

1 **Extraction and quantification of pesticides and metals in palm wines by HS-SPME/GC-**
2 **MS and ICP-AES/MS**

3

4 ¹Juliette Vievard, ^{2*}Tiemele Laurent Simon Amoikon, ²Aminata Nadjoua Coulibaly, ¹Christine
5 Devouge-Boyer, ¹Monica Arellano Gisel Sanchez, ²Aké Francine, ²Theodore N'Dede Djeni,
6 ^{1*}Mélanie Mignot

7 * Correspondence: melanie.mignot@insa-rouen.fr

8 tls.amoikon@gmail.com

9 ¹Normandie University, UNIROUEN, COBRA UMR CNRS 6014, INSA, Avenue de
10 l'Université, 76800 Saint-Etienne-du-Rouvray, France

11 ²Unité de Formation et de Recherche en Sciences et Technologie des Aliments (UFR-STA),
12 Université Nangui Abrogoua, 02 BP 801, Abidjan, 02, Republic of Côte d'Ivoire

13

14 **ABSTRACT**

15 In this study, a HS-SPME/GC-MS and ICP-AES/MS methods are presented to extract and
16 quantify pesticides and metals in palm wines. Various parameters affecting the extraction were
17 investigated: SPME fiber, equilibrium and extraction time, extraction temperature, salinity, and
18 stirring, through an experimental design with 45 trials. The developed method allowed to
19 identify 35 pesticides and quantify 29 of them, from different families of pesticides in 32 palm
20 wine samples. Method performance was evaluated in terms of linearity, repeatability, LOD,
21 LOQ, and accuracy. Among the 32 samples analyzed in 3 replicates, 7 pesticides were detected
22 in 10 samples. Dichlorvos was the only pesticide detected at levels above the European maximal
23 limits. Additionally, 10 of the 19 metals explored by ICP-AES and ICP-MS were found in all

24 samples. Six metals were detected in different samples at levels above the European or OIV
25 maximal limits for drinking water or wine.

26

27 **Keywords:** palm wine, pesticides, metals, headspace solid-phase microextraction, gas
28 chromatography, mass spectrometry, induced coupled plasma mass spectrometry

29

30 **1. Introduction**

31 Palms are believed to be among the oldest flowering plant in the world (Redhead, 1989). The oil
32 palm (*Elaeis guineensis* Jacq.) is one of the major cultivated crops among the economically
33 important palm species. Their growth is so vigorous that they are considered as a “weed” in
34 neglected or abandoned farms. Different parts of the palm tree can be used for various purposes,
35 but one important commercial activity associated with the tree is production of wine from the
36 sap by the local populations (Yaméogo et al. 2008). Yet the oil palm in West Africa is highly
37 valued for palm wine production, and could allow farmers to supplement and diversify their
38 incomes. As an example, in western Ivory Coast, the resumption of wild oil palm in old cocoa
39 farms played an important role in the 1990s, when farmers faced collapsing cocoa prices and
40 inheritance conflicts, which resulted in the abandonment of many cocoa plantations where the
41 density of oil palms trees grew rapidly. Thus, the felling of palm trees made it possible to
42 produce palm wine for sale and for own consumption, thus generating income, reducing risks
43 and creating jobs and new intergenerational and institutional arrangements (Ruf, 2014).

44 Palm wine is the collective name for a group of alcoholic beverages produced by the natural
45 fermentation of the sap obtained from various tropical plants of the Palmae family (Okafor,
46 1978), such as *Elaeis guineensis*, *Raphia hookeri*, *Phoenix dactylifera*, *B. aethiopum* and *Cocos*
47 *nucifera* (Mollet et al. 2000; Sambou et al. 2002; Stringini et al. 2009; Ziadi et al. 2011). It is
48 mainly consumed by more than 10 million people in West Africa (Battcock et al., 1998) but it

49 is also available in Asia, India and Mexico (Chandrasekhar et al., 2012). A study by Fatoye and
50 Morakinyo (2002) on alcoholic drinks (beer, locally fermented wine and locally distilled gins)
51 in West Africa showed it as the most commonly used alcoholic beverages. It serves a variety
52 of purposes, notably in traditional namings and marriage ceremonies, medicine (cure of low
53 sperm count and poor sight), traditional incantation and also acts as catalyst for extraction of
54 juice from herbs and roots for medical application.

55 Generally, the palm sap for palm wine production is obtained through a process known as
56 tapping, operated in open-air, and involving a series of operations on live standing or felled or
57 cut down tree, such as perforation of the trunk, insertion of a tube in the hole and collection of
58 the sap in a container which can be a gourd, clay pot, plastic container, glass bottle or calabash
59 as previously reported (Ouoba et al., 2012). The tapping process between 2 and 8 weeks and
60 palm wine is collected twice a day (early in the morning and in the evening) and either
61 immediately consumed on fields or stored for later sale.

62 Despite the enormous economic, medicinal and socio-cultural importance attached to the
63 production of palm wine in rural African society, the consumption of this drink is becoming
64 increasingly problematic because of two major facts. Indeed, it becomes fearful on the one hand,
65 and this is a recurring observation, to frequent the palm wine from cabarets. This fear is
66 generated by the fact that crooked sellers tend to sell an adulterated version of the drink,
67 resulting from the adulteration of the original palm wine by using water (generally of low
68 quality with a distinct possibility of contamination with undesirable elements such as metals)
69 and artificial sweetness, which sometimes result in diarrhea, abdominal pains, and other
70 stomach problems (Ukhun et al. 2005). In other hand, as palm trees mostly grow concomitantly
71 in cocoa farms in rural areas, the second problem and hence the quieter comes from the potential
72 contamination of this drink during tapping process by pesticides or their residues used in the
73 agrochemical treatments of cocoa fields in which the sap extraction process takes place. Indeed,

74 in an attempt to reduce the incidence of insect pests and diseases on cocoa farms, a large number
75 of pesticides are usually applied on farms. These pesticides are hazardous in very low
76 concentrations and cause immense damage to the ecosystem. In most developing countries like
77 Ivory Coast, consequences are often severe because approved pesticides are not used and
78 recommended frequencies of pesticides application by government agencies for crops are not
79 followed. Farmers however misuse, overuse and apply pesticides indiscriminately with
80 disregard to safety measures and regulations on chemical use as reported by Konradsen (2007)
81 and Sam et al. (2008). In consequence, the drink produced can be contaminated at multiple
82 steps in the tapping process by plant material treated with pesticides, particularly leaves used
83 to cover containers containing the drink in the palm wine processing environment, or insects
84 when flying into bowls. According to the National Pesticide Information Center (2011), an
85 increase in the use of agrochemicals has been observed over the past fifty years making humans
86 more exposed to heavy metals, pesticides and insecticides. Heavy metals and pesticides have
87 carcinogenic, mutagenic and teratogenic potential and exposure can cause irreversible damage
88 to humans, including death, and are therefore considered a serious public health problem
89 particularly in sub-Saharan countries. Although many studies have revealed that pesticides and
90 heavy metals poses threats to the environment, and farmers themselves, their potential presence
91 in palm wine and the risks associated have to be determined.

92 Several methods are available for extraction and quantification of pesticides and metals from
93 complex matrices. For pesticides, liquid extraction with solvents, such as dichloromethane,
94 acetone (Gambacorta et al., 2005), and ethyl acetate (Munawar and Waqas Hameed, 2013) has
95 been used. Pesticide residues have also been extracted from apple juice using a solid phase
96 extraction (SPE) method (Han et al. 2014; Velkoska-Markovska and Petanovska-Ilievska,
97 2013), from berry fruits using an Envi-Carb cartridge (Yang et al, 2011) and using an HS-SPME
98 method, a fast, automatable, and inexpensive method, including in mangoes from Brazil (Filho,

99 Dos Santos, and De Paula Pereira 2010), in water samples from Argentina (Bonansea, Amé,
100 and Wunderlin 2013) and in wines from Italy (Vitali et al. 1998). In general, the analysis of
101 pesticides is done using gas (Han et al., 2014 and Yang et al., 2011) or liquid (Velkoska-
102 Markovska and Petanovska-Ilievska, 2013) chromatography, depending on the polarity,
103 volatility and decomposition risk of the compounds. For the detector, the electron capture
104 detector is commonly used to quantify organochlorines, while the nitrogen-phosphorus detector
105 is used for organophosphates. Mass spectrometry offers the advantage of being able to identify
106 and quantify a wide range of molecules, without being limited to a single class of pesticides
107 (Bonansea, Amé, and Wunderlin 2013). Metals are commonly analyzed by ICP-AES/MS in
108 pharmaceutical compounds (Lewen et al., 2004), textile materials (Rezić and Steffan, 2007), or
109 wines and alcoholic beverages (Płotka-Wasyłka et al., 2018), due to sensitivity, low limit of
110 detection and speed of analysis. Prior to analysis, samples are mineralized to remove organic
111 matter and ensure quantitative dissolution of metals in the case of solid matrices. Most of the
112 time, acids or acid mixtures are used for this step (Lewen et al., 2004): nitric acid, hydrochloric
113 acid, sulfuric acid, or mixtures, with aqua regia being the most famous. Due to the low detection
114 levels required by regulatory agencies, efficient sample preparation and trace-level detection
115 are important aspects in an analytical method.

116 This research was therefore conducted to determine the concentration of heavy metals and
117 pesticides using optimized methods and then raise awareness of the possible health risk while
118 consuming this beverage.

119

120 **2. Materials and methods**

121 2.1 Chemicals

122 Ultrapure water (resistivity 18.2 MW•cm), obtained from a Milli-Q system (Millipore, Bedford,
123 MA, USA), sodium sulfate (GPR Rectapur, VWR Chemicals, Belgium), ethanol (99%, Fisher

124 Scientific, UK), (+) limonene (97%, Accros Organics, USA) decane (99%, Sigma-Aldrich,
125 USA), L-ascorbic acid (99%, VWR Chemicals, Belgium), calcium chloride (98%, VWR
126 Chemicals, Belgium), potassium chloride (99%, Carlo Erba, Italia), magnesium nitrate (98%,
127 Sigma-Aldrich, USA), and sodium chloride (99.5%, Acros Organics, USA), were used for the
128 preparation of the simulative palm wine. For the mineralizations, nitric acid (68%, trace analysis
129 grade, Fisher Scientific, UK) and hydrogen peroxide (30%, VWR Chemicals, Belgium) were
130 employed. Acetone (pesticides residue grade, Fisher Scientific, UK) and methanol (HPLC
131 grade, Fisher Scientific, UK) were used for the preparation of the stock solutions of pesticides.
132 The pesticides standard consisted in six stock solutions. Two solutions were purchased from
133 Restek (USA): an OrganoPhosphorus Pesticides (OPPs) solution in acetone containing
134 acephate ($200 \mu\text{g mL}^{-1}$), azinphos methyl ($400 \mu\text{g mL}^{-1}$), chlorpyrifos ($100 \mu\text{g mL}^{-1}$), demeton-
135 S-methyl ($200 \mu\text{g mL}^{-1}$), dichlorvos ($500 \mu\text{g mL}^{-1}$), dimethoate ($200 \mu\text{g mL}^{-1}$), ethion ($200 \mu\text{g}$
136 mL^{-1}), malathion ($200 \mu\text{g mL}^{-1}$), methamidophos ($500 \mu\text{g mL}^{-1}$), methidathion ($200 \mu\text{g mL}^{-1}$),
137 omethoate ($1,000 \mu\text{g mL}^{-1}$), pirimiphos methyl ($100 \mu\text{g mL}^{-1}$), profenofos ($200 \mu\text{g mL}^{-1}$),
138 prothiofos ($200 \mu\text{g mL}^{-1}$), pyrazophos ($500 \mu\text{g mL}^{-1}$), and tolclofos-methyl ($100 \mu\text{g mL}^{-1}$), and
139 an organohalide pesticides solution in methanol containing aldrin, alachlor, atrazine, γ -BHC
140 (lindane), cis-chlordane, trans-chlordane, dieldrin, endrin, heptachlor, heptachlor epoxide
141 (isomer B), hexachlorobenzene, hexachlorocyclopentadiene, methoxychlor, cis-nonachlor,
142 trans-nonachlor, and simazine, at $200 \mu\text{g mL}^{-1}$ each. Two solutions were purchased from Fisher
143 Scientific (France): an OrganoChloride Pesticides (OCPs) solution in acetone containing aldrin,
144 α , β and δ -BHC, dieldrin, α and β -endosulfan, endosulfan sulfate, endrin, endrin aldehyde,
145 endrin ketone, lindane, heptachlor, heptachlor epoxide (isomer B), methoxychlor, p,p'-DDD,
146 p,p'-DDE, and p,p'-DDT at $200 \mu\text{g mL}^{-1}$ each, and a terbutylazine solution in acetone at 100
147 $\mu\text{g mL}^{-1}$. A methyl parathion solution in acetone at $1000 \mu\text{g mL}^{-1}$, and a diazinon solution in
148 methanol at $100 \mu\text{g mL}^{-1}$ were purchased from Merck (Germany).

149 The stock solution containing all pesticides was prepared in pure acetone and before each serie
150 of analyses. The pesticides standard solutions at different levels were prepared by spiking with
151 the stock solution into 3 mL of the simulative palm wine contained in a 20 mL sealed glass vial.
152 Calibration curves were prepared from dilutions of the stock solution. The concentration of
153 pesticides ranged from 0.25 $\mu\text{g L}^{-1}$ for pirimidiphos methyl and tolclofos-metyl to 1000 $\mu\text{g L}^{-1}$
154 for simazine.

155 A metal solution (VWR Chemicals, Leuven, Belgium) containing aluminum, silver, arsenic,
156 boron, barium, calcium, cadmium, cerium, cobalt, chromium, copper, dysprosium, erbium,
157 europium, iron, gadolinium, holmium, potassium, lanthanum, lithium, lutetium, magnesium,
158 manganese, sodium, neodymium, nickel, phosphorus, lead, rubidium, selenium, samarium,
159 strontium, thallium, thulium, vanadium, and zinc, at 10 mg L^{-1} each, was used. Three mono-
160 element solutions containing bismuth at 10 mg L^{-1} (Spex CertiPrep, USA), molybdenum (Spex
161 CertiPrep, USA) and tin at 1000 mg L^{-1} (Fisher Scientific, France) were used for preparing the
162 stock solutions. Calibration curves were prepared from dilutions of the stock solution. The
163 concentration of metals ranged from 0.05 $\mu\text{g L}^{-1}$ to 100 $\mu\text{g L}^{-1}$ for all metals except for rubidium
164 which ranged from 0.05 to 2 mg L^{-1} and for phosphorus which ranged from 0.5 to 20 mg L^{-1} .

165 2.2 Samples

166 This study was carried out on different palm wines produced in four cities in Ivory Coast
167 (Abidjan, Aboisso, Hiré and Toumodi). Four samples of each city were collected from local
168 producers (P) and four from resellers (R), who could eventually modify the composition of the
169 palm wines. Thirty two palm wines were analyzed (3 replicates) for determination of pesticides
170 and metals. Samples were stored in a freezer at -18°C . Before analysis, samples were kept at
171 4°C during two hours in a fridge and thirty minutes at room temperature. The palm wines
172 presented particles naturally present. To estimate the real concentrations to which the
173 population is exposed, it was decided to let the samples as naturally present and consumed.

174 Consequently, no filtration nor centrifugation was done, and the samples were mixed before
175 analysis in triplicate.

176 2.3 Instrumentation

177 2.3.1 GC-MS

178 Analyses of palm wine were performed using a GC-MS QP-2020 NX (Shimadzu, Germany)
179 equipped with an auto-sampler AOC-6000 (automatic injection). Different GC conditions for
180 analyzing pesticides were tested; to find a good compromise between the analysis time and the
181 resolution. As final GC conditions, a ZB5MS+ (5% polysilarylene and 95%
182 dimethylpolysiloxane, 30 m x 0.25 mm x 0.25 μ m, Phenomenex) column was used to separate
183 pesticides; helium was used as carrier gas (1 mL/min); injection was splitless and at 260°C. The
184 oven temperature was programmed as follows: initially maintained at 40°C (held for 5 min),
185 then increased to 180°C (held for 2 min) at a rate of 10°C/min, a second increase was used to
186 280°C (held for 2 min) at a rate of 5°C/min. The optimal analysis time was 43 minutes.

187 The MS transfer line was kept at 250°C. The mass spectrometer, equipped with a quadrupole
188 mass filter, was in the electron impact ionization mode (70 eV) with an ion source temperature
189 of 200°C. Chromatograms were acquired in Single Ion Monitoring (SIM) scanning mode. The
190 m/z associated with pesticides are specified in Table S1. All compounds could be separated
191 excepted cis and trans chlordane. These isomers were identified on the same peak. Moreover,
192 MS was not able to identify with certainty the three BHC (hexachlorocyclohexane), the three
193 isomers were identified as BHC on chromatograms.

194 2.3.2 ICP-AES and ICP-MS

195 Extraction and mineralization of samples were performed with a Milestone ETHOS 1
196 microwave digestion system equipped with a temperature sensor. TFM reactors were used with
197 a maximum pressure of 100 bar. ICP-AES measurements were performed on an iCAP 6300

198 series instrument (Thermo Fisher Scientific) using the axial view. The quantification of
199 phosphorus and rubidium was performed at 178 nm and 780 nm, respectively.

200 Detection of lithium, boron, chromium, iron, manganese, cobalt, nickel, copper, zinc, strontium,
201 molybdenum, silver, cadmium, tin, barium, lead, and bismuth was performed on an iCAP RQ
202 ICP-MS instrument (Thermo Fisher Scientific) fitted with a collision cell. Two different
203 measurement modes were used: standard mode (STD) and kinetic energy discrimination mode
204 (KED). In the KED mode, Helium gas was injected in the collision cell and an energy
205 discrimination barrier was applied in order to remove unwanted polyatomic interferences. Data
206 processing was performed using ThermoFisher Scientific Qtegra software.

207 As example, optimized conditions for the plasma and nebulizer for ICP-AES and ICP-MS are
208 given in Table S2.

209 2.4 Method optimization

210 2.4.1 The simulative palm wine

211 Because of the low volume of palm wine samples, a surrogate matrix (simulative palm wine)
212 was developed to determine HS-SPME/GC-MS optimal conditions before application on real
213 samples.

214 For this purpose, a palm wine sample was analyzed by GC-MS to determine the main
215 constituents. It was found that palm wine contained around 88 % (m/m) of water, 2 % (m/m)
216 of ethanol, and 10 % (m/m) of sucrose. Based on a previous study on palm wines (Simon
217 Amoikon et al., 2020), the aromatic profile was recreated using 4 mg L⁻¹ of limonene and 10
218 mg L⁻¹ of decane, main compounds found in real palm wines. Calcium (5 mg L⁻¹), potassium
219 (280 mg L⁻¹), magnesium (7 mg L⁻¹) and sodium (4 mg L⁻¹) concentrations were determined in
220 the palm wine sample by ICP-AES. According to Cunningham and Wehmeyer (1988), vitamin

221 C (60 mg L⁻¹) is generally added. Finally, the simulative palm wine was doped to achieve the
222 concentrations generally found in such samples.

223 2.4.2 Optimization of the HS-SPME procedure

224 Experimental parameters were evaluated to determine the optimal conditions for the analysis
225 of pesticides in palm wines. Three fiber types were tested: 100 µm polydimethylsiloxane
226 (PDMS), 85 µm polyacrylate (PA), and 50/30 µm DVB/Carbon WR/PDMS (Restek USA).
227 Fibers were conditioned at 230°C during 30 min before each analysis, according to the
228 manufacturer's recommendations. Three milliliters of the simulative palm wine were placed in
229 a 20 mL sealed headspace glass vial (3 replicates). After extraction, the fiber was automatically
230 retracted and transferred to the GC injector at 260 °C for a desorption time of 3 min.

231 In order to optimize the extraction efficiency, an experimental design (Table S3) with 45 trials
232 was implemented (Ellistat software, Chavanod, France) on a 100 µm PDMS fiber. Some
233 parameters were tested, according to the literature for pesticides extraction by HS-SPME.
234 Extraction temperature between 30 and 60°C (Cincotta et al., 2015), equilibrium time between
235 5 and 30 min (Bernardi et al. 2015, Pellati et al. 2013), and extraction time between 20 and 60
236 min (Pellati et al., 2013) were investigated. Moreover, stirring was studied between 250 and
237 500 rpm (Filho et al., 2010, Bernardi et al. 2015), and salinity (sodium sulfate) between 5 and
238 15 % (m/m) (Vitali et al., 1998, Zhu et al., 2009) of the sample. According to Zhu et al (2009),
239 who worked on the determination of OPPs in textiles using SPME coupled with GC/MS, OPPs
240 were more extracted with sodium sulfate than with sodium chloride.

241 Data processing was performed using the GC-Solution software.

242 2.5 Method validation

243 2.5.1 Analysis of palm wine by HS/SPME-GC/MS

244 For the study of pesticides in complex matrix, an HS-SPME extraction technique was used. As
245 final conditions after optimization, a 20 mL vial was filled with 3 mL of each palm wine
246 samples (3 replicates) and 7.5 % (m/m) of sodium sulfate. Extraction was done in the headspace
247 vial kept at 52.5°C. A DVB/C-WR/PDMS fiber was used for all extractions because it allowed
248 the determination of many pesticides with good repeatability (see results and discussions - fiber
249 coating). The palm wine samples were equilibrated for 11.25 min and extracted for 50 min
250 under continuous stirring (437.5 rpm). The fiber was automatically transferred to the GC
251 injector at 260 °C for desorption time of 3 min. The method performance was evaluated in terms
252 of linearity, limit of detection, limit of quantification, accuracy, and precision (repeatability).

253 The concentration of three stock solutions ranged from 0.5-2.5 $\mu\text{g L}^{-1}$ (for Tolclofos-methyl and
254 Dichlorvos, respectively) to 5-25 $\mu\text{g L}^{-1}$ (for Tolclofos-methyl and Dichlorvos, respectively).
255 These three stock solutions were each injected three times in order to study the repeatability
256 (represented by a relative standard deviation (RSD)). To study the linearity, calibration curves
257 with 3-5 points were established. LOD and LOQ were estimated as concentrations of pesticides
258 which allowed a signal-to- noise ratio (S/N) of 3 and 10, respectively. Additionally, samples
259 were spiked with stock solutions (to obtain three samples with concentrations from 0.5-2.5 μg
260 L^{-1} (for Tolclofos-methyl and Dichlorvos, respectively) to 5-25 $\mu\text{g L}^{-1}$ (for Tolclofos-methyl
261 and Dichlorvos, respectively) three times and relative recoveries were determined.

262 2.5.2 Analysis of palm wine by ICP/AES and ICP/MS

263 In accordance to the French standard « » for the determination of metals in drinking water,
264 ICP-MS is the reference technique and is used after mineralization. No standard exists for palm
265 wine, and it consists of 88% water, so the same methodology was followed and can be
266 assimilated to drinking water.

267 A volume of 2 mL of palm wine was digested with 2 mL of nitric acid and 1 mL of hydrogen
268 peroxide in a microwave system: from room temperature, 30 °C, to 180 °C, 20 minutes at 180

269 °C. After the mineralization, the digest was diluted up to 50 mL with ultrapure water. A blank
270 was analyzed in the same conditions.

271 Samples were analyzed with ICP-AES for the determination of phosphorus and rubidium, and
272 with ICP-MS for the determination of the other metals. To study the linearity, calibration curves
273 with 5 points were established.

274 LOD and LOQ were obtained for the palm wine samples using equations 1 and 2, where BEC
275 is the background equivalent concentration ($\mu\text{g L}^{-1}$), it represents the blank intensity divided by
276 the angular coefficient of the analytical curve and RSD_{blank} is the relative standard deviation of
277 the blank (n=10).

$$278 \quad LOD = \frac{3 \times RSD_{\text{blank}} \times BEC}{100} \quad (\text{Equation 1})$$

$$279 \quad LOQ = \frac{10 \times RSD_{\text{blank}} \times BEC}{100} \quad (\text{Equation 2})$$

280 **3. Results and discussion**

281 3.1 Determination of pesticides in palm wines

282 3.1.1 Determination of SPME conditions

283 Different parameters were tested in order to develop a HS/SPME-GC/MS method with the
284 optimized extraction efficiency. Fiber coating, extraction temperature, equilibrium time,
285 extraction time, stirring, and salinity were studied.

286 3.1.1.1 Extraction temperature, equilibrium and extraction time, stirring and salinity

287 Five parameters have been explored in this study on a 100 μm PDMS fiber: extraction
288 temperature, equilibrium and extraction time, stirring and salinity. Among the 45 trials of the
289 experimental design, one offered the highest peak area for 7/12 extracted pesticides of the OPPs
290 solution (azinphos methyl, chlorpyrifos, ethion, methidathion, pirimiphos methyl, profenofos
291 and tolclofos methyl). This method had a long extraction time and according to the exploitation

292 of the experimental design, the extraction time was the parameter that influences the most the
293 extraction efficiency.

294 Finally, the extraction temperature was set at 52.5°C. Increasing the temperature allows
295 reducing the extraction time, which increases the concentration of semi-volatiles in the vial
296 headspace (Bianchin et al., 2014). Pesticides were mostly obtained with high peak areas for an
297 extraction temperature of 52.5°C. Lower peak areas were obtained with a temperature of
298 37.5°C (comparison of trials 4 and 8 in Table S3, parameters were set at the optimized values,
299 except for extraction temperature), especially for chlorpyrifos (peak areas of 889083 and
300 3829688 for temperatures of 37.5°C and 52.5°C, respectively), ethion (peak areas of 18863 and
301 2459896 for temperatures of 37.5°C and 52.5°C, respectively), and tolclofos methyl (peak areas
302 of 6788178 and 21189553 for temperatures of 37.5°C and 52.5°C, respectively).

303 Equilibrium time was set at 11.25 min. Equilibrium time depends on the chemical nature and
304 physico-chemical parameters of pesticides. The optimum equilibration time was selected as the
305 time ensuring the highest peak area for the majority of pesticides (all other parameters being
306 constant), with a reasonable time. Finally, 11.25 min was selected for equilibration time.

307 The highest extraction time tested with the experimental design was 60 min. A higher extraction
308 efficiency was observed for a higher exposure time between the SPME fiber and the sample
309 headspace (Ho and Hsieh, 2001), but a longer exposure could also damage the fiber and/or the
310 pesticides. The extraction time of 50 min was a compromise between extraction efficiency and
311 fiber lifetime and/or pesticides stability. This extraction time was compared with a lower time
312 of 30 min (trials 6 and 8). All other parameters were constant at the optimized values. Pesticides
313 were mostly obtained with high peak areas with an extraction time of 50 minutes. Ethion (peak
314 areas of 744276 and 2459886 for times of 30 min and 50 min, respectively), and prothiofos
315 (peak areas of 1725649 and 3690958 for times of 30 min and 50 min, respectively) were more
316 extracted with the higher extraction time. However, malathion was more extracted with the

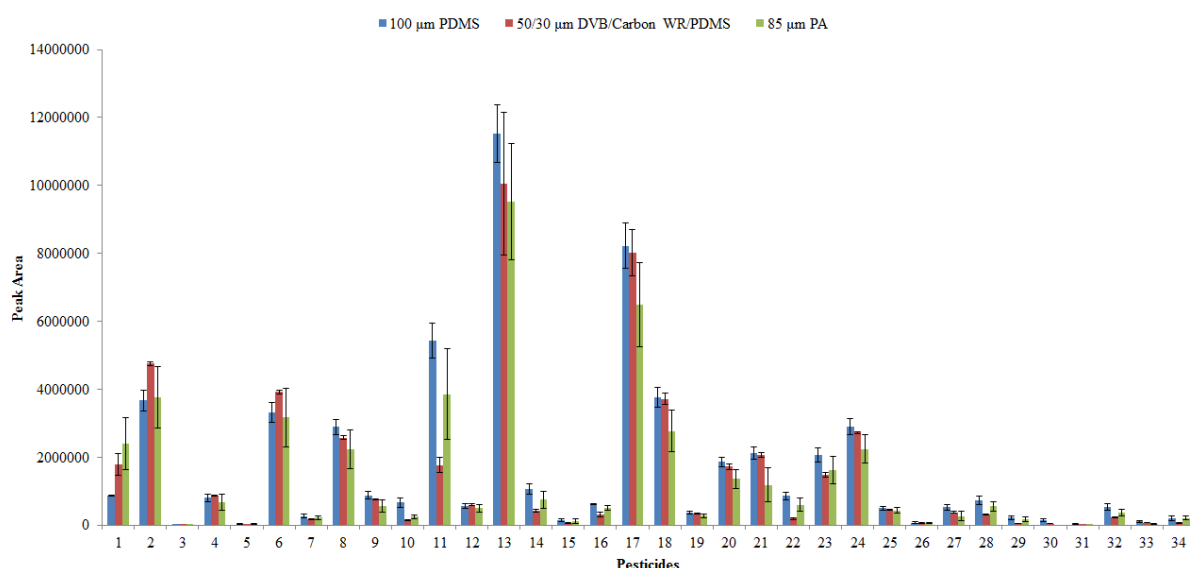
317 lower extraction time (peak areas of 502458 and 198595 for times of 30 min and 50 min,
318 respectively).

319 Stirring and salinity of the sample allows the diffusion layer to reach the equilibrium faster (Ho
320 and Hsieh, 2001). Stirring of 437.5 rpm and 7.5% (m/m) of Na₂SO₄ allowed to reduce the
321 analysis time, by promotion of the pesticides from the liquid phase to the gas phase.

322 Finally, SPME optimized parameters for this study were: an extraction temperature of 52.5°C,
323 an equilibrium time of 11.25 min, an extraction time of 50 min, stirring of 437.5 rpm, and
324 salinity of 7.5% of Na₂SO₄.

325 3.1.1.2 Fiber coating

326 Three SPME fiber coatings were used to extract the pesticides in the simulative palm wine
327 solution: a 100 µm polydimethylsiloxane (PDMS), a 85 µm polyacrylate (PA), and a 50/30 µm
328 DVB/Carbon WR/PDMS. Compounds were spiked between 2.5 to 12.5 µg L⁻¹ triplicate. SPME
329 optimized parameters (see 3.1.1.1 Extraction temperature, equilibrium and extraction time,
330 stirring and salinity) were used.



331
332 **Fig.1.** Comparison of three SPME fiber coatings for the extraction of 35 pesticides. Peaks: (1)
333 dichlorvos, (2) BHC, (3) simazine, (4) BHC, (5) atrazine, (6) lindane, (7) terbutylazine, (8)
334 diazinon, (9) BHC, (10) parathion methyl, (11) tolclofos methyl, (12), alachlor, (13) heptachlor,
335 (14) pirimiphos methyl, (15) malathion, (16) chlorpyrifos, (17) aldrin, (18) heptachlor epoxide,

336 (19) α -endosulfan, (20) chlordane, (21) trans-nonachlor, (22) prothiofos, (23) p,p'-DDE, (24)
337 dieldrin, (25) endrin, (26) β -endosulfan, (27) cis-nonachlor, (28) p,p'-DDD, (29) ethion, (30)
338 endrin aldehyde, (31) endosulfan sulfate, (32) p,p'-DDT, (33) endrin ketone, (34) methoxychlor.
339 Hexachlorobenzene was not represented because its area was too important.

340
341 The three SPME fibers allowed the extraction of all pesticides as it can be observed from Table
342 S4, except for the endrin aldehyde, which was not extracted by the PA fiber. The detector was
343 saturated and no longer able to give an area for hexachlorobenzene using the DVB/Carbon
344 WR/PDMS fiber. Some pesticides had low peak areas no matter the fiber, such as simazine,
345 atrazine, malathion, β -endosulfan, endrin aldehyde, endosulfan sulfate or endrin ketone.
346 Twenty-eight pesticides were extracted with higher area with the PDMS fiber, 5 with the
347 DVB/Carbon WR/PDMS fiber and 2 with the PA fiber. A lot of pesticides were extracted with
348 an important area with PDMS and DVB/Carbon WR/PDMS fibers, such as aldrin (peak area of
349 8228026 for the PDMS fiber and 8021739 for the DVB/Carbon WR/PDMS fiber), diazinon
350 (peak area of 2886678 for the PDMS fiber and 2577749 for the DVB/Carbon WR/PDMS fiber),
351 or heptachlor (peak area of 11522371 for the PDMS fiber and 10058902 for the DVB/Carbon
352 WR/PDMS fiber).

353 However, as shown in Table S4, the DVB/Carbon WR/PDMS fiber exhibited the lowest RSDs
354 for 25 pesticides (RSDs between 0.7 and 18%) and offered 22 RSDs under 10%. Only 14 and
355 1 RSDs were under 10% for the PDMS and PA fibers, respectively. The best repeatability was
356 achieved with the DVB/Carbon WR/PDMS fiber. Therefore, this fiber was selected for the
357 analysis of palm wines.

358 3.1.2 Analysis of palm wine samples

359 3.1.2.1 Method validation

360 3.1.2.1.1 Quadratic or linear behavior of calibration curves

361 Initially, a linear calibration model was developed for all pesticides. This type of calibration is
 362 the most used to obtain an analytical result from the measurement of the signal. According to
 363 Balsini and Parastar (2019), pesticides may have a quadratic behavior. In this study, 12
 364 pesticides (aldrin, chlordane, α -endosulfan, endrin heptachlor, heptachlor epoxide, parathion
 365 methyl, pirimidiphos methyl, prothiofos, p,p-DDD, and simazine), followed a quadratic
 366 calibration curve and 23 of them had a linear behavior. The analytical results were listed in
 367 Table 1 (linear (L) or quadratic (Q) behavior, LODs, and LOQs).

368 **Table 1**

369 Quadratic or linear behavior, LODs, and LOQs of pesticides in this study and results from the
 370 literature

No.	Pesticides	Behavior ^a	Range ($\mu\text{g L}^{-1}$)	R^2	LOQs ($\mu\text{g L}^{-1}$)	LODs ($\mu\text{g L}^{-1}$)	LODs from the literature ($\mu\text{g L}^{-1}$)	LOQs from the literature ($\mu\text{g L}^{-1}$)	Fiber used from literature
1	Dichlorvos	L	1.25-250	0.9999	1.25	0.11	0.05		85 μm PA [27]
							3.80	11.4	65 μm PDMS/DVB [25]
2	BHC	L	1-100	0.9998	0.31	0.09			
3	Hexachlorobenzène	L	0.5-5	0.9971	0.10	0.03			
4	Simazine	Q	100-1000	1	92.1	27.6	0.10		85 μm PA [27]
5	BHC	L	0.5-100	0.9999	0.20	0.10			
6	Atrazine	L	10-1000	0.9993	10.0	3.00			
7	Lindane	L	2-200	0.9999	0.11	0.03			
8	Terbuthylazine	L	1-100	0.9999	1.00	0.30			
9	Diazinon	L	5-100	0.9998	0.58	0.17	3.00		85 μm PA [33]
							0.01		85 μm PA [27]
10	BHC	L	0.5-100	0.9999	0.75	0.22			
11	Parathion methyl	Q	0.5-10	0.9991	0.02	0.01	50.0		85 μm PA [33]
12	Tolclofos-methyl	L	0.25-5	0.9993	0.13	0.04	0.01		85 μm PA [27]
13	Alachlor	L	1-100	0.9999	0.53	0.16			
14	Heptachlor	Q	1-20	0.9994	0.01	0.003			

15	Pirimidiphos methyl	Q	0.25-50	0.9999	0.25	0.08			
16	Malathion	L	5-100	0.9998	1.20	0.36	45.0		85 µm PA [33]
							10.0		85 µm PA [27]
17	Chlorpyrifos	L	0.5-50	0.9997	0.03	0.01	80.0		85 µm PA [27]
							4.50	13.7	65 µm PDMS/DVB [25]
18	Aldrin	Q	1-20	0.9991	0.11	0.03			
19	Heptachlor epoxide	Q	1-20	0.9999	0.08	0.02			
20	α-Endosulfan	Q	0.5-100	0.9999	0.17	0.05			
21	Chlordane	Q	1-20	0.9997	0.16	0.05			
22	trans-Nonachlor	L	1-100	0.9999	0.01	0.002			
23	Prothiofos	Q	0.5-10	0.9999	0.22	0.07			
24	p.p'-DDE	L	0.5-10	0.9963	0.01	0.003			
25	Dieldrine	L	1-20	0.9996	0.09	0.03			
26	Endrin	Q	1-20	0.9999	1.00	0.03			
27	β-Endosulfan	L	0.5-100	0.9999	0.50	0.15			
28	cis-Nonachlor	L	1-100	0.9999	0.06	0.02			
29	p.p'-DDD	Q	0.5-100	0.9998	0.32	0.10			
30	Ethion	Q	1-100	0.9999	0.62	0.18	2.20	6.50	65 µm PDMS/DVB [25]
31	Endrin aldehyde	L	0.5-100	0.9999	0.50	0.15			
32	Endosulfan sulfate	L	1-100	0.9999	1.00	0.30			
33	p.p'-DDT	L	0.5-100	0.9999	0.07	0.02			
34	Endrin ketone	L	0.5-100	0.9999	0.50	0.15			
35	Methoxychlor	L	1-20	0.9997	1.00	0.30			

371

372 ^a Q = quadratic calibration, L = linear calibration

373

374 All calibration curves had correlation coefficients between 0.9963 and 1, therefore, the

375 quantification of pesticides in palm wines was acceptable.

376 3.1.2.1.2 LODs and LOQs

377 The obtained LODs ranged between 0.002 and 27.6 $\mu\text{g L}^{-1}$, for trans-nonachlor and simazine,
 378 respectively, and LOQs ranges between 0.01 and 92.05 for p,p'-DDE and simazine, respectively.
 379 For such compounds, some comparisons can be done with literature. For example, for the
 380 Dichlorvos, LOD with the optimized method was 0.11 $\mu\text{g L}^{-1}$, Sakamoto and Tsutsumi found a
 381 LOD of 0.05 $\mu\text{g L}^{-1}$ in water sample with a 85 μm PA fiber, and Rodrigues et al found a LOD
 382 of 3.8 $\mu\text{g L}^{-1}$ in cow milk sample with a 65 μm PDMS/DVB fiber. Tsoukali et al obtained higher
 383 LODs from whole blood and with a 85 μm PA fiber, as for the Malathion (0.36 $\mu\text{g L}^{-1}$ for palm
 384 wine sample and 45 $\mu\text{g L}^{-1}$ for whole blood sample) or Parathion methyl (0.01 $\mu\text{g L}^{-1}$ for palm
 385 wine sample and 50 $\mu\text{g L}^{-1}$ for whole blood sample). Rodrigues et al obtained higher LOQs with
 386 a LOQ of 6.50 $\mu\text{g L}^{-1}$ for Ethion (LOQ of 0.62 $\mu\text{g L}^{-1}$ for optimized method). The developed
 387 method offered better performances for those compounds.

388 3.1.2.1.3 Relative standard deviation

389 **Table 2**

390 Repeatabilities and recoveries of pesticides

391

No.	Pesticides	Replicate 1 (0.5-2.5 $\mu\text{g L}^{-1}$) ^a		Replicate 2 (2.5-12.5 $\mu\text{g L}^{-1}$) ^a		Replicate 3 (5-25 $\mu\text{g L}^{-1}$) ^a	
		RSD (%) (n = 3)	Relative recovery (%)	RSD (%) (n = 3)	Relative recovery (%)	RSD (%) (n = 3)	Relative recovery (%)
1	Dichlorvos	14.5	116	5.8	95	1	100
2	BHC	10.8	51	11.1	97	3.6	107
3	Hexachlorobenzene	7.9	114	1.4	100	2.5 ^b	75 ^b
4	Simazine	6.2 ^d	102 ^d	36.5 ^e	88 ^e	59.3 ^f	100 ^f
5	BHC	3.9	80	0.9	102	3.7	103
6	Atrazine	11.2 ^d	115 ^d	31.8 ^e	98 ^e	41.7 ^f	117 ^f
7	Lindane	22.1	73	12.2	100	2.7	103
8	Terbutylazine	18.7	94	0.7	98	1.1	102
9	Diazinon	14.7 ^d	95 ^d	11.6	115	6.1	85
10	BHC	22	96	6.9	96	3.1	92
11	Parathion methyl	7.2	110	3.8	99	2.7	102
12	Tolclofos-methyl	15.8	103	15.5	97	5.4	101

13	Alachlor	4	100	4.2	99	6.2	100
14	Heptachlor	7.8	110	3.9	99	0.7	100
15	Pirimiphos methyl	8.9	113	21.4	95	7.1	101
16	Malathion	4.4 ^d	100 ^d	5	82	1.4	109
17	Chlorpyrifos	21.9	44	22.1	93	6.6	110
18	Aldrin	7	109	4.3	99	0.3	100
19	Heptachlor epoxide	17.3	100	11.6	101	6.1	93
20	α -Endosulfan	14.1	102	10.3	105	2.9	99
21	Chlordane	19.1	107	12.9	100	18.2	100
22	trans-Nonachlor	40.4	116	37.6 ^d	90 ^d	13.2	98
23	Prothiofos	18.6	100	20.4	100	6.6	100
24	p,p'-DDE	20.7	113	19.3	92	6.5	102
25	Dieldrin	15	105	15.2	97	4.5	101
26	Endrin	13.5	115	14.2	100	6.5	100
27	β -Endosulfan	20.7	116	23.2	104	9.9	96
28	cis-Nonachlor	36.9	105	31.4 ^d	117 ^d	11.9	99
29	p,p'-DDD	17.9	110	15.1	95	9.3	102
30	Ethion	22	84	38	105	13.6	102
31	Endrin aldehyde	16.4	72	11.4	103	11.6 ^d	100 ^d
32	Endosulfan sulfate	10.6	108	11.9	98	11.3 ^d	100 ^d
33	p,p'-DDT	26.8	120	17.3	99	9.4 ^d	100 ^d
34	Endrin ketone	9.4	97	15.2	97	12.1 ^d	100 ^d
35	Methoxychlor	44 ^c	109 ^c	26.6	90	14.7	102

392

393 ^a range of concentrations

394 ^b at the concentration of 0.5 $\mu\text{g L}^{-1}$

395 ^c at the concentration of 1 $\mu\text{g L}^{-1}$

396 ^d at the concentration of 100 $\mu\text{g L}^{-1}$

397 ^e at the concentration of 500 $\mu\text{g L}^{-1}$

398 ^f at the concentration of 1000 $\mu\text{g L}^{-1}$

399

400 As the pesticides were not at the same concentration in the stock solution, spiking was at three

401 levels and for each level the pesticides were not at the same concentration. For the first level,

402 the concentrations were between 0.5 (for chlorpyrifos and pirimiphos methyl for example) and

403 2.5 $\mu\text{g L}^{-1}$ (for dichlorvos for example), between 2.5 and 12.5 $\mu\text{g L}^{-1}$ for the second level, and

404 between 5 and 25 $\mu\text{g L}^{-1}$ for the final level. Some pesticides were not detected at these

405 concentrations, such as simazine or atrazine, because its LOD were higher than the range of

406 concentrations. Their RSDs and relative recoveries were calculated thanks to higher

407 concentrations (between 100 and 1000 $\mu\text{g L}^{-1}$). Some others were not detected at one
408 concentration of the range tested, such as methoxychlor (replicate 1) or trans and cis-nonachlor
409 (replicate 2), their RSDs and relative recoveries were calculated from another concentrations
410 (at 1 $\mu\text{g L}^{-1}$ instead of 2 $\mu\text{g L}^{-1}$ and at 100 $\mu\text{g L}^{-1}$ instead of 5 $\mu\text{g L}^{-1}$, respectively for
411 methoxychlor, and for trans-nonachlor, and cis-nonachlor).

412 Atrazine, cis-nonachlor, trans-nonachlor, ethion, methoxychlor, and simazine had RSDs higher
413 than 20% for two replicates. Moreover, for these pesticides the calibration curve was only
414 realized with 3 points, except for ethion (with 4 points), so they were identifiable but not
415 quantifiable for this study.

416 The RSDs obtained, for the other pesticides, were between 3.9 and 26.8% for BHC and p,p'-
417 DDT, respectively, at 1 $\mu\text{g L}^{-1}$ for the replicate 1, between 0.7 and 23.2% for terbuthylazine and
418 β -endosulfan, respectively, at 5 $\mu\text{g L}^{-1}$ for the replicate 2, and between 0.3 and 18.2% for aldrin
419 (at 20 $\mu\text{g L}^{-1}$) and chlordane (at 20 $\mu\text{g L}^{-1}$), respectively, for the replicate 3. The results are
420 shown in Table 2. RSDs were mostly under 20% for replicates 1 and 2, respectively for 23/31
421 and 25/31 pesticides, and under 10% for replicate 3 for 25/31 pesticides. Little variation of peak
422 areas used to calculate RSDs for low concentrations could have an important effect, so RSDs
423 were lower for concentrations of the replicate 3. The first BHC (RSD of 3.9% for replicate 1,
424 0.9% for replicate 2, and 3.7% for replicate 3) and parathion methyl (RSD of 7.2% for replicate
425 1, 3.8% for replicate 2, and 2.7% for replicate 3) were pesticides with the lower RSDs for all
426 replicates.

427 3.1.2.1.4 Relative recovery

428 Very good recoveries were obtained for all pesticides at level 3 (75% for hexachlorobenzene
429 and 110% for chlorpyrifos) and level 2 (from 82% for malathion to 115% for diazinon). At
430 level 1, the relative recoveries ranged from 72 to 120% (72% for endrin aldehyde and 120% for

431 p,p'-DDT at $1 \mu\text{g L}^{-1}$, respectively) except for BHC and chlorpyrifos, which showed the
432 applicability of the developed method.

433 3.1.2.2 Pesticides in palm wine samples

434 Thirty-two palm wines were analyzed following the optimized HS-SPME-GC/MS method.
435 Seven pesticides (dichlorvos, endrin ketone, parathion methyl, p,p-DDD, p,p-DDE, p,p-DDT,
436 and pirimidiphos methyl) were identified in 10 palm wine samples (AP1, AP2, AR3, BP4, HR4,
437 TP1, TP2, TR1, TR2, and TR3) as presented in Table 3. Pesticides were found in palm wines
438 from the 4 cities, in 3 samples from Abidjan, 1 from Aboisso, 1 from Hiré, and 5 from Toumodi,
439 with concentrations from 0.26 to $164.7 \mu\text{g L}^{-1}$ for pirimiphos methyl in AP2 and dichlorvos in
440 AR3, respectively. p,p'-DDT was identified in 5 palm wines, in 4 from Toumodi in low
441 concentrations (from 0.503 for TP1 to $0.669 \mu\text{g L}^{-1}$). This study is among the first to detect
442 these pesticides in alcoholic beverages.

443 In AP1, AP2, BP4, HR4, TP1, TP2, TR1, and TR3 palm wine samples, pesticides were
444 quantified at concentrations close to LOQs. EML of pesticides in bunches of grapes are used
445 because in France, the European Maximal Limits (EML) for wine does not exist. Parathion
446 methyl was detected at $0.521 \mu\text{g L}^{-1}$ in AP1, which is a concentration below the EML, as well
447 as a level below the concentration found in wines from different European countries, such as
448 Spain or Portugal (Table 3). In AR3 and TR2, dichlorvos was detected at 164.7 and $38.4 \mu\text{g L}^{-1}$,
449 respectively. As no study has detected this pesticide in alcoholic beverages, results were
450 compared with the concentration of ethion and malathion (organophosphorus pesticides as
451 dichlorvos) in wines from Brazil. Ethion was detected at concentrations between 0.29 and 0.32
452 $\mu\text{g L}^{-1}$ and malathion at $4.4 \mu\text{g L}^{-1}$. Dichlorvos was found at a level above the EML and
453 concentrations found in wines from Brazil (Table 3).

454 **Table 3**

455 Pesticides concentrations in palm wines, European Maximal Limits of pesticides in bunches of
 456 grapes and palm oil and comparison with other studies

Pesticides	European Maximal Limits (ug/kg)		Concentrations ($\mu\text{g L}^{-1}$)										Different countries (Brazil, Portugal, Argentina, Chile)	Spain	Brazil	
	Bunches of grapes ^a	Palm oil ^a	AP 1	A P2	AR 3	BP 4	HR 4	TP 1	TP 2	TR 1	TR 2	TR 3				
Dichlorvos	10	10			164.7							38.4				
Parathion methyl	10	20	0.521										2.80 ^b	3-4 ^c		
Pirimiphos methyl	10	10		0.26												
p,p'-DDE	50	50			0.645											
p,p'-DDD	50	50						0.609								
p,p'-DDT	50	50					0.575	0.503	0.669			0.671	0.669			
Endrin ketone	-	-								0.529						
Ethion	10	20														0.29-0.32 ^d
Malathion	20	20														4.4 ^d

457

458 ^a EU pesticides database

459 ^b Santiago et al., 2021

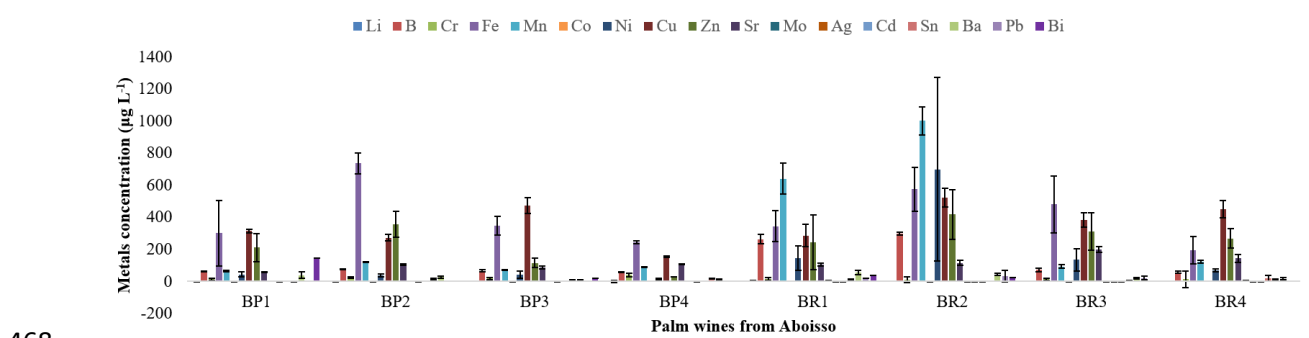
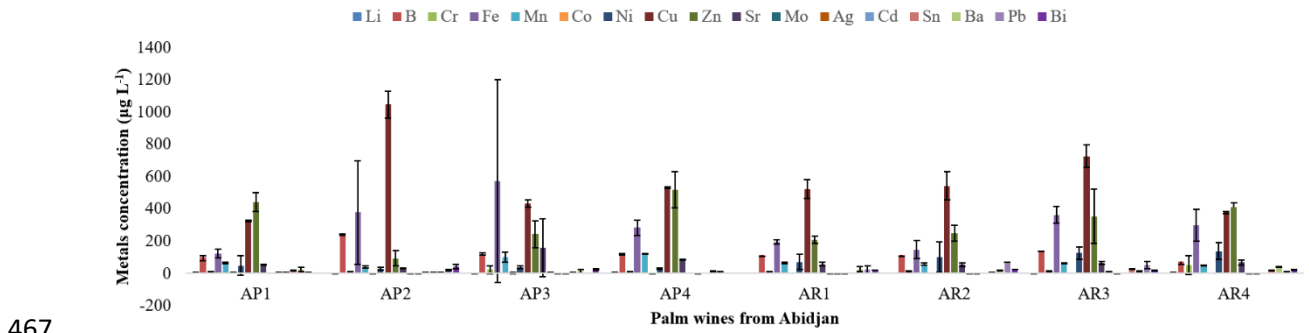
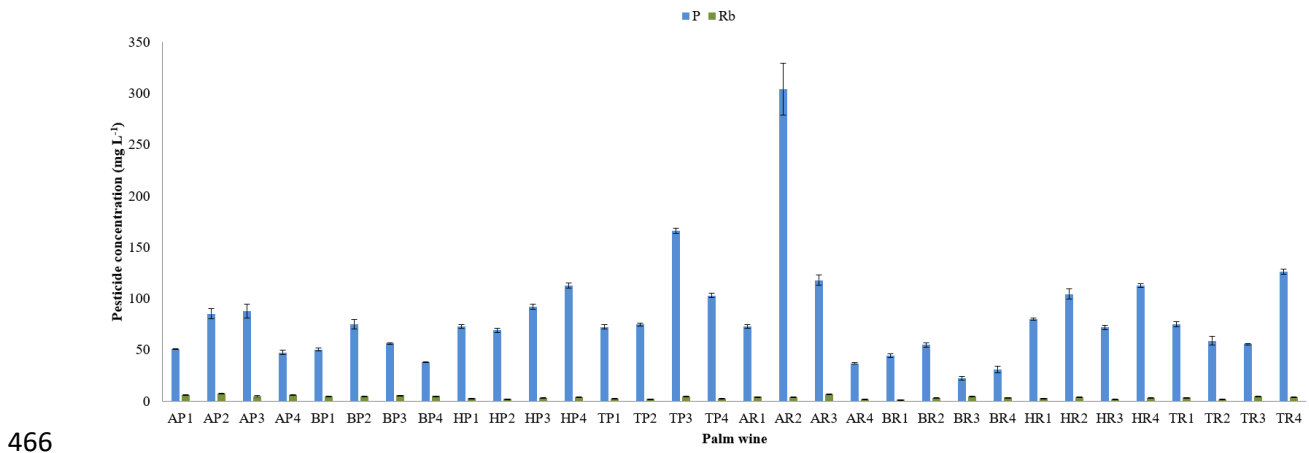
460 ^c Sala et al., 1997

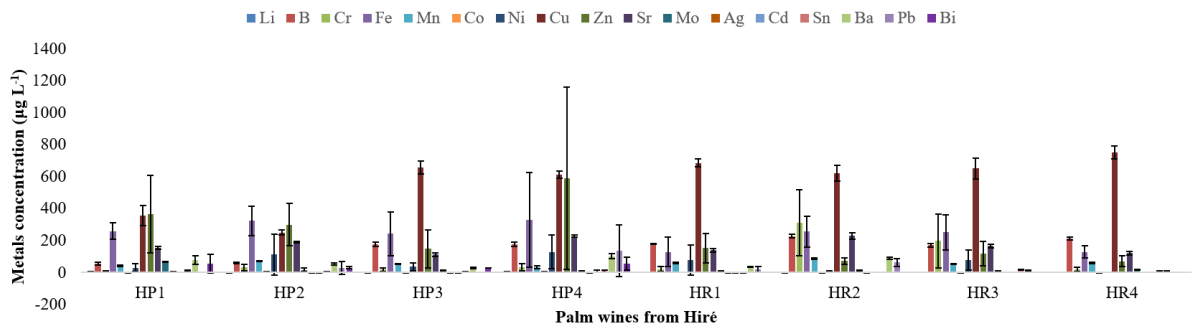
461 ^d dos Anjos & de Andrade, 2015

462

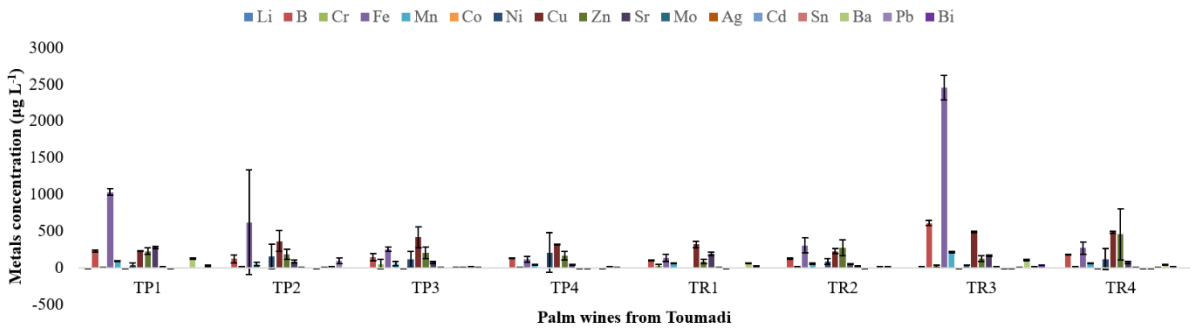
463 3.2 Determination of metals in palm wines

464 Thirty-two palm wine samples were mineralized and analyzed by ICP-AES and ICP-MS. The
 465 results are shown in Figure 2.





469



470

471 **Fig. 2.** Metals concentration in 32 palm wine samples, determined by ICP-AES and ICP-MS

472 The error bars on the graph can be important, because of the heterogeneity of the wine
 473 composition. Indeed, when an identical volume is taken, particles can be present, and those
 474 being certainly richer in metals, that can have an important role on the concentration of metals.

475 First, barium, boron, chromium, copper, iron, lead, manganese, phosphorus, rubidium,
 476 strontium, and zinc were found in all palm wine samples. The International Organization of
 477 Vine and Wine (OIV) recognized maximum limits for only a few elements in wine (Pb, Cd, Cu,
 478 Na, Zn, Ag, As, F, Br, F, and B). The levels of the other metals in palm wine, which is composed
 479 by 88% of water, can be compared with metal limits of drinking water. The higher
 480 concentrations for the most wine samples were found for phosphorus, rubidium, copper and
 481 iron, from 22.2 mg L⁻¹ (BR3) to 303.9 mg L⁻¹ (AR2) for phosphorus, from 1.5 mg L⁻¹ (BR1) to
 482 7.6 mg L⁻¹ (AP2) for rubidium, from 155.6 (BP4) to 1046.9 µg L⁻¹ (AP2, this concentration was
 483 above the OIV maximal limit of 1000 µg L⁻¹) for copper and from 116.4 (TP4) to 2453.5 µg L⁻¹
 484 (TR3) for iron. Manganese was mostly identified in samples from Aboisso, mainly in BR1

485 (641.6 $\mu\text{g L}^{-1}$) and BR2 (1001.3 $\mu\text{g L}^{-1}$) palm wines, at levels above the European maximal
486 limits for drinking water.

487 Then, six metals (cadmium, chromium, copper, iron, manganese, and nickel) were detected in
488 different samples at levels above the European maximal limits for drinking water or above the
489 OIV maximal limits for wine. Cadmium was detected in TP3 (13.8 $\mu\text{g L}^{-1}$) at level above limits
490 of 10 $\mu\text{g L}^{-1}$, and chromium in TP3 (51.4 $\mu\text{g L}^{-1}$), AR4 (52.2 $\mu\text{g L}^{-1}$), HR2 (313.4 $\mu\text{g L}^{-1}$), and
491 HR3 (197.9 $\mu\text{g L}^{-1}$) at level above limits of 50 $\mu\text{g L}^{-1}$.

492 Iron was at level above limits of 200 $\mu\text{g L}^{-1}$ in 24 samples (AP2, AP3, AP4, AR1, AR2, AR3,
493 AR4, BP1, BP2, BP3, BP4, BR1, BR2, BR3, BR4, HP1, HP2, HP3, HP4, HR1, HR2, HR3, HR4,
494 TP1, TP2, TP3, TP4, TR1, TR2, TR3, and TR4), from 242.2 (HP3) to 2453.5 $\mu\text{g L}^{-1}$ (TR3), and
495 nickel at level of 20 $\mu\text{g L}^{-1}$ in 28 samples too (AP1, AP2, AP3, AP4, AR1, AR2, AR3, AR4,
496 BP1, BP2, BP3, BR1, BR2, BR3, BR4, HP1, HP2, HP3, HP4, HR1, HR2, HR3, HR4, TP1,
497 TP2, TP3, TP4, TR1, TR2, TR3, and TR4), from 28.9 (AP4) to 699.8 $\mu\text{g L}^{-1}$ (BR2).

498 Zinc, which was detected at a range of concentrations between 28.6 and 589.9 $\mu\text{g L}^{-1}$, was also
499 found in wines from different European countries, such as Italy or France (Table 4), at similar
500 and higher levels (for example, between 289 and 967 $\mu\text{g L}^{-1}$ for Italian wines). As for Italian
501 and French wines, zinc was not detected at levels above the OIV maximal limits for wine.
502 Moreover, cadmium was detected in one sample (TP3) at concentration above the OIV maximal
503 limit of 10 $\mu\text{g L}^{-1}$. However, its concentration in Italian and French wines was not at levels
504 above the limits.

505 Metals, like copper, iron, and zinc, are essential for human at low concentrations. However, at
506 important concentrations, they can pose a real danger. For example, drinking water
507 contaminated by cadmium and lead can cause renal failure or chromium and nickel can induce
508 hair loss (Singh Sankhla et al., 2016).

509 Finally, the palm wine TP3 from Toumodi was the most polluted sample with metals at levels
 510 above European and OIV maximal limits (cadmium, chromium, iron, manganese and nickel),
 511 given Table 4.

512 **Table 4**

513 KED, or STD mode, LODs and LOQs of metals in palm wines, European and OIV Maximal
 514 Limits of metal in drinking water and wine, and comparison with other studies

Metals	Mode	LOQ ($\mu\text{g L}^{-1}$)	LOD ($\mu\text{g L}^{-1}$)	European Maximal Limits in drinking water ($\mu\text{g L}^{-1}$)	OIV Maximal Limits ($\mu\text{g L}^{-1}$)	Concentrations in French wines (mg L^{-1}) ^a	Concentrations in Italian wines (mg L^{-1}) ^a	Concentrations in Italian wines ($\mu\text{g L}^{-1}$) ^b	This study or (mg L^{-1}) ^c
Barium	KED	1.5	0.45	-	-				3.65- 132.4
Bismuth	STD	17	5.1	-	-				18.5- 146.3
Boron	STD	4	1.2	1500	8000 0			1995- 9489	55.7- 614.4
Cadmium	STD	0.75	0.23	5	10	ND- 0.0002	0.0012- 0.0016	0.17-3.7	0.36- 13.8
Chromium	KED	5	1.5	50	-	0.006- 0.09	0.02-0.05		5.86- 313.4
Cobalt	STD	0.5	0.15	-	-	0.004- 0.005	0.003- 0.006		0.52- 7.35
Copper	KED	3	0.9	2000	1000	ND-0.48	0.001- 1.34	14-456	155.6- 1046.9
Iron	KED	16.5	4.95	200	-	0.81- 2.51	1.35- 27.8		116.4- 2453.5
Lead	KED	8.5	2.5	10	150	0.006- 0.023	0.01- 0.35	3-59	0.38- 135.4
Lithium	STD	0.25	0.08	-	-				0.33- 18.0
Manganese	KED	1	0.3	50	-	0.63- 0.96	0.67-2.5		33.2- 1001.3
Molybdenum	KED	1.3	0.38	-	-				1.36- 69.0
Nickel	KED	9.3	2.9	20	-	ND- 0.052	0.015- 0.21		12.9- 699.8
Phosphorus	-	450	140	-	-				22.2- 303.8 ^c

Rubidium	-	80	20	-	-				1.52- 7.62 ^c
Silver	KE D	0.5	0.15	-	-				0.51- 2.74
Strontium	KE D	2	0.6	-	-				33.2- 281.7
Tin	KE D	1.3	0.38	-	-				2.18- 24.8
Zinc	KE D	12	3.6	-	5000	0.44- 0.74	0.135- 4.8	289-967	28.6- 589.9

515 ^a Płotka-Wasyłka et al., 2018

516 ^b Caridi et al., 2019

517 **4. Conclusion**

518 In this study, the concentration of pesticides and metals in palm wines from Ivory Coast using
519 HS-SPME/GC-MS and ICP-AES/MS were determined. SPME conditions (equilibrium time,
520 extraction time and temperature, stirring, and salinity) were optimized thanks to an
521 experimental design with 45 trials. The developed method used a DVB/C-WR/PDMS fiber and
522 the palm wine samples were equilibrated for 11.25 min and extracted for 50 min under
523 continuous stirring (437.5 rpm) at 52.5°C. The method was validated in terms of linearity,
524 repeatability, LOD, LOQ, and accuracy, and allowed the identification of 35 pesticides and the
525 quantification of 29 in 32 palm wine samples. Additionally, 7 pesticides were identified but
526 dichlorvos was the only one identified in 2 samples at levels above the European maximal limits.

527 In a second step, ICP-AES and ICP-MS were used to study the presence of 19 metals in palm
528 wine samples. Barium, boron, chromium, copper, iron, lead, manganese, rubidium, strontium,
529 and zinc explored were identified in all samples of palm wine. Moreover, 6 metals (cadmium,
530 chromium, copper, iron, manganese, and nickel) were detected in different samples at levels
531 above the European maximal limits for drinking water or above the OIV maximal limits for
532 wine.

533 Finally, pesticides (dichlorvos, endrin ketone, parathion methyl, p,p-DDD, p,p-DDE, p,p-DDT,
534 and pirimidiphos methyl) were found in palm wines from the 4 cities, in 3 samples from Abidjan,

535 1 from Aboisso, 1 from Hiré, and 5 from Toumodi. In addition, one of the palm wine samples
536 from Toumodi was the most polluted sample with metals (cadmium, chromium, iron,
537 manganese and nickel) at levels above European or OIV maximal limits.

538 Maximal limits for metals have been only defined for boron, cadmium, copper, lead and zinc.
539 As shown in this article, samples may contain pesticides and metals in high concentrations. This
540 beverage is largely consumed by local population. It would be important to develop more
541 studies on the composition of palm wine and maximal limits especially for pesticides in wines,
542 in order to be aware of the potential danger of its consumption.

543 **List of abbreviations**

544 PA: polyacrylate

545 BHC: Hexachlorocyclohexane

546 Carbon WR: Carbon Wide Range

547 DVB: Divinylbenzene

548 EML: European Maximal Limits

549 GC: Gas Chromatography

550 GC-MS: Gas Chromatography coupled with Mass Spectrometry

551 HS-SPME: Headspace Solid-Phase Microextraction

552 ICP-AES: Inductively Coupled Plasma - Atomic Emission Spectrometry

553 ICP-MS: Inductively Coupled Plasma - Mass Spectrometry

554 KED: Kinetic Energy Discrimination mode

- 555 LOD: limit of detection
- 556 Log Kow: octanol-water partition coefficient
- 557 LOQ: limit of quantification
- 558 MS: mass spectrometry
- 559 OCPs: OrganoChloride Pesticides
- 560 OIV: International Organization of Vine and Wine
- 561 OPPs: OrganoPhosphorus Pesticide
- 562 PDMS: Polydimethylsiloxane
- 563 p,p'-DDD: Dichlorodiphenyldichloroethane
- 564 p,p'-DDE: Dichlorodiphenyldichloroethylene
- 565 p,p'-DDT Dichlorodiphenyltrichloroethane
- 566 RSD: relative standard deviation
- 567 SIM: Single Ion Monitoring
- 568 STD: Standard mode
- 569 SPME: Solid-Phase Microextraction
- 570 **Authors' contributions**
- 571 Juliette Vievard wrote the paper, performed the analysis, collected the data
- 572 Theodore N'Dede Djeni wrote the paper, gave samples
- 573 Christine Devouge-Boyer contributed to data and analysis

574 Monica Arellano Gisel Sanchez wrote the paper
575 Aké Francine collected the samples
576 Tiemele Laurent Simon Amoikon wrote the paper, gave samples
577 Aminata Nadjoua Coulibaly, wrote the paper, collected the samples
578 Mélanie Mignot oversight the study, reviewed the paper

579 **Acknowledgements**

580 The authors would like to thank Marie Laget who helped for the analyses of metals by ICP-AES. This
581 work has been partially supported by University of Rouen Normandy, INSA Rouen Normandy, the
582 Centre National de la Recherche Scientifique (CNRS), European Regional Development Fund (ERDF)
583 N° HN0001343, Labex SynOrg (ANR-11-LABX-0029), Carnot Institute I2C, the graduate school for
584 research XL-Chem (ANR-18-EURE-0020 XL CHEM) and by Region Normandie. HS-SPME GC/MS
585 has been financed by RIN 2019 FSP-CHEM 18P03656 and ICP/MS though Bioenairgy project (FEDER
586 N° HN0001343). This work was also supported by International Foundation For Science (IFS) project
587 E_037779.

588 **References**

- 589 1. AFNOR - Water quality - Application of inductively coupled plasma mass spectrometry
590 (ICP-MS) - Part 2: determination of selected elements including uranium isotopes
- 591 2. Balsini, P., & Parastar, H. (2019). Development of multi-response optimization and quadratic
592 calibration curve for determination of ten pesticides in complex sample matrices using
593 QuEChERS dispersive liquid–liquid microextraction followed by gas chromatography. *Journal*
594 *of Separation Science*, 42(23), 3553-3562. <https://doi.org/10.1002/jssc.201900823>
- 595 3. Battcock, M., Azam-Ali, S., & Nations, F. and A. O. of the U. (1998). *Fermented Fruits and*
596 *Vegetables: A Global Perspective*. Food & Agriculture Org. (FAO) Agricultural Services
597 *Bulletin N° 134*.

- 598 4. Bernardi, G., Guidetti Vendruscolo, R., dos Santos Ferrão &, T., Smanioto Barin, J., Cichoski,
599 A. J., & Wagner, R. (2015). Jelly Palm (*Butia odorata*) Wine : Characterization of Volatile
600 Compounds Responsible for Aroma. *Chromatography*, 277-292.
601 <https://doi.org/10.1007/s12161-014-9835-5>
- 602 5. Bianchin, J. N., Nardini, G., Merib, J., Dias, A. N., Martendal, E., & Carasek, E. (2014).
603 Screening of volatile compounds in honey using a new sampling strategy combining multiple
604 extraction temperatures in a single assay by HS-SPME–GC–MS. *Food Chemistry*, 145,
605 1061-1065. <https://doi.org/10.1016/j.foodchem.2013.08.139>
- 606 6. Bonansea, R. I., Amé, M. V., & Wunderlin, D. A. (2013). Determination of priority pesticides
607 in water samples combining SPE and SPME coupled to GC–MS. A case study: Suquía River
608 basin (Argentina). 1860-1869. <https://doi.org/10.1016/j.chemosphere.2012.10.007>
- 609 7. Caridi, Pappaterra, Belmusto, Messina, Belvedere, D’Agostino, & Settineri. (2019).
610 Radioactivity and Heavy Metals Concentration in Italian (Calabrian) DOC Wines. *Applied*
611 *Sciences*, 9(21), 4584. <https://doi.org/10.3390/app9214584>
- 612 8. Chandrasekhar, K., Sreevani, S., Seshapani, P., & Pramodhakumari, J. (2012). A Review on
613 palm wine. *International Journal of Research in Biological Sciences*.
- 614 9. Cincotta, F., Verzera, A., Tripodi, G., & Conduro, C. (2015). Determination of
615 Sesquiterpenes in Wines by HS-SPME Coupled with GC-MS. 410-421.
616 <https://doi.org/10.3390/chromatography2030410>
- 617 10. Cunningham, A. B., & Wehmeyer, A. S. (1988). Nutritional value of palm wine
618 from *Hyphaene coriacea* and *Phoenix reclinata* (Arecaceae). *Economic Botany*, 42(3), 301-306.
619 <https://doi.org/10.1007/BF02860158>
- 620 11. dos Anjos, J. P., & de Andrade, J. B. (2015). Simultaneous determination of pesticide
621 multiresidues in white wine and rosé wine by SDME/GC-MS. *Microchemical Journal*, 120,
622 69-76. <https://doi.org/10.1016/j.microc.2015.01.009>

- 623 12. Fatoye FO, Morakinyo O (2002). Substance use amongst secondary school students in rural
624 and urban communities in south western Nigeria. *East Afr. Med. J.*, 79(6): 299–305.
625 <https://doi.org/10.4314/eamj.v79i6.8849>
- 626 13. Filho, A. M., Dos Santos, F. N., & De Paula Pereira, P. A. (2010). Development, validation
627 and application of a methodology based on solid-phase micro extraction followed by gas
628 chromatography coupled to mass spectrometry (SPME/GC–MS) for the determination of
629 pesticide residues in mangoes. 346-354. <https://doi.org/10.1016/j.talanta.2009.12.008>
- 630 14. Gambacorta, G., Faccia, M., Lamacchia, C., Di Luccia, A., & La Notte, E. (2005). Pesticide
631 residues in tomato grown in open field. *Food Control*, 16(7), 629-632.
632 <https://doi.org/10.1016/j.foodcont.2004.07.002>
- 633 15. Han, Q., Wang, Z., Xia, J., Zhang, X., Wang, H., & Ding, M. (2014). Application of
634 graphene for the SPE clean-up of organophosphorus pesticides residues from apple juices.
635 *Journal of Separation Science*, 37(1-2), 99-105. <https://doi.org/10.1002/jssc.201301005>
- 636 16. Ho, W.-H., & Hsieh, S.-J. (2001). Solid phase microextraction associated with microwave
637 assisted extraction of organochlorine pesticides in medicinal plants. *Analytica Chimica Acta*,
638 428(1), 111-120. [https://doi.org/10.1016/S0003-2670\(00\)01219-8](https://doi.org/10.1016/S0003-2670(00)01219-8)
- 639 17. Journal officiel de l'Union européenne (2020): DIRECTIVE (UE) 2020/2184 DU
640 PARLEMENT EUROPÉEN ET DU CONSEIL du 16 décembre 2020 relative à la qualité des
641 eaux destinées à la consommation humaine (refonte)
- 642 18. Konradsen F (2007) Acute pesticide poisoning: a global public health problem. *Dan Med*
643 *Bull* 54(1):58–59. Retrieved from <https://www.ncbi.nlm.nih.gov/>
- 644 19. Lewen, N., Mathew, S., Schenkenberger, M., & Raglione, T. (2004). A rapid ICP-MS
645 screen for heavy metals in pharmaceutical compounds. *Journal of Pharmaceutical and*
646 *Biomedical Analysis*, 35(4), 739-752. <https://doi.org/10.1016/j.jpba.2004.02.023>

- 647 20. Mollet, M., Herzog, F., Behi, Y. et al. Sustainable Exploitation of *Borassus aethiopum*,
648 *Elaeis guineensis* and *Raphia hookeri* for the Extraction of Palm Wine in Côte d'Ivoire.
649 *Environment, Development and Sustainability* 2, 45–59 (2000).
650 <https://doi.org/10.1023/A:1010035915512>
- 651 21. Munawar, A., & Waqas Hameed, S. (2013). Quantification of Pesticide Residues in
652 Vegetables by Different Chromatographic Techniques. *Journal of Chromatography &*
653 *Separation Techniques*, 04(08). <https://doi.org/10.4172/2157-7064.1000200>
- 654 22. National Pesticide Information Center – NPIC. (2011). Lambda-cyhalothrin (General Fact
655 Sheet). Corvallis: NPIC.
- 656 23. Okafor, N. (1978). Microbiology and Biochemistry of Oil-Palm Wine. In D. Perlman (Éd.),
657 *Advances in Applied Microbiology* (Vol. 24, p. 237-256). Academic Press.
658 [https://doi.org/10.1016/S0065-2164\(08\)70642-7](https://doi.org/10.1016/S0065-2164(08)70642-7)
- 659 24. Organisation Internationale de la vigne et du vin (OIV). (2015): International Code of
660 Oenological Practices, Annex: Maximum Acceptable Limits, Issue 2015/01. Paris, France.
- 661 25. Ouoba, L. i. i., Kando, C., Parkouda, C., Sawadogo-Lingani, H., Diawara, B., & Sutherland,
662 J. p. (2012). The microbiology of Bandji, palm wine of *Borassus akeassii* from Burkina Faso:
663 Identification and genotypic diversity of yeasts, lactic acid and acetic acid bacteria. *Journal of*
664 *Applied Microbiology*, 113(6), 1428-1441. <https://doi.org/10.1111/jam.12014>
- 665 26. Pellati, F., Prencipe, F. P., & Benvenuti, S. (2013). Headspace solid-phase microextraction-
666 gas chromatography–mass spectrometry characterization of propolis volatile compounds.
667 *Journal of Pharmaceutical and Biomedical Analysis*, 84, 103-111.
668 <https://doi.org/10.1016/j.jpba.2013.05.045>
- 669 27. Płotka-Wasyłka, J., Frankowski, M., Simeonov, V., Polkowska, Ż., & Namieśnik, J. (2018).
670 Determination of Metals Content in Wine Samples by Inductively Coupled Plasma-Mass
671 Spectrometry. *Molecules*, 23(11), 2886. <https://doi.org/10.3390/molecules23112886>

672 28. Redhead, J. (1989). Utilization of Tropical Foods: Trees. In FAO Food and Nutrition No 43
673 (3) FAO, Rome. pp. 52.

674 29. Rezić, I., & Steffan, I. (2007). ICP-OES determination of metals present in textile materials.
675 *Microchemical Journal*, 85(1), 46-51. <https://doi.org/10.1016/j.microc.2006.06.010>

676 30. Rodrigues, F. de M., Mesquita, P. R. R., de Oliveira, L. S., de Oliveira, F. S., Menezes Filho,
677 A., de P. Pereira, Pedro. A., & de Andrade, J. B. (2011). Development of a headspace solid-
678 phase microextraction/gas chromatography–mass spectrometry method for determination of
679 organophosphorus pesticide residues in cow milk. *Microchemical Journal*, 98(1), 56-61.
680 <https://doi.org/10.1016/j.microc.2010.11.002>

681 31. Ruf François, 2014. Cocoa combined with palm wine in Côte d’Ivoire: An unexpected
682 resilience. *Bois et Forêts des Tropiques* N° 3 2 1 (3).
683 <https://doi.org/10.19182/bft2014.321.a31216>

684 32. Sakamoto, M., & Tsutsumi, T. (2004). Applicability of headspace solid-phase
685 microextraction to the determination of multi-class pesticides in waters. *Journal of*
686 *Chromatography A*, 1028(1), 63-74. <https://doi.org/10.1016/j.chroma.2003.11.066>

687 33. Sala, C., Busto, O., & Guasch, J. (1997). Quick gas chromatographic method for
688 determining common pesticides in musts and wines. 44(5), 5.

689 34. Sam, K. G., Andrade, H. H., Pradhan, L., Pradhan, A., Sones, S. J., Rao, P. G. M., &
690 Sudhakar, C. (2008). Effectiveness of an educational program to promote pesticide safety
691 among pesticide handlers of South India. *International Archives of Occupational and*
692 *Environmental Health*, 81(6), 787-795. <https://doi.org/10.1007/s00420-007-0263-3>

693 35. Sambou, B., Goudiaby, A., Ervik, F., Diallo, D. and Camara, M.C. (2002) Palm wine
694 harvesting by the Bassari threatens *Borassus aethiopum* populations in north-western Guinea.
695 *Biodivers Conserv* 11, 1149–1161. <https://doi.org/10.1023/A:1016005025090>

- 696 36. Simon Amoikon, T. L., Marcotte, S., N'Dede Djeni, T., Celaire N'Sa, K. M., Grondin, C.,
697 Tinsley, C., Casaregola, S., & Dje, M. K. (2020). A study on the potential of yeasts isolated
698 from palm wines to produce flavouring compounds. *LWT*, 128, 109506.
699 <https://doi.org/10.1016/j.lwt.2020.109506>
- 700 37. Santiago, M. A. P. A., dos Anjos, J. P., Nascimento, M. M., da Rocha, G. O., & de Andrade,
701 J. B. (2021). A miniaturized simple binary solvent liquid phase microextraction (BS-LPME)
702 procedure for pesticides multiresidues determination in red and rosè wines. *Microchemical*
703 *Journal*, 167, 106306. <https://doi.org/10.1016/j.microc.2021.106306>
- 704 38. Singh Sankhla, M., Kumari, M., Nandan, M., Kumar, R., & Agrawal, P. (2016). Heavy
705 Metals Contamination in Water and their Hazardous Effect on Human Health-A Review.
706 *International Journal of Current Microbiology and Applied Sciences*, 5, 759-766.
707 <https://doi.org/10.20546/ijcmas.2016.510.082>
- 708 39. Stringini, M., Comitini, F., Taccari, M., & Ciani, M. (2009). Yeast diversity during tapping
709 and fermentation of palm wine from Cameroon. *Food Microbiology*, 26(4), 415-420.
710 <https://doi.org/10.1016/j.fm.2009.02.006>
- 711 40. Tsoukali, H., Theodoridis, G., Raikos, N., & Grigoratou, I. (2005). Solid phase
712 microextraction gas chromatographic analysis of organophosphorus pesticides in biological
713 samples. *Journal of Chromatography B*, 822(1-2), 194-200.
714 <https://doi.org/10.1016/j.jchromb.2005.05.035>
- 715 41. Ukhun, M.E.; Okolie, N.P.; Onyerinde, A.O. Some mineral profile of fresh and bottled palm
716 wine—A comparative study. *Afr. J. Biotechnol.* 2005, 4, 829–832.
- 717 42. Velkoska-Markovska, L., & Petanovska-Ilievska, B. (2013). Optimization and development
718 of SPE-HPLC-DAD method for the determination of atrazine, malathion, fenitrothion and
719 parathion pesticide residues in apple juice. *Macedonian Journal of Chemistry and Chemical*
720 *Engineering*, 32(2), 299-308. <https://doi.org/10.20450/mjce.2013.449>

721 43. Vitali, M., Guidotti, M., Giovinazzo, R., & Cedrone, O. (1998). Determination of pesticide
722 residues in wine by SPME and GC/MS for consumer risk assessment. *Food Additives and*
723 *Contaminants*, 15, 280-287. <https://doi.org/10.1080/02652039809374642>

724 44. Yaméogo, J., Belem-Ouédraogo, M., Bayala, J., Ouédraogo, M.B., Guinko, S., 2008. Uses
725 and commercialization of *Borassus akeassii* Bayton, Ouédraogo, Guinko non-wood timber
726 products in South-Western Burkina Faso, West Africa. *Biotechnol. Agron. Soc. Environ.* 12(1),
727 47–55. <https://popups.uliege.be/1780-4507/index.php?id=2218>.

728 45. Yang, X., Zhang, H., Liu, Y., Wang, J., Zhang, Y. C., Dong, A. J., Zhao, H. T., Sun, C. H.,
729 & Cui, J. (2011). Multiresidue method for determination of 88 pesticides in berry fruits using
730 solid-phase extraction and gas chromatography–mass spectrometry: Determination of 88
731 pesticides in berries using SPE and GC–MS. *Food Chemistry*, 127(2), 855-865.
732 <https://doi.org/10.1016/j.foodchem.2011.01.024>

733 46. Ziadi, M., M'hir, S., Kbaier, N., Hamdi, M. and Ferchichi, A. (2011) Microbiological
734 analysis and screening of lactic acid bacteria from Tunisian date palm sap. *Afr J Microbiol Res*
735 5, 2929–2935. <https://doi.org/10.5897/AJMR11.325>

736 47. Zhu, F., Ruan, W., He, M., Zeng, F., Luan, T., Tong, Y., Lu, T., & Ouyang, G. (2009).
737 Application of solid-phase microextraction for the determination of organophosphorus
738 pesticides in textiles by gas chromatography with mass spectrometry. *Analytica Chimica Acta*,
739 650(2), 202-206. <https://doi.org/10.1016/j.aca.2009.07.050>