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Occurrence of bisphenols and contribution of edible liquids conditioned in plastic packaging to the dietary exposure in Cameroon

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25 **Abstract**

26 Bisphenols are known to be endocrine disruptors. They are ubiquitous in the environment and
27 also in plastic materials such as those used in food packaging. They can lead to the
28 contamination of food in contact with environmental products or plastic packaging. In Africa,
29 there a few studies on the assessment of the amount of bisphenols in foods; hence, most
30 African countries do not have policies guiding on their utilization. The present work is aimed
31 at assessing bisphenol levels in sachet water, bagged whisky, crude palm oil and traditional
32 alcoholic beverages of Cameroon, in Central Africa. A sensitive method based on liquid
33 chromatography tandem mass spectrometry (UPLC-MS/MS) was developed and validated for
34 the simultaneous determination of bisphenol A, B and F (BPA, BPB and BPF). This method
35 showed that recoveries were from 72 to 120% and the limits of quantification for BPA, BFB
36 and BPF from aqueous samples were 0.09, 0.10, and 0.09 $\mu\text{g L}^{-1}$ respectively. Estimation of
37 the contribution to dietary exposure of BPA from these matrices were lower than temporary
38 Tolerable Daily Intake (t-TDI) (4 $\mu\text{g/kg}$ of body weight/day) defined by The European food
39 safety authority (EFSA). One brand of sachet water contained BPF at $14.5 \pm 1.1 \mu\text{g L}^{-1}$.

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41 **Keywords:** Bisphenols, dietary exposure, plastic, foods, Cameroon, UPLC-MS/MS.

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56 **1. Introduction**

57 Bisphenols are chemical compounds which have two phenol groups in their structures. They
58 are added in plastic polymers to ameliorate their physicochemical properties such as
59 flexibility, impermeability, low electrical conductivity and low chemical reactivity.
60 Bisphenols are antioxidants and UV stabilizers in plastic materials. Bisphenol A (BPA) is the
61 most known among bisphenols. BPA is also the monomer of polycarbonate and epoxy resins
62 (glue, paint, etc). Polycarbonate are used to produce baby bottles, water bottles and other
63 products with plastic coating (food and drink cans, dolls, DVD,spoons, etc) (Baluka &
64 Rumbeiha, 2016; Cao & Corriveau, 2008; Omegna et al., 2015).

65 The main source of human exposure to bisphenols is the diet, through packaged foods and
66 beverages (Baluka & Rumbeiha, 2016; Wang et al., 2022). These latter are contaminated by
67 bisphenols migrating from the plastic packaging. Exposition of plastic packaging to
68 physicochemical factors such as UV light, abrasion, aging, storage time, heating, acidity,
69 alkalinity...facilitates migration of their additives or monomers to foods; The contact with
70 hot, polar or fatty food also increase this migration (Hoekstra & Simoneau, 2013;
71 Szczepańska et al., 2019; Xian et al., 2017). Inside the human body, BPA can create health
72 problems to consumers. In fact, BPA is an endocrine disruptor (EDC) (Ortiz-Villanueva et
73 al., 2018). It alters immune functions, increase aggressiveness of teenagers, causes
74 abnormalities in reproduction and cardiovascular disorders (Eng et al., 2013; Jiang et al.,
75 2020; Manfo et al., 2019). BPA is also involved in breast and prostate cancers, and in the
76 morbidity of asthma in children (Quirós-Alcalá et al., 2020; Soto & Sonnenschein, 2010).

77 In response to the devastating effects of BPA as mentioned above, several developed
78 countries have set rules and/or policies regulation their use. From 2008 to 2013, Canada, the
79 United stated and European Union exclude BPA from list of additives used in the
80 manufacturing of baby feeding bottles, toys and coating of infant formula packaging
81 (Almeida et al., 2018; Baluka & Rumbeiha, 2016; Dreolin et al., 2019; Vandenberg et al.,
82 2009). In 2015, The European food safety authority (EFSA) reduced the safe level or
83 Tolerable Daily Intake (TDI) of BPA from 50µg/kg of body weight/day to 4µg/kg of body
84 weight/day; This last value is temporary Tolerable Daily Intake (t-TDI) (EFSA, 2015). The
85 Directive EC N° 213/2018 of European Union specify a new Specific Migration Limit (SML)
86 of BPA from packaging at 0.05 mg/kg of food (The European Commission, 2018)..

87 Some manufacturers have substituted BPA into plastic materials by other bisphenols (B, F,
88 S...); but recent studies show that these bisphenols also have negative effects on human and
89 environmental health (Guo et al., 2021; Rochester & Bolden, 2015; Serra et al., 2019);
90 However there is no regulation concerning these analogues of bisphenol A.

91 In Africa in general and particularly in Cameroon, the topic of bisphenols contamination
92 through plastic leaching is very little discussed (Bousoumah et al., 2021); and there is no
93 regulation on BPA, although it has been shown that polycarbonate (PC) containers are sold
94 there (Baluka & Rumbeiha, 2016; Pouokam et al., 2014). The quality control of plastic
95 products used in food packaging is not implemented in Cameroon, and markets abide with
96 contraband products at low prices. Moreover, there are several practices likely to increase the
97 migration of bisphenols from plastic containers. Empty bottles of mineral water are used to
98 preserve liquid foods with more acidic (natural juice), fatty (palm oil), or alcoholic
99 (traditional alcoholic beverages) characteristics. Some mineral water and whiskies are sold in
100 plastic bags and these are highly consumed by people on the street of cities and villages.

101 Bisphenols analysis in various matrices (foods, beverages, urine, saliva, water, waste water...)
102 has been developed by radioimmunity, competitive ELISA, electrochemistry and
103 chromatography (HPLC-UV, HPLC-FLD, GC or LC-MS, GC or LC-MS/MS) (Manfo et al.,
104 2019; Nemati et al., 2018; Niu et al., 2012; Notardonato et al., 2019; Stefan-van Staden et al.,
105 2014; Xiong et al., 2018). Liquid chromatography coupled to tandem mass spectrometer (LC-
106 MS/MS) is a rapid, sensitive and specific method for the simultaneous quantification of
107 bisphenols. It does not require the additional derivatization step involved in gas
108 chromatography after extraction. Bisphenols are extracted from food matrices by solid phase
109 extraction (SPE) and liquid-liquid extraction (LLE). The recovery in LC-MS/MS values differ
110 from authors (Notardonato et al., 2019; Regueiro & Wenzl, 2015; Xiong et al., 2018). The
111 aim of this study is to assess the level of bisphenols contamination in liquid foods packaged
112 in plastic packaging in Cameroon; in order to identify the exposure of the population to
113 bisphenols from these food sources. The present study will also propose a suitable method of
114 bisphenols extraction from traditional alcoholic beverages in Cameroon.

115 **2. Materials and methods**

116 **2.1. Reagents and standards**

117 Standard of bisphenol A ($\geq 99\%$), bisphenol F ($\geq 98\%$), bisphenol B ($\geq 98\%$) and Bisphenol A-
118 d_{16} (99.1% D, 98% isotopic enrichment) were all purchased from Sigma – Aldrich (Diegem,

119 Belgium). The chemical structures of bisphenols are presented in [Figure 1](#). These standard
120 were used to prepare the stock solution of each analyte and mixture at 1mg/mL of methanol.
121 To perform analysis working standard solutions were prepared by dilution of the stock
122 solutions. Then there were stored at 4°C in amber vials. All organic solvents (ethyl acetate,
123 methanol, dichloromethane and hexane) were HPLC or LC/MS grade and the other reagents
124 were analytical grade. Ammonium hydroxide (28-30%) was purchased from Merck
125 (Darmstadt, Germany). SPE Oasis MAX cartridges (150 mg, 6 mL) were purchased from
126 Waters (Milford, MA). Ultrapure water was obtained by the Milli-Q gradient purification
127 system from Merck Millipore (Bedford, MA, USA).

128 **2.2. Equipment**

129 The ME 235-P balance (Sartorius, Germany) was used for weighing; To degas traditional
130 alcoholic beverages, the Branson 3510 ultrasonic tank (Bransonic, Germany) was used. The
131 pH was determined with the Seven easy pH meter (Mettler Toledo, France). The RII rotary
132 evaporator, (Buchi, Germany) allowed the concentration of the extracts. The IR vortex (Star
133 LAB, France) was used for homogenization, and centrifugation of samples into vials was
134 done by The Universal 320 R Centrifuge (Hettich, Germany). An ACQUITY ultra high
135 performance liquid chromatography system coupled to a Waters XevoTQ triple quadrupole
136 tandem mass spectrometer (Waters Co., USA) was used for bisphenols analysis.

137 **2.3. Samples**

138 Two brands of sachet water, two brands of bagged whiskey, two traditional fermented drinks
139 (palm wine and fermented sorghum beverages called “bili bili”) and crude palm oil, were
140 purchased at the Mokolo market (GPS coordinates: 3.8756; 11.49802) in the city of Yaounde
141 in Cameroon. For each type of sample or brand, five units were purchased. Crude palm oil
142 bottles were made of polyethylene terephthalate (PET). They were purchased in November
143 2020. The brands of sachet water and bagged whiskey were randomly selected. However, it
144 came from street vendors who sold them in wheelbarrows exposed to sunlight. Similarly, the
145 purchase points of crude palm oil and traditional fermented drinks were randomly selected in
146 the Mokolo market. Palm wine and Bili bili were respectively preserved in polypropylene
147 canisters and old polypropylene paint buckets; but with the purchase of each liter the sellers
148 packaged them in old mineral water bottles made in polyethyleneterephthalate (PET). Crude
149 palm oil were preserve in PET bottles. All samples (water, whiskey and wine) were stored at
150 2 – 8°C before analysis, except crude palm oil (25°C).

151 **2.4. Sample treatment: Extraction**

152 *Liquid-liquid extraction of bisphenols from water* was carried out as follow; 100 mL of water
153 sample containing 1 μg (100 μL of BPAd₁₆ at 10 μg per mL of methanol) of internal standard
154 and 10 mL of ethyl acetate were introduced in separating funnel. Then the mixture was stirred
155 for 20 seconds and allowed to stand for 5 min. The organic phase was removed and placed in
156 100 mL round neck flask. The extraction process was repeated twice on the aqueous phase,
157 and the collected organic phases (30 mL) were evaporated through rotary evaporator
158 (evaporation of solvent and water at 40°C). The residue was collected using 2.0 mL of
159 methanol/water (50/50; v/v), then the mixture was transferred to a 2 mL amber glass vial.
160 Finally, the collected extract was centrifuged (4°C, 5000 rpm, 10 min) to eliminate solid
161 particles. The supernatant was stored at 2–8°C before UPLC-MS/MS analysis. To determine
162 the extraction yield, this procedure was applied to water sample spiked with a mix of
163 bisphenols (A, B and F) at 0.1, 0.5 and 2.0 $\mu\text{g/L}$. Every experiment was done in four
164 replicates.

165 *The traditional fermented drinks* were subjected to liquid-liquid extraction (LLE) and to solid
166 phase extraction (SPE). Prior to extraction, beverages were degassed for 30 min using
167 ultrasonic tank. LLE of these beverages was carried out using various solvents namely
168 acetonitrile, hexane, ethyl acetate and dichloromethane. 100 mL of beverages and 1.0 μg of
169 BPAd₁₆ (100 μL of BPAd₁₆ at 10 μg per mL of methanol) were introduced in a separating
170 funnel, and the extraction solvent was added. The extraction procedure from beverages was
171 the same as the one from water; but in this case, the dry extract was recovered with 2 mL of
172 methanol/water (50/50; v/v).

173 The SPE column was washed twice with 6 mL of methanol, then with 6 mL of water and 6
174 mL of methanol/water (50:50, v/v) before use. For solid phase extraction, 1 μg of BPAd₁₆ was
175 initially added to 100 mL of drinks. The mixture was introduced from SPE column at the flow
176 rate of 5 mL/min. The analytes were then eluted from the SPE cartridge using 6 mL of
177 methanol (100%). The eluates collected were dried using a rotary evaporator, and the residue
178 was collected with 2 mL of methanol/water (50:50, v/v). In order to determine extraction
179 yield (recovery), 0.01, 0.05 and 0.2 μg of bisphenols (A, B and F) were added in 100 mL of
180 traditional fermented drinks. Every test was repeated four times.

181 Analysis of *bagged whiskey* did not have the extraction step. Samples were diluted five time,
182 then centrifuged (5000 rpm, 10 min, 4°C) and directly injected to UPLC-MS/MS. Previously,

183 1µg of BPA_{d16} (100µL of BPA_{d16} at 10µg per mL of methanol) was put into 2 mL vial and
184 1900 µL of diluted whiskey was added in the same vial.

185 *The extraction of bisphenols in crude palm oil* was carried out according to the protocol
186 described by Abou et al. (Abou Omar et al., 2017) that was modified to avoid using plastic
187 materials. Briefly, 5g of oil are weighed into 100 mL glass beaker and 1µg of BPA_{d16} (100µL
188 of BPA_{d16} at 10µg per mL in methanol) was added. Then, 15 mL of hexane and 4 mL of
189 methanol/water (50:50, v/v) were added. The mixture was homogenized in a separatory
190 funnel for 1 min, then allowed to stand for 5 min. The aqueous phase was collected in a
191 beaker and transferred to 2mL vials. The vials were vortexed for 1 min, then centrifuged
192 (5000 rpm, 10 min, 4°C). The supernatant was transferred to another vial. Finally, the extract
193 was directly injected in UPLC-MS/MS. The recovery was determined by spiking oil samples
194 with 0.8, 2, 10 and 20 ng of bisphenols per gram of oil. Every test was repeated four times.

195 **2.5. UPLC-MS/MS analysis**

196 Chromatographic separation was carried out using a Waters Acquity™ UPLC™ separation
197 module equipped with a Waters isolator column (P/N: 186003975) located before the
198 injection valve. Waters Acquity UPLC BEH C18 column (2.1 mm×50 mm; particle size, 1.7
199 µm) was used. The mobile phase for the bisphenols analysis without derivatization was a
200 mixture of water with 0.1% ammonium hydroxide and methanol. The flow rate was 0.4
201 mL/min under the following gradient conditions: methanol is maintained at 40% for 1min,
202 increases linearly from 40% to 80% in 3 min and then increased to 100% in 2.1 min, held for
203 1.8 min, and finally returned to the initial composition in 0.1 min. The column was
204 equilibrated for 2 min at the same flow rate before the next injection. The injection volume
205 was 2µL and the column oven was set at 25°C. MS/MS analysis was carried out using a
206 Waters Xevo™ TQD triple-quadrupole mass spectrometer equipped with an Electrospray
207 ionization (ESI) interface operating in negative ion mode (ESI-), with multiple-reaction
208 monitoring (MRM). The capillary voltage was 2.5 kV. Nitrogen gas (purity, 99.9%) was used
209 as the cone gas and desolvation gas at a flow rate of 50L/h and 1200 L/h, respectively. The
210 electrospray source block and desolvation temperatures were held at 150°C and 600°C,
211 respectively. Ion energy 1 was -0.5, and Ion energy 2 was 0.2. Ultra-high-purity argon was
212 used as the collision gas. Two transitions were selected for each bisphenol, and the cone
213 voltage and collision energy were optimized for maximum signal strength.

214 **2.6. Validation of methods and quality assurance**

215 Calibration solutions were prepared by spiking water with bisphenols at 0.1, 0.2, 0.4, 1.0, 1.5,
 216 2.0, 2.5 and 3.0 µg/L. This allows to appreciate the linearity of the methods. Internal standard
 217 curves for BPA, BPB and BPF were obtained by performing a linear regression analysis of
 218 the ratio of standard-solution areas to internal-standard areas versus concentration. The
 219 precision of the method and recovery were determined by spiking samples four times at 3
 220 levels. The concentration of compounds were analyzed by UPLC-MS/MS on the same day,
 221 and the intraday precision was calculated through variance intraday (formula (1)) and
 222 expressed as the relative standard deviation (%). The inter-day precision was quantified
 223 repeating test within three days successively and calculate variance inter-day (formula (2)).
 224 The inter-day precision (reproducibility) was also expressed as the relative standard deviation
 225 (%). Reagent blank was systematically made to ensure that samples were not contaminated by
 226 the medium and materials. After injection of ten samples, reagent blank and standard were
 227 systematically injected in UPLC-MS/MS. The detection and quantification limits (LOD and
 228 LOQ) were obtained by calculating the standard deviation of repeatability carried out with ten
 229 test of sample blank. The LOD was equal to the mean of the tests plus the value of three
 230 standard deviation. The LOQ was the mean of the tests plus the value of ten standard
 231 deviation. Depending on the matrices, three sample blanks were created; Ultrapure water,
 232 palm oil packaged in a glass bottle, and bili bili sold in a large calabash.
 233 The trueness of methods was determined from maximum difference (%) between the mean
 234 obtained from precision test and the expected value from calibration curves.

$$235 \quad V_{intra} = \frac{\sum(n_i-1)S_j^2}{N-K} \quad (1) \quad \text{with} \quad S_j^2 = \frac{\sum(x_{ij}-m_j)^2}{n_j-1}$$

$$236 \quad V_{inter} = \frac{\sum n_j(m_j-M)^2}{K-1} \quad (2)$$

237 Where V_{intra} is the intra-day variance; V_{inter} is the inter-day variance; i varie from 1 to n_j .
 238 (number of days); x_{ij} is the i^{th} value of the j^{th} day; m_j is the mean of the j^{th} day; K was the
 239 number of days; N is the total number of tests carried out in K days; j : 1 to K . M is the
 240 overall mean.

241 The 95% confidence interval (4) was determined through the compound uncertainty (3).

$$242 \quad u_c = \sqrt{U_{mrc}^2 + U_a^2} \quad (3) \quad \text{with} \quad U_a = \frac{s}{\sqrt{n}}$$

$$243 \quad CI = \pm / - k_{student}(\alpha, \gamma) * u_c \quad (4)$$

244 where u_c was the compound uncertainty; U_{mrc} the expanded uncertainty of the reference
245 material ($k_{mrc}=2$); U_a , the random uncertainty obtained from precision tests; s is the overall
246 standard deviation and n , number of tests; CI is the confidence interval; $k_{Student}$ (2.201) is the
247 Student constant. A degree of freedom of 11 ($\gamma = n-1$) and a risk of 5% ($\alpha = 0.05$) were used.

248 **2.7. Data processing and statistical analysis**

249 The Masslynx version 4.1 software allowed the acquisition and processing of
250 chromatographic analysis data; then the data were exported to Excel 2016. All the results
251 were presented with the standard deviation (%), or relative standard deviation.

252 **3. Results and discussion**

253 **3.1. Optimization of UPLC-MS/MS conditions**

254 Optimization of UPLC-MS/MS was done in two steps: identification of the analytes that gave
255 the highest signal intensity in mass spectrometry and improvement of the separation of
256 different analytes by the chromatographic method. As bisphenols are acidic compounds, the
257 highest signal intensity was obtained by electrospray ionization (ESI) mass spectrometry in
258 negative mode (ESI⁻). [Table 1](#) summarizes the result of ionization analyses of bisphenols.
259 These conditions were those which gave the highest signal intensity for the most stable MRM
260 transitions of analytes. The transitions obtained correspond to those presented in the literature
261 ([Xian et al., 2017](#); [Zhao et al., 2016](#)). Bisphenol solutions were then injected in UPLC-
262 MS/MS, to choose the transition with Gaussian curve and the highest signal intensity (best
263 transition).

264 [Figure 2](#) shows that the best transition for BPA correspond to the loss of a methyl radical
265 from of the molecular ion, $[M-H-(CH_3)]^-$, $m/z = 212.001$. That of BPB corresponds to the loss
266 of an ethyl radical from the molecular ion, $[M-H-(CH_2-CH_3)]^-$, $m/z = 212.04$; while the best
267 transition of BPF yields a phenoxide ion, $m/z = 92.992$, $[C_6H_5O]^-$ ([Zhao et al., 2016](#)). Once
268 the best transitions were chosen, the chromatographic separation was optimized by varying
269 the mobile phase gradient, flow rate, and injection volume. The chosen mobile phase for
270 bisphenols analysis (methanol and $H_2O/NH_3.H_2O$ (99.9%: 0.1%; v: v)) is the best for UPLC-
271 MS/MS ([Niu et al., 2012](#)). The presence of $NH_3.H_2O$ enhances bisphenols ionization. The
272 chosen gradient phases (section 2.5) are those with the best peaks (Gaussian shape, separated
273 peaks, less chopped and high signal intensity). The injection rates and injection volumes had
274 been varied. The flow rate of 0.4 min^{-1} was chosen. The injection volume of $2\mu\text{L}$ was used for
275 all analyzes.

276 **3.2. Bisphenols extraction from traditional fermented drinks and crude palm oil.**

277 [Figure 3](#) shows the recoveries of bisphenols (A, B and F) during liquid-liquid extraction with
278 different solvents, and during solid phase extraction (SPE) from traditional fermented drinks.
279 SPE recovery (> 90%) was better than liquid-liquid extraction recovery with polar and non-
280 polar solvents (<80%). The SPE recovery is comparable to those of other authors who worked
281 on wines ([Regueiro & Wenzl, 2015](#)). For liquid-liquid extraction, ethyl acetate (65-77%) has
282 the best recovery, followed by dichloromethane (55-60%), then hexane (20- 50%). The
283 recovery of BPB during hexane extraction is better than those of BPA and BPF. This is due to
284 the fact that BPB is more hydrophobic than the others. Bisphenols also have a hydrophilic
285 phenol function, so they cannot be optimally extracted by hexane when they are in
286 water/alcoholic samples. Ethyl acetate which is more polar than hexane but less than water or
287 alcohols (fermented product), better extracts bisphenols. However, liquid-liquid extraction
288 was difficult to perform due to the formation of a stable emulsion between the extraction
289 solvents and some constituents of these traditional drinks. No study on the extraction of
290 bisphenols from traditional fermented drinks (palm wine and fermented sorghum-based
291 drinks) was found in the literature.

292 [Table 2](#) presents the extraction recoveries, limits of detection and quantification (LOD and
293 LOQ) of bisphenols (A, B and F) in crude palm oil (Liquid-liquid extraction) and traditional
294 fermented drinks (SPE). The recovery in crude palm oil is above 85%. In fact, due to their
295 hydrophobic character, they can easily be extracted from hydrophobic media with a polar
296 solvent. Crude palm oil was first dissolved in hexane before bisphenols were extracted with
297 methanol/H₂O (50/50; v/v). The method used was described by Abou Omar et al., who had
298 not presented the obtained recovery of their study about BPA from refined olive oils ([Abou
299 Omar et al., 2017](#)). Recovery greater than 100% reflects a response due to a matrix effect. The
300 relative standard deviations were generally less than 10%.

301 **3.3. Validation of bisphenol analysis methods in water**

302 [Table 3](#) shows the liquid-liquid extraction recovery, linearity, uncertainty and bias (Trueness)
303 of bisphenols analysis. The recoveries range between 85-120%. These values are close to
304 those observed by other authors for liquid phase micro-extraction and solid phase extraction
305 ([Caban & Stepnowski, 2020](#); [Notardonato et al., 2019](#)). The LOQ were 0.09, 0.10 and 0.09
306 µg/L respectively for BPA, BPB and BPF. The relative uncertainties and maximum bias
307 (trueness) were less than 15% (absolute value). [Table 4](#) presents the intra-day (repeatability)
308 and inter-day (reproducibility) relative standard deviation from the precision tests. Analysis of

309 BPF has a better fidelity than BPA or BPB. The latter has a similar acidity (pKa) and
310 hydrophobicity (log K_{ow}) (Figure 1). BPF is more polar than these two compounds. The
311 intra-day relative standard deviation (RSD) are almost all less than 10%, indicating that the
312 values are not widely dispersed in the same day. The intra-day and inter-day RSD values are
313 close to those of other authors (Xian et al., 2017).

314 The comparison of the current work with previous procedure for the analysis of BPA in oils
315 and beverages is shown in Table 5. The analysis of crude palm oil is done only in two main
316 steps such as liquid-liquid extraction followed by homogenization and centrifugation of the
317 extracts at 4°C. The concentration or filtration step used by other authors (Table 5) were
318 avoided to reduce the risk of contamination. The sensitivity (LOD and LOD) of the developed
319 method is better than that of Xian et al., who also used UPLC-MS/MS (Xian et al., 2017).

320 The method developed for the analysis of bisphenols in alcoholic beverages has fewer steps
321 than those developed by authors who used LC-MS/MS (Table 5). Moreover, the solid phase
322 extraction time is less than 20 min, comparable to that of the Stärker & Welle (2019) method,
323 but less than that of the Regueiro & Wenzl method, (Regueiro & Wenzl, 2015). The method
324 of the present work also has a very good sensitivity (LOQ and LOD) compared to that of
325 other authors (Table 5). The method of Cunha et al., (Cunha et al., 2011) has a smaller LOQ
326 than that of the developed method, but the chromatographic retention time of bisphenol A is
327 6.7 min against 2.4 min in this study; therefore the LC method presented in this work is faster.

328 **3.4. Estimation of the contribution to dietary exposure**

329 The results of the analysis of samples from the Cameroonian market are presented in Table 6.
330 *Sachet waters*: Among the two sachets of mineral water analyzed, one had BPA ($2.2 \pm 0.2 \mu\text{g}$
331 L^{-1}) and BPF ($14.5 \pm 1.1 \mu\text{g L}^{-1}$). According to environmental health criteria (EHC) 240 of
332 WHO, a default body weight is 60 kg, and the maximum of water consumption for exposure
333 assessment must be 6 Liters (WHO, 2020). In this study, large consumers have been
334 considered in order to have maximum exposure conditions. For a concentration of $2.2 \mu\text{g}$ of
335 BPA/L maximum daily exposure through sachet water is $0.22 \mu\text{g kg}^{-1}$ of body weight/ day
336 ($\mu\text{g/kg bw/day}$); thus, 5.5% of the t-TDI ($4 \mu\text{g/kg bw/day}$). The same reasoning for BPF at
337 $14.5 \mu\text{g L}^{-1}$ give a maximum daily exposure of $1.45 \mu\text{g/kg bw/day}$ or 36.25% of the t-TDI (set
338 for BPA). To our knowledge, no study had focus to look for bisphenols in sachet waters,
339 which are nevertheless widely consumed in developing countries due to their availability,
340 accessibility and reduced cost. Several illegal production companies have emerged in cities

341 across Africa, raising doubt about the quality of products (Gake et al., 2022; Hermann et al.,
342 2018; Nyarko & Adu, 2016).

343 *Alcoholic drinks:* No bisphenols were detected in traditional fermented drink. These drinks
344 was packaged in old fuel cans (polyethylene) which was certainly washed or used several
345 times. Bisphenols analysis from traditional fermented drinks (Palm wine and Bili bili) is not
346 reported in the literature. Authors who were interested about bisphenols in other alcoholic
347 drinks obtained BPA and BPF values lower than $1.3\mu\text{g L}^{-1}$ (Cardama et al., 2017; Regueiro &
348 Wenzl, 2015). Among the two brands of bagged whiskey, one had BPA ($1.2 \pm 0.07\mu\text{g L}^{-1}$)
349 (Table 6). The consumption of spirits in Cameroon is about 40 mL per adult per day
350 according to household consumption data (Ingenbleek et al., 2017). On a conservative basis,
351 in order to overestimate the exposure, a consumption of 4 bagged whiskeys (or 200 mL) per
352 day by an adult of 60 kg was considered in this study. The calculated daily intake is 0.004
353 $\mu\text{g/kg}$ of bw/day, or 0.1% of t-TDI. No study on bisphenols from bagged whiskeys has been
354 reported in the literature to our knowledge. Bagged whiskeys would increase consumption of
355 spirits, due to the explosion of cheap supply and the addictive effects that go with ethanol. In
356 front of this situation, several African countries banned these products, but unfortunately are
357 still sold (Otim et al., 2019). A study showed that bagged whiskeys have high levels of heavy
358 metals (Copper, Arsenic, Plomb...) (Otim et al., 2019). The combined effects of BPA with
359 other compounds (Ethanol, heavy metals, etc) must be estimated in order to evaluate the
360 overall risk associated with the consumption of bagged whiskeys.

361 *Crude palm oil:* BPA at 0.4 ng g^{-1} is found in crude palm sold in PET bottles (Table 6). The
362 production and expiration dates are not mentioned on the samples sold in old polyethylene
363 cans or mineral water bottles (PET). From our knowledge, there is no study in the literature
364 on bisphenols with crude palm oil (Ingenbleek, Hu, et al., 2019; Ingenbleek, Sulyok, et al.,
365 2019). However, several studies investigated BPA in various other edible oils. Our results
366 were close to those of Chinese oils ($0.13\text{-}2.19\ \mu\text{g g}^{-1}$) (Liu et al., 2013). They are low
367 compared to edible oils stored in polycarbonate (PC) bottles for 40 days (BPA : $98\text{-}146.2$
368 ng/g) (Xian et al., 2017). Other studies showed an amount of BPA in refined edible oils of
369 $0.5\text{-}4.37\ \mu\text{g g}^{-1}$ and $1.0\text{-}288\ \text{ng g}^{-1}$ (Abou Omar et al., 2017; Nemati et al., 2018). In
370 Cameroon, large consumption of palm oil is around 105 g per Adult male equivalent per day
371 (Ingenbleek et al., 2017). Thus, for a BPA concentration of $0.4\ \text{ng g}^{-1}$ the daily intake is 0.7
372 ng/ kg of bw/day or 0.02% of the t-TDI.

373

374 **4. Conclusion**

375 The aim of this work was to assess the health risk associated with the consumption of liquid
376 foods packaged in plastic materials and sold in Cameroon market. The study was focus on the
377 determination of the amounts of bisphenol A, B and F that migrate from plastic packaging to
378 these foods. The liquid foods selected were crude palm oil, traditional fermented drinks (palm
379 wine and Bili-bili (fermented sorghum drink)), sachet waters and bagged whiskies. Extraction
380 recoveries of bisphenols in water, crude palm oil and traditional fermented drinks were
381 respectively 80-120%, 79-119% and 72-110%. A liquid chromatography tandem mass
382 spectrometry method was developed and validated. For aqueous matrices, limits of
383 quantification (LOQs) were 0.09, 0.10, and 0.09 $\mu\text{g L}^{-1}$, respectively for bisphenol A, B and
384 F. The application of the methods showed BPA content of $2.2 \pm 0.2 \mu\text{g L}^{-1}$, $1.2 \pm 0.07\mu\text{g L}^{-1}$
385 and $0.4 \pm 0.06 \text{ ng g}^{-1}$ respectively in one brand of sachet water, one brand of bagged whisky
386 and crude palm oil. BPA content in traditional drinks was lower than the LOQ. BPF was
387 found in one brand of sachet water at $14.5 \pm 1.1 \mu\text{g L}^{-1}$. BPB was not found in the samples.
388 The estimated BPA daily intakes were $0.22 \mu\text{g/kg bw/day}$, $0.004 \mu\text{g/kg of bw/day}$ and 0.7 ng/
389 kg of bw/day respectively for sachet water, bagged whisky and crude palm oil. All these
390 values were lower than t-TDI ($4 \mu\text{g/kg of body weight/day}$). The estimated BPF daily intake
391 from sachet water was $1.45 \mu\text{g/kg bw/day}$. The risks associated with dietary exposure of
392 bisphenols to the Cameroonian population through these matrices is very low. Nevertheless,
393 the effects of bisphenols at very low doses have to be studied. Similarly, it would be
394 interesting to study the combined effects of bisphenols and other toxic chemicals found in
395 food matrices studied, in order to assess the overall risk associated with their consumption.

396

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405 **Conflicts of Interest:** The authors declare that there is no conflict of interest.

Table 1: Summary of mass spectrometry (MS) conditions for bisphenols analysis.

Compound	Formula	t_R (min)	Parent ions (m/z)	Daughter ions (m/z)	Cone voltage (V)	Collision energy (eV)
BPA	$C_{15}H_{16}O_2$	2.34	226.96	212.0	44	16
				133.0	44	26
BPA _{d16}	$C_{15}D_{16}O_2$	2.34	241.07	223.1	44	18
				142.1	44	30
BPB	$C_{16}H_{18}O_2$	3.04	241.03	212.0	44	18
				147.0	44	26
BPF	$C_{13}H_{12}O_2$	1.17	198.92	93.0	44	22
				105.0	44	18

t_R : rétention time ;

Optimales MS of bisphenols : ES (-),Capillary voltage : 2,5 kV; desolvation temperature : 600°C; Cone flow : 50L/h; desolvation flow : 1200 L/h; Collision energy range : 2 à 80; Minimum fragment size: 30 Da.

Table 2: Extraction of bisphenols (A, B and F) from crude palm oil and traditional fermented drinks.

Compound	Crude palm oil						Traditional fermented drinks				
	Liquid – liquid extraction with MeOH/H ₂ O (50/50)						Solid phase extraction (SPE)				
	Recovery (%) ± SD (n=4)				LOD	LOQ	Recovery (%) ± SD (n=4)			LOD	LOQ
	0.8 ng/g	2 ng/g	10 ng/g	20 ng/g	(ng/g)	(ng/g)	0.1 µg/L	0.5 µg/L	2 µg/L	(µg/L)	(µg/L)
BPA	117.1 ± 3.7	102.6 ± 2.6	109.6 ± 5.4	96.4 ± 13.2	0.17	0.56	95.1 ± 6.8	103.2 ± 6.1	94.5 ± 9.9	0.03	0.11
BPB	103.4 ± 9.5	86.7 ± 8.3	87.8 ± 6.9	79.6 ± 5.2	0.06	0.20	72.2 ± 3.5	109.8 ± 3.2	108.1 ± 4.2	0.01	0.04
BPF	119.5 ± 5.8	79.9 ± 6.9	99.8 ± 12.1	86.5 ± 5.9	0.06	0.20	95.8 ± 9.3	95.9 ± 6.2	105.0 ± 4.5	0.01	0.04

Table 3: Recovery, linearity, LOQ, uncertainty and bias for analysis of bisphenols from water.

Compound	Recovery (%) (\pm SD) (n=4)			LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$)	Linearity ($\mu\text{g/L}$)	R^2	uncertainty %U (k=2.201)	Maxi bias (%)
	0.1 $\mu\text{g/L}$	0.5 $\mu\text{g/L}$	2 $\mu\text{g/L}$						
BPA	121.0 \pm 16.8	96.8 \pm 6.6	83.7 \pm 7.2	0.03	0.09	0.1 - 3	0.9948	12.1	-14.1
BPB	115.8 \pm 12.1	97.1 \pm 12.1	86.5 \pm 6.4	0.04	0.10	0.1 - 3	0.9952	11	-12.4
BPF	99.6 \pm 7.1	87.3 \pm 7.1	81.5 \pm 3.4	0.03	0.09	0.1 - 3	0.9987	10.3	-13.6

SD : Standard deviation ; *Maxi* : Maximum

Table 4: Results of precision with water sample

Compound	RSD (%) intraday			RSD (%) interday		
	0.1 $\mu\text{g/L}$	0.5 $\mu\text{g/L}$	2 $\mu\text{g/L}$	0.1 $\mu\text{g/L}$	0.5 $\mu\text{g/L}$	2 $\mu\text{g/L}$
BPA	7.0	2.5	1.8	13.1	5.5	6.6
BPB	6.4	2.4	5.3	9.5	10.6	5.2
BPF	1.0	3.4	2.4	3.9	7.3	4.0

RSD : Relative standard deviation

Table 5: Comparison of the current work with previous procedure for the analysis of BPA in oils and beverages

Matrice	Extraction method/ Analytic method	Number of steps	Extraction time (min)	LOD ($\mu\text{g}/\text{kg}$ ou $\mu\text{g}/\text{L}$)	LOQ ($\mu\text{g}/\text{kg}$ ou $\mu\text{g}/\text{L}$)	Recovery (%)	^c SRT or ^f TRT (min)	References
Analysis of BPA from oils								
Crude palm oil	^a LLE and UPLC-MS/MS	2	< 35	0.17	0.56	96.4 – 117.1	2.4	This work
Edible oils	^b DMSPE, isotopic dilution and UPLC- MS/MS	4	< 15	0.4	1.0	98.8 – 108.0	2.6	(Xian et al., 2017)
Edible oils	^c DLLME and HPLC- ^g DAD	4	< 5	2.5	6.3	89.5 – 99.7	8.0	(Liu et al., 2013)
Food grade oils	DLLME and HPLC-UV	3	< 20	150	450	65.0	/	(Nemati et al., 2018)
Plant oils	LLE, Gel permeation and LC-MS/MS	4	< 40	/	2.0	94.7 – 106.5	1.8	(Niu et al., 2011)
Analysis of BPA from beverages								
Traditional drinks	^d SPE and UPLC-MS/MS	3	< 20	0.03	0.11	94.5 – 103.2	2.4	This work
Alcoholic beverages	SPE, derivatization and LC-MS/MS	4	> 90	7.1	23.5	77.0 – 81.0	7.6	(Regueiro & Wenzl, 2015)
Beer samples	No extraction step/ LC-MS/MS	2	N.A	10	50	N.A	30.0	(Cardama et al., 2017)
Soft drinks and beers	DLLME and GC-MS	3	< 15	0.005	0.01	82.0 – 93.0	6.7	(Cunha et al., 2011)
Beverages	SPE and LC-MS	3	< 20	0.14	0.99	99.0 – 112.0	/	(Stärker & Welle, 2019)

^a LLE: Liquid-liquid extraction ; ; ^b DSPME: Dispersive solid phase micro-extraction ; ^c DLLME: Dispersive liquid-liquid micro-extraction; ^c DSPME; ^d SPE: Solid phase extraction ; ^e SRT: Specific retention time; ^f TRT : Total run time; ; ^g DAD: Diode array detector.

Table 6: Results of samples analysis.

Type of sample	Code	BPA	BPB	BPF
Concentration in $\mu\text{g/L}$				
Sachet water	BHR	2.2 ± 0.2	N.D	14.5 ± 1.1
	GOL	N.D	N.D	<LOQ
Concentration in ng/g				
Crude palm oil	HR	0.4 ± 0.06	N.D	N.D
Concentration in $\mu\text{g/L}$				
Bagged whisky	Fi	1.2 ± 0.07	N.D	N.D
	Ki	N.D	N.D	N.D
Concentration in $\mu\text{g/L}$				
Traditional fermented drinks	Bili	N.D	N.D	N.D
	VB	N.D	N.D	N.D

*N.D : Not detected

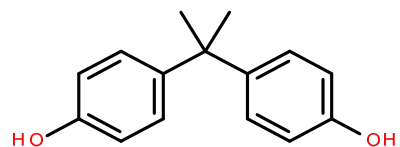
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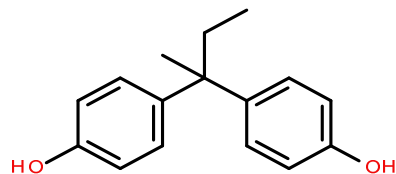
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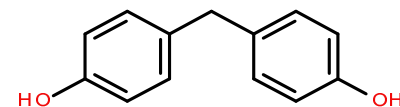
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Bisphenol A or BPA
(Mw : 228.3 ; Log K_{ow} : 4.04 ; pKa : 9.78 – 10.39)



Bisphenol B or BPB
(Mw : 242.3 Log K_{ow} : 4.49 ; pKa : 9.77 – 10.38)



Bisphenol F or BPF
(Mw : 200.2; Log K_{ow} : 3.46 ; pKa : 9.84 – 10.45)

Source : (Xian et al., 2017)

Figure. 1: Structures of bisphenols.

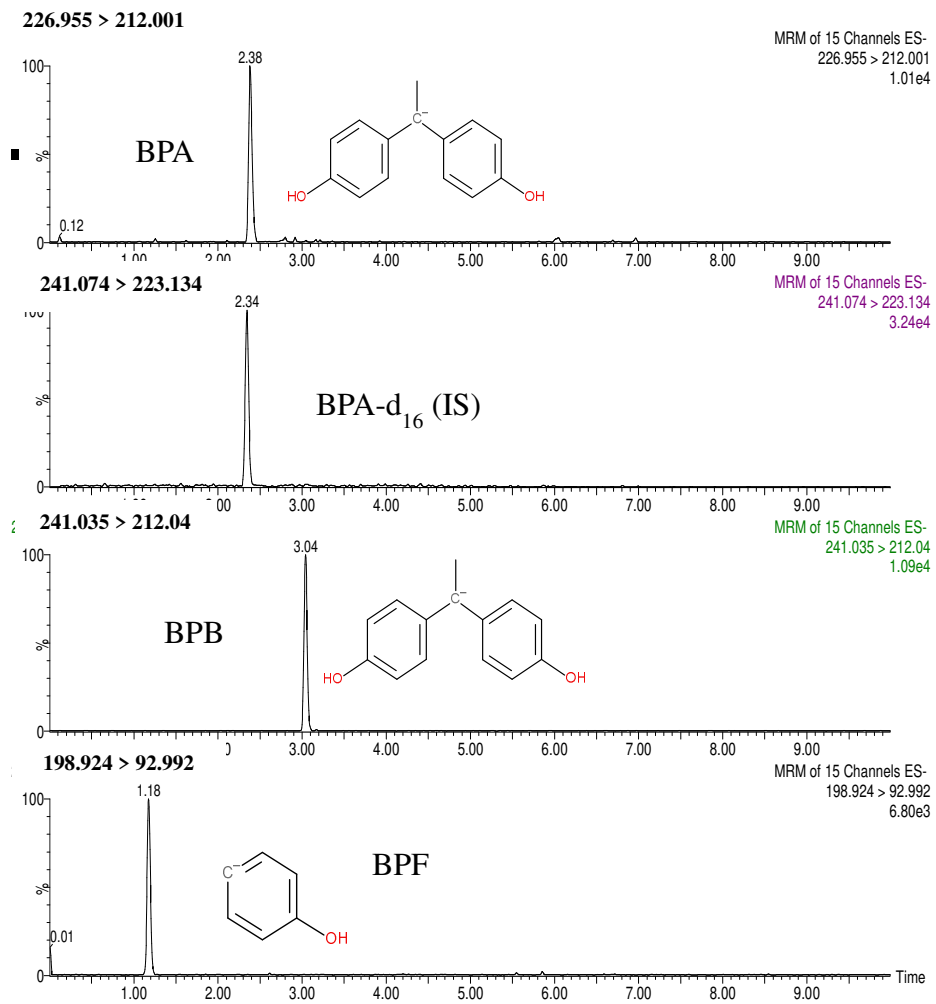


Figure. 2: Selected ion chromatograms of BPA, BPB, BPF and the internal standard (IS).

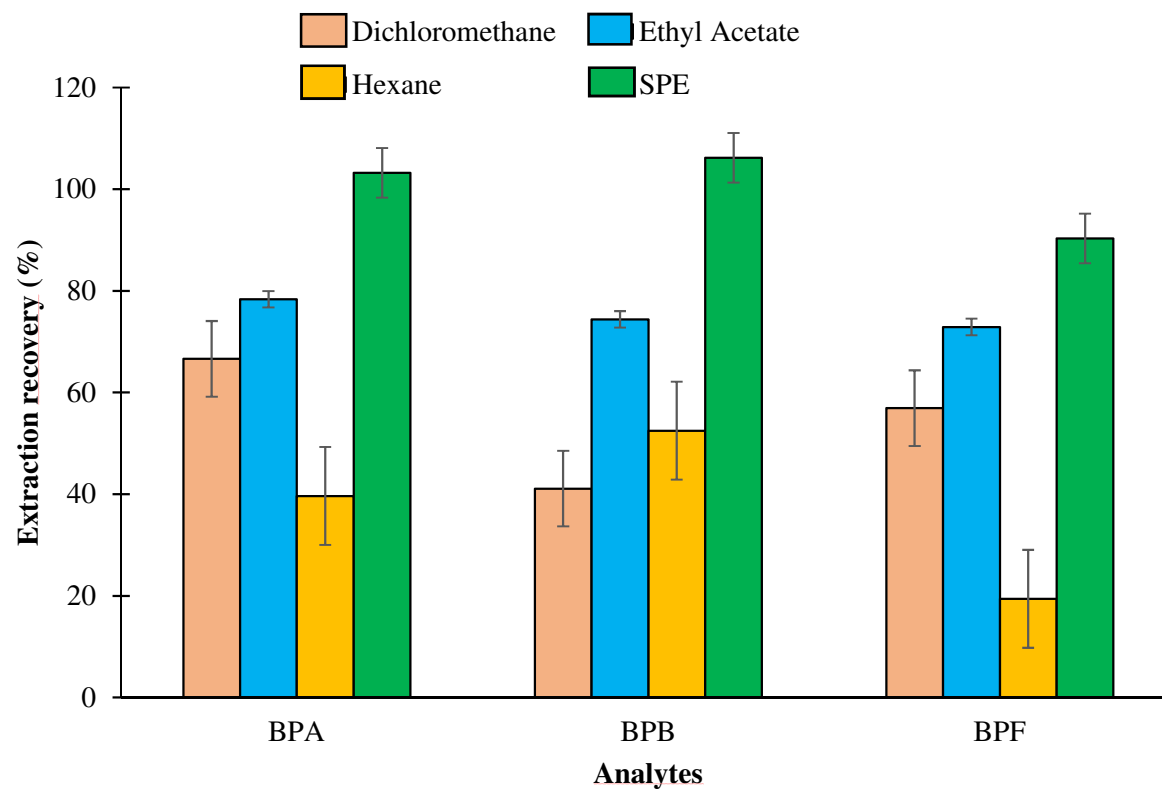


Fig. 3: Extraction yield of bisphenols from traditional fermented drinks using dichloromethane, n-hexane and ethyl acetate, for liquid-liquid extraction, and solid phase extraction (SPE).

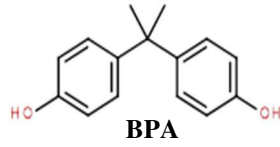
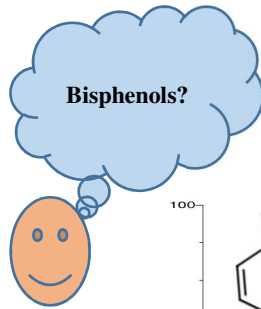
Occurrence of bisphenols and contribution of edibles liquids conditioned in plastic packaging to the dietary exposure in Cameroon



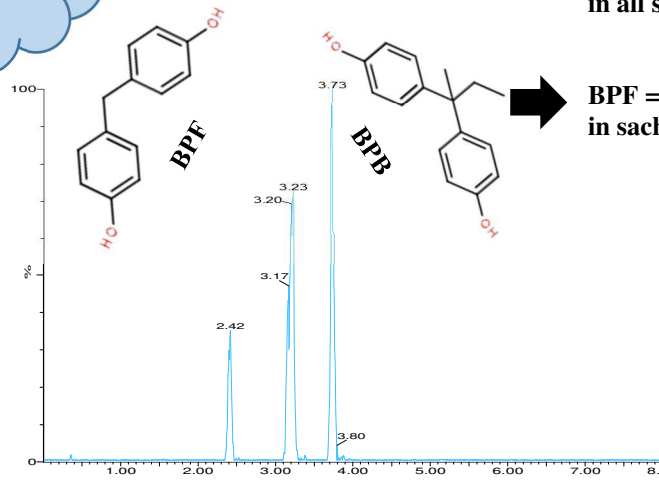
Sachet waters



Bagged whiskey



➔ **BPA < t-TDI**
(4µg/kg bw/day)
in all samples



➔ **BPF = 14.5 ± 1.1 µg/L**
in sachet water



Crude palm oil



Traditional fermented drinks