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

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Abstract

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 HNO_3/H_2O_2 (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.

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

Introduction

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

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

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

In general, the constituents of liqueurs come mainly from the fruits and other inorganic and organic materials used in their elaboration (*4, 10*), but can also be added during the different steps of the preparation process (*3, 11*). Minerals present in fruits depend in turn on factors such as the cultural practices (e.g. the fertilizer addition) and the environmental conditions (e.g. exposure to exhaustion gases, industry wastes and waste waters polluted), which normally affect the content of essential and non-essential minerals, respectively (*12, 13*).

In order to determine the mineral composition in liqueurs, mineralization is a preliminary step necessary to reduce interferences caused by the presence of organic matrix, and so avoid an increment in optical background. This step involves the conversion of the metals associated with the material into a form that can be properly determined[6]. For this purpose, there are two basic techniques: wet mineralization and dry process, also known as calcination. Wet mineralization uses different acids (HNO_3, HCl, H_2SO_4, H_3PO_4, $HClO_4$, and HF), oxidants (H_2O_2), or mixtures thereof to enhance the digestion of the samples due to the reactive ability of the mixture in oxidizing organic matter. The election of conditions and reagents (the strength, purity and safety of the acid, its oxidizing power, boiling point and the salts solubility) will depend on the sample nature and devices used, open or close vessels (*14*). In turn, calcination uses small amounts of reagents and presents high yields and simple instrumental requirements.

Microwave plasma atomic emission spectrometry (MP-AES) is an easy to use technique with high performance, high speed and not requiring hazardous (eliminating flammable and oxidizing gases) and expensive gases (using nitrogen plasma instead the argon plasma used in other techniques), which makes the determination of minerals advantageous in comparison with other spectroscopic and spectrometric techniques. These characteristics make MP-AES improve safety, analytical performance, and reduces operating costs, therefore it has been introduced recently in the mineral characterization of different foods, such as wine (*15*), cheese (*16*), bread (*17*), and plants, such as herbal medicines (*18*), and sunflower (*19*).

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

Materials and methods

Reagents and liqueur samples

All reagents used were of analytical grade: HNO_3 (65%) (Fisher Scientific, Pittsburgh, PA), $HClO_4$ (60%) (Riedel-de Häen, Seelze, Germany), H_2O_2 (30%) (Merck Suprapur, Darmstadt, Germany), Vanadium (V) oxide (98%) (Sigma-Aldrich, United Kingdom). Working standards of the metals Al, Cu, Cd, Pb, Zn, and Mn (stock solution of 50 ppm), Ca, Mg, Na, K, and Fe (stock solution of 100 ppm) and P (stock solution of 10 ppm) were prepared by diluting concentrated stock solutions (Agilent Technologies, Santa Clara, CA) with 5% of nitric acid/Milli-Q water. The ultra-pure water was employed to dilute the samples and standards, and this water was obtained by filtering tap water through a Milli-Q purifier (Millipore Waters, Milford, MA, USA).

A set of 25 commercial liqueurs samples from the principal Portuguese producing regions were evaluated: eleven carob and six fig liqueurs from Algarve region (south of Portugal) and eight almond liqueurs from Algarve and Douro Littoral regions (south and north of Portugal, respectively) (Fig. 1). Most of the samples were kindly provided directly by the producers and a small part was purchased at local markets.

Sample pretreatment: liqueur mineralization

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

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

Wet mineralization in open vessels

Glass digestion vessels were previously cleaned in 10% (v/v) nitric acid solution to avoid cross-contamination. Digestions were carried out in triplicate with uncovered glass tubes using a digital dry bath (Accublock Digital Dry Bath, Labnet International, New Jersey, USA), at the temperatures and times indicated in Table 1. The methods tested mixed HNO_3 with other acids such as $HClO_4$ (method 1 and 2) and HCl (method 3), and the strong oxidant H_2O_2 (method 4). Once the digestion was finished, each sample was removed from the dry bath, cooled to room temperature until next day and then diluted up to 25 mL with Milli-Q water.

Dry mineralization in a muffle furnace

Digestion was carried out in pots as indicated in method 5 (Table 1**)**. Initially, the sample was subjected to low temperatures (80 and 105 ºC) in an oven with assisted air circulation to evaporate and remove all residual water before ashing. Then, the temperature was increased to 450 ºC on a muffle furnace (Thermolyne, Type 1500 Furnace; Sybron Corp., Dubuque, IA) to proceed with the mineralization until the sample acquires whitish colour of ashes. The ashes were dissolved in 10 mL of HNO_3 acid and diluted with Milli-Q water up to 50 mL.

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

Analytical performance of the method

The limits of detection (LOD) and quantification (LOQ) of the method, showed in Table 2, were obtained based on the parameters of the analytical curves using standards with acid matrix in 5% HNO_3. Both limits were calculated according to the following mathematical equations (1 and 2):

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

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

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

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

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

MP-AES: mineral analysis of liqueurs

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

A 6-point calibration curve was carried out for each element in matrix-matched calibration solutions (5% HNO_3) to account for matrix interferences. All the details about calibration curve can be seen in Table 2. After appropriate dilution of samples in 5% HNO_3, they were analyzed in triplicate and the concentrations calculated using the external standard calibration method.

Data analysis

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

Results and discussion

Optimization of the sample digestion procedure

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

In general, no significant differences were observed among the methods when employing the same operating conditions (times and temperatures). This can be observed for Fe concentrations in experiments 1c, 2c, 1e, 3d, 4e, and 2d, 4d; for K in experiments 1d, 2d, 3c, 4d and 1e, 3d, 4e; and for Na in experiments 1e and 4e (Table 1). In the case of Fe concentrations, the major difference lies in maintaining the sample at elevated temperature for longer. Probably, shorter periods are not enough to eliminate the compounds able to complex Fe (*6*). The concentrations of Na and K remain practically unchanged in all methods.

Among all the methods studied, 3 and 4 were those that revealed the best results, with higher concentrations of Fe, K and Na, indicating more complete digestions of carob liqueur. Moreover, the results obtained agree with published data based on the digestion of alcoholic drinks, such as liqueurs, anisettes, cognacs, whiskies, gins, rums and wines using the mixture HNO_3/H_2O_2 (method 4) ([Fe] ≅ <0.01 mg / L to 7.16 mg / L; [K] ≅0.13 mg / L to 1014 mg / L and [Na] ≅ trace to 215.3 mg / L) (*11, 23, 27, 28*) and regarding the digestion of milk samples using the HNO_3/HCl mixture (method 3) ([K] ≅ 1687 mg / Kg to 27253 mg / Kg and [Na] ≅ 407 mg / Kg to 4784 mg / Kg) (*22*). In face of the good performance in digesting the cream carob liqueur and since it requires smaller amounts of acid (higher concentrations may reduce the MP-AES tube lifetime by acid attack), method 4 was selected to be used in the subsequent tests.

Calibration and analytical method performance

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

Under the optimized MP-AES conditions, LOD and LOQ were calculated using the equations described in the section 2.3. Table 2 shows the low values of LOD and LOQ for all the minerals studied. Linear regression analysis for each element was performed by the external standard calibration. The validating parameters of each calibration curve, slope (*a*), intercept (*b*) and correlation coefficient (r^2) are described in Table 2. Good linearity was observed between intensities and concentrations over the range tested (r^2:0.9990-0.9999) for all the analyzed elements.

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

Mineral content in liqueurs: essential elements

Results of the mineral composition of the studied liqueurs (carob, fig and almond liqueurs) are presented in Table 4. There is a high variability in concentrations within each type of liqueur, as demonstrated by the high standard deviations. This is may be due to different manufacturing processes and also to the raw materials (mainly, fruits and water) used to elaborate the liqueurs. The influence of these factors can be verified since some producers were cooperative in sharing their elaboration process. Carob liqueurs 1, 2 and 8 presented the major differences (Table 4). Producer of sample 1 (with high quantities for all elements studied) uses high temperatures (infusion) during the extraction process, the sample 8 (with high quantities of some macroelements) is the only cream-based liqueur, and finally, sample 2 (with the lowest quantity of studied elements) used alcohol of agricultural origin and maceration at room temperature for the liqueur elaboration. Taking this into consideration, the sample 2 and 12 of fig liqueur (respectively, with the lowest and the highest content in macroelements), and samples 1 and 12 of almond liqueur (with K concentrations 3 to 5 and 40 to 61 times highest, respectively) may also indicate the same influence.

Macroelements

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

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

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

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

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

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

Ca concentration in liqueurs from the producers 2, 6 and 12 showed similar quantities, while the values in samples from producers 1, 4 and 5 followed the trend almond $<$ fig $<$ carob, except producer 3 that has the opposite trend. In general, the highest concentrations of Ca were observed in carob liqueurs (112.41 mg/L), close to the double of fig (46.13 mg/L) and almond (50.96 mg/L) liqueurs. If we eliminate sample 8, a liqueur containing cream (rich in Ca), the mean value drops to 89.73 mg/L, still a very high value. Interestingly, the concentration of Ca in sample 8 (339.17 mg/L) is about the double of the value reported by Iwegbue *et al*. (*35*) for a cream liqueur (162.86 mg/L). Mg was the macroelement present in minor amounts and was only detected in seven of the eleven carob liqueurs studied and one of the six fig liqueurs. The mean concentration found in carob liqueurs (16.76 mg/L) was similar to concentrations found in other fruit liqueurs: peach (14.16 mg/L), apple (12.85 mg/L), plum (17 mg/L), banana (11 mg/L), rose hip (18 mg/L), and black currant liqueur (22 mg/L)(*10, 33, 36*). According to the previous discussion, these high concentrations of macroelements present in carob and fig liqueurs can be explained by the contribution of the fruits used in the preparation. The results are consistent with works found on the study of minerals in carob flour and figs, where K is one of the most abundant elements (67.00 % and 68.48 % respectively), followed by Ca (22.46 % in carobs and 20.48 % in figs), Mg (1.51 % in carobs and 5.44 % in figs) and Na (0.84 % in carobs, and 5.46 % in figs) (*37, 38*). In general, this trend was found in carob and fig liqueurs for K (72.03 % and 76.38 %, respectively), Ca (10.13 % and 8.29 %, respectively) and Mg (4.09 % and 0.51%, respectively). In both type of liqueurs, Na (15.61 % in carob and 14.26 % in fig liqueurs) presented highest values. The different proportion of Na, mainly in the case of carob liqueurs, can be due to the addition of acidity regulators such as sodium citrate or sodium bicarbonate (*4*). Since the concentrations of macroelements in almonds are usually high (*39*), the low concentrations of these elements in the almond liqueurs analyzed can be explained by the replacement of fruits by natural flavouring essences (Table 4).

The last macroelement analyzed is phosphorus. Although this element was observed in high concentrations in carob pod (*38*), and fig (*40*), P was only present in two samples of carob liqueurs in small concentrations (3.82 ± 0.09 and 16.19 ± 0.61 µg/L).

Trace elements

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

Usually the traditional stills constructed almost totally with copper provide more of this element (5 ppm) to the produced spirit compared to the new units (with values < 1 ppm) (Soufleros, Mygdalia, & Natskoulis, 2005), which only use copper in some parts of the circuit whilst the rest is made of stainless steel (*41*). Accordingly, carob liqueurs 3, 4, 7, 9 and 10; fig liqueurs 2, 3, 4 and 12 and almond liqueur 12, were probably elaborated with spirits obtained in traditional stills due to the high copper concentrations (1.93 mg/L – 7.60 mg/L) (Table 4), whilst samples of carob liqueurs 1, 2 and 8; fig liqueur 1 and almond liqueur 1, with values below 1 mg/L, probably employed spirits/alcohol elaborated from industrial systems. Finally, in an intermediate position are the carob liqueurs number 5, 6 and 11 with values between 1.27 - 1.66 mg / L and the fig liqueur number 5 with a value of 0.90 mg / L of copper. Despite having these low values we know that they used a traditional system with improvements (good initial cleaning of the equipment, some parts of the equipment are made of stainless still, and/or by reducing its concentration by methods as the use of activated carbon) to obtain a high quality spirit.

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

The trend of the concentration of this element found in the samples in relation to the processing region is as follows: liqueurs from western Algarve have higher concentrations of Fe (> 3.3 mg/L), except sample 11 (1.52 mg/L), than those from eastern Algarve (< 2.6 mg/L). As in the case of copper, the concentrations of Fe present in the tap water from the same regions (year 2015) were always bellow 0.025 mg/L (*30*), indicating again that the tap water cannot be the main source of this mineral. Comparing the presence of this element in carob liqueurs with other liqueurs from literature, similar concentrations were found in samples of cream (1.31 mg/L) and peach liqueurs (1.50 mg/L) and higher values in plum (2.16 mg/L), cherry (2.43 mg/L) and black currant liqueurs (2.79 mg/L) (*10, 23, 33*).

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

As it is shown in Table 4, Zn is present in all carob liqueurs (concentrations ranging 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 (1.24 mg/L). The concentrations in carob liqueurs have lower values (< 0.75 mg/L) in samples from western Algarve, except sample 5 (2.72 mg/L), than those from eastern Algarve (> 1.0 mg/L). According to the literature, this mineral showed lower mean values, ≤ 1 mg / L, in other fruit liqueurs (*10, 23, 33*).

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

Mineral content in liqueurs: non-essential elements

Heavy metals

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

Principal component analysis

Principal component analysis (PCA) was applied using the correlation matrix with the well correlated variables. Consequently, the model was simplified taken into account a more restricted number of variables. Among the set of variables that contributed to obtain the PCA analysis with the two first components are the 7 essential elements (K, Na, Ca, Mg, Fe, Zn and Mn) that were the most representatives of the whole system, and 25 samples (11 of carob, 8 of almond and 6 of fig liqueur). PCA showed that the first two principal components extracted explain 78.54 % of the total variance [F1 (56.67%) and F2 (21.87%)].

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

Conclusions

The digestion pretreatment and MP-AES method proposed in this work to quantify different essential and non-essential minerals in liqueur matrices was optimized. For the digestion of liqueurs, the use of any of the reagents studied would be optimal because with the same operational conditions, the results showed no significant differences. HNO_3/H_2O_2 mixture was chosen because it showed a slight better sample digestion according to the elements of study, with a lower quantity of digesting acid. MP-AES demonstrated to be a good technique for the multielement analysis of these samples, since the method presented good linearities and recoveries, and low LOD and LOQ for the elements analyzed.

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

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

The lack of detection of non-essential elements in 98% of the studied liqueurs and the concentrations of essential elements in allowable ranges indicate the generalization of good manufacturing practices of these drinks. Thus, a moderate consumption of these 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	Reagents					Time/Temperature (min/°C)						
		(mL)	HNO_3(mL)	HNO_3:$HClO_4$ (1:4) (mL)	HCl (mL)	H_2O_2 (mL)	V_2O_5 (µg)	Step 1	Step 2	Step 3	Step 4	Fe	K	Na
1	***a****	***1***	***5***	***5***	-	-	***10***	***20/60***	***45/90***	-	-	***6.10***^{gh}	***670.50***^{e}	***1177.38***^{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	***a***	***1***	-	***10***	-	-	-	***20/60***	***45/90***	-	-	***7.53***^{fg}	***700.5***^{de}	***1286.38***^{bc}
	b	0.5	-	5	-	-	-	20/room	30/80	30/100	30/110	4.19^{hi}	767.33^{ab}	1069.00^{h}
	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	***a*****	***0.5***	***4***	-	***1***	-	-	***20/room***	***30/80***	***30/100***	***30/110***	***8.44***^{f}	***777.07***^{a}	***954.67***^{i}
	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	***a******	***10***	***1***	-	-	***1***	***10***	***20/60***	***45/90***	-	-	-	-	-
	b	0.5	2.5	-	-	2.5	5	20/room	30/80	30/100	30/110	4.42^{hi}	791.00^{a}	1032.50^{h}
	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	*a* (slope)	196713	41.312	371030	2645.8	58.079	4.2475	10.445	21.102	202.01	1.1166	1.0404
y=*a*x+*b*	*b* (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 (%)**
-	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

Table 4 Mineral composition (average concentrations in mg/L or µg/L*) and standard deviations in carob, fig and almond Portuguese liqueurs

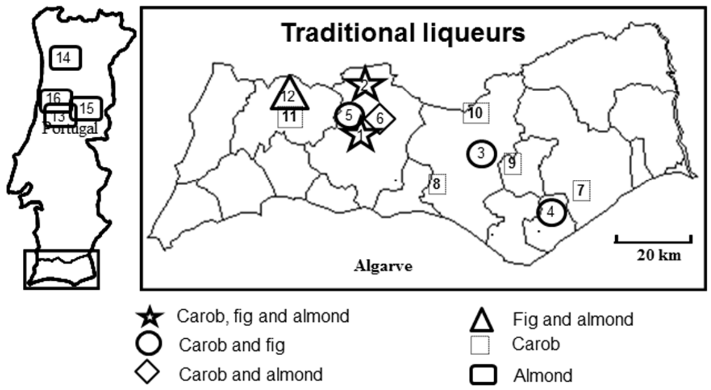
		Macroelements					Trace elements				Heavy metals	
Type liqueur	**Producer†**	**Na**	**K**	**Ca**	**Mg**	**P**	**Cu**	**Fe**	**Zn**	**Mn**	**Cd**	**Pb**
Carob	1	146.83±2.93^{c}	2458.69±44.58^{a}	265.50±6.61^{b}	89.67±5.69^{a}	3.82±0.09^{b}*	0.96±0.08^{g}	2.87±0.29^{bc}	2.89±0.12^{a}	1.20±0.08^{a}	<LOD	<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^{c}	<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^{a}*	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^{c}	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	-	-	-

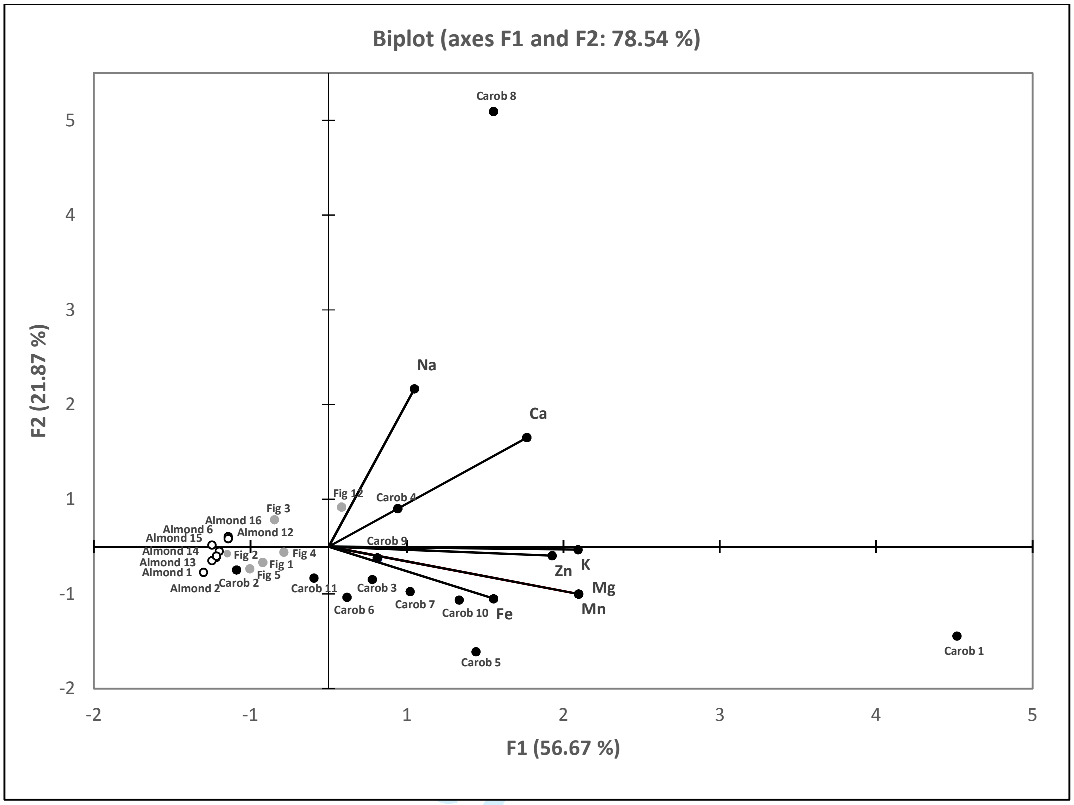
Values of results are mean of 3 replications. Different letters in the same column for each type of liqueur indicate significant differences according to LSD test ($P \leq 0.05$)

$X \pm S_x$ =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


Traditional liqueurs
14
16
15
13
Portugal
12
11
2
5
6
1
10
3
9
8
7
4
20 km
Algarve
Carob, fig and almond
Carob and fig
Carob and almond
Fig and almond
Carob
Almond


Biplot (axes F1 and F2: 78.54 %)
F2 (21.87 %)
F1 (56.67 %)
Carob 8
Na
Ca
Fig 12
Carob 4
Fig 3
Almond 16
Almond 6
Almond 12
Almond 15
Carob 9
Almond 14
Fig 2
Fig 4
Almond 13
Fig 1
Almond 1
Fig 5
K
Zn
Almond 2
Carob 2
Carob 11
Carob 3
Mg
Carob 6
Carob 7
Carob 10
Fe
Mn
Carob 5
Carob 1
5
4
3
2
1
-1
-2
-2
-1
1
2
3
4
5