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Additional Information

Antioxidant characteristics of honey from Mozambique based on specific flavonoids and phenolic acid compounds

ABSTRACT

The most recent guidelines of IUPAC and AOAC recommend the analysis of specific compounds present in antioxidant fractions. For the first time, honey from different provinces of North (Nampula) and Central Mozambique (Sofala, Manica and Zambezia) was analysed considering specific flavonoids and phenolic acid profiles. Seven phenolic acids (chlorogenic, caffeic, ellagic, ferulic, gallic, p-coumaric and sinapic) and eight flavonoids (catechin, chrysin, kaempferol, luteolin, naringenin, pinocembrin, quercetin and rutin) were screened in the samples. Nampula honey had a higher content of most of these compounds and the total antioxidant activity (even reaching up to 40 mg TE/100 g) compared honey from the other provinces. Unlike in other African honeys, luteolin had the greatest impact in the flavonoid content (in some cases up to 72 mg/100 g), representing alone more than 50% of this family. Resulting from a discriminant analysis, specific flavonoids (pinocembrin, kaempferol, rutin and catechin) followed by the chlorogenic phenolic acid were the most important variables that distinguishes Nampula from the other provinces. This work underlines the importance of Mozambiquean honey as a source of natural antioxidants both of which concern the health benefits and its exploitation as a viable and sustainable income for the local population.

Keywords: Phenolic-acids, Flavonoids, Antioxidant activity, Honey characterization, Food analysis, Food Composition.

1. Introduction

Honey production could be considered among one of the potential means to support the creation of sustainable livelihoods in rural African communities (Bradbear, 2005; Serem and Bester, 2012). Several regions of Mozambique have favourable conditions (climate, melliferous flora, and vast forest areas) for exploiting apiculture (Merkel, 2019; Zandamela, 2008). However, despite its potential (estimated at 3,600 tonnes a year, considering the current resources) beekeeping has not reached its full capacity (Jooste and Smith, 2004). According to FAOSTAT, (2016), the honey production in this country stands at 600 tonnes/year but a positive trend has been observed in the last years. Apiculture may produce more benefits to the rural communities of Mozambique than just the actual beekeeping, because it generates other economic revenues related with the assets and resources necessary for this practice (commerce, carpenters, garment makers, packaging processors, among others).

Western societies market trends aimed at searching for healthy foodstuff with antioxidant properties. The requirement for natural antioxidants is growing as they play an important role in human health: avoiding damage produced by oxidising agents and having anti-inflammatory, anti-carcinogenic or anti-atherosclerotic effects, just to cite a few (Samarghandian et al., 2017). Among the most important groups of compounds with antioxidant activity (vitamins, carotenoids and polyphenols), honey is especially rich in polyphenols, and more specifically in flavonoids and phenolic acids (Oroian and Escriche, 2015). Hence, honey is highly valued for its therapeutic characteristics underscoring the importance of the presence of antioxidant compounds which is widely dispersed throughout the plant kingdom (Silici et al., 2010). These compounds could be transferred to honey when bees collecting nectar of blossoms or exudates from plants and trees. It is well known that botanical, geographical and climatic conditions influence the

composition of flavonoids and phenolic acids of honey (Escriche et al., 2014; Gheldof et al., 2002; Sime et al., 2015; Tomás-Barberán et al., 2001). Consequently, specific compounds or relevant content could be found in some types of honey: naringenin, caffeic acid and hesperetin, in citrus blossom honey (Escriche et al., 2011); kaempferol in rosemary honey; quercetin in sunflower honey (Tomás-Barberán et al., 2001) ellagic acid in heather honey (Antony et al., 2000); and caffeic acid, p-coumaric and ferulic in chestnut honey (Merken and Beecher, 2000), among others.

Ample data has been published about polyphenols identified and quantified in different types of honey and countries, including stingless bee honey (Biluca et al., 2017). However, this information is scarce when it refers to African honeys; and when there is, it almost only deals with the total antioxidant capacity (mainly due to the inexpensive spectrophotometric methods, accessible chemical reagents and they do not require sophisticated equipment or highly trained personnel and protocols) (Granato et al., 2016). As an example, several studies could be mentioned of honey from: Ethiopia (Sime et al. 2015), Burkina Faso (Beretta et al., 2005) and South Africa (Serem and Bester, 2012). There are a few exceptions since some flavonoids and other specific antioxidant compounds in honey from Tunisia and Sudan, have been reported (Makawi et al., 2009; Martos, et al. 1997).

Turning the focus on Mozambiquean honey, there is very little scientific data (Escriche et al., 2017; Tanleque-Alberto et al., 2019) and there is no evidence referring to its antioxidant characteristics, neither its specific compounds nor total antioxidant capacity. Therefore, a better understanding of the properties of this uncommon honey centered on its antioxidant characterization is now more than ever of the utmost importance. With this in mind and with the purpose of obtaining the most comprehensive information, it is

recommended to analyse specific compounds of the antioxidant fraction, in line with the most recent statements of IUPAC (International Union of Pure and Applied Chemistry) and AOAC (Association of Official Agricultural Chemists) (Apak, 2013; Editorial, 2017), together with nonspecific analytical methods since the majority of the available data refers to them.

Taking all these factors into consideration, the aim of this work was to determine the specific flavonoids and phenolic acids profiles and the total antioxidant activity in honey from the different provinces of Mozambique.

2. Material and methods

2.1 Collection of honey samples

Honey from Mozambique, collected between 2014-2015 was analysed in this study: 20 samples from Nampula in the North (districts of Moma, Angoche and Ribáuè) and, 15 from Zambezia, 15 from Manica, and 20 from Sofala, in the Centre. In all cases each, sample consisted of 750 g that were obtained in the period of one month personally by one of the coauthors of the present work. In Nampula and Zambezia honey samples were acquired directly from the beekeepers which came from the handling of traditional beehives built with local resources, depending on the availability (twigs, trunks and barks). In Sofala and Manica, samples were purchased at the "Mozambique honey Company". Here, honey comes from beekeepers using more up-to-date modular beehives "Langstroth type". Once all the samples were grouped locally, they were sent to the laboratory, of Universitat Politecnica de Valencia, Spain, and stored at 7-9 ºC until the analyses were carried out.

With the aim of having a more comprehensive understanding of the honey samples used in the present work a parallel characterized from the point of view of their botanical

99 origin, volatile profile, physicochemical and rheological parameters was carried out
100 (Escriche et al., 2017; Tanleque-Alberto et al., 2019).

101 *2.2. Chemical and materials*

102 All the target standards (purity higher than 98%): caffeic acid, chlorogenic acid, ellagic
103 acid, ferulic acid, gallic acid, p-coumaric acid, sinapic acid, catechin, chrysin, kaempferol,
104 luteolin, naringenin, pinocembrin, quercetin, rutin and galangin as well as the 2,2-
105 diphenyl-1-picrylhydracil (DPPH) were obtained from Sigma-Aldrich (St. Louis, USA).
106 These compounds were selected due to their usual presence in honey (Escriche et al.,
107 2014; Lo Dico-Gianluigi et al., 2019).

108 Trolox [(6-hydroxy acid)-2,5,7,8-tetramethylchroman-2-carboxylic acid], was acquired
109 from Scharlab (Barcelona, Spain). Acetonitrile (HPLC grade) and methanol (HPLC
110 grade) were purchased from J. T. Baker (Deventer, Netherlands). Formic acid (97%),
111 hydrochloric acid and sulfuric acid were from Sigma-Aldrich (Steinheim, Germany).
112 Milli-Q water was prepared in-house with a water purification system (Millipore, USA).
113 The SPE cartridges (Strata-X, 33 μm, 3 mL, 200 mg sorbent) were supplied by
114 Phenomenex (Torrance, CA, USA) and syringe filters (13mm, PTFE membrane, 0.45
115 μm) were adquired from Scharlab (Barcelona, Spain).

116 Stock standard solutions of each flavonoid and phenolic acid were obtained at a
117 concentration of 1 mg/mL in methanol. The working standard solutions were prepared by
118 diluting the corresponding stock solution up to a concentration of 100 ng/mL in water.
119 The stock standard solutions were stored at -20ºC and the working at +4ºC.

120 *2.3. Specific phenolic acids and flavonoid compounds analysis*

The extraction of phenolic acids and flavonoid compounds was carried out submitting the honey samples to a solid-phase extraction following the methodology described by Bertoncelj et al. (2011). The honey extracts obtained were then analyzed using a HPLC-Alliance 2695, with a 2996 photodiode array detector (Waters, USA). Phenolic acids and flavonoids compounds were separated on a Brisa-LC, C18 column (250 x 4,6 mm x 5 μm) (Teknokroma, Spain). The binary mobile phase consisted of ACN (acetonitrile) as mobile phase A, and water: formic acid (99:1 v/v) as mobile phase B. The gradient program was: 0 min, 90% B; 25 min, 40% B; 26 min, 20% B; holding up to 30 min; 35 min, 90% B; holding up to 40 min. This means that the total run of each chromatogram was 40 min. The column was maintained at 30ºC. The flow-rate and the injection volume were 0.5 mL/min and 10 μL, respectively. All compounds were identified by comparison of chromatographic retention times and UV spectral characteristics (200-400 nm) of unknown analytes with authentic standards and the available literature (Escriche et al., 2011; Merken and Beecher, 2000).

The quantification was performed through calibration curves constructed via least squares linear regression analyses of the peak area versus their respective concentration. With the aim of avoiding the matrix effect on the quantification, these calibration curves were obtained by spiking the standards in the honey matrix. For this, the curves for chlorogenic acid, catechin, rutin, ellagic and luteolin were made by adding the appropriate amounts of each standard to a final concentration in the sample from 0.2 to 2 mg/100g. While for the curves built for caffeic acid, ferulic acid, gallic acid, p-coumaric acid, sinapic acid, chrysin, kaempferol, naringenin, pinocembrin and quercetin, the standards were added to achieve a concentration in the samples of 0.5 to 15.0 mg/100g. The quantitative results were expressed in mg of compound per 100 g of honey. To check the

145 stability of the chromatographic method, a standard solution was injected at the beginning
146 of each working session.

147 *2.4. Determination of the total antioxidant capacity*

148 The antioxidant activity of the samples and standard (Trolox) was determined by way
149 of the radical scavenging activity method using 2,2-diphenyl-1-picrylhydrazyl radical
150 (DPPH) described by Scherer and Godoy, (2009). The quantification was calculated with
151 the Trolox curve (0.01-0.80 mg/mL), expressed in mg of Trolox equivalent (TE) per 100
152 g of honey. The DPPH was the method of choice, because it was reported as the most
153 commonly applied for the determination of the total antioxidant capacity of African
154 honey. By using the same method, the comparison of results is more feasible (AOAC,
155 2011; Apak et al., 2013 Cicco et al., 2009).

156 All analyses were performed in triplicate.

157 *2.5. Statistical analysis*

158 A one-factor-analysis-of-variance (ANOVA) (using Statgraphics Centurion XVII for
159 Windows) was carried out to evaluate the effect of the honey origin on the flavonoids and
160 phenolic acids compounds. LSD (least significant difference) test and α=0.05 were
161 applied. Moreover, a Principal Component Analysis (PCA) by means of the software
162 Unscrambler (X.10.5 CAMO) was also applied to evaluate the relationship between the
163 quantified compound and the different provinces. The PCA cross validation analysis was
164 performed by previously centering (mean) and scaling (standard deviation) the data. The
165 SPSS 16.0 software was used to carry out the stepwise linear discriminant analyses (by
166 'forward' procedure) and the bivariate Pearson correlations (significance level $\alpha = 0.05$),
167 the latter in order to measure the strength and direction of the linear relationships between

168 pairs of variables. The values of the 70 samples were considered to conduct the statistical
169 analyses.

170 **3. Results and discussion**

171 *3.1. Phenolic acids, flavonoids and total antioxidant activity*

172 In a first step, the goodness of the analytical method was tested; carrying out its
173 validation. The linearity was assessed using matrix-matched calibration curves at the
174 same concentration levels as performed for the quantification. A good linearity was
175 obtained, with R^2 values ranging from 0.9948 for ellagic acid to 0.9996 for sinapic acid,
176 kaempferol and rutin. The accuracy of the method was determined through recovery
177 experiments using the same concentration levels as for linearity. The precision was
178 obtained through the repeatability and reproducibility, expressed as the relative standard
179 deviation (RSD). The repeatability was calculated from the analysis of five samples,
180 performed on the same day, fortifying samples at three of the levels used for the
181 quantification (low, medium and high), and to evaluate the reproducibility these analyses
182 were repeated on three consecutive days. The RSD of the repeatability for all compounds
183 was less than 6% and its reproducibility was always less than 10%. In this work, detection
184 limit (LOD) and quantification limit (LOQ) were calculated as the amount of analyte for
185 which signal-to-noise ratios (S/N) were higher than 3 and 10, respectively. The LOD
186 values ranged from 0.05 for caffeic acid to 0.10 for rutin and LOQ values from 0.2 for
187 caffeic acid to 0.5 for luteolin. The validation results are detailed as Supplementary data
188 (Table S1).

189 Table 1 shows the 7 phenolic acids (caffeic, chlorogenic, ellagic, ferulic gallic, p-
190 coumaric and sinapic) and 8 flavonoids (catechin, chrysin, kaempferol, luteolin,
191 naringenin, pinocembrin, quercetin and rutin) found in the honey samples analysed. Nine

192 of these compounds (caffeic acid, ferulic, gallic, *p*-coumaric acid, chrysin, kaempferol,
193 luteolin, pinocembrin, and quercetin) were also reported in North African honey (Algeria)
194 (Ouchemoukh et al., 2017). These authors found an additional flavonoid (galangin) that
195 was not detected in any Mozambiquean samples, although this was one of the target
196 compounds of the present work.

197 This table also shows the quantitative results of these compounds: average values
198 (expressed as mg of compound per 100 g of honey) with the corresponding standard
199 deviation and the minimum and maximum values for every compound and province. Also
200 included is the total antioxidant activity (expressed as mg TE/100 g honey). In all cases
201 the ANOVA results (with homogeneous groups, F-ratio and significant level) are
202 described. The whole data set corresponding to all honey samples analysed is shown as
203 Supplementary data (Table S2).

204 Honey from Nampula had a total average content of phenolic acids (10.64 mg/100 g);
205 quite high if compared to honey from the other provinces: Sofala (6.62 mg/100 g), Manica
206 (6.83 mg/100 g) and Zambezia (5.67 mg/100 g). This is mainly due to the quantity of
207 chlorogenic acid present in Nampula honey, with an average value of 5.25 mg/100 g,
208 being significantly greater than in the other provinces 0.99 mg/100 g for Sofala, 1.12
209 mg/100 g for Manica and 0.81 mg/100 g for Zambezia. Sinapic was the other phenolic
210 acid with statistical significant differences among provinces, but in this case, the level of
211 this compound was slightly higher in Zambezia. Despite the high content of chlorogenic
212 acid found in this study, its content in honey from other origins is highly variable. It was
213 not reported in other African honey (Makawi et al., 2009; Martos et al., 1997), nor in
214 American tropical honey (do Nascimento et al. 2018), but it was found in significant

amounts in honey from other origins such as Italy (Lo Dico et al., 2019) and even in stingless bee honey (Biluca et al., 2017).

In general, the levels of other phenolic acids compounds quantified in the honey from Mozambique differ considerably with honey from other countries, even when coming from tropical climates. For instance, gallic acid found in American tropical honey (from n.d to 36.18 mg/100 g, do Nascimento et al. 2018) was reported in higher levels than in the present work (up to 1.61 mg/100 g). In other African honey, this compound was not even detected (Makawi et al., 2009; Martos et al., 1997). Intermediate values for gallic acid, were determined in honey from Turkey, ranging from n.d. to 8.2 mg/100 g, depending on the type of honey (Can et al. 2015). Another example could affect the p-coumaric acid, where the contents in Mozambiquean honey varied from 0.2 to 1.9 mg/100 g, in a similar order to what was found in European (0.12-0.81 mg/100 g) (Escriche et al., 2011) or Turkish (n.d.-1.59 mg/100 g) honey (Can et al., 2015, but greater than in Brazilian honey (n.d.-0.20 mg/100 g) (do Nascimento et al., 2018).

Regarding the flavonoids, the total average content had similar behaviour as phenolic acids since the highest level corresponded to samples from Nampula with a value of 30.45 mg/100 g, followed by Sofala (19.54 mg/100 g), Zambezia (15.22 mg/100 g) and Manica (12.95 mg/100 g). In general, in all the provinces the flavonoid content was higher than the phenolic acids. However, in tropical honey from Brazil the situation was the opposite, phenolic acids were higher than flavonoids (Bueno-Costa et al., 2016). Nevertheless, this comparison should be taken with caution, since in the present work the total flavonoid and phenolic content was obtained as the sum of all the compounds resulting from the chromatographic analysis, whereas in the cited reference the spectrometric techniques were used for the same purpose.

239 Four out of seven flavonoid compounds (catechin, kaempferol, pinocembrin and rutin)
240 were significantly higher in Nampula province, whereas the differences were not so
241 remarkable among the other provinces. Luteolin was the major flavonoid in the 4
242 provinces with average values of 15.54; 13.19; 6.65 and 8.54 mg/100 g in Nampula,
243 Sofala, Manica and Zambezia. It is worth mentioning the high values found for this
244 compound in some samples from Nampula and Sofala reaching a maximum value up to
245 72.00 mg/100 g and 22.05 mg/100 g. However, the minimum value for this compound
246 was around 6 or 7 mg/100 g in all the provinces. Luteolin had the greatest impact in the
247 average total flavonoid content in these two provinces since, in some cases, it represented
248 more than 50% of this value. Nevertheless, this compound was found in very low
249 concentration in honey from other origins such as: Tunez (up to 0.011 mg/100g) (Martos
250 et al., 1997), Europe (average value of 0.063 mg/100 g) (Escriche et al., 2011) and it was
251 not even detected in Turkish honey (Can et al., 2015).

252 Among the other flavonoids quantified in Mozambique honey, catechin is remarkable,
253 since it was present in all samples with average values from 2.61 mg/100 g in Manica to
254 6.62 mg/100 g in Nampula. However, this flavonoid was not reported in other African
255 honey (Makawi et al., 2009), although in different varieties of Turkish honeys it reached
256 the level of 2.3 mg/100 g (Can et al., 2015). The concentration of quercetin and
257 kaempferol, was of a similar order as in the present study compared to what was
258 determined in honey from Sudan with different botanical origin (average values 0.54
259 mg/100 g for quercetin and 0.32 mg/100 g for kaempferol) (Makawi et al. 2009). Only in
260 some samples from Nampula the values of these compounds were up to 2.44 mg/100 g
261 and 5.9 mg/100 g, respectively. Lower contents were reported in honey from Tunisia
262 (quercetin 0.013-0.123 mg/100 g and kaempferol 0.009-0.136 mg/100 g) (Martos et al,.
263 1997).

The total antioxidant activity in the analysed samples followed a similar pattern to flavonoids. The mean value of antioxidant activity was also higher for the Nampula samples (21.74 mg TE/100 g). However, in this case it did not show significant differences with those from Sofala (mean value of 18.42 mg TE/100 g). The value of total antioxidant activity ranged from 4.80 mg TE/100 g in a Manica sample to 40.05 mg TE/100 g in a Nampula sample.

In general, certain similarities are observed among the antioxidant characteristics of Sofala, Manica and Zambezia provinces, in contraposition to Nampula, which may be due to the likeness of the flora and climatic conditions of these three provinces (FAO-Governo de Moçambique, 2009; Merkel, 2019; Zandamela, 2008).

The comparison of the total antioxidant activity values among the samples analysed in the present study was valid since the method (DPPH) and the analytical conditions were always the same. Notwithstanding, it is not appropriate to compare the total antioxidant values with other studies because it could have been obtained with other nonspecific analytical methods (ABTS, FRAP, ORAC, TEAC, among others.) based on different mechanisms. Furthermore, even when using the same analytical method, other condition-based factors (pH, solvent, and sample matrix) could have a significant influence in the variability of the results (AOAC, 2011; Apak et al., 2013).

It is important to mention the study performed by Serem and Bester, (2012) since its analytical procedure is comparable to this present work despite using a different way to express the results (µmol TE/g instead of mg TE/100 g). These authors found 1.74 µmol TE/g in South African honey, in the same order as Mozambiquean honey, considering the unit conversion. Other valid examples are the studies conducted by Attanzio, et al. (2016) in European honeys (with average values from 8.5 to 238.4 µmol TE/100 g) or by Rosa

et al. (2011) in Italian honey with a total antioxidant activity average value of 4.8 mmol TE/kg.

3.2. Relationship among antioxidant characteristics

To determine the possibility of a correlation between the different antioxidant data, a Pearson correlation coefficient was obtained for each pair of variables (Table 2). This table shows the correlation matrix obtained together with the corresponding P-value (number in brackets), which indicates the statistical significance of the estimated correlations at 95.0% confidence level. Although the correlations between total antioxidant activity and some specific compounds were significant (since P-values were below 0.05), the linear relationship between each pair of variables is weak as the values are far from +1 or -1. This is the case of 3 phenolic acids: ferulic, chlorogenic, and p-coumaric with values of 0.458, 0.448 and 0.436, respectively and the flavonoid catechin (0.478). Authors do Nascimento et al. (2018) showed in Brazilian honey that the total antioxidant activity (analysed by DPPH) was also positively correlated with p-coumaric (0.415) similar to the present study. As for gallic acid, the same behaviour (no good linear relationship and negative sign) was observed both by these authors and the present work (-0.399 and -0.284).

In Mozambiquean honey the best correlations are shown for some specific compounds. For instance, chlorogenic acid was positively correlated with 4 flavonoids: catechin (0.939), kaempferol (0.639), luteolin (0.473) and rutin (0.364). The strong correlation between chlorogenic acid and catechin (0.939), was considered the best among all variables. Other good correlations were obtained between luteolin/kaempferol (0.901), rutin/pinocembrin (0.816) and luteolin/quercetin (0.790). In general terms, with the only

311 exception of the chlorogenic acid, the greatest correlations are observed between specific
312 flavonoids.

313 Publications about correlation between specific antioxidant compounds in honey are
314 practically non-existing which makes the comparison between the data hereby obtained
315 with previous studies difficult. The only example that could be used for this purpose is
316 the aforementioned study of do Nascimento et al. (2018), where only gallic acid, p-
317 coumaric acid and quercetin compounds were considered and, as in the present study, no
318 significant correlation was reported. However, there is ample data referring to honey from
319 different geographical and botanical origin, regarding the good correlation observed
320 between total antioxidant activity (obtained with different nonspecific analytical
321 methods) and total phenolic/total flavonoid contents (Alvarez-Suarez et al., 2010;
322 Escuredo et al., 2012; Gül and Pehlivanb, 2018; Serem and Bester, 2012). These
323 correlations could make sense, since the latter parameters, although not being solely
324 responsible for the antioxidant capacity of honey, in fact contribute to it (Gheldof et al.,
325 2002).

326 A PCA was carried out to evaluate the global effect that the province of Mozambique
327 has considering 15 variables (7 phenolic acids and 8 flavonoids) of honey based on
328 specific compounds and total antioxidant activity. In this analysis the average values from
329 the three repetitions for each sample of honey were used. Figure 1 shows the PCA biplot
330 obtained (scores and loadings for the two principal components) considering all the
331 antioxidant variables and the different provinces. It was found that three principal
332 components explained 73% of the variations in the data set: PC1 37% of the variability,
333 PC2 23% and PC3 13%. The first principal component clearly differentiates Nampula
334 honey (left quadrant) from the other provinces (right quadrant), the second principal

component slightly separates Manica (upper quadrants) from Sofala and Zambezia (lower quadrants), without noticeable differences between these last two provinces. The third component (not present in the figure), reaffirms the separation of the Nampula samples. The loading plot shows that certain compounds are to some extent responsible for this differentiation.

The information provided by both ANOVA and PCA for all the antioxidant variables indicates (as mentioned above), that some of them are more relevant than others for the differentiation of honeys. To discern which variables contribute the most to this difference, a discriminant analysis was applied. This model was obtained by using the specific antioxidant compounds and the total antioxidant capacity, permitting the classification of 99.9% of the cross-validated cases.

Table 3 shows the standardized canonical discriminant function coefficients obtained. In the construction of the two first discriminant functions, five variables (one phenolic acid and four flavonoids) were used. The most important variables in function 1 (which separates Nampula from the other provinces) were the flavonoids: pinocembrin (1.488 mg/100g), kaempferol (1.441 mg/100g), rutin (1.258 mg/100 g) and catechin 1.104 (mg/100g) showing small differences among them In the second discriminant function the most important variables were catechin followed by chlorogenic acid.

Table 4 shows the classification results (expressed as percentages) of the discriminant analysis, demonstrating a correct classification for Nampula honey (100%). However, 40% of honey from Sofala was incorrectly classified (20% coming from Manica and 20% coming from Zambezia). In the same way honey from Manica was correctly classified with 75%. However, 25% of honey from this province was inaccurately classified as coming from Sofala. The percentage of Zambezia honey (20%) was mistaken for honey from Sofala. The incorrect classification always takes place involving the three provinces

of Central Mozambique (Manica, Sofala and Zambezia). Although there are three different provinces, they share similar botanical and climate conditions (Merkel, 2019). However, Nampula is located in the North of the country where its higher pluviosity leads to the existence of a peculiar melliferous flora different from to the rest of the country. This different vegetation seems to give to the honey originated in this province a certain singularity with respect to its antioxidant properties. In the same way that in other African countries that diverse climatic conditions and flora lead to the existence of different types of honey containing a wide range of total phenols and antioxidant activities (Sime et al., 2015).

4. Conclusion

This research study has set a precedent concerning the antioxidant characteristics of honey from Northern and Central Mozambique, focusing on specific flavonoid and phenolic compounds. In general, flavonoid was higher than phenolic content in honey from all provinces studied. Honey from Nampula (in the North) showed significantly higher values of phenolic acids and flavonoid compounds compared to the other three provinces located in the Centre of the country (Sofala, Manica and Zambezia), where the differences among them were not so noteworthy. Therefore, the climatic and consequently botanical conditions play an important role in the profile of the compounds studied. Luteolin was the most important flavonoid from the quantitative point of view, representing more than 50% of the specific flavonoids in the 4 provinces, being especially abundant in some samples from Nampula. The most important variables, which distinguish Nampula from the other provinces, were the flavonoids in the following order: pinocembrin, kaempferol, rutin and catechin; followed by chlorogenic acid.

383 This study offers the opportunity increase the knowledge of Mozambiquean honey. The
384 specific flavonoid and phenolic compounds analysed could become a powerful tool by
385 putting in value a totally unknown African honey, a result of the health implications of
386 its antioxidant properties. This research could be useful in supporting decision makers
387 when it comes time to successfully market this type of honey.

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392 **Figure caption**

393 **Figure 1.** PCA biplot of score [samples honey from different provinces of Mozambique:
394 Nampula (♦), Sofala (■), Manica (●) and Zambezia (ж)] and loading (specific flavonoids
395 and phenolic compounds and total antioxidant capacity).

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Table 1. Mean (and standard deviation), minimum and maximum values of the phenolic acids, flavonoids and total antioxidant, compounds of the honey samples from different provinces of Mozambique (Nampula, Sofala, Manica and Zambezia), and ANOVA F-ratio for the factor "province".

	Nampula		**Sofala**		**Manica**		**Zambezia**		
	Mean (SD)	Min/Max	Mean (SD)	Min/Max	Mean (SD)	Min/Max	Mean (SD)	Min/Max	**ANOVA F-ratio**
Phenolic acids (mg/100g of honey)									
Caffeic acid	0.83 (0.18)	0.60/1.13	0.89 (0.45)	0.49/1.90	1.23 (0.75)	0.60/2.53	0.49 (0.04)	0.38/0.60	ns
Chlorogenic acid	5.25 (2.31)^{b}	0.72/7.08	0.99 (0.97)^{a}	0.57/4.92	1.12 (0.44)^{a}	0.60/1.89	0.81 (0.02)^{a}	0.78/0.84	13.8***
Ellagic acid	2.00 (0.39)	1.59/2.81	2.31 (1.12)	1.15/5.23	2.12 (0.51)	1.50/2.97	1.86 (0.35)	1.65/2.23	ns
Ferulic acid	0.59 (0.20)	0.31/0.91	0.73 (0.40)	0.04/1.24	0.48 (0.18)	0.24/0.87	0.56 (0.10)	0.44/0.74	ns
Gallic acid	0.53 (0.12)	0.30/0.63	0.60 (0.35)	0.34/1.48	0.82 (0.46)	0.48/1.61	0.49 (0.03)	0.44/0.54	ns
p-coumaric acid	1.14 (0.49)	0.21/1.93	0.73 (0.31)	0.21/1.21	0.70 (0.25)	0.32/1.00	0.88 (0.12)	0.69/1.11	ns
Sinapic acid	0.30 (0.03)^{a}	0.27/0.37	0.37 (0.08)^{a}	0.67/0.54	0.36 (0.06)^{a}	0.27/0.48	0.58 (0.20)^{b}	0.28/0.89	3.8*
Total average of phenolic acids and SD	**10.64 (3.73)**		**6.62 (3.72)**		**6.83 (2.60)**		**5.67 (0.66)**		
Flavonoids (mg/100g of honey)									
Catechin	6.62 (1.88)^{b}	2.95/9.34	3.33 (1.27)^{a}	2.08/6.74	2.61 (0.48)^{a}	2.08/3.56	3.62 (0.48)^{a}	2.74/4.44	12.5***
Chrysin	0.49 (0.18)	0.33/0.86	0.35 (0.04)	0.33/0.43	0.46 (0.12)	0.33/0.76	0.44 (0.04)	0.39/0.48	ns
Kaempferol	2.02 (1.29)^{b}	0.79/5.90	0.57 (0.29)^{a}	0.19/1.02	0.62 (0.20)^{a}	0.38/1.22	0.65 (0.10)^{ab}	0.52/0.82	4.1*

Luteolin	15.54 (15.69)	5.6/72.00	13.19 (5.54)	6.62/22.05	6.65 (0.20)	6.49/7.00	8.54 (1.12)	6.70/11.00	ns
Naringenin	1.20 (1.22)	0.35/4.07	0.63 (0.19)	0.35/0.93	0.71 (0.38)	0.40/1.47	0.436 (0.004)	0.430/0.440	ns
Pinocembrin	0.90 (0.35)[b]	0.46/1.70	0.36 (0.05)[a]	0.32/0.51	0.05 (0.13)[a]	0.36/0.77	0.39 (0.03)[a]	0.34/0.44	8.6***
Quercetin	0.91 (0.75)	0.12/2.44	0.34 (0.27)	0.08/0.90	0.29 (0.24)	0.05/0.75	0.40 (0.07)	0.30/0.54	ns
Rutin	2.73 (1.23)[b]	0.89/4.41	0.78 (0.37)[a]	0.23/1.47	1.15 (0.45)[a]	0.60/1.80	0.76 (0.09)[a]	0.60/0.90	9.4***
Total average of flavonoids acids and SD	**30.45 (22.60)**		**19.54 (8.03)**		**12.95 (2.26)**		**15.22 (1.92)**		
Total antioxidant capacity (mg TE/100 g honey)	21.74 (6.59)[b]	13.06/40.05	18.42 (7.70)[b]	6.25/28.02	10.04 (5.78)[a]	4.80/25.74	15.54 (6.08)[ba]	9.31/26.05	11.9***

Different letters in the same row indicate significant differences at 95% confidence level as obtained by the LSD test.

ns: Non significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table 3. Standardized canonical discriminant function coefficients

Variables	**Function 1**	**Function 2**
	98.5%	**1.4%**
Pinocembrin	1.488	-0.087
Kaempferol	1.441	-0.069

Rutin	1.258	0.102
Catechin	1.104	2.261
Chlorogenic acid	0.439	-1.995

Table 4. Classification results of the discriminant analysis carried out by cross validated procedure. Percentage of samples well classified by the model**.**

Province	**Predicted Group Membership**			
	Nampula	Sofala	Manica	Zambezia
Nampula	100	0	0	0
Sofala	0	60	20	20
Manica	0	25	75	0
Zambezia	0	20	0	80

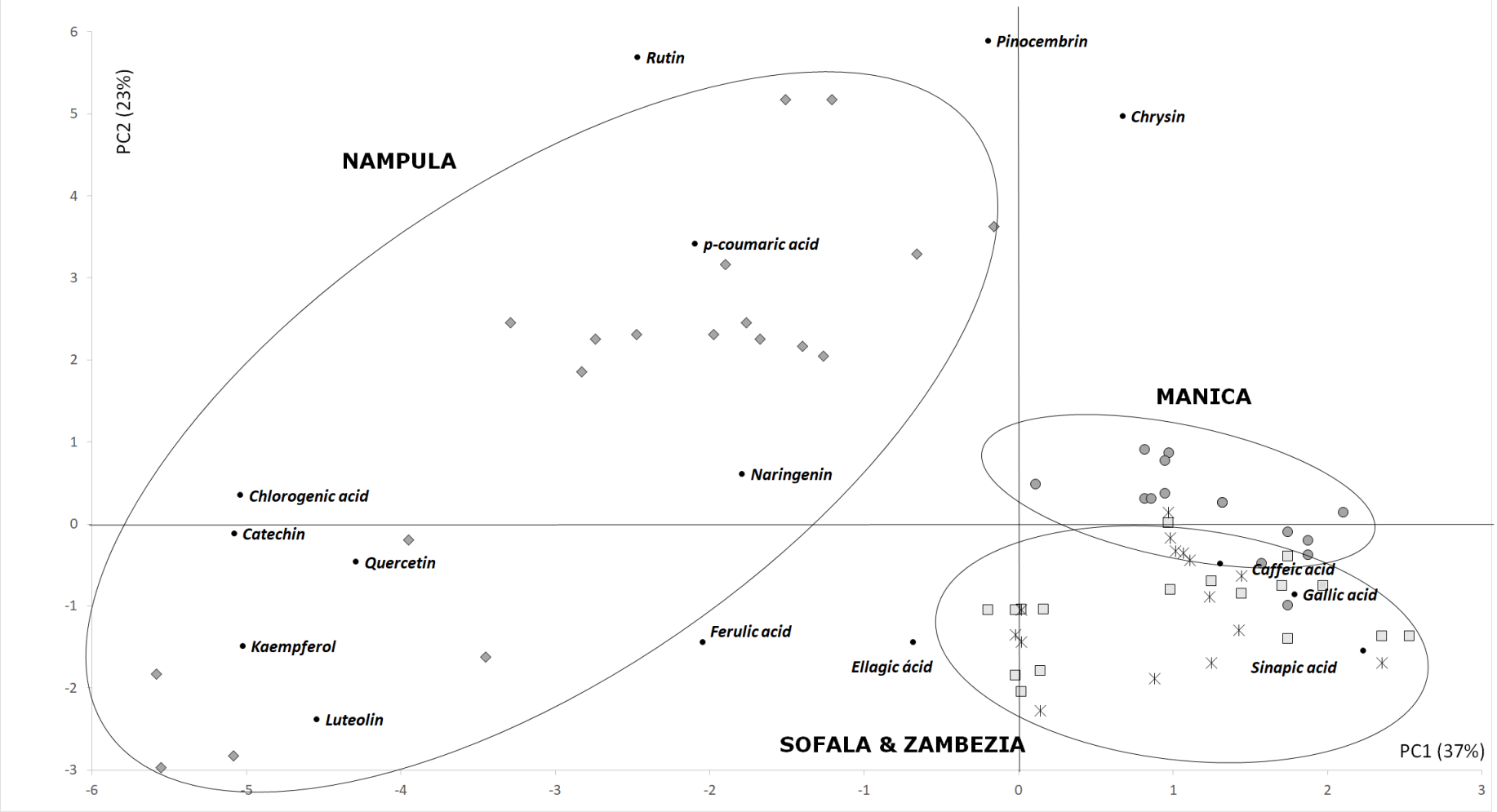
Table 1-Supplementary Material. Validation parameters of the analytical method.

	UV Identifycation λ(nm)	UV Quantifycation λ(nm)	R^2	Recovery (%) (n=5)			Repeatability (%RSD) (n=5)			Reproducibility (%RSD) (n=9)		
Phenolic acids				Low*	Medium*	Low*	Low*	Medium*	Low*	Low*	Medium*	High*
Caffeic acid	288; 298; 318	320	0.9970	94	69	88	3	4	2	3	8	9
Gallic acid	220; 271	290	0.9965	96	94	102	4	5	5	9	7	6
p-coumaric acid	207; 260	320	0.9972	112	107	108	3	2	1	9	9	8
Sinapic acid	220; 280	320	0.9996	105	99	101	3	2	2	5	5	4
Chrysin	313	320	0.9960	93	107	110	5	5	4	6	9	9
Kaempferol	265; 318	360	0.9996	119	118	105	5	4	4	7	8	8
Naringenin	289	290	0.9994	106	115	116	2	5	5	9	9	7
Pinocembrin	290	290	0.9970	93	97	101	2	3	2	4	6	6
Quercetin	226; 350	360	0.9990	118	110	115	1	2	3	3	5	4
Flavonoids				Low**	Medium**	High**	Low**	Medium**	High**	Low**	Medium**	High**
Chlorogenic acid	219; 241; 315	320	0.9981	89	92	89	3	4	3	4	2	1
Ellagic acid	205; 235; 280	290	0.9948	98	102	105	1	1	1	3	1	1
Catechin	220; 289	290	0.9957	102	107	98	5	2	4	2	6	1
Luteolin	268	320	0.9981	101	105	100	3	4	2	3	5	6
Rutin	260; 355	360	0.9996	99	101	103	2	5	4	8	1	6

* Low level = 0.5mg/ 100g; Medium level = 8.0mg/ 100g; High level = 15.0mg/ 100g

** Low level = 0.2mg/ 100g; Medium level = 1.0mg/ 100g; High level = 2.0mg/ 100g

Figure 1


PC2 (23%)
PC1 (37%)
NAMPULA
MANICA
SOFALA & ZAMBEZIA
Rutin
Pinocembrin
Chrysin
p-coumaric acid
Naringenin
Chlorogenic acid
Catechin
Quercetin
Kaempferol
Luteolin
Ferulic acid
Ellagic ácid
Caffeic acid
Gallic acid
Sinapic acid
6
5
4
3
2
1
0
-1
-2
-3
-6
-5
-4
-3
-2
-1
0
1
2
3