/30 µm) was selected.

146 The migration samples were conditioned for 2 min at 80 ºC. Subsequently, the volatile and

147 semi-volatile compounds were extracted by fiber inmersion at 80 ºC and 500 rpm agitation

148 during 15 min and finally SPME fiber was desorbed at 250 ºC for 2 min and. The

149 chromatographic separation was performed on a HP-5MS column (30 m length × 0.25

150 mminner diameter × 0.25 µm film thickness). The oven temperature program was: 40 ºC held

151 for 3 min, ramp 10 ºC min^{-1} to 300 ºC and held for 2 min. Helium was used as carrier gas at a

152 constant flow of 1 mL min^{-1}. The SPME extraction process in biopolymer samples was

153 optimized in a previous work (Osorio, Aznar, & Nerín, 2019).

154 2.5. Analysis by UPLC-Q/ToF

155 The analyses of the non-volatile compounds present in migration solutions were performed

156 using an ACQUITY UPLC chromatograph (Milford, MA, USA). Flow rate was 0.3 mL min^{-}

157 ^{1}. Injection volume was 10 µL. An UPLC BEH C18 column (Waters) of 2.1 × 100 mm, 1.7

158 µm particle size was used at a temperature of 35 °C. Two mobile phases were used for the

159 separation: (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid.

160 Chromatography was performed using the following gradient elution: initial composition

161 98/2 (phase A/ phase B), and at eight min it was changed to 0/100 (phase A/ phase B) with

162 two additional minutes at the final composition.

163 UPLC system was coupled to a Xevo G2 QTOF mass spectrometer from Waters (Milford,

164 MA, USA) with an ESI probe. The conditions of analysis were: positive ionization (ESI+),

165 sensitivity mode, capillary voltage 3.0 kV, sampling cone voltage 30V, extraction cone 3 V,

166 source temperature 150 °C, desolvation temperature 450 °C, cone gas flow rate of 20 L h^{-1},

167   and desolvation gas flow rate of 500 L h^{-1}. Acquisition was carried out in MSE mode in a

168   mass range between 50 and 1200 m/z. The chromatogram was acquired at low and high

169   collision energy (CE) in the collision cell.

170   2.6. Analysis by DART-SVP

171   A direct analysis in real time (DART) couple to Standardised Voltage and Pressure (SVP)

172   100 ion source (IonSense, Saugus, MA) was coupled to an Acquity QDa single quadrupole

173   mass spectrometer (Waters, Wilmslow, UK) via a Vapur interface (IonSense, Saugus, MA).

174   The DART-SVP and Acquity QDa were operated in positive ion mode. The DART-SVP was

175   operated with helium (grade A) in running mode and nitrogen in standby mode. The DART-

176   SVP was operated at several different temperatures between 150 ºC and 450 ºC, the ion-

177   source grid voltage was set to 350 V. The mass spectrometer was operated with the

178   desolvation line temperature set at 250 ºC, heat block 350 ºC, interface voltage 4.5 kV and

179   50-1000 Da scan range. The mass spectrometer was controlled using MassLynx v4.1

180   SCN888 (Waters, Wilmslow, UK). Data were analyzed by MassLynx v4.1.

181   Manual sample injection mode was used. Three microliters of the migration solution were

182   pipette-spotted directly onto the Quick Strip card in the position one. Then, Quick Strip card

183   was simply mounted on the sampling rail. Further, the sample came in contact with the He

184   stream from the DART ion source outlet. Even though the three simulants were tested, only

185   data from EtOH 95% are included in the manuscript. In the other two simulants its high water

186   content provided low quality mass spectra. In DART-SVP, atmospheric moisture is ionized

187   by helium in the 2^3S state with extremmely high efficiency. However, the formation of

188   protonated water clusters is likely to occur from the water present in the samples, while

189   metastable helium atoms are unlikely to survive. For this reason, it has found that moisture

190   should be minimized to avoid competing reactions (Cody, 2009).

2.7. Data processing

In GC-MS analysis, the chromatograms and mass spectra were acquired and processed with MSD ChemStation software version F.01.03 from Agilent Technologies. NIST Standard Reference Database (2018) was used as mass spectra library for compounds identification. Only Match values above 800 were considered acceptable for a candidate confirmation.

In UPLC-Q/ToF, the mass data were analysed with MassLynx software v4.1 from Waters (Milford, MA, USA). Data were acquired in MS^E mode, where 2 functions (low and high energy) were acquired simultaneously. Mass spectra obtained in function 1 provided information about the elemental composition of the precursor ion and the mass spectra in function 2 provided information about the elemental composition of the fragment ions. In UPLC - Q/ToF identification is a more complex process, since there are not spectral libraries. In this case, a structural elucidation of the molecules is necessary. For this purpose, the first step was to determine the elemental composition of the precursor ion, those formulas with the lowest mass error (alwayas below 10 ppm) and the highest isotopic fit were selected. These formulas were introduced in a chemical databases, such as [www. chemspider.com] or [www.scifinder.com] and the possible chemical structures were searched. The criteria for selecting a candidate were based on previous knowledge about the sample together with a chemical background. Finally, the MassFragment® software was used to evaluate if the fragment ions detected in function 2 could fit with the breakage of the proposed candidate. Those candidates with the best match were selected as final candidates (Nerin, Alfaro, Aznar, & Domeño, 2013). In the case of the analysis by DART-SVP, the aim was to determine possible adducts of compounds previously identified by GC-MS or UPLC-MS. The DART-SVP mass spectra were acquired with MassLynx SCN888T software and processed with MassLynx software v4.1

3. Results and discussion

The results showed that overall migration was much higher than in other polymers, close to the Overall Migration Limit (OML) of 60 mg kg^{-1} established by Regulation EU/10/2011 (European Commission, 2011a) in some simulants. The values found for the different simulants were as follows: 50.6 ± 9.2 mg kg^{-1} in EtOH 10%; 13.2 ± 3.2 mg kg^{-1} in AcH 3% and 48.5 ± 4.4 mg kg^{-1} in EtOH 95%. Specific migration results are shown below for each technique.

3.1. Volatile and semi-volatile compounds present in migration from a bamboo-based biopolymer.

Table 1 shows the compounds identified with their retention times, their molecular formula and their presence in the different simulants after the migration tests. A total of twenty-five compounds were identified by comparing their mass spectra with the NIST Standard Reference Database (2018). The identified compounds were checked in the EU/10/2011 positive list (European Commission, 2011a), to confirm if they were authorized and their specific migration limit. Many compounds detected were non-listed substances and among them, only a 2,6,10-trimethyl dodecane had a NOAEL value (1000 mg kg^{-1}day^{-1}) (Agency United States Environmental Protection, 2019). Therefore, their theoretical toxicity level was established according to the TTC (threshold of toxicological concern) and Cramer rules for each compound (Dewhurst & Renwick, 2013; Szczepańska et al., 2018). Table 1 shows the theoretical classification of toxicity for the non listed compounds found.

AcH 3% was the food simulant with the highest number of migrants (15), compared to EtOH 10% (6) and EtOH 95% (6) (Supplementary material 1). Most of the identified compounds were alkanes (Jiong, Fusheng, Chunhong, Mao, & Jianquan, 2014). These compounds are commonly used as raw materials in the production of polymers but they might also come

239 from the degradation of the material. Oxidation can take place because of the interactions

240 between the polymeric material and the oxygen during the manufacture of the material

241 (García Ibarra, Rodríguez Bernaldo de Quirós, Paseiro Losada, & Sendón, 2019). According

242 to Cramer rules, the identified alkanes belong to class I, that means to low toxicity, while

243 class II and class III are of medium and high toxicity respectively. Four compounds were

244 classified as class III: methyl N-hydroxybenzenecarboximidate and three phytosterols (3β-

245 ergost-5-en-3-ol, clionasterol and arundoin). Four phytosterols, stigmasterol, were also

246 detected and classified as class II. Phytosterols, 3β-ergost-5-en-3-ol, stigmasterol, clionasterol

247 and arundoin, were identified only in EtOH 95% food simulant. The presence of phytosterols

248 in young bamboo shoots had been previously demonstrated (Nongdam & Tikendra, 2014).

249 Therefore, these compounds could come from the bamboo used as base material (Moreau et

250 al., 2018). According to the toxicological assays performed by Le Goff et al. and Mea et al.,

251 phytosterols do not have genotoxic potential (Le Goff et al., 2019; Lea, Hepburn, Wolfreys,

252 & Baldrick, 2004). In contrast, phytosterols of bamboo shoot reduces fat and cholesterol

253 levels of blood, and they can inhibit the production of carcinogens and growth of cancer cells

254 (Nongdam & Tikendra, 2014).

255 3.2. Non-volatile compounds present in migration from a bamboo-based biopolymer

256 Table 2 shows the non-volatile compounds found in migration solutions from the bamboo-

257 based biopolymer, their retention time, their accurate mass, the adduct detected ([M+H]$^+$ or

258 [M+Na]$^+$) and their molecular formula. A total of twelve compounds were detected

259 (Supplementary data 1). The amino acid valine was detected in all food simulants. The

260 presence of valine in bamboo shoots was previously reported by different authors (De Silva et

261 al., 2019; Nongdam & Tikendra, 2014). C. Nirmala et al, reported it as one of the most

262 abundant amino acids in the composition of Phyllostachys manii (juvenile bamboo shoots)

263   (Nirmala, Bisht, Bajwa, & Santosh, 2018). Triethanolamine, commonly used as surfactant,

264   was also detected. None of these compounds was present in the EU/10/2011 positive list

265   (European Commission, 2011a). Triethanolamine has a NOAEL value 300 mg kg^{-1}day^{-1} and

266   that of valine is 628 mg kg^{-1}day^{-1} (Agency United States Environmental Protection, 2019).

267   It was also observed that melamine and eight melamine derivatives were identified in all food

268   simulants. This is because in the manufacture of biopolymers it is common to use a polymeric

269   resin, such as melamine, to improve the mechanical properties of the material.

270   Melamine has been reported as a common component in the manufacture of laminates,

271   coatings and mainly, kitchenware utensils (Geueke, 2013; Li, Song, & Wen, 2019).

272   Melamine derivatives can also be formed from the reaction of residual melamine and food

273   simulants (Bradley, Castle, Day, & Leak, 2011; Chien et al., 2011; Karthikraj, Bollapragada,

274   & Kannan, 2018) or during the bamboo based biopolymer manufacture.

275   Melamine is listed in the Regulation EU/10/2011 with a specific migration limit (SML) of 2.5

276   mg kg^{-1}. For this reason, melamine as well as its derivatives were quantified in all food

277   simulants. Since the material evaluated was intended for repeated used, according to

278   EU/10/2011 operating guidelines, the migration was evaluated over three consecutive

279   migration experiments. The results are presented in figure 1. Melamine was the compound

280   with the highest migration values. In all cases, the migration values were above 2.5 mg kg^{-1}

281   in the first, second and third migration experiments, specially in AcH 3%. Therefore, this

282   bamboo-based biopolymer did not comply with the SMLs established by the Regulations

283   (EU) No 10/2011 and No284/2011. According to these results, the material cannot be

284   considered as bamboo, but melamine with bamboo filler. Then, the Regulation 284/2011

285   concerning melamine kitchenware tools applies (European Commission, 2011b). Regarding

286   the food simulants, the highest values were found in the second migration of AcH 3% (144.3

287   ± 1.1 mg kg^{-1}), followed by EtOH10% (48.8 ± 3.4 mg kg^{-1}) and EtOH 95% (4.3 ± 1.0 mg

kg^{-1}), suggesting an important effect of acidity and a high water content in the migration process. The same pattern was observed for all the melamine derivatives analyzed. N-Hydroxymethylmelamine (MD 1) was the melamine derivative with the highest migration values.

The acidic medium of the acetic acid simulant could accelerate the hydrolysis reaction of the melamine resin and as a result, provide high migration values (Bradley et al., 2011; Geueke, 2013). The results also showed that the migration of melamine and its derivatives increased in the second migration experiment and decreased in the third one. This behavior could be attributed to the possible variation in the internal structure of the polymer caused by the simulants, which could have modified the diffusion of the compounds though the polymer and thus affecting the migration. Therefore, the addition of the polymeric resin to the bamboo biopolymer improved its mechanical properties but at the same time, entailed a risk to consumer's health. In addition, melamine is neither a biopolymer nor biodegradable, what means that these materials, promoted as "bamboo food contact materials" can be considered as a fraud to consumers.

3.3. DART-SVP analysis of migration samples

DART-SVP is an electric discharge ambient ionisation technique where excited helium atoms start gas-phase reactions. These reactions result in ions created from atmospheric water-vapor or other gaseous compounds present (Gross, 2014; Navare, Mayoral, Nouzova, Noriega, & Fernández, 2010). As the chemical ionization is the same as in APCI (atmospheric pressure chemical ionization) (Gross, 2014), the formation of adducts according to polarity, molecular weight or volatility of the species present in the samples is very common.

In this study, the ability to implement a DART-SVP method to quickly determine the main migrants and their adducts from bamboo-base biopolymer was investigated. Figure 2 shows a

312   mass spectrum of an EtOH 95% migration sample. Table 3 shows a total of twenty-six

313   masses detected in the DART mass spectrum and that matched with adducts of compounds

314   that were previously detected in migration solutions by GC-MS or UPLC-MS/QToF. The

315   range of m/z values was between 127.0733 and 291.1033. Only peaks with relative intensity

316   higher than 1% of the base peak were considered. The table shows the main adduct ion

317   detected as well as its m/z and relative intensity. In addition, other adducts identified and its

318   relative intensity for each of the compounds are shown. The presence of other adducts

319   confirmed the candidates proposed.

320   Regarding the ions detected, adducts such as $M^{\cdot+}$, $[M+H]^+$, $[M+N]^+$, $[M-H+O]^+$ and $[M+H-$

321    $H_2O]^+$ were observed with major relative intensity. Oxygen, water and ammonia species were

322   detected in the environment (blank spectrum mass), therefore the different adducts identified

323   were the interaction products between gaseous species of the atmosphere and molecular ions

324   from the sample. Other adducts detected were $M^{\cdot+}$, $[M+H]^+$, $[M-H+O]^+$, $[M+H+O]^+$,

325    $[M+NH_4]^+$ and $[M-H+O_2]^+$.

326   In this technique the analytes generate different adducts according to the polarity of specie:

327    $[M+H]^+$, $[M-H+O]^+$, $[M+NH_4]^+$ adducts for medium polar to polar analytes; and $M^{\cdot+}$, $[M-$

328    $H+H_2O]^+$, $[M+H]^+$, $[M-H+O]^+$ adducts for non-polar analytes (Cody, 2009; Gross, 2014).

329   The results suggested that the polarity was an important parameter in the formation of

330   adducts from the migration samples of bamboo-based biopolymer. Melamine and it

331   derivatives formed mainly adducts such as $[M+H]^+$, $[M-H+O]^+$ and $[M-H+O_2]^+$ because these

332   compounds have a high polarity. These adducts had high relative intensity (between 10% -

333   100%) probably because they were at higher concentration than other compounds in the

334   samples (section 3.2). On the other hand, some compounds with low polarity, such as

335   alkanes, formed $[M+N]^+$ and $[M-H+O]^+$ adducts. However, they were in the adduct group

336   with low relative intensities, between 1-10%. Probably, the high volatility of small molecules

promotes a higher number of interactions with the different reactive species and therefore a higher variety of adducts (Bentayeb et al., 2012; Kerpel dos Santos et al., 2018). Among the masses found in DART MS spectrum with relative intensities above 10%, 10 out of them could not be associated to any of the previously identified compounds by GC-MS or UPLC-MS/QToF.

For 5 of these masses, new candidate compounds were proposed (table 4). The candidates were selected according to its elemental composition, adducts detected, and to bibliography of bamboo composition (De Silva et al., 2019; Nirmala et al., 2018; Nongdam & Tikendra, 2014). Other researchers have found in the composition of young bamboo shoots different amino acids, minerals, polyphenols, and/or vitamins, such as serine, coniferyl alcohol, caffeic acid, threonine, paracoumaryl alcohol, histidine and protocatechuic acid, among others (De Silva et al., 2019; Nirmala et al., 2018; Nongdam & Tikendra, 2014; Zhu & Kannan, 2019). Some of the expected adducts for these compounds coincide with the masses detected. However, for 5 of the masses detected in the mass spectrum with intensity values above 10%, it was not possible to propose a candidate: 93.05 (26.8 %), 145.09 (15.0 %), 130.22 (15.0 %), 295.28 (14.5%) and 192.88 (11.5%).

4. Conclusions

Even though some of the compounds found in migration came from bamboo, such as phytosterols, most of the migrants came from the melamine added to it in order to improve the biopolymer properties. Not only melamine but several melamine derivatives were found in migration above the limits established in European legislation. Consequently, this material does not comply with the EU legislation. In fact, the material cannot be identified as bamboo, but as melamine with bamboo filler. As melamine is neither a biopolymer not biodegradable

361 material, the promotion of these kitchenware materials as bamboo can be considered as a

362 fraud to consumers.

363 Regarding to DART-SVP experiments, the analysis allowed detecting the volatile, semi-

364 volatile and non-volatile compounds from migration samples of the bamboo-based

365 biopolymer in a unique analysis of 1.5 minutes duration for each replicate. This technique

366 offers great advantages for rapid detection and routine analysis, without requiring sample

367 preparation.

368 All the compounds identified using DART-SVP were detected in the initial part of this study,

369 where GC-MS and UPLC/QToF methodologies were used. Melamine and its derivatives

370 form the adducts with greater relative intensity. These results demonstrate that the DART-

371 SVP technique is a very useful tool for direct target analysis, where in a few minutes can

372 provide data about the main migrants present in samples.

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537   **Figure captions**

538   **Fig 1.** Bar chart of migration values of melamine and its derivative compounds from

539   bamboo-based biopolymer in three consecu-tive migration assays. a) acetic acid 3% (w/v), b)

540   ethanol 10% (v/v) and c) ethanol 95% (v/v). MD codes are linked to table 2.

541   **Fig.2**. DART-SVP mass spectrum of a migration solution (ethanol 95%) from a bamboo-

542   based biopolymer sample. Numbers in brackets are linked to table 3.

Table 1. Volatile and semi-volatile compounds detected by GC-MS in migration samples from Bamboo-based biopolymer.

#	t_R	MW	Candidate Name	# CAS	MF	Cramer Class	AcH 3%	EtOH 10%	EtOH 95%
1	4.40	164.0473	Phenacyl formate	55153-12-3	$C_9H_8O_3$	I			X
2	7.43	132.0786	3,3-Dimethoxy-2-butanone	21983-72-2	$C_6H_{12}O_3$	I		X	
3	7.65	151.0633	Methyl N-hydroxybenzenecarboximidate	67160-14	$C_8H_9NO_2$	III	X		X
4	10.95	142.1722	Decane	124-18-5	$C_{10}H_{22}$	I		X	
5	10.98	142.1722	3,5-dimethyloctane	15869-93-9	$C_{10}H_{22}$	I	X		
6	12.17	170.2035	2,3-dimethyldecane	17312-44-6	$C_{12}H_{26}$	I		X	
7	12.73	128.1535	2,4-dimethylheptane	2213-23-2	C_9H_{20}	I		X	
8	13.66	170.2035	2-methyl undecane	7045-71-8	$C_{12}H_{26}$	I	X		
9	13.78	212.2504	2,6,10-trimethyl dodecane	3891-98-3	$C_{15}H_{32}$	I	X		
10	13.86	198.2348	4,6-dimethyl dodecane	61141-72-8	$C_{14}H_{30}$	I	X		
11	13.98	226.2661	Hexadecane	544-76-3	$C_{16}H_{34}$	I	X		
12	14.52	170.2035	3,6-dimethyl decane	17312-53-7	$C_{12}H_{26}$	I	X		
13	14.57	184.2191	2,4-dimethyl undecane	17312-80-0	$C_{13}H_{28}$	I	X		
14	14.66	184.2191	4,6-dimethyl undecane	17312-82-2	$C_{13}H_{28}$	I	X		
15	15.27	198.2348	Tetradecane	629-59-4	$C_{14}H_{30}$	I	X		
16	16.47	340.3705	Eicosane, 1-propoxy	281211-96-9	$C_{23}H_{48}O$	I	X	X	
17	16.93	156.1878	2,4,6-trimethyl octane	62016-37-9	$C_{11}H_{24}$	I	X		
18	17.07	212.2504	2,6,11-trimethyl dodecane	31295-56-4	$C_{15}H_{32}$	I	X		
19	17.18	268.313	Nonadecane	629-92-5	$C_{19}H_{40}$	I	X		
20	19.02	380.4382	Heptacosane	593-49-7	$C_{27}H_{56}$	I	X		
21	22.92	256.2402	(S)-12-methylmethy tetradecanoic ester	62691-05-8	$C_{16}H_{32}O_2$	I		X	
22	26.03	400.3705	3β-Ergost-5-en-3-ol	4651-51-8	$C_{28}H_{48}O$	III			X
23	27.27	412.3705	Stigmasterol	83-48-7	$C_{29}H_{48}O$	II			X
24	28.87	414.3862	Clionasterol	83-47-6	$C_{29}H_{50}O$	III			X
25	30.21	440.4018	Arundoin	4555-56-0	$C_{31}H_{52}O$	III			X

t_R: retention time (min). MW: molecular weight. MF: molecular formula. AcH 3%: acetic acid 3% (w/v). EtOH 10%: ethanol 10% (v/v). EtOH 95%: ethanol 95% (v/v). X: compound detected in the simulant.

Table 2. List of compounds non-volatile detected by UPLC-Q/ToF in migration from bamboo-based samples (Repeated use).

#	t_R	Mass (*m/z*)	Adduct	MF	Candidate Name_# CAS	AcH 3%	EtOH 10%	EtOH 95%
						Food simulants		
1	0.65	214.9170	[M+Na]$^+$	$C_2N_4O_3S_2$	Unknown	X	X	
2	0.73	150.1137	[M+H]$^+$ [M+Na]$^+$	$C_6H_{15}NO_3$	Triethanolamine_102-71-6	X	X	
3	0.79	118.0865	[M+H]$^+$	$C_5H_{11}NO_2$	Valine_72-18-4	X	X	X
4	0.80	127.0732	[M+H]$^+$	$C_3H_6N_6$	Melamine_108-78-1	X	X	X
5	0.89	157.0840	[M+H]$^+$	$C_4H_8N_6O$	N-Hydroxymethylmelamine_937-35-9_ **(MD 1)**	X		X
6	0.90	185.1156	[M+H]$^+$	$C_6H_{12}N_6O$	N-Hydroxypropylmelamine_91313-29-0 **(MD 2)**			X
7	0.94	157.0835	[M+H]$^+$	$C_4H_8N_6O$	N-Hydroxymethylmelamine_937-35-9 **(MD 3)**	X	X	
8	1.04	139.0726	[M+H]$^+$	$C_4H_6N_6$	Methylene melamine_85946-83-4 **(MD 4)**	X		
9	1.13	169.0826	[M+H]$^+$	$C_5H_8N_6O$	N-(4,6-Diamino-1,3,5-triazin-2-yl)acetamide_16274-60-5 **(MD 5)**	X	X	
10	2.65	277.1375	[M+H]$^+$	$C_8H_{12}N_{12}$	2,4,6-Pyrimidinetriamine, 5,5'-azobis_ 63436-10-2 **(MD 6)**	X	X	
11	2.83	307.1478	[M+H]$^+$	$C_9H_{15}N_{12}O$	Propanamide, N-(4,6-diamino-1,3,5-triazin-2-yl)-2-[(4,6-diamino-1,3,5-triazin-2-yl)amino]_1421766-78-0_ **(MD 7)**	X		
12	4.54	311.0979	[M+H]$^+$	$C_{14}H_{26}N_6O_2$	Glycine, N-[4-[(1,1-dimethylethyl)amino]-6-(ethylamino)-1,3,5-triazin-2-yl]-N-propyl _2037785-60-5 **(MD 8)**	X	X	X

t_R: retention time (min). AcH 3%: acetic acid 3% (w/v). EtOH 10%: ethanol 10% (v/v). EtOH 95%: ethanol 95% (v/v) X: compound detected in the simulant. MD: melamine derivate.

Journal of Hazardous Materials

Ambient mass spectrometry as a tool for a rapid and simultaneous determination of migrants coming from a bamboo-based biopolymer packaging
--Manuscript Draft--

Editor of Journal of Hazardous Materials

Zaragoza, 21st April 2020

Dear Editor,

Following your recommendation, we are sending you again the manuscript HAZMAT-D-20-02144 entitled **"Ambient Mass Spectrometry as a Tool for a Rapid and Simultaneous Determination of Migrants Coming from a Bamboo-Based Biopolymer Packaging"** by Jazmín Osorio, Margarita Aznar, Cristina Nerín, Nicholas Birse, Christopher Elliott and Olivier Chevallier. The manuscript has been corrected according to the referee's comments. All the suggestions and corrections proposed by the reviewers have been followed. We hope that the paper can be accepted in its present version.

The corresponding author is Prof. Cristina Nerin:
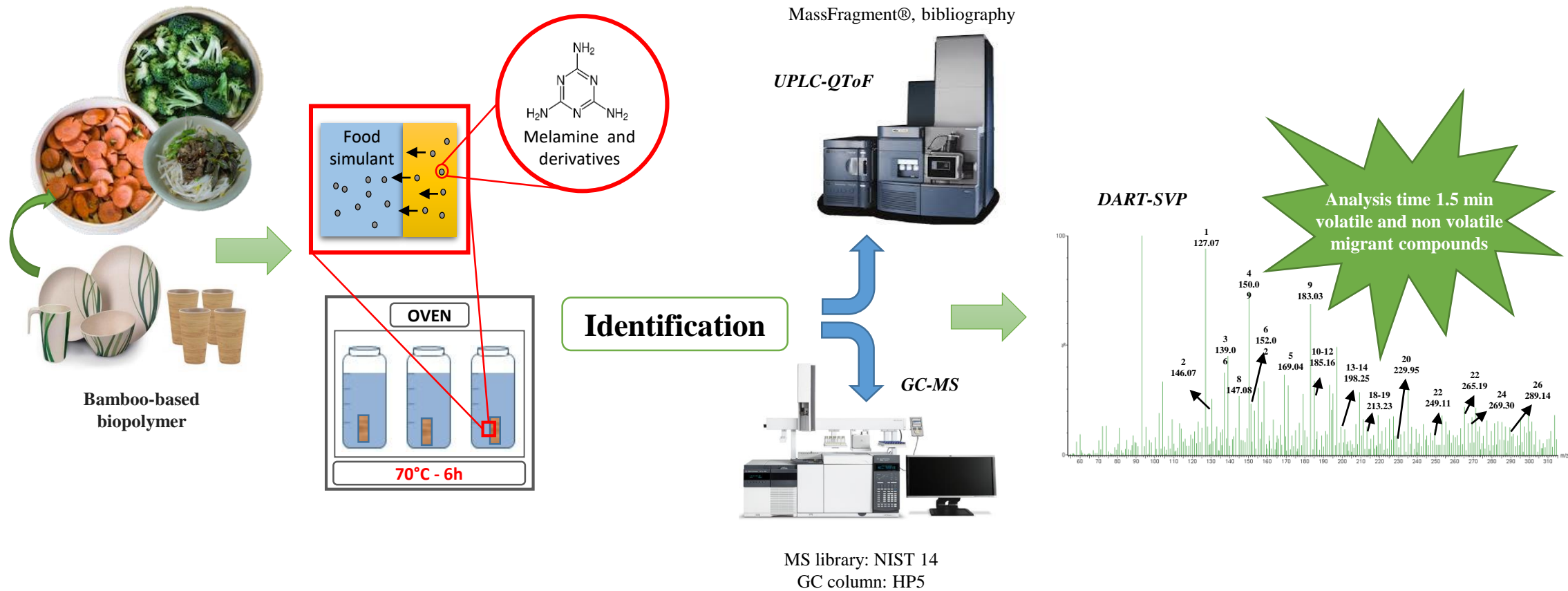
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Best regards,

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Graphical Abstract

Bamboo-based biopolymer

Food simulant

Melamine and derivatives

OVEN

70°C - 6h

Identification

Structural elucidation: MassFragment®, bibliography

UPLC-QToF

GC-MS

MS library: NIST 14
GC column: HP5

DART-SVP

1
127.07

2
146.07

3
139.0
6

6
152.0
2

4
150.0
9

8
147.08

5
169.04

10-12
185.16

9
183.03

13-14
198.25

18-19
213.23

20
229.95

22
249.11

22
265.19

24
269.30

26
289.14

Analysis time 1.5 min volatile and non volatile migrant compounds

Highlights

Migrants coming from a bamboo-based biopolymer packaging were determined

Volatile and non-volatile compounds were analyzed

Ambient mass spectrometry was used for a rapid and simultaneous determination

Most of the migrants detected were melamine and its derivatives

It can be used to test safety and legal compliance of food packaging materials

Answers to Editors and Reviewers:

Reviewer #1:

This article describes a method that can be used for a rapid and simultaneous determination of migrants coming from a bamboo base biopolymer packaging. It's a very nowadays subject that impact not only the food industry but also the cosmetic or household products industries. The emergence of biosourced packaging is an actual new development in the packaging sector. More and more industrial claim the use of biosourced packaging to follow the idea of safe product. However, this work cleary shows that the use of this kind of packaging can be misleading concerning their safety. Authors reminder the necessity of experimental analysis in the evaluation of the safety of emergent packaging, and their compliance with the regulation. In addition to this novel topic, this work proposes to use DART-SVP technology, first time in this kind of application. Both GC and LC MS tools are good choose for screening evaluation.

Reviewer #2

In this manuscript, volatile and non-volatile migrants coming from a bamboo-based biopolymer packaging were determined, and DART-SVP was used for a rapid and simultaneous determination. The topic and the results are interesting and valuable. But there are some questions need to be discussed more clearly.

1 "The results also showed that the migration of melamine and its derivatives increased in the second migration experiment and decreased in the third one." The reason/mechanism need to be discussed.

> ANSWER: When objects are intended for repeated use and in order to predict how migration will behave during the consecutive uses, it is mandatory to perform the migration test 3 consecutive times (10/2011/EU). In the repeated migration essays migration will be affected by different factors such as the initial concentration gradient between the polymer and the simulant, or possible variations in the internal structure of the polymer due to the effects of the simulants, that will modify the diffusion of the compounds through the polymer.

The sentence "This behavior could be attributed to the possible variation in the internal structure of the polymer caused by the simulants, which could have modified the diffusion of the compounds though the polymer and thus affecting the migration." has been added in the manuscript.

2 "25 compounds were identified by GC-MS, 12 compounds were identified by UPLC-Q/ToF, 26 masses detected by DART-SVP matched with adducts of compounds that had been previously detected by GC-MS or UPLC-MS/QToF". How about the compounds detected by GC-MS or UPLC-MS/QToF but not by DART-SVP? If we use DART-SVP as a rapid and simultaneous detection method, some migrants might be missed?

ANSWER: The referee is right, and even though DART-SVP is able to detect a wide range of compounds, some of the migrants detected by GC-MS or UPLC-MS were not detected by DART-SVP. This could be due to different reasons, a lower sensitivity for these compounds or because it was not possible to elucidate the adduct formed. Without chromatographic separation, as it happens in DART, it is more difficult to identify the compounds. DART-SVP as a rapid detection method requires a previous optimization for the analysis of targeted compounds as well as a study of their detection limits.

Some minor considerations:

Some expressions need to be improved:

Line 23: "Twenty-five compounds were detected, alkanes."

ANSWER: The referee is right and the word "alkanes." has been removed from the sentence.

Line 39: "especially those that are abundant, ……"

ANSWER: The sentence has been renamed as "especially those which are abundant"

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Line 101: "adducts, mainly for protonated/deprotonated molecular ion, are commonly formed"

      ANSWER: According to reviewer comment, the sentence has been rewritten to "adducts, mainly protonated/deprotonated molecular ions, are commonly formed."

Line 122: "SPME fiber was purchased to Supelco"

      ANSWER: The sentence has been rewritten to "SPME fibers were purchased at Supelco"

Line 147-148: "The migration samples were conditioned during 2 min…"

      ANSWER: According to referee´s suggestion the sentences "The migration samples were conditioned during 2 min…" has been changed to "The migration samples were conditioned for 2 min"

Line 151: "column of 30 m x 0.25 mm i.d. x 0.25 <mu>m film"

      ANSWER: The sentence has been rewritten as "HP-5MS column (30 m length $\times$ 0.25 mm inner diameter $\times$ 0.25 µm film thickness).

Line 200: "global migration"

      ANSWER: "global migration" has been replaced by "overall migration" which is more accurate, and in accordance with the current legislation.

Line 222-224: "These compounds are commonly used as raw materials in the production of polymers but they might also come from the degradation of the material." How about compounds come from the bamboo?

      ANSWER: Compounds coming from bamboo are discussed on the following lines: 228-234

Line 224-226: "Degradation can happen because of the interactions between the polymeric material and the oxygen present in the air, causing its oxidation"

ANSWER: The sentence has been rewritten as "Oxidation can take place because of the interactions between the polymeric material and the oxygen during the manufacture of the material"

Line 280-281: "the addition the polymeric resin to the bamboo……"

ANSWER: According to referee´s suggestion the sentence has been rewritten as "the addition of the polymeric resin to the bamboo …"

References need to be standardized:

Line 378-379: " Bradley, E. L. (n.d.). Report FD 10/04 FSA PROJECT A03070 Biobased materials used in food contact applications: an assessment of the migration potential."

Line 422-424: "https://comptox.epa.gov/dashboard (accessed Jan.28.2020). (n.d.). United States Environmental Protection Agency https://doi.org/10.2175/193864705783867675"

ANSWER: According to referee´s suggestion, the references have been completed.

Supplementary material:

1 It would be better to merge the supplementary material into the manuscript.

ANSWER: The manuscript already contain a total of 4 tables and 2 figures and the author considered that to include 6 more figures (3 of GC-MS and 3 of UPLC-MS) could be excessive, for this reason they are included as supplementary material.

2 Some compounds listed in table not displayed in the chromatogram. For example, compound 1-3 and 5 can't be found in GC-MS chromatograms.

ANSWER: Not all the compounds present in the table migrated to the three simulants and for this reason some of them are not shown in all the chromatograms. Nevertheless, some changes have been made in the supplementary material for clarity.

Reviewer #3:

Title: Ambient mass spectrometry as a tool for a rapid and simultaneous determination of migrants coming from a bamboo-based biopolymer packaging The work applied GC - MS, UPLC - Q/ToF, DART - SVP techniques to determine the analog compound migration situation in a new type bamboo polymer packaging material. The DART-SVP technique could simultaneously screen the volatile and non-volatile migration, and had the ability of detecting the volatile compounds of GC-MS as well as the ability of detecting the non-volatile compounds of UPLC - Q/ToF. The paper has a certain of innovation and practicability. According to the content of the paper, the following suggestions are given:

Line 23 of the abstract, "Twenty five compounds were detected, alkanes.", the sentence should be rewrite for clarity.

ANSWER: The referee is right and the word "alkanes." has been removed from the sentence.

The error of "calculed" in line 134 should be "calculated"; Line 180 "temperate" should be "temperature".

ANSWER: The referee is right and the words have been changed.

3. There is no space between the number and the unit. For example, the temperature unit on lines 148 and 149, please correct and check other format problems.

ANSWER: According to referee´s suggestion spaces between numbers and units have been corrected throughout the manuscript

4. What is the volume of the three simulators in Migration assays and the temperature at which the simulated solution evaporates to dry?

> ANSWER: In the section 2.3, line 123, the volume of the three simulants was described "Migration assays were carried out by total immersion of the sample (5 cm x 1 cm) into 20 mL of the simulant". The overall volume of the simulant was evaporated to dryness at 110 ºC. In order to clarify the process, this information has been added to the manuscript "The overall simulant solutions were evaporated to dryness at 110 ºC"

5. The symbol "X" in line 151 should be "×".

> ANSWER: The symbol "x" has been changed to "×"

6. Why was it that only one simulation of 95% ethanol (v/v) was tested while the other two instruments tested three simulators when DART-SVP analysis was used?

> ANSWER: . This information has been added to the manuscript in the materials and methods section.

7. "EtOH 95" in line 519 should be "EtOH 95%", and the abbreviations of AcH and EtOH should be explained when they first appeared in the text.

> ANSWER: The referee is right and the abbreviations have been explained in Materials and methods section. Ethanol 95% (v/v) was abbreviated as EtOH 95%, Ethanol 10% (v/v) as EtOH 10% and acetic acid 3% (w/v) as AcH 3%. These abbreviations were used throughout the manuscript.

8. The Graphical Abstract diagram is too simple to fully describe the research content of the paper. The paper used three kinds of instruments to test and compare the results, while the diagram only described a method which is DART-SVP.

> ANSWER: The Graphical Abstract has been modified and completed according to referee´s suggestion.

9. The meaning of X in the table should be explained.

ANSWER: According to referee´s suggestion the meaning of X has been added on tables 1 and 2.

10. In GC-MS, compounds are confirmed through NIST Standard Reference Database. How compounds are confirmed in analysis of UPLC - Q/ToF, DART-SVP, please also explain briefly. Multiple relevant molecular formulas can be matched simply based on a mass number, and how specific compounds are determined.

ANSWER: According to referee´s suggestion, the authors have added a new paragraph on section 2.7 and a reference where the process is widely explained:

"In UPLC-Q/ToF, the mass data were analysed with MassLynx software v4.1 from Waters (Milford, MA, USA). Data were acquired in MS^E mode, where 2 functions (low and high energy) were acquired simultaneously. Mass spectra obtained in function 1 provided information about the elemental composition of the precursor ion and the mass spectra in function 2 provided information about the elemental composition of the fragment ions. In UPLC - Q/ToF identification is a more complex process, since there are not spectral libraries. In this case, a structural elucidation of the molecules is necessary. For this purpose, the first step was to determine the elemental composition of the precursor ion, those formulas with the lowest mass error (alwayas below 10 ppm) and the highest isotopic fit were selected. These formulas were introduced in a chemical databases, such as [www. chemspider.com] or [www.scifinder.com] and the possible chemical structures were searched. The criteria for selecting a candidate were based on previous knowledge about the sample together with a chemical background. Finally, the MassFragment® software was used to evaluate if the fragment ions detected in function 2 could fit with the breakage of the proposed candidate. Those candidates with the best match were selected as final candidates (Nerin, Alfaro, Aznar, & Domeño, 2013). In the case of the analysis by DART-SVP, the aim was to determine possible adducts of compounds previously identified by GC-MS or UPLC-MS. The DART-SVP mass spectra

were acquired with MassLynx SCN888T software and processed with MassLynx software v4.1".

1 **Ambient mass spectrometry as a tool for a rapid and simultaneous determination of**

2 **migrants coming from a bamboo-based biopolymer packaging.**

3

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16

17 **Abstract**

18 New bamboo-based biopolymers are used as food packaging materials, but it must be

19 evaluated to ensure consumers safety. In this study, migration from a commercial bamboo-

20 based biopolymer to ethanol 10% (v/v), acetic acid 3% (w/v) and ethanol 95% (v/v) was

21 studied. The migrants were determined from three different perspectives. Volatile and semi-

22 volatile compounds were analyzed by gas chromatography-mass spectrometry (GC-MS).

23 Twenty-five compounds were detected. In addition, a number of phytosterols were detected

24 in ethanol 95%. Non-volatile compounds were identified and quantified by ultra-performance

25   liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-Q/ToF). Twelve

26   non-volatile compounds were detected in migration solutions, mainly melamine and its

27   derivatives, coming from polymer resins present in the biopolymer. Melamine migration was

28   higher than 50 mg/Kg in the third sequential migration test. Finally, the migration samples

29   were analyzed by DART-SVP (direct analysis in real time coupled to standardized voltage

30   and pressure). This methodology was able to detect simultaneously the main volatile and non-

31   volatile migrants and their adducts in a very rapid and effective way and is shown as a

32   promising tool to test the safety and legal compliance of food packaging materials.

33   **Keywords:** bamboo biopolymer; DART; melamine; migration; food contact material

34   **1. Introduction**

35   There is a great demand for biopolymers in the sector of food contact materials

36   manufacturing. This is because biopolymers are a green alternative to the traditional

37   packaging materials used in the food industry, especially those which are abundant,

38   renewable, biodegradable, and biocompatible (Khlestkin, Peltek, & Kolchanov, 2018).

39   Biopolymers can be classified into two groups, polymers produced from biological products

40   that can be extracted from biomass or produced in fermentation processes, such as

41   carbohydrates, proteins or lipids; and biodegradable and/or compostable polymers (Aznar,

42   Ubeda, Dreolin, & Nerín, 2019; Geueke, 2014; Maisanaba et al., 2018). The addition of

43   additives to the biopolymers is necessary in order to improve their physico-chemical

44   properties. These additives can be for example: polyols, used as non-volatile plasticizers

45   (e.g., glycerol, glycol, and sorbitol); and resins, that mold the material (e.g., urea, melamine,

46   melamine formaldehyde, and amines) (Eckardt, Kubicova, & Simat, 2018; Geueke, 2013).

47   Surprisingly, melamine and other resins are neither biodegradable or come from natural

48   sources (Chien et al., 2011; Geueke, 2013).

49   In recent years, food packaging industries have been highly innovative in the production of

50   new biopolymers derived from various natural resources, such as starch, cellulose, bamboo,

51   and chitosan. Among them, bamboo-base biopolymers are of enormous importance. This

52   fibre is attractive as a natural-based raw material, since it is economical, eco-friendly and

53   biodegradable (Jena, 2018; Xie, Zhou, & Yan, 2019). Bamboo plants are easily available due

54   to its very short life cycle and because bamboo timber can be harvested multiple times from a

55   single planting, which makes its use very sustainable. Bamboo-based biopolymers have better

56   mechanical properties than other biomaterials, such as a good impact resistance, high

57   durability and crack resistance, and good flexural properties (Xie et al., 2019). As any food

58   packaging material or food contact article, the biopolymers must be evaluated to confirm

59   their acceptability and safety. The bamboo-base biomaterials can contain other resins and

60   also non intentionally added substances (NIAS), such as impurities from raw materials,

61   degradation compounds or novel substances coming from the reaction between different

62   reagents (Xu, Xiong, Zhao, Xiang, & Wu, 2020). These compounds, as well as those

63   additives intentionally added, are present in the packaging and could migrate to the food.

64   Migration tests are required in order to guarantee the consumers safety, food preservation and

65   organoleptic properties (Bradley, 2010). These tests require specific conditions of

66   temperature, time and simulants, which should be selected according to the intended use of

67   the material. In the specific migration tests, compounds previously identified in the material

68   as well as new formed compounds can be detected (Nerin, Alfaro, Aznar, & Domeño, 2013).

69   Pure biopolymers are not specifically included in the European plastics regulation and only

70   the frame regulation 1935/2004/CEE applies. However, if they contain some resin or

71   conventional plastic, the regulation on plastics should be applied. European legislation on

72   food contact plastics (Regulation No 10/2011/EU) establishes a positive list with substances

73   that can be used in the manufacturing process and also their specific migration limits (Osorio,

74   Dreolin, Aznar, Nerín, & Hancock, 2019).

75   Since the migrants can be volatile as well as non-volatile compounds, different technologies

76   should be applied in order to have a global view of the safety of the material. Gas

77   chromatography can be applied to the separation of the most volatile and semi-volatile

78   compounds (Biedermann & Grob, 2019) and liquid chromatography to the less volatile ones.

79   Both of them coupled to mass spectrometry detection will allow the identification of the

80   NIAS present in the packaging material. Ambient mass spectrometry techniques can be also

81   used to get a quick confirmation of the presence/absence of the identified compounds.

82   Ambient mass spectrometry (AMS) or ambient ionization techniques (AMI) are ionization

83   sources used in mass spectrometry for direct and rapid analysis of compounds present in solid

84   or liquid samples (Black, Chevallier, & Elliott, 2016; Lu et al., 2018). Additionally, AMS has

85   been utilized as screening method in food safety analysis, criminal investigation,

86   environmental test and other different analytical objectives (Gross, 2014; Lennert & Bridge,

87   2019; Zhang et al., 2018). DESI-MS (Desorption ElectroSpray Ionization Mass

88   Spectrometry) was the first AMS technique developed. Since then, other techniques such as

89   direct analysis in real time (DART), extractive electrospray ionization (EESI), desorption

90   atmospheric pressure chemical ionization (DAPCI), dielectric-barrier-discharge ionization

91   (DBDI) or low-temperature plasma (LTP) have been designed (Barnett & Zhang, 2018).

92   AMS techniques had been previously used in the study of food packaging materials by other

93   groups. For example, Bentayeb et al. determined the compounds present due to print set-off

94   process (Bentayeb et al., 2012; ten Dam et al., 2016) while Aznar et al. analyzed the

95   distribution of ink components in a packaging material and Rothenbacher et al. studied the

96   plasticizers in poly(vinyl chloride) (PVC) (Aznar, Alfaro, Nerín, Jones, & Riches, 2016).

97　　Direct analysis in real time (DART) is one of the most popular ambient pressure ionization

98　　methods. In this technique, the molecules are ionized by excited helium molecules and

99　　adducts, mainly protonated/deprotonated molecular ions, are commonly formed (Black et al.,

100　　2016). Some of its main advantages are: the possibility of direct sampling at ambient

101　　conditions; to generate ions from liquid or solid samples; to be simple to operate and to have

102　　a rapid analysis speed (acquisition time < 60 s) compared to UPLC and GC-MS methods

103　　(Nerin et al., 2013). DART-SVP technique has diverse applications such as food quality and

104　　safety control, drug screening, contamination analysis and environmental monitoring (Barnett

105　　& Zhang, 2018; Djelal, Cornée, Tartivel, Lavastre, & Abdeltif, 2017; Lennert & Bridge,

106　　2018).

107　　In the present study, the migration from a bamboo-based biopolymer intended for food

108　　contact to different food simulants was evaluated. Volatile, semi-volatile and non-volatile

109　　compounds were firstly identified by GC-MS and UPLC-Q/ToF. Additionally, DART-SVP

110　　was used as a tool to assess the presence of all potential migrants in a very short analysis

111　　time.

112　　**2. Materials and methods**

113　　2.1. Chemicals and Reagents

114　　Melamine 99% [108-78-1] (IUPAC name: 1,3,5-triazine-2,4,6-triamine) was from Sigma–

115　　Aldrich (Madrid, Spain). Ethanol absolute (HPLC grade), methanol (UHPLC-MS grade),

116　　acetic acid (HPLC grade) were supplied by Scharlau (Setmenat, Spain) for the analysis in

117　　GC-MS and UPLC-Q/ToF. Ethanol absolute (HPLC grade) supplied by Merck (Darmstadt,

118　　Germany) for the analysis in DART-SVP. Ultra-pure water was obtained from Millipore

119　　Milli-Q system (Billerica, MA, USA). The nitrogen evaporator was a TECHNE sample

120   concentrator (Cole-Parmer Ltd., UK). SPME fibers were purchased at Supelco (Bellefonte,

121   PA, USA).

122   2.2. Samples

123   Bamboo-based biopolymer samples were purchased at a local supermarket in the form of

124   cups, dishes and jugs. All of these were monolayer materials.

125   2.3. Migration assays

126   Migration assays were carried out by total immersion of the sample (5 cm x 1 cm) into 20 mL

127   of the simulant. The process was established in accordance with the European legislation on

128   food contact materials (Regulation No 10/2011/EU) (European Commission, 2011a). Three

129   simulants were evaluated: ethanol 10% (v/v) (EtOH 10%) (simulant A), acetic acid 3% (w/v)

130   (AcH 3%) (simulant B) and ethanol 95% (v/v) (EtOH 95%) (simulant D2 substitute). Overall

131   migration studies were performed during 2 hours at 70 ºC. The overall simulant solutions

132   were evaporated to dryness at 110 ºC. Overall migration was calculated by weighting the dry

133   residue. Specific migration was performed during 6 hours at 70 ºC. Since the food contact

134   materials of this study were intended for repeated use, the migration tests were performed

135   three consecutive times. The assays were carried out in triplicate for each sample and a blank.

136   2.4. Analysis by GC-MS

137   The analyses of volatile and semi-volatile compounds were carried out using a gas

138   chromatograph 6890N equipped with an electron ionization ion source operating at 70 eV,

139   and coupled to a quadrupole mass spectrometry detector 5975D (Agilent Technologies. Santa

140   Clara, CA). It was set in SCAN acquisition mode (50 - 450 m/z). The transfer line heater was

141   set at 280 ºC. The autosampler was a Combi PAL (CTC Analytics, Zwingen, Switzerland).

142   The injector was used in two modes. Liquid injection for migration samples in EtOH 95%, 1

6

143   µL of liquid samples was injected at 250 ℃ in splitless mode. SPME injection was used for

144   aqueous migration samples (AcH 3% and EtOH 10%). The stationary phase of

145   polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR 50/30 µm) was selected.

146   The migration samples were conditioned for 2 min at 80 ℃. Subsequently, the volatile and

147   semi-volatile compounds were extracted by fiber inmersion at 80 ℃ and 500 rpm agitation

148   during 15 min and finally SPME fiber was desorbed at 250 ℃ for 2 min and. The

149   chromatographic separation was performed on a HP-5MS column (30 m length × 0.25

150   mminner diameter × 0.25 µm film thickness). The oven temperature program was: 40 ℃ held

151   for 3 min, ramp 10 ℃ min^{-1} to 300 ℃ and held for 2 min. Helium was used as carrier gas at a

152   constant flow of 1 mL min^{-1}. The SPME extraction process in biopolymer samples was

153   optimized in a previous work (Osorio, Aznar, & Nerín, 2019).

154   2.5. Analysis by UPLC-Q/ToF

155   The analyses of the non-volatile compounds present in migration solutions were performed

156   using an ACQUITY UPLC chromatograph (Milford, MA, USA). Flow rate was 0.3 mL min^{-}

157   ^{1}. Injection volume was 10 µL. An UPLC BEH C18 column (Waters) of 2.1 × 100 mm, 1.7

158   µm particle size was used at a temperature of 35 °C. Two mobile phases were used for the

159   separation: (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid.

160   Chromatography was performed using the following gradient elution: initial composition

161   98/2 (phase A/ phase B), and at eight min it was changed to 0/100 (phase A/ phase B) with

162   two additional minutes at the final composition.

163   UPLC system was coupled to a Xevo G2 QTOF mass spectrometer from Waters (Milford,

164   MA, USA) with an ESI probe. The conditions of analysis were: positive ionization (ESI+),

165   sensitivity mode, capillary voltage 3.0 kV, sampling cone voltage 30V, extraction cone 3 V,

166   source temperature 150 °C, desolvation temperature 450 °C, cone gas flow rate of 20 L h^{-1},

167 and desolvation gas flow rate of 500 L h^{-1}. Acquisition was carried out in MSE mode in a

168 mass range between 50 and 1200 m/z. The chromatogram was acquired at low and high

169 collision energy (CE) in the collision cell.

170 2.6. Analysis by DART-SVP

171 A direct analysis in real time (DART) couple to Standardised Voltage and Pressure (SVP)

172 100 ion source (IonSense, Saugus, MA) was coupled to an Acquity QDa single quadrupole

173 mass spectrometer (Waters, Wilmslow, UK) via a Vapur interface (IonSense, Saugus, MA).

174 The DART-SVP and Acquity QDa were operated in positive ion mode. The DART-SVP was

175 operated with helium (grade A) in running mode and nitrogen in standby mode. The DART-

176 SVP was operated at several different temperatures between 150 ºC and 450 ºC, the ion-

177 source grid voltage was set to 350 V. The mass spectrometer was operated with the

178 desolvation line temperature set at 250 ºC, heat block 350 ºC, interface voltage 4.5 kV and

179 50-1000 Da scan range. The mass spectrometer was controlled using MassLynx v4.1

180 SCN888 (Waters, Wilmslow, UK). Data were analyzed by MassLynx v4.1.

181 Manual sample injection mode was used. Three microliters of the migration solution were

182 pipette-spotted directly onto the Quick Strip card in the position one. Then, Quick Strip card

183 was simply mounted on the sampling rail. Further, the sample came in contact with the He

184 stream from the DART ion source outlet. Even though the three simulants were tested, only

185 data from EtOH 95% are included in the manuscript. In the other two simulants its high water

186 content provided low quality mass spectra. In DART-SVP, atmospheric moisture is ionized

187 by helium in the 2^3S state with extremmely high efficiency. However, the formation of

188 protonated water clusters is likely to occur from the water present in the samples, while

189 metastable helium atoms are unlikely to survive. For this reason, it has found that moisture

190 should be minimized to avoid competing reactions (Cody, 2009).

2.7. Data processing

In GC-MS analysis, the chromatograms and mass spectra were acquired and processed with MSD ChemStation software version F.01.03 from Agilent Technologies. NIST Standard Reference Database (2018) was used as mass spectra library for compounds identification. Only Match values above 800 were considered acceptable for a candidate confirmation.

In UPLC-Q/ToF, the mass data were analysed with MassLynx software v4.1 from Waters (Milford, MA, USA). Data were acquired in MS^E mode, where 2 functions (low and high energy) were acquired simultaneously. Mass spectra obtained in function 1 provided information about the elemental composition of the precursor ion and the mass spectra in function 2 provided information about the elemental composition of the fragment ions. In UPLC - Q/ToF identification is a more complex process, since there are not spectral libraries. In this case, a structural elucidation of the molecules is necessary. For this purpose, the first step was to determine the elemental composition of the precursor ion, those formulas with the lowest mass error (alwayas below 10 ppm) and the highest isotopic fit were selected. These formulas were introduced in a chemical databases, such as [www. chemspider.com] or [www.scifinder.com] and the possible chemical structures were searched. The criteria for selecting a candidate were based on previous knowledge about the sample together with a chemical background. Finally, the MassFragment® software was used to evaluate if the fragment ions detected in function 2 could fit with the breakage of the proposed candidate. Those candidates with the best match were selected as final candidates (Nerin, Alfaro, Aznar, & Domeño, 2013). In the case of the analysis by DART-SVP, the aim was to determine possible adducts of compounds previously identified by GC-MS or UPLC-MS. The DART-SVP mass spectra were acquired with MassLynx SCN888T software and processed with MassLynx software v4.1

3. Results and discussion

The results showed that overall migration was much higher than in other polymers, close to the Overall Migration Limit (OML) of 60 mg kg^{-1} established by Regulation EU/10/2011 (European Commission, 2011a) in some simulants. The values found for the different simulants were as follows: 50.6 ± 9.2 mg kg^{-1} in EtOH 10%; 13.2 ± 3.2 mg kg^{-1} in AcH 3% and 48.5 ± 4.4 mg kg^{-1} in EtOH 95%. Specific migration results are shown below for each technique.

3.1. Volatile and semi-volatile compounds present in migration from a bamboo-based biopolymer.

Table 1 shows the compounds identified with their retention times, their molecular formula and their presence in the different simulants after the migration tests. A total of twenty-five compounds were identified by comparing their mass spectra with the NIST Standard Reference Database (2018). The identified compounds were checked in the EU/10/2011 positive list (European Commission, 2011a), to confirm if they were authorized and their specific migration limit. Many compounds detected were non-listed substances and among them, only a 2,6,10-trimethyl dodecane had a NOAEL value (1000 mg kg^{-1}day^{-1}) (Agency United States Environmental Protection, 2019). Therefore, their theoretical toxicity level was established according to the TTC (threshold of toxicological concern) and Cramer rules for each compound (Dewhurst & Renwick, 2013; Szczepańska et al., 2018). Table 1 shows the theoretical classification of toxicity for the non listed compounds found.

AcH 3% was the food simulant with the highest number of migrants (15), compared to EtOH 10% (6) and EtOH 95% (6) (Supplementary material 1). Most of the identified compounds were alkanes (Jiong, Fusheng, Chunhong, Mao, & Jianquan, 2014). These compounds are commonly used as raw materials in the production of polymers but they might also come

239 from the degradation of the material. Oxidation can take place because of the interactions

240 between the polymeric material and the oxygen during the manufacture of the material

241 (García Ibarra, Rodríguez Bernaldo de Quirós, Paseiro Losada, & Sendón, 2019). According

242 to Cramer rules, the identified alkanes belong to class I, that means to low toxicity, while

243 class II and class III are of medium and high toxicity respectively. Four compounds were

244 classified as class III: methyl N-hydroxybenzenecarboximidate and three phytosterols (3β-

245 ergost-5-en-3-ol, clionasterol and arundoin). Four phytosterols, stigmasterol, were also

246 detected and classified as class II. Phytosterols, 3β-ergost-5-en-3-ol, stigmasterol, clionasterol

247 and arundoin, were identified only in EtOH 95% food simulant. The presence of phytosterols

248 in young bamboo shoots had been previously demonstrated (Nongdam & Tikendra, 2014).

249 Therefore, these compounds could come from the bamboo used as base material (Moreau et

250 al., 2018). According to the toxicological assays performed by Le Goff et al. and Mea et al.,

251 phytosterols do not have genotoxic potential (Le Goff et al., 2019; Lea, Hepburn, Wolfreys,

252 & Baldrick, 2004). In contrast, phytosterols of bamboo shoot reduces fat and cholesterol

253 levels of blood, and they can inhibit the production of carcinogens and growth of cancer cells

254 (Nongdam & Tikendra, 2014).

255 3.2. Non-volatile compounds present in migration from a bamboo-based biopolymer

256 Table 2 shows the non-volatile compounds found in migration solutions from the bamboo-

257 based biopolymer, their retention time, their accurate mass, the adduct detected ([M+H]$^+$ or

258 [M+Na]$^+$) and their molecular formula. A total of twelve compounds were detected

259 (Supplementary data 1). The amino acid valine was detected in all food simulants. The

260 presence of valine in bamboo shoots was previously reported by different authors (De Silva et

261 al., 2019; Nongdam & Tikendra, 2014). C. Nirmala et al, reported it as one of the most

262 abundant amino acids in the composition of Phyllostachys manii (juvenile bamboo shoots)

263   (Nirmala, Bisht, Bajwa, & Santosh, 2018). Triethanolamine, commonly used as surfactant,

264   was also detected. None of these compounds was present in the EU/10/2011 positive list

265   (European Commission, 2011a). Triethanolamine has a NOAEL value 300 mg kg^{-1}day^{-1} and

266   that of valine is 628 mg kg^{-1}day^{-1} (Agency United States Environmental Protection, 2019).

267   It was also observed that melamine and eight melamine derivatives were identified in all food

268   simulants. This is because in the manufacture of biopolymers it is common to use a polymeric

269   resin, such as melamine, to improve the mechanical properties of the material.

270   Melamine has been reported as a common component in the manufacture of laminates,

271   coatings and mainly, kitchenware utensils (Geueke, 2013; Li, Song, & Wen, 2019).

272   Melamine derivatives can also be formed from the reaction of residual melamine and food

273   simulants (Bradley, Castle, Day, & Leak, 2011; Chien et al., 2011; Karthikraj, Bollapragada,

274   & Kannan, 2018) or during the bamboo based biopolymer manufacture.

275   Melamine is listed in the Regulation EU/10/2011 with a specific migration limit (SML) of 2.5

276   mg kg^{-1}. For this reason, melamine as well as its derivatives were quantified in all food

277   simulants. Since the material evaluated was intended for repeated used, according to

278   EU/10/2011 operating guidelines, the migration was evaluated over three consecutive

279   migration experiments. The results are presented in figure 1. Melamine was the compound

280   with the highest migration values. In all cases, the migration values were above 2.5 mg kg^{-1}

281   in the first, second and third migration experiments, specially in AcH 3%. Therefore, this

282   bamboo-based biopolymer did not comply with the SMLs established by the Regulations

283   (EU) No 10/2011 and No284/2011. According to these results, the material cannot be

284   considered as bamboo, but melamine with bamboo filler. Then, the Regulation 284/2011

285   concerning melamine kitchenware tools applies (European Commission, 2011b). Regarding

286   the food simulants, the highest values were found in the second migration of AcH 3% (144.3

287   ± 1.1 mg kg^{-1}), followed by EtOH10% (48.8 ± 3.4 mg kg^{-1}) and EtOH 95% (4.3 ± 1.0 mg

288 kg^{-1}), suggesting an important effect of acidity and a high water content in the migration

289 process. The same pattern was observed for all the melamine derivatives analyzed. N-

290 Hydroxymethylmelamine (MD 1) was the melamine derivative with the highest migration

291 values.

292 The acidic medium of the acetic acid simulant could accelerate the hydrolysis reaction of the

293 melamine resin and as a result, provide high migration values (Bradley et al., 2011; Geueke,

294 2013). The results also showed that the migration of melamine and its derivatives increased

295 in the second migration experiment and decreased in the third one. This behavior could be

296 attributed to the possible variation in the internal structure of the polymer caused by the

297 simulants, which could have modified the diffusion of the compounds though the polymer

298 and thus affecting the migration. Therefore, the addition of the polymeric resin to the bamboo

299 biopolymer improved its mechanical properties but at the same time, entailed a risk to

300 consumer's health. In addition, melamine is neither a biopolymer nor biodegradable, what

301 means that these materials, promoted as "bamboo food contact materials" can be considered

302 as a fraud to consumers.

303 3.3. DART-SVP analysis of migration samples

304 DART-SVP is an electric discharge ambient ionisation technique where excited helium atoms

305 start gas-phase reactions. These reactions result in ions created from atmospheric water-vapor

306 or other gaseous compounds present (Gross, 2014; Navare, Mayoral, Nouzova, Noriega, &

307 Fernández, 2010). As the chemical ionization is the same as in APCI (atmospheric pressure

308 chemical ionization) (Gross, 2014), the formation of adducts according to polarity, molecular

309 weight or volatility of the species present in the samples is very common.

310 In this study, the ability to implement a DART-SVP method to quickly determine the main

311 migrants and their adducts from bamboo-base biopolymer was investigated. Figure 2 shows a

mass spectrum of an EtOH 95% migration sample. Table 3 shows a total of twenty-six

masses detected in the DART mass spectrum and that matched with adducts of compounds

that were previously detected in migration solutions by GC-MS or UPLC-MS/QToF. The

range of m/z values was between 127.0733 and 291.1033. Only peaks with relative intensity

higher than 1% of the base peak were considered. The table shows the main adduct ion

detected as well as its m/z and relative intensity. In addition, other adducts identified and its

relative intensity for each of the compounds are shown. The presence of other adducts

confirmed the candidates proposed.

Regarding the ions detected, adducts such as $M^{.+}$, $[M+H]^+$, $[M+N]^+$, $[M-H+O]^+$ and $[M+H-H_2O]^+$ were observed with major relative intensity. Oxygen, water and ammonia species were

detected in the environment (blank spectrum mass), therefore the different adducts identified

were the interaction products between gaseous species of the atmosphere and molecular ions

from the sample. Other adducts detected were $M^{.+}$, $[M+H]^+$, $[M-H+O]^+$, $[M+H+O]^+$,

$[M+NH_4]^+$ and $[M-H+O_2]^+$.

In this technique the analytes generate different adducts according to the polarity of specie:

$[M+H]^+$, $[M-H+O]^+$, $[M+NH_4]^+$ adducts for medium polar to polar analytes; and $M^{.+}$, $[M-H+H_2O]^+$, $[M+H]^+$, $[M-H+O]^+$ adducts for non-polar analytes (Cody, 2009; Gross, 2014).

The results suggested that the polarity was an important parameter in the formation of

adducts from the migration samples of bamboo-based biopolymer. Melamine and it

derivatives formed mainly adducts such as $[M+H]^+$, $[M-H+O]^+$ and $[M-H+O_2]^+$ because these

compounds have a high polarity. These adducts had high relative intensity (between 10% -

100%) probably because they were at higher concentration than other compounds in the

samples (section 3.2). On the other hand, some compounds with low polarity, such as

alkanes, formed $[M+N]^+$ and $[M-H+O]^+$ adducts. However, they were in the adduct group

with low relative intensities, between 1-10%. Probably, the high volatility of small molecules

14

337 promotes a higher number of interactions with the different reactive species and therefore a

338 higher variety of adducts (Bentayeb et al., 2012; Kerpel dos Santos et al., 2018). Among the

339 masses found in DART MS spectrum with relative intensities above 10%, 10 out of them

340 could not be associated to any of the previously identified compounds by GC-MS or UPLC-

341 MS/QToF.

342 For 5 of these masses, new candidate compounds were proposed (table 4). The candidates

343 were selected according to its elemental composition, adducts detected, and to bibliography

344 of bamboo composition (De Silva et al., 2019; Nirmala et al., 2018; Nongdam & Tikendra,

345 2014). Other researchers have found in the composition of young bamboo shoots different

346 amino acids, minerals, polyphenols, and/or vitamins, such as serine, coniferyl alcohol, caffeic

347 acid, threonine, paracoumaryl alcohol, histidine and protocatechuic acid, among others (De

348 Silva et al., 2019; Nirmala et al., 2018; Nongdam & Tikendra, 2014; Zhu & Kannan, 2019).

349 Some of the expected adducts for these compounds coincide with the masses detected.

350 However, for 5 of the masses detected in the mass spectrum with intensity values above 10%,

351 it was not possible to propose a candidate: 93.05 (26.8 %), 145.09 (15.0 %), 130.22 (15.0 %),

352 295.28 (14.5%) and 192.88 (11.5%).

353

4. Conclusions

355 Even though some of the compounds found in migration came from bamboo, such as

356 phytosterols, most of the migrants came from the melamine added to it in order to improve

357 the biopolymer properties. Not only melamine but several melamine derivatives were found

358 in migration above the limits established in European legislation. Consequently, this material

359 does not comply with the EU legislation. In fact, the material cannot be identified as bamboo,

360 but as melamine with bamboo filler. As melamine is neither a biopolymer not biodegradable

15

361 material, the promotion of these kitchenware materials as bamboo can be considered as a
362 fraud to consumers.

363 Regarding to DART-SVP experiments, the analysis allowed detecting the volatile, semi-
364 volatile and non-volatile compounds from migration samples of the bamboo-based
365 biopolymer in a unique analysis of 1.5 minutes duration for each replicate. This technique
366 offers great advantages for rapid detection and routine analysis, without requiring sample
367 preparation.

368 All the compounds identified using DART-SVP were detected in the initial part of this study,
369 where GC-MS and UPLC/QToF methodologies were used. Melamine and its derivatives
370 form the adducts with greater relative intensity. These results demonstrate that the DART-
371 SVP technique is a very useful tool for direct target analysis, where in a few minutes can
372 provide data about the main migrants present in samples.

373 **Acknowledgements**

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536

Figure captions

Fig 1. Bar chart of migration values of melamine and its derivative compounds from

bamboo-based biopolymer in three consecu-tive migration assays. a) acetic acid 3% (w/v), b)

ethanol 10% (v/v) and c) ethanol 95% (v/v). MD codes are linked to table 2.

Fig.2. DART-SVP mass spectrum of a migration solution (ethanol 95%) from a bamboo-

based biopolymer sample. Numbers in brackets are linked to table 3.

1   **Ambient mass spectrometry as a tool for a rapid and simultaneous determination of**

2   **migrants coming from a bamboo-based biopolymer packaging.**

3

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16

17   **Abstract**

18   New bamboo-based biopolymers are used as food packaging materials, but it must be

19   evaluated to ensure consumers safety. In this study, migration from a commercial bamboo-

20   based biopolymer to ethanol 10% (v/v), acetic acid 3% (w/V) and ethanol 95% (v/v) was

21   studied. The migrants were determined from three different perspectives. Volatile and semi-

22   volatile compounds were analyzed by gas chromatography-mass spec-trometry (GC-MS).

23   Twenty-five compounds were detected, alkanes. In addition, a number of phytosterols were

24   detected in ethanol 95%. Non-volatile compounds were identified and quantified by ultra-

performance liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-Q/ToF). Twelve non-volatile compounds were detected in migration solutions, mainly melamine and its derivatives, coming from polymer resins added to the biopolymer. Melamine concentration values were above the melamine specific migration limit (SML) established by the Regulations (EU) No 10/2011 and (EU) No 284/2011. Finally, the migration samples were analyzed by DART-SVP (direct analysis in real time coupled to standardized voltage and pressure). This methodology was able to detect simultaneously the main volatile and non-volatile migrants, and the adducts in a very rapid and effective way and shows huge promise as a tool to test for the safety and legal compliance of food packaging materials.

Keywords: bamboo biopolymer; DART; melamine; migration; food contact material.

1. Introduction

There is a great demand for biopolymers in the sector of food contact materials manufacturing. This is because biopolymers are a green alternative to the traditional packaging materials used in the food industry, especially those that are abundant, renewable, biodegradable, and biocompatible (Khlestkin, Peltek, & Kolchanov, 2018). Biopolymers can be classified into two groups, polymers produced from biological products that can be extracted from biomass or produced in fermentation processes, such as carbohydrates, proteins or lipids; and biodegradable and/or compostable polymers (Aznar, Ubeda, Dreolin, & Nerín, 2019; Geueke, 2014; Maisanaba et al., 2018). The addition of additives to the biopolymers is necessary in order to improve their physico-chemical properties. These additives can be for example: polyols, used as non-volatile plasticizers (e.g., glycerol, glycol, and sorbitol); and resins, that mold the material (e.g., urea, melamine, melamine formaldehyde, and amines) (Eckardt, Kubicova, & Simat, 2018; Geueke, 2013). Surprisingly,

49 melamine and other resins are neither biodegradable or come from natural sources (Chien et

50 al., 2011; Geueke, 2013).

51 In recent years, food packaging industries have been highly innovative in the production of

52 new biopolymers derived from various natural resources, such as starch, cellulose, bamboo,

53 and chitosan. Among them, bamboo-base biopolymers are of enormous importance. This

54 fibre is attractive as a natural-based raw material, since it is economical, eco-friendly and

55 biodegradable (Jena, 2018; Xie, Zhou, & Yan, 2019). Bamboo plants are easily available due

56 to its very short life cycle and because bamboo timber can be harvested multiple times from a

57 single planting, which makes its use very sustainable. Bamboo-based biopolymers have better

58 mechanical properties than other biomaterials, such as a good impact resistance, high

59 durability and crack resistance, and good flexural properties (Xie et al., 2019). As any food

60 packaging material or food contact article, the biopolymers must be evaluated to confirm

61 their acceptability and safety. The bamboo-base biomaterials can contain other resins and

62 also non intentionally added substances (NIAS), such as impurities from raw materials,

63 degradation compounds or novel substances coming from the reaction between different

64 reagents (Xu, Xiong, Zhao, Xiang, & Wu, 2020). These compounds, as well as those

65 additives intentionally added, are present in the packaging and could migrate to the food.

66 Migration tests are required in order to guarantee the consumers safety, food preservation and

67 organoleptic properties (Bradley, n.d.). These tests require specific conditions of temperature,

68 time and simulants, which should be selected according to the intended use of the material. In

69 the specific migration tests, compounds previously identified in the material as well as new

70 formed compounds can be detected (Nerin, Alfaro, Aznar, & Domeño, 2013). Pure

71 biopolymers are not specifically included in the European plastics regulation and only the

72 frame regulation 1935/2004/CEE applies. However, if they contain some resin or

73 conventional plastic, the regulation on plastics should be applied. European legislation on

74   food contact plastics (Regulation No 10/2011/EU) establishes a positive list with substances

75   that can be used in the manufacturing process and also their specific migration limits (Osorio,

76   Dreolin, Aznar, Nerín, & Hancock, 2019).

77   Since the migrants can be volatile as well as non-volatile compounds, different technologies

78   should be applied in order to have a global view of the safety of the material. Gas

79   chromatography can be applied to the separation of the most volatile and semi-volatile

80   compounds (Biedermann & Grob, 2019) and liquid chromatography to the less volatile ones.

81   Both of them coupled to mass spectrometry detection will allow the identification of the

82   NIAS present in the packaging material. Ambient mass spectrometry techniques can be also

83   used to get a quick confirmation of the presence/absence of the identified compounds.

84   Ambient mass spectrometry (AMS) or ambient ionization techniques (AMI) are ionization

85   sources used in mass spectrometry for direct and rapid analysis of compounds present in solid

86   or liquid samples (Black, Chevallier, & Elliott, 2016; Lu et al., 2018). Additionally, AMS has

87   been utilized as screening method in food safety analysis, criminal investigation,

88   environmental test and other different analytical objectives (Gross, 2014; Lennert & Bridge,

89   2019; Zhang et al., 2018). DESI-MS (Desorption ElectroSpray Ionization Mass

90   Spectrometry) was the first AMS technique developed. Since then, other techniques such as

91   direct analysis in real time (DART), extractive electrospray ionization (EESI), desorption

92   atmospheric pressure chemical ionization (DAPCI), dielectric-barrier-discharge ionization

93   (DBDI) or low-temperature plasma (LTP) have been designed (Barnett & Zhang, 2018).

94   AMS techniques had been previously used in the study of food packaging materials by other

95   groups. For example, Bentayeb et al. determined the compounds present due to print set-off

96   process (Bentayeb et al., 2012; ten Dam et al., 2016) while Aznar et al. analyzed the

97   distribution of ink components in a packaging material and Rothenbacher et al. studied the

98   plasticizers in poly(vinyl chloride) (PVC) (Aznar, Alfaro, Nerín, Jones, & Riches, 2016).

99 Direct analysis in real time (DART) is one of the most popular ambient pressure ionization

100 methods. In this technique, the molecules are ionized by excited helium molecules and

101 adducts, mainly for protonated/deprotonated molecular ion, are commonly formed (Black et

102 al., 2016). Some of its main advantages are: the possibility of direct sampling at ambient

103 conditions; to generate ions from liquid or solid samples; to be simple to operate and to have

104 a rapid analysis speed (acquisition time < 60 s) compared to UPLC and GC-MS methods

105 (Nerin et al., 2013). DART-SVP technique has diverse applications such as food quality and

106 safety control, drug screening, contamination analysis and environmental monitoring (Barnett

107 & Zhang, 2018; Djelal, Cornée, Tartivel, Lavastre, & Abdeltif, 2017; Lennert & Bridge,

108 2018).

109 In the present study, the migration from a bamboo-based biopolymer intended for food

110 contact to different food simulants was evaluated. Volatile, semi-volatile and non-volatile

111 compounds were firstly identified by GC-MS and UPLC-Q/ToF. Additionally, DART-SVP

112 was used as a tool to assess the presence of all potential migrants in a very short analysis

113 time.

114 **2. Materials and methods**

115 2.1. Chemicals and Reagents

116 Melamine 99% [108-78-1] (IUPAC name: 1,3,5-triazine-2,4,6-triamine) was from Sigma–

117 Aldrich (Madrid, Spain). Ethanol absolute (HPLC grade), methanol (UHPLC-MS grade),

118 acetic acid (HPLC grade) were supplied by Scharlau (Setmenat, Spain) for the analysis in

119 GC-MS and UPLC-Q/ToF. Ethanol absolute (HPLC grade) supplied by Merck (Darmstadt,

120 Germany) for the analysis in DART-SVP. Ultra-pure water was obtained from Millipore

121 Milli-Q system (Billerica, MA, USA). The nitrogen evaporator was a TECHNE sample

122 concentrator (Cole-Parmer Ltd., UK). SPME fiber was purchased to Supelco (Bellefonte, PA,

123 USA).

124 2.2. Samples

125 Bamboo-based biopolymer samples were purchased at a local supermarket in the form of

126 cups, dishes and jugs. All of these were monolayer materials.

127 2.3. Migration assays

128 Migration assays were carried out by total immersion of the sample (5 cm x 1 cm) into 20mL

129 of the simulant. The process was established in accordance with the European legislation on

130 food contact materials (Regulation No 10/2011/EU) (European Commission, 2011a). Three

131 simulants were evaluated: ethanol 10% (v/v) (simulant A), acetic acid 3% (w/v) (simulant B)

132 and ethanol 95% (v/v) (simulant D2 substitute). Global migration studies were performed

133 during 2 hours at 70 ºC. The simulant solutions were evaporated to dryness. Global migration

134 was calculated by weighting the dry residue. Specific migration was performed during 6 hours

135 at 70 ºC. Since the food contact materials of this study were intended for repeated use, the

136 migration tests were performed three consecutive times. The assays were carried out in

137 triplicate for each sample and a blank.

138 2.4. Analysis by GC-MS

139 The analyses of the volatile and semi-volatile compounds were carried out using a gas

140 chromatograph 6890N equipped with an electron ionization ion source operating at 70 eV,

141 and coupled to a quadrupole mass spectrometry detector 5975D (Agilent Technologies. Santa

142 Clara, CA). It was set in SCAN acquisition mode (50 - 450 m/z). The transfer line heater was

143 set at 280 ºC. The autosampler was a Combi PAL (CTC Analytics, Zwingen, Switzerland).

144 The injector was used in two modes: liquid injection (migration samples: ethanol 95%)) and

145 SPME injection (aqueous migration samples: acetic acid 3% and ethanol 10%). The

146 stationary phase of polydimethylsiloxane/ divinylbenzene/carboxen (PDMS/DVB/CAR 50/30

147 µm) was used for the SPME extraction. The migration samples were conditioned during 2

148 min at 80ºC. Subsequently, the volatile and semi-volatile compounds were extracted at 80ºC

149 and 500 rpm agitation during 15 min. SPME fiber was desorbed at 250ºC for 2 min and 1 µL

150 of liquid samples was injected at 250 ºC in splitless mode. The chromatographic separation

151 was performed on a HP-5MS column of 30 m x 0.25 mm i.d. x 0.25 µm film thickness. The

152 oven temperature program was: 40 ºC held for 3 min, ramp 10 ºC/min to 300 ºC and held for

153 2 min. Helium was used as carrier gas at a constant flow of 1 mL/min. The SPME extraction

154 process in biopolymer samples had been optimized in a previous work (Osorio, Aznar, &

155 Nerín, 2019).

156 2.5. Analysis by UPLC-Q/ToF

157 The analyses of the non-volatile compounds present in migration solutions were performed

158 using an ACQUITY UPLC chromatograph (Milford, MA, USA). Flow rate was 0.3 mL/min.

159 Injection volume was 10 µL. An UPLC BEH C18 column (Waters) of 2.1 × 100 mm, 1.7 µm

160 particle size was used at a temperature of 35 °C. Two mobile phases were used for the

161 separation: (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid.

162 Chromatography was performed using the following gradient elution: initial composition

163 98/2 (phase A/ phase B), and at eight min it was changed to 0/100 (phase A/ phase B) with

164 two additional minutes at the final composition.

165 UPLC system was coupled to a Xevo G2 QTOF mass spectrometer from Waters (Milford,

166 MA, USA) with an ESI probe. The conditions of analysis were: positive ionization (ESI+),

167 sensitivity mode, capillary voltage 3.0 kV, sampling cone voltage 30V, extraction cone 3 V,

168 source temperature 150 °C, desolvation temperature 450 °C, cone gas flow rate of 20 L/ h,

169   and desolvation gas flow rate of 500 L/h. Acquisition was carried out in MSE mode in a mass

170   range between 50 and 1200 m/z. The chromatogram was acquired at low and high collision

171   energy (CE) in the collision cell.

172   2.6. Analysis by DART-SVP

173   A direct analysis in real time (DART) couple to Standardised Voltage and Pressure (SVP)

174   100 ion source (IonSense, Saugus, MA) was coupled to an Acquity QDa single quadrupole

175   mass spectrometer (Waters, Wilmslow, UK) via a Vapur interface (IonSense, Saugus, MA).

176   The DART-SVP and Acquity QDa were operated in positive ion mode. The DART-SVP was

177   operated with helium (grade A) in running mode and nitrogen in standby mode. The DART-

178   SVP was operated at several different temperatures between 150 ºC and 450 ºC, the ion-

179   source grid voltage was set to 350 V. The mass spectrometer was operated with the

180   desolvation line temperate set at 250 ºC, heat block 350 ºC, interface voltage 4.5 kV and 50-

181   1000 Da scan range. The mass spectrometer was controlled using MassLynx v4.1 SCN888

182   (Waters, Wilmslow, UK). Data were analyzed by MassLynx v4.1.

183   Manual sample injection mode was used. Three microliters of the migration solution were

184   pipette-spotted directly onto the Quick Strip card in the position one. Then Quick Strip card

185   was the simply mounted on the sampling rail. Posteriorly, the sample came in contact with

186   the He stream from the DART ion source outlet.

187   2.7. Data processing

188   In GC-MS analysis, the chromatograms and mass spectra were acquired and processed with

189   MSD ChemStation software version F.01.03 from Agilent Technologies. NIST Standard

190   Reference Database (2018) was used as mass spectra library for compounds identification.

191   Only Match values above 800 were considered acceptable for a candidate confirmation.

192   In UPLC-Q/ToF, the mass data were analysed with MassLynx software V 4.1 from Waters

193   (Milford, MA, USA). Mass spectra obtained in function 1 provided information about the

194   elemental composition of the precursor ion and the mass spectra in function 2 provided

195   information about the elemental composition of the fragment ions. MassFragment® software

196   from Waters was used for checking the feasiability of the candidates proposed (Ubeda,

197   Aznar, & Nerín, 2018). The DART-SVP mass spectrums were acquired with MassLynx

198   SCN888T software and processed with MassLynx software V 4.1.

199   **3. Results and discussion**

200   The results showed that global migration was higher when compared to other polymers, but

201   below Overall Migration Limit (OML) of 60 mg kg^{-1} established by Regulation EU/10/2011

202   (European Commission, 2011a) in all simulants. The values found for the different simulants

203   were as follows: 50.6 ± 9.2 mg kg^{-1} in ethanol 10%; 13.2 ± 3.2 mg kg^{-1} in acetic acid 3% and

204   48.5 ± 4.4 mg kg^{-1} in ethanol 95%. Specific migration results are shown below for each

205   technique.

206   3.1. Volatile and semi-volatile compounds present in migration from a bamboo-based

207   biopolymer.

208   Table 1 shows the compounds identified with their retention times, their molecular formula

209   and its presence in the different simulants in migration samples. A total of twenty-five

210   compounds were identified by comparing their mass spectra with the NIST Standard

211   Reference Database (2018). Compounds identified were checked in the EU/10/2011 positive

212   list (European Commission, 2011a), to confirm if they were authorized and their specific

213   migration limit. All the compounds detected were non-listed substances and only a 2,6,10-

214   trimethyl   dodecane   had   a   NOAEL   value   (1000   mg   kg^{-1}day^{-1})

215   (https://comptox.epa.gov/dashboard (accessed Jan.28.2020), n.d.). Therefore, their theoretical

216   toxicity level was established according to the TTC (threshold of toxicological concern) and

217   Cramer rules for each compound (Dewhurst & Renwick, 2013; Szczepańska et al., 2018).

218   Table 1 shows the theoretical classification of toxicity for the rest of non listed compounds

219   found.

220   Acetic acid 3% was the food simulant with the highest number of migrants (15), compared to

221   ethanol 10% (6) and ethanol 95% (6) (Supplementary material 1). Most of the identified

222   compounds were alkanes (Jiong, Fusheng, Chunhong, Mao, & Jianquan, 2014). These

223   compounds are commonly used as raw materials in the production of polymers but they

224   might also come from the degradation of the material. Degradation can happen because of the

225   interactions between the polymeric material and the oxygen present in the air, causing its

226   oxidation (García Ibarra, Rodríguez Bernaldo de Quirós, Paseiro Losada, & Sendón, 2019).

227   According to Cramer rules, the identified alkanes belong to class I, that means to low

228   toxicity, while class II and class III are of medium and high toxicity respectively. Four

229   compounds were classified as class III: methyl N-hydroxybenzenecarboximidate and three

230   phytosterols (3β-ergost-5-en-3-ol, clionasterol and arundoin). Four phytosterols, stigmasterol,

231   were also detected and classified as class II. Phytosterols, 3β-ergost-5-en-3-ol, stigmasterol,

232   clionasterol and arundoin, were identified only in ethanol 95% food simulant. The presence

233   of phytosterols in young bamboo shoots had been previously demonstrated (Nongdam &

234   Tikendra, 2014). Therefore, these compounds could come from the bamboo used as base

235   material (Moreau et al., 2018). According to the toxicological assays performed by Le Goff et

236   al. and Mea et al., phytosterols do not have genotoxic potential (Le Goff et al., 2019; Lea,

237   Hepburn, Wolfreys, & Baldrick, 2004). In contrast, phytosterols of bamboo shoot reduces fat

238   and cholesterol levels of blood, and they can inhibit the production of carcinogens and

239   growth of cancer cells (Nongdam & Tikendra, 2014).

240   3.2. Non-volatile compounds present in migration from a bamboo-based biopolymer

241   Table 2 shows the non-volatile compounds found in migration solutions from the bamboo-

242   based biopolymer, their retention time, their accurate mass, the adduct detected ([M+H]$^+$ or

243   [M+Na]$^+$) and their molecular formula. A total of twelve compounds were detected

244   (Supplementary data 1). The amino acid valine was detected in all food simulants. The

245   presence of valine in bamboo shoots has been previously reported by different authors (De

246   Silva et al., 2019; Nongdam & Tikendra, 2014). C. Nirmala et al, reported it as one of the

247   most abundant amino acids in the composition of Phyllostachys manii (juvenile bamboo

248   shoots) (Nirmala, Bisht, Bajwa, & Santosh, 2018). Triethanolamine, used commonly as

249   surfant, was also detected. None of these compounds was present in the EU/10/2011 positive

250   list (European Commission, 2011a). Triethanolamine had a NOAEL value 300 mg kg^{-1}day^{-1}

251   and valine, with NOAEL value 628 mg kg^{-1}day^{-1} (https://comptox.epa.gov/dashboard

252   (accessed Jan.28.2020), n.d.).

253   It was also observed that melamine and eight melamine derivatives were identified in all food

254   simulants. This is because in the manufacture of biopolymers it is common to use a polymeric

255   resin, such as melamine, to improve the mechanical properties of the material.

256   Melamine has been reported as a common component in the manufacture of laminates,

257   coatings and mainly, kitchenware utensils (Geueke, 2013; Li, Song, & Wen, 2019).

258   Melamine derivatives can also be formed from the reaction of residual melamine and food

259   simulants (Bradley, Castle, Day, & Leak, 2011; Chien et al., 2011; Karthikraj, Bollapragada,

260   & Kannan, 2018) or during the bamboo based biopolymer manufacture.

261   Melamine is listed in the Regulation EU/10/2011 with a specific migration limit (SML) of 2.5

262   mg kg^{-1}. For this reason, melamine as well as its derivatives were quantified in all food

263   simulants. Since the material evaluated was intended for repeated used, according to

264   EU/10/2011 operating guidelines, the migration was evaluated over three consecutive

265   migration experiments. The results are presented in figure 1. Melamine was the compound

266 with the highest migration values. In all cases, the migration values were above 2.5 mg kg^{-1}

267 in the first or second migration experiment and therefore, this bamboo-based biopolymer did

268 not comply with the SMLs established by the Regulations (EU) No 10/2011 and No284/2011

269 . According to these results, the material cannot be considered as bamboo, but melamine with

270 bamboo filler. Then, the regulation 284/2011 applies (European Commission, 2011b).

271 Regarding the food simulants, the highest values were found in the second migration of acetic

272 acid 3% (w/V) (144.3 ± 1.1 mg kg^{-1}), followed by ethanol 10% (v/v) (48.8 ± 3.4 mg kg^{-1}) and

273 ethanol 95% (v/v) (4.3 ± 1.0 mg kg^{-1}), suggesting an important effect of acidity and a high

274 water content in the migration process. The same pattern was observed for all the melamine

275 derivatives analyzed. N-Hydroxymethylmelamine (MD 1) was the melamine derivative with

276 the highest migration values.

277 The acidic medium of the acetic acid simulant could accelerate the hydrolysis reaction of the

278 melamine resin and as a result, provide high migration values (Bradley et al., 2011; Geueke,

279 2013). The results also showed that the migration of melamine and its derivatives increased

280 in the second migration experiment and decreased in the third one. Therefore, the addition the

281 polymeric resin to the bamboo biopolymer improved its mechanical properties but at the

282 same time, entailed a risk to consumer's health.

283 3.3. DART-SVP analysis of migration samples

284 DART-SVP is an electric discharge ambient ionisation technique where excited helium atoms

285 start gas-phase reactions. These reactions result in ions created from atmospheric water-vapor

286 or other gaseous compounds present (Gross, 2014; Navare, Mayoral, Nouzova, Noriega, &

287 Fernández, 2010). As the chemical ionization is the same as in APCI (atmospheric pressure

288 chemical ionization) (Gross, 2014), the formation of adducts according to polarity, molecular

289 weight or volatility of the species present in the samples is very common.

290 In this study, the ability to implement a DART-SVP method to quickly determine the main

291 migrants and their adducts from bamboo-base biopolymer was investigated. Figure 2 shows a

292 mass spectrum of an ethanol 95% migration sample. Table 3 shows a total of twenty-six

293 masses detected in the DART mass spectrum and that matched with adducts of compounds

294 that had been previously detected in migration solutions by GC-MS or UPLC-MS/QToF. m/z

295 values ranged between 127.0733 and 291.1033. Only peaks with relative intensity higher than

296 1% of the base peak were considered. The table shows the main adduct ion detected as well

297 as its m/z and relative intensity. In addition, other adducts identified and its relative intensity

298 for each of the compounds are shown. The presence of other adducts confirmed the

299 candidates proposed.

300 Regarding the ions detected, adducts such as $M^{\cdot+}$, $[M+H]^+$, $[M+N]^+$, $[M-H+O]^+$ and $[M+H-$

301 $H_2O]^+$ were observed with major relative intensity. Oxygen, water and ammonia species were

302 detected in the environment (blank spectrum mass), therefore the different adducts identified

303 were the interaction products between gaseous species of the atmosphere and molecular ions

304 from the sample. Other adducts detected were $M^{\cdot+}$, $[M+H]^+$, $[M-H+O]^+$, $[M+H+O]^+$,

305 $[M+NH_4]^+$ and $[M-H+O_2]^+$.

306 In this technique the analytes generate different adducts according to the polarity of specie:

307 $[M+H]^+$, $[M-H+O]^+$, $[M+NH_4]^+$ adducts for medium polar to polar analytes; and $M^{\cdot+}$, $[M-$

308 $H+H_2O]^+$, $[M+H]^+$, $[M-H+O]^+$ adducts for non-polar analytes (Cody, 2009; Gross, 2014).

309 The results suggested that the polarity was an important parameter in the formation of

310 adducts from the migration samples of bamboo-based biopolymer. Melamine and it

311 derivatives formed mainly adducts such as $[M+H]^+$, $[M-H+O]^+$ and $[M-H+O_2]^+$ because these

312 compounds have a high polarity. Additionally, these adducts had high relative intensity

313 (between 10% - 100%) probably because they were at higher concentration than other

314 compounds in the samples (section 3.2). On the other hand, some compounds with low

13

315 polarity, such as alkanes, formed $[M+N]^+$ and $[M-H+O]^+$ adducts. However, they were in the

316 adduct group with low relative intensities, between 1-10%. Probably, the high volatility of

317 small molecules promotes a higher number of interactions with the different reactive species

318 and therefore a higher variety of adducts (Bentayeb et al., 2012; Kerpel dos Santos et al.,

319 2018). Among the masses found in DART MS spectrum with relative intensities above 10%,

320 10 out of them could not be associated to any of the previously identified compounds by GC-

321 MS or UPLC-MS/QToF.

322 For 5 of these masses, new candidate compounds were proposed (table 4). The candidates

323 were selected according to its elemental composition, adducts detected, and to bibliography

324 of bamboo composition (De Silva et al., 2019; Nirmala et al., 2018; Nongdam & Tikendra,

325 2014). Researchers have found in the composition of young bamboo shoots different amino

326 acids, minerals, polyphenols, and/or vitamins, such as serine, coniferyl alcohol caffeic acid,

327 threonine, paracoumaryl alcohol, histidine and protocatechuic acid, among others (De Silva et

328 al., 2019; Nirmala et al., 2018; Nongdam & Tikendra, 2014; Zhu & Kannan, 2019). Some of

329 the expected adducts for these compounds coincide with the masses detected. However, for 5

330 of the masses detected in the mass spectrum with intensity values above 10%, it was not

331 possible to propose a candidate: 93.05 (26.8 %), 145.09 (15.0 %), 130.22 (15.0 %), 295.28

332 (14.5%) and 192.88 (11.5%).

333

334 **4. Conclusions**

335 Even though some of the compounds found in migration came from bamboo, such as

336 phytosterols, most of the migrants came from the melamine added to it in order to improve

337 the biopolymer properties. Not only melamine but several melamine derivatives were found

338 in migration above the limits established in European legislation. Consequently, this material

339 does not comply with the EU legislation. In fact, the material cannot be identified as bamboo,

340 but as melamine with bamboo filler.

341 Regarding to DART-SVP experiments, the analysis allowed detecting the volatile, semi-

342 volatile and non-volatile compounds from migration samples of the bamboo-based

343 biopolymer in a unique analysis of 1.5 minutes duration for each replicate. This technique

344 offers great advantages for rapid detection and routine analysis, without requiring sample

345 preparation.

346 All the compounds identified using DART-SVP were detected in the initial part of this study,

347 where GC-MS and UPLC/QToF methodologies were used. Melamine and its derivatives

348 form the adducts with greater relative intensity. These results demonstrate that the DART-

349 SVP technique is a very useful tool for direct target analysis, where in a few minutes can

350 provide data about the main migrants present in samples.

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515

Figure captions

Fig 1. Bar chart of migration values of melamine and its derivative compounds from

bamboo-based biopolymer in three consecu-tive migration assays. a) AcH 3%, b) EtOH 10%

and c) EtOH 95. MD codes are linked to table 2.

Fig.2. DART-SVP mass spectrum of a migration solution (ethanol 95%) from a bamboo-

based biopolymer sample. Numbers in brackets are linked to table 3.

Abstract

New bamboo-based biopolymers are used as food packaging materials, but it must be evaluated to ensure consumers safety. In this study, migration from a commercial bamboo-based biopolymer to ethanol 10% (v/v), acetic acid 3% (w/v) and ethanol 95% (v/v) was studied. The migrants were determined from three different perspectives. Volatile and semi-volatile compounds were analyzed by gas chromatography-mass spectrometry (GC-MS). Twenty-five compounds were detected. In addition, a number of phytosterols were detected in ethanol 95%. Non-volatile compounds were identified and quantified by ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-Q/ToF). Twelve non-volatile compounds were detected in migration solutions, mainly melamine and its derivatives, coming from polymer resins present in the biopolymer. Melamine migration was higher than 50 mg/Kg in the third sequential migration test. Finally, the migration samples were analyzed by DART-SVP (direct analysis in real time coupled to standardized voltage and pressure). This methodology was able to detect simultaneously the main volatile and non-volatile migrants and their adducts in a very rapid and effective way and is shown as a promising tool to test the safety and legal compliance of food packaging materials.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jazmín Osorio: Investigation, Methodology, Writing- Original draft preparation; **Margarita Aznar**: Conceptualization, Methodology, Writing- Reviewing; **Cristina Nerín**: Supervision, Writing- Reviewing; **Nicholas Birse**: Resources; **Christopher Elliott**: Supervision; **Olivier Chevallier**: Supervision, Writing- Reviewing

*Novelty Statement (maximum limit:100 words)

An ambient pressure ionization method (direct analysis in real time) coupled to mass spectrometry (DART- SVP) was used for the first time to detect simultaneously the main volatiles and non-volatile migrants from a bamboo-based biopolymer. Due to the increasingly use of this biopolymer in food packaging manufacturing, and the necessity of performing a risk assessment in food packaging materials, the results obtained in this work are of great relevance. The analysis allowed detecting the volatile, semi-volatile and non-volatile compounds from migration in a unique analysis of 1.5 minutes duration. This technique offers great advantages for rapid detection and routine analysis

1   **Ambient mass spectrometry as a tool for a rapid and simultaneous determination of**

2   **migrants coming from a bamboo-based biopolymer packaging.**

3

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16

17   **Abstract**

18   New bamboo-based biopolymers are used as food packaging materials, but it must be

19   evaluated to ensure consumers safety. In this study, migration from a commercial bamboo-

20   based biopolymer to ethanol 10% (v/v), acetic acid 3% (w/v̶V̶) and ethanol 95% (v/v) was

21   studied. The migrants were determined from three different perspectives. Volatile and semi-

22   volatile compounds were analyzed by gas chromatography-mass spec-trometry (GC-MS).

23   Twenty-five compounds were detected, alkanes. In addition, a number of phytosterols were

24   detected in ethanol 95%. Non-volatile compounds were identified and quantified by ultra-

performance liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-Q/ToF). Twelve non-volatile compounds were detected in migration solutions, mainly melamine and its derivatives, coming from polymer resins ~~added to~~present in the biopolymer. Melamine ~~concentration~~ migration was higher than 50 mg/Kg in the third sequential migration test. ~~values were above the melamine specific migration limit (SML) established by the Regulations (EU) No 10/2011 and (EU) No 284/2011.~~ Finally, the migration samples were analyzed by DART-SVP (direct analysis in real time coupled to standardized voltage and pressure). This methodology was able to detect simultaneously the main volatile and non-volatile migrants~~,~~ and their adducts in a very rapid and effective way and is shown as ~~as huge~~ promising~~e as a~~ tool to test ~~for~~ the safety and legal compliance of food packaging materials.

Keywords: bamboo biopolymer; DART; melamine; migration; food contact material,

1. Introduction

There is a great demand for biopolymers in the sector of food contact materials manufacturing. This is because biopolymers are a green alternative to the traditional packaging materials used in the food industry, especially those ~~that~~ which are abundant, renewable, biodegradable, and biocompatible (Khlestkin, Peltek, & Kolchanov, 2018). Biopolymers can be classified into two groups, polymers produced from biological products that can be extracted from biomass or produced in fermentation processes, such as carbohydrates, proteins or lipids; and biodegradable and/or compostable polymers (Aznar, Ubeda, Dreolin, & Nerín, 2019; Geueke, 2014; Maisanaba et al., 2018). The addition of additives to the biopolymers is necessary in order to improve their physico-chemical properties. These additives can be for example: polyols, used as non-volatile plasticizers (e.g., glycerol, glycol, and sorbitol); and resins, that mold the material (e.g., urea, melamine, melamine formaldehyde, and amines) (Eckardt, Kubicova, & Simat, 2018; Geueke, 2013).

Surprisingly, melamine and other resins are neither biodegradable or come from natural sources (Chien et al., 2011; Geueke, 2013).

In recent years, food packaging industries have been highly innovative in the production of new biopolymers derived from various natural resources, such as starch, cellulose, bamboo, and chitosan. Among them, bamboo-base biopolymers are of enormous importance. This fibre is attractive as a natural-based raw material, since it is economical, eco-friendly and biodegradable (Jena, 2018; Xie, Zhou, & Yan, 2019). Bamboo plants are easily available due to its very short life cycle and because bamboo timber can be harvested multiple times from a single planting, which makes its use very sustainable. Bamboo-based biopolymers have better mechanical properties than other biomaterials, such as a good impact resistance, high durability and crack resistance, and good flexural properties (Xie et al., 2019). As any food packaging material or food contact article, the biopolymers must be evaluated to confirm their acceptability and safety. The bamboo-base biomaterials can contain other resins and also non intentionally added substances (NIAS), such as impurities from raw materials, degradation compounds or novel substances coming from the reaction between different reagents (Xu, Xiong, Zhao, Xiang, & Wu, 2020). These compounds, as well as those additives intentionally added, are present in the packaging and could migrate to the food.

Migration tests are required in order to guarantee the consumers safety, food preservation and organoleptic properties (Bradley, 2010). These tests require specific conditions of temperature, time and simulants, which should be selected according to the intended use of the material. In the specific migration tests, compounds previously identified in the material as well as new formed compounds can be detected (Nerin, Alfaro, Aznar, & Domeño, 2013). Pure biopolymers are not specifically included in the European plastics regulation and only the frame regulation 1935/2004/CEE applies. However, if they contain some resin or conventional plastic, the regulation on plastics should be applied. European legislation on

74 food contact plastics (Regulation No 10/2011/EU) establishes a positive list with substances

75 that can be used in the manufacturing process and also their specific migration limits (Osorio,

76 Dreolin, Aznar, Nerín, & Hancock, 2019).

77 Since the migrants can be volatile as well as non-volatile compounds, different technologies

78 should be applied in order to have a global view of the safety of the material. Gas

79 chromatography can be applied to the separation of the most volatile and semi-volatile

80 compounds (Biedermann & Grob, 2019) and liquid chromatography to the less volatile ones.

81 Both of them coupled to mass spectrometry detection will allow the identification of the

82 NIAS present in the packaging material. Ambient mass spectrometry techniques can be also

83 used to get a quick confirmation of the presence/absence of the identified compounds.

84 Ambient mass spectrometry (AMS) or ambient ionization techniques (AMI) are ionization

85 sources used in mass spectrometry for direct and rapid analysis of compounds present in solid

86 or liquid samples (Black, Chevallier, & Elliott, 2016; Lu et al., 2018). Additionally, AMS has

87 been utilized as screening method in food safety analysis, criminal investigation,

88 environmental test and other different analytical objectives (Gross, 2014; Lennert & Bridge,

89 2019; Zhang et al., 2018). DESI-MS (Desorption ElectroSpray Ionization Mass

90 Spectrometry) was the first AMS technique developed. Since then, other techniques such as

91 direct analysis in real time (DART), extractive electrospray ionization (EESI), desorption

92 atmospheric pressure chemical ionization (DAPCI), dielectric-barrier-discharge ionization

93 (DBDI) or low-temperature plasma (LTP) have been designed (Barnett & Zhang, 2018).

94 AMS techniques had been previously used in the study of food packaging materials by other

95 groups. For example, Bentayeb et al. determined the compounds present due to print set-off

96 process (Bentayeb et al., 2012; ten Dam et al., 2016) while Aznar et al. analyzed the

97 distribution of ink components in a packaging material and Rothenbacher et al. studied the

98 plasticizers in poly(vinyl chloride) (PVC) (Aznar, Alfaro, Nerín, Jones, & Riches, 2016).

99   Direct analysis in real time (DART) is one of the most popular ambient pressure ionization

100   methods. In this technique, the molecules are ionized by excited helium molecules and

101   adducts, mainly for protonated/deprotonated molecular ions, are commonly formed (Black et

102   al., 2016). Some of its main advantages are: the possibility of direct sampling at ambient

103   conditions; to generate ions from liquid or solid samples; to be simple to operate and to have

104   a rapid analysis speed (acquisition time < 60 s) compared to UPLC and GC-MS methods

105   (Nerin et al., 2013). DART-SVP technique has diverse applications such as food quality and

106   safety control, drug screening, contamination analysis and environmental monitoring (Barnett

107   & Zhang, 2018; Djelal, Cornée, Tartivel, Lavastre, & Abdeltif, 2017; Lennert & Bridge,

108   2018).

109   In the present study, the migration from a bamboo-based biopolymer intended for food

110   contact to different food simulants was evaluated. Volatile, semi-volatile and non-volatile

111   compounds were firstly identified by GC-MS and UPLC-Q/ToF. Additionally, DART-SVP

112   was used as a tool to assess the presence of all potential migrants in a very short analysis

113   time.

114   **2. Materials and methods**

115   2.1. Chemicals and Reagents

116   Melamine 99% [108-78-1] (IUPAC name: 1,3,5-triazine-2,4,6-triamine) was from Sigma–

117   Aldrich (Madrid, Spain). Ethanol absolute (HPLC grade), methanol (UHPLC-MS grade),

118   acetic acid (HPLC grade) were supplied by Scharlau (Setmenat, Spain) for the analysis in

119   GC-MS and UPLC-Q/ToF. Ethanol absolute (HPLC grade) supplied by Merck (Darmstadt,

120   Germany) for the analysis in DART-SVP. Ultra-pure water was obtained from Millipore

121   Milli-Q system (Billerica, MA, USA). The nitrogen evaporator was a TECHNE sample

122 concentrator (Cole-Parmer Ltd., UK). SPME fibers ~~was~~ were purchased ~~at~~to Supelco

123 (Bellefonte, PA, USA).

2.2. Samples

125 Bamboo-based biopolymer samples were purchased at a local supermarket in the form of

126 cups, dishes and jugs. All of these were monolayer materials.

2.3. Migration assays

128 Migration assays were carried out by total immersion of the sample (5 cm x 1 cm) into 20 mL

129 of the simulant. The process was established in accordance with the European legislation on

130 food contact materials (Regulation No 10/2011/EU) (European Commission, 2011a). Three

131 simulants were evaluated: ethanol 10% (v/v) (EtOH 10%) (simulant A), acetic acid 3% (w/v)

132 (AcH 3%) (simulant B) and ethanol 95% (v/v) (EtOH 95%) (simulant D2 substitute). Overall

133 ~~Global~~ migration studies were performed during 2 hours at 70 ºC. The ~~same~~overall simulant

134 solutions were evaporated to dryness at 110 ºC. Overall ~~Global~~ migration was calculated by

135 weighting the dry residue. Specific migration was performed during 6 hours at 70 ºC. Since

136 the food contact materials of this study were intended for repeated use, the migration tests

137 were performed three consecutive times. The assays were carried out in triplicate for each

138 sample and a blank.

2.4. Analysis by GC-MS

140 The analyses of ~~the~~ volatile and semi-volatile compounds were carried out using a gas

141 chromatograph 6890N equipped with an electron ionization ion source operating at 70 eV,

142 and coupled to a quadrupole mass spectrometry detector 5975D (Agilent Technologies. Santa

143 Clara, CA). It was set in SCAN acquisition mode (50 - 450 m/z). The transfer line heater was

144 set at 280 ºC. The autosampler was a Combi PAL (CTC Analytics, Zwingen, Switzerland).

145 The injector was used in two modes~~.:~~ ~~L~~liquid injection <u>for (</u>migration samples <u>in</u>~~:~~ ~~ethanol~~

146 <u>EtOH 95%,)</u> 1 µL of liquid samples was injected at 250 ºC in splitless mode. ~~and~~ SPME

147 injection <u>was used for (</u>aqueous migration samples~~:~~ ~~acetic acid(~~AcH 3% and ~~ethanol~~ <u>EtOH</u>

148 10%). The stationary phase of polydimethylsiloxane/~~—~~divinylbenzene/carboxen

149 (PDMS/DVB/CAR 50/30 µm) was ~~used for the SPME extraction~~<u>selected</u>. The migration

150 samples were conditioned <u>for</u>~~during~~ 2 min at 80 ºC. Subsequently, the volatile and semi-

151 volatile compounds <u>-</u>were extracted <u>by fiber inmersion</u> at 80 ºC and 500 rpm agitation during

152 15 min <u>and finally</u>. SPME fiber was desorbed at 250 ºC for 2 min and ~~1 µL of liquid samples~~

153 ~~was injected at 250 ºC in splitless mode~~. The chromatographic separation was performed on a

154 HP-5MS column ~~of(~~-30 m <u>length ×</u>~~x~~ 0.25 mm ~~i.d.~~<u>inner diameter ×</u>~~x~~ 0.25 µm film thickness<u>)</u>.

155 The oven temperature program was: 40 ºC held for 3 min, ramp 10 ºC <u>/</u>min^{-1} to 300 ºC and

156 held for 2 min. Helium was used as carrier gas at a constant flow of 1 mL <u>/</u>min^{-1}. The SPME

157 extraction process in biopolymer samples ~~had been~~<u>was</u> optimized in a previous work (Osorio,

158 Aznar, & Nerín, 2019).

2.5. Analysis by UPLC-Q/ToF

160 The analyses of the non-volatile compounds present in migration solutions were performed

161 using an ACQUITY UPLC chromatograph (Milford, MA, USA). Flow rate was 0.3 mL <u>/</u>min^{-}

162 ^{1}. Injection volume was 10 µL. An UPLC BEH C18 column (Waters) of 2.1 × 100 mm, 1.7

163 µm particle size was used at a temperature of 35 °C. Two mobile phases were used for the

164 separation: (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid.

165 Chromatography was performed using the following gradient elution: initial composition

166 98/2 (phase A/ phase B), and at eight min it was changed to 0/100 (phase A/ phase B) with

167 two additional minutes at the final composition.

168 UPLC system was coupled to a Xevo G2 QTOF mass spectrometer from Waters (Milford,

169 MA, USA) with an ESI probe. The conditions of analysis were: positive ionization (ESI+),

170 sensitivity mode, capillary voltage 3.0 kV, sampling cone voltage 30V, extraction cone 3 V,

171 source temperature 150 °C, desolvation temperature 450 °C, cone gas flow rate of 20 L h^{-1},

172 and desolvation gas flow rate of 500 L h^{-1}. Acquisition was carried out in MSE mode in a

173 mass range between 50 and 1200 m/z. The chromatogram was acquired at low and high

174 collision energy (CE) in the collision cell.

175 2.6. Analysis by DART-SVP

176 A direct analysis in real time (DART) couple to Standardised Voltage and Pressure (SVP)

177 100 ion source (IonSense, Saugus, MA) was coupled to an Acquity QDa single quadrupole

178 mass spectrometer (Waters, Wilmslow, UK) via a Vapur interface (IonSense, Saugus, MA).

179 The DART-SVP and Acquity QDa were operated in positive ion mode. The DART-SVP was

180 operated with helium (grade A) in running mode and nitrogen in standby mode. The DART-

181 SVP was operated at several different temperatures between 150 ºC and 450 ºC, the ion-

182 source grid voltage was set to 350 V. The mass spectrometer was operated with the

183 desolvation line temperature set at 250 ºC, heat block 350 ºC, interface voltage 4.5 kV and

184 50-1000 Da scan range. The mass spectrometer was controlled using MassLynx v4.1

185 SCN888 (Waters, Wilmslow, UK). Data were analyzed by MassLynx v4.1.

186 Manual sample injection mode was used. Three microliters of the migration solution were

187 pipette-spotted directly onto the Quick Strip card in the position one. Then, Quick Strip card

188 was the simply mounted on the sampling rail. PosteriorlyFurther, the sample came in contact

189 with the He stream from the DART ion source outlet. Even though the three simulants were

190 testeds, only data from EtOHh 95% are included in the manuscript. In the other two simulants

191 its high water content generatedprovided low quality mass spectra. In DART-SVP,

8

192   atmospheric moisture is ionized by helium in the 2^3S state with extremmely high efficiency.

193   However, the formation of protonated water clusters is likely to occur from the water present

194   in the samples, while metastable helium atoms are unlikely to survive. For this reason, it has

195   found that moisture should be minimized to avoid competing reactions (Cody, 2009).

196   2.7. Data processing

197   In GC-MS analysis, the chromatograms and mass spectra were acquired and processed with

198   MSD ChemStation software version F.01.03 from Agilent Technologies. NIST Standard

199   Reference Database (2018) was used as mass spectra library for compounds identification.

200   Only Match values above 800 were considered acceptable for a candidate confirmation.

201   In UPLC-Q/ToF, the mass data were analysed with MassLynx software v4.1 from Waters

202   (Milford, MA, USA). Data were acquired in MS^E mode, where 2 functions (low and high

203   energy) were acquired simultaneously. Mass spectra obtained in function 1 provided

204   information about the elemental composition of the precursor ion and the mass spectra in

205   function 2 provided information about the elemental composition of the fragment ions. In

206   UPLC - Q/ToF identification is a more complex process, since there are not spectral libraries.

207   In this case, a structural elucidation of the molecules is necessary. For this purpose, the first

208   step was to determine the elemental composition of the precursor ion, those formulas with the

209   lowest mass error (alwayas below 10 ppm) and the highest isotopic fit were selected. These

210   formulas were introduced in a chemical databases, such as [www. chemspider.com] or

211   [www.scifinder.com] and the possible chemical structures were searched. The criteria for

212   selecting a candidate were based on previous knowledge about the sample together with a

213   chemical background. Finally, the MassFragment® software was used to evaluate if the

214   fragment ions detected in function 2 could fit with the breakage of the proposed candidate.

215   Those candidates with the best match were selected as final candidates (Nerin, Alfaro, Aznar,

216 & Domeño, 2013). In the case of the analysis by DART-SVP, the aim was to determine

217 possible adducts of compounds previously identified by GC-MS or UPLC-MS. The DART-

218 SVP mass spectra were acquired with MassLynx SCN888T software and processed with

219 MassLynx software v4.1

3. Results and discussion

221 The results showed that overall global migration was much higher when compared tothan in

222 other polymers, close to the but below Overall Migration Limit (OML) of 60 mg kg^{-1}

223 established by Regulation EU/10/2011 (European Commission, 2011a) in someall simulants.

224 The values found for the different simulants were as follows: 50.6 ± 9.2 mg kg^{-1} in ethanol

225 EtOH 10%; 13.2 ± 3.2 mg kg^{-1} in acetic acidAcH 3% and 48.5 ± 4.4 mg kg^{-1} in ethanol EtOH

226 95%. Specific migration results are shown below for each technique.

227 3.1. Volatile and semi-volatile compounds present in migration from a bamboo-based

228   biopolymer.

229 Table 1 shows the compounds identified with their retention times, their molecular formula

230 and its their presence in the different simulants after thein migration samplestests. A total of

231 twenty-five compounds were identified by comparing their mass spectra with the NIST

232 Standard Reference Database (2018). The identified cCompounds identified were checked in

233 the EU/10/2011 positive list (European Commission, 2011a), to confirm if they were

234 authorized and their specific migration limit. All theMany compounds detected were non-

235 listed substances and among them, only a 2,6,10-trimethyl dodecane had a NOAEL value

236 (1000 mg kg^{-1}day^{-1}) (Agency United States Environmental Protection, 2019). Therefore, their

237 theoretical toxicity level was established according to the TTC (threshold of toxicological

238 concern) and Cramer rules for each compound (Dewhurst & Renwick, 2013; Szczepańska et

239 al., 2018). Table 1 shows the theoretical classification of toxicity for the ~~rest of~~ non listed

240 compounds found.

241 ~~Acetic acid~~AcH 3% was the food simulant with the highest number of migrants (15),

242 compared to ~~ethanol~~ EtOH 10% (6) and ~~ethanol~~ EtOH 95% (6) (Supplementary material 1).

243 Most of the identified compounds were alkanes (Jiong, Fusheng, Chunhong, Mao, &

244 Jianquan, 2014). These compounds are commonly used as raw materials in the production of

245 polymers but they might also come from the degradation of the material. ~~Degradation~~

246 Oxidation can ~~happen~~ take place because of the interactions between the polymeric material

247 and the oxygen during the manufacture of the material~~present in the air, causing its oxidation~~

248 (García Ibarra, Rodríguez Bernaldo de Quirós, Paseiro Losada, & Sendón, 2019). According

249 to Cramer rules, the identified alkanes belong to class I, that means to low toxicity, while

250 class II and class III are of medium and high toxicity respectively. Four compounds were

251 classified as class III: methyl N-hydroxybenzenecarboximidate and three phytosterols (3β-

252 ergost-5-en-3-ol, clionasterol and arundoin). Four phytosterols, stigmasterol, were also

253 detected and classified as class II. Phytosterols, 3β-ergost-5-en-3-ol, stigmasterol, clionasterol

254 and arundoin, were identified only in ~~ethanol~~ EtOH 95% food simulant. The presence of

255 phytosterols in young bamboo shoots had been previously demonstrated (Nongdam &

256 Tikendra, 2014). Therefore, these compounds could come from the bamboo used as base

257 material (Moreau et al., 2018). According to the toxicological assays performed by Le Goff et

258 al. and Mea et al., phytosterols do not have genotoxic potential (Le Goff et al., 2019; Lea,

259 Hepburn, Wolfreys, & Baldrick, 2004). In contrast, phytosterols of bamboo shoot reduces fat

260 and cholesterol levels of blood, and they can inhibit the production of carcinogens and

261 growth of cancer cells (Nongdam & Tikendra, 2014).

262 3.2. Non-volatile compounds present in migration from a bamboo-based biopolymer

263   Table 2 shows the non-volatile compounds found in migration solutions from the bamboo-

264   based biopolymer, their retention time, their accurate mass, the adduct detected ([M+H]$^+$ or

265   [M+Na]$^+$) and their molecular formula. A total of twelve compounds were detected

266   (Supplementary data 1).

267   The amino acid valine was detected in all food simulants. The presence of valine in bamboo

268   shoots ~~has been~~was previously reported by different authors (De Silva et al., 2019; Nongdam

269   & Tikendra, 2014). C. Nirmala et al, reported it as one of the most abundant amino acids in

270   the composition of Phyllostachys manii (juvenile bamboo shoots) (Nirmala, Bisht, Bajwa, &

271   Santosh, 2018). Triethanolamine, commonly used ~~commonly~~ as surfactant, was also detected.

272   None of these compounds was present in the EU/10/2011 positive list (European

273   Commission, 2011a). Triethanolamine ha~~s~~d a NOAEL value 300 mg kg^{-1}day^{-1} and that of

274   valine~~, with NOAEL value~~is 628 mg kg^{-1}day^{-1} (Agency United States Environmental

275   Protection, 2019).

276   It was also observed that melamine and eight melamine derivatives were identified in all food

277   simulants. This is because in the manufacture of biopolymers it is common to use a polymeric

278   resin, such as melamine, to improve the mechanical properties of the material.

279   Melamine has been reported as a common component in the manufacture of laminates,

280   coatings and mainly, kitchenware utensils (Geueke, 2013; Li, Song, & Wen, 2019).

281   Melamine derivatives can also be formed from the reaction of residual melamine and food

282   simulants (Bradley, Castle, Day, & Leak, 2011; Chien et al., 2011; Karthikraj, Bollapragada,

283   & Kannan, 2018) or during the bamboo based biopolymer manufacture.

284   Melamine is listed in the Regulation EU/10/2011 with a specific migration limit (SML) of 2.5

285   mg kg^{-1}. –For this reason, melamine as well as its derivatives were quantified in all food

286   simulants. Since the material evaluated was intended for repeated used, according to

287   EU/10/2011 operating guidelines, the migration was evaluated over three consecutive

288   migration experiments. The results are presented in figure 1. Melamine was the compound

289   with the highest migration values. In all cases, the migration values were above 2.5 mg kg^{-1}

290   in the first, ~~or~~ second and third migration experiments, specially in AcH 3%. ~~and T~~therefore,

291   this bamboo-based biopolymer did not comply with the SMLs established by the Regulations

292   (EU) No 10/2011 and No284/2011~~–~~. According to these results, the material cannot be

293   considered as bamboo, but melamine with bamboo filler. Then, the R~~r~~egulation 284/2011

294   concerning melamine kitchenware tools applies (European Commission, 2011b). Regarding

295   the food simulants, the highest values were found in the second migration of ~~acetic aci~~AcH~~d~~

296   3% ~~(w/V)~~ (144.3 ± 1.1 mg kg^{-1}), followed by ~~ethanol~~ EtOH10% ~~(v/v)~~ (48.8 ± 3.4 mg kg^{-1})

297   and ~~ethanol~~ EtOH 95% ~~(v/v)~~ (4.3 ± 1.0 mg kg^{-1}), suggesting an important effect of acidity

298   and a high water content in the migration process. The same pattern was observed for all the

299   melamine derivatives analyzed. N-Hydroxymethylmelamine (MD 1) was the melamine

300   derivative with the highest migration values.

301   The acidic medium of the acetic acid simulant could accelerate the hydrolysis reaction of the

302   melamine resin and as a result, provide high migration values (Bradley et al., 2011; Geueke,

303   2013). The results also showed that the migration of melamine and its derivatives increased

304   in the second migration experiment and decreased in the third one. This behavior could be

305   attributed to the possible variation in the internal structure of the polymer caused by the

306   simulants, which could have modified the diffusion of the compounds though the polymer

307   and thus affecting the migration. Therefore, the addition of the polymeric resin to the bamboo

308   biopolymer improved its mechanical properties but at the same time, entailed a risk to

309   consumer's health. In addition, melamine is neither a biopolymer nor biodegradable, what

310   means that these materials, promoted as "bamboo food contact materials" can be considered

311   as a fraud to consumers.

312   3.3. DART-SVP analysis of migration samples

313   DART-SVP is an electric discharge ambient ionisation technique where excited helium atoms

314   start gas-phase reactions. These reactions result in ions created from atmospheric water-vapor

315   or other gaseous compounds present (Gross, 2014; Navare, Mayoral, Nouzova, Noriega, &

316   Fernández, 2010). As the chemical ionization is the same as in APCI (atmospheric pressure

317   chemical ionization) (Gross, 2014), the formation of adducts according to polarity, molecular

318   weight or volatility of the species present in the samples is very common.

319   In this study, the ability to implement a DART-SVP method to quickly determine the main

320   migrants and their adducts from bamboo-base biopolymer was investigated. Figure 2 shows a

321   mass spectrum of an ~~ethanol~~ EtOH 95% migration sample. Table 3 shows a total of twenty-

322   six masses detected in the DART mass spectrum and that matched with adducts of

323   compounds that ~~had been~~ were previously detected in migration solutions by GC-MS or

324   UPLC-MS/QToF. The range of m/z values ~~ranged~~ was between 127.0733 and 291.1033.

325   Only peaks with relative intensity higher than 1% of the base peak were considered. The table

326   shows the main adduct ion detected as well as its m/z and relative intensity. In addition, other

327   adducts identified and its relative intensity for each of the compounds are shown. The

328   presence of other adducts confirmed the candidates proposed.

329   Regarding the ions detected, adducts such as $M^{\cdot+}$, $[M+H]^+$, $[M+N]^+$, $[M-H+O]^+$ and $[M+H-$

330    $H_2O]^+$ were observed with major relative intensity. Oxygen, water and ammonia species were

331   detected in the environment (blank spectrum mass), therefore the different adducts identified

332   were the interaction products between gaseous species of the atmosphere and molecular ions

333   from the sample. Other adducts detected were $M^{\cdot+}$, $[M+H]^+$, $[M-H+O]^+$, $[M+H+O]^+$,

334    $[M+NH_4]^+$ and $[M-H+O_2]^+$.

335   In this technique the analytes generate different adducts according to the polarity of specie:

336    $[M+H]^+$, $[M-H+O]^+$, $[M+NH_4]^+$ adducts for medium polar to polar analytes; and $M^{\cdot+}$, $[M-$

337 H+H₂O]⁺, [M+H]⁺, [M-H+O]⁺ adducts for non-polar analytes (Cody, 2009; Gross, 2014).

338 The results suggested that the polarity was an important parameter in the formation of

339 adducts from the migration samples of bamboo-based biopolymer. Melamine and it

340 derivatives formed mainly adducts such as [M+H]⁺, [M-H+O]⁺ and [M-H+O₂]⁺ because these

341 compounds have a high polarity. Additionally, Tthese adducts had high relative intensity

342 (between 10% - 100%) probably because they were at higher concentration than other

343 compounds in the samples (section 3.2). On the other hand, some compounds with low

344 polarity, such as alkanes, formed [M+N]⁺ and [M-H+O]⁺ adducts. However, they were in the

345 adduct group with low relative intensities, between 1-10%. Probably, the high volatility of

346 small molecules promotes a higher number of interactions with the different reactive species

347 and therefore a higher variety of adducts (Bentayeb et al., 2012; Kerpel dos Santos et al.,

348 2018). Among the masses found in DART MS spectrum with relative intensities above 10%,

349 10 out of them could not be associated to any of the previously identified compounds by GC-

350 MS or UPLC-MS/QToF.

351 For 5 of these masses, new candidate compounds were proposed (table 4). The candidates

352 were selected according to its elemental composition, adducts detected, and to bibliography

353 of bamboo composition (De Silva et al., 2019; Nirmala et al., 2018; Nongdam & Tikendra,

354 2014). Other rResearchers have found in the composition of young bamboo shoots different

355 amino acids, minerals, polyphenols, and/or vitamins, such as serine, coniferyl alcohol, caffeic

356 acid, threonine, paracoumaryl alcohol, histidine and protocatechuic acid, among others (De

357 Silva et al., 2019; Nirmala et al., 2018; Nongdam & Tikendra, 2014; Zhu & Kannan, 2019).

358 Some of the expected adducts for these compounds coincide with the masses detected.

359 However, for 5 of the masses detected in the mass spectrum with intensity values above 10%,

360 it was not possible to propose a candidate: 93.05 (26.8 %), 145.09 (15.0 %), 130.22 (15.0 %),

361 295.28 (14.5%) and 192.88 (11.5%).

362

4. Conclusions

Even though some of the compounds found in migration came from bamboo, such as phytosterols, most of the migrants came from the melamine added to it in order to improve the biopolymer properties. Not only melamine but several melamine derivatives were found in migration above the limits established in European legislation. Consequently, this material does not comply with the EU legislation. In fact, the material cannot be identified as bamboo, but as melamine with bamboo filler. As melamine is neither a biopolymer not biodegradable material, the promotion of these kitchenware materials as bamboo can be considered as a fraud to consumers.

Regarding to DART-SVP experiments, the analysis allowed detecting the volatile, semi-volatile and non-volatile compounds from migration samples of the bamboo-based biopolymer in a unique analysis of 1.5 minutes duration for each replicate. This technique offers great advantages for rapid detection and routine analysis, without requiring sample preparation.

All the compounds identified using DART-SVP were detected in the initial part of this study, where GC-MS and UPLC/QToF methodologies were used. Melamine and its derivatives form the adducts with greater relative intensity. These results demonstrate that the DART-SVP technique is a very useful tool for direct target analysis, where in a few minutes can provide data about the main migrants present in samples.

Acknowledgements

385 GUIA T53_17R. ~~J. Osorio thanks the financial help from~~ ERASMUS + program for the

386 ~~financing of the doctoral~~ research stay.

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545

546 **Figure captions**

547 **Fig 1.** Bar chart of migration values of melamine and its derivative compounds from

548 bamboo-based biopolymer in three consecu-tive migration assays. a) ~~acetic acid 3% (w/v),~~

549 ~~AcH 3%,~~ b) ~~ethanol 10% (v/v)~~ ~~EtOH 10%~~ and c) ~~ethanol 95% (v/v)~~~~EtOH 95~~. MD codes are

550 linked to table 2.

551 **Fig.2**. DART-SVP mass spectrum of a migration solution (ethanol 95%) from a bamboo-

552 based biopolymer sample. Numbers in brackets are linked to table 3.

Table 1

Table 1. Volatile and semi-volatile compounds detected by GC-MS in migration samples from Bamboo-based biopolymer.

#	t_R	MW	Candidate Name	# CAS	MF	Cramer Class	Food simulant AcH 3%	EtOH 10%	EtOH 95%
1	4.40	164.0473	Phenacyl formate	55153-12-3	$C_9H_8O_3$	I			X
2	7.43	132.0786	3,3-Dimethoxy-2-butanone	21983-72-2	$C_6H_{12}O_3$	I		X	
3	7.65	151.0633	Methyl N-hydroxybenzenecarboximidate	67160-14	$C_8H_9NO_2$	III	X		X
4	10.95	142.1722	Decane	124-18-5	$C_{10}H_{22}$	I		X	
5	10.98	142.1722	3,5-dimethyloctane	15869-93-9	$C_{10}H_{22}$	I	X		
6	12.17	170.2035	2,3-dimethyldecane	17312-44-6	$C_{12}H_{26}$	I		X	
7	12.73	128.1535	2,4-dimethylheptane	2213-23-2	C_9H_{20}	I		X	
8	13.66	170.2035	2-methyl undecane	7045-71-8	$C_{12}H_{26}$	I	X		
9	13.78	212.2504	2,6,10-trimethyl dodecane	3891-98-3	$C_{15}H_{32}$	I	X		
10	13.86	198.2348	4,6-dimethyl dodecane	61141-72-8	$C_{14}H_{30}$	I	X		
11	13.98	226.2661	Hexadecane	544-76-3	$C_{16}H_{34}$	I	X		
12	14.52	170.2035	3,6-dimethyl decane	17312-53-7	$C_{12}H_{26}$	I	X		
13	14.57	184.2191	2,4-dimethyl undecane	17312-80-0	$C_{13}H_{28}$	I	X		
14	14.66	184.2191	4,6-dimethyl undecane	17312-82-2	$C_{13}H_{28}$	I	X		
15	15.27	198.2348	Tetradecane	629-59-4	$C_{14}H_{30}$	I	X		
16	16.47	340.3705	Eicosane, 1-propoxy	281211-96-9	$C_{23}H_{48}O$	I	X	X	
17	16.93	156.1878	2,4,6-trimethyl octane	62016-37-9	$C_{11}H_{24}$	I	X		
18	17.07	212.2504	2,6,11-trimethyl dodecane	31295-56-4	$C_{15}H_{32}$	I	X		
19	17.18	268.313	Nonadecane	629-92-5	$C_{19}H_{40}$	I	X		
20	19.02	380.4382	Heptacosane	593-49-7	$C_{27}H_{56}$	I	X		
21	22.92	256.2402	(S)-12-methylmethy tetradecanoic ester	62691-05-8	$C_{16}H_{32}O_2$	I		X	
22	26.03	400.3705	3β-Ergost-5-en-3-ol	4651-51-8	$C_{28}H_{48}O$	III			X
23	27.27	412.3705	Stigmasterol	83-48-7	$C_{29}H_{48}O$	II			X
24	28.87	414.3862	Clionasterol	83-47-6	$C_{29}H_{50}O$	III			X
25	30.21	440.4018	Arundoin	4555-56-0	$C_{31}H_{52}O$	III			X

t_R: retention time (min). MW: molecular weight. MF: molecular formula. AcH 3%: acetic acid 3% (w/v). ~~si simulant.~~ EtOH 10%: ethanol 10% (v/v). ~~simulant.~~ EtOH 95%: ethanol 95% (v/v) ~~simulant~~. X: compound detected in the simulant.

Table 2

Table 2. List of compounds non-volatile detected by UPLC-Q/ToF in migration from bamboo-based samples (Repeated use).

#	t_R	Mass (m/z)	Adduct	MF	Candidate Name_# CAS	Food simulants		
						AcH 3%	EtOH 10%	EtOH 95%
1	0.65	214.9170	[M+Na]$^+$	$C_2N_4O_3S_2$	Unknown	X	X	
2	0.73	150.1137	[M+H]$^+$ [M+Na]$^+$	$C_6H_{15}NO_3$	Triethanolamine_102-71-6	X	X	
3	0.79	118.0865	[M+H]$^+$	$C_5H_{11}NO_2$	Valine_72-18-4	X	X	X
4	0.80	127.0732	[M+H]$^+$	$C_3H_6N_6$	Melamine_108-78-1	X	X	X
5	0.89	157.0840	[M+H]$^+$	$C_4H_8N_6O$	N-Hydroxymethylmelamine_937-35-9_(**MD 1**)	X		X
6	0.90	185.1156	[M+H]$^+$	$C_6H_{12}N_6O$	N-Hydroxypropylmelamine_91313-29-0 (**MD 2**)			X
7	0.94	157.0835	[M+H]$^+$	$C_4H_8N_6O$	N-Hydroxymethylmelamine_937-35-9 (**MD 3**)	X	X	
8	1.04	139.0726	[M+H]$^+$	$C_4H_6N_6$	Methylene melamine_85946-83-4 (**MD 4**)	X		
9	1.13	169.0826	[M+H]$^+$	$C_5H_8N_6O$	N-(4,6-Diamino-1,3,5-triazin-2-yl)acetamide_16274-60-5 (**MD 5**)	X	X	
10	2.65	277.1375	[M+H]$^+$	$C_8H_{12}N_{12}$	2,4,6-Pyrimidinetriamine, 5,5'-azobis_ 63436-10-2 (**MD 6**)	X	X	
11	2.83	307.1478	[M+H]$^+$	$C_9H_{15}N_{12}O$	Propanamide, N-(4,6-diamino-1,3,5-triazin-2-yl)-2-[(4,6-diamino-1,3,5-triazin-2-yl)amino]_1421766-78-0_(**MD 7**)	X		
12	4.54	311.0979	[M+H]$^+$	$C_{14}H_{26}N_6O_2$	Glycine, N-[4-[(1,1-dimethylethyl)amino]-6-(ethylamino)-1,3,5-triazin-2-yl]-N-propyl _2037785-60-5 (**MD 8**)	X	X	X

t_R: retention time (min). AcH 3%: acetic acid 3% (w/v). ~~simulant.~~ EtOH 10%: ethanol 10% (v/v). ~~simulant.~~ EtOH 95%: ethanol 95% (v/v) ~~simulant.~~ X: compound detected in the simulant. MD: melamine derivate.

Table 3

Table 3. List of compounds detected in specific migration to ethanol 95% (v/v) from Bamboo-based samples by DART-SVP.

#	MW	Candidate Name	MF	Main adduct			Other adducts (Int)
				m/z	Ion	Int	
1	127.0732	Melamine[b]	$C_3H_6N_6$	127.07	$[M+H]^+$	87.7	$[M-H+O_2]^+$ (3.9)
2	132.0786	3,3-Dimethoxy-2-butanone[a]	$C_6H_{12}O_3$	146.07	$[M+N]^+$	6.0	$[M-H+O]^+$ (6.0)
3	139.0726	Methylene melamine[b]	$C_4H_6N_6$	139.06	$[M+H]^+$	29.9	$[M-H+O_2]^+$ (7.8); $[M-H+O]^+$ (4.0); $M^{.+}$ (3.2); $[M+NO]^+$ (2.7); $[M+N]^+$ (2.6)
4	150.1137	Triethanolamine[b]	$C_6H_{15}NO_3$	150.09	$[M+H]^+$	100.0	$[M-H+O]^+$ (8.1)
5	151.0633	Methyl N-hydroxybenzenecarboximidate[a]	$C_8H_9NO_2$	169.04	$[M+NH_4]^+$	7.8	$[M+H+O]^+$ (2.7)
6	156.1878	2,4,6-trimethyl octane[a]	$C_{11}H_{24}$	152.02	$[M+H]^+$	2.6	
7	157.0835	N-Hydroxymethylmelamine[b]	$C_4H_8N_6O$	139.06	$[M+H-H_2O]^+$	29.9	$[M+H]^+$ (3.9); $[M+H+O]^+$ (1.3)
8	164.0473	Phenacyl formate [a]	$C_9H_8O_3$	147.08	$[M+H -H_2O]^+$	2.7	
9	169.0826	N-(4,6-Diamino-1,3,5-triazin-2-yl)acetamide	$C_5H_8N_6O$	183.03	$[M-H+O]^+$	19.3	$[M+H]^+$ (7.8); $M^{.+}$ (2.7); $[M+NH_4]^+$ (2.5)
10	170.2035	2,3-dimethyldecane[a]	$C_{12}H_{26}$	185.16	$[M-H+O]^+$	13.3	$[M+H]^+$ (7.5); $[M+NH_4]^+$ (2.1)
11	170.2035	2-methyl undecane[a]	$C_{12}H_{26}$	185.16	$[M-H+O]^+$	13.3	$[M+H]^+$ (7.5); $[M+NH_4]^+$ (2.1)
12	170.2035	3,6-dimethyl decane[a]	$C_{12}H_{26}$	185.16	$[M-H+O]^+$	13.3	$[M+H]^+$ (7.5); $[M+NH_4]^+$ (2.1)
13	184.2191	2,4-dimethyl undecane[a]	$C_{13}H_{28}$	198.25	$[M+N]^+$	4.8	$[M+NH_4]^+$ (2.5); $[M+H+O]^+$ (1.6)
14	184.2191	4,6-dimethyl undecane[a]	$C_{13}H_{28}$	198.25	$[M+N]^+$	4.8	$[M+NH_4]^+$ (2.5); $[M+H+O]^+$ (1.6)
15	185.1156	N-Hydroxypropylmelamine[b]	$C_6H_{12}N_6O$	185.16	$[M+H]^+$	13.3	$[M+NO]^+$ (2.3)
16	198.2348	4,6-dimethyl dodecane[a]	$C_{14}H_{30}$	198.25	$M^{.+}$	4.8	$[M+H -H_2O]^+$ (2.3); $[M-H+O]^+$ (2.0)
17	198.2348	Tetradecane[a]	$C_{14}H_{30}$	198.25	$M^{.+}$	4.8	$[M+H -H_2O]^+$ (2.3); $[M-H+O]^+$ (2.0)
18	212.2504	2,6,10-trimethyl dodecane[a]	$C_{15}H_{32}$	213.23	$[M+H]^+$	2.0	
19	212.2504	2,6,11-trimethyl dodecane[a]	$C_{15}H_{32}$	213.23	$[M+H]^+$	2.0	
20	214.9171	Unknown[b]	$C_2N_4O_3S_2$	229.95	$[M-H+O]^+$	2.0	$M^{.+}$ (1.8)
21	226.2661	Hexadecane[a]	$C_{16}H_{34}$	240.26	$[M+N]^+$	3.1	
22	249.1123	Glycine, N-[4-[(1,1-dimethylethyl)amino]-6-(ethylamino)-1,3,5-triazin-2-yl]-N-propyl [b]	$C_{10}H_{12}N_6O_2$	265.19	$[M-H+O]^+$	17.1	$[M+NO]^+$ (3.1); $M^{.+}$ (2.6) $[M+NO]^+$ (2.4)
23	256.2400	(S)-12-methylmethy tetradecanoic ester[a]	$C_{16}H_{32}O_2$	239.26	$[M+H -H_2O]^+$	6.1	$[M-H+O]^+$ (3.6); $[M+H+O]^+$ (3.5); $[M-H+O_2]^+$ (3.3); $[M+N]^+$ (2.6)
24	268.3130	Nonadecane[a]	$C_{19}H_{40}$	269.30	$[M+H]^+$	3.9	$[M+H+O]^+$ (3.8)
25	277.1395	2,4,6-Pyrimidinetriamine, 5,5'-azobis [b]	$C_8H_{12}N_{12}$	291.10	$[M-H+O]^+$	2.5	$M^{.+}$ (2.0)
26	307.1478	Propanamide, N-(4,6-diamino-1,3,5-triazin-2-yl)-2-[(4,6-diamino-1,3,5-triazin-2-yl)amino] [b]	$C_9H_{15}N_{12}O$	289.14	$[M+H-H_2O]^+$	2.1	

MW: molecular weight. MF: Molecular formula. Int: relative intensity.
[a] Compound identified by GC-MS. Average atomic mass ("*m/z* M+·") was calculated using the MassLynx software. [b] Compound identified by UPLC-Q/ToF.

Table 4

Table 4. Adducts detected only by DART-SVP in specific migration samples in ethanol 95% (v/v) from Bamboo-based samples.

m/z $M^{+\cdot}$	Candidate Name	MF	Principal adduct			Other adducts
			m/z	Ion	Int	
150.0681	Paracoumaryl alcohol	$C_9H_{10}O_2$	151.14	$[M+H]^+$	15.4	$[M+H+O]^+$ (5.3)
154.0266	Protocatechuic acid	$C_7H_6O_4$	137.16	$[M+H -H_2O]^+$	12.0	$[M+N]^+$ (2.7); $[M+NO]^+$ (4.8)
155.1546	L-Histidine	$C_6H_9N_3O_2$	155.14	$M^{+\cdot}$	9.9	$[M-H+O_2]^+$ (2.5)
180.0423	Caffeic acid	$C_9H_8O_4$	197.16	$[M+H+O]^+$	21.0	
180.0786	Trans-coniferyl alcohol	$C_{10}H_{12}O_3$	197.16	$[M+H+O]^+$	21.0	

Figure 1

Figure 1

Figure 2

Figure 2

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