

Multi-element characterization of carob, fig and almond liqueurs by MP-AES

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| Complete List of Authors: | Rodríguez Solana, Raquel; Faculty of Science and Technology, Centre for Mediterranean Bioresources and Food Carlier, Jorge; Marine Science Center, University of Algarve Costa, Maria Clara; Marine Science Center, University of Algarve Romano, Anabela; Faculty of Sciences and Technology, Centre for Mediterranean Bioresources and Food |
| Keywords: | Essential mineral elements, Toxic metals, Liqueurs, MP-AES |
| Abstract: | <p>Carob pod, fig and almond liqueurs are a good source of income in different Mediterranean regions. This manuscript aimed to characterize the mineral content of these traditional beverages and evaluate the influence of the raw material on the mineral composition. A total of 25 fruit liqueurs from sixteen producers were analyzed. A simple open-vessel sample mineralization by wet digestion using the mixture HNO₃/H₂O₂ (1:1) was selected before spectrometric analysis. Nine essential elements (Cu, Ca, Mg, Na, K, Fe, Zn, Mn and P) and two non-essentials (Cd and Pb) were quantified by MP-AES. Carob liqueurs presented the broader profile of minerals. It was the only fruit liqueur that presented Fe in 72.7 % of samples, and P and Mn in 18.2 %, and also showed low amounts of the non-essential element, Pb, in two of the eleven samples analyzed. Conversely, almond liqueurs presented the lowest mineral content with only 5 elements detected. Fruit liqueurs analyzed presented great variability in the mineral content even within the same type of liqueur due to the different manufacturing processes. Despite this variability, application of principal component analysis (PCA) to essential mineral concentrations (K, Na, Ca, Mg, Mn, Fe and Zn) resulted in satisfactory classification (PC1 and PC2 account for 78.54 % of the total variance) of Portuguese liqueurs in terms of the type of liqueur studied.</p> |
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1 **Multi-element characterization of carob, fig and almond liqueurs by MP-AES**

2 Raquel Rodríguez-Solana¹, Jorge Dias Carlier², Maria Clara Costa², Anabela Romano^{1*}

3 ¹Faculty of Sciences and Technology, Centre for Mediterranean Bioresources and Food,
4 Ed. 8, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

5 ²Marine Science Center, University of Algarve, Campus de Gambelas, 8005-139 Faro,
6 Portugal

7 *Corresponding author. E-mail address: aromano@ualg.pt

For Review Only

8 Abstract

9 Carob pod, fig and almond liqueurs are a good source of income in different
10 Mediterranean regions. This manuscript aimed to characterize the mineral content of
11 these traditional beverages and evaluate the influence of the raw material on the mineral
12 composition. A total of 25 fruit liqueurs from sixteen producers were analyzed. A
13 simple open-vessel sample mineralization by wet digestion using the mixture
14 $\text{HNO}_3/\text{H}_2\text{O}_2$ (1:1) was selected before spectrometric analysis. Nine essential elements
15 (Cu, Ca, Mg, Na, K, Fe, Zn, Mn and P) and two non-essentials (Cd and Pb) were
16 quantified by MP-AES. Carob liqueurs presented the broader profile of minerals. It was
17 the only fruit liqueur that presented Fe in 72.7 % of samples, and P and Mn in 18.2 %,
18 and also showed low amounts of the non-essential element, Pb, in two of the eleven
19 samples analyzed. Conversely, almond liqueurs presented the lowest mineral content
20 with only 5 elements detected. Fruit liqueurs analyzed presented great variability in the
21 mineral content even within the same type of liqueur due to the different manufacturing
22 processes. Despite this variability, application of principal component analysis (PCA) to
23 essential mineral concentrations (K, Na, Ca, Mg, Mn, Fe and Zn) resulted in satisfactory
24 classification (PC1 and PC2 account for 78.54 % of the total variance) of Portuguese
25 liqueurs in terms of the type of liqueur studied.

26 **Keywords** Essential mineral elements · Toxic metals · Liqueurs · MP-AES

27 **Introduction**

28 Carob, fig and bitter almond liqueurs are among the traditional liqueurs from Portugal.
29 These liqueurs are produced mainly by maceration of different parts of plants, such as
30 leaves (fig tree), fruits (figs, carob pods or almonds) or natural flavouring essences
31 (bitter almond oil) in fig or strawberry tree fruit distillates or ethanol of agricultural
32 origin as is defined in annex II of Regulation (EC) No 110/2008 (1).

33 The mineral composition of these beverages is important because of the implications in
34 the organoleptic characteristics of the liqueurs, and the nutritional/toxicological
35 implications on human health (2, 3). Trace elements, such as Cu, Fe, Mn, and Zn,
36 influence the organoleptic properties of liqueurs (4). Cu and Fe can produce turbidity or
37 changes in colour due to the formation of compounds and because they act as catalysts
38 in the oxidation processes involved in aging (5, 6), and Ca and Mg can form compounds
39 that precipitate and help the clouding of the finished product.

40 From a nutritional point of view, around 25 minerals (essential elements) play an
41 important role in proper mechanism in human body and hence their deficiency in the
42 diet leads to many diseases: rickets, anaemia, etc (7). Generally, a recommended intake
43 to keep these elements at healthy levels is necessary. However, some of these essential
44 elements can also have toxic effects as is the case of Fe and Zn (with minimal safety
45 concern) with a permitted daily exposure (PDE) of 13000 µg/day and Mn and Cu (with
46 low safety concern) with a PDE of 2500 µg/day, established by the European Medicines
47 Agency (8). Other elements, such as Pb and Cd, are cumulative and toxic for human
48 health, whose chronic exposure may even cause death (9).

49 In general, the constituents of liqueurs come mainly from the fruits and other inorganic
50 and organic materials used in their elaboration (4, 10), but can also be added during the
51 different steps of the preparation process (3, 11). Minerals present in fruits depend in

52 turn on factors such as the cultural practices (e.g. the fertilizer addition) and the
53 environmental conditions (e.g. exposure to exhaustion gases, industry wastes and waste
54 waters polluted), which normally affect the content of essential and non-essential
55 minerals, respectively (12, 13).

56 In order to determine the mineral composition in liqueurs, mineralization is a
57 preliminary step necessary to reduce interferences caused by the presence of organic
58 matrix, and so avoid an increment in optical background. This step involves the
59 conversion of the metals associated with the material into a form that can be properly
60 determined⁶. For this purpose, there are two basic techniques: wet mineralization and
61 dry process, also known as calcination. Wet mineralization uses different acids (HNO₃,
62 HCl, H₂SO₄, H₃PO₄, HClO₄, and HF), oxidants (H₂O₂), or mixtures thereof to enhance
63 the digestion of the samples due to the reactive ability of the mixture in oxidizing
64 organic matter. The election of conditions and reagents (the strength, purity and safety
65 of the acid, its oxidizing power, boiling point and the salts solubility) will depend on the
66 sample nature and devices used, open or close vessels (14). In turn, calcination uses
67 small amounts of reagents and presents high yields and simple instrumental
68 requirements.

69 Microwave plasma atomic emission spectrometry (MP-AES) is an easy to use technique
70 with high performance, high speed and not requiring hazardous (eliminating flammable
71 and oxidizing gases) and expensive gases (using nitrogen plasma instead the argon
72 plasma used in other techniques), which makes the determination of minerals
73 advantageous in comparison with other spectroscopic and spectrometric techniques.
74 These characteristics make MP-AES improve safety, analytical performance, and
75 reduces operating costs, therefore it has been introduced recently in the mineral

76 characterization of different foods, such as wine (15), cheese (16), bread (17), and
77 plants, such as herbal medicines (18), and sunflower (19).

78 Despite the long tradition of the Portuguese carob, fig and almond liqueurs, they have
79 not been studied to date. This study constitutes the first chemical characterization of
80 these liqueurs in terms of mineral constituents. It may be of great importance in order to
81 evaluate the relation between their mineral composition and the type of fruit employed
82 in their production. In addition, this work shows the performance of MP-AES for the
83 quantification of the essential elements Cu, Ca, Mg, Na, K, Fe, Zn, Mn and P, and non-
84 essential elements Cd, Pb, in liqueurs, after a simple wet digestion in open vessels to
85 eliminate possible interferences due to their organic matrix.

86 **Materials and methods**

87 **Reagents and liqueur samples**

88 All reagents used were of analytical grade: HNO₃ (65%) (Fisher Scientific, Pittsburgh,
89 PA), HClO₄ (60%) (Riedel-de Häen, Seelze, Germany), H₂O₂ (30%) (Merck Suprapur,
90 Darmstadt, Germany), Vanadium (V) oxide (98%) (Sigma-Aldrich, United Kingdom).
91 Working standards of the metals Al, Cu, Cd, Pb, Zn, and Mn (stock solution of 50
92 ppm), Ca, Mg, Na, K, and Fe (stock solution of 100 ppm) and P (stock solution of 10
93 ppm) were prepared by diluting concentrated stock solutions (Agilent Technologies,
94 Santa Clara, CA) with 5% of nitric acid/Milli-Q water. The ultra-pure water was
95 employed to dilute the samples and standards, and this water was obtained by filtering
96 tap water through a Milli-Q purifier (Millipore Waters, Milford, MA, USA).

97 A set of 25 commercial liqueurs samples from the principal Portuguese producing
98 regions were evaluated: eleven carob and six fig liqueurs from Algarve region (south of
99 Portugal) and eight almond liqueurs from Algarve and Douro Littoral regions (south

100 and north of Portugal, respectively) (Fig. 1). Most of the samples were kindly provided
101 directly by the producers and a small part was purchased at local markets.

102 **Sample pretreatment: liqueur mineralization**

103 A previous step of optimization was carried out with the sample that is probably the
104 most difficult to digest, due to its cream based (fat) and small visible pieces of fruit
105 (carob pod flour) undissolved, a carob cream liqueur. The elements under study in the
106 optimization step were the two major elements Na and K, with importance in the
107 regulation of blood pressure in human body (20) and the trace element Fe, related to
108 deterioration in sensory quality of liqueurs (5).

109 The optimization study shown in Table 1 was carried out using open-vessels, with dry
110 and wet digestion procedures. All the “a” experiments of methods 1, 3 and 4, followed
111 the guidelines found in the literature. The other (b-e) are modifications performed in the
112 reaction volume and/or in the temperature ramps to improve results of the previous “a”
113 experiments. Initially, low temperatures were applied to degrade the organic matter
114 present in the matrix, and then the temperature was raised to proceed to the
115 decomposition of inorganics, which are difficult to dissolve (24). These modifications
116 were necessary since the procedures described in the literature did not lead to a
117 complete mineralization of the samples, as indicated by the elemental analysis results
118 and by the light yellow colour of the digested liquid (25).

119 *Wet mineralization in open vessels*

120 Glass digestion vessels were previously cleaned in 10% (v/v) nitric acid solution to
121 avoid cross-contamination. Digestions were carried out in triplicate with uncovered
122 glass tubes using a digital dry bath (Accublock Digital Dry Bath, Labnet International,
123 New Jersey, USA), at the temperatures and times indicated in Table 1. The methods

124 tested mixed HNO₃ with other acids such as HClO₄ (method 1 and 2) and HCl (method
125 3), and the strong oxidant H₂O₂ (method 4). Once the digestion was finished, each
126 sample was removed from the dry bath, cooled to room temperature until next day and
127 then diluted up to 25 mL with Milli-Q water.

128 *Dry mineralization in a muffle furnace*

129 Digestion was carried out in pots as indicated in method 5 (Table 1). Initially, the
130 sample was subjected to low temperatures (80 and 105 °C) in an oven with assisted air
131 circulation to evaporate and remove all residual water before ashing. Then, the
132 temperature was increased to 450 °C on a muffle furnace (Thermolyne,
133 Type 1500 Furnace; Sybron Corp., Dubuque, IA) to proceed with the mineralization
134 until the sample acquires whitish colour of ashes. The ashes were dissolved in 10 mL of
135 HNO₃ acid and diluted with Milli-Q water up to 50 mL.

136 The optimal digestion conditions, chosen from a comparative analysis of mineral
137 measures obtained in the different tested methods, were applied for the analysis of the
138 mineral composition of all carob, fig and almond liqueurs studied.

139 **Analytical performance of the method**

140 The limits of detection (LOD) and quantification (LOQ) of the method, showed in
141 Table 2, were obtained based on the parameters of the analytical curves using standards
142 with acid matrix in 5% HNO₃. Both limits were calculated according to the following
143 mathematical equations (1 and 2):

$$144 \quad LOD = \frac{3S_{y/x}}{m} \quad (1)$$

$$145 \quad LOQ = \frac{10S_{y/x}}{m} \quad (2)$$

146 where $S_{y/x}$ =the estimation of the standard deviation of the regression line, and m = slope
147 of the calibration curve.

148 The linear regression analysis for each element was performed by the external standard
149 calibration. The validating parameters of each calibration curve, slope (a), intercept (b)
150 and correlation coefficient (r^2) are described in Table 2.

151 Finally, the calculation of the recovery was performed to test trueness of the developed
152 method and thus determine whether analyte detection is affected by the influence of the
153 matrix on digestion procedure. The recovery assays were performed in triplicate using a
154 spiked liqueur sample by adding two different quantities of standard (0.5 mg/L and 1
155 mg/L for phosphorous and 2 mg/L and 2.5 mg/L for the other elements) to known
156 amount of sample (see Table 3). Recovery was calculated as follows: a known amount
157 of an analyte, using pure 5% HNO_3 standard, was spiked into a liqueur sample and this
158 value was subtracted from one of unspiked liqueur sample. Subtraction was divided by
159 the spiked sample and multiplied per 100.

160 **MP-AES: mineral analysis of liqueurs**

161 The mineral composition of liqueurs was measured by microwave plasma atomic
162 emission spectrometry (Agilent 4200 MP-AES, Santa Clara, CA) using multi-element
163 analysis. The operational conditions of MP-AES method were firstly optimized by
164 evaluation of MP-AES quantitative operational mode for multi-element analysis. Each
165 element was monitored at a specific wavelength to ensure interference-free detection.
166 Optimum instrumental conditions for MP-AES measurements are summarized in Table
167 2. The instrument viewing position was optimized using the standard of maximum
168 concentration.

169 A 6-point calibration curve was carried out for each element in matrix-matched
170 calibration solutions (5% HNO_3) to account for matrix interferences. All the details

171 about calibration curve can be seen in Table 2. After appropriate dilution of samples in
172 5% HNO₃, they were analyzed in triplicate and the concentrations calculated using the
173 external standard calibration method.

174 **Data analysis**

175 Descriptive statistical analysis (mean and standard deviation), principal components
176 analysis (PCA), and one-way analysis of variance (ANOVA), were evaluated.
177 Separation of the means was performed by the Fisher's least significant difference at P =
178 0.05 when ANOVA showed significant differences (P < 0.05). ANOVA were analyzed
179 using Statistix 9 (Analytical Software, Tallahassee, FL, USA). Principal components
180 analysis (PCA) was used in data reduction to identify a small number of factors that
181 explain most of the variance observed in a much larger number of manifest variables
182 (26). Specifically, it was used to evaluate the relationship between the mineral
183 composition and samples, and was performed using the commercial software XLstat-
184 Pro (Addinsoft) for windows.

185 **Results and discussion**

186 **Optimization of the sample digestion procedure**

187 The different digestion methods tested with a cream carob liqueur sample are shown in
188 Table 1. The general trend of the results shows that the methods reaching higher
189 temperatures and being kept at this temperature for longer periods (1e, 1d, 2d, 3d, 3e,
190 and 4c, 4d), revealed higher concentrations of the elements analyzed, especially in terms
191 of Fe concentration. This suggests that longer periods at higher temperatures allowed
192 the complete digestion of the samples.

193 In general, no significant differences were observed among the methods when
194 employing the same operating conditions (times and temperatures). This can be

195 observed for Fe concentrations in experiments 1c, 2c, 1e, 3d, 4e, and 2d, 4d; for K in
196 experiments 1d, 2d, 3c, 4d and 1e, 3d, 4e; and for Na in experiments 1e and 4e (Table
197 1). In the case of Fe concentrations, the major difference lies in maintaining the sample
198 at elevated temperature for longer. Probably, shorter periods are not enough to eliminate
199 the compounds able to complex Fe (6). The concentrations of Na and K remain
200 practically unchanged in all methods.

201 Among all the methods studied, 3 and 4 were those that revealed the best results, with
202 higher concentrations of Fe, K and Na, indicating more complete digestions of carob
203 liqueur. Moreover, the results obtained agree with published data based on the digestion
204 of alcoholic drinks, such as liqueurs, anisettes, cognacs, whiskies, gins, rums and wines
205 using the mixture $\text{HNO}_3/\text{H}_2\text{O}_2$ (method 4) ($[\text{Fe}] \cong <0.01 \text{ mg / L to } 7.16 \text{ mg / L}$; $[\text{K}]$
206 $\cong 0.13 \text{ mg / L to } 1014 \text{ mg / L}$ and $[\text{Na}] \cong \text{trace to } 215.3 \text{ mg / L}$) (11, 23, 27, 28) and
207 regarding the digestion of milk samples using the HNO_3/HCl mixture (method 3) ($[\text{K}] \cong$
208 $1687 \text{ mg / Kg to } 27253 \text{ mg / Kg}$ and $[\text{Na}] \cong 407 \text{ mg / Kg to } 4784 \text{ mg / Kg}$) (22). In face
209 of the good performance in digesting the cream carob liqueur and since it requires
210 smaller amounts of acid (higher concentrations may reduce the MP-AES tube lifetime
211 by acid attack), method 4 was selected to be used in the subsequent tests.

212 **Calibration and analytical method performance**

213 To check the method performance, the limits of detection and quantification (LOD and
214 LOQ, respectively), and the linearity range using standards with acid matrix in 5%
215 HNO_3 , and spike-and-recovery using a spiked liqueur sample, were studied.

216 Under the optimized MP-AES conditions, LOD and LOQ were calculated using the
217 equations described in the section 2.3. Table 2 shows the low values of LOD and LOQ
218 for all the minerals studied. Linear regression analysis for each element was performed

219 by the external standard calibration. The validating parameters of each calibration
220 curve, slope (a), intercept (b) and correlation coefficient (r^2) are described in Table 2.
221 Good linearity was observed between intensities and concentrations over the range
222 tested (r^2 :0.9990-0.9999) for all the analyzed elements.

223 Finally, the calculation of the recovery was performed to test trueness of the developed
224 method and thus determine whether analyte detection is affected by the influence of the
225 matrix on digestion procedure. Table 3 reports the recovery data that were obtained with
226 values between 97.93-120.00 %. Considering the results of the recovery test, the
227 method is deemed to be accurate. Only the value of P (120%) was at the limit, so in
228 future measurements of this element the use of the internal standard method or method
229 of standard additions is recommended.

230 **Mineral content in liqueurs: essential elements**

231 Results of the mineral composition of the studied liqueurs (carob, fig and almond
232 liqueurs) are presented in Table 4. There is a high variability in concentrations within
233 each type of liqueur, as demonstrated by the high standard deviations. This is may be
234 due to different manufacturing processes and also to the raw materials (mainly, fruits
235 and water) used to elaborate the liqueurs. The influence of these factors can be verified
236 since some producers were cooperative in sharing their elaboration process. Carob
237 liqueurs 1, 2 and 8 presented the major differences (Table 4). Producer of sample 1
238 (with high quantities for all elements studied) uses high temperatures (infusion) during
239 the extraction process, the sample 8 (with high quantities of some macroelements) is the
240 only cream-based liqueur, and finally, sample 2 (with the lowest quantity of studied
241 elements) used alcohol of agricultural origin and maceration at room temperature for the
242 liqueur elaboration. Taking this into consideration, the sample 2 and 12 of fig liqueur
243 (respectively, with the lowest and the highest content in macroelements), and samples 1

244 and 12 of almond liqueur (with K concentrations 3 to 5 and 40 to 61 times highest,
245 respectively) may also indicate the same influence.

246 *Macroelements*

247 In general, the amounts of Na, K, Mg and Ca are related with the water used in the
248 dilutions to elaborate the product (3, 29), but in some cases the concentrations of these
249 elements can increase due to the raw materials with which the alcoholic base is in
250 contact, the fruits (3, 4, 10).

251 The liqueurs used in this work can be distributed in three groups according to the
252 regions where they are produced: western Algarve (samples 1, 2, 5, 6, 11, 12), eastern
253 Algarve (samples 3, 4, 7, 8, 9, 10) and North of Portugal (samples 13, 14, 15, 16) (Fig.
254 1). The public data for the quality of the water along the year 2015 in the Municipalities
255 where the liqueurs are produced (Monchique and Silves in the western side of Algarve;
256 Loulé, Olhão and Tavira in the eastern Algarve, and Rio Tinto and Anadia in the north
257 of Portugal) reveals in all cases the same type of water concerning the macroelements
258 with concentrations between 17-48 mg/L for Ca, 5-25 mg/L for Mg and between 8-27
259 mg/L for Na (30-32). However, it is likely that some producers use bottled soft water
260 with lower contents of these elements, because it is advantageous in the production of
261 liqueurs to have the guarantee that the finished product is not clouded.

262 Assuming the maximum quantity of macroelements found in tap water as zero (48 mg/L
263 for Ca, 25 mg/L for Mg and 27 mg/L for Na), it can be concluded that this factor does
264 not justify the major differences found in the liqueurs (Table 4). Thus, it seems that the
265 materials (carob, fig or almond) and the methods of production have greater influence
266 on the concentrations of these elements in the liqueurs. Moreover, when the liqueurs of
267 each type are organized in order of increasing concentration for each of the studied

268 macroelements, it is not visible any stratification related with the geographical locations
269 of the producers.

270 K concentration is found in highest concentration in all samples of carob liqueurs, with
271 intermediate values in fig liqueurs (66.7% of the samples) and lowest in almond
272 liqueurs [only in one sample (12.5%)], with mean values of 799.44 mg/L, 425.01 mg/L
273 and 54.53 mg/L (eliminating sample 12, the mean value drops to 9.94 mg/L),
274 respectively (Table 4). High quantities of potassium have been reported for other fruit
275 liqueurs, 600 mg/L in raspberry liqueurs, and 505 mg/L in cherry liqueurs (10).

276 Regarding Na, the highest concentration was observed in carob liqueurs (173.27 mg/L),
277 mainly in samples 1, 4, 5, 6 and 12, followed by fig liqueurs (79.35 mg/L), and then
278 almond liqueurs (45.28 mg/L) (Table 4). The exception was in liqueurs 2 and 3, from
279 the same producer, which showed similar concentrations. These concentrations are in
280 the range of results previously reported, as raspberry liqueurs with a concentration of 94
281 mg/L and 88 mg/L in apple liqueurs (10).

282 The dietary values of K/Na ratio found in populations intake that eat natural foods are
283 between 3 and 10 (20). Our study revealed that range of K/Na in samples 4, 5, 6, 7 and
284 9 of carob liqueurs, in samples 4, 5 and 12 of fig liqueur, and only in sample 12 of
285 almond liqueur. Similar ratios were found also in other fruit beverages, such as juices
286 (black mulberry and grape juices) and liqueurs (peach, plum, cherry, strawberry,
287 raspberry and black currant liqueurs) (10, 33, 34).

288 Ca concentration in liqueurs from the producers 2, 6 and 12 showed similar quantities,
289 while the values in samples from producers 1, 4 and 5 followed the trend almond < fig <
290 carob, except producer 3 that has the opposite trend. In general, the highest
291 concentrations of Ca were observed in carob liqueurs (112.41 mg/L), close to the double
292 of fig (46.13 mg/L) and almond (50.96 mg/L) liqueurs. If we eliminate sample 8, a

293 liqueur containing cream (rich in Ca), the mean value drops to 89.73 mg/L, still a very
294 high value. Interestingly, the concentration of Ca in sample 8 (339.17 mg/L) is about the
295 double of the value reported by Iwegbue *et al.* (35) for a cream liqueur (162.86 mg/L).

296 Mg was the macroelement present in minor amounts and was only detected in seven of
297 the eleven carob liqueurs studied and one of the six fig liqueurs. The mean
298 concentration found in carob liqueurs (16.76 mg/L) was similar to concentrations found
299 in other fruit liqueurs: peach (14.16 mg/L), apple (12.85 mg/L), plum (17 mg/L),
300 banana (11 mg/L), rose hip (18 mg/L), and black currant liqueur (22 mg/L)(10, 33, 36).

301 According to the previous discussion, these high concentrations of macroelements
302 present in carob and fig liqueurs can be explained by the contribution of the fruits used
303 in the preparation. The results are consistent with works found on the study of minerals
304 in carob flour and figs, where K is one of the most abundant elements (67.00 % and
305 68.48 % respectively), followed by Ca (22.46 % in carobs and 20.48 % in figs), Mg
306 (1.51 % in carobs and 5.44 % in figs) and Na (0.84 % in carobs, and 5.46 % in figs) (37,
307 38). In general, this trend was found in carob and fig liqueurs for K (72.03 % and 76.38
308 %, respectively), Ca (10.13 % and 8.29 %, respectively) and Mg (4.09 % and 0.51%,
309 respectively). In both type of liqueurs, Na (15.61 % in carob and 14.26 % in fig
310 liqueurs) presented highest values. The different proportion of Na, mainly in the case of
311 carob liqueurs, can be due to the addition of acidity regulators such as sodium citrate or
312 sodium bicarbonate (4). Since the concentrations of macroelements in almonds are
313 usually high (39), the low concentrations of these elements in the almond liqueurs
314 analyzed can be explained by the replacement of fruits by natural flavouring essences
315 (Table 4).

316 The last macroelement analyzed is phosphorus. Although this element was observed in
317 high concentrations in carob pod (38), and fig (40), P was only present in two samples
318 of carob liqueurs in small concentrations (3.82 ± 0.09 and 16.19 ± 0.61 $\mu\text{g/L}$).

319 *Trace elements*

320 Cu was quantified in all carob (2.39 mg/L) and fig (2.85 mg/L) liqueurs. Similar
321 concentrations were detected on raspberry liqueurs (1.28 mg/L) and cherry liqueurs
322 (2.68 mg/L) (10). This element was nearly undetectable in samples of almond liqueurs
323 (only present in samples 1 and 12). The Cu content, discarding samples (1, 2, 8, 12-16)
324 with different production methods, reveals a trend that seems to separate the liqueurs by
325 production region: liqueurs produced in western Algarve (samples 5, 6 and 11) have
326 lower concentrations of Cu (< 1.7 mg/L) than those produced in eastern Algarve
327 (samples 3, 4, 7, 9 and 10) (> 2.3 mg/L). The fact that this element is characteristic of
328 the distillation system employed (3), and knowing that the water supplied to these
329 regions has concentrations always below 0.30 mg/L (30), or even lower when the
330 producers used bottled water, makes us think that the greatest contribution found
331 between regions may be influenced predominantly by the system used.

332 Usually the traditional stills constructed almost totally with copper provide more of this
333 element (5 ppm) to the produced spirit compared to the new units (with values < 1 ppm)
334 (Soufleros, Mygdalia, & Natskoulis, 2005), which only use copper in some parts of the
335 circuit whilst the rest is made of stainless steel (41). Accordingly, carob liqueurs 3, 4, 7,
336 9 and 10; fig liqueurs 2, 3, 4 and 12 and almond liqueur 12, were probably elaborated
337 with spirits obtained in traditional stills due to the high copper concentrations (1.93
338 mg/L – 7.60 mg/L) (Table 4), whilst samples of carob liqueurs 1, 2 and 8; fig liqueur 1
339 and almond liqueur 1, with values below 1 mg/L, probably employed spirits/alcohol
340 elaborated from industrial systems. Finally, in an intermediate position are the carob

341 liqueurs number 5, 6 and 11 with values between 1.27 - 1.66 mg / L and the fig liqueur
342 number 5 with a value of 0.90 mg / L of copper. Despite having these low values we
343 know that they used a traditional system with improvements (good initial cleaning of
344 the equipment, some parts of the equipment are made of stainless still, and/or by
345 reducing its concentration by methods as the use of activated carbon) to obtain a high
346 quality spirit.

347 Fe is an element present in figs (0.05%) (37) and carob pods (0.11%) (38) but only was
348 detected and quantified in carob liqueurs (1.62 mg/L). The processing of carob or the
349 different steps in the liqueur elaboration probably makes it more accessible during the
350 extraction step to the alcoholic base used.

351 The trend of the concentration of this element found in the samples in relation to the
352 processing region is as follows: liqueurs from western Algarve have higher
353 concentrations of Fe (> 3.3 mg/L), except sample 11 (1.52 mg/L), than those from
354 eastern Algarve (< 2.6 mg/L). As in the case of copper, the concentrations of Fe present
355 in the tap water from the same regions (year 2015) were always bellow 0.025 mg/L
356 (30), indicating again that the tap water cannot be the main source of this mineral.

357 Comparing the presence of this element in carob liqueurs with other liqueurs from
358 literature, similar concentrations were found in samples of cream (1.31 mg/L) and peach
359 liqueurs (1.50 mg/L) and higher values in plum (2.16 mg/L), cherry (2.43 mg/L) and
360 black currant liqueurs (2.79 mg/L) (10, 23, 33).

361 In this study, Mn concentration was only detected and quantified in two carob liqueurs.
362 This is probably due to this element being present in low concentrations in figs (0.03%)
363 and carob pods (0.08%) (37, 38). In general, concentrations of manganese found in
364 liqueurs are low (< 1 mg/L). Nevertheless, raspberry (2.55 mg/L) and black currant (2.22
365 mg/L) liqueurs presented slightly higher concentrations (10).

366 As it is shown in Table 4, Zn is present in all carob liqueurs (concentrations ranging
367 from 0.39 to 2.89 mg/L), but just in two of fig (0.61 and 0.81 mg/L) and one of almond
368 (1.24 mg/L). The concentrations in carob liqueurs have lower values (< 0.75 mg/L) in
369 samples from western Algarve, except sample 5 (2.72 mg/L), than those from eastern
370 Algarve (> 1.0 mg/L). According to the literature, this mineral showed lower mean
371 values, ≤ 1 mg / L, in other fruit liqueurs (10, 23, 33).

372 Finally, we can conclude that different contents of such elements for liqueurs produced
373 in the western or in the eastern side of Algarve suggest the possibility of some
374 differences in the process of production in these two regions. The presence of trace
375 elements at high concentrations in liqueurs is usually associated with incorrect
376 manufacturing processes (42). Therefore, the concentrations found in the samples
377 corroborate good processing practices in the studied regions.

378 **Mineral content in liqueurs: non-essential elements**

379 *Heavy metals*

380 In general, there was no trace of Cd and Pb in the analyzed samples, which highlights
381 the good manufacturing practices of most of the traditional liqueurs producers. Only
382 samples 4 and 7 of carob liqueur presented lead in low concentrations. This presence
383 could derive from the reparations performed in distillation equipment or from polluted
384 water employed in the dilution step (3).

385 **Principal component analysis**

386 Principal component analysis (PCA) was applied using the correlation matrix with the
387 well correlated variables. Consequently, the model was simplified taken into account a
388 more restricted number of variables. Among the set of variables that contributed to
389 obtain the PCA analysis with the two first components are the 7 essential elements (K,

390 Na, Ca, Mg, Fe, Zn and Mn) that were the most representatives of the whole system,
391 and 25 samples (11 of carob, 8 of almond and 6 of fig liqueur). PCA showed that the
392 first two principal components extracted explain 78.54 % of the total variance [F1
393 (56.67%) and F2 (21.87%)].

394 Figure 2 shows the biplot scores from the first two principal components. The samples
395 were differentiated according to the mineral composition as can be deduced from the
396 high percentage of the total variance of the observations. Three main groups of samples
397 are observed corresponding to each of the types of liqueurs studied. Fig and almond
398 liqueurs are on the left side of the chart and these samples were not defined by any of
399 the studied elements. On the other hand, samples of carob liqueurs were characterized
400 by all the elements shown in the PCA and in general cover the entire right side of the
401 graph. Specifically, samples 1, 6 and 9 of carob liqueurs and sample 3 of fig liqueur
402 were correlated by Na and Ca content, while carob samples, 2, 3, 6, 7, 8, 11 and 4 were
403 characterized by the presence of the trace elements Fe, Zn, and Mn, and the
404 macronutrients K and Mg. Finally, the elements showed in the graph had great
405 importance in the results of the samples, being Mg (20.10%), Mn (20.09%), K (20.00%)
406 and Zn (16.08%) the elements with most influence in F1 and Na (56.43%) and Ca
407 (26.96%) in F2.

408 **Conclusions**

409 The digestion pretreatment and MP-AES method proposed in this work to quantify
410 different essential and non-essential minerals in liqueur matrices was optimized. For the
411 digestion of liqueurs, the use of any of the reagents studied would be optimal because
412 with the same operational conditions, the results showed no significant differences.
413 HNO₃/H₂O₂ mixture was chosen because it showed a slight better sample digestion
414 according to the elements of study, with a lower quantity of digesting acid. MP-AES

415 demonstrated to be a good technique for the multielement analysis of these samples,
416 since the method presented good linearities and recoveries, and low LOD and LOQ for
417 the elements analyzed.

418 The results obtained in this work indicate that the fruits employed in the elaboration of
419 each liqueur markedly influence the final mineral content of these beverages and permit
420 a differentiation among them, as showed in the PCA. Differences in the liqueurs
421 production methods may contribute to the great variability in minerals, even within the
422 same type of liqueur.

423 Among the Portuguese liqueurs studied, carob liqueurs are those presenting the highest
424 values for all the minerals analyzed whereas those of almond showed the lowest
425 contents. The mineral content compared to other fruit liqueurs reported in the literature,
426 showed the highest macroelement profile of carob liqueurs and similar quantities of
427 trace elements.

428 The lack of detection of non-essential elements in 98% of the studied liqueurs and the
429 concentrations of essential elements in allowable ranges indicate the generalization of
430 good manufacturing practices of these drinks. Thus, a moderate consumption of these
431 liqueurs can contribute positively to human requirements of essential elements.

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Table 1 Optimization of sample digestion carried out with a carob liqueur: wet (method 1, 2, 3 and 4) and dry (method 5) digestion conditions and Fe, K and Na average concentrations

| Method [†] | Digestion conditions | | | | | | Digestion results (mg/L) | | | | | | | |
|---------------------|-------------------------|-----------------------|--|-----------|------------------------------------|------------------------------------|---------------------------|----------------|--------------|---------------|---------------|---------------------------|----------------------------|-------------------------------|
| | Sample (mL) | Reagents | | | | | Time/Temperature (min/°C) | | | | Fe | K | Na | |
| | | HNO ₃ (mL) | HNO ₃ :HClO ₄ (1:4) (mL) | HCl (mL) | H ₂ O ₂ (mL) | V ₂ O ₅ (µg) | Step 1 | Step 2 | Step 3 | Step 4 | | | | |
| 1 | <i>a</i> [*] | <i>1</i> | <i>5</i> | <i>5</i> | - | - | <i>10</i> | <i>20/60</i> | <i>45/90</i> | - | - | <i>6.10</i> ^{gh} | <i>670.50</i> ^e | <i>1177.38</i> ^{def} |
| | b | 0.5 | 2.5 | 2.5 | - | - | 5 | 20/room | 30/80 | 30/100 | 30/110 | 3.43 ⁱ | 749.33 ^{abc} | 1096.13 ^{gh} |
| | c | 0.5 | 2.5 | 2.5 | - | - | 5 | 20/room | 30/80 | 30/100 | 120/110 | 11.61 ^e | 697.20 ^{de} | 1218.13 ^{cdef} |
| | d | 0.5 | 2.5 | 2.5 | - | - | 5 | 20/room | 30/80 | 30/100 | 240/110 | 23.71 ^c | 725.83 ^{bcd} | 1240.83 ^{cde} |
| | e | 0.5 | 2.5 | 2.5 | - | - | 5 | 20/room | 30/80 | 30/100 | 270/110 | 25.02 ^{bc} | 767.20 ^{ab} | 1276.20 ^{bc} |
| 2 | <i>a</i> | <i>1</i> | - | <i>10</i> | - | - | - | <i>20/60</i> | <i>45/90</i> | - | - | <i>7.53</i> ^{fg} | <i>700.5</i> ^{de} | <i>1286.38</i> ^{bc} |
| | b | 0.5 | - | 5 | - | - | - | 20/room | 30/80 | 30/100 | 30/110 | 4.19 ^{hi} | 767.33 ^{ab} | 1069.00 ^b |
| | c | 0.5 | - | 5 | - | - | - | 20/room | 30/80 | 30/100 | 120/110 | 11.75 ^e | 722.53 ^{cd} | 1161.60 ^{fg} |
| | d | 0.5 | - | 5 | - | - | - | 20/room | 30/80 | 30/100 | 240/110 | 26.39 ^b | 693.50 ^{bcd} | 1171.75 ^{ef} |
| 3 | <i>a</i> ^{**} | <i>0.5</i> | <i>4</i> | - | <i>1</i> | - | - | <i>20/room</i> | <i>30/80</i> | <i>30/100</i> | <i>30/110</i> | <i>8.44</i> ^f | <i>777.07</i> ^a | <i>954.67</i> ^j |
| | b | 0.5 | 4 | - | 1 | - | - | 20/room | 30/80 | 30/100 | 120/110 | 18.52 ^d | 702.27 ^{de} | 1248.67 ^{bcd} |
| | c | 0.5 | 4 | - | 1 | - | - | 20/room | 30/80 | 30/100 | 240/110 | 29.61 ^a | 730.00 ^{bcd} | 1247.17 ^{cde} |
| | d | 0.5 | 4 | - | 1 | - | - | 20/room | 30/80 | 30/100 | 270/110 | 26.94 ^b | 777.40 ^a | 1321.40 ^{ab} |
| 4 | <i>a</i> ^{***} | <i>10</i> | <i>1</i> | - | - | <i>1</i> | <i>10</i> | <i>20/60</i> | <i>45/90</i> | - | - | - | - | - |
| | b | 0.5 | 2.5 | - | - | 2.5 | 5 | 20/room | 30/80 | 30/100 | 30/110 | 4.42 ^{hi} | 791.00 ^a | 1032.50 ^b |
| | c | 0.5 | 2.5 | - | - | 2.5 | 5 | 20/room | 30/80 | 30/100 | 120/110 | 13.74 ^e | 716.00 ^{cd} | 1226.40 ^{cdef} |
| | d | 0.5 | 2.5 | - | - | 2.5 | 5 | 20/room | 30/80 | 30/100 | 240/110 | 26.81 ^b | 730.67 ^{bcd} | 1215.67 ^{cdef} |
| | e | 0.5 | 2.5 | - | - | 2.5 | 5 | 20/room | 30/80 | 30/100 | 270/110 | 26.29 ^b | 790.80 ^a | 1277.60 ^{bc} |
| 5 | a | 3 | - | - | - | - | - | 30/80 | 120/105 | 240/450 | - | 11.96 ^e | 757.75 ^{abc} | 1387.42 ^a |

Values of digestion results are mean of 3 replications. Different letters in the same column indicate significant differences according to LSD test ($P \leq 0.05$)

[†]All the “a” experiments of methods 1, 3 and 4 followed the guidelines found in the literature. The others (b, c, d, etc.) are modifications made to improve results of the previous “a” experiments

Literature: *Navarro-Alarcon *et al.* (21); **Tanabe *et al.* (22); ***Iwegbue *et al.* (23)

Table 2 MP-AES operating and method conditions (linearity and limits of detection (LOD) and quantification (LOQ))

| Operating conditions* | | Na | K | Ca | Mg | Cu | Fe | Zn | Mn | P | Cd | Pb |
|---|----------------------|------------|------------|-------------|---------|---------|---------|---------|----------|----------|---------|---------|
| Wavelength (nm) | | 588.995 | 766.491 | 393.366 | 383.829 | 324.754 | 371.993 | 213.857 | 403.076 | 213.618 | 226.502 | 368.346 |
| Nebulizer flow (L/min) | | 0.95 | 0.75 | 0.60 | 0.90 | 0.7 | 0.65 | 0.45 | 0.9 | 0.35 | 0.5 | 0.75 |
| Viewing position | | 10 | 10 | 0 | 0 | 20 | -10 | 10 | -10 | 20 | 0 | 0 |
| Method conditions | | Na | K | Ca | Mg | Cu | Fe | Zn | Mn | P | Cd | Pb |
| Calibration range (mg/L) | | 0.025-5 | 0.005-5 | 0.5-10 | 0.025-5 | 0.001-5 | 0.010-5 | 0.001-5 | 0.0025-5 | 0.0025-5 | 0.050-5 | 0.025-5 |
| Calibration curve | <i>a</i> (slope) | 196713 | 41.312 | 371030 | 2645.8 | 58.079 | 4.2475 | 10.445 | 21.102 | 202.01 | 1.1166 | 1.0404 |
| | <i>b</i> (intercept) | +1080,8 | 370.2 | 86958 | -40.875 | -274.9 | -53.409 | 81.188 | -258.79 | 5582.7 | -8.5072 | -17.418 |
| Correlation coefficient (r^2) | | 0.9994 | 0.9992 | 0.9990 | 0.9999 | 0.9999 | 0.9997 | 0.9997 | 0.9998 | 0.9993 | 0.9997 | 0.9996 |
| Dilution sample range (v/v) | | Up to 1/50 | Up to 1/25 | No dilution | | | | | | | | |
| LOD (mg/L) | | 0.15 | 0.07 | 0.52 | 0.05 | 0.05 | 0.10 | 0.10 | 0.08 | 0.14 | 0.11 | 0.13 |
| LOQ (mg/L) | | 0.49 | 0.22 | 1.74 | 0.17 | 0.17 | 0.33 | 0.32 | 0.25 | 0.46 | 0.38 | 0.42 |

*Common MP-AES operating conditions to all samples: read time (s): 3; number of replicates: 5; sample uptake time (s): 70; stabilization time (s): 15; pump speed (rpm): 15; sample uptake fast pump: on; background correction: auto; rinse time: 40

Table 3 Recovery tests for minerals added to diluted carob liqueur sample (N:3)

| Essential elements: macroelements | | | | | | | | | | | |
|-----------------------------------|--------------|--------------|-----------------|--------------|-----------------|--------------|----------------|--------------|----------------|--------------|--------------|
| Ca (393.366 nm) | | | Mg (383.829 nm) | | Na (588.995 nm) | | K (766.491 nm) | | P (213.618 nm) | | |
| Added | Found (mg/L) | Recovery (%) | Found (mg/L) | Recovery (%) | Found (mg/L) | Recovery (%) | Found (mg/L) | Recovery (%) | Added | Found (mg/L) | Recovery (%) |
| - | 1.00±0.19 | - | 0.74±0.03 | - | 1.01±0.08 | - | 7.54±0.08 | - | - | 0.00±0.00 | - |
| + 2 mg/L | 3.13±0.01 | 105.57 | 2.83±0.01 | 103.53 | 3.30±0.07 | 109.65 | 9.43±0.05 | 98.86 | + 0.5 mg/L | 0.60±0.00 | 120.00 |
| +2.5 mg/L | 3.66±0.04 | 101.39 | 3.32±0.01 | 102.15 | 3.77±0.08 | 106.09 | 9.92±0.04 | 98.94 | +1 mg/L | 1.18±0.01 | 117.53 |

| Essential elements: trace elements | | | | | | | | | |
|------------------------------------|--------------|--------------|-----------------|--------------|-----------------|--------------|-----------------|--------------|--------------|
| Fe (371.993 nm) | | | Cu (324.754 nm) | | Zn (213.857 nm) | | Mn (403.076 nm) | | |
| Added | Found (mg/L) | Recovery (%) | Found (mg/L) | Recovery (%) | Found (mg/L) | Recovery (%) | Found (mg/L) | Recovery (%) | Recovery (%) |
| - | 0.06±0.01 | - | 0.02±0.00 | - | 0.07±0.01 | - | 0.03±0.00 | - | - |
| + 2 mg/L | 2.19±0.01 | 106.73 | 2.15±0.02 | 106.54 | 2.16±0.02 | 104.65 | 2.26±0.03 | 111.65 | |
| +2.5 mg/L | 2.76±0.0 | 108.15 | 2.71±0.02 | 107.60 | 2.69±0.03 | 104.51 | 2.87±0.04 | 113.69 | |

| Non-essential elements: heavy metals | | | | |
|--------------------------------------|--------------|--------------|-----------------|--------------|
| Cd (226.502 nm) | | | Pb (368.346 nm) | |
| Added | Found (mg/L) | Recovery (%) | Found (mg/L) | Recovery (%) |
| - | 0.00±0.00 | - | 0.00±0.00 | - |
| + 2 mg/L | 1.96±0.02 | 97.93 | 2.22±0.02 | 110.97 |
| +2.5 mg/L | 2.51±0.02 | 100.26 | 2.82±0.04 | 112.65 |

Essential elements

Non-essential elements

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Table 4 Mineral composition (average concentrations in mg/L or $\mu\text{g/L}^*$) and standard deviations in carob, fig and almond Portuguese liqueurs

| Type liqueur | Producer† | Macroelements | | | | | Trace elements | | | | Heavy metals | |
|--------------|---------------------------|----------------------------|----------------------------|---------------------------|-------------------------|--------------------------|------------------------|-------------------------|-------------------------|------------------------|--------------|------------------------|
| | | Na | K | Ca | Mg | P | Cu | Fe | Zn | Mn | Cd | Pb |
| Carob | 1 | 146.83±2.93 ^c | 2458.69±44.58 ^a | 265.50±6.61 ^b | 89.67±5.69 ^a | 3.82±0.09 ^{bc*} | 0.96±0.08 ^g | 2.87±0.29 ^{bc} | 2.89±0.12 ^a | 1.20±0.08 ^a | <LOD | <LOD |
| | 2 | 36.83±3.01 ^e | 86.00±2.78 ^g | 21.33±3.33 ^g | <LOD | <LOD | 0.85±0.16 ^g | <LOD | 0.72±0.17 ^d | <LOD | <LOD | <LOD |
| | 3 | 31.67±1.04 ^e | 738.25±45.69 ^d | 67.17±1.89 ^e | 15.00±0.50 ^d | <LOD | 4.23±0.04 ^b | 0.49±0.15 ^e | 1.44±0.08 ^b | <LOQ | <LOD | <LOD |
| | 4 | 187.67±4.62 ^b | 855.65±17.80 ^c | 141.33±3.33 ^c | <LOD | <LOD | 3.10±0.12 ^c | 2.52±0.13 ^c | 1.03±0.20 ^c | <LOD | <LOD | 2.09±0.35 ^a |
| | 5 | 50.00±8.05 ^{de} | 384.50±9.64 ^f | 46.50±2.00 ^f | 20.50±1.32 ^e | <LOD | 1.27±0.07 ^f | 3.33±0.58 ^b | 2.72±0.11 ^a | 0.27±0.01 ^b | <LOD | <LOD |
| | 6 | 75.83±7.78 ^d | 352.83±5.35 ^f | 72.50±1.32 ^e | 4.41±2.31 ^e | <LOD | 1.66±0.15 ^e | 3.89±0.76 ^a | 0.39±0.01 ^e | <LOD | <LOD | <LOD |
| | 7 | 57.83±6.11 ^{de} | 495.50±8.72 ^e | 61.83±2.84 ^e | 3.91±1.08 ^e | <LOD | 2.37±0.42 ^d | 1.84±0.84 ^d | 2.85±0.38 ^a | <LOQ | <LOD | 1.53±0.38 ^b |
| | 8 | 1165.31±73.62 ^a | 773.90±34.81 ^d | 339.17±25.27 ^a | <LOD | 16.19±0.61 ^{**} | 0.29±0.04 ^h | <LOD | 1.65±0.21 ^b | <LOD | <LOD | <LOD |
| | 9 | 86.67±2.02 ^d | 833.95±17.80 ^c | 89.00±3.28 ^d | 13.00±0.00 ^d | <LOD | 7.60±0.41 ^a | 1.38±0.46 ^d | 1.07±0.08 ^c | <LOD | <LOD | <LOD |
| | 10 | 37.67±3.33 ^e | 1313.56±65.18 ^b | 66.83±2.47 ^e | 37.83±3.55 ^b | <LOD | 2.61±0.12 ^d | <LOD | 1.54±0.11 ^b | <LOQ | <LOD | <LOD |
| | 11 | 29.67±1.26 ^e | 501.00±11.65 ^e | 65.33±2.93 ^e | <LOD | <LOD | 1.31±0.06 ^f | 1.52±0.15 ^d | 0.53±0.04 ^{de} | <LOQ | <LOD | <LOD |
| | $\bar{X} \pm S_{\bar{X}}$ | 173.27±332.90 | 799.44±639.39 | 112.41±99.68 | 16.76±26.89 | 1.94±4.87 [*] | 2.39±2.07 | 1.62±1.40 | 1.53±0.92 | 0.18±0.35 | - | 0.33±0.74 |
| Fig | 1 | 43.17±6.51 ^{bc} | 661.72±15.42 ^b | 31.50±2.29 ^d | <LOD | <LOD | 0.46±0.00 ^e | <LOD | <LOQ | <LOD | <LOD | <LOD |
| | 2 | 36.67±4.01 ^{cd} | 7.93±0.23 ^f | 12.00±0.87 ^f | <LOD | <LOD | 5.55±0.30 ^a | <LOD | <LOD | <LOD | <LOD | <LOD |
| | 3 | 30.67±6.79 ^d | 66.17±0.29 ^e | 117.50±3.28 ^a | <LOD | <LOD | 2.43±0.24 ^b | <LOD | 0.81±0.15 ^a | <LOD | <LOD | <LOD |
| | 4 | 47.50±2.50 ^b | 197.83±5.25 ^d | 42.17±2.75 ^c | <LOD | <LOD | 5.76±0.10 ^a | <LOD | <LOD | <LOD | <LOD | <LOD |
| | 5 | 36.25±2.47 ^{cd} | 341.67±12.27 ^c | 24.25±3.89 ^e | <LOD | <LOD | 0.90±0.04 ^d | <LOD | 0.61±0.07 ^b | <LOD | <LOD | <LOD |
| | 12 | 281.83±6.66 ^a | 1274.72±17.08 ^a | 49.33±0.76 ^b | 17.00±1.80 ^a | <LOD | 1.93±0.03 ^c | <LOD | <LOD | <LOD | <LOD | <LOD |
| | $\bar{X} \pm S_{\bar{X}}$ | 79.35±99.37 | 425.01±477.35 | 46.13±37.36 | 2.83±6.94 | - | 2.85±2.28 | - | 0.30±0.35 | - | - | - |
| Almond | 1 | 31.25±0.35 ^{de} | 27.83±0.76 ^b | 42.50±6.36 ^c | <LOD | <LOD | 0.40±0.03 ^b | <LOD | <LOD | <LOD | <LOD | <LOD |
| | 2 | 35.00±2.78 ^{cd} | 7.46±0.49 ^c | 34.67±4.37 ^d | <LOD | <LOD | <LOQ | <LOD | <LOD | <LOD | <LOD | <LOD |
| | 6 | 39.00±0.50 ^e | 6.57±0.07 ^c | 77.00±5.29 ^a | <LOD | <LOD | <LOQ | <LOD | <LOD | <LOD | <LOD | <LOD |
| | 12 | 55.25±3.18 ^b | 366.67±8.02 ^a | 50.00±9.90 ^{bc} | <LOD | <LOD | 3.46±0.11 ^a | <LOD | 1.24±0.12 ^a | <LOD | <LOD | <LOD |
| | 13 | 22.50±2.60 ^f | 5.91±1.46 ^c | 48.50±3.61 ^{bc} | <LOD | <LOD | <LOQ | <LOD | <LOD | <LOD | <LOD | <LOD |
| | 14 | 29.50±3.91 ^e | 5.87±0.42 ^c | 54.50±4.24 ^b | <LOD | <LOD | <LOQ | <LOD | <LOD | <LOD | <LOD | <LOD |
| | 15 | 121.50±2.18 ^a | 8.92±0.31 ^c | 22.25±1.06 ^e | <LOD | <LOD | <LOQ | <LOD | <LOD | <LOD | <LOD | <LOD |
| 16 | 28.25±1.77 ^e | 7.00±0.10 ^c | 78.25±4.60 ^a | <LOD | <LOD | <LOQ | <LOD | <LOD | <LOD | <LOD | <LOD | |
| | $\bar{X} \pm S_{\bar{X}}$ | 45.28±32.31 | 54.53±126.34 | 50.96±19.29 | - | - | 0.56±1.18 | - | 0.16±0.44 | - | - | - |

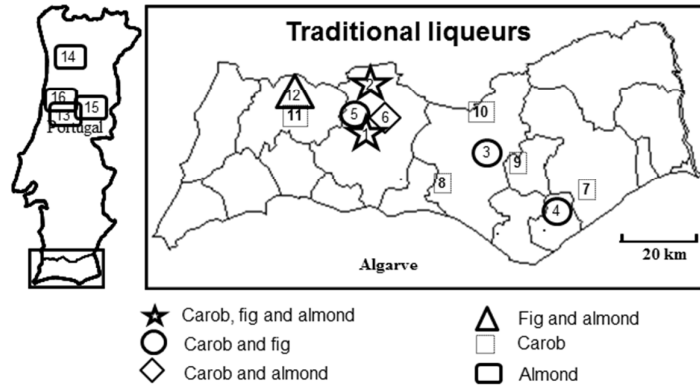
Values of results are mean of 3 replications. Different letters in the same column for each type of liqueur indicate significant differences according to LSD test ($P \leq 0.05$)

$\bar{X} \pm S_{\bar{X}}$ = mean concentration of 3 replications and standard deviation; LOD: Limit of detection, LOQ: Limit of quantification. Different letter in the same column indicate significant differences according to LSD test ($P \leq 0.05$); † the same numbers indicate the same producers

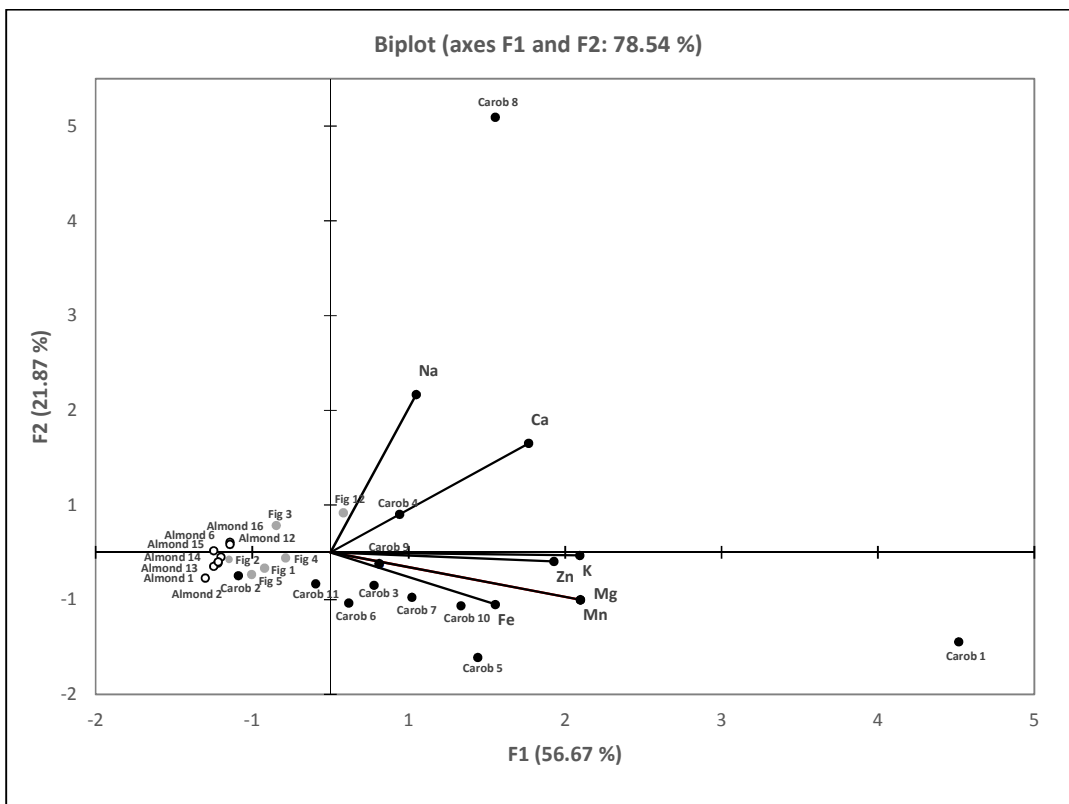
Figure 1 Geographical location of different carob, fig and almond liqueurs studied in this work

Figure 2 Principal Component Analysis (PCA) score plot for commercial carob, fig and almond liqueurs based on mineral composition of the digested samples

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